

**Cuyo  
Biology Society**

**XXXVII  
Scientific Meeting  
5-6 dec 2019 - San Luis**

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<https://sbccuyo.org.ar>

performed MTT assays and to evaluate synergism and antagonism between drugs, we used Computer Software Program CompuSyn. Our results indicate that 72 h-treatments with different doses of TZ, TD, LP, and their combinations are effective decreasing SKBR3 and BT-474 cell proliferation, and LP has the greatest effect decreasing it. The SKBR3 cells are more sensitive to treatments than BT-474 cells. The analysis of pharmacological interaction in SKBR3 cells showed synergism in the combination TZ+LP (10:1 µg/mL) and TZ+LP (10:10 µg/mL), and in these concentrations, is possible a 10-fold TZ reduction achieving the same therapeutic effect. Additionally, TD+LP exhibited synergism in all doses tested, and in TD+LP (10:1 µg/mL) and TD+LP (10:10 µg/mL), is possible a 10-fold TD reduction achieving the same therapeutic effect. On the other hand, in BT-474 cells, we observed synergism between TD+LP in all doses tested, and only in the lowest dose for TZ+LP (1:0.1 µg/mL). A favorable 10-fold TZ or TD dose reduction is possible for combination 10:1 µg/mL and 10:100 µg/mL in TZ+LP and TD+LP. In conclusion, the coadministration of anti-HER2 therapies with different mechanism action in patients 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.

## A19

### 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 the 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's 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.

## A20

### 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 an 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 (LD<sub>50</sub>/mg protein) and electrophoretic characteristics under non-denaturing conditions were determined. MCF7 cells were cultured on coverslips and incubated with 250 and 500 LD<sub>50</sub> 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-Golgi 97 and as secondary anti-mouse-Alexa 488. The preparations visualized by fluorescence microscopy. At 90 min incubation with 250 LD<sub>50</sub>, it was observed that ~90% of the cells were taken off and deformed by the action of the BoNT A 1935 and ~40% for the BoNT A Hall, while with 500 LD<sub>50</sub> 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.

#### A21

### **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/ $\beta$ -catenin signaling serves a role in the development of breast cancer, while others have concluded that abnormal regulation of the 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/ $\beta$ -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<sub>50</sub> values were obtained after 48 h of CisPt or Doxo exposition.  $\beta$ -catenin and Hsp27 gene expression were measured by qPCR. Total and active  $\beta$ -catenin, phospho ant total HSP27, phospho and GSK3 $\beta$ , phospho and total p38 expressions were measured by western blot and immunofluorescence. 3D cell culture from MDA MB231 cells was treated with increasing concentrations of CisPt and Doxo for 48h. Results: MDA-MB231 cells showed higher IC<sub>50</sub> values for CisPt and Doxo than the MCF10A cell line. In MDA-MB231 cells, the expression of  $\beta$ -catenin, active  $\beta$ -catenin, total and phospho-GSK3 $\beta$ , and total HSP27 significantly decreased in the CisPt group ( $P < 0.05$ ). No changes were observed in the Doxo-treated group. In MCF10A cells, the expression levels of total and active  $\beta$ -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 GSK3 $\beta$  in comparison with control ( $P < 0.05$ ). In contrast, CisPt administration significantly increased phospho-GSK3 $\beta$  expression, respect to the control group ( $P < 0.05$ ). Interestingly, in MDA-MB231 cells the nucleolus appeared disaggregated, and active  $\beta$ -catenin increased at this subcellular localization after CisPt and Doxo treatment. In contrast, total  $\beta$ -catenin was preferentially localized in the Golgi. On 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  $\beta$ -catenin and HSP27. While, in Doxo-treated cells, as related to stable levels of  $\beta$ -catenin and increased expression of HSP27. The differential expression and localization of  $\beta$ -catenin and HSP27 could be related to a differential cellular response depending on the cytotoxicity mechanism of the chemotherapeutic agent used, which in turn affect the cell fate decision. Our preliminary data indicate that  $\beta$ -catenin and HSP27 may be potential therapeutic targets in TNBC.

#### A22

### **EFFECT OF A PPARGAMMA SYNTHETIC AGONIST ASSOCIATED WITH RETINOIC ACID ON THE 24-HOUR RHYTHMS OF BMAL1 AND ROR $\alpha$ PROTEINS IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE**

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Alzheimer's disease (AD) pathogenesis is associated with cognitive deficit and alterations in the circadian rhythms. Recently, PPAR- $\gamma$  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 A $\beta$ (1-42) modified the daily rhythms of A $\beta$  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 A $\beta$ (1-42) peptide on the 24-h rhythms of ROR $\alpha$  protein levels in the rat TC; second, to evaluate the effect of the PPAR $\gamma$  agonist, pioglitazone, along with the RXR ligand, retinoic acid, on those temporal patterns. Groups were defined as (1) control (saline solution), (2) A $\beta$ -injected (A $\beta$  aggregates-10  $\mu$ g), (3) A $\beta$ -injected treated with Pio-RA (A $\beta$  aggregates-10  $\mu$ g) 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. A $\beta$ , BMAL1 and ROR $\alpha$  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 Tukey's test, a  $P < 0.05$  was considered to be significant. Daily rhythms were assessed by the Chronos-Fit software. We found that the injection of A $\beta$ (1-42) modified the daily rhythms of ROR $\alpha$  protein level in the rat TC. The treatment of Pio-RA reestablished the daily rhythms of A $\beta$ , BMAL1, and ROR $\alpha$  protein levels. These findings would emphasize the importance of Pio-RA in the modulation of the daily rhythmicity of clock genes in AD.