BIO_17 - Evaluation of the *in vitro* antitumor effect of the association between doxorubicin and crotamine toxinAssessment of the *in vitro* antitumor effect of the association between doxorubicin and crotamine toxin

Vanessa Pinheiro Gonçalves Ferreira[1]; Juliana Ramos de Oliveira[2]; Cláudia do Ó Pessoa[3]; Andreimar Martins Soares[4]; Roberto Nicolete[5].
[1]Fundação Oswaldo Cruz (Fiocruz);
[2]Programa de Doutorado em Biotecnologia Rede Nordeste de Biotecnologia (RENORBIO – UECE);
[3]Núcleo de Pesquisa e Desenvolvimento de Medicamentos (UFC);
[4]Fundação Oswaldo Cruz Rondônia;
[5]Fundação Oswaldo Cruz Ceará.

Introduction: Doxorubicin (Dox), an anticancer drug long used in clinical practice, is indicated in the treatment of several types of cancer. However, like most chemotherapy drugs currently used, its effects end up reaching not only tumor cells, leading to side effects.

Objective: In this work, the crotamine toxin (Cta) (a polypeptide with a cell-penetrating protein attribute) isolated from the snake *Crotalus durissus terrificus* (rattlesnake) was used in combination with the drug doxorubicin to evaluate the antitumor effect *in vitro*.

Methodology: Colorimetric assay with MTT salt was performed to assess cytotoxicity using B16F10 murine melanoma cells. Tumor cells were plated (0.5×10^6) in a 96-well plate in RPMI medium supplemented with 10% FBS in a 5% CO_2 incubator $_{at}$ 37°C. After 24 h of incubation, the cells were treated with Cta (200, 1,000 and 5,000 nM), Dox (0.02 - 1 nM) and association (Cta + Dox) and subsequently incubated for 72 h in a 5% CO_2 incubator at 37°C. In another analysis, we used the CellASIC ONIX Microfluidic Platform (Merck), as a form of dynamic monitoring, in real time from start to finish, of phenotypic events of B16F10 tumor cells treated with DOX (0.2 nM) and in association with Cta toxin (200 nM) for more than 72h.

Results: The toxin used alone at the minimum concentration of 200 nM exerted 40% toxicity and at higher concentrations (1000 and 5000 nM) decreased cell viability more significantly. Dox, in turn, at concentrations (0.02 – 0.1 nM) showed toxicity between 20 and 80%. On the other hand, and surprisingly, the pharmacological association between Cta (200 nM) and Dox (0.2 nM) was able to exert cytotoxicity around 60%.

Conclusion: The combination of the nanocarrier toxin Cta with the chemotherapeutic Doxorubicin in minimal concentrations was able to improve the antiproliferative activity (potentiating effect) observed in B16F10 cells, thus contributing to new studies involving alternative/complementary therapies for the control of cell replication in different tumor lineages and /or *in vivo* models.

Keywords: Crotamine; Crotalus durissus terrificus; Cytotoxicity