Cuyo Biology Society

XXXVII Scientific Meeting

5-6 dec 2019 - San Luis

A23

SESQUITERPENE LACTONES AFFECT THE REDOX SYSTEM OF *TRYPANOSOMA CRUZI*

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Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) and affects millions of people worldwide, mostly in Latin America. Despite its sanitary importance, there are currently only two drugs available for its treatment: benznidazole and nifurtimox, both exhibiting serious adverse effects on patients. In order to complete its life cycle, *T. cruzi* faces extreme environmental conditions –i.e. oxidative stress- as it propagates from an insect vector to a mammalian host, driving the transition from non-infective epimastigote to the infective form trypomastigote. It is known that the antioxidant defense system in the trypanosomatids is different from that in mammalian cells since the parasites have exclusive molecules and reducing enzymes. Because of this, the parasite redox machinery is an attractive target for antiparasitic therapies. The sesquiterpene lactone dehydroleucodine (DhL), is a trypanocidal molecule –containing an alpha-methylene group that could react with sulfhydryl groups of key redox enzymes. This study was focused on elucidating the DhL mechanism of action and extended to ten DhL derivatives (DC-X1 to DC-X10) obtained by chemical substitutions on the methylene group. We firstly confirmed an antiproliferative effect of DhL and its chemical derivatives, being DC-X6 one of the most active. The effect of DhL and DC-X6 was blocked by reduced glutathione, suggesting that compounds are reactive to sulfhydryl groups of certain molecules. Moreover, parasites overexpressing reducing enzymes, such as Tc-CPX, showed a protective effect against these STLs. Consistent with these results, both STLs increased ROS concentration in the wild type parasites. These results indicate that STLs induce oxidative stress on the parasites, possibly by affecting some crucial enzymes of the redox system.

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A SEMI-SYNTHETIC MOLECULE DERIVED FROM DEHYDROLEUCODINE AFFECTS THE *TRYPANOSOMA CRUZI* CELL CYCLE

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Trypanosoma cruzi is a parasite causing Chagas disease, which is endemic in Latin America, but in the last 20 years, it has expanded worldwide. The current treatment is restricted to Nifurtimox and Benznidazole, but both are relatively toxic and have limited efficacy on the patients. The development of new effective therapeutic agents is urgently needed. The sesquiterpene lactones (STLs) are natural compounds purified from native plants of Argentina with multiple pharmacological applications. The STL dehydroleucodine (DhL), has an alpha-methylene group that could react with multiple sulfhydryl group-containing proteins, affecting cellular functions such as proliferation, the activity mitochondrial, leading to the cell death/apoptosis. This study is focused on elucidating the action mechanisms of DhL and its derivative DC-X11, obtained by chemical substitution, on *T. cruzi* epimastigotes (strain Dm28c). We observed that DhL and DC-X11 have antiproliferative and cytostatic effects on the parasites. By morphological and ultrastructural studies, we observed an increase of parasites with multiple cell nuclei, kinetoplasts, or flagella after the treatment with DC-X11, suggesting an effect on late steps of the cell cycle (i.e., cellular division). These results were confirmed with parasites synchronized with hydroxyurea (HU 20 mM) for 24 h, and then they were treated with the compound. We concluded that the derivative DC-X11 inhibits *T. cruzi* proliferation by delaying the progression of the cell division. Further studies are necessary to identify the molecular targets affected by DC-X11.

A25

THE NEUROTOXIN OF AN AUTOCHTHONOUS *CLOSTRIDIUM BOTULINUM* AFFECTS THE ACTIN CYTOSKELETON IN BREAST CANCER CELLS

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Botulism is a neuroparalitic disease caused by botulinum neurotoxins (NTBo, serotypes A-G) produced by *Clostridium botulinum*, whose main reservoir is the soil (Su). Infant botulism is a toxi-infection, caused by the ingestion of spores, subsequent colonization, and the production of toxins *in situ*. The autochthonous NTBo would correspond to subtype A2 and have higher toxicity than A1

(Botox®), so in the future, they could be used as a therapeutic agent. The NTBos mechanism of action on certain pathologies is still to be clarified. Previous results from our laboratory showed that autochthonous NTBo 1935 from Su, degrades actin of rat brain homogenates, suggesting this protein could be an active target of NTBos. In this work, the action of this NTBo on the actin cytoskeleton in mammary tumor cells was evaluated. The NTBos of Su from strain 1935 and strain A Hall (both serotype A) were purified by saline precipitation. MCF7 cells (breast cancer cells) were cultured in Petri dishes or coverslips with 250 LD_{50} of the NTBos for 25, 45, or 90 min. After incubations, cells were processed for Western blot or immunofluorescence in order to evaluate the distribution and expression of actin. NTBo 1935 produced higher actin degradation and an increased location of this protein at the plasma membrane in comparison with A Hall in a time-dependent manner. However, at 90 min of treatment, we observed 90% of cytotoxicity, and further studies at this time were not evaluated. These results provide new insights about the NTBo mechanism of action and its possible use in the fight against breast cancer.

A26
A MELANOMA CELL LINE EXPOSED TO EXTREMELY LOW FREQUENCY MAGNETIC FIELDS: ASSESSMENT OF PROLIFERATION

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Extremely low-frequency magnetic fields (ELF-MFs) have been the axis of heated discussions for decades for their possible causal link to childhood leukemia. However, the ELF-MFs are interesting for the opposite reason: a possible therapeutic use. Indeed, there are several *in vitro* experiments reporting inhibition of cancer cell proliferation, and some *in vivo* studies also point in the same direction: significant reduction of tumor growth has been reported in mice with induced breast cancer tumors, sarcoma, and melanoma. In order to elucidate the effect of magnetic fields on the B-16 cell line (murine melanoma), we built two identical systems of coils of cubic geometry. Each one consisted of a triaxial system of 3 pairs of coils in an orthogonal arrangement. Then we proceeded to perform three experiments (each repeated three times). In all of them, cells were seeded in 96-well microplates (one "control" and one "exposed"), and cell viability was measured by the MTT assay at t = 72 h (beginning of exposure was considered time zero, t = 0 h). A negative control (or sham-exposure) was first conducted were both plates were subjected to the same field (static, vertical 50 microTeslas 'µT', "MF_{ref}"). In a second experiment, one of the plates was exposed to a 50 Hz 100 μT_{peak} alternating current (AC) field plus MF_{ref} while the other one was kept at MF_{ref}. In the third experiment, a gradient of the direct current (DC) field was evaluated. No significant differences were found between both plates in any of the three experiments. In summary, the combinations of AC/DC magnetic fields that we tested, for an exposure time of 1h did not affect the viability in the B-16 cell line. Probably, different field parameters, exposure durations and intermittence, as well as cyclic exposure patterns are necessary to obtain results of biological relevance and a possible therapeutic effect.

A27
EFFECTS OF PIOGLITAZONE-RETINOIC ACID ON DAILY RHYTHMS OF APO E IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) late onset, which constitutes 90% of cases, could be mainly attributable to deficiencies in the clearance of the Aß. Apolipoprotein E (Apo E) is associated with age-related risk for Alzheimer's disease and plays a key role in facilitating the proteolytic clearance of Aβ from the brain. ApoE expression is transcriptionally induced by PPARγ in coordination with RXRs. Taking into account those observations, the objectives of this study were: first, to analyze the effect of an i.c.v. injection of Aβ(1-42) on the 24-h rhythms of Aβ, BMAL1, RORα, and ApoE protein levels in the rat prefrontal cortex (PC); second, to evaluate the effect of pioglitazone-retinoic acid (Pio-RA) on those temporal patterns. Four-month-old male Holtzman rats were divided into three groups defined as: control, Aβ-injected (Aβ) and Aβ-injected treated with Pio-RA. Rats were maintained under 12 h-light:12 h-dark conditions before the sacrifice. Aβ, BMAL1, RORα, and ApoE proteins levels were analyzed by immunoblotting in PC samples isolated every 6 h throughout a 24-h period. The regulatory region of Apo E was scanned for E-box, RORE, RXRE, and PPRE sites. We found that an i.c.v. injection of Aβ (1-42) modified the daily variation of ApoE, BMAL1, RORα, and Aβ protein in the rat prefrontal cortex. Also, we found E-box, RXRE, and PPRE sites on the regulatory region of the Apo E gene. The treatment of Pio-RA reestablished the rhythmicity of those temporal patterns. These findings might constitute, at least in part, the molecular basis of the restoration of daily rhythmicity of Apo E by the administration of Pio-AR in AD.

A28
THE *IN VIVO* EFFECT OF NATURAL COMPOUNDS ON *LEISHMANIA* (L.) *AMAZONENSIS*

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