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(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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