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Targeting Tumour Survival and Angiogenic Migration after Radiosensitization with an Estrone Analogue in an in vitro Bone Metastasis Model

Authors : Jolene M. Helena, Annie M. Joubert, Peace Mabeta, Magdalena Coetzee, Roy Lakier, Anne E. Mercier

Abstract : Targeting the distant tumour and its microenvironment whilst preserving bone density is important in improving the outcomes of patients with bone metastases. 2-Ethyl-3-O-sulphamoyl-estra1,3,5(10)16-tetraene (ESE-16) is an in-silico-designed 2- methoxyestradiol analogue which aimed at enhancing the parent compound's cytotoxicity and providing a more favourable pharmacokinetic profile. In this study, the potential radiosensitization effects of ESE-16 were investigated in an in vitro bone metastasis model consisting of murine pre-osteoblastic (MC3T3-E1) and pre-osteoclastic (RAW 264.7) bone cells, metastatic prostate (DU 145) and breast (MDA-MB-231) cancer cells, as well as human umbilical vein endothelial cells (HUVECs). Cytotoxicity studies were conducted on all cell lines via spectrophotometric quantification of 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide. The experimental set-up consisted of flow cytometric analysis of cell cycle progression and apoptosis detection (Annexin V-fluorescein isothiocyanate) to determine the lowest ESE-16 and radiation doses to induce apoptosis and significantly reduce cell viability. Subsequent experiments entailed a 24-hour low-dose ESE-16-exposure followed by a single dose of radiation. Termination proceeded 2, 24 or 48 hours thereafter. The effect of the combination treatment was investigated on osteoclasts via tartrate-resistant acid phosphatase (TRAP) activity- and actin ring formation assays. Tumour cell experiments included investigation of mitotic indices via haematoxylin and eosin staining; pro-apoptotic signalling via spectrophotometric quantification of caspase 3; deoxyribonucleic acid (DNA) damage via micronuclei analysis and histone H2A.X phosphorylation (γ-H2A.X); and Western blot analyses of bone morphogenetic protein-7 and matrix metalloproteinase-9. HUVEC experiments included flow cytometric quantification of cell cycle progression and free radical production; fluorescent examination of cytoskeletal morphology; invasion and migration studies on an xCELLigence platform; and Western blot analyses of hypoxia-inducible factor 1-alpha and vascular endothelial growth factor receptor 1 and 2. Tumour cells yielded half-maximal growth inhibitory concentration (GI50) values in the nanomolar range. ESE-16 concentrations of 235 nM (DU 145) and 176 nM (MDA-MB-231) and a radiation dose of 4 Gy were found to be significant in cell cycle and apoptosis experiments. Bone and endothelial cells were exposed to the same doses as DU 145 cells. Cytotoxicity studies on bone cells reported that RAW 264.7 cells were more sensitive to the combination treatment than MC3T3-E1 cells. Mature osteoclasts were more sensitive than pre-osteoclasts with respect to TRAP activity. However, actin ring morphology was retained. The mitotic arrest was evident in tumour and endothelial cells in the mitotic index and cell cycle experiments. Increased caspase 3 activity and superoxide production indicated pro-apoptotic signalling in tumour and endothelial cells. Increased micronuclei numbers and γ-H2A.X foci indicated increased DNA damage in tumour cells. Compromised actin and tubulin morphologies and decreased invasion and migration were observed in endothelial cells. Western blot analyses revealed reduced metastatic and angiogenic signalling. ESE-16-induced radiosensitization inhibits metastatic signalling and tumour cell survival whilst preferentially preserving bone cells. This low-dose combination treatment strategy may promote the quality of life of patients with metastatic bone disease. Future studies will include 3-dimensional in-vitro and murine in-vivo models.

Keywords : angiogenesis, apoptosis, bone metastasis, cancer, cell migration, cytoskeleton, DNA damage, ESE-16, radiosensitization.
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