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Poster Presentations Abstracts

P-001

Methyl jasmonate ameliorates testosterone propionate-induced prostatic hyperplasia in castrated Wistar rats

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Keywords: Benign prostate hyperplasia; Castration; methyl jasmonate; antioxidants; prostate

Benign prostate hyperplasia (BPH) is a progressive disease that is related to age. The search for therapeutic agents to treat BPH is ongoing. We investigated the ameliorative effects of methyl jasmonate (MeJA) in testosterone propionate (TP)-induced BPH in castrated rats. Castration was performed by removing both testes through the scrotum sack under ketamine anaesthesia. Rats were assigned into 7 groups of 7 animals as follows: non-castrated control, castrated control, castrated rats that received TP only, castrated rats that received TP and MeJA, castrated rats that received TP and Finasteride (Positive control), castrated rats that received MeJA only and castrated rats that received Finasteride only. Results indicate that BPH rats had significantly ($p < 0.05$) elevated prostate weight and relative weight of prostate. BPH rats had significantly ($p < 0.05$) increased activities of prostatic acid and alkaline phosphatases, levels of zinc and malondialdehyde. Levels of enzymic and non-enzymic antioxidative indices were significantly ($p < 0.05$) reduced in BPH rats. Histology revealed hyperplasia of prostatic transition lobe and, increased expression of PSA & Ki67. Treatment with MeJA and finasteride reduced metabolic stress via attenuation of activities of the phosphatases & levels of antioxidants. MeJA ameliorates TP-induced prostatic hyperplasia via antioxidative mechanism.

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P-003

Ambient UV-B exposure attenuate the binding affinity of ofloxacin with bacterial DNA gyrase and induced apoptosis in human keratinocytes via Reactive Oxygen Species mediated pathway

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Keywords: Ofloxacin; UV-B; Reactive oxygen species(ROS); DNA damage; apoptosis

Ofloxacin (OFLX) is a known synthetic broad spectrum antibiotics which inhibit bacterial DNA topoisomerase activity. Previous studies showed reduced antimicrobial activity of photosensitized OFLX and its photo-products. Here, we have addressed the binding affinity of OFLX and its photo-products against DNA gyrase to measure the antimicrobial activity. Further, the study was extended to explore the molecular mechanism of photogenotoxicity on human keratinocyte cells (HaCaT) under environmental UV-B irradiation. Photochemical experiments showed the generation of Reactive Oxygen Species (ROS) such as $^1O_2$, $O_2^{\bullet-}$ and $^{\bullet}OH$ by photosensitized OFLX. OFLX shows a concentration dependent decrease in cell viability of HaCaT through MTT and NRU tests. Significant intracellular ROS generation was measured by DCFDA assay. ROS caused an oxidative DNA damage via single stranded DNA breaks, micronuclei and CPD formation. OFLX induced cell cycle arrest in G1 phase with appearance of sub-G1 peak. OFLX triggered apoptosis via permeabilization of mitochondrial membrane with the downregulation of anti-apoptotic Bcl-2 and caspase-3 whereas, upregulation of pro-apoptotic Bax and Cyto-C proteins. Our study illustrated that binding affinity of

photo-products of OFLX with DNA gyrase is mainly responsible for attenuated antimicrobial activity. Thus, study suggests that sunlight exposure should avoid by drug users during peak hours.

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P-005

Oxidative stress markers in Cuban centenarians

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Keywords: Oxidative stress; Aging; Centenarians; Oxidation

Human longevity is a multifactorial condition with both genetic and environmental contribution. There exist a whole variety of hypotheses which try to elucidate the mechanisms of aging. One of them, free radical theory of aging, supports a role for oxidative stress as a determinant of the rate of aging. The aim of this work has been to evaluate some oxidative stress markers in Cuban centenarians. In the present study we include 350 centenarians from four different provinces of Cuba. Plasma levels of MDA, AOPPs and free thiol groups as well as the activities of SOD1 and CAT were measured. All techniques were performed using spectrophotometric methods. Levels of plasma MDA and AOPPs were highest in centenarians compared to the respective younger groups. Additionally SOD1 activity differs between the groups, showing significantly decreased in individuals between 61 to 99 years. However, this result did not apply to the centenarians group. Interestingly, CAT activity did not significantly differ between these older groups. Plasma free thiols in centenarian were significantly lower than all age group. In the present study, we report the age-dependent alterations in biomarkers of oxidative damage and confirming the occurrence of oxidative damage to biomolecules during the aging process.

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P-006

Cytochrome c- cardiolipin complex: from peroxidase to Fenton chemistry

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Keywords: Cardiolipin; cytochrome c; lipid modification; Fenton chemistry

The small heme protein cytochrome c (cyt c) has important functions as redox carrier in the mitochondria. While located in the intermembrane space, it is closely associated with the mitochondria-specific lipid cardiolipin (CL). Upon release from stressed mitochondria, cyt c is also involved in apoptosis induction. However, in functioning mitochondria the physiological significance of alterations in cyt c-CL interaction remains largely unknown. Our studies have demonstrated a hydrogen peroxide-dependent iron release from cyt c, which resulted in “Fenton-like” lipid modifications in CL-containing liposomes in the presence of reducing agents, like ascorbic acid or glutathione.

By using HPTLC and MALDI-TOF MS we could identify phosphatidic acid and phosphatidylhydroxyacetone as the fragmentation products of free radical transformations of the polar head group of cardiolipin. Moreover, in the hydrophobic part, we observed the formation of CL aldehydes, lyso-CL and lyso-CL aldehydes. The named products could have physiological significance as phosphatidic acid is a secondary messenger in mitochondria, while lyso-CL is involved in the reactions with the apoptosis-related protein tBid. Therefore, our results may suggest a non-enzymatic pathway for the production of CL-derived biologically active compounds.

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P-007

Loss of KRIT1 causes a sustained activation of an adaptive cellular allostatic response that counteracts intrinsic oxidative stress but sensitizes cells to further oxidative challenges

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Keywords: Cerebrovascular disease; KRIT1 (CCM1); NRF2/HO-1; NRF2/GLO1; Oxidative stress and adaptive cellular responses

KRIT1 (CCM1) is a disease gene responsible for Cerebral Cavernous Malformations (CCM), a major cerebrovascular disease affecting 0.3–0.5% of the population.

Previously, we demonstrated that KRIT1 loss-of-function is associated with altered ROS homeostasis, activation of the redox-sensitive transcription factor c-Jun, and increased cell sensitivity to oxidative stress, raising the possibility that KRIT1 dysfunction exerts pleiotropic effects on multiple redox-sensitive mechanisms.

To address this possibility, we investigated redox-sensitive systems that play a critical role in cellular responses to oxidative stress, including the master NRF2 antioxidant defense pathway and its downstream targets, including Heme Oxygenase-1 (HO-1) and Glyoxalase 1 (GLO1).

The outcomes of our experiments showed that KRIT1 loss-of-function induces a redox-sensitive adaptive upregulation of NRF2, HO-1 and GLO1, resulting in a sustained cellular allostatic response that counteracts intrinsic oxidative stress but sensitizes cells to further oxidative challenges.

While extending the pleiotropic functions of KRIT1, these findings shed new light on the mechanistic relationship between KRIT1 loss-of-function and enhanced cell susceptibility to oxidative damage, thus providing valuable new insights into CCM pathogenesis and

novel options for the development of preventive and therapeutic strategies.

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P-008

Formation of cyanogen iodide by heme peroxidases

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Keywords: Lactoperoxidase; myeloperoxidase; immune defense; cyanogen iodide

The heme-containing proteins lactoperoxidase (LPO) and myeloperoxidase are important enzymes involved in immune defence reactions. They are well known for their ability to oxidise in a hydrogen peroxide-driven reaction thiocyanate, iodide and in case of myeloperoxidase also bromide and chloride to the corresponding hypo(pseudo)halous acids. Here we describe the formation of cyanogen iodide (ICN) as a novel product of these enzymes. The formation of ICN was evidenced by both ^{13}C-nuclear magnetic resonance spectroscopy by applying ^{13}C-labeled thiocyanate and by head-space gas-chromatography mass spectrometry. This product is only formed when iodide is used in excess over thiocyanate during these reactions.

Furthermore, the formation of bactericidal components by the LPO-hydrogen peroxide-thiocyanate/iodide system was investigated on a luminescent *E. coli* strain. This approach delivered time-resolved data about the killing of these microorganisms. In the mixed presence of thiocyanate and iodide (with an excess of iodide) a considerably higher killing rate was observed than in the sole application of either ion species. These data correspond well with the direct apllication of ICN. Control experiments of the determination of colony-forming units confirmed the results. Our data offer a novel product pathway that may be important for numerous biotechnological application of LPO.

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P-009

Oxygen levels and free-radical processes in biosystems

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Keywords: Oxygen; radicals; oxidation; fragmentation; inhibitors

Free-radical processes play an important role in functioning of living organisms. The probability of occurrence of the free-radical reactions is significantly affected by oxygen. Depending on the organ type and the nature of tissues and cells, the oxygen levels vary within the range of about 1 to 15%. The diffusion-limited reaction of O_2 with the carbon-centered radicals of biomolecules leads to their oxidation and oxidative destruction, accompanied with formation of cytotoxic products, which is deemed to be the main cause of the damage to the lipids, nucleic acids and proteins. In our studies, we have shown that the radicals formed from hydroxyl-containing molecules under conditions of low oxygen levels undergo various fragmentation reactions before interaction with O_2 takes place. These reactions result in causing damage to the initial substances and formation of bioactive products. Such reactions prevail when the reactive oxygen species interact with hydrocarbons, hydroxyl-containing glycerophospholipids, amino acids, peptides and sphingolipids.

In the report, the issues will be discussed associated with the influence of oxygen and the structure of biologically relevant substances on the probability of various free-radical transformations to occur, the possible consequences of such processes and the ways of controlling them.

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P-010

UV-induced free radical transformations of sphingolipids

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Keywords: Photolysis; sphingolipids; nitrogen-centered radicals

Photochemical transformations of membrane lipids can lead to their modification and/or destruction, and consequently cause changes in their biological functions. Among the most studied photoinduced reactions of glycerophospholipids are the processes of lipid oxidation and oxidative degradation. Sphingolipids unlike glycerophospholipids resistant to peroxidation processes. At the same time, these lipids are part of the plasma membranes of skin cells and exposed to UV light.

We have sown that direct action of UV irradiation on sphingomyelins in water dispersions induces the Norrish type I decomposition with the formation and subsequence rupture of nitrogen-centered radicals. In the case of sphingosine, containing a free amino group, its photolysis can lead to the photodissociation and formation of N-centered radicals due to n,σ^* - transition. The main products of sphingolipids photodestruction were unsaturated aldehydes, which possess a wide spectrum of biological activity. The photolysis of sphingosine and sphingomyelin with the addition of sensitizers expands the range of photolysis products via the possible formation of N- and C-centered radicals.

The results of this study can broaden the frontier of research regarding to the mechanisms of photochemical reactions of sphingolipids that may be involved in the development of UV-erythema of the skin, radiation damages.

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P-011

Mycosporine-like amino acid activation of the Keap1-Nrf2 pathway

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Keywords: Antioxidant; Nrf2; Natural Products; Oxidative stress; Mycosporine-like amino acid

Oxidative stress is a contributing factor in the progression of numerous pathological conditions including neurodegeneration, cancer and ageing. The Keap1-Nrf2 pathway is a master regulator of oxidative stress: reactive oxygen species are sensed by Keap1 to release Nrf2 for transcriptional activation of protective genes controlled by the nuclear antioxidant response element. We have identified homologs of Nrf2 in early eukaryotes and used virtual screens to predict natural products able to activate Nrf2 by competitive inhibition of Keap1-Nrf2 binding.

Mycosporine-like amino acids (MAAs) are water-soluble metabolites produced by taxonomically diverse organisms, particularly marine algae and seaweeds. These compounds absorb UV radiation – thus acting as “primary sunscreens” – and reported also to protect against oxidative damage.

We have tested the MAAs, porphyra-334, shinorine, and palythine for in-vitro antioxidant activity using the DPPH free-radical quenching assay and report also their ability to activate the Keap1-Nrf2 pathway using fluorescence polarization and thermal shift assays to detect Keap1 receptor antagonism. Our results demonstrate that shinorine and porphyra-334 are competitive inhibitors of Keap1-Nrf2 binding having potential to protect against UVR via sunscreen absorption and transcriptional activation of endogenous defenses against UV-induced oxidative damage.

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P-012

The Enzymatic Nature of Ascorbate Recycling

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Keywords: Ascorbate; Dehydroascorbate Reductase; X-ray Crystallography; Structure; Normal Mode Analysis

Dehydroascorbate reductase (DHAR) catalyzes the glutathione (GSH)-dependent reduction of dehydroascorbate and plays a direct role in regenerating ascorbic acid, an essential plant antioxidant vital for defense against oxidative stress. DHAR enzymes bear close structural homology to the glutathione transferase (GST) superfamily of enzymes and contain the same active site motif, but most GSTs do not exhibit DHAR activity. The presence of a cysteine at the active site is essential for the catalytic functioning of DHAR, as mutation of this cysteine abolishes the activity. Here we present the crystal structure of DHAR2 from Arabidopsis thaliana with GSH bound to the catalytic cysteine. This structure reveals localized conformational differences around the active site which distinguishes the GSH-bound DHAR2 structure from that of DHAR1. We also unraveled the enzymatic step in which DHAR releases oxidized glutathione (GSSG). To consolidate our structural and kinetic findings, we investigated potential conformational flexibility in DHAR2 by normal mode analysis and found that subdomain mobility could be linked to GSH binding or GSSG release.

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P-013

Copper(II)-induced Cytotoxicity and Oxidative Stress in Human Blood Cells and its Attenuation by Carnosine

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Keywords: Copper; carnosine; PMRS; oxidative stress

Copper (Cu) is widely present in environment, mainly in +1 and +2 oxidation states. Exposure to excess Cu has adverse effects on human health. Cu toxicity is thought to be mediated through oxidative stress. The protective effect of carnosine, a dipeptide with antioxidant and antiglycating properties, against Cu(II) induced cytotoxicity was examined. Human erythrocytes and lymphocytes were incubated with 0.5 mM $CuCl_2$ for 1 h in presence and absence of carnosine. Cell lysates were prepared and analyzed for several parameters. Treatment of cells with $CuCl_2$ elevated carbonyl content, lipid peroxidation and methemoglobin levels and decreased glutathione. Impaired antioxidant power and increased formation of reactive oxygen species were observed. The activities of major antioxidant enzymes were prominently altered. The plasma membrane redox system (PMRS) of erythrocytes was inhibited and there was substantial change in membrane morphology. Preincubation of cells with carnosine attenuated the oxidative damage induced by $CuCl_2$, restored the enzyme activities and antioxidant power and also prevented inactivation of erythrocyte PMRS. Carnosine protected human erythrocytes and lymphocytes from $CuCl_2$-induced oxidative damage. It fortified the enzymatic and non-enzymatic antioxidant systems of these cells. The protection by carnosine can be attributed to its antioxidant nature.

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P-014

Rapid ascorbate response to bacterial elicitor treatment in *Arabidopsis thaliana* cells

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Keywords: Oxidative burst; ROS; harpin proteins; ascorbate; glutathione; hypersensitive response; plant-pathogen interaction

An early event of the incompatible plant-pathogen interactions is an oxidative burst. The major water soluble, low molecular weight antioxidant, ascorbic acid plays a crucial role in Reactive Oxygen Species (ROS) balancing (scavenging). The regulation of ascorbate level therefore can be an important point of the fine tuning of ROS level during the early phase of plant-pathogen interaction. To evaluate how this interaction affects the biosynthesis, the recycling and the level of ascorbate, we challenged *Arabidopsis thaliana* cells with 2 different harpin proteins (HrpZpto and HrpWpto).

HrpZpto and HrpWpto treatments caused a well-defined ROS peak. The expression of the alternative oxidase (AOX1a) and vtc5, one of the paralog genes that encode the rate limiting enzyme of ascorbate biosynthesis followed the elevation of ROS. Similarly the activity of ascorbate-peroxidase and galactono-1,4-lactone dehydrogenase (GLDH), the enzyme catalyzing the ultimate, mitochondria coupled step of ascorbate biosynthesis and the level of ascorbate and glutathione also followed the elevation of ROS due to harpin treatment. The enhanced expression of AOX1a, the elevated activity of GLDH and the increased level of ascorbate and glutathione all can contribute to the mitigation or absence of programmed cell death. Finally a new function, the fine tuning of redox balance during plant-pathogen interaction can be proposed to vtc5.

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P-015

Cellular redox status mediates adaptive response to ionizing radiation in human peripheral blood mononuclear cells

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Keywords: Ionizing radiation; Human PBMC; Radioadaptive response; DNA damage; Oxidative stress

Radioadaptive response refers to the phenomenon where a small priming dose of ionizing radiation (IR) reduces the detrimental effects of subsequent higher IR dose. In this study, we demonstrated that adaptive response in human peripheral blood mononuclear cells (PBMCs) is associated with altered cellular oxidative stress. DNA strand breaks, analysed with comet assay and γH2AX, was used as the endpoint to demonstrate adaptive response in PBMCs exposed to a priming dose of 10 cGy Co60 γ-rays followed by a challenge dose of 2 Gy γ-rays (dose rate 0.3 Gy/min) after 4 hours. Primed cells showed significantly lesser DNA damage accompanied with decreased levels of ROS and increased antioxidant responses. This included changes in the expression for genes like SOD2, GPx, CAT and TRX. In addition, increased activity of enzymatic defense system, mainly SOD and TXNRD, but not CAT and GPx, was observed in the primed cells. These responses were shown to be mediated through increased DNA binding of stress responsive transcription factors Nrf2 and NFκB. The primed cells also showed early activation of ERK, p38 and JNK MAP kinases. Label-free LCMS analysis revealed unique pattern of differentially expressed proteins in the primed cells. A better understanding of adaptive response will contribute towards refinement of radiation protection strategies and radiotherapy protocols.

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P-016

Cytoprotective effects of Chilean wild currants (Ribes spp.) against oxidative stress mediated by enhancing the activity of cellular antioxidant enzymes

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Keywords: Ribes; Cytoprotection; Oxidative Stress; AGS cells; Phenolics

The South American Ribes species are very appreciated for their sweet taste. Continuing our research on Ribes species, we decided to investigate the cytoprotective activity of this fruits against oxidative stress. Phenolic-enriched extracts (PEE), the anthocyanin fraction (AF) and copigments fraction (CF) were prepared in the lab. The cytoprotective effect of the Ribes was evaluated using human gastric epithelial cells (AGS). Cells were incubated overnight with the PEE, AF or CF at different concentrations. Then, AGS cells were exposed to H_2O_2 for 2 hour and viability was measured by means of the MTT reduction assay. In another experimental approach, cells were scrapped, collected by centrifugation and lysed to determine the activity of intracellular antioxidant enzymes. A significant cytoprotective effect was observed in a dose-dependent manner for all species studied. The highest activity was found for R. punctatum with 55.0 $\pm$ 1.8% of cell viability against 33.0 $\pm$ 0.4% of survival of untreated cells. No significant difference between PEE, AF or CF in the cytoprotective effect. A significant increase in the activity of superoxide dismutase by all Ribes species was found in a dose dependent manner. Our results on the protective potential of South American Ribes species provides

evidence on the potential of native currants to be developed as functional foods.

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P-017

Changes in expression of NLRP3 inflammasome components and oxidative parameters of mice subjected to high-fat diet and rosa mosqueta oil supplementation

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Keywords: Rosa mosqueta oil; NLRP3 inflammasome; oxidative stress; insulin resistance

Introduction: Rosa mosqueta oil (RM) is high in alpha-linolenic acid (ALA) and tocopherols (α-, γ-), which have anti-inflammatory, antioxidant and insulin sensitizing properties, thus preventing the high-fat diet (HFD)-induced damage in mice.
Objective: To evaluate changes in NLRP3 inflammasome components expression and oxidative parameters in HFD-fed mice supplemented with RM.
Methods: C57BJ/6 J mice (n = 9/group) were fed for 12 weeks and divided into: (i) control diet (CD, 20% proteins, 70% carbohydrates, 10% lipids); (ii) CD+RM (0.01 mL/g bodyweight/day); (iii) HFD (20% protein, 20% carbohydrate, 60% lipids); (iv) HFD+RM. Oxidative stress (carbonylated proteins, MDA content and Nrf2 levels) and NLRP3 inflammasome components (NLRP3, ASC, Caspase-1, IL-1beta) expression in liver and visceral adipose tissue were determined. Results: HFD+RM group showed significantly decreased (two-way ANOVA, bonferroni test, $P < 0.05$) liver and adipose tissue NLRP3 inflammasome expression, along with decreased oxidative stress compared to the HFD group.
Conclusion: Dietary RM supplementation decreases NLRP3 inflammasome expression and oxidative stress. This data could be associated with the prevention of metabolic syndrome. FONDECYT 1140547.

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P-018

Free radical pathway of 2-hexadecenal formation in cells and its biological role

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Keywords: 2-hexadecenal; astroglial cells; reactive chlorine and oxygen species

In our earlier studies it has been established that the action of gamma-, UV-irradiation and HOCl on aqueous deaerated sphingolipids dispersions causes destruction of studied biomolecules with the formation of 2-hexadecenal. HOCl has powerful cytotoxic properties because it is a strong oxidizer and a source of reactive chlorine species.

We investigated the effect of HOCl on human erythrocytes, HEK293 and astroglial cells. For the sensitive quantitative analysis of 2-hexadecenal in cells we used extraction procedure and applied the method based on HPLC with fluorescence detector. We found that the HOCl-treatment of cells at concentrations from 10 μM to 1 mM provokes 2-hexadecenal formation.

It has been found that 2-hexadecenal at micromolar concentrations regulates reactive oxygen species generation in human peripheral blood neutrophils stimulated by adhesion and fMLP, through reallocation of myeloperoxidase, phospholipase A2, cyclooxygenase and 5-lipoxygenase contributions to this process. 2-Hexadecenal modifies the functions of astroglial cells in culture by changing their morphological characteristics. This is associated with the redistribution of F-actin and the subsequent cytoskeleton reorganization. It results in cells' mitotic and proliferative activity reduction through the initiation of apoptosis involving JNK and p38 mitogen-activated protein kinase pathways.

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P-019

Alterations of cultured myotubes and fasting plasma metabolite profiles related to mitochondrial dysfunction in Type 2 diabetes subjects

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Keywords: Mitochondrial dysfunction; blood plasma; skeletal muscle; metabolomics; biomarkers

The current study sought to determine whether circulating metabolite signatures in cultured myotubes and fasting plasma of T2D (Type 2 diabetes) subjects are associated with mitochondrial dysfunction. A cellular disease model of human myotubes with mitochondrial dysfunction was first established. The intracellular-defined metabolites was analyzed. Further, a targeted metabolic profiling of fasting blood plasma from normal (n = 83) and T2D (n = 92) subjects in a cross sectional study was validated. Multivariable-adjusted conditional logical regression analysis was computed to verify differentiating metabolites correlated with T2D. Several metabolites were considerably altered in cultured myotubes. We further tested whether these cellular metabolites are linked to the plasma metabolites of T2D subjects. Targeted analysis of plasma metabolites adjusted for several confounders revealed 20 significant robust metabolites ($P < 0.05$) comprised primarily of branched chain amino acids (leucine, isoleucine and valine), medium-chain acylcarnitine (C6, C8, C10:2, C10:1 and C1^{2}1), free fatty acids (C16:0, C18:0, C18:2, C20:5) and sphingomyelin (d18:2/16:0). In summary, our finding yields a valuable insight on the identification of circulating selective metabolite signals

associated with mitochondrial dysfunction in skeletal muscles that are distinctly found in the blood plasma of T2D subjects.

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P-020

Anti-oxidative potential and biocompounds of five Lamiaceae family herbal species

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Keywords: Carotenoid; phenolic compounds; vitamin; tannins; flavonoid

The present study was designed to evaluate the antioxidants compound and activities in *Salvia officinalis, Mentha spicata, Lavandula angustifolia, Satureja hortensis* and *Origanum vulgare* methanol extracts. Antioxidant activities of plant extracts were assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ability to reduce Fe^{3+} to Fe^{2+} assay. Free radical scavenging activity of *S. officinalis* was recorded as high as 90.1% followed by *L.angustifolia* (88.7%) at 300 μg/ml. This value was found close to the activity of synthetic antioxidant, butylated hydroxytoluene (BHT) (94.0%) at the same concentration. In this study, *Salvia officinalis* and *L. angustifolia* had higher total phenolic contents (21.7 ± 1.18 and 19.2 ± 1.06 mg GA/100 g DW) whereas *S. officinalis* had the largest flavonoid contents (4.8 ± 0.12 mg CE / 100 g DW). Results showed strong correlation between antioxidant activity and carotenoids content (r=0.82), phenolic compounds (r= 0.92), vitamin C (r= 0.81), vitamin E (r= 0.79) and tannins content (r= 0.81). It can be concluded from the current results that antioxidative potency of *L. angustifolia* and *S. officinalis* is attributed to the presence of higher contents of biocompound like carotenoids, phenolics, vitamins and flavonoids.

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P-021

Chemical constituents and antioxidant properties of *Matricaria recutita* and *Chamaemelum nobile* essential oil growing in south west of Iran

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Keywords: Chamomile; radical scavenging; chamazulene

The composition of essential oil isolated from *Matricaria recutita* and *Chamaemelum nobile*, growing wild in Iran, was analyzed by GC and GC-MS. The *Matricaria recutita* essential oil was characterized by chamazulene (29.80%), α-pinene (8.0%), β-Pinene (7.93%), ά-bisabolol (5.76%), ά-bisabololoxide A (5.51%), trans-b-farnesene (5.51%) and Chrysanthenone (4.88%). The *Chamaemelum nobile* essential oil was characterized by Chamazulene (31.12%), β-Pinene (10.11%), ά-bisabolol (7.32), a pigenin-7-glucoside (6.20%), ά -bisabololoxide A (5.98%), α-pinene (5.97%) and β–thujone (4.84%). Antioxidant activity was analyzed using the 2,2-diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging method and Reducing power antioxidant (the ability of oil to reduce iron +3). Comparing the DPPH scavenging activity of chamomile essential oil from *Matricaria recutita* (137.2 μg/ml) and *Chamaemelum nobile* (195.8 μg/ml) and those expressed by BHT (100.0 μg/ml), it was shown that the essential oil from *Matricaria recutita* exhibited the good antioxidant effects than *Chamaemelum nobile*. Results showed *Matricaria recutita* essential oil absorbance value (0.94%) was close to synthetic antioxidant BHT (1.12%) obtained at 100 μg/ml. *Matricaria recutita* essential oil displayed the stronger antioxidant activity compared to *Chamaemelum nobile* essential oil.

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P-022

Antioxidants for the stabilization of flaxseed oil

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Keywords: Flaxseed oil; Lipid oxidation; Ascorbyl palmitate; Plant stabilizers; Storage stability

Efficacy of a number of synthetic and natural antioxidants and their compositions used as inhibitors of the oxidative processes in flaxseed oil was investigated. Liposoluble esters of ascorbic acid were found to be excellent oxidation inhibitors for the flaxseed oil, while displaying much lower activity in other vegetable oils. Using ascorbyl palmitate alone as a stabilizing additive provided a reliable and safe protection of flaxseed oil from oxidation. The efficiency of using a number of medicinal and aromatic plants, such as sage, St Johns's wort, thyme, rose hips, cumin, ginger, turmeric and clove, as well as haricot beans and soybeans, to improve oxidative stability of flaxseed oil was also studied. Adding to flaxseed oil some vegetable compositions, e.g. those containing legume beans, sage or cumin, was shown to be effective in slowing down oxidation of the oil, ensuring thereby a substantial increase of its storage period. These findings enabled the authors to develop an industrial technology for manufacturing oxidation-resistant edible flaxseed oil and biologically food supplements on its basis, in which ascorbyl palmitate or plant stabilizers were used.

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P-023

Differential antioxidant protection against oxidants by esculetin or quercetin in human leukemia NB4 cells

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Keywords: Esculetin; quercetin; leukemia; hydrogen peroxide; tert-butyl hydroperoxide

Esculetin (6,7-dihydrocoumarin) and the flavonoid quercetin (3,5,7,3',4' pentahydroxyflavone) show different pharmacological properties probably related to effects on redox homeostasis. Human leukemia NB4 cells were preincubated for 30 min or 2 h with either 100 μM esculetin or 25 μM quercetin. The cells were then treated with 1 mM H_2O_2 or 250 μM tert-butyl hydroperoxide (t-BHP) for 1 h. Pretreatments with quercetin prevented loss of cell viability (impermeability to PI) induced by H_2O_2 or t-BHP. Treatment with 1 mM H_2O_2 for 1 h produced lower apoptotic cells (26%) than 250 μM t-BHP (38%). Pretreatments with esculetin increased the apoptosis induced by H_2O_2 but reduced significantly the apoptosis produced by t-BHP. Quercetin reduced apoptosis produced by H_2O_2 and wholly protects against apoptosis induced by t-BHP. Superoxide was increased by treatment with H_2O_2 or t-BHP. Esculetin or quercetin increased the levels of superoxide in cells treated with H_2O_2 but reduced them in cells treated with t-BHP. Esculetin but not quercetin almost prevented peroxide production by H_2O_2. Our results show different effects of antioxidant treatment on leukemia cells and possible differential applications for antitumor therapy with either of those antioxidant compounds used.

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P-024

Nrf 2 and NFkB involvement in the antioxidant action of esculetin or quercetin in human leukemia NB4 cells

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Keywords: Esculetin; quercetin; leukemia; NFkB; Nrf2

Esculetin (6,7-dihydrocoumarin) and the flavonoid quercetin (3,5,7,3',4' pentahydroxyflavone) are compounds that could change the balance of redox homeostasis. NB4 leukemia cells treated with 25 μM quercetin for 24 h and with esculetin at either 100 or 500 μM for different times. Quercetin increased the levels of pro-inflammatory NFkB p65 in the nucleus correspondingly reducing them in the cytosol. The levels of NFkB p65 decreased in the nucleus at high esculetin concentration treatments for long times (19 h), concomitantly, increasing the levels of anti-inflammatory NFkB p50 in the nucleus. This could suggest formation of inhibitory p50 homodimers possibly related with anti-inflammatory response. Lipoxygenase expression was reduced either by esculetin or quercetin. A significant increase of Nrf2 in the nucleus of NB4 cells treated with 100 μM esculetin for 19 hours was observed. Quercetin increased the levels of Nrf2 in the cytosol reducing them in the nucleus. Superoxide dismutase (SOD) expression increased in NB4 cells treated with esculetin in contrast with quercetin. All these data support a relevant differential role for NFkB and Nrf2 in antiinflamatory and redox response when apoptosis was induced by esculetin or quercetin in human leukemia NB4 cells.

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P-025

Oxidative damage, antioxidant defense and DNA repair capacity in patients with Primary Dyslipidemia

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Introduction: Primary Dyslipidemia is a group of genetic disorders of lipid metabolism. Biomolecules oxidative damage has been reported in those patients, although DNA repair capacity is less reported. The aim of this work is evaluate oxidative stress biomarkers and DNA repair capacity in dyslipidemic patients.
Material and Methods: Plasma levels of malondialdehyde, advanced oxidation products of proteins and GSH, as well as the activities of SOD1, catalase, glutathione peroxidase and glutathione reductase were measured in patients from a Hospital in Havana. Comet assay was used to evaluate single strand breaks, oxidation-induced DNA damage, and repair capacity in isolated lymphocytes.
Results: Levels of plasma MDA and GSH were higher in patients, as well as the enzymatic activity of GPx and GR. However, CAT and SOD1 activities did not significantly differ between patients and healthy controls. Additionally, no differences in DNA damage were observed between groups, but DNA repair capacity was significantly slower in patients, mainly in patients with Familiar Hypercholesterolemia (FH). Combined Hyperlipoproteinemia patients showed normal DNA repair.
Conclusions: These findings show alterations in biomarkers of oxidative stress in patients. The deficiencies in the repair mechanisms could be related with the clinic evolution and the severity of complications in patients with FH.

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P-026

Moderate maternal caloric restriction affects mitochondrial biogenesis and redox homeostasis in the pups' hypothalamus

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Keywords: Caloric restriction; Mitochondria; Redox status; Maternal effect; Hypothalamus

Caloric restriction (CR) is known as the best strategy to promote health and life span in various animal models. However, its effects during pregnancy remain to be elucidated. Our objective was to evaluate the effect of maternal CR on pups' hypothalamus mitochondrial and redox status. Adult female Wistar rats underwent 20% CR during pregnancy, supplemented with a micronutrient mix. Pups were euthanized in postnatal day (PND) 0 and 7, and hypothalamus was dissected. The protocol was approved by an ethical commission under the n° 30044. Our results showed mitochondrial biogenesis was reduced in PND0 and PND7. In PND0, mitochondrial superoxide levels were decreased while dichlorofluorescein oxidation increased. In PND7, alterations in reactive oxygen species concentrations were abolished. Superoxide dismutase activity and reduced glutathione content were diminished in PND0, while catalase activity was enhanced. In PND7, the antioxidant system was shifted. Catalase, glutathione peroxidase, and glutaredoxin activities, as well as reduced glutathione content increased. Apparently, pups born to restricted dams present unbalanced antioxidant system and diminished mitochondrial function. The former was restored to normal function in PND7, the latter was only partially restored. Our data suggest a huge impact on pups' hypothalamus elicited by maternal CR.

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P-027

Naringin supplementation during pregnancy reduces mitochondrial function and modifies redox network system in offspring's cerebellum and striatum

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Keywords: Naringin; Maternal Effect; Mitochondria

Consumption of polyphenol-rich diets is known to improve brain's redox homeostasis parameters and cognitive function, however, little is known about the effects of polyphenols consumption during pregnancy in offspring's brain. Moreover, we sought to evaluate the effects of maternal supplementation with naringin during pregnancy in offspring's cerebellum and striatum. Naringin was supplemented by oral gavage (100 mg/kg) from the first to the last day of pregnancy. On postnatal day one, offspring's brain was collected and evaluated. The protocol was approved by an ethical commission under the n° 31397. Maternal naringin supplementation decreased offspring's mitochondrial electron transport system (METS) activity in cerebellum, along with increased levels of reduced glutathione. Moreover, the METS activity was also reduced in offspring's striatum, accompanying a tendency of increased levels of nitric oxide and dichlorofluorescein oxidation. Concerning other redox network components, we also observed increased catalase activity and a tendency of increased glutathione peroxidase activity in striatum. Maternal naringin supplementation disrupted offspring's cerebellum and striatum redox network, which might have been triggered by a specific negative adaptive response of each brain region evaluated in this work.

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P-029

Free radical transformations of hydroxyl-containing amino acids and related compounds in aqueous solutions

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Keywords: N-centered radicals; free radical destruction; serine; threonine; glucosamine

It is well known that reactive oxygen species (ROS), generated via various enzymatic and non-enzymatic pathways, can cause the damage of biomolecules. It was established that ROS, interacting with amino acids, initiated mainly their deamination and decarboxylation, the probability of which was largely determined by the molecule structures and their forms in the solutions. Using γ-irradiation for $^{\bullet}$OH generation we have shown that serine (Ser), threonine (Thr) and related dipeptides as well as glucosamine (GlcN) and 1-amino-2-propanol undergo $^{\bullet}$OH-induced carbon skeleton destruction in aqueous solutions. It was shown that destruction of Ser and Thr proceeded via formation of N-centered radicals of starting compounds. As a result of such reactions glycine and formaldehyde (or acetaldehyde) were formed. The non-enzymatic free radical Ser conversion, resulting in the formation of

glycine and formaldehyde, is similar to the Ser bioconversion driven by serine hydroxymethyltransferase. Among radiolysis products of Ser and Thr in oxygenated solutions glyoxal and methylglyoxal, which are known to possess high toxicity, have been found. The obtained data are recommended to consider during estimation of ROS-induced pathophysiological processes involving such biomoleculs as Ser, Thr and the corresponding peptides as well as GlcN, and, possibly, their complex macromolecular derivatives.

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P-030

Radical-regulatory and anti-tumor properties of quinone derivatives

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Keywords: Derivatives of quinones; free-radical reactions; anti-tumor properties

Redox-active derivatives of quinones participate in many biochemical processes. Based on such compounds, a large number of drugs have been developed, which are widely used in medicine, including anticancer treatment. Most of the processes in biological systems, where quinones are involved, proceed with participation of free-radical intermediates and this makes actual the study of their mechanisms.

The data obtained indicate that the tested benzoquinones effectively interact with carbon-centered radicals of hydroxyl-containing organic compounds and inhibit thereby the reactions of their oxidation, deamination and fragmentation. It has been shown that quinone derivatives, including doxorubicin, inhibit reactive oxygen species (ROS)-induced fragmentation of glycerophospholipids, suppressing the formation of phosphatidic acid. As it is known, phosphatidic acid promotes proliferation of cancer cells.

It has been found that the action of benzoquinones on glioma cells in vitroleads to decrease of reduced glutathione pool, to modification of ROS production in the cytoplasm and mitochondria, and also to suppression of proliferation of this type cells.

Possible relationship between radical-regulatory and anti-tumor properties of quinoid-type compounds in question is proposed.

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P-031

Antioxidant supplementation during pregnancy enhances mitochondrial function and alters redox status on offspring's cerebellum

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Introduction: Pregnancy is an important period for the fetus metabolic programming that can be modulated by several factors, such as the maternal diet. Polyphenol rich-diets are related to better brain and cognitive function, through the reduction of oxidative processes in the brain. Our objective was to evaluate the effect of maternal naringenin supplementation during pregnancy on offspring's' cerebellum redox homeostasis.
Material and Methods: Naringenin was orally administered to female Wistar rats, 5 days a week, during pregnancy. The offspring was evaluated at 7 days of life.
Results and Discussion: Maternal naringenin supplementation increased the mitochondrial electron transport chain activity, and agreeing with this finding, there was also increased mitochondrial superoxide content, dichlorofluorescein oxidation, and malondialdehyde levels. Although there was no effect on the antioxidant enzymes activities, maternal naringenin consumption caused an increase in the reduced glutathione content.
Conclusion: Maternal naringenin ingestion caused important alterations in the offspring's cerebellum, suggesting a pro-oxidant effect.

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P-032

Modulation of Melanoma Cell Proliferation and Spreading by Novel Small Molecular Weight Antioxidants

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Keywords: Melanoma; Antioxidant; Molecular Pathways; Free Radical; Invasion

Melanoma, the most dangerous skin cancer, originates from the melanocytes and has a high tendency to invade neighboring tissues, and metastasize. Free radicals appear to be involved in the melanocyte transformation and melanoma progression as well as invasion. Consequently, potent antioxidants may prevent transformation and

progression of the tumor. We have recently synthesized some small molecular weight antioxidant compounds of eleven different synthetic classes. These compounds were previously found to posses free radical quenching activity in an in vitro antioxidant assay. Compounds 1 and 2 were found to be most potent among them with IC50=42.9 ± 0.31, and 55.6 ± 2.1 μM, respectively, as compared to standard butylated hydroxy toluene with IC50=128.8 ± 2.1 μM. We are now testing these compounds for their intracellular free radical quenching role in skin melanoma cells in vitro and their ability to reduce proliferation and spreading. We are currently analyzing the influence of these compounds on the molecular pathways, involved in the proliferation-invasion inhibition.

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P-033

Oxidative and nitrosative stress damage induced by Tacrolimus in brain of rat: Protective effect of Mycophenolate mofetil

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Keywords: Tacrolimus; Mycophenolate mofetil; Acetylcholinesterase activity; Oxidative/Nitrosative stress; Apoptosis

Tacrolimus (TAC) and mycophenolate mofetil (MMF) are used effectively to prevent immunologic rejection after solid-organ transplantation. Some immunosuppressive drugs contribute to the pathophysiology of cerebral injury. In this study, we investigate the efficacy of MMF against TAC induced neurotoxicity in the brains of rats. Statistical analyses showed that rats treated with TAC by oral gavage at a dose of 60 mg/kg b.w. caused an alteration of acetylcholinesterase (AChE) activity, a potential induction of oxidative/nitrosative stress mediated by an increase of malondialdehyde (MDA), protein carbonyls (P.C.) and a decrease of the nitric oxide (NO). In addition, TAC provoked significantly caspase-3 activity in brains of rats. A significant correlation between caspase-3 levels and oxidative/nitrosative markers was observed. However, the co-treatment with MMF at a dose of 50 mg/kg is able to restore the AChE activity and all stress markers and regulated apoptosis in brains. These data indicated that MMF plays a neuroprotective role in brain tissue damage.

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P-034

By downregulating transcription of PARP1, CDK4/6 inhibitors sensitise human lung cancer cells to oxidative stress-induced DNA damage triggered by WP631 and etoposide

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Keywords: CDK4/6 inhibitors; cancer; PARP1; DNA damage; histone epigenetic marks

Inhibitors of CDK4/6 – LEE011 and PD0332991 have been tested in clinical trials in combination with other anticancer drugs for their use in cancer treatment. These compounds are documented to arrest cell cycle progression. We show that in human lung cancer cells these agents cause cell accumulation in G1 phase and repression of PARP1 transcription by triggering the recruitment of RB-E2F1-HDAC1-EZH2 repressive complex to the promoter of PARP1 gene, which encodes the protein involved in among other things several mechanisms of DNA damage repair. PARP1 repression could be reversed by administration of inhibitors of histone acetyltransferases and polycomb repressive complex 2 in growth arrested cells. Moreover, cells pre-incubated with LEE011 and PD0332991, in which PARP1 expression was downregulated, were more sensitive to cell death resulting from their treatment with WP631, etoposide and hydrogen peroxide than corresponding cells, in which PARP1 expression was sustained by their stable transfection with an expression vector carrying cDNA for PARP1. Downregulation of PARP1 transcription in the lung cancer cells by treatment with CDK4/6 inhibitors was followed by the increase in DNA damage in consequence of oxidative stress generated by WP631, etoposide and hydrogen peroxide.

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P-035

Melatonin as an antioxidant treatment for oxidative blood disorders: validation of a red blood cell auto incubation model

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We investigated melatonin effects on red blood cell (RBC) metabolism in an H_2O_2-induced oxidative stress model. The study was carried out on three healthy adult donors by incubating RBCs in their own plasma at 37 °C, or under the influence of 1 mM H_2O_2 and 100 μM melatonin at different times (0, 1, 3 and 6 hours). We assessed incubation period, treatment, as well as interaction effects on oxidative stress markers, and adenine nucleotide and oxypurine levels. We found positive correlations between incubation times and hemolysis degree for each treatment. However, we did not observe any influence on RBC osmotic fragility and antioxidants tested. On the other hand, we found an increasing effect of incubation period on lipid peroxidation levels. Furthermore, oxidation induction regardless time more than doubled protein carbonyl groups in plasma but melatonin neutralized this H_2O_2 effect. Unexpectedly, we did not find any relevant alterations on energy expenditure or adenylate nucleotide metabolism regarding to treatments or incubation periods investigated. The results obtained for markers of lipid and protein injury validated the auto incubation model, as well indicated a protection effect of melatonin. This effect along with its exceptional multiplicity actions reinforced the hypothesis of pharmacological use of melatonin in oxidative blood disorders.

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P-036

Effect of urea in the reaction of nucleosides with hypobromous acid

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Keywords: Hypobromous acid; HOBr; urea; nucleoside

Hypobromous acid (HOBr) is generated by eosinophil peroxidase or myeloperoxidase using hydrogen peroxide, chloride, and bromide in the host defense system of humans, protecting against invading bacteria. Generally, HOBr can react with amines (R-NH2), resulting in bromamines (R-NHBr). Urea (CO(NH2)2) is a ubiquitous molecule with high concentrations (5 mM in plasma, 285 mM in urine) in humans. However, there is little information on the effect of urea in the reaction of HOBr. In the present study, we examined the reaction of nucleosides with HOBr in the absence and presence of urea using HPLC. Without urea, nucleosides immediately reacted with HOBr in the order of dG > dC > dT > dA. In the presence of 100 mM urea, the reaction was slow and carried out for several hours. 8-Br-dG, a reaction product from dG, increased by the addition of urea. Whereas, 100 mM lysine suppressed the reaction almost perfectly. The results suggest that urea reacts with HOBr resulting in urea bromamine, and which reacts with nucleosides slowly. Urea may have an importance for mutagenesis caused by the reaction of HOBr.

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P-037

Effect of Neutral Sphingomyelinase Inhibition on ER Stress and Apoptosis in Liver Ischemia–Reperfusion Injury

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Keywords: Liver; ischemia-reperfusion injury; ceramide; neutral sphingomyelinase

This study determined the role of selective neutral sphingomyelinase (N-SMase) inhibition on endoplasmic reticulum (ER) stress and apoptotic markers in a rat model of liver ischemia reperfusion (IR) injury. Liver IR injury was created by clamping blood vessels supplying the median and left lateral hepatic lobes for 60 min, followed by 60 min reperfusion. Sphingmyelin and ceramide levels in liver tissue were determined by tandem mass spectrometry. Spingomyelin levels were significantly increased in all IR groups compared to controls. Treatment with a specific N-SMase inhibitor significantly decreased all measured ceramides in IR injury. A significant increase was observed in ER stress markers C/EBP-homologous protein (CHOP) and 78 kDa glucose-regulated protein (GRP78) in IR injury, which was not significantly altered by N-SMase inhibition. Inhibition of N-SMase caused a significant reduction in phospho-NF-kB levels, hepatic TUNEL staining, cytosolic cytochrome c and caspase-3, -8 and -9 activities which were significantly increased in IR injury. Data herein confirm the role of ceramide in increased apoptotic cell death and highlight the protective effect of N-SMase inhibition in down-regulation of apoptotic stimuli responses occurring in hepatic IR injury.

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P-038

Role of the myeloperoxidase oxidant hypothiocyanous acid (HOSCN) in the adaption of cells to oxidative stress during inflammation

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Keywords: Inflammation; atherosclerosis; myeloperoxidase; protein oxidation; glycolysis

A host of chronic inflammatory diseases are accelerated by the formation of the powerful oxidant hypochlorous acid (HOCl) by myeloperoxidase (MPO). In the presence of thiocyanate (SCN^-), the production of HOCl by MPO is decreased in favour of the formation of a milder oxidant, hypothiocyanous acid (HOSCN). Unlike HOCl, HOSCN reacts selectively with thiols to result in reversible

modifications that can be repaired, potentially reducing the extent of MPO-induced damage during inflammation. In this study, we show that exposure of macrophages, a key inflammatory cell type, to HOSCN results in the reversible modification of multiple metabolic proteins, leading to decreased glycolysis, oxidative phosphorylation and reduced formation of ATP, NADH and lactate. HOSCN was able to re-route the glycolytic flux through the pentose phosphate pathway, which elevated the production of NADPH by increasing glucose 6-phosphate dehydrogenase activity. This glycolytic switch was not observed on exposure of macrophages to HOCl. These results suggest that HOSCN may induce an adaptive response in the macrophages, whereby the increased NADPH could increase the capacity of the cellular enzymatic antioxidant systems to deal with an oxidative insult. These data provide new insight into pathways by which SCN^- could modulate MPO-induced damage in inflammatory diseases, including atherosclerosis.

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P-039

Mass Spectrometric Identification of Collagen Alpha-1 (III) Chain and Chondroitin Sulfate Proteoglycan-4 Nitration in Patients with Acute Pulmonary Embolism: A Preliminary Study

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Keywords: Pulmonary embolism; nitrotyrosine; human collagen alpha-1 (III) chain; human chondroitin sulfate proteoglycan

Pulmonary embolism (PE)-induced oxidative stress can lead to the accumulation of nitroproteins that may play a role in disease progression. We used a proteomic approach to analyze nitrated plasma proteins in patients diagnosed with acute PE. Nitrotyrosine (NO2Tyr)-containing proteins were immunoprecipitated with a NO2Tyr affinity sorbent. Precipitated proteins were separated by SDS-PAGE and visualized by either Coomassie Blue staining or western blotting with mouse monoclonal anti-NO2Tyr antibody. Immunoreactive bands observed in disease patients were in-gel digested and analyzed by MALDI-TOF mass spectrometry. Mass fingerprint data sets obtained from the 138 kDa peptide fragment ions matched human collagen alpha-1 (III) chain (CO3A1)with Mascot algorithm analysis giving a score of 65 ($p < 0.05$). Mass fingerprint data sets obtained from the 250 kDa peptide fragment ions matched human chondroitin sulfate proteoglycan 4 (CSPG4) with Mascot algorithm analysis giving a score of 57 ($p < 0.05$). Nitration-induced alterations of CSPG4 activity can possibly lead to decreased fibrin degradation and enhanced complement system activity. In vivo characteristics of these nitroproteins could be significant with regards to biomarker studies and understanding of disease mechanism in patients with PE.

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P-040

A role for chlorinated nucleosides in the promotion of inflammation and endothelial dysfunction in atherosclerosis?

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Keywords: Atherosclerosis; myeloperoxidase; DNA; hypochlorous acid; inflammation

Release of myeloperoxidase (MPO) at sites of inflammation plays a key role in atherosclerotic lesion development. MPO produces the highly reactive oxidant hypochlorous acid (HOCl), which modifies RNA and DNA forming different chlorinated nucleoside products. Concurrent release of DNA during lesion development either from necrotic cells or via extracellular traps results in the presence of chlorinated nucleosides within atherosclerotic plaques. In this study, we show that exposure of macrophages and human coronary artery endothelial cells (HCAEC) to chlorinated nucleosides results in the incorporation of these materials into the RNA and DNA. Treatment of macrophages with 5-chlorocytidine (5ClC) and 8-chloroguanosine (8ClG) results in the activation NF-kB and increased release of the pro-inflammatory cytokine IL-1b, whereas HCAEC are highly sensitive to 8-chloroadenosine (8ClA), which decreases metabolic function and ATP levels via the accumulation of the chlorinated analogue 8ClATP. HCAEC exposure to 8ClA also caused a marked increase in the expression of thioredoxin interacting protein (TXNIP), a regulator of cellular metabolism within cells, and triggered activation of the unfolded protein response, consistent with ER stress. These data provide new insight into pathways of lesion development and highlight a potential biomarker for the diagnosis and treatment of the disease.

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P-041

Redox control of renal metabolism and transport function by the NADPH oxidase Nox4

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Keywords: NADPH oxidase 4; redox proteomics; metabolomics

The NADPH oxidase Nox4 produces H_2O_2, a potent modulator of cellular metabolism. It oxidizes iron and cysteines and directly influences metabolic enzymes or the dynamics of metabolites. Nox4 is highly expressed in the kidney and shows a protective role in renal disease whereas its expression is lost in renal pathology. However, its physiological function in the kidney is still unknown.

In situ hybridization (RNAscope) showed Nox4 exclusively in proximal tubular cells, which reabsorb and secrete Na^+, Cl^-, metabolites and $HCO3^-$. We have investigated a redox-dependent tubular function and metabolism by metabolite profiling by 1H-NMR and LC/MS combined with redox proteomics (BIAM assay for oxidized cysteines) in models of gain/loss of function of Nox4.

A unique decrease of α-ketoglutarate (KG) in plasma and urine in contrast to an increased concentration in renal tissue was identified in Nox4-/-. As other TCA cycle metabolites were not altered, this suggests a redox-dependent transport of KG. Redox proteomics identified Slc4A7 (sodium-bicarbonate co-transporter) and glutamine synthetase, as oxidation targets of Nox4. Accordingly, glutamine concentration is increased in cells overexpressing Nox4.

Our results using metabolomics and redox proteomics add insights on the redox regulation of physiological renal metabolic function by Nox4.

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P-042

The protective effect of hesperetin on UVA-induced matrix metalloproteinase-1 (MMP-1) in primary human dermal fibroblasts and mouse skin through modulation of nuclear factor erythroid 2- related factor 2 (NRF2)-regulated antioxidant defenses

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Keywords: Hesperetin; Matrix metalloproteinase-1 (MMP-1); Nuclear factor E2-related factor 2 (Nrf2); Primary human dermal fibroblast (HDF); Ultraviolet A (UVA)

Skin aging is an accumulation of collagen degradation caused by matrix metalloproteinase-1 (MMP-1) mainly present in human dermal fibroblasts (HDFs). Increased activity of Nrf2, the transcription factor of antioxidant genes, to counteract oxidative stress is suggested to delay aging process and thus compounds targeting Nrf2 might represent a promising strategy for skin photoprotection. In addition, natural products containing hesperetin were reported to provide photoprotective effects on UVA-induced MMP-1 in HDFs.

Aims: This study aims to investigate anti-photoaging effects of hesperetin on UVA-mediated MMP-1 induction and collagen depletion through activation of Nrf2-mediated antioxidant defenses (GCLC and NQO-1) in HDFs and the skin of BALB/c mice.

Results: This study revealed that hesperetin (up to 15 μM) could reduce MMP-1 activity and increase collagen levels in UVA (8 J/cm2)-irradiated HDFs. In vivo immunofluorescence staining showed that treatment with hesperetin (up to 60 μM/cm2) protected against UVA (60 J/cm2)-mediated MMP-1 induction and collagen reduction in association with increase in Nrf2 nuclear localization and its target proteins (GCLC and NQO-1) in mouse skin. In summary, hesperetin exerted anti-photoaging effects on UVA-induced MMP-1 induction possibly through activation of Nrf2-regulated antioxidant defenses in HDFs and mouse skin.

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P-043

Oxidative stress is related to frailty, not to age or sex, in a geriatric population

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Age-associated frailty is a geriatric syndrome. Frail individuals are vulnerable and lack capacity to manage external stressors. Frailty is associated with oxidative stress, but it has not been addressed in a large controlled human cohort. We aimed to ascertain whether indicators of oxidative damage to lipids and proteins are biomarkers of frailty, after adjusting for age, sex, and other possible confounders. We measured lipid and protein oxidation in the Toledo Study for Healthy Aging participants (N = 742, aged 65–95), classified as frail, prefrail and nonfrail according to the Fried criteria. We found that age- and sex-adjusted levels of MDA and protein carbonylation in plasma proved to be related to frailty, even after including possible independent confounders. In conclusion, circulating oxidative damage biomarkers, such as MDA and protein carbonylation, are related to frailty and not to age or sex. These parameters may be considered as potential biomarkers of frailty in the context of a multidisciplinary health-promoting approach for older adults.

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P-044

Thai herbal antipyretic 22 formula inhibits melanogenesis through activation of NRF2-regulated antioxidant defense in UVA-irradiated B16 melanoma cells

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Keywords: Antioxidant defense; Melanogenesis; Nuclear factor E2-related factor 2 (Nrf2); Polyherbal formula; Ultraviolet A

Thai herbal antipyretic 22 formula (APF22), a polyherbal formula, has been used to treat dermatologic problems including hyperpigmentation. Exposure to ultraviolet A (UVA) causes abnormal melanin production induced by photooxidative stress in the skin. Our previous study suggests that pharmacological actions of the APF22 may be attributed to gallic acid (GA), a possible active ingredient of this formula.
Aims: This study investigated the protective effects of the APF22 extracts, ferulic acid (FA) and GA (used as reference compounds), on melanogenesis through modulation of Nrf2 signaling and promotion of antioxidant defenses in mouse melanoma (B16F10) cells exposed to UVA (8 J/cm2).
Results: The APF22 extracts, FA and GA provided anti-melanogenic effect on UVA-irradiated B16F10 cells by suppression of melanin synthesis as well as tyrosinase activity and protein levels. Moreover, APF22 extracts, FA and GA were able to activate Nrf2-ARE signaling and induce mRNA levels of antioxidant defenses including glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx) and the glutathione-s-transferase (GST) in UVA-irradiated cells. Our study concluded that APF22 extracts suppressed UVA-mediated melanogenesis possibly via redox mechanisms involving activation of Nrf2-ARE signaling. Moreover, pharmacological action of the APF22 extracts may be attributed to FA and GA.

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P-045

The activation of the endoperoxide ascaridole in Leishmania

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Keywords: Ascaridole; endoperoxides; Leishmania; electron spin resonance; iron

Endoperoxides (EP) are successfully used in malaria therapy. It was shown in a mouse model that the EP ascaridole (Asc) is useful for the treatment of cutaneous leishmaniasis. The current study explored the activation of Asc and artemisinin (Art) in biomimetic systems and inside *Leishmania tarentolae* promastigotes (LtP) as model for pathogenic Leishmania in comparison to J774 macrophages. Using ESR spin-trapping we identified isopropyl radicals arising from reduction by Fe^{2+} in cell-free systems. Combined GC/NMR analysis confirmed the loss of isopropyl residues from Asc. In LtP carbon-centered radicals were identified in the presence of Asc and Art by ESR spin-trapping. Both Asc and Art inhibited the viability in LtP with IC50 values in the low μM range, while IC50 values for J774 macrophages were considerably higher. A similar structure without EP bridge (1,4-cineole) resulted in no detectable radicals and less cytotoxicity in LtP. The IC50 values for LtP viability in the presence of Asc or Art were increased by the spin trap DMPO. ICP-OES measurements revealed that in LtP the total iron concentration was twice as high as values in J774 macrophages. Studies of the labile iron pool (LIP) in LtP by low temperature ESR revealed an oxidation of the LIP by Asc. These data demonstrate that radical formation from Asc/Art in LtP is an essential part of their antileishmanial mechanism.

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P-046

Cordycepin induced MA-10 mouse Leydig tumor cell apoptosis by regulating p38 MAPKs and PI3K/AKT signaling pathways

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Keywords: Cordycepin; MA-10 cell; Leydig tumor; apoptosis; caspase; p38; AKT; cell cycle

The p38 MAPKs play important roles in the regulation of balance between cell survival and cell death on the development of various cancers. However, the roles of p38 MAPKs regulating apoptotic effects on Leydig tumor cells remain unclear. In the present study, we showed that cordycepin (3′-deoxyadenosine) selectively induced apoptosis in MA-10 mouse Leydig tumor cells through regulating the p38 MAPK and PI3K/AKT signaling pathways. Cordycepin reduced viability in MA-10 and TM4 cells, but not cause cell death of primary mouse Leydig cells on moderate concentration. Cordycepin increased reactive oxygen species (ROS) levels, which is associated with the induction of apoptosis as characterized by positive Annexin V binding, activation of caspase-3, and cleavage of PARP. Inhibition of p38 MAPKs activity by SB203580 significantly prevented cordycepin-induced apoptosis in MA-10 cells. Co-treatment with wortmannin or the autophagy inhibitor 3-methyladenine (3-MA) elevated levels of apoptosis in cordycepin-treated MA-10 cells. Moreover, cordycepin activated p53, p21 and TGFß; and downregulated CDK2. The antitumor activity of cordycepin-treated MA-10 cells was significantly distinct in severe combined immunodeficiency (SCID) mice in vivo. These results suggested that cordycepin is a highly selective treatment to induce MA-10 cells apoptosis via p38 MAPKs signaling.

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P-047

The mechanisms of histamine N-methyltransferase (HNMT)-mediated Herceptin® drug-resistance in breast cancer cells

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Keywords: Histamine-N-methyltransferase; breast cancer; Her2

In this study, reverse-transcription polymerase chain reaction (RT-PCR) was used to examine histamine-N-methyltransferase (HNMT) expression in 50 randomly selected human breast cancer samples and to compare HNMT expression between 363 breast tumor and normal tissue pairs isolated by surgical and laser capture microscope dissections. Stable inhibition of HNMT protein expression was established in the SKBR3 breast cancer cell line using short interfering RNA (siRNA). Additionally, a high HNMT expressing cell line was established using MDA-MB-231 human breast cancer cells. Cell proliferation assays, soft agar assays and tumor growth assays were used as measures of the tumorigenic effects of HNMT in nude mice. All statistical tests were two-sided. In 236 of the 363 (65%) paired samples, HNMT mRNA was expressed at higher levels in breast cancer tissue than in the surrounding normal tissue (mean 7.8-fold). Stable knockdown of HNMT by siRNA in SKBR3 cells inhibited tumor cell proliferation and growth in soft agar and completely abolished tumor growth when the cells were introduced as xenografts in SCID mice (n=5 mice per group; mean tumor volume 6 weeks post-injection of si-HNMT cells=0 mm3 vs. post-injection of parental cells=2993.2 mm3; difference=1997.6 mm3; 95% CI=1705 to 2290.2 mm3; P=0.001). HNMT is important for the proliferation of human breast cancer cells.

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P-048

Garcinia kola – African ethno medication with anti-atherosclerotic effects?

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Keywords: Garcinia kola; garcinoic acid; macrophages; inflammation; atherosclerosis

The plant *Garcinia kola* is used in African ethno medicine to treat various diseases. Anti-inflammatory effects are one of the several beneficial properties described for this plant. We hypothesized that garcinoic acid (GA), as one of the phytochemicals isolated from *Garcinia kola*, is responsible for these effects. Therefore, we investigated the capacity of GA to block the inflammatory response in LPS-activated murine macrophages and the inflammatory disease atherosclerosis. We found that the LPS-induced upregulation of the expression of iNos and Cox2 and the formation of respective signaling molecules nitric oxide, thromboxanes and prostaglandins was significantly diminished by GA. Further, application of GA (1 mg/kg, i.p.) affected the composition, but not the size of developed atherosclerotic plaques in aortic roots of ApoE-/-mice fed with high-fat Western-type diet. In brief, stability of the plaques was improved by increased collagen content and smaller necrotic core size under GA treatment. Based on these data we predict that GA blocks inflammatory response and plays a pivotal role in the formation of atherosclerotic plaques, in particular, affecting the degree of stability. If the proposed anti-atherosclerotic properties of GA are supported by further studies, this compound is a promising new therapeutic lead molecule against atherosclerosis and its complications.

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P-049

Hydrogen peroxide formation by Nox4 limits malignant transformation

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Keywords: Nox4 and Cancer

Through the constitutive production of H_2O_2 the NAPDH Oxidase Nox4 promotes differentiation of cells. Previous observations suggested that the absence of Nox4 promotes inflammation and de-differentiation. Chronic inflammation and lack of differentiation are pre-carcinogenic states. We therefore hypothesized that lack of Nox4 promotes tumor development.

Tumor formation was studied in two murine tumor models. In both models tumor burden was massively enhanced in Nox4-deficient mice. Genetic deletion of Nox4 resulted in increased DNA damage. In contrast, the DNA damage marker γH2AX was significantly reduced in Nox4-/- mice. As underlying mechanism we identified that Nox4 oxidizes Akt, which subsequently retains the phosphatase PP2A in the cytosol. In the absence of Nox4 PP2A accumulates in the nucleus. As a consequence, H2AX is dephosphorylated before DNA repair can take place resulting in genomic instability. In line with this, phosphorylated Akt accumulates in

the cytosol promoting proliferation and survival of tumor cells as observed. Overexpression of all three Akt isoforms could rescue the described phenotype whereas overexpression of Akt mutants lacking the redox-sensitive cysteines, had no effect on PP2A translocation, percentage of DNA damage and γH2AX phosphorylation.

Taken together these results suggest a protective role of endogenous Nox4 for malignant transformation.

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P-050

S-nitrosoglutathione potentiates protein S-nitrosation under oxidative stress, a potential improvement of NO storage into smooth muscle cells

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Keywords: S-nitrosoglutathione; Oxidative stress; Protein S-nitrosation

Cardiovascular diseases are associated with oxidative stress and reduced nitric oxide (NO) bioavailability. The ability of NO donors like S-nitrosoglutathione (GSNO) to regulate NO bioavailability under oxidative stress is poorly studied. Here, we monitored protein S-nitrosation (Pr-SNO), a post-translational protein modification in smooth muscle cells exposed to GSNO under oxidative stress. Intracellular thiol redox status in relation with the extent and distribution of GSNO-induced intracellular Pr-SNO (LC-MALDI MS) were assessed. The role of the gamma-glutamyl transferase (GGT), a redox enzyme metabolizing GSNO, in Pr-SNO formation was also studied. GSNO prevented the oxidation of proteins SH groups. Concomitantly, a 2-fold increase of GSNO-dependent Pr-SNO formation still depending on GGT activity was observed. Mass spectrometry identified 51 proteins S-nitrosated by GSNO under oxidative stress (vs 32 in basal condition), including a higher number of cytoskeletal proteins (17 vs 8 in basal condition) related to cell morphogenesis and movement. Furthermore, additional proteins belong to cell adhesion and protein trafficking were S-nitrosated under oxidative stress. Oxidative stress modifies the extent and distribution of GSNO induced Pr-SNO formation, a NO storage form in tissue. Further studies will likely elucidate the pathophysiological significance of these observations.

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P-051

Intracerebroventricular injection of glycine alters enzymatic antioxidant defenses in rat striatum: prevention by bezafibrate

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Keywords: Glycine; striatum; bezafibrate; antioxidant enzyme

Non-ketotic hyperglycinemia (NKH) is an inborn error of metabolism caused by deficiency in the glycine (GLY) cleavage system and characterized by a high cerebrospinal fluid / plasma GLY ratio. NKH often results in early death, and therapeutical approaches are limited to controlling the symptoms, mainly seizures and hypotonia, and detoxifying GLY. Therefore, we evaluated antioxidant defenses in striatum, cerebral cortex and hippocampus of young rats following an intracerebral administration of GLY, as well as the beneficial effects of a pre-treatment with bezafibrate (BEZ), a potential neuroprotective compound, on the alterations caused by GLY. GLY administration increased the activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase in striatum, and decreased reduced glutathione concentrations in hippocampus. No significant alterations were verified in cerebral cortex. BEZ totally prevented GLY-induced increase of SOD and GR activities, and attenuated the increase of GPx activity. Our data show that GLY intracerebral administration disturbs antioxidant defenses in brain. In addition, since some alterations caused by GLY were prevented by BEZ, this compound may be considered as adjuvant therapy for NKH.

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P-052

Thioredoxins, glutaredoxins and peroxiredoxins in redox-dependent formation of cancer cell resistance

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Keywords: Thioredoxin; glutaredoxin; peroxiredoxin; cancer cell resistance; gene expression

Thioredoxin (Trx), glutaredoxin (Grx), peroxiredoxin (Prx) which can play significant role in adaptation to oxidative stress and in maintaince of cell proteostasis.
Aims: Here we studied the expression of Trx, Grx and Prx isoforms under the formation of cancer cell resistance to cisplatin (cis-diamine dichloroplatinum, CDDP); its cytostatic effect, apart from alkylating mechanism, is associated with activation of generation of reactive oxygen species.
Results: Under development of resistance of human mammary adenocarcinoma (MCF-7/CDDP) and ovarian carcinoma (SKOV-3/CDDP) cells to CDDP co-ordinative enhanced expression of genes encoding isoforms of Trx (TRX1,TRX2), Grx (GLRX1, GLRX2), Prx (PRDX1, PRDX2, PRDX3, PRDX6) was found in both types of resistant cells in compare with wild cells. Decrease of growth of apoptotic cell death was observed under exposure to hydrogen peroxide in the CDDP-resistant cells which possessed elevated antioxidant status, as evidenced by the high level of GSH/GSSG and low generation of ROS. There was a much smaller increase in the level of phosphorylated forms of ASK1 and JNK1 in the resistant cells of each line compared with the sensitive cells. It can be concluded that the data obtained confirm the important contribution of Trxs, Grxs, Prxs into redox-dependent mechanisms for the development of cancer cells resistance to CDDP.

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P-053

Piceatannol exerts anti-obesity effect through modulating adipogenic proteins and gut microbiota in C57BL/6 mice

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Keywords: Obesity; Piceatannol; High-fat diet; C57BL/6; AMPK; gut microbiota

Obesity is a global health concern. Piceatannol (Pic), an analog of resveratrol (Res), has lots of biological actives. In this study, we investigated the anti-obesity effect of Pic in the high-fat diet (HFD) induced obese animal model. The results showed that Pic significantly reduced mice body weight in a dose-dependent manner without affecting food intake. Pic significantly decreased the weight of liver, spleen, perigonadal and retroperitoneal fat compared with HFD group. Pic significantly reduced the cell size of perigonadal fat and decreased in both the size and number of lipid droplets accumulated in the liver. Pic-treated mice showed higher phosphorylatedadenosine 5 ´-monophosphate-activated protein kinase (pAMPK) and phosphorylated acetyl-CoA carboxylase (pACC) expression and decreased expression of CCAAT/enhancer-binding proteins (C/EBP) α, peroxisome proliferators activated receptors (PPAR) γand (FAS) , resulted in decreased lipid accumulation tin adipocyte and liver. Although, HFD group had lower Firmicutes (F)/Bacteroidetes (B) ratio, but Pic could increase Lactobacillus and Clostridiales and decreased Sphingobacteriales compared with HFD group. Collectively, these results suggested that Pic could be a candidate for obesity treatment.

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P-054

Mitochondrial dynamics impairment leads to adipogenesis failure

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The white adipose tissue (WAT) is crucial for maintaining metabolism homeostasis by storing lipids and/or by secreting adipokines. A healthy WAT relies on continuous renewal, through a multi-step process called adipogenesis. Upon failure, the risk for lipotoxicity across the organism is higher and insulin resistance can arise in several tissues. A likely contributor for adipogenesis failure is mitochondrial dysfunction and subsequently cellular redox changes. Using 3T3-L1 cells, we have found that exposing them to hyperoxia (as a source of oxidative stress) for 8 days led to adipogenesis impairment, confirmed by oil red staining and transcription factors qPCRs. In our model, oxidative stressed preadipocytes exhibited mitochondrial dynamics diminishment (live cell imaging), Reactive Oxygen Species (ROS) production (fluorescence probe), less mitochondrial mass (WB and qPCR), smaller ATP/ADP ratio (HPLC) and less respiration capacity (Seahorse). In fact, challenging cells with antimycin, under normoxic conditions, showed similar features to hyperoxic treated cells in what adipogenesis is concerned. Recently, we found NFkB to be activated during the 8 days of hyperoxia in contrast to NFkB levels of differentiating cells which decreased after 2 days. We are convinced that mitochondrial dysfunction during aging/oxidative stress activates NFkB, blocking the main transcription factors and impairing adipogenesis.

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P-055

Inhibition of glycogen synthase kinase-3β reduces ROS production and alters antioxidant enzyme activities in MPP+-induced neuronal cell death

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Keywords: Glycogen synthase kinase-3β; Parkinson's disease; oxidative stress

Oxidative stress plays a central role in the pathogenesis of Parkinson's disease. Recently, it has been shown that glycogen synthase kinase 3beta (GSK-3β) is activated in various MPP+/MPTP models of Parkinsonism and some potent GSK-3β inhibitors such as lithium and TDZD-8 protect against MPP+-induced cell death in SH-SY5Y cells. Tideglusib, a thiazolidinedione compound, is a non-ATP competitive inhibitor of GSK-3β and an agonist of peroxisome proliferator-activated receptor gamma (PPARγ). Recently, dual effect of tideglusib is thought to have a role in neuroprotection.

The aim of the present study was to evaluate the antioxidant effects of tideglusib in MPP+-induced dopaminergic neuronal loss in SH-SY5Y cells. Reactive Oxygen Species (ROS) production was measured by DCF-DA assay and SOD, catalase and GPx enzyme activities were analyzed following treatments.

In the present study, MPP+-induced cell death and ROS generation were significantly attenuated by tideglusib. Although we could not observe any significant alteration in SOD activities following tideglusib treatment against MPP+, both catalase and GPx enzyme activities were significantly increased in tideglusib-treated cells.

Our study provides the evidence that both GSK-3β inhibition and PPARγ activation may exert neuroprotection through increasing endogenous cellular antioxidant defenses in cell culture model of Parkinson's disease.

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P-056

Novel redox-targets of NADPH oxidase 4 identified by the BIAM switch assay

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Keywords: Nox4; ROS; oxidation

NADPH oxidases of the Nox family are important sources of reactive oxygen species. Only a limited number of Nox-differentially oxidized proteins have been identified so far. We set out to identify redox-targets of Nox4 by redox-proteomics. Tetracycline-inducible HEK293 cells overexpressing Nox4 (HEK-TET-Nox4) and podocytes of WT and Nox4-/- mice were used. Redox-modified proteins were identified by the BIAM switch assay in combination with mass spectrometry and western blot analysis.

Increased Nox4 expression in response to TGFβ-1 was detected by western blot analysis in podocytes of WT mice. In response to TGFβ-1, oxidation of 138 proteins increased in podocytes of wild-type but not in podocytes of Nox4-/- mice. Identified proteins clustered in different groups of cellular processes like “cellular oxidant detoxification” and “activation of protein kinase activity”. As a second approach, HEK-TET-Nox4 cells were used in which Nox4 overexpression and Reactive Oxygen Species (ROS) production can be stimulated by tetracycline. In this protocol, an overlap in the oxidized proteins to the WT/KO system was found for proteins with antioxidant capacity as well for some interesting novel redox-targets of Nox4.

The BIAM-Switch assay coupled to Mass spec is a powerful and versatile tool to identify differentially oxidized proteins. Nox4 is a source of ROS which changes the redox-state of numerous proteins.

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P-057

Mechanosensitive microRNAS in endothelial responses to shear stress and Nrf2-mediated redox signalling

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Keywords: Shear stress; microRNAs; Nrf2

Atherosclerotic lesions develop in regions of the vasculature with complex fluid shear stress (FSS) patterns. Endothelial cells (EC) in atheroprone and atheroprotected regions are exposed to low oscillatory (OS) or high unidirectional (US) shear stresses respectively. Oxidative stress and altered activity of the transcription factor Nrf2 have been shown to contribute to atherogenesis. Using in vitro culture of human umbilical vein EC (HUVEC) under FSS, we have confirmed that expression and nuclear translocation of Nrf2 are enhanced by US (15 dyn/cm^2) compared with OS ($\pm$5 dyn/cm^2), enhancing expression of antioxidant genes such as heme oxygenase-1 (HO-1) and glutathione levels. This study investigates whether mechanosensitive microRNAs may contribute to FSS-dependent Nrf2 regulation. A screen of microRNAs known or predicted to target antioxidant genes revealed that microRNAs miR-21-3p, miR-320a, and miR-409-5p are significantly differentially regulated in HUVEC exposed to OS or US for 24 hours. Overexpression of miR-320a in static HUVEC using miRNA mimics significantly enhanced protein expression of HO-1, recapitulating observations in EC under US. This study suggests that modulation of microRNAs may ameliorate deficits in redox signalling in EC under atheroprone FSS.

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P-058

PGC-1α downregulation in steatotic liver enhances ischemia-reperfusion injury and impairs ischemic preconditioning

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Keywords: Mitochondria; oxidative metabolism; antioxidants; oxidative stress; reactive oxygen species

Liver steatosis is associated with mitochondrial dysfunction and elevated Reactive Oxygen Species (ROS) levels together with enhanced sensitivity to IR injury and limited response to preconditioning protocols. Here, we sought to determine whether the downregulation in the steatotic liver of PGC-1a, a master regulator of mitochondrial metabolism and ROSl, could be responsible for the sensitivity of steatotic liver to ischemic damage. PGC-1a was induced in normal liver following exposure to an IR protocol concomitant with an increase in the levels of antioxidant proteins. By contrast, its induction was severely blunted in steatotic liver, resulting in a modest induction of antioxidant proteins. Livers of PGC-1a-/- mice on chow diet were normal, but they exhibited an enhanced sensitivity to IR injury and also a lack of response to ischemic preconditioning, a phenotype that recapitulated the features of steatotic liver in terms of liver damage. Utilizing an in vitro model of IPC, we found that PGC-1a expression was downregulated in hepatic cells cultured at 1% O_2 whereas it was induced following reoxygenation (3% O_2), and was responsible for the recovery of antioxidant gene expression following the ischemic period. We concluded that PGC-1a plays an important role in the protection against IR injury in the liver, which is likely associated with its capacity to induce antioxidant gene expression.

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P-059

Anti-oxidant and anti-inflammatory effects of a flavonoid-rich extract from orange juice in experimental colitis

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Keywords: Citrus sinensis; Colitis; Oxidative stress Inflammation; Orange juices; Flavonoids

Flavonoids may be effective in preventing chronic degenerative diseases linked to oxidative stress, which in turn is involved in the etiopathogenesis of inflammatory bowel disease. This study was designed to assess the effects of a flavonoid-rich extract of orange juice (OJe) in mice exposed to experimental colitis. Four days of intracolonic injection of dinitrobenzene sulfonic acid (DNBS) caused colitis in mice. Daily oral administration of OJe decreased the loss of body weight caused by DNBS, lowered neutrophil infiltration, nitrotyrosine and PAR stainings, both TNF-α and IL-1β generation, nuclear NF-κB translocation, levels of ICAM-1 and P-selectin as well as increased MPO activity, enhanced MnSOD expression and modulated that of Bcl-2 and Bax. Our results indicate that OJe may be used as functional food in case of colonic oxidative stress-induced inflammation, representing a new healthy approach to avoid ulcerative colitis.

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P-060

New radical-regulatory properties of tryptophan and its derivatives

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Keywords: Tryptophan derivatives; organic radicals; free-radical reactions; steady-state radiolysis; chemical initiation

The effects provoked by ionizing radiation or metabolic disturbances in a living organism result in hyperproduction of reactive species, including O-, C- and N-centered organic and inorganic radicals. Further transformations of such radicals lead to activation of free-radical processes causing damage to biologically relevant molecules. Such processes account for and/or participate in the development of cardiovascular, neurodegenerative, oncological and inflammatory diseases. So, radical-regulatory activity of substances should be mainly associated with their high reactivity towards different types of organic radicals. The subject of this study was investigation of new type tryptophan and its derivatives interactions with O- and C-centered radicals being formed during radiation- and peroxide-induced transformations of organic compounds and with N-centered 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Tryptophan and its derivatives displayed the antioxidant properties due to the ability of these compounds to reduce O-centered organic radicals via the reaction of electron transfer from the lone pair of nitrogen atoms. We have found that

substances under study can add and oxidize C-centered organic radicals. Serotonin and 5-hydroxytryptophan are characterized by moderate reactivity toward the N-centered DPPH radical as compared with that of classical antioxidants.

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P-061

N,N.N′,N′-tetramethylhydroethidine (TMHE) - in search for better probes for the detection of superoxide radical anion

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Keywords: Superoxide radical anion; hydroethidine

Superoxide radical anion is a product of one-electron reduction of molecular oxygen. In vivo superoxide radical anion rapidly reacts with nitric oxide with the formation of peroxynitrite ($ONOO^-$) or undergoes spontaneous/SOD-catalyzed dismutation with the formation of hydrogen peroxide (H_2O_2) and thus it is a precursor of other biologically relevant oxidants playing an important role in various pathologies. Due to its reactivity and short lifetime, its detection and quantitation is difficult and demands special techniques. One of the approaches is the use of fluorogenic probes, the compounds which themselves are not fluorescent but in the reaction with superoxide radical anion are oxidatively transformed into the fluorescent products that can be directly detected. Among various profluorescent probes available hydroethidine (HE) seems to be a gold standard for detection of superoxide in biological systems. Here we present a spectroscopic and chemical characterization of new analogue of hydroethidine - N,N.N',N'-tetramethylhydroethidine (TMHE).

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P-062

In vivo recording of epidermal stem cell redox state

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Keywords: Skin; keratinocytes; redox; roGFP; in vivo

Oxidative stress and reactive oxygen species (ROS) are signaling factors thought to be important drivers of ageing due to their unique ability to damage DNA, as aging and cancer are driven by the accumulation of mutations in stem cells of proliferative tissue. They are an attractive target for interventions, but the development of such interventions is hampered by the lack of sensitive detection methods usable in live animals. Consequently, little is known about the dynamics of oxidative stress in vivo, and antioxidant treatments have failed spectacularly.

We generated mice expressing a ratiometric redox-sensitive fluorescent protein (roGFP1) in epidermal keratinocytes, which are located just 20–40 μm below the skin surface. Keratinocyte stem cells continuously regenerate the epidermis but are also the origin of skin basal and squamous cell carcinomas. Non-invasive recording of stem cell redox state is possible over hours or even days in live animals at single cell resolution using conventional confocal microscopy. We will present redox state recordings from live skin in response to DNA damage, oncogenic and proliferative stimuli and after injury.

Direct measurements of glutathione redox state in stem cell compartments of live animals are a powerful and convenient tool for evaluating interventions aimed at modulating oxidative stress in vivo.

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P-063

Violation of reproductive function in female rats at an intoxication dust aerosols Aral Sea

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Keywords: Aral Sea; reproductive function

In recent years, the Aral Sea region has more than doubled the incidence of hematopoietic organs, endocrine and reproductive systems.Purpose of the study:The study of oxidative stress in blood in female rats during the action of dust-salt aerosols of the Aral Sea in an experiment.Materials and methods of research: The object of the study was white mongrel female rats with a starting weight of 160–180 g in the number of 27 individuals 7 of them in the control group. Malonic dialdehyde (MDA) was determined in rat blood by the method of E.N. Korobeynikova, the activity of glutathione peroxidase (GPO) was carried out according to the method of Vlasova SN. Statistical analysis of the STATISTICA 6.0 package.The critical level of significance was assumed to be 0.05 ($p < 0.05$).Results and discussion: Experimental studies have shown that inhalation seeding with dust-salt aerosols at a dose of 2 mg / m3 for 30 days leads to an increase in the MDA content in blood in female rats and is reliable in comparison with the control (control group 0.71 $\pm$ 0.03 and Experimental group 1.83 $\pm$ 0.26). The activity of glutathione peroxidase decreases compared to the control value (control group 0.96 $\pm$ 0.08 and test group 0.86 $\pm$ 0.07). In our opinion, disturbances in the processes of free radical oxidation in cells affect the processes of oogenesis and the violation of reproductive function in female rats.

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P-064

Zero-valent Iron Nanoparticles Inhibited Head and Neck Cancer Cells Growth: A Pilot Evaluation and Mechanistic Characterization

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Keywords: Zero-valent Iron; ROS stress; lipid peroxidation

Nanomaterials were already identified to exhibit anti-cancer property without carrying drugs. We previously revealed the selective anti-cancer activity of Fe@Au nanoparticles (NP) by impairing mitochondria. The zero-valent iron (ZVI) core contributes the major cytotoxicity. Here, we screen oral cancer cells for resistance profile to ZVI NP and identify OECM1, OC3 and SCC9 to be sensitive, while HSC3, SAS and OC2 tolerate ZVI NP. Resistance clones of OECM1 also can be derived by pulsed NPs treatment. We identify that ZVI NP initiated Fenton reaction and induced free radicals with distinct difference in the mitochondria or cytosol between the refractory and sensitive cancer cells. Lipid peroxidation plays critical roles in the ZVI NP derived ROS induction and subsequent declined mitochondrial respiration. We further discovered the decrease of GPx upon ZVI treatment. GPx inhibitors and glutathione deprivation are able to sensitize ZVI refractory cancer cells accompanied by enhancing mitochondrial depolarization. These results reveal a potential anti-cancer mechanism of ZVI. We also demonstrate how to manipulate the ZVI resistance by combination therapy. This study provides biomarkers that predicts ZVI NP efficacy and new strategies to overcome ZVI resistance through integration of small molecular inhibitors in such new generational anti-cancer nanomedicine.

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P-065

Effect of GCEE or GCEE-loaded microspheres Administration on the Levels of Glutathione and Thiol Redox Molecules in Rat Brain

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Keywords: γ-glutamylcysteine ethyl ester; glutathione; thiol redox molecules; drug delivery to brain

It is known that activity of thiol redox molecules such as Trx, TrxR and Ref-1 is regulated by glutathione (GSH). γ-glutamylcysteine ethyl ester (GCEE) is a precursor of GSH biosynthesis and neuroprotective agent. Little is known about boosting or regulating capacity of GCEE or its pharmaceutical preparations for GSH levels or thiol redox molecules in rat brain under normal physiological conditions. Experimentally GCEE (10 mg/kg/daily) was administered to the rats as acute or chronic (five consecutive days). GSH levels determined in brain tissues by DTNB-GSH reductase assay. Trx, TrxR and Ref-1 mRNAs were quantified by qPCR. Lipid/water/lipid technique was used for the preparation of GCEE- loaded microspheres and administered to the rats with the dose of 2 mg/ml. Following the administration of GCEE- loaded microspheres, the levels of GSH in hippocampus and cortex regions were determined at 12 different time periods ranging 0–96 h by HPLC pharmacokinetically. Acute or chronic treatments with GCEE significantly increased GSH levels, and Trx mRNA expression was the most regulated parameter. Pharmacokinetically the most remarkable change in GSH levels in brain tissues was observed at 4–6 h. As a conclusion, GCEE is able to regulate the levels of GSH and thiol redox parameters, and GCEE-loaded microspheres can be used to deliver this agent to the brain successfully.

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P-066

Validation study of a food frequency questionnaire for the measurement of food consumption in polyphenols: use of a urinary bioamarker. Preliminary results

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Keywords: Polyphenol; FFQ; urinary biomarker

The purpose of our study was to assess the validity of the food frequency questionnaire (FFQ) used in the NESCaV study (Nutrition, Environment and Cardiovascular Health) for the measurement of food polyphenols intake.

53 volunteer adults, aged between 20 and 60, were included in the Stroxybio study*. The nutritional intake of total polyphenols estimated from the FFQ was compared with the results obtained from a 3-day dietary recording (EA) as well as the total polyphenol urine concentrations determined by the Folin-Ciocalteu method. The polyphenol feed composition was estimated using the Phenol-Explorer database. The median intakes of polyphenols estimated from FFQ and EA were 2270.92 mg per day and 1203.63 mg per day, respectively. The median urinary concentrations of polyphenols are 10678.57 μg of gallic acid equivalent per gram of creatinine.

The results show that there was a strong association between the results of the two dietary questionnaires, FFQ and EA (r=0.633,

p < 0.0001). A moderate but significant association was observed between FFQ results and urinary polyphenol concentrations (r=0.30, p=0.0319).

In conclusion, this study shows all the interest to combine both food survey and nutritional biomarker data to estimate exposure to polyphenols in a given population.

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P-067

Protective mechanism of Eucommia ulmoids flavone (EUF) on enterocyte damage induced by LPS

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Keywords: Eucommia ulmoids flavone; Inflammation; enterocytes and LPS

The study was carried out to explore the anti-oxidative properties of Eucommia ulmoids flavone. IPEC-J2 cells was cultured in DMEM medium containing 0, 10 μg/ml EUF, 0, 40ng/ml LPS and 0 or 10μmol/L LY29400 (PI3K inhibitor) for 2 days. The results reveal that LPS decrease DNA synthesis ($P < 0.05$) and viable number of cells ($P < 0.05$) arrest cell cycle at S-phase and induce apoptosis ($P < 0.05$), influence mitochondrial function ($P < 0.05$), decrease SOD activity, polyamine capacity ($P < 0.05$) and induce inflammation by phosphorylation of p-IKKα/β and p-NF-KB pathways ($P < 0.05$). In addition, Eucommia ulmoids flavone, confirms beneficial effects on DNA synthesis, cell viability, mitochondrial function, SOD activity and polyamine capacity ($P < 0.05$). This action proved by activation of p-Akt pathway which revert LPS induced inflammatory response. In the view of current evidences, EUF flavones activates the transcription factors and cell survival signaling pathways such as p-Akt pathways which restores mitochondrial functions and inhibiting the productions of free radicals. The dietary EUF flavones could be helpful in implication of mitochondrial diseases in humans and animals.

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P-068

Effect of dust-salt aerosols of the Aral Sea on biochemical indices in the rats' testicular homogenate

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Keywords: Effect of dust-salt aerosols of the Aral Sea on biochemical indices in the rats' testicular homogenate

The influence of adverse social and ecological factors leads to stressful situations, which cause a deterioration in health and an increase in the frequency of pathologies of various genesis and disorders at the molecular-cellular level. The aim of the study was the investigation of the products of lipoprocessing in testicular homogenate in rats under the effect of dust-salt aerosols of the Aral Sea in an experiment. Materials and methods of research: the objects of study were white juvenile rats - males, the initial mass of 180–200 g in the number of 27 individuals, 7 of them in the control group. Considering circadian mechanisms of regulation of biorhythms, all experiments were conducted at the same time of day and seasons (autumn, winter). Results and discussions: Experimental studies have shown that acute inhalation primer of dust-salt aerosols at a dose of 2 mg/m^3 after 60 days induces significant impairment of products of lipoprocessing cascades and enzymes of AOP and ADA. Thus, with the activation of LPO processes, free radicals with high reactivity can interact with lipids, proteins, nucleic acids, as a result of which destructive processes occur, leading to the appearance of oxidation products and degradation of polymers.

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P-069

Reductive stress in pathophysiology

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Oxidative stress, as defined by Sies more than thirty years ago, has received much attention and has served as an important intellectual tool to understand the pathophysiology of many diseases and also of normal processes like ageing.

However, recently the idea that the cells might suffer from reductive rather than oxidative stress and that such stress may be relevant in pathophysiology has gained momentum.

Some time ago we defined reductive stress as a "as a pathophysiological situation in which the cell becomes more reduced than in the normal, resting state". We postulated that reductive stress might be due, at least in part to a "small but persistent generation of oxidants that results in a hormetic overexpression of antioxidant enzymes that leads to a reduction in cell compartments".

Experiments showing reductive stress in experimental myocardial ischaemia in swine, in clinical studies in Alzheimer's

disease patients and in normal individuals at high risk of developing Alzheimer's (because they carry the ApoE4 allele), will be discussed to highlight the role of reductive stress in these pathophysiological processes.

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P-070

Environmental noise and particulate matter exposure, oxidative stress and vascular function - the underestimated cardiovascular risk factors

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Keywords: Environmental risk factors; traffic noise exposure; particulate matter exposure; mental stress; stress hormones; endothelial dysfunction; oxidative stress; hypertension

Epidemiological studies have demonstrated that traffic noise and particulate matter exposure is associated with cardiovascular diseases such as arterial hypertension, myocardial infarction and stroke. Persistent chronic noise and particulate matter exposure increases the risk of cardiovascular and metabolic diseases such as arterial hypertension, coronary artery disease, diabetes and stroke. Data of the Heinz Nixdorf Recall Study (Kälsch et al. Eur. Heart J. 2014) but also large epidemiological studies (reviewed in Münzel et al. Eur. Heart J. 2017) point towards a link between the incidence of ischemic heart diseases and exposure to noise and/or particulate matter, supporting their role as independent cardiovascular risk factors, suggesting synergistic effects by both stressors and warranting further detailed studies to understand the mechanistic basis of this association. Recently, the underlying molecular mechanisms leading to noise-dependent adverse effects on the vasculature were characterized in an animal model of aircraft noise exposure identifying oxidative stress as a central player in mediating vascular dysfunction, which shares striking similarities with the pathomechanisms reported for particulate matter exposure. With the present overview the mechanistic parallels in the pathophysiological processes induced by noise and particulate matter exposure will be discussed.

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P-071

Taking up the cudgels for the traditional reactive oxygen and nitrogen species detection assays and their use in the cardiovascular system

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Keywords: Oxidative stress; redox signaling; fluorescence and chemiluminescence-based assays; dihydroethidium oxidative fluorescence microtopography; lucigenin-enhanced chemiluminescence; L-012-enhanced chemiluminescence

Reactive oxygen and nitrogen species (RONS such as H_2O_2, nitric oxide) confer redox regulation of essential cellular functions, initiate and catalyze adaptive stress responses. In contrast, excessive formation of RONS may lead to appreciable impairment of cellular function and in the worst case to cell death, organ dysfunction and severe disease phenotypes of the entire organism. Therefore, the knowledge of the severity of oxidative stress and tissue specific localization is of great biological and clinical importance. However, at this level of investigation quantitative information may be enough, whereas for the development of specific drugs, the cellular and subcellular localization of the sources of RONS or even the nature of the reactive species may be of great importance, and accordingly, more qualitative information is required. These two different philosophies currently compete with each other and their different needs (also reflected by different detection assays) often lead to controversial discussions within the redox research community. With the present overview we discuss these different philosophies and needs, but also to defend some of the traditional assays for the detection of RONS that work very well in our hands. We will also provide an overview on the “new assays” with a brief discussion on their strengths but also weaknesses and limitations.

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P-072

Oxidative modifications of α- and β- caseins induced by AAPH-derived peroxyl radicals: Role of tryptophan and tyrosine residues

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Keywords: Caseins; peroxyl radicals; protein aggregation/fragmentation; tryptophan oxidation; tyrosine oxidation

In whole milk, as consequence of lipid-peroxidation, α- and β-caseins (α-Cn and β-Cn) can be oxidized leading to protein aggregation and/or fragmentation. The present work was designed to address the hypothesis that changes in the molecular mass of caseins induced by AAPH-derived peroxyl radicals (ROO•) would be related to Trp and/or Tyr residue oxidation. SDS-PAGE results showed that the exposition of β-Cn to a low AAPH concentration (20 mM), leaded exclusively to the formation of two fragments. In addition, the kinetic profile of the decrease of β-Cn monomer was in agreement with the consumption of its single Trp residue suggesting that β-Cn is fragmented by a process related to Trp oxidation. By contrast, at a high AAPH concentration (100 mM), β-Cn oxidation showed only the formation of protein aggregates. No relationship was observed between the kinetic profiles of consumption of the monomer and Trp. Nonetheless, consumption of Tyr and Lys residues was evidenced by UPLC-methodology. When α-Cn was exposed to different concentrations of AAPH, only aggregates of proteins were generated and the consumption of Tyr and Lys residues was detected. These results show that both caseins have a different susceptibility to reaction with ROO•. These difference are probably associated with their content of Trp and Tyr residues; α-Cn has 2 Trp, and 10 Tyr, while β-Cn has 1 Trp and 4 Tyr.

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P-073

Validated routine-ready UHPLC/MS-MS method for the reference range determination in human plasma of 15-f2t-isoprostane, biomarker of the oxidative stress

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Keywords: 15-F2t-isoprostane; routine way

Background: Isoprostanes are considered as being "gold standard" markers of lipid peroxidation although large differences have been observed in normal plasma reference values.
Material and Methods: After blood collection on EDTA, plasma samples were purified through an original solid-liquid extraction protocol. After evaporation to dryness and reconstitution, samples were directly injected into an UHPLC/MS-MS system for 15-F2t-isoprostane analysis.
Results: A population of 61 subjects aged between 18 and 60 years (29 men and 32 women) was selected in our study *. The mean value in plasma 15-F2t-isoprostane obtained was 260 pg / mL with a standard deviation of 55 pg / mL (minimum and maximum values of 294.7 and 403.3 pg / mL). The normal distribution observed gave a reference interval between 151.96 and 368.16 pg/mL. This observation was confirmed by the Shapiro-Wilk test result ($p=0.1125$). Interestingly, we have observed a moderate but significant positive correlation with the copper/zinc ratio ($r=0.34$; $p=0.01$) and a moderate but significant negative correlation with total glutathione ($r=-0.28$; $p=0.34$), both markers being considered as reflecting the presence of in vivo oxidative stress.
Conclusion: Our study has allowed to measure plasma 15-F2t-isoprostane in a routine way.

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P-074

Identification of nitration sites in the extracellular matrix protein laminin

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Keywords: Nitration; Extracellular matrix; Mass Spectrometry

Peroxynitrous acid (ONOOH), produced by activated leukocytes can nitrate Tyr and Trp residues on proteins. These modifications are used as biomarkers and may contribute to human pathologies. Extracellular matrix (ECM) proteins are abundant, long-lived and exposed to high oxidant fluxes; they may therefore accumulate damage. Nitration has been detected in human atherosclerotic lesions, and this co-localizes with the major ECM protein laminin. Nitration also affects laminin assembly and decreases cell binding Aims: To identify laminin nitration sites, and understand how and why nitration alters laminin structure and function Results: Using mass spectrometry we detected nitration at 126 Tyr and 20 Trp sites in laminin exposed to a 500-fold ONOOH molar excess. The degree of nitration varies greatly between sites, with median occupancy of ~4%, but 29 sites had >10% occupancy. 18 sites were also seen with a 5-fold molar excess, indicating that these sites are major targets. These occur within 3 cell attachment sites, as well as regions involved in laminin polymerization and ECM assembly Conclusions: Laminin is readily nitrated by ONOOH, and this occurs selectively at sites in functionally-important domains, consistent with biological data. Laminin nitration may contribute to human disease, through decreased cell adhesion and altered ECM integrity, and be a sensitive marker of damage.

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P-075

Modulation of oxidative stress response in neurodevelopment disorders. The case of the Rett syndrome variants: MECP2 and CDKL5

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Rett syndrome (RTT) variants are rare neurodevelopmental disorders caused by mutations in the X-linked MECP2 or CDKL5 genes. Because CDKL5 mediates MeCP2 phosphorylation and CDKL5 is a MeCP2-repressed target gene, it is possible that these proteins share similar molecular pathways. Altered redox status and subclinical inflammation seem to be common denominators between the two RTT variants: increased systemic levels of 4-HNE protein adducts and dysregulated cytokine profiles were reported. The altered redox homeostasis appears to be a critical player in the posttranslational modifications of SRB1, a specific high-density lipoproteins receptor, leading to altered serum lipid profiles in both variants. Although these parallels, Nrf2 system shows an impaired function in both disorders, but with some divergent aspects. CDKL5 show an inability to induce a proper defensive response with a significantly low nuclear Nrf2 translocation after oxidative challenge, while in MECP2 a sustained Nrf2 activation observed in basaline and upon oxidative stress stimulus. These findings reveal common defective pathways related to redox homeostasis between MECP2 and CDKL5. At present, the molecular basis of the discrepancy in the Nrf2 is unknown and requires further investigation in the light of the possibility to develop new therapeutic strategies for these devastating disorders without a cure.

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P-076

The importance of culturing primary cells under physiological conditions: proliferation, senescence, pluripotency

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Keywords: Stem cell; pluripotent; senescence; oxidative stress; aging

Mesenchymal stem cells (MSCs), such as human dental pulp stem cells (hDPSCs), are currently a source for cell therapy. However, cell therapy protocols require 10–400 million cells per treatment, and consequently, they need to be expanded in vitro before implantation, with the inconvenience that MSCs undergo senescence following a certain number of cell expansion passages, loosing their stem cell qualities. Ambient oxygen tension (21% pO_2) is normally used for in vitro culture, but physiological levels in vivo range between 3% and 6% pO_2. We previously demonstrated that hDPSC proliferation rate is significantly lowered at 21% pO_2 due to enhanced oxidative stress, which led to the activation of p38/p21/NRF-2 pathway, upregulating antioxidant defenses. Moreover, long-term in vitro culture of hDPSCs at 21% pO_2 caused an oxidative stress-related premature senescence, as evidenced by increased β-galactosidase activity and lysil oxidase expression, which is mediated by p16INK4a pathway. This was accompanied by a downregulation of OCT4, SOX2, KLF4 and c-MYC factors, which was recued by BMI-1 silencing. Thus, p16INK4a and BMI-1 might play a role in the oxidative stress-associated premature senescence. For all these reasons, we show that it is important for clinical applications to culture cells at physiological pO_2 to retain their stemness characteristics and to delay senescence.

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P-077

Clearing Amyloid-β through PPARγ/ApoE Activation by Genistein is a Treatment of Experimental Alzheimer's Disease

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Keywords: Alzheimer's disease; astrocytes; genistein; ApoE; PPARg

Amyloid-b (Ab) clearance from brain, which is decreased in Alzheimer's disease, is facilitated by apolipoprotein E (ApoE). ApoE is upregulated by activation of the retinoid X receptor moiety of the RXR/PPAR dimeric receptor. As we have previously demonstrated, estrogenic compounds, such as genistein, have antioxidant activity, which can be evidenced by increased expression of manganese superoxide dismutase (MnSOD). Furthermore, genistein is a non-toxic, well-tested, and inexpensive drug that activates PPARg receptor. We isolated and cultured cortical astrocytes from dissected cerebral cortices of neonatal mice (C57BL/6 J). Preincubation with genistein (5 mM) for 24 hours, prior to the addition of Ab (5 mM) increased ApoE release to the culture medium in a concentration dependent manner. This effect is mediated by activation of PPARg as its inhibition significantly prevents the increase in genistein- induced ApoE release. Finally, treatment of an Alzheimer's disease mouse model with genistein was associated with a lowering of Ab levels in brain, in the number and the area of amyloid plaques (confirmed in vivo by positron emission tomography) as well as in microglial reactivity. Our results strongly suggest that controlled

clinical trials should be performed to test the effect of genistein as treatment of human Alzheimer's disease.

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P-078

DNA damage, repair of single strand breaks, total antioxidant capacity and reduced glutathione levels in female patients with a family history of cancer

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Keywords: Oxidative DNA damage; DNA repair capacity; Total antioxidant capacity of plasma; Reduced glutathione; cancer

Introduction: Oxidative DNA damage, a biomarker used in peripheral blood studies related to cancer and other diseases, may be modulated by plasma antioxidant capacity (TAC), the levels of reduced glutathione (GSH) and the DNA repair ability.
Objectives: To determine endogenous and oxidative DNA damage, lymphocyte DNA repair capacity against hydrogen peroxide, plasma levels of TAC and GSH in female patients with a family history of cancer.
Methods: We studied 40 patients (38,9 $\pm$ 14,6 years) and 21 healthy women (39.4 $\pm$ 12.5 years). The alkaline comet assay was used for determination of markers of DNA damage and repair ability. Total antioxidant capacity and plasma glutathione levels were determined by spectrophotometric methods.
Results: Endogenous and oxidative DNA damage were increased 1.5 and 1.3 times in patients, although without statistical significance. Residual DNA damage after challenging cells with hydrogen peroxide was increased 1.2 times in this group, reflecting a lower efficiency of DNA repair, but without statistical significance. Plasma levels of GSH were significantly elevated in patients, whereas plasma TAC was not significantly modified.
Conclusions: Patients showed a tendency for genomic instability that could be modulated by an increase in plasma GSH levels. These markers could be used in clinical follow-up of patients with genetic risk of developing cancer.

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P-079

Contribution of NADPH oxidase isoforms to angiotensin II-mediated oxidative stress and DNA damage in the mouse kidney

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Keywords: Hypertension; angiotensin II; DNA damage

Hypertension caused by elevated levels of the blood pressure-regulating hormone angiotensin II (AngII) leads to oxidative stress and DNA damage in kidneys of mice. In vitro-studies showed a role of the free radical-generating NADPH oxidase (Nox) in this damage. Here, we studied the contribution of the isoforms Nox1, 2 and 4 to AngII-induced oxidative stress and DNA lesions in the kidney.

Male wildtype (WT) C57BL/6, Nox1-, Nox2- and Nox4-knockout (KO) mice were infused with either vehicle or AngII during 28 days. Afterwards, urine and kidneys were analyzed for markers of oxidative stress and DNA damage.

In WT mice, AngII induced hypertension, elevated urinary albumin levels and formation of ROS and DNA damage in the kidney. In Nox2- and Nox4-KO mice blood pressure was as high as in WT mice but lower in Nox1-KO mice. A rise in urinary albumin and systemic oxidative stress due to AngII was observed in all KO mice. In kidney tissue, only Nox1-KO mice showed no significant ROS formation. Basal values of genomic damage were higher in Nox2- and Nox4-KO mice. Only in Nox1-KO mice AngII did not cause significantly more DNA double strand breaks than in the controls.

Nox1 seems to be mostly responsible for the AngII-induced generation of ROS in mouse kidney. Surprisingly, genomic damage in kidneys of Nox2- and Nox4-KO mice was already increased in the absence of AngII.

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P-080

Effect of ozone oxidative stress on chloride current in human lung cells: Chemical mediators and protective role of catalase

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Keywords: Oxidative stress; Cl channel; catalase; hydrogen peroxide; 4HNE

In this work we have investigated the effects of ozone (O_3), one of the most noxious pollutants to which respiratory tract is organ

most exposed, on Cl currents in human cultured lung epithelial cells (A549 line).

Biological and electrophysiological technique was applied to study the action of O_3 and its main bio-products (4HNE and H_2O_2) on Cl currents in A549 cells, which simulates the first barrier encountered by oxidants.

O_3 exposure (0.1 ppm, 30') significantly affects Cl current inducing a large outward rectifier component. While 4HNE (up to 25 μM) was not able to reproduce the effect, H_2O_2 produced by the cell (Glucose Oxidase 10mU) mimicked O_3 damage.

Then we analyzed the effect of G.O. treatment on ClC-2 and ORCC gene expression, the two Cl channels mainly involved in current alteration. RT-PCR showed the ability of oxidative stress due to H_2O_2 to modulate the gene expression of these channels.

Eventually, we verified the protective effect of catalase (1 mM, 1.30 h). The results showed the ability of catalase in suppressing the outward rectifier component activated by O_3, bringing back the current values to the control level.

This study brings new insights on the mechanisms involved in O_3 induced lung tissue damage, confirming its ability to modify the cellular redox homeostasis, and to highlight a new aspect such as the alteration of Cl channel functionality via H_2O_2 formation.

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P-081

Identification and characterization of inter-species aging-related transcriptomic regulators

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Keywords: Aging; mitochondria; ROS

Life expectancy in industrialized countries has significantly increased in recent years. The aim to extend healthy lifespan, a.k.a healthspan, requires the identification and characterization of molecular pathways mechanistically linked to the aging process.

To identify conserved transcriptional patterns involved in decline of organisms associated with aging we analyzed deep sequencing data from 3 species (the nematode *C. elegans*, the zebrafish *D. rerio*, and *M. musculus*) at three time-points in their lifetime. Using bio-informatics pipeline of motif search through scanning transcription factor binding sites in promoter regions of genes associated with age-related changes with known position weight matrix sets, we identified a number of highly conserved transcription factors which may regulate longevity. Lifespan assay results show that when their activity is compromised in adult *C. elegans* nematodes by RNAi, the change in expression profile indeed affects lifespan of the animals.

Through RNA next generation sequencing and epistasis analyses we found novel candidate downstream effectors suggesting a crucial role of redox sensing and energy homeostasis in lifespan regulation. Further experimental tests will provide information on such longevity-related genes in *C. elegans* and higher organisms contributing to better understanding of the general paradigm of aging in humans.

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P-082

Nrf2 and NF-κB have a role in aldosterone-mediated oxidative damage

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Hypertension caused by elevated levels of the blood pressure-regulating hormone aldosterone (Ald) leads to oxidative stress and DNA damage in kidneys of rats. In vitro studies showed a protective role of the transcription factor Nrf2 and adverse effects of NF-κB activation. Here we studied the impact of Nrf2 activation and NF- κB inhibition on Ald-mediated damage. Further, the kidneys were studied for markers of epithelial-mesenchymal transition (EMT).

Sprague-Dawley rats were infused with either vehicle or Ald during 28 days. Some rats were additionally treated with sulforaphane (Sulf), an Nrf2-activator or with pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB.

Ald-treatment led to increased blood pressure, which was lowered by Sulf and PDTC, respectively. Loss of kidney function induced by Ald was ameliorated by either Sulf or PDTC. Additionally, Ald caused higher levels of double strand breaks, which were reduced by Sulf and also by PDTC. Increased levels of apoptosis in cortex of Ald-treated rats were also reduced by both substances. We could not observe any differences in epithelial or mesenchymal markers after 28 days Ald-treatment.

In summary, activation of Nrf2 and as well as inhibition of NF-κB protected from Ald-induced hypertension, kidney damage and DNA lesions.

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P-083

The effect of a bespoke home based physical activity intervention on markers of oxidative stress and markers of general health in older adults

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Keywords: Physical activity; community; dementia

Home-based unsupervised exercise training has been shown to improve health in older adults. Assessing adherence, especially using a self-report format, in unsupervised exercise interventions is problematic. The aim of this pilot study was to objectively assess the effect of an unsupervised home-based exercise intervention in community dwelling older adults. Markers of oxidative stress and measures of health were assessed, including cerebral hemodynamics and cognitive function. Physical activity was measured

continuously using a wrist watch type accelerometer with optical heart rate monitoring. A secondary aim of the study was to test the feasibility of a wrist activity monitor as a tool to assess physical activity in this population. The intervention failed to increase the level of physical activity in all the participants, although changes were observed. No significant changes were observed in markers of oxidative stress or other measures of health in response to the exercise intervention. Progressive goals, based on adherence measurement, are potentially important for the success of an unsupervised exercise intervention. Wrist-based accelerometers may be useful tools for the assessment of physical activity in sedentary older adults, but further development of specialist software may be necessary.

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P-084

Therapeutic assessment of tirapazamine-induced oxidative stress on gastric cancers

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Keywords: Tirapazamine; Catalase; Glutathione S-transferase P; Gold nanoparticles

Tirapazamine (TPZ) has been considered as a therapeutic candidate for cancers in the future. It selectively activates multiple reductases to release free radicals from hypoxic cells such as cancer cells, leading to DNA damages and cell death. This unique character of TPZ may impose less cytotoxicity on normal organs or tissue, leading to reduce the side effects on cancer patients. In this study, we conjugated TPZ onto gold nanoparticles (AuNPs) for increased stability in serum and more efficiently assessing to tumors via Enhanced Permeability and Retention (EPR) Effect. Our results demonstrated the TPZ is significantly cytotoxic to MNK45 hypoxic gastric cancer cells, but less harmful to cells growing under normoxia condition. We noticed that TPZ-induced cell death may result from oxidative stress in cells.Several detoxification enzymes such as catalase and Glutathione S-transferase P in hypoxic MNK45 cells were increased after exposure to TPZ. Moreover, our data suggested TPZ-induced cell death is mediated by apoptotic program. Furthermore, we found intravenously injected TPZ-AuNPs were largerly retained in tumors and significantly reduced the tumor volume in xenografts, suggestion TPZ-AuNP may directly impact on tumor cell proliferating and growing. Taking together, TPZ-AuNPs may be a putative candidate for cancer therapy

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P-085

Characterization of vimentin-zinc interaction and its impact on the response to electrophilic and oxidative stress

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Vimentin is an intermediate filament protein expressed in mesenchymal cells, playing a key role in organelle positioning, cell migration and signalling. Several electrophiles and oxidants target vimentin, mainly through its cysteine residue C328, causing network reorganization. Our previous studies suggest that cellular zinc availability may function as a reversible switch controlling vimentin dynamics and susceptibility to oxidants. Thus, we aimed to characterize the interaction between vimentin and zinc, and to understand its protective role against oxidative and electrophilic stress.

We have observed that micromolar zinc induces vimentin polymerization in vitro, as assessed by centrifugation and light scattering assays, which is reversed by zinc chelators, such as EDTA and TPEN. Several crosslinking agents induce vimentin oligomerization. Remarkably, preincubation with zinc selectively protects vimentin from cysteine crosslinking with dibromobimane (DBB), whereas amino group crosslinking by disuccinimidyl tartrate (DST) is not affected. Incubation with zinc also protects vimentin from modification by several electrophilic lipids, an effect that is not mimicked by other divalent cations, like magnesium. These results illustrate an avid interaction between vimentin and zinc in vitro, which could be important for vimentin dynamics and response to oxidants and electrophiles.

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P-086

Oxidative modifications cross-talk in redox regulation of cellular physiology

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Redox balance plays an important role in the regulation of cellular physiology via orchestrated action of electron donors/acceptors, reactive oxygen and nitrogen species (RONS) and antioxidant defence mechanisms. Recently postulated hypothesis of "redox switches" via ROS-induced protein post-translational modifications (PTMs) acknowledge its importance in cellular signaling events. Importantly, impairment of redox homeostasis is a crucial factor in the development of numerous human pathologies including metabolic and cardiovascular diseases. However, the role of redox regulated modifications and PTM cross-talk is poorly investigated, mostly due to the analytical challenges in their high-throughput detection and quantification. Using state-of-the-art bioanalytical methods, a detailed investigation of different lipid and protein PTMs was performed using dynamic cardiomyocyte model of nitroxidative stress. Fluorescent microscopy revealed significant alterations in subcellular distribution of main cytoskeletal proteins – actin, vimentin and tubulin. Using in-depth proteomics approach over 35 different post-translational modifications were mapped and relatively quantified for cytoskeletal proteins. This allowed to identify “hot spots”, like the single cysteine residue of vimentin, which might play an important role in PTM cross-talk and thus take part in redox regulation.

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P-087

NRF2 controls proteostasis through the transcriptional regulation of autophagy

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Keywords: Autophagy; chaperone mediated autophagy; NRF2; proteostasis; transcriptional regulation

Cells control the abundance and quality of the proteome through a wide network that integrates signaling pathways, gene expression and protein degradation systems. Degradation of cytosolic components inside lysosomes is carried out by specific types of autophagy in mammals: macroautophagy, chaperone mediated autophagy (CMA) and microautophagy. Considering autophagy as a proteostatic and defensive mechanism, we sought to determine if this process was regulated by the transcription factor NRF2, classically considered the master regulator of the antioxidant cell response. A bioinformatics analysis allowed us to identify putative NRF2 binding sequences in macroautophagy and CMA related genes. Several were further validated as NRF2-regulated genes by ChIP assays and quantitative PCR in Nrf2-deficient cells. Consequently, Nrf2-knockout cells exhibited impaired macroautophagy flux in response to H_2O_2. Oxidative stress also up-regulates CMA through transcriptional induction of Lamp-2a, but to a lesser extent in Nrf2-deficient cells. Moreover, Nrf2-knockout cells showed reduced LAMP-2A lysosomal levels, the limiting step for CMA. Pharmacological activation of NRF2 led to the perinuclear accumulation of LAMP-2A positive lysosomes, indicative of CMA activation. Overall, these results point to a novel role of NRF2 in the regulation of autophagy and suggest a new strategy to combat proteinopathies.

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P-088

p38α and NF-κB regulate antioxidant defense in the liver through an age-dependent mechanism

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Keywords: p38; nf-kappab; oxidative stress; aging

p38α MAPK is a sensor of oxidative stress. The aim of this work was to assess the role of p38α in the regulation of the antioxidant defense in the liver with aging. Livers ofyoung and old wild type (WT) and p38α liver-specific knock out (KO) mice were used to determine glutathione redox status by mass spectrometry; malondialdehyde (MDA) levels by HPLC; mRNA expression of glutamate cysteine ligase (Gclc), Sod1, Sod2 and catalase by RT-PCR and nuclear levels of NF-κB subunit p65 by western-blotting. Chromatin immunoprecipitation (ChIP) assay of p65 was performed. Young KO liver exhibited increased in GSSG/GSH ratio and MDA levels when are compared with young WT mice. However, old KO mice had lower hepatic GSSG/GSH ratio and MDA levels than young KO mice. The mRNA expression of Gclc, Sod1, Sod2 and catalase was decreased in young KO mice compared with WT young mice. In contrast, these antioxidant enzymes were up-regulated in KO old mice regard to WT. p65 was markedly phosphorylated in the nucleus of WT young and old KO mice. ChIP assay of p65 confirmed the recruitment of p65 to the promoters of antioxidant genes in WT young and KO old mice. Deficiency of p38α in the liver causes dramatic down-regulation of antioxidant enzymes in young mice, but triggers adaptive up-regulation in the long term via NF-κB to enhance the antioxidant defense in old p38α KO mice.

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P-089

NRF2/KEAP1-mediated antioxidant defence pathway regulates skeletal muscle circadian clock function

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Keywords: NRF2; Circadian; FDB; muscle; COPD

The circadian clock is an intrinsic timing mechanism which regulates most physiological processes within the body. Disruption of circadian rhythms has been identified as a risk factor for several chronic diseases with altered redox control. We investigated the hypothesis that NRF2, a master regulator of antioxidant defence, is a clock-controlled gene in skeletal muscle, which can modulate the circadian clock function. Using genetic, pharmacological and real-time imaging approaches, we identified a feedback mechanism between the circadian clock machinery and the NRF2/KEAP1 antioxidant pathway. Pharmacological manipulation of NRF2 exerted robust effects on both the amplitude and periodicity of circadian clock oscillations. Moreover, single muscle fibre isolation has confirmed cell-autonomous changes in core clock gene expression in fibres from Nrf2 KO mice. Interestingly, similar effects on clock gene expression were also evident in muscle fibres isolated from old wild-type mice. Loss of Nrf2, or ageing led to diminished clock gene cycles but activated rhythmic gene cycles of genes involved in inflammation and stress resistance. All together, these findings implicate NRF2 as an important therapeutic target which may be utilised in future to reset or re-align disrupted circadian rhythms seen in several chronic diseases associated with muscle wasting including COPD and sarcopenia.

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P-090

Lipofuscin effects in *Caenorhabditis elegans* ageing model

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Keywords: Ageing; Lipofuscin; Proteostasis; *Caenorhabditis elegans*

Ageing is a multidimensional process involving many variables that affect all levels of life. Upon ageing a significant increase of damaged biomolecules, such as accumulated proteins occurs with age pigments being such molecules. The “age pigment” lipofuscin is naturally produced throughout the life of an organism and it is aggregated in post-mitotic cells as ageing progresses resulting in age-dependent degeneration of various cellular systems. Lipofuscin is non-degradable and consists of oxidized, cross-linked proteins, lipids and saccharides. Its accumulation seems to be associated with the cellular proteolytic mechanisms inability to degrade it during ageing. Lipofuscin accumulation promotes ageing as it acts as proteasome inhibitor by directly binding on proteasome complexes. Our aim is to study the effects of lifelong exposure to lipofuscin on organismal lifespan and physiology by exploiting the ageing model of *Caenorhabditis elegans*. Our preliminary results demonstrated that the nematode lifespan is altered upon treatment with artificial lipofuscin and that the two major cellular degradation mechanisms (proteasome and autophagy) are affected. Detailed analysis of the involved antioxidant mechanisms and metabolic pathways has been performed and additional experimentation will elucidate the molecular and biochemical basis of the above-mentioned effects.

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P-091

N-acetylcysteine, an antioxidant with anti-adipogenic effect on adipocytes

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Keywords: N-Acetylcysteine; Obesity; Triglyceride; Adipogenic factor

Reports about antioxidant effect in obesity are contradictory. We showed that N-acetylcysteine (NAC) inhibits cellular lipid accumulation during adipocyte differentiation, through the inhibition of adipogenic transcriptor factors expression, such as PPARγ and, MAPKs phosphorylation (Pieralisi et al.,Redox Biol 2016; Soto el al, Redox Rep 2016). Here we evaluated NAC on fully differentiated cells (3T3-L1 adipocytes: AC). Treatments with 0.01 to 5 mM NAC, included in culture media for 5 days, were not toxic. 5 mM NAC treatment on AC (ACN) provoked a decrease of 60% in cellular Triglycerides (Tg) content (1.22+0.09 gTg/g protein [AC] vs 0.49+0.03 gTg/g protein [ACN], $p < 0.05$). We evaluated Oil-Red-O stained lipids content in ACN comparing to AC, which is set to 100 (100+4 [AC] vs 80+2 [ACN] arbitrary units (AU), $p < 0.05$), lipid protein perilipin (Pl) mRNA levels (Pl/ Rplp0:100+13 [AC] vs 72+9 [ACN] AU, $p < 0.05$) and PPARγ: protein expression (PPARγ/GAPDH:100+6 [AC] vs 70+4 [ACN] AU; $p < 0.05$) and mRNA levels (PPARγ/ Rplp0:100+7 [AC] vs 74+14 [ACN] AU, $p < 0.05$). These NAC treatments produced a decrease of 20 – 30%, suggesting that NAC could

inhibit new lipid production. We developed liposome of 100 nm diameter (LIP) including 5 mM NAC, as better tool for NAC administration. Phase transition enthalpy decreased from 3.86 J/g in LIP to 3.25 J/g in LIP+NAC, indicating NAC incorporation.

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P-092

Coordinated alteration of expression of redox-dependent genes in development of adaptive antioxidant response under formation of drug resistance of cancer cells

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Keywords: Adaptive antioxidant response; redox-dependent genes; superoxide dismutase; glutathione peroxidase; glutathione transferase; thioredoxin

Aims: Here we studied the expression of isoforms of redox-dependent antioxidant enzymes and a rise of adaptive antioxidant response under development of cancer cells resistance to anticancer agent cisplatin (CDDP) possessed pro-oxidant action.
Results: Under development of resistance of human erythroleukemia K562 and ovarian carcinoma SKOV-3 cells to CDDP co-ordinative enhanced expression of genes encoding isoforms of superoxide dismutase (SOD1, SOD2), glutathione peroxidase (GPx1, GPx4), glutathione transferase (GSTP1-1, GSTM1-1), glutaredoxin (GLRX1, GLRX2), thioredoxin (TRX1,TRX2) was found in both types of resistant cells in compare with wild cells. In addition, growth of GSH/GSSG ratio as index of cellular redox state as well as elevated level of transcription factor Nrf2 were observed in resistant K562/DOX and SKVLB cells. Decrease of apoptotic cell death was observed under exposure to hydrogen peroxide in the CDDP-resistant cells possessed elevated antioxidant status, as evidenced by low generation of ROS. It can be concluded that the mechanism of the formation of cancer cells resistance to CDDP involves the development of adaptive antioxidant response as key in stress adaptation.

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P-093

Localized redox relays as a privileged mode of cytoplasmic hydrogen peroxide signaling

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Keywords: Redox signaling; redox relays; peroxiredoxins; hydrogen peroxide; mitogenic signaling

Hydrogen peroxide's (H_2O_2) best characterized signaling actions in mammalian cells involve the oxidation of thiols in cytoplasmic targets within minutes of stimulation. However, these redox targets are orders of magnitude less H_2O_2-reactive and abundant than cytoplasmic peroxiredoxins (Prx). How can they be oxidized in minutes? Our computational results show that at H_2O_2 supply rates commensurate with mitogenic signaling a H_2O_2 concentration gradient of a few tenths of μm is established. Even near supply sites H_2O_2 concentrations are too low to oxidize typical targets in minutes. Further, any Prx inhibition or increase in H_2O_2 supply able to strongly increase the local H_2O_2 concentration would collapse the gradient and/or extensively oxidize PrxI/II, inconsistent with observations. In turn, the local concentrations of Prx sulfenate and disulfide forms strongly exceed those of H_2O_2. Redox targets reacting with these forms at rate constants much lower than that for thioredoxin could be oxidized in < 10 s. Moreover, the spatial distribution of these Prx forms allows them to reach targets within 1 μm from the H_2O_2 sites while maintaining signaling localized. Altogether, these results suggest that H_2O_2 signaling is mediated by localized redox relays whereby Prx are oxidized to sulfenate and disulfide forms at H_2O_2 supply sites and these forms in turn oxidize the redox targets nearby.

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P-094

Scavenger receptor B1, a cutaneous sensor of pollution-induced oxidative damage

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Keywords: SR-B1; environmental stressors; skin; wound healing; epidermal lipids

Scavenger receptor B1 (SR-B1) is a cell membrane receptor expressed in multiple tissues and exerts functions such as cholesterol and vitamins uptake, vesicles trafficking and pathogens recognition. SR-B1 is expressed also in human skin, especially in

epidermis and sebaceous glands. Recent studies have shown the ability of environmental stressors (i.e. cigarette smoke, CS) to affect SR-B1 levels in cultured keratinocytes. Skin acts as a barrier against outdoor environment and its physical integrity together with its physiological composition are crucial to accomplish its functions. In this study we aimed to evaluate SR-B1 role in cutaneous barrier maintenance. We attested that SR-B1 redox susceptibility not only applies to 2D cultured cells, but also to wholly differentiated skin, by using 3D reconstructed epidermis. CS and other pollutants like ozone and particulate matter decreased SR-B1 levels within the tissue. We demonstrated SR-B1 implication in cutaneous integrity preservation; indeed, it appeared actively involved in skin wound healing, by regulating keratinocytes proliferation, migration and pro-migratory cytoskeleton rearrangement. By using SR-B1 knockdown organotypic skin model, we showed a striking change in lipids organization within the epidermal layers. Our findings suggest that SR-B1 loss upon external insults may influence cutaneous repair and lipid homeostasis.

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P-095

Application of Resonance Raman spectroscopy for the direct detection of manganese porphyrins and their redox state in endothelial cells

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Manganese porphyrins (MnPs) can act as efficient catalytic antioxidants. MnPs can reach mitochondria and confer pharmacological protection in different models of disease where oxidants such as peroxynitrite participate. MnPs quantitation and subcellular distribution by LC-MS/MS has been reported; however, a direct method to evaluate their uptake and redox state in living cells has not been developed. Here, we applied Resonance Raman (RR) confocal microscopy and confirmed that the MnPs-based lipophilic SOD mimics, MnTnBuOE-2-PyP5+ and MnTnHex-2-PyP5+ were incorporated into endothelial cells, and that Mn(III)P can be reduced by intracellular components such as the mitochondrial electron transport chain. Experiments with isolated mitochondria revealed the reduction to Mn(II)P, which was in part affected by the inhibitors of electron transport chain, supporting the action of MnPs as efficient redox active compounds in mitochondria. We have also characterized the reaction kinetics of MnTnBuOE-2-PyP5+ with peroxynitrite and evaluated the cytoprotective capacity of MnPs by exposing the endothelial cells to nitro-oxidative stress induced by peroxynitrite. This data introduce a novel application of the RR spectroscopy for the direct detection of MnPs and their redox state in living cells, and helps to rationalize its antioxidant capacity in biological systems.

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P-096

Peroxiredoxins and the pro-inflammatory immune response

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Keywords: Peroxiredoxins; inflammation; ER; JAK/STAT; relish

Accumulating evidence suggests that compromised redox signaling could be responsible for development of hyperactive, pro-inflammatory immune responses, which ultimately affect healthy aging and shorten longevity. Here we show that the lifespan shortening effects observed in flies with altered peroxiredoxin activity in the ER (dPrx4) and mitochondrial (dPrx3 and dPrx5) compartments are associated with activation of the NF-κB –dependent immunity-related/inflammatory genes, which are normally induced in response to infection and constitutively overproduced in old animals. The NF-κB targets could also be activated under conditions of ER stress. In transgenic flies expressing the ER-localized dPrx4 in the absence of Relish, a Drosophila NF-κB ortholog, the pro-inflammatory effects typically elicited by dPrx4 overexpression were absent. The absence of Relish also significantly rescued the severe shortening of lifespan normally observed in dPrx4 overexpressors. Furthermore, the overactivation of immune/inflammatory responses triggered by aberrant dPrx4 activity was mediated by JAK/STAT signaling, as revealed in epistatic analysis using mutants representing the JAK/STAT pathway. We have also found that the proinflammatory response provoked by the oxidant paraquat required dPrx4 activity in the ER, as this response was abrogated in the dprx4 mutant.

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P-097

Is Twin Pregnancy being a Medical Boon? – Comparative Evaluation on Oxidative Stress in Multiple Pregnancy

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Keywords: Nitric oxide synthase; Oxidative stress; Red Blood Cells; Twin pregnancy and Umbilical cord

Pregnancy, a state of metabolic challenge to be met both by the mother and the developing fetus with a high demand of oxygen. An overproduction of reactive oxygen species (ROS) with greater susceptibility to oxidative stress condition causes impaired blood flow and restricted growth of the fetus. The umbilical cord vessels lack innervations, and therefore the endothelial cells play a determinative role in the control of the blood flow by endothelial nitric oxide (NO) production which gets regulated by the endothelial nitric oxide synthase (NOSIII). In contrast to the beneficial aspect of NO, it also acts as a pro-oxidant molecule, reacts with superoxide anion forming the potent peroxynitrite ($ONOO^-$) that enhances oxidative macromolecular damages. We aimed to evaluate the redox status of neonatal human red blood cells (RBCs) and endothelial cells derived from umbilical cord vessels, in cases of multiple versus single pregnancy to follow a correlation between the NOSIII activity with maturation of the neonates. In twins, we detected endothelial dysfunction and altered NO production in the cord vessels. As a compensatory mechanism, an increased RBC NOSIII-dependent NO production in the circulating RBCs might improve the blood flow to the fetus. However, the increased NOSIII expression is not necessarily beneficial in the highly oxidative background as a consequence of twin pregnancy.

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P-098

Protein thiol modifications in the development of the 'preeclampsia phenotype'

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Keywords: Thiol; Preeclampsia; oxidative; stress; Hydrogen sulphide; Glutaredoxin; redox switch

Introduction: High soluble Flt1 (sFlt1) is pivotal in the development of 'preeclampsia phenotype' of hypertension and proteinuria. Disruption of endogenous protective pathways and increased oxidative stress are hallmarks.of preeclampsia. Yet the underlying molecular mechanisms remains unclear. During high oxidative stress, thiols on key proteins are reversibly modified by S-glutathionylation, which can be enzymatically removed by Glutaredoxin-1(Glrx). We investigated the role of Glrx in preeclampsia.
Results: Glrx mRNA and protein expression were increased while -SSG adducts were lower in placenta from early onset preeclamptic (<34 wks) compared to gestational matched placenta (35 wks). During pregnancy mice overexpressing Glrx (TG) developed the "preeclampsia phenotype" with worse maternal and fetal outcome including; hypertension (day17.5), marked collapsed of glomeruli indicating kidney dysfunction and decreased placental weight compared to wildtype littermates. sFlt1 levels were higher in TG plasma and placenta. Thiol modification of key proteins that are part of the endogenous protective pathways regulating placenta sFlt1 expression were identified by Tandem Mass Tag analysis in endothelial cells overexpressing Glrx and confirmed by Biotin switch assay in placenta.
Summary: These data support the proposed hypothesis that Glrx regulates sFlt1 expression to promote the 'PE phenotype'.

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P-100

Isothiocyanates trigger early disruption of mitochondrial function in cells overexpressing Bcl-2

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Keywords: Isothiocyanates; Bcl-2; Mitochondrial function; apoptosis

Epidemiological evidence indicates that increased consumption of isothiocyanates is associated with reduced incidence of cancer. One mechanism to explain this is the ability of isothiocyanates to trigger apoptosis, including in cells that overexpress the anti-apoptotic protein Bcl-2, which is overexpressed in a number of cancers. This study aimed to examine the mechanisms by which phenethyl isothiocyanate (PEITC) induces apoptosis in Bcl-2 overexpressing (Bcl-2 OE) cells.

Bcl-2 OE cells were more sensitive than wild-type cells to PEITC (LC50=8 μM vs. 12.5 μM). Bax-/-Bak-/-and Bim-/-Bid-/- cells were resistant to PEITC, indicating a classical apoptotic cell death. Cell death occurred more rapidly in the Bcl-2 OE cells, and increased levels of caspase activity and earlier activation were observed. PEITC had a greater effect on mitochondrial metabolic activity in the Bcl-2 OE cells; they had higher basal mitochondrial respiration, however, this was susceptible to inhibition by PEITC. There was also a significant decline in mitochondrial spare respiratory capacity. These effects occurred prior to caspase activation and cell death. Together, these results suggest that isothiocyanates bypass the action of Bcl-2 by acting directly on mitochondria to promote Bax/Bak-dependent apoptosis. This information may be valuable for designing novel isothiocyanates to target Bcl-2 OE cancer cells.

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P-101

Macrophages from xCT-deficient mice survive under low cysteine/glutathione redox conditions with high oxidative stress

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Keywords: Macrophage; xCT; cystine; glutathione; nitric oxide

Murine macrophages produce a large body of reactive oxygen species and nitric oxide (NO) in response to inflammatory stimuli and confer protection against bacterial infection. xCT transports cystine into cells in combination with glutamate discharge and supports glutathione synthesis. We performed characterization of macrophages from xCT-knockout (KO) mice from the aspect of redox homeostasis. Cystine uptake was observed in the wild-type (WT) macrophages at 24 h after isolation and elevated by stimulation with lipopolysaccharide (LPS) but was not detected in xCT-KO macrophages. Intracellular glutathione levels were lower in the xCT-KO macrophages compared to WT macrophages and further decreased by stimulation with LPS. xCT-KO macrophages maintained viability at least for 5 days but was more vulnerable to prooxidant menadione than WT macrophages. In response to stimulation with LPS, xCT-KO macrophages produced less nitric oxide compared to the WT macrophages, although levels of the NOS2 protein as well as arginine uptake rate were not significantly different. Inhibition of glutathione synthesis further decreased glutathione levels but only slightly affected nitrite levels. These data imply that macrophages possess a unique mechanism against oxidative insult, independently from the glutathione system, to cope with the bacterial infection.

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P-102

Interaction of nitrated/nitroxidized phospholipids with vimentin

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Keywords: Lipoxidation; nitrated phospholipids; vimentin; cysteine

Nitrated and nitroxidized derivatives of phosphatidylcholine (PC) were recently identified and characterized using LC-MS-based approaches and have been detected in cardiac mitochondria from diabetic rats and cardiomyoblasts subjected to starvation [1, 2]. Nitrated fatty acids (NO2-FA), like nitrated oleic acid (NO2-OA) can exert important biological effects through posttranslational modification of proteins [3]. Therefore, we aimed to evaluate the ability of nitrated palmitoyl oleyl PC (NO2-POPC), synthesized through mimetic nitration of POPC with NO2BF4, to interact with vimentin, a well-known lipoxidation target. In vitro, NO2-POPC, but not POPC, shielded vimentin from modification by biotinylated iodoacetamide. In cells, NO2-POPC induced a marked rearrangement of the vimentin network with condensation at the cell periphery, which was attenuated in cells expressing a vimentin mutant lacking the single cysteine residue. NO2-POPC also induced cell rounding that was mimicked by NO2-FA, whereas NO donors only partially reproduced this effect. These results indicate that NO2-POPC can interact with vimentin and modulate the organization of vimentin network.

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P-103

Proteomic analysis of rutin effect on human skin fibroblasts exposed to UVA or UVB irradiation

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Keywords: fibroblasts; UV radiation; rutin; proteome

Rutin, due to its polyphenolic structure reveals antioxidant properties and may be used as a cytoprotective compound against UV-induced effects on skin cells. Therefore, the aim of this study was to examine the rutin effects on UV-induced changes in proteome profile of human skin fibroblasts cultured in vitro.

PCA analysis of results obtained by QExactive OrbiTrap mass spectrometer show clear differentiation only between control and UVA treated cells, although significant changes in the individual proteins level are also observed between the rest of tested fibroblast groups. Rutin treatment prevents UVA-induced, even 2-fold, increase in the total expression of proteins involved in antioxidant (e.g. superoxide dismutases, disulfide isomerase) and inflammatory response (e.g. IL-17, PAK2, YWHAZ), what is not observed in fibroblasts after rutin treatment following UVB irradiation. However, rutin treatment of fibroblasts after UVB radiation promotes rutin-Keap1 adducts formation thus activation of Nrf2 - responsible for cytoprotective proteins synthesis. Rutin also prevents UV-induced

apoptosis, through restoring the pro-apoptotic proteins level (e.g. p53 and cytochrome c) that is enhanced after the irradiation.

In conclusion, our results show that rutin effectively prevents UV-induced damages associated with proinflammatory/prooxidative and proapoptotic activity.

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P-104

SOD mimic M40403 improves sperm fertilizing potential through activating NO/NRF2 signaling pathway

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Keywords: Nrf2; Nitric oxide; SOD mimic; infertility

Global rise in male infertility give importance to find a new approach to increase fertility. Redox signaling emerged recently as important for spermatozoa functioning. Accordingly, we just showed great therapeutic potential of redox modulator SOD mimic M40403. Molecular mechanisms underlying beneficial effects of M40403 indicate NO involvement, but remain unsolved; we aimed to reveal this herein.

Compared to the control, incubation of spermatozoa in Tyrode's medium for 3 h, decreased sperm motility, mitochondrial membrane potential (MMP), level of NO, expression of NO synthases (NOSs), and completely abolished nuclear localization of NF-E2-related nuclear factor 2 (Nrf2) that was followed by decrease in SODs, catalase and GPX expression. Treatment with M40403 decreased levels of $O_2^{\bullet-}$, increased level of NO, restored expression of NOSs and MMP, and triggered marked nuclear translocation of Nrf2 followed by up-regulation of SODs, catalase and GPX and increase in sperm motility. In turn, M40403 + L-NAME treatment nullified all beneficial effects of M40403.

These data reveal the redox based mechanisms of beneficial effects of M40403 SOD mimic in sperm cells, suggesting NO/Nrf2 signaling as a central pathway. Utilization of a redox modulator, M40403, as an inducer of Nrf2 is a promising pharmacological approach for the improvement of sperm fertilizing potential and beat infertility.

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P-105

A lesson from the oxidative metabolism of a hibernator's heart: strategy for cardioprotection

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Keywords: hibernation; heart; oxidative metabolism; mitochondria

Maintaining heart viability in states of unfavorable metabolite/oxygen supply has become a prime challenge in cardiac medicine. Mitochondria have been recognised as key actors in cardioprotection. Our goal was to reveal the myocardial protective phenotype related to mitochondrial bioenergetic pathways in the state of limited metabolite supply and low temperature using the physiological model of mammalian hibernation.

European ground squirrels (Spermophilus citellus) were exposed to cold (4 ± 1 °C) and divided into two groups: (1) animals that fell into torpor (hibernating group) and (2) animals that stayed active and euthermic for 1, 3, 7, 12, or 21 days (cold-exposed group). We found increased protein levels of electron transport chain components and ATP synthase in late cold acclimation and in hibernation. Also, protein level of PGC-1α was upregulated. Phospho-AMPKα protein content was increased during early cold acclimation. HIF1α protein level was unchanged. Protein levels of manganese and copper-zinc superoxide dismutase and glutathione peroxidase were increased in hibernation.

The hibernation related phenotype of the heart is characterised by controlled improvement of mitochondrial energy and antioxidative capacity. Its reproduction could have broad implications, from myocardial protection in ischaemia/reperfusion to the hypothermic survival and cold preservation of organs.

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P-106

Targeting of $O_2^{\bullet-}$/NO ratio as a strategy to improve energy metabolism in diabetes

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Keywords: Diabetes; Energy metabolism; Redox regulation; L-arginine; SOD mimic

Redox homeostasis disturbance, mainly caused by increased $O_2^{\bullet-}$ level and/or decreased NO bioavailability, represents an important contributing factor in the (ethio)pathology of diabetes. We examined whether targeting of $O_2^{\bullet-}$/NO ratio by two redox-modulating compounds, L-arginine (substrate for NO synthases) and Mn(II) pentaazamacrocyclic mimics of SOD, could improve diabetic state and associated impairment of energy metabolism.

Multiple beneficial effects of L-arginine and SOD mimics in alloxan-induced diabetes were observed. First of all, acting directly on pancreas, L-arginine and SOD mimic induce β-cells regeneration. In skeletal muscle, those treatments restore diabetes-induced impairment in mitochondrial energy metabolism (OXPHOS) and glucose transport (GLUT4) by targeting AMPKα signaling. Similarly, SOD mimic improves energy metabolism in hippocampus of diabetic rats. L-arginine and SOD mimic stabilize diabetes-induced redox disbalance in diabetic skin acting on NO producing (NOS) and $O_2^{\bullet-}$/H_2O_2 removing (MnSOD and GPx) systems.

The data suggest that fine settings of $O_2^{\bullet-}$/NO ratio by L-arginine and SOD mimic could have beneficial implications for several pathological hallmarks of diabetes: impaired insulin synthesis and sensitivity as well as accompanying metabolic complications and speak in favor of therapeutic potential of these redox-active agents in diabetic conditions.

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P-107

A mass spectrometry approach for the identification and localization of acrolein modifications of proteins

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Keywords: Oxidative stress; lipid oxidation; lipoxidation; unsaturated aldehydes

Lipids containing polyunsaturated fatty acids are primary targets of oxidation, producing reactive products including a variety of short-chain aldehydes which can covalently modify proteins in a process called lipoxidation. These post-translational modifications influence cell behaviour and can be involved in inflammatory diseases. The exact nature of many of these adducts, and their relationship with cellular effects are still unclear. There is a need to develop better mass spectrometry (MS) methods for the identification of these adducts in complex biological systems. Reduced lysozyme was used as a model system to investigate the formation of short-chain aldehyde-containing lipoxidation products. The protein was modified using acrolein, the adducts formed stabilized by NaBH4 reduction, and the intact protein and tryptic digests analysed using MS. Analysis of intact modified lysozyme showed that multiple sites (up to 8) could be modified, all resulting from Michael addition (+58 Da in the reduced form). Analysis of tryptic digests allowed the localization of the adducts to specific cysteine and lysine residues and the identification of amino acid-specific fragmentations. MS methods provide a powerful tool for the identification and localization of aldehyde protein adducts, and further aldehydes and proteins are now being investigated.

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P-108

Identification and localization of protein-pentanal adducts, a potential lipoxidation marker

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Keywords: oxidative stress; saturated aldehydes; lipid oxidation; lipoxidation

Oxidative stress has been linked to several inflammatory diseases; this may be due to oxidative damage to biomolecules. Oxidized phospholipids have been shown to be related to several pathophysiological pathways, as antigens or ligands, and more recently through protein modification creating protein-phospholipid adducts. The literature on protein-phospholipid adducts is sparse, although this type of protein modification could be of interest as potential biomarkers for disease. The aim of this project is to study the formation of these adducts, using both intact protein and bottom-up mass spectrometry techniques. For this work, two model proteins were modified with pentanal, a saturated breakdown product of phospholipid oxidation and a model for phospholipid aldehydes, and the adducts stabilized by reduction and analysed by ESI MS. The modified proteins were also subjected to SDS-PAGE, in-gel tryptic digestion, and LC-MSMS analysis. The results of the top-down and bottom-up analysis show the presence of pentanal adducts, and identified the sequence of the modified peptides. The mass shifts observed were consistent with the formation of Schiff's base adducts, and only modifications to lysine were observed. Product ions that might be useful as diagnostic ions for the modification were identified. Future work will investigate adduct formation using oxidized phospholipids.

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P-109

Effect of URB597 on phospholipid metabolism in the heart of hypertensive rats

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Keywords: hypertension; anandamide; endocannabinoid system; URB597

Since hypertension is involved in redox and endocannabinoid systems disturbances, chronic administration of URB597, a FAAH inhibitor, that modulates the level of endocannabinoids, particularly anandamide, may regulate hypertensive metabolic consequences. Therefore, the aim of this study was to compare the effects of chronic administration of URB597 to SHRs and DOCA-salt rats on cardiac metabolism associated with redox system and lipid metabolism. It was shown that both primary and secondary hypertension is associated with enhanced cardiac inflammation and redox imbalance, resulting in increased lipid peroxidation products and endocannabinoids generation. URB597 administration decreasing FAAH activity enhanced endocannabinoids level, particularly in DOCA-salt heart. However in SHRs heart additional increase in MDA level was observed. Lipid mediators were involved in inflammatory response by PPARa expression in DOCA-salt rats and by PPARg expression in SHRs hearts. URB597 administration to normotensive rats also affected cardiac oxidative metabolism and endocannabinoids system, resulting in an enhanced level of MDA and endocannabinoids in Wistar rats. It can be concluded that lipid metabolism in heart rats with secondary hypertension and their control rats is more susceptible to chronic URB597 action and may lead to stronger cardiac disorders.

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P-110

Glutathione deprivation improves oxidative capacity but disrupts endocrine role of white adipose tissue in overall metabolic homeostasis

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Keywords: White adipose tissue; cold; BSO; browning; adipokines

Clarification of molecular mechanisms underlying lipid buffering capacity of white adipose tissue (WAT) may aid in the improvement of therapeutic options for obesity and diabetes prevention. Results of our studies have shown that cold-induced regression of WAT involves transient increase of tissue lipid oxidation, uncoupling capacity, an occurrence of browning allsynchronized with increase in adiponectin and resistin synthesis and profound glutathione (GSH) depletion. This GSH decrease prompted us to examine if a decrement of GSH a priori recapitulates cold-induced favorable phenotype of WAT. To this end, room-temperature and cold-exposed rats were treated with L-buthionine-S,R-sulfoximine (BSO). Indeed, BSO treatment reduced rat body weight and lipid content in fat tissue on account of increased OXPHOS and uncoupling capacity of WAT in both, cold-exposed and room-temperature maintained rats. However, in comparison to control, BSO decreased expressions of adiponectin and resistin at room temperature and attenuated their adaptive increase on cold exposure. Decrement of GSH raise lipid burning phenotype in WAT, but it may interfere with endocrine role of WAT in overall metabolic homeostasis maintenance.

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P-111

The Comparison of Lipid Peroxidation, Glutathione Levels and Antioxidant Enzyme Activities in Blood Obtained from Captive and Wild Northern Bluefin Tuna (*Thunnus thynnus L.*, 1758)

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Keywords: Northern Bluefin Tuna; *Thunnus thynnus*; blood; tissue; antioxidant enzymes; LPO; Glutathione; Cortisol

Just like in many countries, in Turkey, Northern Bluefin Tuna (NBTs) are considered to be high profitable fish species. NBTs, which are the members of Scombridae family, are of an average 120–250 cm fork–tailed length and weight 26–250 kg. NBTs have been grown in cages and exported from Turkey to far East Countries, since 2002.

In this case, it leads to increase the fishing of NBT, therefore wild NBT population stock is under the threat of overfishing in Turkey. Furthermore, there is not enough research on high stocking stress effects on NBTs and he flesh quality of the fish.

In this study, the antioxidant enzymes; [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat)], lipid peroxidation (LPO), glutathione (GSH) and cortisol levels, which have taken from blood samples of captive and wild NBT, have been investigated. All the data is grouped according to weight, sex and age. The student's t test, the one-way ANOVA and the statistical confidence tests ($p < 0.05$ and $p < 0.001$) have been applied to the results of the study.

According to results, a high activity of antioxidant enzymes, LPO, cortisol have been found in the blood of the captive NBT samples, but the GSH levels of blood have turned out to be low in comparison to the wild NBT samples. These two findings show that captive NBTs suffer from stress more than wild NBTs.

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P-112

The influence of female hormones and gestational diabetes on DNA damage

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Keywords: Gestational diabetes; female hormones; DNA damage

Gestational diabetes (GD) is a disorder in pregnant characterized by high blood glucose and insulin levels and associated with changes in female hormones. Previous in vitro studies showed a high insulin level stimulates ROS production resulting in DNA damage and mutation. Herein, we aim to investigate the effect of female hormones and diabetes during pregnancy on mediating DNA damage.

The effect of progesterone, estradiol (100 nM) and insulin (10 nM) was investigated in vitro using HL60 and ex vivo with stimulated peripheral blood mononuclearPBMC and PBMC's. The study included 4 main groups (non-pregnant with/without hormonal contraceptives, diabetic, pregnant, and GD women). The genomic damage was examined using comet assay and micronucleus frequency test.

The female hormones mediated DNA damage in HL60 and stimulated PBMC's. Hormonal contraceptive users showed no significant DNA damage compared with non-pregnant women control. The DNA damage was significantly induced in pregnant compared with non-pregnant women. The DNA damage seems to be significantly induced in GD women, while reduction in DNA damage is observed after delivery.

The higher DNA damage in pregnant women could be due to the changes of female hormones and in gestational diabetes due to high insulin levels in the blood. Altogether, mechanistic reasons of the alterations concerning cancer risk need to be investigated.

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P-113

Antioxidant activity of *Crataegus Monogyna L* flowers

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Keywords: Flowers of *Crataegus Monogyna*; TEAC; CUPRAC; Rutin

Crataegus species are known by their biological effects for many years. *Crataegus monogyna Jacq*. flowers have been ethnomedically used in different parts of Turkey for their many biological effects such as against tachycardia and sedative, antihypertensive, antispasmodic and diuretic. In addition to the widespread use of Crataegus species, many pharmacopoeias and monografts are registered, and a large number of in vitro, in vivo and clinical studies have been carried out with respect to bioactivity and mechanism of action. In vitro, leaves and fruits of Crataegusspecies have also been shown to exhibit antioxidant. However, these properties are influenced by the type of plant organ, the harvest season, the species and the geographical origin. In this contex, different extracts of ripe flowers of *Crataegus monogyna* (CM) collected from natural habitats on the limit of Konya/Seydişehir-Turkey at September 2015 were evaluated for their antioxidant capacity. Rutin contents was determined as 2.68 μg/ml in ethanolic extract of *Crataegus monogyna Jacq*., % ABTS· radical scavenging activity of the extract is 78.80 ± 4.09 and 1.080 ± 0.08 μg trolox/mg extract in CUPRAC method. In conclusion, the results of this study indicate that flowers of *Crataegus monogyna* provide considerable antioxidant protection and can be suitable raw material for the medicine, food and cosmetic industries.

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P-114

High cholesterol diet-mediated unfolded protein response activation enhances autophagic cell death in heart tissue

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Keywords: Hypercholesterolemia; Autophagy; ER stress

The role of ER in the proper synthesis and correct folding of protein, to establish the homeostasis of organism, is well documented by various studies. Following excessive oxidative stress, the accumulation of unfolded/misfolded proteins increase in the lumen of ER which blocks the inhibitory effect of GRP78 on UPR regulatory proteins; IRE1, PERK and ATF6.

It is clear that ER stress mediated UPR is a potent inducer of proteasomal and autophagic activities which serves as a degradation process for maintaining stress tolerance, limited damage and viability under stress conditions. Interestingly, aberrantly activation of autophagy can result in destroying major portions of the cytosol and organelles, which will lead to autophagic death of cardiac myocytes, and development of cardiac failure.

In the present study Grp78, Grp94, pIRE1, pPERK, derlin-1, proteasome β5 subunit, VCP, beclin-1 and LC3-II, well known markers of ER stress, proteasomal and autophagic activities, are investigated in the heart tissue of rabbits. Furthermore, autophagic activity in heart tissue is observed in hypercholesterolemic vs control rabbits by electron microscopy. Additionally, colocalization studies of mitochondria and p62 in cryo sections of heart tissue were applied to understand the levels of mitochondria degradation by autophagy.

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P-115

Cell cycle arest and regulation of Nrf2 by Ganoderma lucidum in hepatocellular carcinoma

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Keywords: Ganoderma lucidum; Nrf2; cell cycle arrest; hepatocellular carcinoma cell-line

Currently, hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide. However, the studies focusing on

molecular etiology of HCC are limited. The Nrf2 signaling pathway can protect cells from a variety of toxicants and carcinogens by increasing the expression of a number of cytoprotective genes.The idea of using G. lucidum for cancer treatment is based on numerous laboratory and preclinical studies with cancer and immune cells as well as animal models demonstrating various biological activities in vitro and in vivo. The aim of our study is to evaluate the potential antioxidant role of G. lucidum in HCC. For this purpose, analysis of Nrf2 levels and cell cycle arrest were done. The most effective concentration of G. lucidum were found at 1/5 dilution. The changes in cytoplasmic/nuclear Nrf2 protein levels following G. lucidum extracts (1:5 or 1:10) treatments for 24, 48 or 72 h were observed. 1/10 and 1/5 diluted of G. lucidum induced G0/G1 cell cycle arrest in HCC. Consistently, the cells distributed in S phase were significantly reduced.In conclusion, our findings suggest that G. lucidum has a potential as an anticancer agent and. our data support the importance of the clarification of the molecular mechanisms of phytochemicals-induced Nrf2 activation.

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P-116

Crosstalk between insulin resistance and oxidative stress in the development of Alzheimer-like neurodegeneration

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Keywords: down syndrome; insulin signaling; alzheimer disease; protein oxidation

Down Syndrome (DS) individuals by the age of 40ys develop a type of dementia that has the same characteristics as Alzheimer disease (AD). Previous studies in DS and AD brain suggest common neurodegenerative pathways including mitochondrial dysfunction, oxidative stress (OS) and reduced glucose metabolism. In addition, several studies suggest a link between insulin resistance and cognitive dysfunction in AD.

The present study aims to analyze the crosstalk between the onset of brain insulin resistance (BIR) and OS as possible contributing factors to the neurodegenerative process in tg mouse model of DS (Ts65Dn).

We longitudinally analyze (at 1–3-9–18 months) changes of i) IR/IRS1/ERK1/2/Akt levels and activation state iii) oxidative stress markers and iii) biliverdin reductase-A (BVR-A), SIRT1 and PTEN protein levels and activation, in the cortex of Ts65Dn mice. In parallel, changes of APP/Abeta levels have been analyzed.

Our results show the mutual interaction between increased OS and BIR in Ts65dn, which does not correlate with Abeta levels. We found that OS negatively impacts the activation of insulin cascade since postnatal age that also persists with age. These findings highlight the role of BIR in the onset of AD-like neurodegeneration and suggest that aberrant insulin signaling strongly contributes to cognitive decline also in DS.

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P-117

HyPer biosensor to monitor intracellular hydrogen peroxide in skeletal muscle cells

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Keywords: HyPer; hydrogen peroxide; myoblast; myotubes; skeletal muscle fibres

Hydrogen peroxide (H_2O_2) is one of the reactive oxygen species (ROS) that seems to play an essential role in cellular signalling pathways coupled to frequent pathophysiological processes. However, using traditional methodology it is virtually impossible to identify and quantify H_2O_2 flux in cells.

We have developed methodological approaches based on the use of a hydrogen peroxide biosensor, HyPer, in skeletal muscle cells: myoblasts and myotubes C2C12, and individual matured muscle fibres isolated from the mouse muscle. Using transfection techniques with chemical agents and microinjection/electroporation techniques, we have achieved the expression of HyPer biosensor in those cells. In combination with live cell fluorescence microscopy image analysis we have monitored the intracellular flow of H_2O_2 in situ and in real time in those skeletal muscle cells that expressed HyPer. The HyPer fluorescence emitted by cells was registered during a period in which cells were exposed either to extracellular hydrogen peroxide or to a reducing agent, dithiothreitol, in the medium.

Conclusion: i) it is possible the expression of hydrogen peroxide biosensor HyPer in myoblasts and myotubes C2C12 and in single isolated skeletal muscle fibres, and ii) HyPer biosensor is functional and detects changes in the intracellular concentration of hydrogen peroxide in situ and in real time in skeletal muscle cells.

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P-118

Non-photonic sensing of membrane-delimited reactive species with a Na^+ channel protein containing selenocysteine

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Keywords: Cysteine and selenocysteine; Redox sensors; Phototoxicity; Voltage-gated Na^+ channels; Inactivation motif

Genetically encoded redox-sensitive fluorescent proteins are very useful tools for investigating the occurrence and consequences of reactive species (RS) in living cells. However, light-induced side effects are serious confounding factors [Edwards, A.M. & Silva, E.,2001; Eichler, M. et al.,2005]. Therefore, we have previously introduced a genetically engineered redox-sensitive sodium channel (rNaV1.4 mutant M1305C; roNaV1) as a non-photonic tool for measuring membrane-delimited cellular RS [Ojha, N.K. et al.,2014]. Here we introduce a second-generation NaV-channel based RS sensor, containing selenocysteine in its inactivation motif (rNaV1.4 mutant M1305U; roNaV2), with strongly increased RS sensitivity. Kinetics of loss of channel inactivation was assayed for application of 1 μM chloramine T: While the ratiometric signal of roNaV1 was basically insensitive to that low concentration, roNaV2 responded with a time constant of 300 s. Likewise, roNaV2 responded rapidly to 500 μM H_2O_2 (ca. 50 s). Most importantly, roNaV2 responds to blue light (470 nm) induced chemical modification with a time constant of about 300 ms, while roNaV1 was 1,000- and wild-type NaV1.4 even 10,000-times slower. Thus, roNaV2 allows for the assessment of chemical modification induced in fluorescence microscopy settings with high sensitivity and time resolution.

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P-119

ER stress related lipid accumulation and apoptotic cell death in nonalcoholic fatty liver diesease

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Keywords: Hypercholesterolemia; Endoplasmic Reticulum Stress; NAFLD; Apoptosis

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease that ranges from benign steatosis to nonalcoholic steatohepatitis (NASH) . The role of cholesterol alterations in NAFLD progression have been shown in various studies. Additionally, activation of ER stress, induces inflammation, insulin resistance and apoptotic cell death which results in NAFLD/NASH transition.

UPR is mediated by the activation of three ER proteins: IRE1, PERK and ATF-6 . These proteins are inactivated by binding to the ER chaperone GRP78 under normal conditions. Following dissociation of GRP78, each of these sensor proteins (PERK, ATF6 and IRE1) use a unique mechanism to induce transcription factors. If the stress is too severe and excess the capacity of defense mechanisms, cells switch to apoptotic cell death pathways which is mainly driven by CHOP.

The aim of this study is to identify the effect of ER stress on the molecular mechanisms of apoptosis in hypercholesterolemia induced NAFLD. In this direction, lipid accumulation and protein expressions of GRP78, GRP94, IRE1, PERK, Bax, CHOP, Caspase 3 has been investigated in the liver tissues of NAFLD rabbit model. Our model demostrated that high cholesterol diet increase ER stress, that triggers apoptosis in the progresion of NAFLD.

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P-120

Dietary Sulforaphane supplementation induces Nrf2, attenuating hypoxia-induced vascular smooth muscle cell proliferation and remodelling following carotid artery ligation

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Keywords: Vascular; Nrf2; smooth muscle; sulforaphane

We sought to assess whether dietary sulforaphane (SFN) supplementation to C57BL/6 J mice attenuates injury following carotid artery ligation, in which the endothelium remains intact. We also sought to establish whether SFN-induced induction of Nrf2 redox defence genes underlies hypoxia-induced proliferation of vascular smooth muscle cells and may therefore contribute towards vascular injury protection. Pre-treatment with a physiologically achievable (0.5 mg/kg) dose of SFN and continued administration 2 and 4 weeks post ligation was associated with Nrf2-dependent gene expression (e.g. heme oxygenase 1, HO-1) and reduced neointimal formation assessed by Van Gieson staining. Using an oxygen regulated work station, exposure of primary mouse aortic smooth muscle cells (MASMC, P2) to hypoxia (1% O2) enhanced proliferation when assessed by cell count and protein expression. In SFN (2.5 μM) treated MASMC, proliferation was attenuated following serum challenge (10% FCS) for 72 h and correlated with Nrf2 target gene induction. Overall our results suggest that independent of endothelial oxidative damage, reduced oxygen availability enhances vascular smooth muscle cell proliferation. SFN supplementation induces Nrf2 defences and reduces smooth muscle cell proliferation, suggesting dietary Nrf2 supplementation may provide a useful therapeutic intervention to promote vascular health.

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P-121

Metabolic activity of radish sprouts derived isothiocyanates in *Drosophila melanogaster*

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We used *Drosophila melanogaster* as a model system to study the absorption, metabolism and potential health benefits of plant bioactives derived from radish sprouts (*Raphanus sativus* cv. Rambo), a Brassicaceae species rich in glucosinolates and other phytochemicals. Flies were subjected to a diet supplemented with lyophilized radish sprouts (10.6 g/l) for 10 days, containing high amounts of glucoraphenin and glucoraphasatin, which can be hydrolyzed by myrosinase to the isothiocyanates sulforaphene and raphasatin, respectively. We demonstrate that *Drosophila melanogaster* take up and metabolize isothiocyanates from radish sprouts through the detection of the metabolite sulforaphane-cysteine in fly homogenates. Moreover, we report a decrease in the glucose content of flies, an upregulation of spargel expression, the Drosophila homolog of the mammalian PPARγ-coactivator 1 α, as well as the inhibition of α-amylase and α-glucosidase in vitro. Overall, we show that the consumption of radish sprouts affects energy metabolism in Drosophila melanogaster which is reflected by lower glucose levels and an increased expression of spargel, a central player in mitochondrial biogenesis. These processes are often affected in chronic diseases associated with aging, including type II diabetes mellitus.

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P-122

The effects of taurine on the levels of GSH and LPO on *in vitro* glucose-induced cataractous rabbit lenses

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Keywords: Taurine; cataract; lipidperoxidation; reduced glutathione

In this our study, we try to determine whether taurine has anticataractogenic action by the levels of glutathione (GSH) and lipid peroxidation (LPO) in vitro glucose-catarct induced rabbit lenses. With this aim lens samples were divided into two parts as cortex and nucleus, then the GSH and LPO levels were determined under the headings of control, cataract and taurine study groups. According to our results, in the nucleus samples the GSH levels in the cataract and taurine groups showed no remarkable differences as compared to the controls (respectively $p=0.413$ and $p=0.792$). however, in the cortex samples the GSH levels in the cataract group decreased significantly relative to the controls ($p=0.033$). There was no considerable difference between the GSH levels in the taurine and the control groups of the cortex samples ($p=0.295$). Concerning the LPO levels, the control, cataract and taurine groups of the nucleus samples displayed no significant differences, whereas in the cortex samples, the cataract group was found to have significantly increased LPO levels as compared to the controls ($p=0.022$). LPO levels of the taurine group tended to yield similar results to those of the control group. According to GSH and LPO results in the taurine groups, it can be postulated that taurine may protect lens membrane lipids against oxidative destruction.

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P-123

Circadian clock as possible protective mechanism to pollution induced skin damage

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Ozone (O_3) is among the most toxic stressors to which living organisms are continuously exposed and the skin is one of the most susceptible tissues to environmental damage. Many data suggests a significant role of the circadian system in the regulation of protein involved in the cellular response to oxidative stress. However, there is still an incomplete understanding of the molecular mechanism linking circadian rhythms and cellular defensive pathway. Here we investigated a possible protective role of the circadian system to O_3 induced damage in human keratinocytes. Our results showed that, mimicking environmental condition of O_3 exposure, synchronized keratinocytes exhibited a more efficient antioxidant response, attested by a faster activation of NRF2, compared to the arrhythmic ones.Analysis of clock gene mRNA level in rhythmic cells reveals a more rapid induction of Bmal1. Tthese data suggested that in keratinocytes,Bmal1 regulates the expression of Nrf2. Based on this findings, we suppose that an adequate coordination of the circadian system and antioxidant pathway might be essential to maintain the homeostasis in the skin. Alteration of metabolic pathways as occurs in many diseases or irregular schedule of life activity (shift work, transcontinental journey) could negatively influencegene expression and associated organ physiology via its effect on the circadian system.

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P-124

Attenuation of skeletal muscle oxidative stress in atherosclerotic mice

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Keywords: NADPH oxidase 2; muscular oxidative stress; atherosclerosis; diet

Background: NADPH oxidase 2 (NOX2) is a major enzymatic source of reactive oxygen species (ROS) that contributes to systemic atherosclerosis in apolipoprotein E null (ApoE-/-) mice. The aim of this study was to investigate the role of Nox2 inhibition in skeletal muscle pathophysiology and cellular oxidative stress of ApoE-/- mice administered a Western diet (WD).
Methods: ApoE-/- mice were maintained on either a chow or a WD for 12 weeks and were treated with the Nox2ds-tat inhibitor or control peptide for the last 8 weeks of feeding. Skeletal muscles and the liver were dissected for molecular, biochemical and histological studies.
Results: There was perturbed gene expression for antioxidant genes followed by increased oxidative stress as shown by lipid peroxidation and protein oxidation in the skeletal muscle of WD-fed mice. Pharmacological inhibition of NOX2 decreased superoxide production both in the muscle and liver and protein carbonylation in the muscle of WD-fed ApoE-/- mice.
Conclusions: These data indicate that ApoE-deficiency and western diet independently induce oxidative damage in skeletal muscle that is attenuated by NOX2 inhibition. This study provides key evidence to better understand the pathophysiology of skeletal muscle in peripheral atherosclerosis and identifies potential therapeutic targets for decreasing oxidative stress in skeletal muscle.

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P-125

Acute effect of phosphodiesterase type 5 inhibitor tadalafil on plasma redox status in healthy men

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The phosphodiesterases type 5 (PDE5) inhibitors (PDE5i) (e.g., sildenafil, tadalafil) widely used to treat erectile dysfunction, and for recreational purpose such as sports supplements, may enhance the cGMP-dependent metabolic effects of NO. An increase in NO production, following tadalafil administration, could generate peroxynitrite, the most reactive free radical species causing oxidative injury. We investigate whether the acute supplementation with PDE5i could affect plasma antioxidant status in healthy, physically active humans.

A crossover study has been carried out with male volunteers (n=6) supplemented with a single dose of 20 mg tadalafil. Plasma total antioxidant status (TAS) and glutathione (GSH) homeostasis were evaluated immediately before and after 2, 6 and 24 hours of the acute administration.TAS values increased after 2 h (1.01 ± 0.13 Trolox eq. mM) to decrease after 24 h (0.85 ± 0.06) compared to baseline (0.92 ± 0.09). Oxidized glutathione (GSSG) increased from 6 h (3.68 ± 0.55, 4.27 ± 0.97 and 5.09 ± 0.82 GSSG 10-5 M for baseline, 6 h and 24 h respectively). GSH/GSSG ratio decreased (16.85 ± 5.28 and 12.85 ± 2.00 for 6 and 24 h) compared to baseline (18.36 ± 4.92).

Our preliminary results show that an acute tadalafil administration affects plasma antioxidant status in healthy physically active men.

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P-126

Aminophospholipid oxidation and glycation in immunity: good or bad?

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Keywords: Aminophospholipids; Oxidation; glycoxidation; inflammation; mass spectrometry

It is well recognized that modified lipids can have a role in immunity. Oxidized phospholipids are important intermediaries of cellular and inflammatory events although this is a field far from being completely elucidated. The aminophospholipids (APL), phosphatidylethanolamine (PE) and phosphatidylserine (PS), are components of cell membranes and biofluids, playing diverse roles in cell signaling events. Research work developed in our laboratory pinpointed fragmentation pathways specific of new oxidized APL, due to modifications in both polyunsaturated fatty acyl chains and free amino group in polar head. These oxidation products were detected in cells and organelles upon oxidative conditions. Depending on the APL precursor and on the type of modification, modified APL can display either pro- or anti-inflammatory effects. Oxidized, glcated and glycoxidized PE can stimulated monocytes a dendritic cells, mediating pro-inflammatory events, which could have a role in the lower grade inflammatory in diabetes. On the other hand oxidized PS can display anti-inflammatory events, modulating inflammatory signaling in macrophages.More effort is needed to unveil the role of APL modifications in inflammatory-associated conditions such as cardiovascular, depression and neurodegenerative diseases.

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P-127

Is NADPH oxidase activity regulated by free radicals?

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Keywords: NADPH oxidase; superoxide; OH radicals

NADPH oxidase is a ubiquitous enzyme that is the main producer of superoxide anions in cells. In phagocytes, it is constituted by the assembly of four cytosolic (p67phox, p47phox, p40phox and Rac) and two membrane (p22phox and Nox2) proteins. In response to pro-inflammatory mediators, the NADPH oxidase complex is activated. In cells, arachidonic acid (cis-AA), released by activated phospholipase A2, plays also a role in activation of the NADPH oxidase.

We are currently investigating how the production of superoxide, precursor of all ROS and hence of oxidative stress generated by the NADPH oxidase are regulated. After having investigated the role of arachidonic acid and protein assembly, we turned to the regulation by free radicals. We used gamma and pulse radiolysis to produce $O_2^{\bullet-}$ and OH radicals. On a cell-free system, we showed that during its assembly, the system passes through sub-states of different sensitivities. The regulatory activity of each sub-unit varies with their oxidation state. We are now investigating the sensitivity of human neutrophils toward oxidation by free radicals.

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P-128

Synergy between UV and pollutants induce redox imbalance in skin cell

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Keywords: Pollution; Polycyclic Aromatic Hydrocarbons; UV; skin model; glutathione; metabolism

Since low concentrations (nanomolar range) of Polycyclic Aromatic Hydrocarbons (PAH, common pollutants) were measured in the blood of people living in a polluted environment, skin could be exposed to PAH either by systemic distribution (food, breath) or topical expsoure. In the same time, skin is exposed to solar radiation, especially UV wavelength (UVA represents 70% of solar wavelength). Here, we compared in vitro the biological effects of PAH on normal human keratinocytes and reconstructed skin model exposed either to daily UV (d-UV 300–400 nm) or to UVA1 (350–400 nm). UVA1 was often as potent as d-UV in inducing a strong phototoxic impact. Moreover, benzo[a]pyrene (BaP) and indeno[1,2,3-cd]pyrene (IcdP) were phototoxic at very low concentrations (nanomoles per liter) and impaired keratinocytes clonogenic potential. At sub-phototoxic doses BaP and IcdP induce redox imbalance and involved a change of metabolism: glutathione-metabolism appear as a key mechanism in the cellular defence, with activation of Xc- system.

PAH were well known for their carcinogenic potential at micromolar concentration, but for the first time we demonstrate the phototoxicity of some particular PAH at realistic concentration (nanomolar range).

In such experimental conditions mimicking skin contamination, our results suggest that chronic exposure to photo-polluting stress might impair cutaneous homeostasis.

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P-129

Alteration of phospholipidome profile in the heart of an animal model of acute myocardial infarction

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Keywords: Acute myocardial infarction; ischaemia; phospholipids; mass-spectrometry

Acute myocardial infarction (AMI) is one of the main causes of mortality and health care cost in the world. Development of valuable clinical tools can help in diagnosis in the early stage of disease or predicting recovery. The pathogenesis of AMI is multifactorial and lipids can have an active role in this pathology and be good candidates as biomarkers of initial stages of the disease and extent of tissue damage. Changes in phospholipidome profile were observed in cardiomyoblasts as model of AMI. However, lipidomics studies in AMI are scarce. In this work, a mass spectrometry-based lipidomics approach was used to evaluate changes in phospholipid profile in heart of murine models under ischemia, ischemia-reperfusion and starvation conditions. Differences in fatty acids and phospholipid profiles, related to the course of AMI were observed. An increase of C16:0, C18:0, C20:4 and C22:6, and a decrease of C18:2 were observed in ischemia-reperfusion and starvation. An increment of PL bearing FA 22:6, such as PC(18:0/22:6), PE(16:0/22:6), and PE(18:0/22:6), occurred in the same conditions. These PL can be precursors of anti-inflammatory lipids (e.g. resolvins) involved in AMI recovery. Therefore, lipidomics profiling by mass spectrometry can offer valuable information to understand AMI that can be exploited for diagnosis and prognosis.

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P-130

Oxidative metabolism of phosphatidylethanolamines predicted by electrochemistry-mass spectrometry

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Keywords: Phospholipids; phosphatidylethanolamine; oxidation; electrochemistry; lipoxygenase; metabolism

Phosphatidylethanolamines (PEs) are a major class of phospholipids in cellular membranes and lipoproteins. Oxidation of PEs generates products exerting a vast number of biological functions, not totally unveiled yet. In vitro biomimetic models have been used to identify oxidized PEs and to develop analytical strategies for their targeted in vivo detection. Most models are based on radical oxidation, but the oxidative metabolism of PE also relies on controlled reactions catalyzed by enzymes as lipoxygenase (LOX), which can be mimicked by electrochemical (EC) oxidation. In this study, three PE standards were oxidized using an EC flow-through cell as a biomimetic model of oxidative injury. The oxidation products were identified by on-line EC-electrospray ionization mass spectrometry (ESI-MS and MS/MS). Long chain and short chain oxidation products were identified as modifications in the sn-2 acyl chain, whereas the oxidation pattern was dependent on the unsaturation level. Some of these oxidized species have already been observed during the modification of PEs driven by radical oxygen species (ROS), and among these products it was possible to characterize the oxidized isomers synthesized by 15-LOX in human immune cells. This EC-MS platform was, therefore, able to mimic the oxidative metabolism of PEs mediated by both ROS and enzymes.

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P-131

Altered protein O-GlcNAcylation profile revealed by proteomics: Novel insights on protein signalling mechanisms in AD

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Keywords: Alzheimer disease; glucose metabolism; O-GlcNacylation

PET studies have demonstrated the early deficiency of cerebral glucose metabolism in AD patients. Reduced glucose utilization lead to altered protein O-GlcNAcylation, which might represent a link between glucose hypometabolism and the progression of AD. Increasing evidence support, in AD brain, a general decrease of protein O-GlcNAcylation coupled with a mutual inverse increased phosphorylation on Ser/Thr residues. Several redox signalling proteins are aberrantly regulated by O-GlcNacylation, during AD contributing to decreased cell survival and increased stress conditions. In this study, we investigated, by 1D and 2D SDS-PAGE, the levels of total and protein specific O-GlcNAcylation levels in the cortex of 12 months-old 3xTg-AD compared with age-matched non-Tg mice. Our data demonstrate a general decrease of total levels of O-GlcNAcylation in 3xTg-AD together with the impairment of O-GlcNacylation cycling enzymes. Data from proteomics analysis led to the identification of several proteins with differential O-GlcNAc levels between transgenic and WT animals which belongs to key pathways involved in the progression of AD, such as, neuronal structure, degradation processes and energy metabolism. Our findings may contribute to understand the effects of altered protein O-GlcNAcylation profile during AD, identifying novel mechanisms of disease progression

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P-132

SIRT3 protects against palmitate-induced neuronal lipotoxicity

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Keywords: Sirtuins; mitochondrial dysfunction; lipotoxicity; diabetes; neurodegeneration

The mitochondrial NAD+-dependent deacetylase Sirtuin3 orchestrates adaptive responses to metabolic stresses and exerts antioxidative capacities. As a result, SIRT3 knockout mice exhibit mitochondrial dysfunction and are prone to neurodegeneration. High levels of circulating free fatty acids are associated with mitochondrial dysfunction, oxidative stress and insulin resistance (IR), features of metabolic and neurodegenerative diseases. We

show that neurons treated with the saturated fatty acid palmitic acid exhibit reduced SIRT3 expression, increased protein acetylation and mitochondrial dysfunction. We hypothesized that SIRT3 protects neurons from lipid-induced mitochondrial dysfunction and IR as observed in metabolic disorders. Thus, we overexpressed SIRT3 in mouse neurons (SIRT3OE) and analyzed its function on neuronal metabolism under basal and lipotoxic conditions. Strikingly, SIRT3OE in neurons caused IR, mitochondrial dysfunction and ER stress, compared to control. Yet in palmitate-induced lipotoxic conditions, SIRTOE rescued palmitate-induced IR as well as palmitate-induced ER stress and mitochondrial dysfunction. In summary, in an unstressed state, elevated SIRT3 levels in neurons cause IR and cell stress, while protecting against palmitate-induced cell stress and improving insulin sensitivity, pointing to a crucial role for the precise regulation of SIRT3 in the brain.

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P-133

Label-free chemiluminescence imaging of oxidative processes in human skin

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Keywords: Oxidative stress; chemiluminescence; imaging; skin; EPR

Oxidative processes present across all types of organisms, including humans, cause chemical formation of electron excited species with subsequent endogenous ultra-weak photon emission. Thus, imaging of this endogenous chemiluminescence using ultrasensitive devices potentially enables label-free monitoring of oxidative stress in optically accessible areas of human body, such as human skin. However, no quantified imaging of oxidative processes in human skin has been performed until now using endogenous chemiluminescence under controlled extent of oxidative stress conditions. Furthermore, the mechanisms and dynamics of endogenous chemiluminescence is not fully explored. Here we demonstrate that different degrees of oxidative processes on skin can be spatially resolved through non-invasive label-free endogenous chemiluminescence imaging in a quantitative manner. Additionally, to obtain insight into the underlying mechanisms, we developed and employed a minimal chemical model of skin based on a mixture of lipid (linoleic acid) / melanin / water to show that it reproduces essential features of the response of a real skin to oxidative stress. Our results contribute to novel non-invasive label-free methods for quantitative monitoring of oxidative processes and oxidative stress.

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P-134

Nrf2-pathway alteration in RTT syndrome

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Rett syndrome (RTT) is a rare neurodevelopmental disorder that affects almost exclusively females; 90% of the classic form is caused by mutations in the X-linked MeCP2 gene and patients exhibits neurological but also multisystemic symptoms after 6–18 months. Oxidative stress (OS) seems to play a role in the pathogenic mechanisms of RTT but the link is still unclear. Several enzymes involved in the antioxidant defense are regulated by Nrf2 (Nuclear erythroid related factor 2), an essential transcription factor activated in response to OS. The aim of our study was to evaluate the activation of Nrf2 pathway in fibroblasts isolated from RTT patients after challenging the cells with 4-HNE, aldehyde which levels have been found significantly high in RTT patients plasma. We found that 5 μM of 4-HNE was able to increase the nuclear translocation of Nrf2 in RTT fibroblasts as demonstrated by DNA binding assay and nuclear Western blot assay. Surprisingly, while 4-HNE treatment increased the gene expression of antioxidant enzymes such as GPX (glutathione peroxidase) and GR (glutathione reductase), their activities were significant corrupted in RTT cells suggesting a possible post-translational modification leading to the inability of RTT patients to fully counteract an oxidative stress challenge and bringing new insights on the possible link between this pathology and an OS state.

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P-135

HO-1 down-regulation increases the efficacy of BRAFV600E inhibition-based therapy in primary melanoma cells

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Keywords: HO-1; melanoma; cell resistance; NK; oxidative stress

Heme oxygenase 1 (HO-1) plays a key role in maintaining cell redox balance. Under stress conditions, HO-1 is up-regulated and generates bilirubin, ferritin and carbon monoxide, with antioxidant, antiapoptotic and anti-inflammatory properties [Jozkowicz et al., 2007]. HO-1 induction favors cancer progression [Furfaro et al., 2016] and its involvement in tumor resistance to therapy [Was et al., 2006] and immune-escape has been highlighted in melanoma-bearing mice [Di Biase et al., 2016].

In this work, the role of HO-1 in melanoma cell resistance to Vemurafenib/PLX4032, a selective inhibitor of mutant BRAFV600 has been investigated, as well as HO-1 involvement in natural killer (NK)-mediated killing.

BRAFV600E mutant primary melanoma cells isolated in our lab have been treated for 24 h with 1 μM PLX4032. The treatment reduced viability (-23%), increased HO-1 mRNA level of two-fold, and decreased the ability of IL-15 activated NK cells to degranulate in response to PLX4032-treated cells, when compared to untreated melanoma cells. HO-1 silencing increased PLX4032 efficacy further reducing cell viability to 47% in comparison to cells treated with PLX4032 alone and restored the degranulation potential of NK. Thus, we hypothesize that HO-1 inhibition can effectively improve the efficacy of mutant-BRAF inhibitors and favors NK-depending killing of PLX4032-treated melanoma cells.

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P-136

Acute effect of Thai Chi on marker of oxidative stress and flow-mediated dilation among healthy young and elderly volunteers

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Keywords: Tai Chi; oxidative stress; inflammation

Tai Chi is an ancient exercise originated from China. The slow movements of Tai Chi are not thought to evoke an increase in heart rate above 60% HRM, and yet Tai Chi has been found to improve vascular health. We are interested in the response to a single session of Tai Chi, as a mild physical stressor. The acute response to exercise is very important as a stimulus for adaptation, but to our knowledge, no previous studies have assessed the acute response to Tai Chi. The aim of this study was to investigate the response to a single session of Tai Chi. Blood markers of oxidative stress (lipid peroxidation, antioxidant capacity) and inflammation (IL-6) were assessed along with flow-mediated dilation (FMD) in young (18-25years old) and elderly participants (65–75 years old). Participants visited the laboratory twice to undertake Tai Chi or a control visit. Blood withdrawal and FMD assessment were performed every hour, for 4 consecutive hours. Inflammation at baseline was increased in older participants, compared to young and MDA and IL-6 increased immediately after Tai Chi in both groups. Antioxidant capacity increased immediately and post one hour after Tai Chi in both groups. A single bout of Tai Chi was seen to promote cytokine release and increased antioxidant capacity, and may have a beneficial effect in improving human vasculature via these mechanisms in both old and young.

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P-137

Expression patterns of peroxiredoxins in the rat bone and their changes after ovariectomy—an implication in aging?

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Keywords: Peroxiredoxins; bone; immunohistochemistry

Peroxiredoxins (Prxs, 20–30 kDa) belong to the thioredoxin protein family that is characterized by a common structural motif, the thioredoxin fold, and the presence of one or two cysteines in their active site. These proteins are recognized as key molecules in redox signaling and are expressed in various tissues of mice and humans. The growing evidence for a role of oxidative stress in bone pathologies, prompted us to systematically analyze the expression patterns of Prxs (1–6) in the femur of rats and their changes after ovariectomy. All analyzed Prxs were abundantly expressed in different cell types of the bone of sham operated rats. Strong signals for Prx1 and 3 were observed in osteocytes' lacunae, osteoblasts, bone lining cells, and in the epiphyseal cartilage. Both Prx1 and Prx3 showed a characteristic staining pattern in the canalicular system of the bone. Ovariectomy in rats induced down-regulation of Prxs in almost all cell types of the bone and bone marrow compartment. Because these redox proteins are crucial not only for maintaining a reduced environment, but also for many other cellular processes, the results shown here might have an implication in diseases associated with estrogen deficiency, for instance, osteoporosis and the immune response in aging.

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P-138

A correlation between redox imbalance and altered mitochondrial quality control pathway in Autistic Spectrum Disorder

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Keywords: Autism; Oxidative stress; mitochondria

The Autistic Spectrum Disorder (ASD) refers to a range of conditions classified as neurodevelopmental disorders characteristic of the early childhood.

Over the last 50 years, considerable efforts have been made to understand pathogenesis of ASD: it is generally accepted that a lot of causes could trigger the disease.

Since growing evidences described an association between oxidative stress and mitochondrial dysfunction in ASD, we investigated the possible defects of mitochondrial quality control pathway as contributing factors to the altered redox homeostasis.

Using freshly skin fibroblasts isolated from ASD patients and healthy subjects, we demonstrated in autistic cells an increased levels of 4HNE protein adducts - a major secondary product of lipid peroxidation - and catalase, one of the main enzymes of the cellular defensive system.

Transmission electron microscope (TEM) analysis demonstrated an altered mitochondrial morphology in autistic fibroblasts. An increased expression of the genes that encode for molecules involved in mitochondrial fission/fusion processes, was also detected. In addition, overregulation of PINK1, but decreased Parkin expression, both associated to mitophagy process, were observed.

Taken together our findings provide new insights on the possible contribution of the altered mitochondrial quality control pathway in the redox imbalance of ASD patients.

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P-139

Functional and structural characterization of a novel class of MAP-kinase inhibitors

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Keywords: p38; MAPKs; JNK; ERK

p38 MAP kinases are a class of Ser/Thr kinases that link a number of extracellular stimuli to intracellular signaling pathways with effects on cellular processes such as cell cycle regulation, cell death, differentiation, senescence, cytokine production, etc.

p38 kinase activation is nvolved in the pathogenesis of several inflammatory and oxidative stress diseases so that developing novel and more efficient pharmacological modulators of p38 activity is of great importance. In this context, we tested in HT29 human colorectal adenocarcinoma cells two novel pyrazolobenzothiazine inhibitors (COXP4M12 and COXH11) of p38 MAPK activity previously characterized in vitro in cell-free experiments. The inhibitory activity of the two test compounds was confirmed during the stimulation of cellular p38 activity with either LPS or H_2O_2.

Interestingly enough, in LPS stimulated cells, these compounds were also found to be efficient inhibitors of JNK stress kinase, but not of ERK1/2 kinase. Of the two test compounds COXH11 showed the better inhibitory efficacy combined with low cytotoxicity and thus it was selected for further biochemical investigation that included successful crystallographic experiments for structural and molecular characterization of the protein-inhibitor complex. These preliminary data encourages us to further investigate COXH11 MAPK inhibitor in vivo.

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P-140

GSTP expression influences the metabolism and redox of cellular glutathione

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Keywords: GSTP; Nrf2; Se-compound; thiol; GSH

Recently we described the role of glutathione-S-transferase P-1 (GSTP) expression in regulating Nrf2 transcriptional activity by protein-protein interaction. A functional interplay between Nrf2 and GSTP to control glutathione (GSH) biosynthesis, detoxification function and signal transduction through protein Cys glutathionylation can be hypothesized. To explore such interplay the effect of GSTP gene manipulation on the cellular levels and redox of main thiols in murine embryonic fibroblasts (MEFs) was investigated. GSTP knockout MEFs showed increased levels of all the identified forms of thiols that included GSH, GSSG, GSSP, Cys, Cyss, CyssP, HCys, HCyss, HCyssP, γ-GluCys and CysGly.

The exposure to the Se-organic thiol-peroxidase compound Ebselen, further and massively up-regulated GSH, Cys and HCys and their corresponding disulfides in GSTP-KO, but not in wild-

type (WT) cells. GSSP (protein glutathionylation), and to a lower extent HCyssP, increased after treatment with Se-organic compounds only in WT cells (confirmed by both HPLC and immunoblot analysis).

These findings confirms the role of GSTP as a feedback component of Nrf2 pathway (control of GSH levels) and identify with an unbiased approach the role of this gene in mediating protein glutathionylation signalling during the exposure to Se-organic thiol-peroxidases.

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P-141

Peripheral oxidative profile and specific advanced glycation end products can be a signature of cognitive decline in Alzheimer's disease

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Keywords: Blood; Biomarkers; Carboxylmethyl lysine; Protein Carbonyl; Neurodegenerative diseases; Methylglyoxal

An early diagnosis of Alzheimer's disease (AD) is required for early and more effective treatment. Oxidative stress plays an early role in AD and could promote the formation of advanced glycation end products (AGEs) in AD brains. However, the peripheral association of specific AGEs and oxidative markers with cognitive decline remains to be defined in order to establish a clinical signature for an early diagnosis.
Objectives: To measure the total antioxidant capacity (TAC), the levels of protein carbonyl (PC), AGEs precursors (methylglyoxal, MG; glyoxal, GO) and some specific AGEs (pentosidine; carboxylmethyl lysine, CML) and their association with clinical scores (MMSE and MoCA).
Methods: These markers were measured in blood from patients with MCI (mild cognitive impairment) and AD at different stages and from age-matched controls by Western blot, ELISA or HPLC.
Results: A decrease of TAC and an increase of AGEs precursor's levels (MG and GO) are observed in MCI patients. PC and CML levels raised later in AD groups. Pentosidine levels were not different. Interestingly, PC and CML levels are correlated with cognitive scores.
Conclusion: Our results demonstrate that TAC decreases early in MCI stage while some oxidative markers and AGEs precursors increase and are correlated with cognitive decline. Overall, some oxidative stress markers have a potential as biomarkers for AD.

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P-142

TRAMP mice overexpressing SOD2 develop poorly differentiated neuroendocrine tumors and lower survival

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Keywords: SOD2; cancer; progression; prostate

In addition of being a primary defense against $O_2^{\bullet-}$ SOD2, the mitochondrial antioxidant enzyme was claimed for years to have a tumor suppressor role. However, in the last few years numerous reports indicate that, rather on the contrary this enzyme is associated with poor patient outcome and a more aggressive disease in a wide variety of tumors. We used murine model C57BL/6-Tg (TRAMP) 8247Ng/J as well as Sod2+/− knockdown and Sod2+/++ overexpressing mice to generate 2 additional phenotypes TRAMP/Sod2+/− and TRAMP/Sod2+/++ to study the influence of systemic modification of expression levels of SOD2 in prostate cancer. We studied the tumor progression at different ages analyzing genitourinary system weight, tumor diagnosis, androgen receptor (AR), BCL-2, matrix metalloproteinases (MMPs), VEGF and redox enzymes levels. We found that an overexpression of SOD2 not only brought a more malignant tumor diagnosis (mostly poorly differentiated) but also compromised the survival of these mice. Moreover, castration at 12 weeks of age of the three phenotypes showed that TRAMP/Sod2+/++ mice had a relapse rate dramatically higher. These mice mostly developed poorly differentiated neuroendocrine tumors with multiple metastasis, and its survival rate was very low.

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P-143

Air particulate matter as enhancer of ozone-induced skin damage

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Keywords: Pollution; skin; damage; CAPs; ozone; oxidative stress; skin diseases; aging

The skin is a biological shield against environmental stressors, such as O_3, UV and CAPs (concentrated air particles). It is well known that chronic exposure of the cutaneous tissue to the pollutants causes oxidative stress (OS) and inflammation and this has been associated with several skin diseases and premature aging. On the other hand, many studies have been performed using only one pollutant although we are daily exposed to several stressors. Therefore, the purpose of the present work was to analyze the combined effects of CAPs and O_3 on skin by the use of reconstructed human epidermis (RHE). Results showed that the combination of the two stressors induced a further up-regulation of OS markers (4HNE), increased tissue defense (HO-1) and inflammatory responses (COX-2 and MMP-9), when compared to the single treatment. Interestingly, these effects were more evident when treatment with CAPs preceded O_3, probably due to the capability to penetrate RHE. Moreover, these results were confirmed by H&E staining: the histological examination allowed us to note that the exposure to CAPs and then O_3 was able to perturb the skin structure. Our study underlines how important is trying to reduce CAPs levels since they are able to additively affect the skin when in the presence of other pollutants.

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P-144

Clinical evaluation of wound healing capacity of isopod *Ceratothoa oestroides* oil extract

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A very significant health issue worldwide with great impact at personal, professional and social levels, is non-healing ulcers with massive cost in terms of human and material resources. A chronic ulcer is characterized by a persistent inflammation associated with high oxidative stress.

A very significant health issue worldwide with great impact at personal, professional and social levels, is non-healing ulcers with massive cost in terms of human and material resources. A chronic ulcer is characterized by a persistent inflammation associated with high oxidative stress.

Significant pre-clinical wound healing data of an ointment containing isopod *Ceratothoa oestroides* (sea lice) oil extract combined with gentamycin, polymyxin B and acetic acid (formula A) leaded to a clinical trial realized to Andreas Syggros Hospital of Cutaneous & Venereal Diseases. The trial was conducted in comparison with a formula containing hyaluronic acid and silver sulfadiazine (formula B).

Adult patients with venous or diabetic foot ulcers meeting the required criteria, randomized to receive daily dressing containing either formula A or formula B. Every 2 weeks, patients were clinically evaluated for wound healing progress according to Bates Jensen or PUSH criteria. Measurements of pH, temperature, wound area, oxygen saturation and glucose blood levels were regularly obtained.

Formula A demonstrates remarkable wound healing activity.

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P-145

Effect of anti-inflammatory/antioxidant agent on diabetic hairless mouse skin

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Keywords: Skin; inflammation; diabetes; antioxidants

Skin inflammation is associated with almost all cutaneous disorders. Diabetes mellitus also influences skin physiology producing dryness and accelerating ageing. The development of effective and safe topical anti-inflammatory/antioxidant agents may be beneficial in such cases. We hereby evaluated the effect of a novel multifunctional derivative (AK1) that combines structural moieties of tolfenamic acid and L-cysteine.

Thirteen groups of male hairless mice were exposed to acute UVR (2,5 MED). 1% tolfenamic acid, L-cysteine ethyl ester (as reference agents), AK1 and excipient were topically applied daily on streptozotocin-induced diabetic (D) or non diabetic (ND) mice. The examined parameters were TEWL, skin hydration, redness, thickness, elasticity, oxidative stress and hydrophilic antioxidants.

In ND mice, AK1 significantly reduced TEWL on the 4th and 11th day while hydration levels were higher on day 14 in the AK1 group compared to control groups ($p < 0,05$). Concerning skin antioxidant capacity, no significant differences among groups were observed. In D mice, administration of AK1 lowered levels of TEWL compared to cysteine or control groups.

Application of AK1 on the skin of both D and ND mice appears to exhibit an effective anti-inflammatory activity.

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P-146

The effect of anti-aging agents in skin oxidative stress induced by UV Radiation in vivo

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Keywords: Skin; photoaging; antioxidants; oxidative stress

UV radiation is related to oxidative stress and skin aging. UV induced reactive oxygen species lead to progressive degradation of dermal collagen and elastin.

Four of the most known anti-aging agents were examined in the photoaged skin of hairless SKH-hr2 mice. 50 mice were

irradiated with increasing UVR doses (0.5–2 MED) 3 times per week for 4 weeks. After the end of the irradiations, 5 formulations containing retinoic acid 0.05%, lactic acid 10%, salicylic acid 3%, epidermal growth factor 3.3% and the vehicle were applied on the back of the mice for 3 weeks.

Hydration, transepidermal water loss (TEWL), elasticity and skin thickness, were frequently measured. After the end of applications, oxidative stress was evaluated in skin biopsies and strippings of stratum corneum using fluorescence spectroscopy, as well endogenous antioxidants using HPLC–EC detection.

Epidermal thickening was evident in all mice after UV exposure. After treatment with lactic acid the thickness decreased significantly. Highest TEWL was observed in the retinoic acid group. The skin treated with salicylic acid demonstrated the lowest levels of ascorbic and uric acid. In strippings, lactic acid icreased the levels of antioxidants. Highest elasticity was measured in the retinoic acid group. In conclusion, retinoic and lactic acids seem to be the most effective treatments for the photodamaged skin.

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P-147

In vitro evaluation of pine extracts antioxidant protection

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Oxidative stress is known to be responsible for a wide range of pathological conditions. Many skin diseases are initiated or/and promoted by oxidative stress. Oxidative stress could be caused by insufficient fuctioning of the antioxidant defence mechanisms or increased exposure to reactive oxygen species (ROS).

The antioxidant and protective efficacy of aqueous extracts of various parts of the plants *Pinus halepensis*, *Pinus brutia*, *Pinus nigra,* and *Eucalyptus globulus* in comparison with aqueous extract of green tea dry leaves (*Camelia sinensis*, Theaceae), was evaluated. The plant selection was based on their content in phenolic antioxidant molecules. The comparison was made by carrying out in vitro assays of the plant extracts and observing their effects on cell culture of fibroblasts from mice fibroblasts, which were exposed to chemical oxidative stress.

The resutls of this research showed the importance of the concentration of phenolic antioxidants contained into the extracts. The lower dose of 0,1 μg/ml of extract decrease oxidative stress and viability, while 1 μg/ml enhace oxidative stress and fibroblast viabilty. It seems that rates of mitosis is dependent of oxidative stress.

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P-148

Identification of novel carbonylated amino acids in proteins from human plasma

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Keywords: Human plasma; carbonylation; peroxyl and alkoxyl radicals

In order to fully understand the role of protein carbonylation in health and disease, identification of carbonylated protein(s), and their detailed characterisation is required.

Mass spectrometry is a particularly suited for such studies due to its specificity and sensitivity. We have used biotin-hydrazide and mass spectrometry based approach for identification of up to 14 different types of carbonylated amino acids.

In native human plasma we have observed 133 carbonylated sites in 36 proteins. The approach identified 10 hitherto undetected types of carbonylated amino acids in proteins: aldehyde and ketone modifications of leucine, valine, alanine, isoleucine, glutamine, lysine and glutamic acid (+14 Da), an oxidised form of methionine - aspartate semialdehyde (-32 Da) - and decarboxylated glutamic acid and aspartic acid (-30 Da). The carbonyl compounds reported are consistent with the chemistry of peroxyl and alkoxyl radicals generated on proteins.

The consequence of the formation of these products has yet to be understood. However, it is important to note that some of these carbonyls can introduce changes to protein charge, give rise to Schiff base cross-links, and can lead to changes in residues that define protein structure (e.g. ring opening of P residues). These events may affect protein function (e.g. interaction with other) or protein conformation and activity.

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P-149

Roles of branched-chain amino acids regulation in oxidative stress revealed by fibroblasts from classic Maple Syrup Urine Disease patients

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Keywords: Oxidative stress; mitochondrial superoxide; protein carbonylation; Maple Syrup Urine Disease; branched-chain amino acid catabolism

Branched-chain amino acids (BCAAs) are essential amino acids commonly used in clinical procedures to improve patients outcome.However, their roles and cellular mechanisms are not clear. One effect of BCAAs supplementation is reduction of oxidative stress. BCAAs levels are regulated by their catabolism and the rate limiting enzyme is branched chain a-ketoacid dehydrogenase

(BCKDH).Blockage in BCKDH activity leads to classic Maple Syrup Urine Disease (MSUD).To study the roles of BCAAs, we used cells with a single gene defect in BCKDH as a cellular model.We studied fibroblasts from four unrelated patients with null mutations in BCKDH and from controls.Fibroblasts from patients showed 2-fold increase in mitochondrial superoxide levels and 1.5-fold increase in protein carbonylation levels respect to controls.No changes in SOD2 protein levels were detected, indicating an increase production of mitochondrial superoxide and not an increase detoxification.Eleven proteins related to oxidative stress were differentially regulated in MSUD (p-value 0.05).Including up-regulated peroxiredoxin-4(PRDX4) and protein disulphide-isomerase(P4HB), as well as down-regulated prostaglandin G/H synthase 1 (PTGS1), also known as cyclooxygenase-1.These results correlate specific proteins to known effects of BCAAs in oxidative stress and shed light in the pharmacological mechanisms of BCAAs supplementation.

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P-150

Chronic UV irradiation induced oxidative stress in the skin of diabetic hairless mice

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Keywords: Skin; inflammation; diabetes; antioxidants; oxidative stress; ultraviolet radiation

Diabetic mellitus induces many pathophysiological changes in skin. The effect of UV light on chronic exposure in diabetic and normal skin was investigated.

Hairless mice skin, diabetic (D) and non-diabetic (ND), were exposed to UVA and UVB radiation 3 times per week for 18 weeks. The irradiation dose was equal to 0.75 M.E.D during the first week and increased by 25% each week until the maximal dose was 3.5 M.E.D. Diabetes was induced by streptozotocin injection. Stratum corneum hydration of D mice was significantly lower than ND ($p < 0.01$). The transepidermal water loss of D mice was less than ND mice due to the dryness of the skin of D mice. The elasticity of ND mice's skin was significantly higher ($p < 0.05$). Skin sebum in the D mice was much lower in relation to ND ($p < 0.05$). Skin of D mice is more pigmented and thinner than this of ND. Hydrophilic antioxidants and oxidative stress in both SC and total skin of D and ND mice is in process of evaluation.

This results suggest that D mice presented more severe inflammation, photoageing and hyperpigmentation after chronic UV irradiation.

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P-151

Glutathione and the switch of aerobic metabolism collaborate for multi-drug resistance of neuroblastoma

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Keywords: GSH; neuroblastoma; BSO; chemoresistance; aerobic metabolism

The availability of antioxidants is recognized as one of the critical factors able to make cancer cells resistant to chemotherapy. In this context, it has been demonstrated that many chemoresistant cancers display high levels of glutathione (GSH) and consequently, its depletion by L-buthionine sulfoximine (BSO), has been proposed as a chemosensitizing therapy. To investigate the role of GSH and of tumor metabolism in multi-drug resistance (MDR), HTLA-230 neuroblastoma cells were chronically treated with etoposide at a concentration that in vitro mimics the clinically-used dose. The selected cells (HTLA-Chr) were highly tumorigenic and acquired MDR, becoming less sensitive to etoposide or doxorubicin compared to parental cells. Moreover, HTLA-Chr cells, while having an efficient aerobic metabolism, owing to a favourable P/O ratio and a decreased formation of lactic acid, were also characterized by an up-regulation of catalase and higher levels of GSH. BSO treatment of HTLA-Chr cells markedly reduced their tumorigenicity that was, instead, enhanced by N-Acetylcysteine, able to promote GSH synthesis. Collectively, our results show that GSH and the switch of aerobic metabolism collaborate for the acquisition of MDR, providing pre-clinical evidence that may drive future therapeutic approaches for sensitizing neuroblastoma to conventional therapies.

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P-152

Skin Inflammation and Oxidative Stress

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Keywords: Skin inflammation oxidative stress antioxidants

Enhanced oxidative stress is related to enhanced inflammation. The behavior of endogenous antioxidants in inflammatory phenomena is not quite clear. In a trial to elucidate the relation between skin inflammation, oxidative stress and antioxidants in vivo and in vitro data will be discussed.

Using keratinocytes and fibroblasts producing oxidative stress either by UV light or xanthine – xanthine oxidase or tobacco smoke, the protection of antioxidants is evaluated. In vivo, in hairless mice, oxidative stress is estimated under different conditions as acute and chronic exposure to UV light or to tobacco smoke in both normal and diabetic skin, evaluating inflammation, oxidative stress and endogenous antioxidants.

It seems that the dose of administered antioxidants is much important as a light pro-oxidant activity could be beneficial for the regeneration and protection of skin. In vivo, after UV light exposure, endogenous antioxidants as glutathion at the level of stratum corneum decreased while uric acid is increased. Administration of exogenous non antioxidant substances as lactic acid could also increase uric acid. Stratum corneum is much more susceptible to oxidative stress and antioxidant alterations in relation to living epidermis and dermis.

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P-153

SIRT3 knockdown increases oxidative stress and sensitivity to cytotoxic treatments in SW620 cancer cells

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Keywords: Sirtuin 3; colon cancer; oxidative stress; oxaliplatin

Sirtuin 3 (SIRT3) is the major deacetylase in mitochondria and is activated under oxidative stress conditions. SIRT3 regulates mitochondrial metabolism and the antioxidant response to lower reactive oxygen species (ROS) production. Thus, SIRT3 could allow cells to counteract the effect of anticancer therapies, which increase ROS levels. The aim of this study was to determine whether SIRT3 knockdown in colon cancer cells could increase oxidative stress and therefore make them more sensitive to cytotoxic treatments. A stable SIRT3 silencing in SW620 human cell line was achieved through a specific shRNA. Expression of antioxidant enzymes and mitochondrial proteins was analyzed, as well as protein levels by Western Blot and MnSOD enzymatic activity. Cells were also treated with oxaliplatin to study ROS production by Amplex Red®. SIRT3 silencing resulted in a decrease of both antioxidant enzymes and proteins related to mitochondrial function, as well as a reduction in MnSOD activity. Furthermore, SIRT3 knockdown produced a significant increase in ROS production, which was greater with oxaliplatin treatment. Overall, these results suggest that SIRT3 is a key factor regulating mitochondria under oxidative stress. SIRT3 knockdown could be a therapeutic strategy for colon cancer, since it improves the effectiveness of cytotoxic treatments.

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P-154

Diabetic skin and UV light: protection by *Pinus halepensis* pine bark extract

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Keywords: Diabetes mellitus; skin; UVR; *pinus halepensis*; vitamin C

Diabetic skin is characterized by great sensitivity when compared to normal skin. Ultraviolet radiation is directly related to the oxidative stress affecting the skin. Diabetic skin, when compared to the normal one, is greatly damaged by ultraviolet radiation resulting in significant photoaging. Despite the fact that the long-term effect of UV light on normal skin has been partially investigated, for the diabetic skin data is missing. Antioxidants, such as *Pinus halepensis* seem to prevent photoaging. On the skin of hairless male diabetic mice the effects of UV radiation and the possible protection of *Pinus halepensis* pine bark extract topical application were studied, showing the significant antioxidant role of pine.

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P-155

The role of aging and senescence on pancreatic β-cell function and proliferation

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Keywords: Aging; senescence; pancreatic β-cell; type-2-diabetes

Aging and age-related diseases are associated with the impairment of tissue function and their regenerative capacity. In terms of pancreatic β-cells, it is well known that insulin resistance in peripheral tissues increases with age due to metabolic changes caused by adiposity or physical inactivity. But in the presence of functional β-cells, insulin resistance alone is insufficient to lead to type-2-diabetes. However, in combination with an insulin

secretory dysfunction and a decreased β-cell mass coincided with advanced age, this leads to worsening of glucose tolerance and finally to hyperglycemia and the onset of type-2-diabetes. Investigations regarding the molecular basis of the limited proliferation capacity of aged endocrine pancreas revealed that cellular senescence seems to be involved. Senescence is characterized by an upregulation of tumor suppressor proteins such as p16Ink4a and p53, severe morphological diversifications or widespread changes in protein expression and secretion. But the impact of senescence-related changes on β-cell impairment has not yet been described. Therefore, by comparing lean C57Bl/6 J mice and adipose, diabetes-prone New Zealand Obese mice with diabetes-resistant B6.V-Lepob/J mice in different stages of age, we aim to characterize senescent β-cells and investigate the role of cellular senescence on β-cell functionality and proliferation.

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P-156

A "multi-omic" investigation of the effects of long wavelength ultraviolet light on primary human keratinocytes identifies NUPR1 as central stress response mediator

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Keywords: UV-stress; oxidized phospholipids; poly-omics; Nrf2; Nupr1

Long wavelength ultraviolet (UVA) light is the dominant oxidative stressor for the skin and promoting skin aging by yielding oxidation derived deleterious compounds. The dynamics of UVA/ROS induced reactive lipids and the responses to such stress in keratinocytes (KC), the major epidermal cell type, is only poorly understood.

The aims of this study were to assess the photooxidation of phospholipids in KC, their contribution to transcriptional and translational responses, and to identify involved signaling pathways.

We investigated the oxidized phospholipidome of cultured primary KC 0 h and 24 h post stress (UVA-1 or in vitro UV-oxidized phospholipids) with HPLC-MS/MS. We investigated the transcriptomic response 7 h after stress using microarrays and deep sequencing and changes in the proteome 24 h post stress using HPLC-MS.

Lipidomics identified 173 UV-induced lipid species of which 89 declined to baseline levels after 24 h, and of which 141 were also induced by or contained in UVPAPC. We identified carbonylated, hydroxy and lysoPC species as regulated. The transcriptomic response showed a shared NRF2 signature for both stressors, and UPR/ER stress upon UVA. Upstream regulator analysis identified NUPR1 as novel high level stress response regulator, as knockdown of NUPR1 resulted in dysregulated expression of antioxidant, lipid detoxifying and cell cycle regulation genes.

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P-157

Potential Healing Properties of Bee Products to Thermal and Sun Burns

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Keywords: Burn skin uv thermal beeswax cocoons royal jelly

Burn injuries are to be considered and treated as a disease. They cause severe damage on skin tissue and affect all the systems of the human body. Bee products, such as honey and propolis, are known for their healing and antibacterial properties when applied on injured skin. In order to explore the possibility of other bee products having similar properties, royal jelly, bee cocoons, and beeswax are isolated and tested on 64 lab mice type SKH-1. Half of them are exposed to 8 MED of UV radiation, causing severe sun burn, and on the other half a second degree thermal burn is induced using a metal rod heated up to 60 °C. The parameters which are studied to evaluate their pharmacological action are hydration, transepidermal waster loss, elasticity, skin thickness, antioxidants (ascorbic acid, uric acid, GSH) and oxidative stress, as well as histological analysis. Based on the results, royal jelly and bee cocoons showed significant effects on the burned skin, while all three of them improved the visual outcome of the scar tissue. Further studies are to be made on the properties of their mixtures.

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P-158

GLP-2 in the capacity of (-)-epicatechin and anthocyanidins to improve insulin sensitivity

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Keywords: Obesity; insulin resistance; glucagon-like peptide-2 (GLP-2); anthocyanidins

Glucagon-like peptide-2 (GLP-2) is an incretin involved in energy balance through the regulation of postprandial glycaemia. GLP-2 is generated from the post-translational cleavage of proglucagon, being rapidly degraded in the bloodstream by dipeptidyl peptidase IV (DPP-IV). We currently investigated the effects of (-)-epicatechin (EC) and anthocyanidins (AC) to modulate GLP-2 using in vivo and in vitro approaches.

To assess in vivo effects on proglucagon mRNA and plasma GLP-2 concentrations, male C57BL/6 J mice were fed for 14 w control (C) or high fat (HF) diets without or with 20 mg EC/kg or 40 mg AC/kg BW.

Supplementation with EC and AC mitigated HF-induced insulin resistance (improved GTT, ITT, plasma insulin and glucose levels). Plasma GLP-2 levels were 148 and 200% higher in C mice supplemented with EC and AC compared to non-supplemented C mice, and 83 and 46% higher in HF mice supplemented with EC and AC compared to non-supplemented HF mice. Colon proglucagon mRNA levels were higher (84%) in AC-supplemented vs unsupplemented C mice, while EC had no effect. In vitro, cyanidin, but not EC, inhibited DPP-IV activity.

The improvement of insulin sensitivity by EC and AC in a model of high fat diet-induced type 2 diabetes could be in part due to GLP-2 upregulation. The underlying mechanisms for AC involve an increase in proglucagon expression and decreased GLP-2 degradation.

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P-159

Role of NADPH oxidase on TNFα-induced intestinal permeabilization

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Increased intestinal barrier permeability is a major event in the pathophysiology of diseases with an inflammatory background. Tumor necrosis factor alpha (TNFα) plays a central role promoting barrier dysfunction. NADPH oxidase (NOX) is co-activated by TNFα and increased superoxide anion formation could be involved in barrier disruption. In human Caco-2 cells differentiated into intestinal epithelial monolayers we investigated: i- the relative abundance of NOX family members, ii) the effects of TNFα on NOX activation/expression, iii) the effects of epicatechin (EC) and cyanidin (CN) on NOX activity and Caco-2 monolayer protection. Assessment of NOX1, NOX2, NOX3, NOX4 and NOX5 mRNA levels showed NOX1 and NOX4 as predominant. TNFα treatment for 6 h increased (circa 32% and 30%) NOX1 and NOX4 expression; and for 10 min increased (circa 49%) NOX activity. From a group of flavonoids tested, EC and CN were the most effective inhibiting basal and TNFα-stimulated NOX activity. The extent of inhibition was different between basal and stimulated conditions suggesting differential effects on different NOX members. EC- and CN-mediated NOX inhibition was associated with their capacity to protect barrier permeability. NOX can play a major role in the development of inflammation-mediated intestinal permeabilization, suggesting a beneficial effect of EC/CN-rich diets on intestinal pathologies.

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P-160

Microglia based Alzheimer therapy

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Keywords: Alzheimer; microglia; aging; inflammation

Alzheimer's disease (AD) is an age-related neurodegenerative disease associated with the formation of amyloid plaques, tau aggregation and oxidative/inflammatory damage. Microglia play an important role in the early phase of the disease and are known to be involved in AD progression. Senescent microglia accumulate in AD causing inflammation, neuronal damage and increasing the Aß load due to a failing protein degradation system.

This study examines the transplantation of in vitro derived young microglia from wild type mice (aged ~3 months) to the brain of AD mice (APP/PS1 transgenic model: > 12 months).

Microglia could be detected in the brain for four weeks after transplantation. Analysis of amyloid content shows a significant reduction as well as improved inflammation markers. Microglia and astrocyte morphology and activation status was analysed using histology. Changes in the short term memory of the recipient animals was analysed using a maze and found to be improved. Current analysis focuses on the quality and M1/M2 status of the microglia produced for transplantation to better understand if the therapeutic effect can be improved.

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P-161

Developmental expression and dysregulation of miR146a and miR155 in Down's syndrome and mouse models of Down's syndrome and Alzheimer's disease

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Keywords: Inflammation; miRNAs; down syndrome; Alzheimer Disease

Increasing evidence supports the involvement of inflammation-related miRNAs in neural development and function under pathological conditions. We hypothesized that an dysregulation of miR146a and miR155, key regulators of the innate immune response, may occur in patients with Down syndrome (DS) and Alzheimer's disease (AD).

The miRNA expression patterns were investigated in developing hippocampus from DS and in DS-AD adults. Quantitative RT-PCR was employed to evaluate the miRNA levels in the AD hippocampus at different stage of disease (sAD) and in DS (Ts65Dn) and AD (APP/PS1) mouse models.

Both miRNAs were expressed in prenatal human hippocampus. In DS we detected increased miR146a expression in reactive astrocytes, which was also found in hippocampus of sAD and negatively correlated with its target IRAK1. APP/PS1 mice showed a significant expression increase of both miRNAs at 11–13 months of age as compared to wt and mice at 3 months. A negative correlation between miR146a levels and its target TRAF6 was observed in both mice models.

These findings suggest a possible involvement of miR146a and miR155 in brain development and neurodegeneration. In particular, we provide evidence of a dysregulation of these two immunomodulatory miRNAs in AD with a potential therapeutical implication, deserving further investigation.

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P-162

Impact of the age-related protein aggregate lipofuscin on β-cell functionality

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Keywords: Lipofuscin; aging; β-cell

The formation of cross-linked protein aggregates such as lipofuscin is a typical hallmark of cellular aging and especially affects long-living and postmitotic cells such as neurons and skeletal muscle cells. Lipofuscin can neither be degraded nor exocytosed, it is a prominent source of oxidants and able to inhibit proteolytic systems such as the 20 S proteasome and the autophagy-lysosomal pathway.

Adult pancreatic β-cells are also considered to be long-living cells which may last for years or even a life-time. It was shown, that these cells are also characterized by an age-dependent increase in lipofuscin content, but little is known about the process of accumulating protein aggregates and their impact on β-cell functionality.

We used the pancreatic β-cell line MIN6 with a well-established artificial lipofuscin to investigate different parameters of β-cell functionality and the impact of lipofuscin on the insulin-degrading enzyme (IDE).

Interestingly, we observed both, a significantly higher insulin amount and secretion in the aggregate-fed MIN6 cells. While the expression of insulin does not seem to be affected by lipofuscin treatment, the presence of lipofuscin is able to decrease the activity of IDE by an unknown mechanism.

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P-163

Bcl-2 modulates ER/SR calcium uptake by interaction with SERCA and heat shock proteins

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Bcl-2 is an anti-apoptotic protein, which is involved in the interaction with multiple proteins at various cellular locations, including mitochondria and the ER. We show here, that Bcl-2 interacts efficiently with various isoforms of the sarco/endoplasmic reticulum Ca-ATPase (SERCA), including SERCA1, SERCA2 and SERCA3, which leads to SERCA inactivation, conformational changes, and translocation of SERCA between specific membrane microdomains. These functional effects of Bcl-2 are demonstrated both in vitro, and in cells overexpressing Bcl-2 and SERCA. Co-immunoprecipitation experiments confirm the interaction of Bcl-2 and SERCA in vitro and in cell culture. Amino acids critical for the functional interaction of Bcl-2 with SERCA have been identified ny mutagenesis. One example is the mutation of Gly145 to Glu145, which reduces the ability of Bcl-2 to inactivate SERCA. This mutation had previously been shown by others to lower the anti-apoptotic activity of Bcl-2, providing an interesting parallel between the anti-apoptotic activity of Bcl-2 and its potency to inactivate SERCA. Heat shock proteins prevent the Bcl-2-induced inactivation of SERCA, even when heat shock proteins carry oxidative post-translational modifications such 3-nitrotyrosine and/or 4-hydroxynonenal adducts (i.e., modifications accumulated under conditions of oxidative stress).

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P-164

Circulating mtDNA levels as an early marker for metabolic syndrome

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Keywords: mtDNA; metabolic syndrome; glucose; BMI; ccf-mtDNA

Chronic diseases, infection and physical activity are conditions related with metabolic or oxidative stress and the releasing of mitochondria to circulation by cells. This might be due to apoptosis or transference of mitochondria from one cell to another, either as survival or as tissue repair process. Circulating levels of cell-free mitochondrial DNA (ccf-mtDNA), although controversial, have been used for cancer and sepsis diagnose. The objective of this study was to investigate the relationship between ccf-mtDNA and metabolic syndrome. In this sense, after IRB approval, healthy subjects (n=32) and adults with diabetes (n=6) were invited to participate. Body mass index (BMI) was calculated and fasting glucose measured as biomarker of metabolic syndrome. In addition, DNA was extracted from serum and real time PCR used to detect ccf-mtDNA and both parameters were compared. Our data showed that ccf-mtDNA values ranged from 10 to 100 ng/ml in subjects with normal BMI while there were no detectable levels of ccf-mtDNA in samples from overweight subjects and diabetic patients. Glucose levels did not show differences independently of their BMI, but there was an inverse relationship between ccf-mtDNA and BMI. In conclusion, our results suggest that ccf-mtDNA might represent an early biomarker to identify people with metabolic syndrome or at risk for future develpment of diabetes.

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P-165

The role of mitochondrial reactive oxygen species in the response of the pulmonary vasculature to hypoxia and right heart remodeling

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Keywords: MitoQ; mitochondrial ROS; pulmonary hypertension; hypoxic pulmonary vasoconstriction

Introduction: Increased release of mitochondrial superoxide has been suggested to mediate acute hypoxic pulmonary vasoconstriction (HPV) as well as chronic hypoxia-induced pulmonary hypertension (PH) and right heart remodeling. Thus, we investigated the superoxide release during HPV, chronic hypoxia-induced PH and after pulmonary arterial banding (PAB), as well as the effect of the mitochondria-targeted antioxidant MitoQ on these processes.
Results: Superoxide levels were increased in PASMC during acute hypoxia, and decreased after 5 days of hypoxia. In parallel MitoQ, but not its inactive carrier substance, TPP+, significantly inhibited acute HPV and the rise in superoxide concentration induced by acute hypoxia. However, MitoQ application did not affect the hypoxia-induced proliferation of PASMC or the development of chronic hypoxia-induced PH. In contrast, MitoQ application attenuated right ventricular remodeling after chronic hypoxic exposure as well as after PAB with regard to development of right heart hypertrophy and dilatation. Accordingly, superoxide levels were increased in the RV after PAB.
Conclusion: Increased superoxide concentration mediates acute HPV, while decreased superoxide levels were detected in chronic hypoxia-induced PH. MitoQ may be beneficial under conditions of exaggerated acute HPV and to prevent the development of right heart remodeling.

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P-166

Ultrastructural Assessment of Mitochondrial Network in the Cultured Skin Fibroblasts from Patients Harboring tRNA Mutations

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Keywords: Mitochondria; MERRF; MELAS; TEM

Single-point mutation of mitochondrial DNA (mtDNA) has been confirmed to be involved in some inheritance of mitochondrial myopathy such as Mitochondrial Encephalopathy, Lactic acidosis, And Stroke-like syndrome (MELAS) and Myoclonic Epilepsy and Ragged-Red Fibers (MERRF). The A-to-G mutation at nucleotide 8344 accounts for 80 to 90% of MERRF syndrome. More than 80% of MELAS patients carry the A3243G mutation. The location of these two point mutations were identified in the transfer RNA (tRNA). It remains unknown why these particular tRNA point mutations caused the abnormal phenotypes. Some studies have demonstrated that Reactive Oxygen Species (ROS) production, alteration in antioxidant defenses and detoxification enzymes is involved in the pathogenesis of MERRF and MELAS syndrome. More and more reports revealed a potential role of oxidative stress within dysregulated mitochondrial network in lesion tissue. However, structural integrity and appropriate distribution and dynamics of mitochondria have to be maintained to execute normal physiological functions. Therefore, we integrated light and electron microscopy to illuminate mosaic mechanisms of the altered mitochondrial distribution and dynamics in cultured skin fibroblasts from patients with mitochondrial tRNA mutations.

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P-167

NoxO1 contributes to the differentiation of intestinal stem cells

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The intestinal epithelium is one of the most rapidly renewing tissues in the body. Low constitutive ROS formation is needed for cellular differentiation in different tissues. In the intestine, Nox1 is the predominant Nox and the cytosolic scaffolding protein NoxO1 enables its constitutive activity. We hypothesize that NoxO1 by enabling a constitutive low Reactive Oxygen Species (ROS) formation impacts on proliferation of intestinal stem cells.

In the mouse colon Nox1 mRNA was expressed at the bottom of the crypts where NoxO1 mRNA was expressed throughout the whole epithelium with reduced abundance toward the top of the crypt. Interestingly NoxO1 protein expression was restricted to the bottom of the crypts, indicating that Nox1 may impact on the protein stability of NoxO1. In Noxo1 knockout mice, Nox1 expression was increased in the crypts whereas the expression of a potential substitute for NoxO1, namely p47phox, was not changed. Accordingly superoxide anion production in intestinal crypts isolated from NoxO1 knockout mice as measured by LO12 chemiluminescence was reduced. In cells isolated from the crypt bottom of NoxO1-/- mice PCNA was decreased, indicating a decreased cell proliferation. Interestingly, Hes-1 was significantly reduced in colons of NoxO1-/- mice.

We conclude that loss of NoxO1 may impact on the proliferation of epithelial cells in the gut, mediated by the formation of superoxide anions.

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P-168

Intestine permeability of S-nitrosoglutathione as a potential nitric oxide donor via oral administration

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Keywords: S-nitrosoglutathione; nitric oxide donor; oral route; intestine permeability

Nitric oxide (NO) is a gaseous messenger which plays an important role in the vascular system homeostasis. Its origin includes both endogenous (catabolism of arginine catalyzed by endothelial NO synthase) and exogenous (intake of drinking water and foods) sources. The endogenous NO production decreases within ageing and cardiovascular diseases (CVDs) related to endothelial dysfunction, thus exogenous source of NO has to supply. Drugs actually used as NO donors have major drawbacks such as oxidative stress induction and tolerance phenomenon. Numerous studies highlight the potency of low molecular weight S-nitrosothiols as NO donors because they do not exhibit the previously cited side effects. However, very few reports have been focused on their bioavailability after oral administration which represents the most convenient route for the chronic treatment of CVDs.

We presently study the intestine permeability of S-nitrosoglutathione (GSNO) as a NO donor by using an ex vivo model: monolayer of differentiated Caco-2 cells. NO species (nitrite, nitrate and S-nitrosothiols) apparent permeabilities were measured with the help of a fluorogenic probe (i.e. 2,3-diaminonaphthalene) and compared with reference drugs commonly used to evaluate intestine permeability. Some perspectives to improve the oral bioavailability of GSNO are indicated.

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P-169

A study of the role of oxidative stress and low-grade inflammation in development of *Helicobacter pylori*-induced insulin resistance in asymptomatic sedentary young men

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Keywords: Helicobacter pylori; insulin resistance; oxidative stress; sedentary lifestyle; HOMA index

Low grade inflammation due to *Helicobacter pylori* infection is likely causative factor for insulin resistance in infected patients(1). The study analyzes effects of *H. pylori* contamination on low-grade inflammation and oxidative stress in asymptomatic sedentary males. We enrolled 30 apparently healthy asymptomatic young subjects (18 *H. pylori* negative and 12 positive). We studied whole blood glucose, glycated hemoglobin, insulin, C-peptide, cortisol, aldosterone, testosterone, thyroid stimulating hormone, C-reactive protein, interleukins 6 and 10, TNF-alpha, comet-assay, urine levels of 1,4-dihydroxynonane mercapturic acid (DHN-MA) and F2-isoprostanes. The results showed two-fold elevation of fasting insulin level and HOMA index in *H. pylori*–positive subjects ($p < 0.05$). Determination of inflammatory parameters, DHN-MA, F2-isoprostanes and monocyte DNA damage did not show significant differences between the groups. The early stage of *H. pylori*-triggered metabolic changes include development of insulin resistance in some of the *H. pylori*-positive subjects, however inflammatory and OS related changes are limited to the gut-liver axis with a little systemic impact.

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resistance but not inflammation or oxidative stress. Croat Med J 2016;57:141-9.

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P-170

Pro-oxidant tumor therapy in murine melanoma and pancreatic cancer

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Keywords: Reactive species; immunogenic cell death; macrophages

Despite recent advances in oncology, cancer is still among the most life threatening diseases. This requires furthering therapeutic approaches. Cold physical plasma is an ionized gas. Its biological active components are reactive oxygen and nitrogen species (ROS, RNS). Such species receive increasing recognition in pro-oxidant tumor therapies. They are evident in chemo and radiation therapy in that antitumor immunity is often observed. We tested the efficacy of plasma-derived oxidants in murine melanoma and pancreatic cancer. In vitro, B16 melanoma cells showed a decrease in metabolic activity, viability, cell growth, and cell migration. Cell death was confirmed in melanoma spheroid cultures. Plasma treatment upregulated MHC class I expression and calreticulin exposure. The latter is known to be important in conferring immunological cell death. Extended the findings to another cancer model, plasma-treated murine PDA6606 pancreatic cancer cells also gave decrease in cellular activity and viability. In co-culture, macrophage motility was enhanced. In a syngeneic pancreatic cancer mouse model, peritoneal tumor nodes were frequently lavaged with plasma-treated liquid. Treatment decreased lesion number and size, and increased cell apoptosis in tumors, macrophage infiltration, and animal survival. These results argue for role of pro-oxidants in tumor therapy.

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P-171

Silymarin and silybin in suppression of UVA-induced oxidative stress in normal human dermal fibroblasts

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Keywords: UVA radiation; flavonolignan; photoprotection; primary culture

A standardized extract from the seeds of *Silybum marianum*, silymarin (SM) and its main component silybin (SB) have been studied for 3 decades for their potential to reduce skin carcinogenesis induced chemically and by chronic exposure to UVB (280–320 nm) light as they possess several biological activities including anti-oxidant, anti-inflammatory, immunomodulatory and cellular regeneration. However, protection of SM/SB against UVA radiation (320–400 nm) has not been almost studied. Previously, we only demonstrated the regenerative effects of SM and SB on UVA-damaged HaCaT keratinocytes. Thus here we studied possible UVA (320–400 nm) photoprotective effects of SM and SB on primary human dermal fibroblasts (HDF).

HDF were isolated from superfluous skin of donors undergoing plastic surgery operations (Department of Plastic and Aesthetic Surgery). Cells were pre-treated with the SM/SB for 1 h and then were exposed to UVA radiation using a solar simulator. Our results demonstrated that HDF pre-treatment with SM and SB resulted in reduction in ROS production, depletion in intracellular GSH level, caspase-3 activity, DNA single strand breaks formation. Effectiveness of SM and SB was comparable.

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P-172

Two putative selenium binding proteins as modulators of *C. elegans* stress response and life span

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Keywords: Selenium; *C. elegans*; oxidative stress; daf-16; skn-1

Selenium-binding proteins do not contain selenium in the form of selenocysteine or selenomethionine but as inorganic selenium bound to the protein. The C. elegans genome encodes only one selenocysteine-containing selenoprotein, TrxR-1. However, at least two ORFs encode putative selenium-binding proteins, CeSELENBP1 and CeSELENBP2. These are 54% and 36% homologous to human selenium-binding protein-1 (SELENBP1), respectively, and both contain a cysteine residue hypothesized to bind selenite as in SELENBP1.

Considering the role of selenium in antioxidant defence, we hypothesized that CeSELENBP1 and CeSELENBP2 may modulate the response of C. elegans to oxidative stress. Unexpectedly, lifelong knock-down of either of the genes significantly increased life span and stress resistance to the redox cycler paraquat.

DAF-16 and SKN-1 are key players in the C. elegans stress response that are known to mediate many of the published life-extending effects of chemical compounds, phytochemicals and nutrition regimens. However, RNAi against CeSELENBP1 and CeSELENBP2 significantly increased life span of the mutants to an extent similar to that found in wild type worms exposed to RNAi targeting either of the putative selenium binding proteins. This suggests that neither DAF-16 nor SKN-1 are involved in life span extension elicited by knock-down of CeSELENBP1 and CeSELENBP2.

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P-173

Anti-aging activity of silymarin and its components

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Keywords: Flavonolignan; Collagenase; Elastase; Tyrosinase; Carbonyl proteins

(Photo)aging is a term used for functional and aesthetic changes in skin after chronic exposure to UV radiation causing oxidative modification of proteins and modulation of extracellular matrix degrading enzymes and tyrosinase. Silymarin (SM), a seed extract from *Silybum marianum* and its components (silybin, dehydrosilybin, silydianin, isosilybin and silychristin) seem to be suitable candidates to modulate (photo)aging due to their antioxidant properties.

To test their effects the isolated enzymes (collagenase from *Clostridium histolyticum*, human leukocytes elastase, bovine testes hyaluronidase and mushroom tyrosinase) were used. After incubation of enzymes with compounds, the reactions were started with specific substrates and colour/fluorescence changes were monitored at specific wavelength. After 1 h treatment with studied compounds dermal fibroblasts were irradiated with UVA and the carbonyl protein level was estimated by using fluorescein-5-thiosemicarbazide.

SM and its components have anti-collagenase and anti-elastase effect, decrease carbonyl protein level and activate tyrosinase, but no effect on hyaluronidase activity was found. SM and its components can be recommended in skin protection and prevention of (photo)aging.

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P-174

Placental and mitochondrial Q10 content after CoQ10 supplementation during pregnancy

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Keywords: Pregnancy; preeclampsia; Q10 supplementation; mitochondria; placenta

Pre-eclampsia is a common disorder of human pregnancy (about 7% of all pregnancies) in which the normal hemodynamic response to pregnancy is compromised. In 2009, we demonstrated that supplementation with CoQ10 reduces significantly the risk of developing pre-eclampsia in women at risk for the condition. From these women, placental and mitochondrial Q10 levels, in women receiving either placebo or 200 mg CoQ10 daily were measured. Results showed that before supplementation, at week 20 of pregnancy, plasma CoQ10 levels showed no difference between the control (0.134 ± 0.05 umol/l) and supplemented (0.139 ± 0.06 umol/l) groups. Interestingly, at delivery, placental tissue showed no differences in the placebo group between women with normal pregnancy and those with preeclampsia; while in the Q10 group, women with preeclampsia showed significantly higher placental levels (0.31 ± 0.20 ug/mg of protein) compared to normal pregnant women (0.18 ± 0.08 ug/mg of protein; $p=0.005$). However, mitochondrial levels of Q10 in placenta from pregnant women with preeclampsia receiving placebo did not show differences compared to those receiving CoQ supplementation (0.82 ± 0.41 vs. 0.77 ± 0.42 ug/mg of protein). These results suggest that in women with preeclampsia, although CoQ10 reduced preeclampsia and increased its content in placental tissue, it is not able to increase the mitocondrial levels of CoQ10.

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P-175

Punicalagin of Pomegranate and (-)-Epigallocatechin-3-gallate of Green Tea Rescue the Cell Viability and Attenuate Inflammatory Responses of Human Epidermal Keratinocytes Exposed to Airborne Particulate Matter PM10

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Keywords: Airborne particulates; PM10; keratinocytes; punicalagin; epigallocatechin-3-gallate; inflammation

The skin is directly exposed to atmosphere and airborne particulate matter with a diameter of < 10 μm (PM10) is an important contributor to oxidative damage, inflammation, and premature aging of the skin. The purpose of this study is to test a hypothesis that PM10-induced skin cell damages might be attenuated by polyphenolic antioxidants. Cultured human epidermal keratinocytes were exposed to PM10 in the absence or presence of punicalagin and (-)-epigallocatechin-3-gallate (EGCG), which are natural polyphenols found in pomegranate and green tea, respectively. The treated cells were subjected to assays for viability and reactive oxygen species (ROS) production. Expression levels of NADPH oxidases (NOX), inflammatory cytokines and matrix metalloproteinase (MMP) 1 were determined by quantitative real time-polymerase chain reaction or Western blotting. Pretreatment of cells with punicalagin ($3 \sim 30$ μM) and (-)-EGCG ($3 \sim 10$ μM) at non-toxic concentrations rescued the viability of the cells exposed to PM10 (100 μg mL-1). They also suppressed ROS production, and the expression of the NOX1, NOX2, dual oxidase (DUOX)2, interferon (INF)-γ, tumor necrosis factor-α, interleukin (IL)-1β, IL-6, IL-8 and MMP-1 stimulated by PM10. This study suggests that polyphenolic antioxidants, such as punicalagin and (-)-EGCG, may be useful for the protection of the skin from the airborne particulates.

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P-176

Screening of Marine Plants for Phenolic Antioxidants Mitigating Oxidative Stress in Keratinocytes Exposed to Airborne Particulate Matter PM10

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Keywords: Marine plants; *Ecklonia cava*; airborne particulatate matter; PM10; phenolic antioxidants

Airborne particulate of less than 10 μm (PM10) has a major health impact to humans. PM10 consists of the broad range of toxic compounds, such as transition metals, endotoxins, and ultrafine components and can induce oxidative stress and inflammation. The purpose of this study was to identify phenolic antioxidants from marine plants, and to examine their protective effects in keratinocytes exposed to PM10. Of the extracts of 50 marine plants tested, the extracts of *Ecklonia cava* Kjellman showed the highest 'total phenol content' and the highest 'free radical-scavenging activities' against of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl radicals in vitro. Therefore, the extract of *Ecklonia cava* Kjellman was tested in the subsequent cell experiments. When HaCaT keratinocytes were exposed to PM10, reactive oxygen species production and lipid peroxidation increased, in a dose-dependent manner. The extract of *Ecklonia cava* Kjellman attenuated reactive oxygen species production and lipid peroxidation in HaCaT keratinocytes exposed to PM10 at its non-toxic concentrations. Of its solvent fractions, ethyl acetate fraction was the most effective at inhibiting cellular lipid peroxidation. This study suggests that marine plants are useful sources of polyphenolic antioxidants which mitigate oxidative stress due to airborne particulates.

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P-177

αB-crystallin activation in cardiac muscle by acute exercise mirrors the sHSP kinetic in oxidative skeletal muscle fibers: animal and cellular study

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Keywords: Redox; Oxidative Stress; Heat Shock Protein; Muscles; Exercise

Alpha-B-Crystallin (CRYAB), a Small Heat Shock Protein sensitive to oxidative stress, is implicated in various biological processes in many tissues. In cardiac muscle, CRYAB exerts a cardio protective role in ischemia-induced damage preventing apoptosis and necrosis.

We aimed to study αB-crystallin' response in mouse cardiac tissue (H), at different time of recovery from an acute aerobic exercise (1 hour), correlating its modulation with oxidative stress level.

We found that a single bout exercise lead to a specific short-term increase of phospho-αB-crystallin level (pCRYAB), without changes of its total expression. Further, the level of 4-hydroxynonenal, a marker of lipidic peroxidation, has shown a similar trend of pCRYAB enhancement. This may indicate that CRYAB in cardiac muscle is activated and it has a putative role in oxidative stress during exercise. These results are supported by our previous data obtained in mouse skeletal tissues (i.e. gastrocnemius, soleus) and in H_2O_2-treated C2C12 myotubes. In particular, we observed not only a fiber-dependent response of pCRYAB, but also its translocation into myofibrillar compartment.

Experiments are in progress to further investigate on CRYAB role during exercise and its interactions with cytoskeletal structures.

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P-178

Rosemary (*Rosmarinus officinalis L*) extract increases ROS and modulates Nrf2 pathway in human colon cancer cell lines

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Keywords: Rosemary; ROS; pro-oxidant; Nrf2; colon cancer

Carnosic acid, carnosol and betulinic acid are the main compounds present in rosemaryextract (RE) and have shown antiproliferative effects on various tumor cell lines. The objective of this work was to study the anticancer effects of rosemary extract on different human colon cancer cell lines (HGUE-C-1, HT-29 and SW480).

For this purpose, intracellular free radical generation and modulation of nuclear factor erythroid 2 [NF-E2]-related factor 2 (Nrf2) pathway was explored. Cells were treated with RE (20–40 μg/mL) for 24 h at 37 °C and cell viability was determined by MTT assay. Reactive oxygen species (ROS) production was measured with DCF-DA. In addition, cells were transfected with Nrf2-targeting siRNA to determine the potential role of this transcription factor.

The inhibition of cell proliferation was correlated to an increase of intracellular ROS content by RE. Furthermore, Nrf2 silencing in siRNA experiments decreased cell viability in all cell lines, then the molecular mechanism seems to involve activation Nrf2/ARE pathway as a response for cell survival at least in this cell line.

In conclusion, the antiproliferative effects of a rosemary extract obtained by CO2-supercritical fluid extraction may be related to their pro-oxidative capability by increasing the intracellular generation of ROS, which leads to the activation of Nrf2 as a response for cancer cell survival.

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P-179

Antioxidant activity and intestinal absorption of apigenin and its potassium salt derivative in Caco-2 cell monolayers

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Keywords: Apigenin; TEAC; FRAP; antioxidant activity; intestinal permeability

Apigenin is a common bioactive flavonoid found in a variety of fruits, plants and vegetables. Many pharmacological activities of apigenin have been identified, but its low water solubility limits clinical evidence on its oral bioavailability. This study estimated the antioxidant activity of apigenin and its potassium salt derivative (apigenin-K) and the intestinal permeability of both compounds in Caco-2 cell monolayers.

The antioxidant activity was evaluated with different methods: trolox equivalent antioxidant capacity (TEAC) and ferric reducing-antioxidant power (FRAP). Intestinal permeability coefficient was estimated in Caco-2 cell monolayer model with the addition of apigenin or apigenin-K to the apical (AP) or basolateral (BL) site.

Apigenin exhibited a higher antioxidant activity than apigenin-K. Intestinal absorption evaluation corroborated the low permeability of both apigenin and apigenin-K.

These results indicate that apigenin and apigenin-K may be considered as antioxidant ingredients for oral administration. Their low absorption estimation suggests that encapsulation may be necessary to enhance apigenin bioavailability for oral applications.

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P-180

Associations between oxidative stress markers, antioxidants and age in a large cross-sectional study

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Keywords: Oxidative stress; antioxidants; cross-sectional study; aging

From animal and cell culture studies it is known that oxidative stress is involved in the aging process. However, there are only few large human studies in healthy individuals related to this issue and these focused mostly on single biomarkers. Since it is recommended to measure a set of different biomarkers to evaluate the oxidative stress status of an individual we analyzed protein carbonyls, 3-nitrotyrosine, malondialdehyde and the antioxidants ascorbic acid, uric acid, α-tocopherol, lycopene, glutathione and cysteine in 1,559 participants (aged 55–75 years) of the European multicenter study MARK-AGE. Our data suggest that older participants have elevated oxidative stress and reduced antioxidants. We observed differences in protein carbonyls, malondialdehyde,

3-nitrotyrosine, α-tocopherol, cysteine, and glutathione between the three study groups after adjustment for relevant co-factors which emphasize the influence of lifestyle rather than genetics. Protein carbonyls, 3-nitrotyrosine, cysteine and uric acid were positively associated with age while lycopene was inversely associated with age. We conclude that lifestyle may be a more important contributor to redox biomarkers than genetics and should be studied in the future with special emphasis on the diet since this may also offer a promising approach for intervention studies.

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P-182

Genetic Polymorphism of methylenetetrahydrofolate reductase (MTHFR) C 677 T and A1298C gene and risk of head and neck squamous cell carcinoma-A qualitative analysis

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Keywords: MHTFR Polymorphism; gentics of HNSCC; risk factors of HNSCC; methylation pathway; folate metabolism

Background and Aim: Functional polymorphisms in genes encoding enzymes involved in folate metabolism might modulate head and neck squamous cell carcinoma (HNSCC) risk of which Methylenetetrahydrofolate reductase gene (MTHFR) C677T and A1298C polymorphism is important due to changes in folate levels that can induce disorders in methylation pathway, which results in carcinogenesis.
Materials & Methods: 25 samples of genomic DNA extracted from whole blood of both HNSCC cases and 25 age matched controls taken & subjected to PCR amplification using specific primers followed by restriction fragment length polymorphism for detection of MTHFR C677T & 1298 C. Interactions with clinical histopathological parameters were also evaluated.
Results: 1298 AC/CC genotypes are associated with age ≥ 49 years, tobacco and alcohol ($P < 0.05$). Regarding clinical histopathological parameters, A1298C polymorphism more frequent in patients with oral cavity as primary site ($P < 0.05$). No association found between MTHFR C677T polymorphism and HNSCC($p = 0.50$).
Conclusion: Variables like age ≥ 49 years, male gender, tobacco and alcohol were associated with MTHFR 1298AC or CC genotypes, indicating that individuals with these variables have higher risk. However further longitudinal studies are required to confirm these as established risk factors. No association between MTHFR C677T polymorphism and HNSCC was possible in this study.

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P-183

Differentiation modifies Bach1 dependent regulation of HO-1 expression and increases sensitivity to oxidative stress in neuroblastoma cells

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Keywords: Differentiation; HO-1; oxidative stress; Bach1; Nrf2

Neuronal adaptation to oxidative stress is crucial to prevent degenerative diseases. The role of the Nrf2/HO-1 system in cell response to hydrogen peroxide (H_2O_2) has been investigated using SH-SY5Y neuroblastoma cells as undifferentiated or after differentiation with all-trans retinoic acid (ATRA). We showed that undifferentiated cells resisted to oxidative stimulus and up-regulated HO-1 which was responsible for their survival, since its silencing decreased viability in cells exposed to H_2O_2. On the contrary, in ATRA-treated cells the viability decreased in response to increasing concentration of H_2O_2 and HO-1 was not induced. However, bilirubin supplementation restores cell viability, underlining the role of HO-1-derived bilirubin in cell resistance to oxidative stress. Investigating the mechanisms of HO-1 induction, we showed that in undifferentiated cells the nuclear level of Bach1, repressor of HO-1 transcription, decreased as well as its binding to the promoter of HO-1. In the same condition, Nrf2 binding to the same DNA region increased. Yet, in differentiated cells Bach1 nuclear level was not modified by the exposure to H_2O_2 as well as its binding to HO-1 promoter and, as a consequence, the binding of Nrf2 to HO-1 promoter was not modified by H_2O_2. In conclusion, our findings highlight the role of Bach1/HO-1/bilirubin in neuronal response to oxidative stress.

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P-184

Functional state and morphology of mesenchymal stem cells after oxidative stress

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Keywords: MSCs; ROS; oxidative stress

Mesenchymal stem cells (MSCs) are an important member of the stem cell family and can be found in most postnatal organs and tissues. Due to multilineage differentiation potential, broad range of bioactive molecules secretion and other properties, these cells are being studied for their potential use in different modes of therapy. But a number of reports point to oxidative stress have been hypothesized to lead to loss of transplanted MSCs from the

injured sites. Therefore, the understanding of the contributions of oxidative stress to MSC biology is required. The purpose of this study was to investigate the functional state of adipose-derived MSCs after oxidative stress.

The cells treated with hydrogen peroxide (50, 200, 500 mkM) for 6 h were washed and further incubated in fresh medium for 4 days. We found cell viability was less 5% at 500 mkM H_2O_2 and exclude this concentration from analysis. In other samples a part of living cells was high (more 85%), but cell grow rate was significantly decreased after oxidative stress. Also the treated MSCs were enlarged in size and displayed more granular cytoplasm. Intracellular reactive oxygen species (ROS) level, activity of lysosome and mitochondrion compartments were increased.

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P-185

Low-fluence photodynamic treatment modifies immunogenicity of mesenchymal stromal cells

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Keywords: mesenchymal stromal cells; photodynamic treatment; immunogenicity; peripheral blood mononuclear cells

Mesenchymal stromal cells (MSCs) represent one of the most important component of tissue microenvironment. They can be characterized by high proliferative and regenerative potential as well as low immunogenicity that serves MSC application for regenerative therapy. The same properties make them the key players in the pathogenesis of different diseases. Most of these physiological and pathological processes involving MSCs are accompanied by reactive oxygen species (ROS). However, the ROS effects on MSC functional activity are still poorly investigated. In present study we demonstrated that ROS can modify MSC immunogenicity.

MSCs were obtained from stromal vascular fraction of human adipose tissue. Intracellular ROS were induced by low fluence (0.25 J/cm2) photodynamic treatment (PDT) with Al-phthalocynine.

We demonstrated that low-fluence PDT significantly increase MSC immunogenicity as manifested by decreased MSC viability after co-culture with human peripheral blood mononuclear cells PBMNs. It was accompanied by enhanced FasL expression in MSCs. In addition, it was found out that intracellular ROS induction resulted in dramatic decrease of CCL2 and increase of IL8 but not IL6 production by MSCs. Low-fluence PDT did not affect Fas, MICA/B, Nectin-2, HLA-ABC expression in MSC as well as co-cultivation with PDT-MSC did not influence the expression of DNAM-1, NKG2D and CD69 in NK.

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P-186

Mechanisms involved in chronic effects of doxorubicin in rat hearts

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Keywords: doxorubicin; heart; cell signaling; oxidative stress; quercetin

Doxorubicin (DOX) is chemotherapeutic agent frequently used in treatment of many types of malignancies. Limitation of its use is toxicity on several organs. The aim of study was to investigate mechanisms involved in chronic effects of DOX on rat hearts and to search for effects of flavonoid quercetin (QCT) on DOX-induced changes. In the study, male Wistar rats were used. DOX was administered by i.p. injection of seven doses (cumulative dose 15 mg. kg-1). QCT (20 mg/kg/day) was administered 3 weeks during and 3 weeks after DOX treatment. DOX induced subcellular alterations of cardiomyocytes and disorganizations of cardiac extracellular space. Effects of DOX were associated with MMP-2 activation, decreased SOD activity, increased superoxide content, caspase-3 activation, and iNOS induction. QCT prevented the deleterious effects of DOX on ultrastructure of the tissue of left ventricle and reversed the DOX-induced effects on MMP-2 activation. QCT application also prevented effects of DOX on apoptosis induction and SOD inhibition. Moreover, QCT increased myocardial resistance to ischemia/reperfusion injury in DOX-treated rat hearts. These effects of QCT were linked to Akt kinase/GSK-3ß/beta-catenin signaling and associated with connexin-43 up-regulation.

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P-187

Prevention of UVB-induced Oxidative Stress and DNA damage in human keratinocytes by citrus and olive formulations

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Keywords: Citrus; olive; rosemary; UVB radiation; photoprotection

Solar ultraviolet (UV) radiation, especially UVB (290 nm-320 nm) component, causes DNA damage, pyrimidine dimmers, 8-hydroxy-2´-deoxyguanosine (8-OHdG), p53 induction, protein oxidation and generation of reactive oxygen species (ROS). Some polyphenols show the ability to protect the skin from the adverse effects of UVB radiation, including the risk of skin cancers. This study evaluated the protective effects of formulations containing citrus, rosemary and olive polyphenols (F1 and F2) against UVB-induced damage in human keratinocytes. F1 contained citrus, rosemary diterpenes and olive polyphenols and F2 citrus and olive flavones.

The antioxidant capacity was determined using the ROS-sensitive dye 2',7'-dichlorofluorescein diacetate (DCF-DA). Late apoptosis, mitochondrial membrane potential (MMP) and H2A.X histone phosphorylation were measured using Muse Cell Analyzer. The treatment with both formulations suppressed UVB-induced ROS production, and decreased late apoptosis, cell depolarization and DNA damage by H2A.X assay.

In conclusion, these results suggest that the compounds present in both formulations might contribute to the observed protective effect. Formulations may be considered as candidates for oral or topical photoprotection and their mechanism of action may deserve further attention.

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P-188

Antioxidant activity and photoprotective effect of citrus and olive formulations on UVB-induced damage in human keratinocytes

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Keywords: Citrus; olive; rosemary; UVB radiation; antioxidant activity

UV radiation, absorbed by the epidermis, is the major cause of a variety of cutaneous disorders including photoageing and skin cancers. In recent years, an increase in the use of botanicals with antioxidant and anti-inflammatory properties as skin photoprotective agents is emerging. This study evaluated the antioxidant activity and the protective effect of citrus and olive formulations (F1 and F2), against UVB-induced damage in human keratinocytes. F1 contained citrus, rosemary diterpenes and olive polyphenols and F2 citrus and olive flavones.

Total phenolic content was determined with Folin-Ciocalteu. The antioxidant activity was evaluated with different methods: trolox equivalent antioxidant capacity (TEAC), ferric reducing-antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays. Photoprotection study was performed determining the viability of HaCaT cells exposed to UVB through MTT assay.

The F1 showed higher phenolic content and antioxidant capacity. Both formulations also exhibited high protective effects on cell survival upon UVB radiation, finding a higher effect for F1 when both were used at same concentration.

These results indicate that F1 may be considered as a better ingredient for oral or topical photoprotection. This suggests that, probably not only citrus and olive polyphenols but also rosemary compounds act in the protective action.

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P-189

PPAR gamma activation can improve aberrant redox regulation in hypertension

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Keywords: PPAR gamma; Nrf2; redox regulation; hypertension

Dysregulation of redox- sensitive signaling plays an important role in develompment of hypertension. PPAR gamma belongs to nuclear receptors and is a potent nutritional sensor and redox regulator. In our study we focused on the role of PPAR gamma activation of RAS/ROS regulation, and antioxidant response in spontaneously hypertensive rats (SHR). Treatment was performed by gavage with PPAR gamma agonist pioglitazone (PIO - 10 mg/kg/day, 10 days). Treatment with PPAR gamma agonist PIO in influenced blood pressure, redox- sensitive responses, lipid profile and homocystein level in age- dependent manner. The improvement parameters in hypertension and redox regulation have been observed in young animals, but not in adult SHR rats. PIO treatment influenced redox regulation (gene expression of Nrf2 and SOD isoforms) correlated with SOD a CAT activity and NO bioavailibility. PPAR gamma activation has been corelated with downregulation of and AT1R - RAS axis and „up-regulation" of AT2R and Mas receptor. This most sensitive tissue responses in RAS/ROS signaling and NO level have been found primary in kidney of young hypertensive rats.

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P-190

The interplay between nuclear and cytoplasmic distribution of methionine cycle enzymes in acute liver injury

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Keywords: oxidative stress; GSH/GSSG; methionine cycle enzymes; nuclear translocation

The hepatic methionine cycle comprises specific isoenzymes and proteins classically ascribed to the cytoplasm, with metabolites being putatively transported to other subcellular locations as required. This hypothesis was challenged by the identification of several enzymes in the nuclear compartment; hence new questions emerged regarding their nuclear role and the putative link between anomalous subcellular localization and disease. Using models of acute liver failure (D-galactosamine and acetaminophen intoxications) we have identified an opposite regulation for nuclear and cytoplasmic protein levels. Expression of liver-specific genes (Mat1a, Bhmt, Gnmt) decreases in hepatic intoxications, whereas the mRNA levels for the other genes of the cycle increase. Total AdoMet levels decrease in hepatic intoxications, while nuclear accumulation of MATα1 (encoded by Mat1a), BHMT, GNMT and SAHH is observed. These nucleo/cytoplasmic changes of localization correlate with specific epigenetic modifications and/or protein N-homocysteinylation. Subcellular distribution is controlled by the ratio of glutathione species, according to the data obtained using inhibitors or precusors of glutathione synthesis alone or in combination. Altogether, our results support the existence of a nuclear branch of the methionine cycle in order to provide or eliminate metabolites close to the nuclear machinery.

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P-191

Grape pomace extract, rich in polyphenols, stimulate the emergence of brown-like cells in white adipose tissue in spontaneously hypertensive rats and in 3T3-L1 adipocytes

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Keywords: Grape pomace extract; polyphenols; brown like-adipocytes; mitochondrial biogenesis; high-fat diet

This study investigated the effect of grape pomace extract (GPE) on brown-like induction in epididymal white adipose tissue (eWAT) from spontaneously hypertensive rat (SHR) receiving a high-fat diet (HF) and in 3T3-L1 adipocytes. HF increased eWAT adiposity and diameter in Wistar Kyoto (WKY) but not in SHR, while GPE (300 mg/kg/day) supplementation reduced adipocyte diameter in WKY and attenuated adipose inflammation in both strain. In contrast, GPE increase protein levels involved in the "browning" such as PGC-1a, PPARγ and UCP-1 from SHR eWAT. In differentiated adipocytes from SHR eWAT, UCP-1 protein levels significantly decreased in cells pre-treated with SB203580 (p38 inhibitor) or U0126 (ERK inhibitor) but not in cells treated with GPE alone suggesting that GPE enhance UCP-1 in part via p38 and ERK. In 3T3-L1 adipocytes treated with palmitate the pretreatment with GPE (30 mM) enhances the activation of the downstream of β-adrenergic signaling cascade (PKA, AMPK, p38, ERK) in part involved in mitochondrial biogenesis (UCP-1, PPARγ and PGC-1α) and in fatty acid oxidation (ATGL). These effects were comparable when epicatechin or quercetin (1 μM), the main flavonoids found in GPE, was used. Overall, GPE could induce brown-like adipocytes in white adipocytes supporting the utilization of winemaking residues, rich in polyphenols, to treat obesity or related diseases.

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P-192

Comparison of anti-inflammatory and antioxidant activities of an anthocyanin-rich fraction from Portuguese blueberries (*Vaccinium corymbosum L.*) with 5-aminosalicylic acid, in a co-culture model of inflammatory bowel disease

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Keywords: Inflammatory bowel disease; Anthocyanins; Anti-inflammatory; Anti-oxidant; Co-cultures

The overproduction of reactive oxygen species (ROS) and pro-inflammatory mediators is closely related with inflammatory diseases, such as inflammatory bowel disease (IBD). Despite the advances in therapeutics, new and safer strategies are still required. In this context, anthocyanins, highly available in human diet and recognized as health-promoting agents, have been a focus of interest.

Thus, the aim of this work was to study the anti-inflammatory potential of a characterized anthocyanin-rich fraction from Portuguese blueberries, as compared with 5-aminosalicylic acid

(ASA), a standard anti-inflammatory drug in IBD, by using a co-culture system of intestinal epithelial Caco-2 cells and RAW 264.7 macrophage cells, as a model of colitis.

The compounds were pre-incubated in the apical side of a Transwell plate containing a differentiated and polarized Caco-2 cell monolayer, for 3 h, followed by addition of 1 μg/mL lipopolysaccharide to the basolateral side to stimulate the RAW 264.7 cells. After 24 h, the inflammatory process was evaluated in terms of nitric oxide, IL-6 and IL-1β contents in culture supernatants, as well as of intracellular ROS. A monoculture of RAW 264.7 was used for comparison.

Our data showed that the production of pro-inflammatory mediators and ROS in both compartments was significantly decreased by anthocyanins, in a higher extent than by 5-ASA.

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P-193

Redox control of the 20 S proteasome gating: Implications on the chronological life span of yeast cells

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Keywords: proteasome; S-glutathionylation; life span; redox modification

Two Cys residues located in the α5-subunit of the yeast 20 S proteasome (20SPT) are post-translationally modified by S-glutathionylation. Such modification implies on the opening of the 20SPT gate when compared to the DTT-reduced purified preparations. The site-specific mutation of the highly conserved (yeast to human) α5-Cys76 residue induced either decreased cell viability or life span when compared to the wild type strain. The α5-C76S-mutated strain presented decreased resistance to oxidative stress without any alteration on major redox parameters. The closed gate conformation of the 20SPT pool in that short-lived strain represents 70% of total 20SPT pool against 35% in the wild type counterpart. Mass spectrometry analysis of the α5-subunit in the α5-C76S-mutated strain showed S-glutathionylation of the remaining α5-C221 residue suggesting that redox modification of the conserved α5-C76, but not of the α5-C221, is the one involved in the gate opening. Circular dichroism analyses of recombinant α5-WT and α5-C76S subunits showed similar pattern between them regarding their secondary and tertiary structures. The differences between the wild type and short-lived strains regarding prevalence of 20SPT gate conformation, clearly demonstrate that the 20SPT opening is relevant to maintain cellular homeostasis and life span.

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P-194

Induction of mitochondrial reactive oxygen species by a novel STAT3 inhibitor triggers apoptosis in human glioblastoma cells

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Keywords: mitochondria; STAT3; apoptosis; glioblastoma

Glioblastoma multiforme (GBM) is the most malignant of all the gliomas, with no effective treatments. Signal Transducer and Activator of Transcription 3 (STAT3) plays a key role in GBM tumor growth, apoptosis inhibition, and chemotherapeutic drug resistance. Thus, targeting STAT3 in GBM appears to be promising. Our previous studies identified phospho-valproic acid (P-V), as a new STAT3 inhibitor that possesses significant anticancer efficacy in GBM models. However P-V's exact mechanism of action remains elusive. The objective of this work was to determine the mechanism on how P-V induced apoptosis in human GBM cells, particularly focusing on the effect of P-V on mitochondria. Using human GBM cell lines (LN18, U87, and U118), we explored the effects of P-V on apoptosis, mitochondrial reactive oxygen species and mitochondrial membrane potential. P-V reduced p-STAT3 levels in GBM cells, leading to a lower levels of STAT3 in the mitochondria. P-V treatment selectively increased the levels of superoxide anion in mitochondria, led to a collapse of the mitochondrial membrane potential and triggered apoptosis. P-V treatment caused GBM apoptotic cell death within 24 h, as evidenced by PARP cleavage and caspase 9 activation. In conclusion, our results indicate that P-V targets the mitochondria and induces mitochondrial-dependent apoptosis.

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P-195

Nuclear factor (erythroid-derived-2)-like 2 (Nrf2) signalling is involved in transdifferentiation of hepatocyte-like cells

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Keywords: Nrf2; liver progenitor cells; redox signaling

The identification of key regulators in hepatic progenitor cells differentiation is determinant for organ regeneration and may improve stem cell transplantation for end-stage liver disease. Mesenchymal, embryonic and pluripotent stem cells have been investigated as potential sources for hepatic differentiation, but their unstable function and atypical morphology limit the usefulness for cell treatment. The human bipotent liver progenitor cell line HepaRG is applied for toxicity screening, viral hepatitis research, hepatocyte differentiation and transplantation testing.

Since redox-dependent signaling molecules are involved in the regulation of stem cell self-renewal and differentiation, we studied

the role of Nrf2 – the main transcription factor involved in the oxidative stress response – in HepaRG transdifferentiation. Nrf2 was upregulated in undifferentiated HepaRG, which exhibited higher free radical production as compared to transdifferentiated cells. Pharmacological and genetic inhibition of Nrf2 led to morphological, phenotypical and functional patterns characteristic of transdifferentiated elements.

To conclude, we show a determinant role of Nrf2 in the process of HepaRG transdifferentiation, suggesting that this redox-dependent transcription factor represents a potential target to regulate the commitment of undifferentiated hepatic progenitor cells into specific lineages.

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P-196

The intestinal/liver axis in the capacity of anthocyanidins to mitigate high fat diet-induced insulin resistance

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Keywords: High fat diet; anthocyanidins; insulin resistance; steatosis; intestinal permeabilization

An increased permeability of the intestinal barrier is proposed as one underlying factor of obesity-associated pathologies. Consumption of high fat diet (HFD) is associated with intestinal permeabilization and increased paracellular transport of endotoxins, which can promote steatosis and insulin resistance. We investigated the capacity of dietary anthocyanidins (AC) to mitigate steatosis and improve insulin sensitivity in HFD-fed mice. Male C57BL/6 J mice were fed for 14 w control (C) or high fat (HF) diet, with or without supplementation with 2, 20, or 40 mg/kg BW of a blend of AC-rich plant extracts (ACE). The consumption of HFD caused overweight and insulin resistance, which were mitigated by ACE consumption. Concurrently, ACE prevented HFD-induced intestinal permeabilization, altered expression of ileum tight junction proteins (decreased ZO-1 and occludin) and endotoxemia. AC protection of barrier permeability is in part due to the inhibition of HFD-induced ileum ERK1/2 and AMPK activation, increased GLP-2 levels, and mitigation of dysbiosis. Consistently, the highest AC dose prevented steatosis and improved blood and liver inflammation and oxidative stress. Findings suggest that ACE supplementation could be an important strategy to mitigate obesity and Western style diets-associated insulin resistance in part through the preservation of intestinal barrier integrity.

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P-197

Role of mitochondrial NADP+-dependent isocitrate dehydrogenase (IDH2) on cisplatin-induced nephrotoxicity

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Keywords: IDH2; cisplatin; nephrotoxicity

Mitochondrial NADP+-dependent isocitrate dehydrogenase (IDH2) is a major producer of NADPH, which is a critical factor for the maintenance of intracellular redox balance, in the mitochondria. Nephrotoxicity of cisplatin, an anticancer drug, limits the use of cisplatin by impairing glutathione (GSH)-mediated antioxidant system in the cell. Here, we investigated the involvement of IDH2 in cisplatin-induced nephrotoxicity using IDH2 gene-deleted (IDH2-/-) mice. IDH2 deficiency aggravated renal functional and morphological impairments after cisplatin administration. Cisplatin decreased the level of NADPH which is required for the reduction of oxidized glutathione (GSSG) by glutathione reductase (GR) in the kidney. This reduction of NADPH levels were greater in the IDH2-/- mouse kidneys than wild type (IDH2+/+) mouse kidneys. Cisplatin-induced reactive oxygen species (ROS) production and oxidative stress was greater in the IDH2-/- mouse kidneys than IDH2+/+ mouse kidneys as evaluated by hydrogen peroxide level, lipid peroxidation, and GSSG/total GSH ratio. In addition, mitochondrial fragmentation and renal cell death after cisplatin injection were greater in the IDH2-/- than IDH2+/+ mouse kidneys. These results indicate IDH2 deficiency worsens cisplatin-induced nephrotoxicity by increasing mitochondrial and cellular oxidative stresses.

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P-198

Role of ATF3 in mediating lipid-induced stress signaling in brain microvascular endothelial cells

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Keywords: triglyceride-rich lipoproteins (TGRL); activating transcription factor (ATF3); vascular inflammation; vascular dementia (VaD)

Elevation of blood triglycerides, primarily triglyceride-rich lipoproteins (TGRL), is an independent risk factor for atherosclerotic cardiovascular disease and Vascular Dementia (VaD). The accumulating evidence indicates that the development of atherosclerosis and VaD are linked to vascular inflammation. Previous studies suggest a relationship of blood lipids and/or lipoproteins to vascular inflammation leading to dementia.

The objective of this study is to characterize human brain

microvascular endothelial cells (HBMVEC) responses to postprandial TGRL and determine the pathways that have potential to increase risk for vascular dementia.

To address this question, we treated HBMVEC with TGRL lipolysis products and then we used NGS-based RNA-Seq methods for transcriptional profiling experiments. Our data demonstrated upregulation of activating transcription factor 3 (ATF3)-dependent inflammatory pathways in HBMVEC. Additionally, system biology analysis exhibited putative lipid-related targets and corresponding isoforms of ATF3 that may be implicated in hypertriglyceridemia-induced vascular inflammation in brain. Furthermore, RNA-Seq analysis determined specific isoforms of key transcriptional regulator ATF3 and stress response signaling pathway in lipolysis products-mediated brain vascular inflammation which may lead to vascular dementia.

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P-199

Plasma 3-Methylhistidine as Marker for Muscle Status: Impact of Diet and Meat Intervention

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Keywords: aging; biomarker; muscle status; sarcopenia; diet

An increased loss of muscle mass during aging can lead to frailty and subsequently to disability which is associated with a higher mortality. An objective biomarker is needed for the identification of elevated muscle degradation and the diagnosis of frailty since current clinical tools may be biased. 3-Methylhistidine (3MH) is postulated as potential biomarker for muscle status but its plasma concentration is influenced by meat consumption. Therefore, a meat-free period is recommended before blood sampling; however this may be a limitation for clinical trials.

To better understand the influence of the diet and meat consumption on plasma concentrations of 3MH, 1-Methylhistidine (1MH) and creatinine we analyzed these biomarkers by UPLC-MS/MS in 35 young participants, divided into a vegetarian (n = 16) and an omnivore (n = 19) group. After baseline measurements, the latter group adhered to a meat-free diet for six days, with a defined bolus of meat on day four. Data on anthropometry, body composition, grip strength, nutrition and physical activity were collected and will be assessed together with biomarker results.

This study will help to objectively estimate the influence of nutrition on 3MH and test the suitability of 3MH as a biomarker of sarcopenia.

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P-200

Amaranth oil reduces accumulation of 4-hydroxynonenal-histidine adducts in gastric mucosa and improves heart rate variability in duodenal peptic ulcer patients undergoing *Helicobacter pylori* eradication

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Keywords: peptic ulcer; oxidative stress; *Helicobacter pylori*; Amaranth oil; 4-hydroxynonenal; heart rate variability

H. pylori-induced oxidative stress (OS) in gastric mucosa (GM) is associated with accumulation of the 4-hydroxynonenal-histidine adducts (HNE)[1] and represents a milieu for chronic gastritis, duodenal peptic ulcer (DPU), gastric cancer and some extragastric diseases [2]. We aimed to test if a 4 week supplementation of amaranth seed oil (AO) influences accumulation of HNE in GM and heart rate variability (HRV) in *H. pylori*-positive DPU patients. 75 DPU patients were randomly split into two groups with standard medical treatment (n = 39) and standard treatment plus 1 ml of AO per os, once a day, n = 36). Clinical data and HRV records, endoscopy with biopsies from GM were performed before and after the treatment. Supplementation of AO significantly reduced accumulation of HNE in GM and increased HRV in DPU patients ($p < 0.05$). Improvement of clinical symptoms and ulcer healing was observed in both groups. We conclude, that the reduction of OS in GM of DPU patients is related to modulation of redox homeostasis by AO.

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P-201

Role of redox-potential decreasing in the chronic isoproterenol-induced cardiac hypertrophy and its pharmacological correction

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This study investigated the efficacy of preventive role of NAD-containing cardiotrophic drug Adenocine® in chronic isopreterenol (ISO)-induced cardiac hypertrophy (CH) on tissue antioxidant status. On the 14 days of ISO-induced CH (10 mg/kg daily) all male chinchilla rabbits were randomly assigned into 3 groups: control - infusion of 0,9% NaCl; main I - receive 10 mg/kg nebivolol per os in combination with lisinopril (10 mg/kg) infusion (traditional treatment (TT)); and main II - receive intravenously 15 mg/kg of Adenocin dissolved in water for injection during 7 days. The increases of heart weight by 18,6% and heart-to-body weight ratio by 35,5% observed in CH were significantly suppressed in Adenocin-treated group by 15,8 and 31,5% and only by 6,4 and 9,7% respectively after TT. ISO-induced CH is associated with loss of NAD from 6,4 ± 0,3 to 5,1 ± 0,4mMol/mg. TT didn't cause the restoration NAD/NADH and NAD content in LV myocardium and decrease level of BNP. Adenocin treatment restored the normal level of NAD+/NADH, increased the activity of CuZn–SOD, Mn–SOD,superoxide anion production, catalase activities and decreased the content of MDA while the TT did not.
Conclusion: The results described the benficial cardioprotective effect of Adenocin on the the activities of both enzymatic and non-enzymatic antioxidants LV myocardium of ISO-induced CHF.

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P-202

Role of Redox Signaling in cardioinflammation and chronic heart failure

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Disruptions in the physiologic maintenance of the redox potential in cardiomyocytes with several biological processes, ultimately leading to cell death. A progressive rise of oxidative stress due to altered reduction–oxidation (redox) homeostasis appears to be one of the hallmarks of the processes that regulate gene transcription in cardiac hypertrophy and heart failure development. Reactive metabolites and NAD particularly, serve as signaling messengers for the evolution and perpetuation of the inflammatory process that is often associated with cell death, degeneration, alteration transcription factors in many human disease states and inflammatory-related injury. These proinflammatory factors act as potent stimuli in cardiac inflammation through up-regulation of diverse inflammatory genes, including matrix metalloproteinases (MMPs), cytosolic phospholipase A2 (cPLA2), cyclooxygenase-2 (COX-2), and adhesion molecules. In this review, we discuss the mechanisms underlying the intracellular signaling pathways, especially ROS, involved in the expression of several inflammatory proteins induced by proinflammatory factors in cardiomyocytes. The present review elaborates on the role of the redox-sensitive and oxygen-sensitive transcription factor NF-κB and tumor necrosis factor alpha in mediating cardiac hypertrophy and chronic heart failure.

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P-203

Quercetin derivatives from *Hibiscus sabdariffa* reduce lipid content and increase mitochondrial biogenesis in hypertrophied 3T3-L1 adipocytes

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Keywords: Quercetin; *Hibiscus sabdariffa*; obesity; mithocondrial biogenesis

There is evidence that *Hibiscus sabdariffa* (HS) extracts ameliorate metabolic disturbances related to obesity. Our recent studies postulate quercetin derivatives as candidates to be responsible for such effects.

The aim of this study was to determine the effect of several quercetin-related molecules (quercetin, quercetin-3-glucuronide and quercetin-3-glucoside) and other representative antioxidant compounds on triglyceride accumulation in hypertrophied 3T3-L1 adipocytes. In addition, we also studied the effect of these quercetin derivatives on mitochondrial mass and the possible mechanism involved by analyzing key metabolic targets in energy metabolism.

The results show that, among all the phenolic compounds from HS tested, quercetin derivatives had the highest capacity to reduce triglyceride accumulation in hypertrophied adipocytes. Furthermore, both HS complete extract and quercetin compounds increased the mitochondrial mass in adipocytes. Specially, quercetin-3-glucuronide seemed to be the most bioactive compound and increased AMPK and PPARα expression and decreased FASN expression.

In conclusion, these data suggest that quercetin derivatives from HS may reduce lipid content of hypertrophied adipocytes through the activation of AMPK and inhibition of FASN. Moreover, these flavonoids increased mitochondrial biogenesis which may be also mediated through the modulation of PPARα.

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P-204

Differences in antioxidant enzymes in tumour tissue and non-tumour adjacent tissue in colorectal cancer patients at different stages (III and IV)

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Keywords: CRC; oxidative stress; antioxidant enzimes; non-tumour adjacent tissue

Colorectal Cancer (CRC) is one of the leading causes of death by cancer worldwide; its high mortality rate is mainly attributable to metastases. Oxidative stress has been classically related to cancer due to the harmful effects of free radicals, both in their onset and in their evolution. Recent studies have revealed the influence of non-tumour adjacent tissue on the progression and metastasis of CRC. Based on this, we have studied the protein levels of antioxidant enzymes by western blot comparing samples of non-tumour adjacent tissue and tumour tissue of CRC patients in advanced stages (III and IV). In the results obtained, higher levels of antioxidant enzymes have been observed in the tumour tissue than in the non-tumour adjacent tissue, although these differences are shortened in stage IV, due to the fact that stage IV patients present a significant increase in levels of antioxidant enzymes in non-tumour adjacent tissue. In addition, higher levels of antioxidant enzymes have been observed in the non-tumour adjacent tissue of patients in IV stage than in III This study emphasizes the importance of non-tumour adjacent tissue as a prognostic factor in the evolution of CRC cancer patients.

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P-205

Effects of metabolites derived from *Hibiscus sabdariffa* on high glucose-induced oxidative stress and inflammation in hypertrophied 3T3-L1 adipocytes

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Keywords: quercetin; Hibiscus sabdariffa; oxidative stress; inflammation; hypertrophied adipocyte

Recent evidences indicate that polyphenols from *Hibiscus sabdariffa* L. (HS) may become an alternative against metabolic disturbances associated to obesity. Our findings strongly support that quercetin derivatives from HS are absorbed and metabolized in vivo and suggests that these metabolites are the major blood compounds accounting for these effects.

The purpose of this work was to determine the effect of several quercetin derivatives (quercetin, quercetin-3-glucuronide and quercetin-3-glucoside) compared with others of recognized capacity to reduce oxidative stress and inflammation in a high-glucose hypertrophied adipocyte model.

The results indicate that HS and quercetin derivatives reduce glucolipotoxicity-induced oxidative stress in 3T3-L1 adipocytes and INS832/13 β-cells. Furthermore, among all the polyphenolic compounds tested, quercetin derivatives were the more active compounds reducing ROS generation in hypertrophied 3T3-L1 adipocytes. These flavonoids also modulated pro-inflammatory adipokine secretion and reduced the expression of MCP-1 cytokine at transcriptional level in the hypertrophied cell model.

In conclusion, these data suggest that quercetin derivatives seem to be the main responsible compounds of the antioxidant and anti-inflammatory activities of HS extract.

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P-206

Quercetin supplementation decreases erythrocytes oxidative damage at resting and after an acute bout of eccentric exercise in humans

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Quercetin (Q) functions as antioxidant in vitro, but its effect have been minimally examined in combination with exercise in humans. The purpose of this investigation was to determine the effects of a diet supplemented with 1 g per day of Q for 2 weeks on the erythrocytes oxidative balance before and after an acute bout of eccentric exercise (EE). Fourteen volunteer males were randomly assigned, in a double-blind crossover design, to a placebo or experimental supplemented groups. Blood samples were taken before and after 2 weeks of supplementation under resting and post-exercise conditions. Erythrocytes glutathione (GSH, GSSG, GSH/GSSG), malonaldehyde (TBARs), enzymes antioxidant activities as well as time of hemolysis were evaluated in ex vivo.

Quercertin per se did not affect redox homeostasis but increased the time of hemolysis and decreased TBARs levels. Following the EE the Q group displayed a higher GSH/GSSG ratio and a less pronounced increase in TBARs, compared to placebo group. Moreover, we found that GPx enzyme activity were induced after EE only in Q group, while any significant modification of this parameter was detected in placebo group.

In conclusion, the Q supplementation may be used as a countermeasure against oxidative stress inducing, in erythrocytes, a cellular adaptation allowing subjects to better cope with the oxidative stress induced by an acute exercise.

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P-207

The NADPH oxidase Nox4 promotes endothelial differentiation from murine induced-pluripotent stem cells

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Keywords: Nox4; iPSCs; H3K27me3; JmjD3

Nox4 is the only constitutively active NADPH oxidase, producing H_2O_2. It is highly expressed in endothelial cells, where it plays an important role in differentiation. It is therefore hypothesized that Nox4 induces differentiation in endothelial cells and by deficiency preserves stemness.

Using Yamanaka factors, MEFs from wildtype and Nox4-/- mice were reprogramed into iPSCs and then differentiated into endothelial cells. In the course of differentiation, Nox4 expression increased in wildtype cells. Absence of Nox4 resulted in a prolonged expression of stem cell markers and in a diminished expression of endothelial markers in differentiated cells. On the functional level a lower tube formation and sprouting capacity of Nox4-deficient ECs was observed. Using an in vivo matrigel plug assay, a lower capacity of Nox4-/- iPSC-ECs integrated in a newly formed vascular network. As a potential mechanism we observed, increased H3K27me3 in Nox4-/-, which leads to decreased CD31 and VEGFR2 expression. Demethylation of this histone site is mediated by JmjD3, which is not differentially expressed in both cell strains. A BIAM switch assay revealed that JmjD3 was less oxidized in Nox4-/- than in WT cells. Therefore we conclude that Nox4 oxidizes and activates JmjD3. In conclusion Nox4 via an epigenetic modification promotes the differentiation of endothelial cells out of iPSCs.

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P-209

C-terminal domain of tetanus toxin changes the apoptosis- and autophagy-related protein levels following spinal cord injury in rat brain

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Keywords: spinal cord injury; cell death mechanisms; tetanus toxin

Traumatic spinal cord injury (SCI) can lead to post-traumatic inflammation, oxidative stress, motor neuron apoptosis, necrosis and autophagy of tissue. To promote and enhance recovery after SCI, recent development of devices and therapeutic interventions are needed. The aim of the present study was to investigate the possible role of C-terminal domain of tetanus toxin (Hc-TeTx) on cell death mechanisms including apoptosis and autophagy following SCI.

Thirty five adult rats were divided into five groups (n=7 each) as follows: control, sham, trauma (SCI), SCI+Hc-TeTx and SCI +methylprednisolone groups. The functional neurological deficits due to the SCI were assessed by behavioral analysis using the BBB open-field locomotor test. The alterations in pro-/anti-apoptotic and autophagy related-protein levels were measured by western blotting technique.

In our study, Hc-TeTx promotes locomotor recovery and motor neuron survival of SCI rats. Hc-TeTx decreased expression of bax, bad, bak, cleaved caspase-3, Ask1 and autophagy-related proteins including Atg5 and LC3II in brain. Our study provides an evidence that cell death mechanisms play critical roles in SCI and non-toxic peptides may exert protective effect and decrease cell death following SCI.

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P-210

Phenolic concentration and antioxidant activity of Mediterranean plants extracts

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Keywords: Antioxidant activity; phenolic content; plants extracts

Oxidative stress can produce skin damage, photoaging, inflamation or carcinogenesis related to its effect in cells. Phenolic compounds have been used in cosmeceuticals due to their antioxidant effect. Antioxidant effect of phenolic compounds is related to their bencenic ring and their functional groups.

This study evaluated the content of phenolic compounds with the Folin-Ciocalteu assay and the antioxidant activity with the trolox equivalent antioxidant capacity (TEAC) of 36 Mediterranean plants extracts. In addition, we studied the absorbance spectrum of the extracts between 220 and 820 nm.

The extracts showed different phenolic content, antioxidant capacity and absorbance spectrum. Some extracts showed a high phenolic content, over 10% GAE w/w and a high antioxidant capacity, over 100 mmol Trolox Eq/100 g extract.

These results indicate that the selected extracts have a good potential antioxidant activity and it would be interesting to continue the study of these extracts.

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P-211

Antioxidant activity and photoprotection of Mediterranean plants extracts

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Keywords: Antioxidant activity; mediterranean plants; photoprotection; UVB radiation

Ultraviolet radiation causes DNA damage, protein oxidation and generation of reactive oxygen species. This oxidative stress can produce skin damage, photoaging, inflammation or carcinogenesis. Some polyphenols, present in plant extracts, have shown antioxidant and photoprotective effects. This study evaluated the antioxidant effect of 9 Mediterranean plants extracts and their photoprotective effect against UVB radiation in human keratinocytes.

The antioxidant activity of the extracts was evaluated in vitro with two different methods: ferric reducing-antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays.

Photoprotection study was carried out determining the viability of HaCaT cells (keratinocytes) exposed to UVB through MTT assay. The cells were treated with 100-200 μg/mL of the different extracts.

In addition, the antioxidant activity was determined in the photoprotection assay, using the ROS-sensitive dye 2', 7'-dichlorofluorescein diacetate (DCF-DA).

The extracts showed high antioxidant capacity and a photoprotective effect against UVB-induced ROS production in HaCaT cells.

These results show that these Mediterranean extracts may be considered as a potential ingredient for photoprotection.

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P-212

The second order mechanism of copper induced lipid peroxidation

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In spite of the great interest and intensive research, the actual mechanism of copper-induced peroxidation is debatable. The accepted paradigm is that the two step mechanism (r.1,r.2) can be ruled out because reaction r.1 is "thermodynamically unfavorable", the redox potential of $LO2\cdot$ (range 0.77 V - 1.44 V) being higher than the redox potential of Cu(II) (0.16 V)

$LOOH + Cu2+ = LO2\cdot + H+ + Cu1+$ r.1

$LOOH + Cu1+ = LO\cdot + OH- + Cu2+$ r.2

The sum of reactions r.1 and r.2 gives r.3

$2LOOH = LO2\cdot + LO\cdot + H2O$ r.3

We think that the latter argument is not valid for systems far from equilibrium, particularly when the overall process (r.3) is thermodynamically favorable. Therefore the reaction may occur via the two steps mechanism. Alternatively, (i) additional reducing agents may reduce Cu(II) (ii) r.3 may involve intramolecular dismutation of an intermediate complex Cu(LOOH)x.

The different mechanisms can be expected to result in different overall reaction orders. Experimentally, the dependence of peroxidation on the concentration of hydroperoxides accords with second order kinetics which is indicative for intermediate complex formation. This is also consistent with continuous peroxidation of lipids within any given lipoprotein particle only if the particle contains at least two hydroperoxide molecules. It also accords with the effects of deuteration of LDL on its peroxidation.

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P-213

Mitochondrial Biogenesis, Autophagy and Mitochondrial UPR Co-operate in Modulating Ionizing Radiation Induced Cellular Damage

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Keywords: Autophagy; Ionizing radiation; Mitochondrial Stress; Oxidative Imbalance

This study aims to understand the important role of mitochondria in relaying ionizing radiation induced cellular insult. We exposed normal human dermal fibroblast cells to X-rays and investigated the changes in mitochondrial structure/function and related cellular parameters. We show that X-rays mediated oxidative outburst majorly contribute for mitochondrial fission, augmented mitochondrial DNA damage and lowered OXPHOS activity and ATP levels. We also demonstrate that irradiated cells exhibit a G2/M arrest with a modest percentage of cells undergoing cell death. In fact, our data show the activation of cytoprotective autophagy (observed by elevated levels of Beclin-1, LC3b, Atg5/16 L proteins) as a protecting mechanism against X-ray mediated cellular insults, blocking which (using chloroquine/3-methyladenine) led to activation of caspase-3 mediated apoptosis. Upregulation of mitochondrial biogenesis factors Nrf1/ PGC-1α was observed post irradiation. Apart from mitochondrial biogenesis, Nrf1 also forms an integral component of mitochondrial unfolded protein response. Hence, we infer that autophagy, mitochondrial biogenesis and mitochondrialUPR co-operate in order to maintain cellular integrity and impede radiation induced cell damage.

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P-214

Influence of drying temperature and harvesting season on phenolic content and antioxidant activity of olive (*Olea europaea*) leaf extracts

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Keywords: Polyphenols; Antioxidant activity; Olive leaf extract; Extraction

Oxidative stress occurs due to the overproduction of oxygen and nitrogen free radicals, which may cause damage to cells and tissues by their ability to react with different biomolecules. In this sense, reverting oxidative stress damage has been considered as a promising strategy in different diseases with an altered oxidative status.

Antioxidant compounds relevance has been increased because recent evidence regarding their role in human health. In fact, antioxidants present in dietary products, such as polyphenols, have been associated with preventive effects in various diseases such as cancer or inflammation.

In this study, up to 30 olive leaf extract have been obtained using different drying techniques and the influence on total polyphenolic content (measured using FOLIN assay) and antioxidant activity (using Trolox Equivalent Antioxidant Capacity and Ferric Reducing Ability of Plasma) has been tested.

The influence of drying temperature and harvesting season on the results are discussed and the best season and extracting procedure have been identified.

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P-215

Antioxidant enriched olive leaf extracts show antiproliferative activity in cellular models of breast cancer

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Keywords: Polyphenols; Antiproliferative; Olive leaf extract; Breast cancer

Most polyphenols present relevant antioxidant activity and modulate the activity of various kinases, enzymes that regulate energy metabolism, protein regulatory pathways proliferation and signaling. Therefore, it is logical to think that treatment with extracts rich in these compounds, such olive leaf extract, may be a good option to treat tumors.

Many studies support the antitumor activity of antioxidant naturally compounds in different models. Specifically, for breast cancer, interesting results have been obtained with olive extracts in previous results of our group.

In this study, olive leaves were collected in different seasons and dried using different conditions (25, 40, 60, 80, 100 or 120 °C)

before polyphenols were extracted. Then, total polyphenolic content and antiproliferative activity of extracts by cell viability assay with MTT reagent in two breast cancer cell models, JIMT-1 and MCF7, were carried out.

Results showed a correlation between the phenolic content of each extract and their antiproliferative capacity. In conclusion, the extracts with the highest phenolic content are also the ones with the highest antiproliferative activity.

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P-216

The antiproliferative effects of four marine invertebrate ectracts in colon cancer cells in relation to the modulation of oxidative stress-related pathways

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Keywords: ROS; Marine invertebrates; Anticancer compounds; Cytotoxicity

The goal of this study was to correlate the anti-proliferative activity of four marine invertebrate extracts on different colon cancer cell lines with free radical generation and modulation of oxidative stress-related pathways.

Three human colorectal carcinoma cell lines (HGUE, HT29 and SW480) were treated with four marine extracts (10-100 μg/mL) for 24 h at 37 °C. Cell viability (IC50 values)was determined by MTT. Reactive oxygen species (ROS) production was measured with DCF-DA. Mitochondrial functionality was tested by MitoTracker Red and MitoTracker Green fluorescent probes.

Marine extracts reduced cell viability of colon cancer cells starting at 10 μg/mL after 24 h the exposition. From 4 tested extracts, several exhibited pronounced cytotoxic effect, at least in one of the cell lines. The inhibition of cell proliferation was correlated to an increase of intracellular ROS content and a decrease of the mitochondrial membrane potential.

Our results suggest that the cytotoxic properties of the tested extracts observed in this preliminary study are due to the presence of pro-oxidant activity compounds which are responsible of the anti-proliferative effect in colon cancer cell lines. These extracts present pharmacological potential and further investigations to determine which are responsible compounds and the mechanism are currently ongoing.

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P-217

Apoptosis and necrosis induced by hydrogen peroxide and cyanate in human lymphocytes

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Keywords: apoptosis; lymphocytes; carbamylation; oxidative stress; carbonyls

Chronic kidney disease is associated with the modifications of biological molecules and macromolecules, which are caused by both processes – carbamylation and oxidation. In blood an equilibrium between urea, cyanate and isocyanate exists. Isocyanate and hydrogen peroxide lead to carbamylation and oxidation, respectively of peptides, proteins, lipids, enzymes and other biological molecules.

The apoptotic/necrotic changes in isolated human peripheral blood mononuclear cells (MNCs) subjected to hydrogen peroxide (H_2O_2), cyanate (NaOCN) and their combination were examined. The activities of caspases (3 and 9) and the levels of carbonyls and amino groups in proteins as well as genotoxicity were determined.

Treatment of MNCs with NaOCN, alone and in combination with H_2O_2, led to a decrease in the content of amine groups and an increase in the carbonyl level of MNCs in comparison with the control. We observed increases in the activities of caspases-3 and -9 in cells exposed to H_2O_2 and its combination with NaOCN. An increase of single- and double strand DNA breaks was found in cells treated with H_2O_2 and its combination with NaOCN in comparison to the control.

These findings suggest that sustained carbamylation and oxidative stress may be a major cause of lymphocyte apoptosis in chronic kidney disease patients, which depends on the mitochondrial pathway.

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P-218

Effects of NFKB1 gene polymorphism and frequently used drugs on the activation of nuclear factor κB (NF-κB)

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Keywords: Nuclear factor NF-κB; genetic polymorphism NFKB1 gene; drugs; age; gender

The NF-κB signaling pathway plays a central role as an important interface between oxidative stress and inflammation. NF-κB activation is of high interest in various human diseases and in response to bioactive food compounds.

The aim of this study was to elucidate effects of genetic background, age, gender and use of drugs on NF-κB activation.

In the BIOCLAIMS cohort (1310 subjects, 606 M, 704 F, 18-85 y) (i) activation of p65 and p50, the most abundant NF-κB heterodimers; (ii) -94 ATTG insertion/deletion polymorphism in the promotor region of the NFKB1 gene; (iii) use of drugs with effects on NF-κB activation (renin-angiotensin-aldosterone system inhibitors, calcium channel blockers, statins, oral antidiabetics, acetylic salicylic acid; on-drug subjects, n=403), were determined.

While p65 and p50 activation did not differ between genotypes in the entire study population (38.9% INS/INS, 46.3% INS/DEL and 14.8% DEL/DEL), there was a 14% higher p50 activation in DEL/DEL than in INS/DEL when on-drug subjects were excluded ($P < 0.05$). On-drug subjects showed lower activation of p65 (17%, $P < 0.001$) and p50 (9.7%, $P=0.004$) and an incremental decrease with cumulative numbers of used drugs. There were no age or gender effects.

In conclusion, DEL/DEL carriers and subjects on frequently used drugs exhibit alterations in NF-κB activation that need to be taken into account in human studies.

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P-219

Serum from patients affected by Alzheimer disease shows a paraoxonase-dependent pro-apoptotic effect on endothelial cells

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High-density lipoprotein (HDL) protects, among others, endothelial cells from oxidative challenge. The functionality of HDL closely depends on enzymes such as paraoxonase-1 (PON-1), PAF-AH and myeloperoxidase (MPO). Decreased activities of these enzymes have been found in association with higher risk of endothelial dysfunction (ED). Growing evidence suggests that this vascular abnormality is implicated in Alzheimer's disease (AD) development. We have recently shown that decreased PON-1 is an early event in AD development. However, the mechanism underlying the axis HDL dysfunction-ED-AD has not been proved yet. Since it is now clear that endothelial apoptosis is one of the hallmarks of ED, we sought to determine whether serum from AD patients affects this death process in human umbilical vein endothelial cells (HUVEC). Sera of 10 patients (n=4 controls, n=6 AD) were assessed for MPO, PON-1 and incubated for 48 h with HUVEC. Apoptosis levels were assessed with the Annexin V-FITC binding assay (flow cytofluorimetry). AD patients had 42 and 10% lower levels of PON-1, PAF-AH, respectively and 39% higher MPO than controls. Regarding in vitro assay, cells treated with AD serum presented a significant ($p < 0.05$) increase of (mostly early) apoptosis compared to controls. Our findings suggest that dysfunctional HDL-driven endothelial apoptosis may be implicated in AD pathogenesis.

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P-220

The intracellular metabolites of quercetin derivatives correlate with oxidative stress in hypertrophied 3T3-L1 adipocytes

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Keywords: Adipocyte; Quercetin; metabolites; ROS

Quercetin (Q) is one of the most abundant flavonoids in human dietary sources and has demonstrated that might ameliorate obesity-related pathologies. Quercetin-3-glucuronide (Q3GA) is

supposed to be the main metabolite in blood circulation, but the intracellular final effectors for its activity are still unknown. Here we investigate the uptake and metabolism of quercetin and its metabolite quercetin-3-glucuronide by hypertrophied 3T3-L1 adipocytes and their effect on oxidative stress.

Cytoplasmic fractions were obtained and quercetin metabolites were determined by liquid chromatography coupled to a time-of-flight mass detector with electrospray ionization (HPLC-DAD-ESI-TOF). Intracellular ROS generation was measured by a ROS-sensitive fluorescent probe.

Both Q and Q3GA were efficiently absorbed by hypertrophied adipocytes and metabolized to some extent to Q3GA and Q, respectively, but Q absorption was more efficient (1.92 ± 0.03 μg/μg protein) and faster than that of Q3GA (0.12 ± 0.0015 μg/μg protein), leading to a higher intracellular concentration of the aglycone.

The intracellular decrease of ROS in a hypertrophied adipocyte model treated with Q or Q3GA is correlated with the intracellular metabolite for the first time. Both compounds might be able to reach other intracellular targets, thus contributing to their bioactivity.

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P-221

Olive leaf polyphenols alleviate oxidative stress and improve mitochondrial function in high glucose-induced 3T3-L1 hypertrophic adipocytes

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Keywords: obesity; 3T3-L1; olive leaf extract; polyphenols

Recent scientific evidences show that polyphenols derived from the olive tree leaf (*Olea europaea*) may have applications in obesity-related pathologies through AMPK activation. We aimed to explore the effect of olive leaf extract (OLE) on lipid accumulation, oxidative stress and mitochondrial potential in adipocyte model.

In the present work, we used a glucotoxicity-induced insulin-resistant hypertrophic 3T3-L1-adipocytes model. Intracellular ROS generation and mitochondrial function was measured by ROS-sensitive fluorescent probes.

Our results indicate that OLE showed the capacity to reduce triglyceride accumulation in hypertrophic adipocytes. In addition, the extract exhibited a dose-dependent decrease of intracellular ROS generations as well as restored the mitochondrial membrane potential in adipocytes. Therefore, olive leaf polyphenols may become for the dietary intervention focused on the management of obesity-associated disturbances.

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P-222

Regulation of Nrf2 signaling during the antitumoral activity of Sorafenib in hepatoma cells

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Keywords: Hepatocarcinoma; Sorafenib; Nrf2; autophagy; oxidative stress; cell death

Introduction: Sorafenib is the accepted treatment for patients in advanced stage of hepatocarcinoma.
Goal: The study evaluated the regulation of the endoplasmic reticulum stress (ERS), Nrf2, oxidative/nitrosative stress, autophagy, cell proliferation and apoptosis by Sorafenib.
Methods: The study includes in vitro and in vivo experimental designs.
Results: Sorafenib induced ERS, Thr183JNK/JNK and transient autophagy that was followed by apoptosis, and reduction of cell proliferation. Sorafenib reduced dose-dependent nitric oxide, $O_2^{\bullet-}$ and H_2O_2 generation, and S-nitrosylated, carbonylated and tyrosine nitrated proteins. Sorafenib reduced S-nitrosylation of cell death receptors that shifted caspase-8- to caspase-3-related apoptosis. Sorafenib decreased luciferase activity in control, VEGF- and PDGF-stimulated ARE-Luc-transfected HepG2, and went down the expression of Nrf2-related redox genes. The reduction of Nrf2 signaling was related to increased Ser9GSK3β/Tyr216GSK3β. The in vivo study confirmed the antitumoral properties and molecular signaling of Sorafenib in xenograft mice model.
Conclusions: 1) Sorafenib induced ERS, autophagy and apoptosis in Sorafenib-treated HepG2. 2) Sorafenib reduced Nrf2-dependent signaling through regulation of GSK3β activity. 3) Nrf2 signaling and thioredoxin were involved in the reduction of S-nitrosylation of cell death receptor by Sorafenib.

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P-223

Induction of FGF21 by CO/PERK/ATF4 Pathway Mediates Metabolic Homeostasis

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The rate of metabolic disease including type 2 diabetes, obesity, and cardiovascular disease has rapidly increased, but the exact mechanisms of metabolic syndrome are under investigation. We suggest carbon monoxide (CO) plays an important role in alleviating of metabolic dysfunction. Even though anti-inflammatory, anti-proliferative, and anti-apoptotic effects of CO on a variety of cellular injury model has been known well, the effects of CO on complex pathways of metabolic disease remains unknown. We demonstrated that CO increased FGF21 expression and secretion in hepatocyte and liver. CO stimulated PERK activation and then enhanced the levels of FGF21 through eIF2a-ATF4 signal pathway. CO-induced FGF21 attenuated ER stress or diet-induced obesity (DIO)-mediated hepatic steatosis Moreover, CO lowered blood glucose levels, enhanced insulin sensitivity, and promoted energy expenditure by stimulating beiging. Finally, we suggest that CO is a potent inducer of FGF21 expression and that CO critically depends on FGF21 to regulate metabolic homeostasis.

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P-224

Regular exercise participation improves genomic stability in diabetic patients: an exploratory study to analyse telomere length and DNA damage

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Keywords: Telomeres; DNA damage; Apoptosis; Oxidative stress

Physical activity demonstrated to be effective as prevention and treatment for different chronic conditions, including type 2 diabetes. In particular, several studies highlighted as its beneficial effects may be related to a major stability of the DNA molecule, such as the telomeric ends.

Here we analyze the effect of exercise training on telomere length, spontaneous and H_2O_2-induced DNA damage, as well as the apoptosis level in leukocytes from untrained or trained type 2 diabetic (T2D) patients or age-matched healthy subjects (HS). Moreover, the expression analysis of selected genes belonging to DNA repairs systems, cell cycle control, as well as to antioxidants and defence systems was performed.

Our findings demonstrate that diabetic patients participating to a regular exercise program show a longer telomere sequence and a substantial reduction of spontaneous DNA damage. Further, ex vivo treatment of leukocytes with H_2O_2 highlighted: 1) a preferential telomere attrition; 2) a decreased susceptibility to DNA damage in leukocytes of trained T2D patients with respect to untrained ones, and 3) a relationship between DNA damage and cell apoptosis. Finally, the gene expression analysis in T2D subjects suggests an adaptive response to prolonged exercise training aimed at improving the response of specific genes.

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P-225

Impact of lipotoxic stress on proteolytic systems in human liver cells

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Keywords: Lipotoxicity; ubiquitin-proteasome system; lysosomal system

Type 2 diabetes is a chronic metabolic disorder characterized, among other features, by elevated levels of circulatory free fatty acids. Several studies in animal and cellular models indicate that increased accumulation of lipids leads to oxidative and ER stress, mitochondrial dysfunction and alteration of proteostasis, resulting in cellular dysfunction.

Proteostasis is mainly regulated by two pathways, the "ubiquitin-proteasome system" and the "autophagy-lysosomal system". The proteolytic systems degrade un-/misfolded proteins and regulate the functional protein pool. However, it remains elusive if a hyperlipidemic environment leads to cellular dysfunction via alterations of both proteolytic systems.

The purpose of this study is to investigate the role of the proteolytic systems in lipotoxic conditions. Therefore, we induced lipid accumulation in HepG2 cells by exposing them to palmitic acid. According to preliminary data palmitic acid induced lipid droplet formation and alters the cellular redox state, iNOS levels and induces ER stress. Under these conditions, further experiments are being performed to elucidate the impact of lipotoxic stress on the proteolytic systems in HepG2 cells.

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P-226

Hibiscus and lemon verbena polyphenols: Assessment for weight management in overweight volunteers. Appetite control and satiety

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Keywords: *Hibiscus sabdariffa*; *Lippia citriodora*; polyphenols; obesity; appetite

Obesity is one of the most pervasive in chronic diseases where therapeutic approaches have failed. Emerging scientific evidence indicates that dietary supplementation may be an alternative in the treatment of obesity and other metabolic disorders.

A previous study showed that the consumption of 500 mg/day of MetabolAid® dietary ingredient (*Lippia citriodora* extract + *Hibiscus sabdariffa* extract) for two months in overweight women decreased weight, improved anthropometric parameters, decreased systolic blood pressure and heart beat and improved blood lipid profile. Although appetite assessment was not strictly evaluated, most individuals consuming MetabolAid® experienced a satiating effect. The primary objective of this study assessed the feelings of appetite, hunger and satiation in the group having MetabolAid compared to placebo in a double-blind, placebo-controlled and randomized trial in 54 overweight women (BMI 24-35 kg/m2).

The results of the study showed that the consumption of 500 mg/day of MetabolAid® significantly increased satiety and fullness, decreasing hunger and prospective food consumption compared to placebo after 1 month, being these differences greater after 45 days and 2 months. Moreover, people having the active ingredient improve anthropometric parameters, decrease blood pressure and heartbeat, confirming the results of the previous study.

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P-227

The role of cholesteryl ester transfer protein expression on endothelial cells: oxidative stress and vascular dysfunction

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Keywords: CETP; Superoxide, Hydrogen peroxide; eNOS; endothelium

High-density lipoprotein cholesterol (HDL-C) levels are inversely correlated with development of atherosclerotic coronary heart disease and studies demonstrate that cholesteryl ester transfer protein (CETP) deficiency is associated with markedly increased HDL levels. HDL may exert atheroprotective activity by preventing endothelial dysfunction, a key step in the development of atherosclerosis. However, the CETP effects per se on endothelial function needs to be clarified. This study evaluates the role of CETP in the setting of early atherosclerosis and its contribution to endothelial dysfunction. In vitro, knockdown of Human Aortic Endothelial Cells (HAECs) with siCETP, prevented expression of adhesion molecules. Notably, eNOS activity was augmented in aortas of CETP-transgenic mice and correlated with decreased levels of inhibitory interaction with caveolin-1. Furthermore, the presence of CETP in aortas coincided with an increase in vascular production of reactive oxygen species evidenced by augmented oxidized to reduced glutathione ratio (GSSG/GSH), increased superoxide and hydrogen peroxide production and a marked decrease in endothelium-dependent vasorelaxation. Together, these findings suggest a role for CETP in promoting endothelial dysfunction and highlight the role in promoting oxidative stress.

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Fapesp

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P-228

Green Rooibos Extract improves plasma lipid profile and oxidative status in diabetic non-human primates

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Rooibos,a shrub-like leguminous bush endemic to South Africa, is traditionally used as a herbal tea. Unfermented (green) rooibos presents with a much higher polyphenol content, in particular aspalathin which constitutes a major metabolically bioactive compound. Recent studies, both in vitro and ex vivo, highlighted an enhanced glucose uptake effect of aspalathin, associated with increased GLUT 4 translocation to the plasma membrane via AMPK activation. In high fat fed mice, aspalathin also showed a plasma lipid lowering effect. In the present study, we evaluated the effect of 4 weeks of supplementation (90 mg/kg body weight t.i.d) with a pharmaceutical grade aspalathin-enriched green rooibos extract containing ca. 12.8% aspalathin, on gluco-lipidic and oxidative indexes in high fat diet-induceddiabetic and normal non-human primates (*Chlorocebus aethiops*). Diabetic models were characterized by glucose intolerance and increased total cholesterol and LDL. Moreover, decreased plasma CoQ10 and increased oxidative status were associated with higher levels of oxLDL. Green rooibos supplementation of the diabetic animals significantly improved all listed indices, highlighting the potentialities of this natural extract in modulating some risk factors associated with cardiovascular disease associated with type 2 diabetes.

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P-229

Ubiquinol supplemenetation in elderly patients undergoing aortic valve replacement: Biochemical and clinical effects

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There is a steady rise in the mean age of patients affected by heart disease undergoing cardiac surgery. However senescent myocardium has reduced tolerance to ischemic stress and there are clear indications about age-associated deficit in myocardial performance after operatory stress. CoQ10 improve several conditions related to bioenergetic deficit or increased exposure to oxidative stress. The use of ubiquinol, the reduced form of CoQ10, is promising in these clinical settings, on the basis of its superior bioavailability and of the alleged impaired CoQ10-reducing capacity in the elderly. In this study clinical and biochemical effects of ubiquinol were evaluated in 46 patients undergoing aortic valve replacement. They, affected by severe aortic stenosis, were randomized into 2 groups, placebo and CoQ10 group supplemented with 400mg/day of ubiquinol starting, 7 days before surgery and continuing for 1 month after cardiac intervention. CoQ10 levels, its oxidative status and IL-6, TNF-alpha and S100 protein concentration in plasma were assessed. Moreover, main cardiac adverse effects in the postoperatory phase, NYHA class, contractility and myocardial hypertrophy were evaluated. Ubiquinol treatment improve basal (before surgery) oxidative status of CoQ10 and considerably mitigate increased oxidation related to the operation.

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P-230

Simvastatin differently effect oxidative status in cultured myocytes from young and old donors

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Sarcopenia is a condition of age-related loss of muscle mass and strength which affects balance, gait and overall ability to perform tasks of daily living, with increased risk of falls. While mitochondrial dysfunction and associated oxidative stress contribute to muscle ageing, the interplay between ROS and protective antioxidant response in the cell is regarded as a key factor in maintaining muscle strength and physical performance. CoQ10 is a lypophilic endogenous cofactor with both antioxidant and bioenergetics function whose levels decrease with age. Moreover also lipid-lowering drugs targeting HMG-CoA reductase (statin) are able to effect CoQ10 synthesis. In the present study we show that murine myocytes (C2C12) exposed to simvastatin 0.3-40 μM for 72 h show a reduced content of CoQ associated with increased ROS production and cytotoxicity. Moreover exposure in the same conditions of primary human myocytes from young and old donors show an enhanced susceptibility to oxidative stress in the elderly a condition that might suggest an enhanced detrimental side effects of statins with potential implications in sarcopenia development.

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P-231

Combination of Ubiquinol intake and moderate physical activity efficiently counteracts myocytes mitochondrial dysfunctions and apoptosis in a mouse model of sarcopenia

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Sarcopenia is a age-related condition characterized by loss of muscle mass and strength with important societal implication in light of the growing elderly population. Mitochondrial dysfunction plays a central role in the pathogenesis of sarcopenia. In this scenario, physical exercise on the one hand is able to enhance mitochondrial capacity and biogenesis via PGC-1a induction, while on the other hand could promote oxidative stress via mitochondrial ROS production limiting the benefits of physical activity on aged skeletal muscles. In the present study we evaluate the effects of both physical activity and coenzyme Q10 intake, alone or in association in the SAMP8 mouse model of sarcopenia. Our data show that the combination of the treatments promotes mitochondrial functionality and biogenesis, while limiting muscular hypertrophy produced by physical activity alone that could be deleterious in the context of aged muscles. Such modulation was associated with a lower activation of AKT and ERK pathway along with the prevention of muscle apoptotic death. Moreover, in mice undergoing combined treatment, coenzyme Q10 seems to synergize with physical activity by modulating several factors involved in metabolism and longevity, including SIRT proteins that might play a key role in mediating treatment-induced benefits.

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P-232

Role of inflamma-mitomiRs miR-146a, miR-181a and miR-34a in regulating mitochondrial dysfunction during replicative senescence of human endothelial cells

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The aging process is characterized by a drastic metabolic change, intimately associated with impaired mitochondrial functions and homeostasis: alteration in autophagic clearance and apoptotic rates, oxidative stress and an inflammatory and secretory phenotype (SASP). Current research suggests that some microRNAs can play a direct role in regulating mitochondrial activity (mitomiRs). Ingenuity Pathway Analysis of mitomiRs targets has disclosed that miR-146a, miR-34a and miR-181a are closely linked to each other and to Bcl-2 family linking them to important cell functions (proliferation, death, survival, maintenance) and age-related diseases. We validated this bio-informatic analysis in human senescent endothelial cells (HUVEC) compared to young cell. Selected mitomiRs were up-regulated in senescent cells while a decreased in Bcl-2 protein expression was observed as well as permeability transition pore opening. Transfection of these miRs in young cells produced similar effects at Bcl-2 level and PTP opening, demonstrating a direct link between this miRNA set and mitochondrial dysfunction in senescent cells. Due to Bcl-2 role as metabolic sensors/messengers involved in apoptosis, autophagy and anti-oxidative pathways, the proposed set of regulatory miRs represent an intriguing novel mechanism controlling mitochondrial function and dysfunction during cellular aging.

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P-233

The protective antioxidant effect of Lemon balm extract against UVB-induce damage in a skin cell model

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Keywords: Antioxidant; ROS; Lemon balm; Polyphenol; Keratinocyte

Solar radiation exposure is the main cause of a variety of cutaneous disorders, including photoageing and skin cancers. Ultraviolet radiation (UV), especially UVB, causes DNA damage, pyrimidine dimmers, 8-hydroxy-2´-deoxyguanosine, p53 induction, protein oxidation and generation of reactive oxygen species (ROS) formation. Polyphenol species of Mediterranean plants have shown photoprotective effects. This study evaluated the protective action of lemon balm extract (LB) against UVB-induced damage in human keratinocytes (HaCaT cells) and correlates both activities.

Antioxidant activity has been measured using up to four in vitro different assays (Folin-Ciocalteu assay, trolox equivalent antioxidant capacity, ferric reducing-antioxidant power and oxygen radical absorbance capacity assays). On the other hand, cell viability of HaCaT exposed to UVB was determined by MTT assay. Finally antioxidant activity by ROS-sensitive dichlorofluorescein diacetate was measured confirming the correlation between antioxidant and protective activities.

In conclusion, our results suggest that LB extract present a potential photoprotective effect in human skin cell model against UVB-induced damage mediated by its antioxidant capacity, however, further research should be developed to elucidate molecular mechanisms.

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P-234

Glut 1 overexpression prevents glucose deprivation-induced prostate cancer cell death by increasing pentose phosphate pathway and glutathione

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Keywords: prostate cancer; Glut 1; apoptosis; oxidative stress; glutathione

Although glucose metabolism was override in prostate cancer, it is recognized that there are metabolic differences between androgen-sensitive and castration-resistant phenotypes that might be responsible of progression or treatment success. Among the targets of glucose metabolism, glucose uptake is on the top of the cascade. The increase of glucose uptake by GLUTs overexpression in oncogenesis or the androgenic regulation of some GLUT transporters in other tissues is demonstrated. The prostate, at the beginning of carcinogenesis, depends on OXPHOS but then, at aggressive stages, tumors become again glycolytic like other cancers. Therefore, their resistance to glucose deprivation is different at the beginning or at later stages. In this work, we demonstrated by using androgen sensitive and insensitive cells that facilitative GLUT1 transporter overexpression protect prostate cancer cells from apoptosis caused by glucose removal. Glucose removal caused an increment in oxidative stress that stimulated androgen receptor activity and GLUT1 overexpression. This protection is mediated by a derivation towards pentose phosphate metabolic pathway and an increment of glutathione in androgen dependent prostate cancer cells.

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P-235

A novel nutrient blend mimics calorie restriction transcriptomics differentially in multiple tissues of mice

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Keywords: calorie restriction mimetic; transcriptomics; phytonutrients; anti-aging; nutrition

Identification of caloric restriction mimetics (CRMs), compounds that mimic the beneficial effects of caloric restriction (CR) without restriction of dietary energy would be an advancement in anti-aging science. The present study investigated whether the transcriptional profiles of a putative CRM nutrient blend could mimic that of CR in diverse tissues following long-term feeding in B6C3F1 male mice. Study design: Young Controls (YC; 5 mo.) and 3 groups treated from 14-30 mo.: Old Controls (OC), Old CR (OCR; 25% CR) and Old Supplemented (OS); n = 7/group. Gene expression profiling in cerebral cortex (CCT), skeletal muscle (gastrocnemius) (SKL), heart (HRT), white adipose tissue (WAT) and liver (LVR) was performed using Affymetrix Mouse 2.0ST arrays. Principal component analysis revealed that gene expression profiles of YC and OC were distinct from one another in all tissues at 30 months. Using differential analysis, genes commonly expressed in OCR and OS groups compared to the OC group were identified in CCT (3,468), SKL (2,386), HRT (3,523), LVR (1,276) and WAT (683). The OS group mimicked OCR transcriptional profiles most dramatically in CCT, HRT and SKL, tissues highly relevant to aging and age-associated diseases. These CRM effects, elicited by a mid-life intervention, may have positive implications for healthy human aging or 'youthspan' and warrant further investigation.

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P-236

Oxidative Stress Markers of Alzheimer's Disease in Peripheral Cell Mitochondria

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Keywords: Alzheimer's Disease; oxidative stress; protein oxidation; mitochondria

Alzheimer's disease (AD) represents a neurodegenerative disease leading to progressive dementia in the elderly. AD has been associated with increased oxidative stress and mitochondrial dysfunction. Hence, markers for oxidative stress are expected to accumulate not only in brain mitochondria, but also in mitochondria of peripheral cells. We have developed and optimized a method for the rapid and selective fluorogenic derivatization and enrichment of specific protein oxidation products, 3-nitrotyrosine and 3,4-dihydroxyphenylalanine (DOPA), in order to quantitatively compare protein oxidation in the mitochondria of peripheral, white blood cells obtained from healthy donors, patients with mild cognitive impairment (MCI), and AD patients. Double blind studies reveal a ca. two-fold increase of protein nitration/oxidation between mitochondrial proteins derived from healthy volunteers and MCI patients, with no further increase between MCI and AD patients. Proteomic studies will show whether specific proteins are target for nitration/oxidation during the pathogenesis of MCI and AD. Mitochondrial protein nitration/oxidation may be used for early screening of patients with the genetic risk for the development of AD or as companion diagnostic for pharmacological interventions.

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P-237

Site-specific cysteine oxidation regulates 26S proteasomes

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Keywords: lipotoxicity; ubiquitin-proteasome system; thiol

The main machinery responsible for cellular protein maintenance is the ubiquitin-proteasomal system. The main task of the system is a fast and efficient degradation of proteins not needed anymore in cellular metabolism. It is accepted that upon oxidative stress, the proteasome suffers a series of functional alterations, including a reversible, oxidation-triggered 26 S proteasome disassembly into its catalytic (CP) and regulatory (RP) particles. Formation of low fluxes of oxidants were stimulated with antimycin or palmitic acid in pancreatic MIN6 cells, inducing a partial increase of dimerization of mitochondrial and citosolic peroxiredoxins. Under this conditions, no significant increase of total protein thiol oxidation was detected. However, ATP-stimulation of 26 S proteasomes was inhibited while the particle remained fully assemblied. Discrete cysteine residues of both CP and RP were identified oxidized by a cysteine-targeted proteomics approach. Such results may rely on differential reactivity of cysteines in the subunits and suggest specific regulation of particles by discrete cysteine oxidation. Further studies will elucidate the type of modification on each cysteine residue and the impact. Our results suggest a novel mechanism of redox regulation of 26 S proteasome that precedes oxidation-driven 26 S particle disassembly.

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P-238

Mitochondrial adaptive response in a model of CoQ10 deprived human dermal fibroblasts

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CoQ10 is a ubiquitous isoprenilated quinone with a key role in cellular bioenergetic and as antioxidant in membranes. The organism produces adequate amount to support physiologic demand although biosynthesis could be influenced by genetic background, nutrition and lifestyle. Moreover, ageing and of HMG-CoA reductase inhibitors may play an important role in further lowering synthesis. At skin level CoQ10 decrease is known to be associated with senescence manifestation. In the present study we developed a model of CoQ10 deprivation in human dermal fibroblast to mimic processes associated with ageing in order to elucidate the role of this cofactor in mitochondrial remodeling and adaptive response. Flow cytometric analysis of mitochondrial function and ROS production was associated with respirometry profiling and molecular markers of mitochondrial biogenesis in cells exposed for 72hrs to a log range of simvastatin concentrations. Analysis outlined a series of complex adaptative response where statin dose dependently affected CoQ10 levels and oxidative status steering either hormetic responses or mitochondrial PTP opening associated to selective elimination of defective mitochondria or overt cellular toxicity stressing the central role of this molecule in the biochemistry of cell ageing

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P-239

Effects of persimmon extract in 3T3-L1 cells

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Keywords: Persimmon extract; bioactive components; oxidative stress; 3T3-L1; ROS

There is growing evidence that natural products are important in the relationship between nutrients and healthin humans. Currently, one of the biggest problems in the food industries is the waste management that is generated in food production. In the present work, we have obtained a persimmon extract (PE) rich in bioactive compounds from the industrial sub-product of natural persimmon juice. We present here the characterization of bioactive components of PE and document their effects in hypertrophic and insulin resistant adipocytes.

High-performance liquid chromatography coupled to mass spectrometry (HPLC-ESI-MS) was used for detected and identified carotenoids such as α-carotene, β-carotene and β-criptoxantin and polyphenols such as quercetin, procyanidin, hesperidin/neohesperidin, among others. To determine the properties of the PE, the antioxidant status was measured by the TEAC, FOLIN, FRAP and ORAC tests, showing a strong antioxidant activity.

The intracellular ROS generation and triglyceride accumulation was measured by sensitive fluorescent probes in a hypertrophied adipocyte model. The results show that the PE exhibited an effective dose-dependent decrease of intracellular ROS generation as well as triglyceride accumulation in hypertrophic adipocytes.

Therefore, PE extract can be used for preventive purposes against oxidative stress and obesity.

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P-240

Anti-oxidative effects of a white grape juice extract on lymphocytic mitochondrial functions

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Keywords: Oxidative stress; grape juice; lymphocytes; mitochondrial functionality; natural products

Due to the pivotal role of mitochondria in cell physiology and pathology, recently, a great deal of data has been acquired on mitochondrial dysfunction in aging and in age-related diseases. This stimulated the search for compounds able to promote mitochondrial functionality.

Our study was designed to evaluate the antioxidant property of a white grape juice extract (WGJe). First, we assessed its potentiality in cell-free experimental models and we assayed its capability of preventing the AAPH-induced reactive oxygen species (ROS) in HepG2 cells. Then we investigated WGJe effects in an ex vivo experimental model consisting of activated lymphocytes of both young and elder subjects, where pre-treatments of phytohemagglutinin/interleukin 2-activated lymphocytes with WGJe (0.05 and 0.1 μg/mL) for 24 and 48 h improved the mitochondrial functionality. In particular, WGJe steadily decreased the mitochondrial mass of older subjects, without a relevant effect in younger counterpart. In addition, WGJe significantly enhanced $\Delta\psi m$ in both groups investigated. Finally, we found that WGJe reduced the endogenous mitochondrial production of H_2O_2, more in young subjects rather than in elders.

Overall, our data indicate that WGJe may be used to improve mitochondrial functions in oxidative injured cell, and strengthen the potential use of WGJe as an anti-oxidative remedy for maintaining human health.

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P-241

Catalase localization in Duchenne-Becker patients' erythrocytes

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Keywords: Catalase; erythrocyte; Duchene-Becker muscular dystrophy; immunogold

Duchenne-Becker muscular dystrophy (DBMD) might be caused by a widespread genetic defect in surface membranes, which could be expressed in membranes not pathologically involved in DBMD. This hypothesis was supported by a substantial amount of evidence of abnormalities in erythrocytes from patients with DBMD. Catalases are well studied enzymes that play critical roles in protecting cells against the toxic effects of hydrogen peroxide. In previous years a lot of papers on oxidative status in DBMD are mostly confirmed increased activity of enzymes involved in the elimination of reactive oxygen species in order to protect the cells from damage, including superoxide dismutase, catalase and glutathione peroxidase. Immunohistochemistry and immunogold labeling were used to study erythrocytes from patients with Duchenne-Becker muscular dystrophy and from age-matched normal boys. There were significant differences in the catalase localization of erythrocytes from Duchenne patients when compared to controls. Hence, the internal catalase localization in the erythrocyte is atypical in DBMD, supporting the concept that a membrane and cytoskeletal defect involving multiple tissues is present in this disorder.

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242

Cerebral protection during fetal-to-neonatal transition under hypoxic atmosphere

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Keywords: Hypoxia; oxidative stress; biomarkers; brain; mitochondria

Newborn asphyxia is a complication during the perinatal period. The use of O_2 for resuscitation has been broadly used in clinic. However, it has been demonstrated that oxygen overexposure induces oxidative stress (OS). We speculate that delaying postnatal in the extrauterine oxygenation status would preserve reducing equivalents, enhance redox adaptation, and protect oxyregulator tissues. The objective is evaluated OE status, induced by Fetal-Neonatal Transition (FNT) under different FiO_2 conditions, in brain. FiO_2 in pregnant mice was reduced from 21% to 14% or not the night before of delivery (G19). 8 hours after birth both group were led to room air (Hx14/21 and Nx21/21 groups) or hyperoxia (FiO_2=100%) (Hx14/100 and Nx21/100 groups) and reset to 21% after 1 hour. The pups were sacrificed P1. We have determinated OS biomarkers (GSH/GSSG, Cysteine/ Cystine, Homocysteine/ Homocystine), protein (m-tyr and o-tyr/Phe, 3NO2-tyr and 3Cl-tyr/tyr), and DNA (8OH-dG/2-dG) damage by HPLC-MS/MS and we have done a mitocondrial morphology study using ME. The results show a higher OS in the Nx21/100 group compared to the Hx14/100 group that decreases. The group Hx14/21 and Hx14/100 present a better mitochondrialcharacteristics compared to environmental conditions and their subsequent reoxygenation. Our results support that FNT under hypoxic conditions could be protective to face a possible event of newborn resuscitation.

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P-243

Mitochondrial respiratory profile in human dermal fibroblast treated with HMG-coa reductase inhibitor

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Coenzyme Q10 is a endogenous isoprenilated quinone with antioxidant and bioenergetic activity. Its cellular levels and oxidative status are effected in ageing and synthetic rate is influenced by simvastatin, a widely used lipid lowering drug inhibiting HMG-coA reductase, a key enzyme in the mevalonate pathway. In light of its central role in mitochondrial bioenergetic and its relevance to the senescence process we evaluated how CoQ10 deprivation affected mitochondrial respiration in terms of oxygen consumption rate (OCR), evaluated using extracellular flux analyzer Seahorse bioscience, either in basal condition or following the incubation of selective inhibitors of different respiratory complexes. Human dermal fibroblast incubated at sublethal concentration of simvastatin, in the range 0.6 – 10 μM for 72 hours, showed a significant decrease in basal mitochondrial respiration at all tested concentrations associated with a significant decrease in ATP production. Moreover, at concentration higher than 2.5 μM significant decrease of maximal respiration and spare respiratory capacity suggest a decrease of the mitochondrial mass and/or poor ETC integrity that parallelled CoQ10 deprivation stressing the role of this molecule in cellular bioenergetics. Overall the data support the use of this experimental model for the study of age-related CoQ10 decrease in human skin.

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P-244

Impact of lipo- and glucotoxic stress on proteolytic systems in murine liver

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Keywords: Metabolic syndrome; liver; proteasomal system; inflammation; glucotoxicity; lipotoxicity

Metabolic syndrome (MS) is a chronic pathology characterized (amongst others) by dysregulated plasma glucose and high serum triglycerides. One of the central organs affected is the liver, causing increasing fat accumulation and inflammation.

To investigate the impact of chronic inflammation and fat accumulation on the main mammalian proteolytic and -static system – the ubiquitin-proteasomal system (UPS) – the liver of NZO mice suffering either from hyperlipidemia or from both hyperlipidemia and hyperglycemia at different ages was investigated.

Our interest was in changes/redistributions of UPS-compounds (depending on age and/or metabolic state) as well as its response to the chronic inflammation associated with MS. First experimental results revealed changes in the distribution of UPS-compounds (especially of its central protease, the 20 S proteasome, responsible for proteolytic degradation of oxidatively damaged proteins) as well as expression of the so-called "immunoproteasome" (i20S), a special form induced by inflammation. Furthermore, the proteasomal 19 S regulator (enabling degradation of native proteins) was significantly redistributed between nucleus and cytosol of the investigated liver cells, as well as the amounts of oxidatively damaged proteins.

Summarizing our results, we hypothesize that the cellular dysfunctions induced by MS are in part due to changes of the UPS.

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P-245

Compromised proteasomal function in endothelial cell senescence

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Keywords: Senescence; proteasome; endothelial

Senescence is thought to contribute to endothelial dysfunction, which ultimately leads to development of cardiovascular disease. The current project characterizes proteasomal protein degradation in endothelial cells undergoing replicative or stress-induced senescence in vitro or chronological ageing in vivo.

Our results demonstrate that human endothelial cells become senescent in vitro within 25 cumulative population doublings. Senescence was verified by increased number of senescence-associated β-galactosidase (SA-β-gal)-positive cells and increased cell size and granularity. In addition, protein carbonylation and nitration as well as reduced GSH levels were observed. Studies on the expression of proteasomal subunits revealed a decline of the β5 and β2 subunits in senescent cells. In parallel, trypsin- and chymotrypsin-like activities were decreased. Similar observations were made in primary endothelial cells derived from old mice (24 months old) when compared to young mice (4 months old). Transient inhibition of proteasome in endothelial cells led to premature senescence. In parallel, persistent accumulation of carbonylated proteins was observed indicating a role of oxidative stress in the development of senescence. From these results we suggest a relationship between proteolytic insufficiency and senescence, which may contribute to vascular ageing.

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P-246

Spectra of ultra-weak photon emission

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Organisms undergoing oxidative metabolism or stress chemically generate electronically excited species through dioxetane and tetroxide pathways. Consequently, luminescence is emitted which could be exploited for non-invasive label-free monitoring of oxidative stress. Spectral analysis of this endogenous chemiluminescence can serve as a tool for identification of the emitters as products of oxidation.

Here we employ quantum mechanical calculations and sensitive photon counting methods to obtain deeper understanding of the emitter molecules both from theoretical and experimental perspective. We focused on the analysis of carbonyl groups of simple model species (formaldehyde, acetone, etc.) and carbonyls formed by fragmentation of linoleic acid since they are common product of oxidation and also one of major emitters of endogenous chemiluminescence. We calculated emission spectra of carbonyl groups using TURBOMOLE code for quantum mechanical calculations. We used TDDFT method for excitation and emission and DFT method for optimization of the ground state. The generalized gradient approximation of Perdew, Burke and Ernzerhof (PBE) were chosen for the exchange correlation density functional. We used def2-SV(P) as basis set and the corresponding Coulomb fitting bases. We found emission spectra of individual carbonyls in water environment and in vacuum.

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P-247

Free radical production and detoxification in complex IV deficient cancer cells

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Keywords: Mitochondrial complex IV deficiency; F1Fo ATP synthase reversal; free radical detoxification; cell bioenergetics

Mitochondrial membrane potential (DYm) is one of the key drivers of free radical production. Changes in DYm strongly affect free radical turnover in both mitochondria and cytosol. We found

that in cytochrome c oxidase deficient HCT116 cells F1Fo ATP synthase (mATPase) is reversed in order to maintain DYm polarisation. For small energy costs, mATPase enables mitochondrial protein import, Ca^{2+} turnover and Krebs cycle activity. Although glycolysis is the main source of ATP in COX-deficient cells, the DYm does not decrease upon inhibition of the adenine nucleotide translocators, suggesting their redundant role. The ATP-Mg/Pi carriers and substrate level phosphorylation can continuously support the mATPase activity and hence DYm polarisation. Intriguingly, in COX-deficient cells complex III contributes to DYm maintenance, redox state, and free radical (H_2O_2) production. An enlarged mitochondrial free radical detoxification machinery (e.g. elevated SOD2) protects the cells from oxidative damage. Although cytosol is known as the main area of free radical synthesis in COX-deficient HCT116 cells, and NAD(P)H levels are increased, the expression of genes involved in glutathione-dependent reactions does not change, suggesting an involvement of alternative mechanisms of free radical quenching. An observed large increase in fatty acid deposition is likely to contribute to cell protection.

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P-248

A study on mitochondrial stress responses due to OXPHOS inhibition

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Keywords: Mitochondria; stress; adaptation; OXPHOS; quality control

Optimal mitochondrial function is maintained by fission and fusion processes that shape the mitochondrial network and morphology, the elimination of dysfunctional mitochondrial through mitophagy and the generation of new through biogenesis. These processes are crucial for adaptation during stress conditions, and have been linked to diseases. In this study we investigated mitochondrial stress related protein expression after exposing cultured SH-SY5Y neuroblastoma cells to sub-lethal doses of OXPHOS inhibitors for 72 hours. Our focus was to investigate proteins that are involved in the dynamics, quality control and biogenesis. We found that exposure to electron transport chain inhibitors resulted in clear changes in expression of mitochondrial shaping proteins. These changes were accompanied by increased in the levels of PINK1 and p62, most apparent in the cells exposed to complex III inhibitors. The finding that inhibition of different complexes caused different responses, suggests that the effects are not caused by reduced bioenergetics function alone, and are likely to involve reactive oxygen species (ROS). Our data suggest that mitochondrial stress responses caused by OXPHOS inhibition depend on which complex that is affected. The effects on mitochondrial shaping and autophagy protein suggested that central parts of the mitochondrial quality control system were compromised, or possibly overcharged.

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P-249

Oxidative Stress Suppresses the Expression of 15-Hydroxyprostaglandin Dehydrogenase through Epigenetic Modulation in Human Colon Epithelial Cells

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Keywords: Oxidative Stress; 15-PGDH; Hypermethylation; Colon Epithelial Cells

15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is an enzyme that catalyzes the conversion of prostaglandin E2 (PGE2)to a biologically inactive 15-keto-PGE2. 15-PGDH has been considered to be a tumor suppressor as its expression is frequently repressed in human malignancies. In our study, we observed that H_2O_2 suppresses the expression of 15-PGDH in both concentration and time dependent manners. H_2O_2 induced methylation of the 15-PGDH promoter as determined by methyl specific PCR. H_2O_2–induced down-regulation of 15-PGDH expression as well as enhanced methylation of 15-PGDH promoter was abrogated by the DNA methyltransferase (DNMT) inhibitor, 5-Aza-2′-deoxycytidine (5-Aza). The antioxidant, N-acetylcysteine (NAC) attenuated the H_2O_2-induced upregulation of DNMT3 expression and subsequently methylation of 15-PGDH promoter, thereby restoring the expression levels of 15-PGDH. Taken together, these finding suggest that oxidative stress down-regulates the expression of 15-PGDH through hypermethylation of CpG island in the 15-PGDH promoter.

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P-250

A quantitiative LC-MS/MS method for the measurement of tocopherols, polyinsaturated fatty acids and their metabolites in human plasma and serum

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Keywords: Tocopherols; polyunsaturated fatty acids; metabolites; LC-MS/MS; human plasma

A sensitive and selective LC-MS/MS method for the quantification of vitamin E (α- and γ-tocopherol), polyunsaturated fatty acids (arachidonic, eicosapentaenoic, docosahexaenoic and α-linolenic acids) and their metabolites in human plasma and serum was developed and validated. The sample treatment consisted of an enzymatic hydrolysis followed by a deproteinization and a liquid-liquid extraction. The separation was achieved by reversed phase chromatography. For each analyte, two MRM transitions were monitored in order to confirm identity. All determined compounds showed good linearity over the investigated concentration range. Method accuracy was satisfactory according to the international guidelines. Our data show that this LC-MS/MS method is suitable for the quantification of a diverse set of bioactive lipids and tocopherols in human plasma of adult and children subjects. The determined levels are in agreement with the literature, thus providing a flexible method to explore pathophysiological processes in which changes of the investigated compounds are involved.

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P-251

Adaptation of the nematode *C. elegans* to hypoxia and reoxygenation stress reveals an unexpected function of the neuroglobin GLB-5 in innate immunity

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Keywords: C. elegans; globin 5; hypoxia; reoxygenation stress; innate immunity

Deprivation of oxygen (hypoxia) followed by reoxygenation (H/R stress) is a major component in several pathological conditions such as vascular inflammation, myocardial ischemia, and stroke. However how animals adapt and recover from H/R stress remains an open question. Previous studies showed that the neuroglobin GLB-5(Haw) is essential for the fast recovery of the nematode *Caenorhabditis elegans* (*C. elegans*) from H/R stress. Here, we characterize the changes in neuronal gene expression during the adaptation of worms to hypoxia and recovery from H/R stress. Our analysis shows that innate immunity genes are differentially expressed during both adaptation to hypoxia and recovery from reoxygenation stress. Moreover, we reveal that the prolyl hydroxylase EGL-9, a known regulator of both adaptation to hypoxia and the innate immune response, inhibits the fast recovery from H/R stress through its activity in the O2-sensing neurons AQR, PQR, and URX. Finally, we show that GLB-5(Haw) acts in AQR, PQR, and URX to increase the tolerance of worms to bacterial pathogenesis. Together, our studies suggest that innate immunity and recovery from H/R stress are regulated by overlapping signaling pathways.

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P-252

Oxidised lipids affect miR expression by microvascular endothelial cells

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Keywords: Epigenetic; Alzheimer's disease; lipid peroxidation; redox

Alzheimer's disease (AD) aetiology is complex with gene and environmental risk factor interaction. Our recent studies have confirmed that oxLDL is higher in vascular dementia patients and correlated with cognitive function.

We hypothesised that endothelial cells of the blood-brain barrier are critical mediators of systemic nutrient effects within the brain. Therefore we have studied the effect of 27 hydroxycholesterol and F2alpha isoprostane on microvascular endothelial cell redox state, inflammatory cytokines and regulatory microRNA (miR) profile.

We showed that lipids from patients with dementia or hypercholesterolaemia release directional inflammatory molecular signatures via an endothelial redox state-dependent mechanism.

miR expression in endothelial cells with and without oxidised lipid treatment was undertaken using an Agilent DNA microarray scanner and microarray data was analysed using GeneSpring GX software. Upregulated miR showed an increase in oxidative stress

and inflammatory pathways. Downregulated pathways included growth factor signalling. Using qPCR, we determined that miR-144 and 146 which are anti-inflammatory and redox regulating modulators were decreased by oxidised lipids. A neurotrophic factor-targeting miR was increased in expression.

These data highlight that oxidised lipids have important regulatory effects on endothelial microvascular cell function.

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P-253

Integrative omics-defined redox, metabolic and functional responses to environmental metal exposure

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Our previous studies showed that low dose cadmium (Cd) altered protein redox states resulting in inflammatory signaling, actin cytoskeleton disruption, and fibrosis. However, little is known about low-level Cd effects on the redox proteomic and metabolic regulation in pulmonary fibroblasts and potential impact on pulmonary health. To address this issue, we investigated low dose Cd effects using an integrative omics approach with biochemical and functional analyses. Redox proteomics was performed on lung fibroblasts and lung tissues of C57BL/6 male mice exposed to Cd (3.3 mg/L, 16 weeks). Lung tissues were also analyzed for metabolomics. Both redox proteomics and metabolomics identified a large number of mitochondrial proteins and metabolites altered by Cd, suggesting that mitochondria are sensitive to Cd-induced oxidation. Cd also increased nuclear translocation of thioredoxin-1 and stimulated myofibroblast differentiation and fibrosis as shown by smad transcription factor activity and subsequent differentiation marker proteins. Pathway analysis showed that PIP2 metabolism, carbohydrate metabolism with pyruvate and TCA metabolism, are significantly affected by Cd. The integrative redox proteomics and metabolomics with mechanistic and functional pathways provide a powerful approach to understand complex mechanisms of low environmental Cd in lung disease.

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P-254

Towards Magnetic Mapping of Cellular Organelles using Fluorescent Nanodiamonds

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Keywords: Fluorescent NanoDiamonds; Magnetometry; ROS visualization; Organelle Isolation; Biocompatibility

Recently, Fluorescent NanoDiamonds (FNDs) have gained attention in the physical, chemical and biological fields. Their fluorescence is influenced by the magnetic surrounding. This allows magnetic resonance imaging on the nanoscale. Since the read out is optical only a microscope is needed and the technique is so sensitive that even single electron spins are visible.

We show that nanodiamonds are non-toxic in mammalian cell lines, which freely take up these particles. In addition, we show the effects of different concentrations, size, surface termination, shape and aggregation state on the biocompatibility at the level of genetic and protein changes as well as overall viability and reactive oxygen species (ROS) production.

Yeast cells can be stimulated to ingest FNDs after chemical or electrical transformation. Altogether, Fluorescent Nanodiamonds show much promise to become a sensitive and direct detector for a wide range of molecules, among which free radicals are a major contender because of their high magnetic moment.

In order to understand a spectrum from the interior of a cell, we isolate organelles and analyze these using our highly sensitive homebuilt confocal setup. In addition, we target cell organelles using an antibody targeting with biotin-streptavidin interactions. Here we present the results of our pilot experiments and give an overview of single organelle magnetometry implications.

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P-255

Oxysterols are involved in colorectal carcinogenesis by damaging intestinal layer

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Colorectal cancer (CRC) is one of the most common tumors world wild. High cholesterol diet is considered a risk for CRC development.

Cholesterol oxidation products, namely oxysterols, have been shown to have a role in human degenerative diseases, mainly for their ability to favor inflammatory reactions. Therefore, they could be involved in inducing intestinal inflammation, a process strongly associated to colorectal carcinogenesis.

Dietary oxysterols could derange intestinal epithelial barrier by inducing the activation of metalloproteinases (MMPs) as well as

the decrease of tight junctions (TJs), which are essential in mucosa barrier maintenance. Enterocyte-like CaCo-2 cells were treated with a mixture of oxysterols representative of a hyper-cholesterol diet. The time course study of MMPs activity (MMP-9 and MMP-2) showed their significant increase, reaching the maximum at 72 hours treatment. Moreover, the dietary oxysterols decreased the TJ production, in particular of junctional adhesion molecule (JAM), zonula occludens (ZO) and occludin. These two events appeared to be associated. In fact, cell pretreatment with specific MMPs inhibitors restored TJ levels.

Therefore, dietary oxysterols could be actually implicated in tumor progression towards a more aggressive phenotype by inducing extracellular matrix destabilization and intestinal barrier disruption.

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