ANTITUMOR ACTIVITY OF METHYLCARBAMATES WITH MANNICH ADDUCTS AGAINST ORAL SQUAMOUS CELLS CARCINOMA

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Introduction

Oral squamous cell carcinoma (OSCC) is currently considered a public health problem in Brazil. Carboplatin and Cisplatin are the drugs most used in chemotherapy for this type of cancer, however, they have many side effects, such as nephrotoxicity and acquired resistance. Thus, the search for more effective chemotherapy drugs is justified. The present study aims to show the synthesis of eighteen synthetic substances from fused naphthoquinones Mannich-based adducts and testing them on OSCC models.

Material and methods

Compounds synthesis occurred through the fusion of lawsone to a Mannich reaction (MB1-18). Cytotoxicity and selectivity were evaluated by MTT assays in OSCC (SCC4, SCC9, SCC25) and fibroblast. The following assays were also carried out: hemolysis assays; clonogenic assay; *in vivo* acute toxicity (CEUA: 982); *in silico* prediction; molecular docking; caspase inhibitor assay; necroptosis inhibition; autophagy inhibition; flow cytometry and cell cycle assays.

Results and discussion

Among the eighteen 1,4-naphthoquinones tested, only the methyl benzyl (4-chlorophenyl) (3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl) methyl) carbamate (MB10) demonstrated a selectivity index higher than 2 (SI: IC_{50} on normal cells / IC_{50} on tumor cells) considering the 3 strains of OSCC (SCC9; SCC4 and SCC25) and normal mouth cells (human fibroblasts). The most selective substance (MB10) reduced the formation of clones in SCC9 via clonogenic assay. The tests for reactive oxygen species (ROS) demonstrated that the substance MB10 does not produce ROS in SCC9. *In vivo* tests showed that the LD_{50} of MB10 was around 150 mg/kg and signs of toxicity were observed in the lungs and kidneys in higher concentrations. *In silico* predictions of MB10 indicated that their chemical and biological characteristics by Lipinski's rule of five exhibit better drug profile than carboplatin and doxorubicin. The computational target fishing strategy used indicated that the MB10 could exert its anticancer action by inhibiting topoisomerase I and IIβ and hPKM2. Autophagy cell death followed by latte apoptosis was observed by the presence of autophagosomes and their inhibition through fluorescence microscopy, which is related to the Docking assay molecular structure suggesting that inhibition of hPKM2 leads to energy deprivation and consequently autophagy. The stress generated by the autophagic process leads to apoptotic cells, active caspases 3/7 intense DNA fragmentation, phosphatidyl serine

exposure and activation of effector caspases were observed latte. For the cell cycle analysis, it was shown that the substance does not cause an arrest in the cell cycle.

Conclusion

Furthermore, inhibition of autophagy by 3MA partially inhibited cell death that was further inhibited by co-treatment with pan-caspase inhibitor (ZVAD). The results indicated that 1 of the 18 Mannich bases synthetic naphthoquinone compounds has a good profile for the treatment of OSCC.

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