A COMPARATIVE STUDY OF THE ANTIPROLIFERATIVE ACTIVITY OF THIOSEMICARBAZONE IRON CHELATORS IN SKIN CELLS
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Although the promise of antitumour iron chelation therapy of cancer (ICT) is widely recognised, to date the potential of iron−chelators (ICs) in skin cancer has not been properly explored. A key challenge to be addressed is the observation in both animal and clinical trials that the antitumour properties of currently available ICs may be accompanied by severe toxic side effects upon repeated systemic administration. Further optimization of the dose, period of treatment and mode of administration therefore seems crucial for the success of ICT. The lipophilic nature of thiosemicarbazone ICs of the di−2-pyridyl− (i.e. Dp) and 2−benzoylpyrindine− (i.e. Bp) series makes them potentially attractive for treatment of non−melanoma skin cancer via topical application (1). As a first step towards this goal, in the present study, we investigated the antiproliferative potential of the Dp− and Bp− thiosemicarbazone analogues Dp44mT, Dp4pT, Bp4eT and Bp4pT using the human primary fibroblast cell line FEK4 and the spontaneously immortalised human keratinocyte cell line, HaCaT as models. The HaCaT cell line has proved a useful and reliable in vitro model of human skin cell carcinoma. This cell line is hyperproliferative and shows a significantly higher proliferation rate compared to normal human skin keratinocytes and FEK4 fibroblasts. The time−course and dose response studies with MTT assay revealed that both Bp− and Dp− analogues have a more pronounced growth inhibitory effect in HaCaT cancer cells than in normal FEK4 fibroblasts. The average IC50 values of the compounds in HaCaT cells were in the nanomolar range (i.e. 0.05−0.5 uM), while in FEK4 cells the average IC50 values were in the micromolar range (i.e. 1−5 uM). In HaCaT cells, Bp4pT appeared to be the most potent antiproliferative chelator with IC50 = 0.05 uM. In contrast in FEK4 cells, both Bp analogues and DP4pT yielded the same IC50 value of 1 uM. Moreover in FEK4 cells, Dp44mT appeared to be the least effective antiproliferative chelator (IC50 = 5uM). Nevertheless the IC50 values of the Bp− and Dp− thiosemicarbazones in both cell lines used in this study were 50−200 times lower than those obtained with desferrioxamine (DFO) and salicylaldehyde isonicotinoyl hydrazone (SIH) chelators with the same cell lines in this laboratory. In an attempt to relate these findings to an in vivo setting, clone forming assays were performed in parallel and the results showed that in agreement with MTT data, both Bp− and Dp−analogues have substantially higher antiproliferative activity than DFO and SIH. We also analysed the impact of the chelators on the cell cycle by flow cytometry, using bromodeoxyuridine incorporation. The results showed that both Dp− and Bp− analogues had an effective impact on the cell cycle in HaCaT cells with concentrations that were 100−200− fold lower than those necessary to obtain the same effect with SIH and DFO. The strong antiproliferative activities observed for both Dp− and Bp− chelators, together with their lipophilicity, therefore provide a rationale for their use in topical ICT of non−melanoma skin cancer.