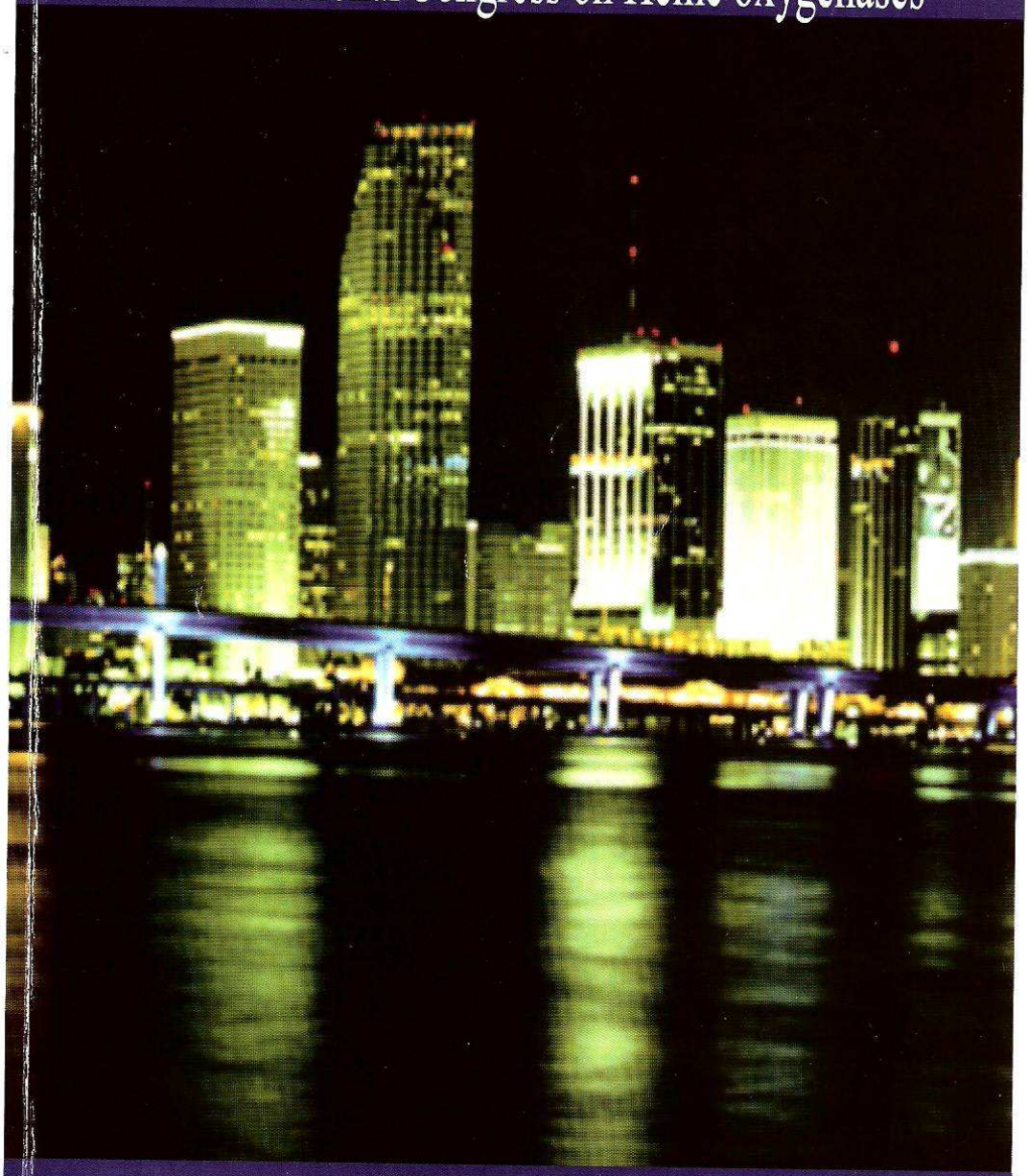
Heme Oxygenases in Biology & Medicine

6th International Congress on Heme oxygenases



September 30- October 4, 2009

Miami Beach, Florida

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INVITED SPEAKERS

Paolo Arosio, Università di Brescia, Italy
Fritz Bach, Harvard Medical School, Boston, USA
Eugenio Barone, Catholic University, Rome, Italy
Marcelo Torres Bozza, Universidade Federal do Rio de Janeiro, Brazil
Ioav Cabantchick, Hebrew University of Jerusalem, Israel
Augustine M. Choi, Brigham and Women's Hospital, Boston, USA
Michael Cooreman, Ikaria
Melissa Cudmore, University of Birmingham, UK
Victor Darley-Usmar, University of Alabama at Birmingham, Birmingham, USA
Phyllis Dennery, University of Pennsylvania, Philadelphia, USA
Sylvain Dore, Johns Hopkins, Baltimore, USA
John Eaton, University of Louisville, USA
Bruce A. Freeman, University of Pittsburgh, Pittsburgh, USA
Feng Guo, UCLA School of Medicine, Los Angeles, USA
Craig Hersh, Brigham and Women's Hospital, Boston, USA
Toshinori Hoshi, Johns Hopkins, USA
Thomas Kietzmann, University of Kaiserslautern, Germany
Kazu Igarashi, Tohoku University School of Medicine, Japan
Stephan Immenschuh, Hannover Medical School, Germany
Ken Itoh, Hirosaki University, Japan
Jonathan Jaggar, University of Tennessee, Memphis, USA
Charles Leffler, University of Tennessee, Memphis, USA
Giovanni Li Volti, University of Catania, Italy
Mahin D. Maines, University of Rochester, Rochester, NY, USA
Anthony F. McDonagh, Univ of California, San Francisco, USA
Roberto Motterlini, Italian Institute of Technology, Genoa, Italy
Martina Muckenthaler, University of Heidelberg, Germany
Chris Peers, Leeds, UK
Stephen Peterson, New York Medical College
Claude Piantadosi, Duke University Medical Center, Durham, USA
Scott Plevy, University North Carolina, Raleigh-Durham, USA
Robert Poole, University of Sheffield, United Kingdom
Raymond F. Regan, MD, Thomas Jefferson University, Philadelphia, USA
Tracey Rouault, NIH/NICHHD, Bethesda, USA
D.J. Schaer, Washington DC, USA
Michal Schwartzman, New York University, New York, USA
Laura Silvestri, Milan, Italy
M. Celeste Simon, University of Pennsylvania, Philadelphia, USA
Miguel Soares, Instituto Gulbenkian de Ciência, Portugal
Young-Joon Surh, Seoul, South Korea
Emanuela Tolosano, University of Torino, Italy
Libor Vitek, Charles University, Prague
Barbara Wegiel, Harvard Medical School, Boston, USA
William Wilkinson, Cardiff, UK
Ronald J Wong, Stanford University, USA
Weiling Xu, Cleveland Clinic, USA
Maria Zenclussen, Otto-von-Guericke-University, Germany

NO. 42 -- Maria Marta Facchinetti, Instituto de Invetigaciones Bioquimicas Bahia Blanca
EXPRESSION OF HEME OXYGENASE-1 IN HUMAN GLIOMAS

Norberto A Gandini[1], Debora Salomon[1], Maria E Fermento[1], Jean C Zenklusen[2], Ana Robles[3], Alejandro Curino[1]& Maria Marta Facchinetti[1] - Laboratorio de Biologia Básica del Cáncer, Instituto de Investigaciones Bioquímicas Bahia Blanca (INIBIBB-CONICET), Bahía Blanca, Argentina; Office of Cancer Genomics, National Cancer Institute, National Institutes of Health, Bethesda, MD; Laboratory of Human Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD.

The vast majority of experiments performed in different tumor cells indicate that HO-1 is a potent cytoprotective and antiapoptotic enzyme which improves survival of cancer cells subjected to different kinds of therapy. In glioma tumors HO-1 has been shown to be upregulated as compared with normal brain, although the significance of this upregulation is not clear. Therefore, the aim of our study was to perform a wide screening of heme oxygenase-1 (HO1) expression in gliomas by using tissue microarrays (TMA) containing astrocytomas (18), oligodendrogliomas (29), mixed tumors (12), glioblastoma multiforme (GBM, 57) and normal brain (18) and to correlate protein expression with patient clinic pathological data.. HO-1 showed cytoplasmic localization, although a few cells also presented nuclear staining. Nuclear HO-1 was confirmed in T98 and U87 cells. HO-1 was positive in 51% of GBM, 62% astrocytomas, 61% oligodendrogliomas, 42% of mixed and 22% of normal brain tissue. We found differences in HO-1 positivity rates between normal brain and oligodendrogliomas ($p=0.012$) and astrocytomas ($p=0.027$). Comparison by the Kaplan-Meier method revealed a significant decrease in overall survival of astrocytoma patients with HO-1 positivity ($p=0.03$, log rank test). We also performed RT-PCR for mRNA quantification, although no significant differences were observed between normal ($n=5$) and malignant samples ($n=5$). In conclusion, our results corroborate higher frequency of HO-1 protein expression in gliomas than in normal brain and show a correlation of HO-1 expression with patient survival.

NO. 43 -- Alejandro Curino, Instituto de Investigaciones Bioquímicas Bahia Blanca
HEME OXYGENASE-1 MODULATES BREAST CANCER PROGRESSION

Norberto A Gandini; Maria E. Fermento; Maria M. Facchinetti & Alejandro C. Curino - Instituto de Investigaciones Bioquímicas Bahia Blanca (INIBIBB-CONICET), Bahía Blanca, Argentina.

An increasing amount of evidence indicates that HO-1 activation may play a role in carcinogenesis and can potently influence the growth and metastasis of tumors. In breast cancer it has been shown to decrease proliferation and invasion. The aim of this work was to study the role of HO-1 in breast cancer progression. As a first approach we tested HO-1 expression in human breast cancer specimens evaluating its correlation with patient survival. Immunohistochemistry for HO-1 was performed in tissue samples from 60 patients with primary breast cancer. We found positive staining in 73% of specimens and protein expression was stronger in tumor than in non-malignant adjacent area (Mann-Whitney U Test; $p<0,05$). Interestingly, HO-1 was detected in the nuclei in 22% of the samples expressing HO-1. Analysis of prognostic significance revealed that HO-1 positivity associates with better patient outcome as assessed by Kaplan Meier (log-rank test; $p<0.05$). In order to better study the significance of HO-1 in breast cancer, we assessed the role of HO-1 on cellular survival and migration by using primary tissue cultures from breast tumors belonging to a DMBA-induced rat mammary carcinoma model and also breast cancer cell lines. Upregulation of HO-1 by hemin resulted in reduced cell survival as assessed by MTT, whereas inhibition of enzyme activity by SnPP had the opposite effect. Flow cytometry was used to confirm these results. No significant modulation of either cyclin D or cyclin E levels was observed. Nuclear localization was also observed in these cells. Additionally, cell motility was affected by HO-1 modulation. Taken together, these results show that HO-1 expression is upregulated in human breast cancer specimens and suggest that HO-1 may play a role in breast cancer progression by modulating cell proliferation and migration.

NO. 44 -- Maria José Alcaraz, Department of Pharmacology, University of Valencia
HEME OXYGENASE-1 DOWN-REGULATES HMGB1 IN OSTEOARTHRITIC SYNOVIOCYTES

Isabel Garcia-Arnandis[1], Isabel Guillén[1,2], Miguel Angel Castejón[3], María José Alcaraz[1] - [1]Department of Pharmacology, University of Valencia, [2]Cardenal-Herrera CEU University, Moncada, [3] Department of Orthopaedic Surgery and Traumatology, Hospital Universitario de la Ribera, Alzira, Spain.

Extracellular high mobility group box B1 (HMGB1) potentiates the effects of pro-inflammatory cytokines and could be involved in synovitis and arthritic diseases. We have examined the interactions between heme oxygenase-1 (HO-1) and HMGB1 in osteoarthritic synoviocytes in primary culture. Human synoviocytes from osteoarthritic patients were treated with human recombinant HMGB1 (5-50 ng/ml) in the presence or absence of IL-1 . HO-1 was induced by cobalt protoporphyrin IX (CoPP). Lentiviral HO-1.flag vector was also used for HO-1 overexpression. HO-1 gene silencing was achieved by using a specific siRNA. IL-1 stimulation of synoviocytes increased HMGB1 protein and mRNA expression,

Several lines of *in vitro* evidence supported the idea of a therapeutic role of dietary supplements in free radical-related diseases, such as neurodegenerative disorders, and the main contribution of heme oxygenase in this cytoprotective effect has been often emphasized. However, recent randomized, double-blind, placebo-controlled clinical trials clearly demonstrated that nutritional compounds do not have any significative effect on the cognitive performance in patients suffering from Alzheimer's disease (AD). In particular, curcumin at doses of 1-4 g/day for 6 months did not reduce both the lack in cognitive performance and the peripheral biomarkers of inflammation measured (serum Aβ-peptide and isoprostanes). Furthermore, in these patients, curcumin did not ameliorate the plasma lipid profile as hypothesized, rather a slight increase in cholesterol plasma level occurred. Oral *lichen planus* is a mucocutaneous inflammatory disease on which the therapeutic potential of curcumin was claimed. Thirty-three patients affected by oral *lichen planus* were treated with 2 g/day curcuminoids for 7 weeks and the change in symptoms and occurrence of side effects measured. After the first *ad-interim* analysis, no difference between the placebo and curcuminoid groups was detected and the trial was ended for futility. Gingko biloba is a nutritional supplement with neuroprotective effects *in vitro*. Recent clinical trials and meta-analysis clearly demonstrated that gingko biloba did not have any beneficial effect on the cognitive performance in AD patients. An important point to be considered when herbal nutrients are given to patients, is their interaction with drug-metabolizing enzymes. Both curcumin and gingko biloba was shown to affect the activity of different isoforms of cytochrome-P-450, glutathione-S-transferase and UDP-glicuronosyltransferase thus changing blood concentrations of several drugs. An important scientific and ethical issue is not to create false illusions in people affected by severe diseases, such as AD, regarding the therapeutic use of herbal nutrients, rather their potential toxic effects should be highlighted.

NO. 67 – Maria M Facchinetti, de Biología Básica del Cáncer, Instituto de Investigaciones Bioquímicas Bahía Blanca (INIBIBB-CONICET)

EXPRESSION OF HO-1 IN HUMAN LUNG CANCER AND ITS CORRELATION WITH CLINICAL DATA

Javier E Mendizabal[1&], Sol Degese[1&], Norberto A Gandini[2], Omar Coso[1], Alejandro Curino [2] & Maria M Facchinetti[2] - [1]Laboratorio de Fisiología y Biología Molecular, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina. [2]Laboratorio de Biología Básica del Cáncer, Instituto de Investigaciones Bioquímicas Bahía Blanca (INIBIBB-CONICET), Argentina & These authors contributed equally to this study.

An increasing amount of evidence indicates that HO-1 activation may play a role in carcinogenesis, potently influencing the growth and metastasis of tumors. Although several reports show a role for HO-1 in lung cancer, little is known about enzyme expression in human lung cancer specimens. Therefore, the aim of this work was to study HO-1 expression in human lung carcinoma samples and its correlation with clinicopathological data. As a first approach we tested HO-1 expression in 16 paraffin-embedded tumor specimens. Positive protein immunostaining was observed in 75 % (12) of the samples analyzed and HO-1 was mainly expressed by carcinoma cells. In order to perform a wide screening of HO-1 expression in lung cancer we used a tissue microarray (TMA) consisting of 330 cores including non-malignant lung tissue, adenocarcinoma, squamous cell carcinoma, carcinoid tumors, small cell carcinoma and large cell carcinoma. 92 % of tumor samples were positive for HO-1 and protein expression correlated with advanced stages ($p=0.021$, χ^2), lymph node involvement ($p=0.025$), and gender ($p=0.036$). Additionally, we performed RT-PCR in 19 samples of human lung carcinomas, detecting HO-1 mRNA in 84% of them. A significant correlation was observed between HO-1 mRNA levels and smoking habit ($p=0.004$) and T staging (TNM, $p=0.001$). In summary, these results present additional evidence of an important role for HO-1 in lung cancer.

NO. 69 – May F. Mrad, Department of Biochemistry, American University of Beirut

STATIN INDUCTION OF THE ANTI INFLAMMATORY HO-1 IN MURINE MACROPHAGES: TRANSCRIPTIONAL REGULATION AND ROLE OF NO

Charbel A. Mouawad, May F. Mrad, Mona S. Nasrallah, Georges Nemer, Jawed Alam* and Aida Habib - American University of Beirut, Dept. of Biochemistry, Beirut, Lebanon and * Dept. of Molecular Genetics, Ochsner Clinic Foundation, New Orleans, LA 70121, USA

Statins have pleiotropic effects independent of their capacity to lower cholesterol. Lipopolysaccharide (LPS) and nitric oxide (NO) are strong inducers of HO-1 in macrophages. The purpose of this study is to investigate the effect of statins on HO-1 expression in Raw 264.7, J774A.1 and in primary elicited murine peritoneal macrophages (eMPM) and to explore transcriptional regulation of HO-1 by statins. Induction of HO-1 was obtained with 25 μM simvastatin and 10 μM fluvastatin. Co-treatment of cells with simvastatin and increasing concentrations of spermine NONOate (SPNO), a nitric oxide donor or LPS resulted in an additional important expression of HO-1. Statins alone increased NO formation and iNOS expression in Raw 264.7 and J774A.1 and pretreatment with inhibitors of NO synthase blocked this effect. This induction was NO independent in primary eMPM prepared from BALB/C and C57BL/6. In Raw 264.7,