Doxorubicin and quercetin combined effect on SAOS-2 cells grown in 2D and 3D model systems

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INTRODUCTION: Osteosarcoma (OS) is a highly aggressive primary malignant bone tumor that most commonly affects children, adolescents, and young adults. The standard treatment for OS consists of surgical resection and chemotherapy, whereas radiation therapy is recommended for the unresectable tumor. Due to its easy metastasis and recurrence, the 5-year overall survival rate is only 66.5 % [1]. Thus, there is a critical need to recognize the molecular mechanisms underlying OS development and pathogenesis. Traditionally, two-dimensional (2D) cells are widely used in cancer biology and pre-clinical studies. However, 2D models are unable to mimic cell–cell and cell-extracellular matrix interactions which are crucial for adequate cellular function. Three-dimensional (3D) model systems are able to recapitulate key features of human cancer and are recognized as a promising platform for fundamental and translational research [2]. In the present work, we established an osteosarcoma 3D model based on alginate microbeads and studied the effect of combined treatment with doxorubicin (Doxo), widely used chemotherapeutic, and quercetin (Quer), a plant pigment with anticancer properties, on OS model systems.

EXPERIMENTAL: In our research, human permanent cell lines derived from osteosarcoma, SAOS-2 (ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (fetal bovine serum) and 1 % AA (antibiotic-antimycotic) at 37°C with 10 % CO_2. Cells were treated with doxorubicin (Ebewe), quercetin (Sigma), and their combination. The cells were immobilized in 1.5 wt.% alginate in the form of microbeads by manual extrusion followed by cultivation up to 21 days. Cell viability was determined using the MTT test, and viability rates were compared using Student's t-test with Graphpad Prism software. The experiment was performed in at least 3 technical replicates.

RESULTS AND DISCUSSION: Cells were successfully immobilized in alginate microbeads (diameter: ~1230 μm) and their viability significantly increased during the cultivation up to 21 days. Literature data have shown that quercetin could enhance chemotherapeutic effect of doxorubicin on cancer cells. Therefore, osteosarcoma cells were treated with both Doxo and Quer. Experimental results have shown that the combination of 1 μg/ml Doxo and 5 μM Quer significantly decreased the viability of SAOS-2 cells cultured in 2D conditions compared to cells treated with 1 μg/ml Doxo. On the other hand, viability of the cells cultured in 3D conditions treated with the same combination of Quer and Doxo did not show any statistically significant effect on cell viability. We can hypothesize that microenvironment-based mechanisms modulate doxorubicin sensitivity and increase resistance to treatment of osteosarcoma cells cultured in 3D conditions [2].

CONCLUSION: Collectively, quercetin sensitized osteosarcoma cells to doxorubicin in 2D model. However, in an *in-vivo* like 3D model system, the effect on of the combined treatment was not observed. Further research is needed to investigate the possible role of quercetin in tumor treatment.

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