BIO087

Evaluation of cytotoxicity and apoptosis induction of Oxo-monastrol in human hepatoma cell line C3A.

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Introduction: Chemotherapeutic agents used for cancer treatment have as one of their principal aims mitosis arrest. Despite the great advances obtained in cancer treatment with these agents is necessary to obtain new substances having different targets of not microtubules in order to reduce side effects and thus optimize chemotherapy and quality of life those in need. With the understanding of cell cycle and its chemical mediators began the search for new targets for anti-mitotic not involving the microtubules, thereby preventing some side effects such as neurotoxicity. Kinesins stood out in this quest. Among the motor kinesins related to mitosis, the protein EG5 has been studied for their potential for cancer therapy because of its importance for cell division. This protein is overexpressed in tumor cells compared to non-tumor cells. Due to the specificity of Monastrol to act on targets that are specific to tumor cells, were developed some analogues of this compound in order to enhance its effect and understand its mechanism of action. One of these analogues is the Oxo-monastrol, a precursor of Monastrol which the sulfur atom was replaced by an oxygen atom. It is believed that this analog, as well as monastrol, inhibits EG5 motor activity by allosteric inhibition which prevents ATP, no movement of centrossomos toward the poles, the spindle pole is not formed, and then, the cell is arrested in G2-M phase of cell cycle and is programmed cell death (apoptosis). **Objective:** Therefore, this study aims to evaluate the genotoxic potential of synthetic compound Oxo-monastrol in human liver carcinoma line, C3A, by cytotoxicity assay (MTT) and evaluate induction of apoptosis *in situ* by Hoechst 33342 staining. **Methods:** For the cytotoxicity assay (MTT) the cells were plated in 96 well plates and exposed to Oxo-monastrol (1, 10, 100, 200, 300, 400 and 500 µM) for 24 and 48 hours. The analysis of induction of apoptosis was based in pattern of nuclear fragmention DNA and chromatin condensation after 24 hours of exposure of C3A cells to Oxo-Monastrol (1, 10 and 100 µM). The assays was performed in three independent experiments and in the cell induction assay, 500 cells were analysed per treatment via fluorescence microscopy. Doxorubicin (1µM) was used as cytotoxic-inducing agent and Camptothecin (200 µM) as apoptosis-inducing agent. The data obtained were analyzed by analysis of variance (ANOVA) followed by Dunnett's test, using the program GraphPad Instat version 5.00. **Results:** In the cytotoxicity assay at 24 hours just concentration of 500 µM was cytotoxic as compared to control ($p < 0.001$), and after 48 hours of exposure, 300, 400 and 500 µM were also cytotoxic. In the analysis of the induction of apoptosis *in situ* any of the concentrations tested induced apoptosis. **Conclusion:** The data obtained showed that Oxo-Monastrol has a low cytotoxicity and did not induce apoptosis after 24 hours of exposure, suggesting that this molecule has not anti-tumor activity against hepatoma cell line C3A under the conditions tested, however more studies are needed.

Keywords: Monastrol, C3A, Apoptosis, Cytotoxicity

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