ACTIVITY OF DOXORUBICIN AND 5-FLUOROURACIL IN HUMAN MELANOMA A375 SPHEROIDS FORMED BY DIFFERENT METHODS

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Melanoma is the third most common form of skin cancer and is associated with a high risk of metastasis. Incidence and mortality rates are increasing each year, and it is predicted that the number of melanoma cases will continue to rise rapidly in the coming decades [1]. For these reasons, further research is required to improve our understanding of melanoma biology and to identify new effective anticancer drugs that could improve patient survival [2].

In this study, we evaluated the impact of in vitro 3D culture modelling of the human melanoma A375 on the activity of doxorubicin (DOX) and 5-fluorouracil (5-FU). These drugs were selected based on their differences in mechanism of action. DOX is a cytotoxic drug that inhibits topoisomerase II, causing DNA damage and cell death [3]. In contrast, 5-FU is a cytostatic drug that inhibits DNA and RNA synthesis, thereby suppressing cell proliferation [4]. Two of the most commonly used 3D culture formation methods were selected: the hanging drop method and the non-adhesive surface formation method [5].

Spheroid growth dynamics were assessed by phase-contrast microscopy through analysis of changes in spheroid diameter. To compare the effects of anticancer drugs in spheroids of different sizes, spheroids were generated using two initial cell numbers (250 and 2000 cells per spheroid). Following treatment with DOX and 5-FU,spheroids were incubated for 8 days. Drug activity was evaluated based on changes in spheroid diameter and cell viability, measured using the MTT assay. DOX transport into spheroids was assessed by measuring DOX fluorescence intensity over the total spheroid area using fluorescence microscopy.

In general, A375 spheroids formed using the non-adhesive surface method were more sensitive to DOX than spheroids formed using the hanging drop method. In smaller spheroids, 10 µM 5-FU inhibited viability more efficiently when spheroids were formed using the hanging drop method. DOX transport into spheroids varied depending on the concentration of DOX used. When spheroids were incubated with 10 µM DOX, accumulation was higher compared to incubation with 2 µM DOX. No statistically significant differences in DOX transport were determined in spheroids formed by different methods, nor in spheroids of different diameters (200 µm and 400 µm).

In conclusion, A375 spheroids formed using the non-adhesive surface method are more sensitive to DOX, while the effect of 5-FU varies depending on its concentration and the size of the spheroids. DOX transport into A375 spheroids does not depend on the formation method but is dependent on the DOX concentration used.

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