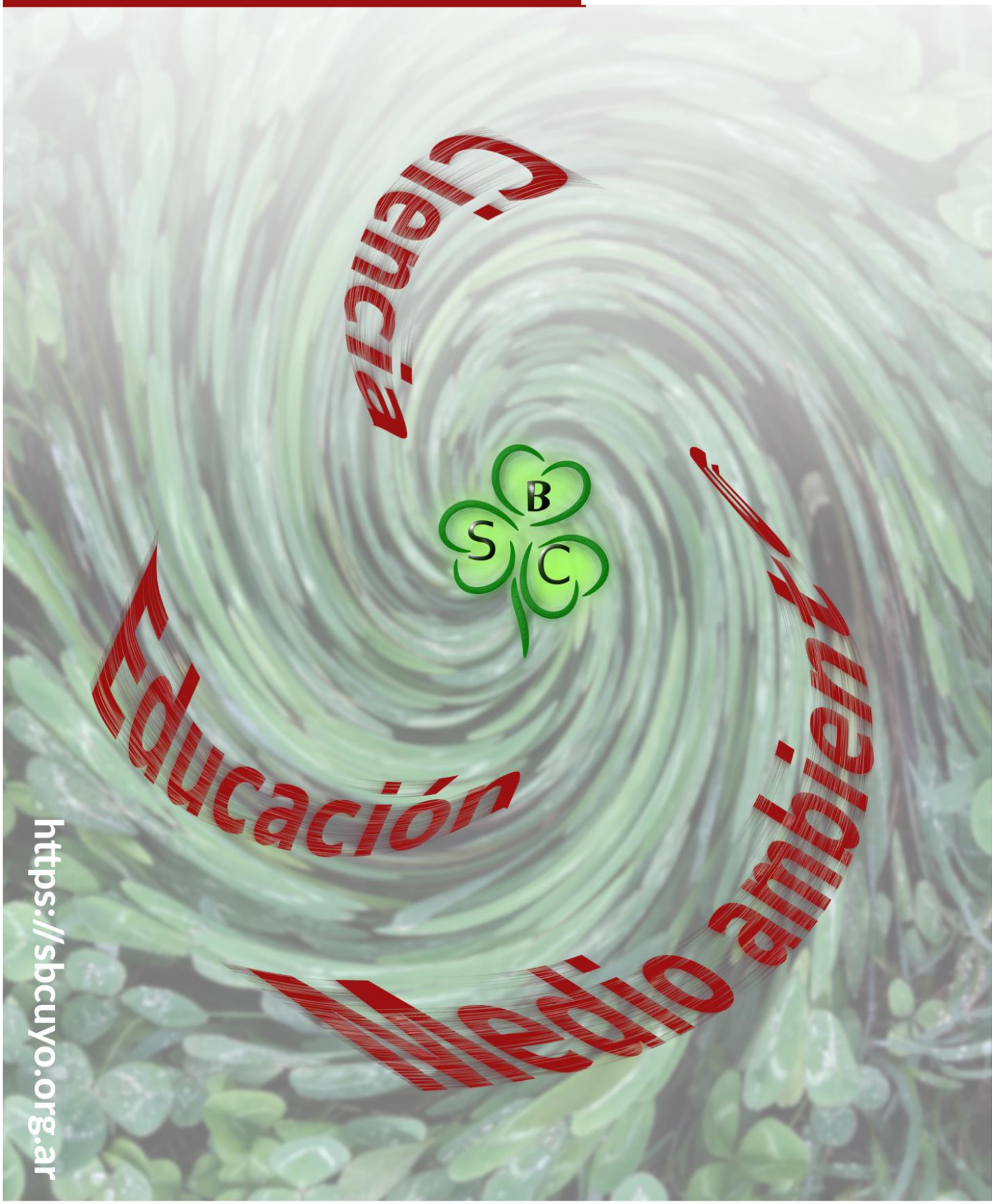
Sociedad de Biología de Cuyo

XXXVII Reunión Científica Anual

5 y 6 dic 2019 - San Luis



with HER2 positive BC could contribute to improve their prognosis and reduce the adverse effects of therapy because the TZ or TD doses applied would be lower due to the adjuvant effect of LP.

6. SYNERGISTIC ANTITUMOR ACTIVITY BY COMBINING RETINOIC ACID WITH FOCAL ADHESION KINASE INHIBITOR

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Breast cancer (BC) is a common malignant disease worldwide. Retinoids are currently being used in clinical trials to treat or prevent cancer progression and have been proposed as an adjuvant treatment of breast carcinoma because of its ability to inhibit cell growth. We have previously demonstrated that long retinoic acid (RA) treatment (10^{-6}M) reduced cellular adhesion and migration in BC cells. In addition, we verified that the administration of Trastuzumab (TZ) in combination with RA synergistically decreased cell survival, adhesion/migration/invasion in BC cells. TZ+RA strongly reduced Focal Adhesion Kinase (FAK) expression and induced nuclear FAK translocation. We speculate that RA+FAK inhibitor (FAKi) could reduce tumor growth and tumor formation by preventing tumor adhesion. We used LM3 cell line, derived from a murine mammary adenocarcinoma, with tumorigenic and metastatic capacity in BALB/c mice treated or not with FAKi for 72 h. We performed an orthotopic assay evaluating LM3 tumor growth in the mammary gland of female BALB/c bearing or not a slow-release RA (10 mg)-containing subcutaneous silastic pellet or an empty pellet as control. RA and FAKi separately reduce the tumor growth but the combined treatment induced a stronger inhibition in tumor volume. In addition, each drug seems to increase mice survival but only the combination of drugs is statistically significant. Furthermore, we also performed an experimental metastatic assay. Then LM3 cells pretreated or not with FAKi for 72 h were injected into the tail vein of mice bearing or not RA-containing pellet. RA significantly reduced lung metastatic dissemination. FAKi and the combination RA+FAKi presented a lower, but non-significant, number of lung nodules than the control group. In conclusion, the sensibility to RA therapies could be increased with FAKi coadministration in BC tumors.

7. EFFECT OF BOTULINUM NEUROTOXIN OF MENDOZA *Clostridium botulinum* STRAIN ON TUBULIN IN BREAST CARCINOMA CELLS (MCF 7)

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Botulinum neurotoxin serotype A (BoNT A) produced by *Clostridium botulinum*, causes botulism and is used to treat multiple diseases. Its potential action in cancer therapy is currently being evaluated. In precedent studies we have shown that BoNT A from native soil strains (Su), they have different characteristics to that of the prototype A Hall strain such as; greater specific toxic activity (AE) and differences in molecular structure and enzymatic activity against SNARE proteins.
In this work we study the action of a autochthonous BoNT A compared to the A Hall on tubulin in the MCF-7 cell line of breast carcinoma.
The autochthonous BoNTs of the strain Su 1935 (Tupungato) and A Hall were used in their native form, purified by saline precipitation. The values of AE (LD50 / mg protein), and electrophoretic characteristics under non-denaturing conditions were determined. MCF7 cells were cultured on coverslips and incubated with 250 and 500 LD_{50} of the BoNTs for 10, 25, 45 and 90 min. Later, the cells were fixed and processed for immunodetection. As primary antibodies were used anti-tubulin or anti-Golgina 97 and as secondary anti-mouse-Alexa 488. The preparations visualized by fluorescence microscopy. At 90 min incubation with 250 LD_{50} it was observed that ~ 90% of the cells were take off and deformed by the action of the BoNT A 1935 and ~ 40% for the BoNT A Hall, while with 500 LD_{50} both toxins were deleterious to the cells. When the cells were incubated with the toxins for 25 min, a disruption of microtubules with both toxins was observed, the effect being greater with the BoNT A1935. This effect was accompanied by a redistribution of the Golgi apparatus. Western blotting showed the shape of new tubulin bands, possibly due to protein degradation. This effect was also greater in the BoNT 1935. These results show a cytotoxic action of BoNT A with disorganization of cell microtubules, being observed with greater intensity in the cells treated with the autochthonous BoNT A. The degradation of tubulin and its intracellular reorganization would be part of the deleterious action of this toxin on tumor cells, opening new perspectives for therapy against solid tumors.

8. DIFFERENTIAL EXPRESSION AND LOCALIZATION OF BETA-CATENIN AND HSP27 AFTER CISPLATIN/DOXORUBICIN TREATMENT IN TRIPLE NEGATIVE BREAST CANCER CELLS

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The treatment of triple-negative breast cancers involves the administration of the conventional chemotherapeutic drug doxorubicin, given the lack of specific targeted agents. Novel therapeutic strategies, such as cisplatin, are currently being tested for these patients. Many studies have demonstrated that aberrant Wnt/β-catenin signaling serves a role in the development of breast cancer, while others have concluded that abnormal regulation of Wnt pathway induces tumor cell chemoresistance. The small heat shock protein 27 (HSP27)

is overexpressed in human breast cancer cells. As a result, cancer cells may suppress apoptosis and develop resistance to antineoplastic agents, such as doxorubicin. The present study sought to examine the role of the Wnt/β-catenin and HSP27 signaling pathway in response to cisplatin (CisPt)/doxorubicin (Doxo) treatment in human triple-negative (TN) breast cancer cell lines. Material and Methods: MDA-MB231 (TN) and MCF10A cell lines were used. Cell viability was measured using MTT assay and IC_{50} values were obtained after 48 h of CisPt or Doxo exposition. β-catenin and Hsp27 gene expression were measured by qPCR. Total and active β-catenin, phosphor ant total HSP27, phospho and GSK3β, phosphor and total p38 expressions were measured by western blot and immunofluorescence. 3D cell culture from MDA MB231 cells were treated with increasing concentrations of CisPt and Doxo for 48h. Results: MDA-MB231 cells showed higher IC_{50} values for CisPt and Doxo than the MCF10A cell line. In MDA-MB231 cells, the expression of β-catenin, active β-catenin, total and phospho-GSK3β and total HSP27 significantly decreased in the CisPt group ($p<0.05$). No changes were observed in Doxo-treated group. In MCF10A cells, the expression levels of total and active β-catenin did not modify with CisPt treatment, but in the Doxo group the proteins evaluated showed a tendency to increase. Also in MCF10A Doxo treatment significantly decreased the expression of GSK3β in comparison with control ($p<0.05$). In contrast, CisPt administration significantly increased phospho-GSK3β expression respect to the control group ($p<0.05$). Interestingly, in MDA-MB231 cells the nucleolus appeared disaggregated and active β-catenin increased at this subcellular localization after CisPt and Doxo treatment. In contrast, total β-catenin was preferentially localized in the Golgi. In the other hand 3D cell culture was more resistant to Doxo-treatment than 2D cell culture. CisPt induced a decrease in 3D cell culture growth. Conclusions: CisPt treatment was associated with decreased expression of β-catenin and HSP27. While, in Doxo-treated cells, as related to stable levels of β-catenin and increased expression of HSP27. The differential expression and localizations of β-catenin and HSP27 could be related to a differential cellular response depending on the cytotoxicity mechanism of chemotherapeutic agent used., that in turns affect the cell fate decision. Our preliminary data indicate that β-catenin and HSP27 may be potential therapeutic targets in TNBC.

9. EFFECT OF A PPARGAMA SYNTHETIC AGONIST ASSOCIATED WITH RETINOIC ACID ON THE 24-HOUR RHYTHMS OF BMAL1 AND RORα PROTEINS IN AN EXPERIMENTAL MODEL OF ALZHEIMER DISEASE

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Alzheimer's disease (AD) pathogenesis is associated to cognitive deficit and alterations in the circadian rhythms. Recently, PPAR-γ agonists have shown neuroprotective effects in neurodegenerative disorders. Previous studies indicate a role of retinoic acid in cognitive activities and anti-amyloidogenic properties. Previously, we found that an intracerebroventricular (i.c.v) injection of Aβ(1-42) modified the daily rhythms of Aβ and BMAL1 in the rat temporal cortex (TC). Continuing with that study, the objectives of this study were: first, to analyze the effect of an i.c.v. injection of Aβ(1-42) peptide on the 24h rhythms of RORα protein levels in the rat TC; second, to evaluate the effect of the PPARγ agonist, pioglitazone, along to the RXR ligand, retinoic acid, on those temporal patterns. Groups were defined as: 1) control (saline solution) 2) Aβ-injected (Aβ aggregates-10 µg) 3) Aβ-injected treated with Pio-RA (Aβ aggregates-10 µg) and (Pio 10mg/kg, ip)/AR (1mg/kg, ip) by 15 days. Rats were injected into the lateral ventricle (coordinates: AP:-1 mm, L:1.5 mm, and DV:-3.5 mm). TC samples were isolated every 4 h during a 24h period. Aβ, BMAL1and RORα proteins levels were determined by immunoblotting. To analyze the daily rhythmicity, 12 rats from each group were used. The data were analyzed by one-way ANOVA followed by the Tukey, a $p<0.05$ was considered to be significant. Daily rhythms were assessed by the Chronos-Fit software. We found that injection of Aβ(1-42) modified the daily rhythms of RORα protein level in the rat TC. The treatment of Pio-RA reestablished the daily rhythms of Aβ, BMAL1 and RORα protein levels. These findings would emphasize the importance of Pio-RA in the modulation of daily rhythmicity of clock gene in AD.

10. SESQUITERPENE LACTONES AFFECT THE REDOX SYSTEM OF *TRYPANOSOMA CRUZI*

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Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) and affects to 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 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 which could reacts 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.