SELECTION AND CHARACTERIZATION OF A HUMAN OVARIAN CANCER CELL LINE RESISTANT TO AURANOFIN

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Introduction

Auranofin, an oral gold (I) compound developed as an anti-arthritic drug, also exerts potent antitumor activity in in vitro and in vivo tumour models. The mechanisms of action of auranofin in cancer is not fully understood. The aims of this study were to develop and characterize an auranofin-resistant human ovarian cancer cell line from an auranofin-sensitive parental cell line (A2780) to improve the understanding of molecular mechanisms of auranofin cytotoxicity as well as to unravel the mechanisms responsible for resistance to gold compounds.

Materials and Methods

The auranofin resistant cell line was established from the parent ovarian cancer cell line A2780 by a continuous exposure to stepwise increasing auranofin concentrations. The growth inhibitory effects of auranofin as well as of other cytotoxic drugs and gold compounds against auranofin-resistant cells were determined by the sulforhodamine B assay. Determination of cellular gold accumulation in auranofin resistant and sensitive cell lines was performed by a Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). The mRNA expression levels of 24 candidate genes involved in cellular transport and/or trafficking pathways were evaluated by quantitative real-time PCR. Glutathione content in auranofin resistant and sensitive cells was determined by a spectrophotometric method. t-Tests were used for pairwise comparisons and p values <0.05 were considered statistically significant (Graph Pad Prism version 5).

Results

From the auranofin-sensitive human ovarian cancer cell line A2780 (IC50 0.73±0.19 M after 72 h drug exposure), a highly resistant (>20-fold) subline A2780/AF-R was developed (IC50 15.75±4.36 M after 72 h drug exposure). Marked reduction of gold accumulation occurred in auranofin-resistant A2780 cells compared with A2780 cells (338.0 pmol/106 cells vs 4630.0 pmol/106 cells at 480 min, respectively) while no changes in intracellular glutathione content were observed. Resistance to auranofin was associated with a low level of cross-resistance to some investigational gold compounds (cross resistance index, i.e. ratio between IC50 in A2780/AF-R and IC50 in A2780, ranging from 3.6 to 2.5) as well as to oxaliplatin and other anticancer drugs with different mode of action (i.e. melphalan, vinblastine, etoposide) (cross resistance index ranging from 3.9 to 1.9). Reduced gold accumulation was associated to substantial gene expression changes in various influx (e.g. SLC22A1, SLC47A1, SLCO1B1) and efflux (e.g. ABCB1, ABCC2, ABCC3) transporters. Verapamil partially reverted the resistant phenotype in A2780/AF-R cells. Co-exposure of A2780/AF-R cells to auranofin and verapamil 5, 10, 20 µM resulted in a 2.7-, 2.9- and 4.8-fold

increase in the cytotoxicity of auranofin, respectively. No substantial differences in the growth inhibitory effects of auranofin were observed after co-exposure with verapamil in the auranofin sensitive cell line.

Conclusions

These data provide evidence that multiple drug transporters may act as mediators of transport of auranofin and other gold compounds in cancer cells. Further investigation into the molecular mechanisms mediating transport of auranofin and new gold complexes in view of their potential clinical application in the treatment of cancer is warranted.