ASSESSMENT OF BIOLOGICAL ACTIVITY OF SYNTHETIC COUMARIN-BASED HYBRIDS WITH NAPHTHOQUINONE AND TRIAZOLE SYSTEMS AGAINST ORAL SQUAMOUS CELL CARCINOMA

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Introduction

Cancer is one of the leading causes of death worldwide in the last decade [1]. One important type of cancer is Oral Squamous Cell Carcinoma (OSCC), which accounts for approximately 90% of oral cancer cases [2]. It is a cancer characterized by a low overall 5-year survival rate in metastatic cases and a higher incidence in men than in women [3]. A relevant aspect in OSCC treatment is the aggressiveness of its treatment, which may involve the use of chemotherapeutic agents recognized for their side effects or surgical removal of malignant tissue, which can lead to functional loss, factors that reduce adherence to treatment and cause suffering in patients [4]. In this scenario, the synthesis of new hybrid compounds combining natural products and synthetic molecules with anticancer properties offers a promising approach to develop anticancer drugs with improved pharmacological activity and lower toxicity [5]. Examples of molecules used for synthesizing new compounds include coumarin, naphthoquinones and triazole systems which have been extensively studied for their biological properties. The objective of this work is to evaluate the *in vitro* cytotoxicity and selectivity of twelve newly synthesized hybrids that incorporate at least two of the three mentioned systems against OSCC.

Material and Methods

Cell Viability Assay. Cytotoxicity and selectivity of the hybrid compounds was evaluated using MTT cell viability assay. The OSCC lines SCC4, SCC9, SCC25 and primary normal human gingival fibroblast were treated with twelve different coumarin hybrids (CH 1-12) for 48 hours. Statistical analysis and determination of inhibitory concentration of 50% (IC_{50}) for the cells were performed using GraphPad Prism software. Cell viability was assessed after incubation with 0.5 mg/mL MTT reagent for 3.5 hours and absorbance was measured at 560 nm. Carboplatin and Shikonin were used as positive controls and DMSO negative control. *Hemolysis assay*. Surfactant potential of the coumarin hybrids was determined through a hemolysis assay using human erythrocytes, which was approved by the Research Ethics Committee of Universidade Federal Fluminense (CAAE: 43134721.4.0000.5626). Erythrocytes were incubated with 500 µM of coumarin hybrids for 45 minutes. The results were obtained by measuring the absorbance at 540 nm. PBS with glucose 10% and 1% Triton X-100 were negative and positive controls, respectively. *ROS production assay*. The induction of reactive oxygen species (ROS) was measured after treating SCC9 with the most selective coumarin hybrids. Cells were treated for 12 and 24 hours, and H_2O_2 production was detected using the ROS-Glo™ H_2O_2 assay kit (Promega) in bioluminescence assay. Result was obtained in the luminometer (Turner Design TD 20/20). Menadione served as positive control.

Results and Discussion

Cytotoxicity and Selectivity. Cytotoxicity of coumarin hybrids was assessed using SCC9, followed by SCC4 and SCC25. Among all compounds tested, only CH 1, 2 and 6 exhibited cytotoxicity (IC_{50} < 100 µM) with average IC_{50} values of 61.72 µM, 10.43 µM and 24.81 µM, respectively. Subsequently, these compounds were tested on primary normal human gingival fibroblasts, where the IC_{50} values for CH 1, 2 and 6 were determined as 177.70 µM, 22.77 µM and 30.90 µM. Using this data, the selectivity index (S.I.) was calculated, representing the degree of selective toxicity of the tested compounds against cancer cells. It is calculated as the ratio between the IC_{50} in cancer cells and the IC_{50} in normal cells. Compounds with S.I. greater than 2 are considered selective [6]. The selective hybrids were CH 1 (S.I.= 2.93) and CH 2 (S.I.= 2.25). CH 6 was not selective (S.I.= 1.24). Carboplatin had an average IC_{50} 210.2 µM in SCC and 347,70 µM in fibroblast. resulting in a S.I. of 1.65. Shikonin's average IC_{50} in SCC was 2.13 µM and in fibroblast, it was 1.17 µM, resulting in a S.I. of 0.54. *Hemolysis assay.* Erythrocytes are important subjects of study in the assessment of new compounds which enables the prediction of the toxicity of molecules [7]. After incubation, none of the coumarin hybrids exhibited significant hemolysis (<5%). *H_2O_2 production assay.* Induction of ROS production in cancer cells is one of the mechanisms underlying anticancer therapy as it enhances oxidative stress and leads to cell death, a process in which coumarin and naphthoquinone derivatives may be involved [8-9]. Treatment of SCC9 with coumarin hybrids for 12 hours significantly increased H_2O_2 production. CH 1 enhanced ROS production 2-fold, and CH 2 showed a 4-fold increase in ROS detection while control Menadione showed a 3-fold increase in ROS.

Conclusion

The results demonstrated that coumarin hybrids CH 1 and 2 were more selective than Carboplatin and Shikonin. Additionally, the coumarin hybrids exhibited lower IC_{50} than Carboplatin, drug used in the clinics. This suggests that lower doses of these hybrids may inhibit OSCC effectively, potentially reducing toxicity. Hemolysis assay indicated that the tested hybrid compounds lack surfactant potential, thus, they do not interact in a non-specific manner with biological membranes. ROS production assay suggests that the induction of H_2O_2 may be one of the mechanisms of action of CH 1 and 2. Ongoing studies involve the assessment of acute toxicity in C57BL/6 mice, investigation of cell death pathway, and conducting *in silico* assays to determine pharmacological profiling.

Acknowledgments

The authors thank CNPq, PROPPI, FAPERJ (E-26/210.068/2021 and E-26/210.915/2021) and CAPES (Finance Code 001).

Bibliographic References

[1] WHO: 'Global health estimates: Leading causes of death', 2019, Available in: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death.
[2] NHS: 'Overview Mouth Cancer', 2023, Available in: <https://www.nhs.uk/conditions/mouth-cancer/>
[3] Siegel, R., Miller, K., Fuchs, H., et al.: 'Cancer Statistics, 2022', CA: Can. J. Clin., 2022, 72, (1), pp. 1-93.
[4] ACS: 'Chemotherapy for Oral Cavity and Oropharyngeal Cancer', 2023, Available in: <https://www.cancer.org/cancer/types/oral-cavity-and-oropharyngeal-cancer/treating/chemotherapy.html>
[5] Bérube, G.: 'An overview of molecular hybrids in drug discovery', Exert Opin. Dru. Discov., 2016, 11, (3), pp. 281-305.
[6] Chipoline, I., Fonseca, A., Costa, G., et al.: 'Molecular mechanisms of action of new 1,4-naphthoquinones tethered to 1,2,3-1H-triazoles with cytotoxicity and selective effect against oral squamous cell carcinoma, Bioorg. Chem., 2020, 101, pp. 103984.
[7] Podsiedlik, M., Markowicz-Piasecka, M., Sikora, J.: 'Erythrocytes as model cells for biocompatibility assessment, cytotoxicity screening of xenobiotics and drug delivery', Chem. Biol. Interact., 2020, 332, pp. 109305.
[8] Wang, J., Huangfu, M., Li, X., et al.: 'Osthole Induces Apoptosis and Caspase-3/GSDME-Dependent Pyroptosis via NQO1-Mediated ROS Generation in HeLa Cells', Oxid. Med. Cell. Longev., 2022, 2022, pp. 1-22.
[9] Zhang, Y., Luo, Y., Piao, X., et al.: 'The design of 1,4-naphthoquinone derivatives and mechanisms underlying apoptosis induction through ROS-dependent MAPK/Akt/STAT3 pathways in human lung cancer cells', Bioorg, Med. Chem., 2019, 27, (8), pp. 1577-1587.