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Proadifen sensitizes resistant ovarian adenocarcinoma cells to cisplatin

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HIGHLIGHTS

- Proadifen potentiated the effect of cisplatin in resistant ovarian cancer cells.
- Proadifen decreased the levels of reduced glutathione primarily in A2780cis cells.
- Proadifen inhibited the activity of MRP1 and MRP2 primarily in A2780cis cells.
- Proadifen inhibited the expression of survivin in A2780cis cells.

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ABSTRACT

Proadifen (SKF-525A) is a P450 monooxygenase inhibitor with potential anti-proliferative activity and the ability to potentiate the toxicity of hypericin-mediated photodynamic therapy and mitoxantrone *via* alteration of ABC transport proteins. Elevated expression of some ABC transporters may also determine the efficacy of cisplatin-based chemotherapy. Thus, the purpose of this study was to investigate the ability of proadifen to sensitize A2780 and A2780cis ovarian cancer cells to cisplatin (CDDP). Herein, we show for the first time that proadifen sensitized resistant ovarian cancer cells to CDDP-induced cell death. The chemosensitizing effect of proadifen on CDDP action was also confirmed by MTT assays in multicellular spheroids. The possible mechanisms responsible for the enhanced cytotoxicity of proadifen/CDDP combined treatment may be attributed to a decrease of reduced relative glutathione levels, downregulation of multidrug resistance-associated proteins 1 and 2 (MRP1, MRP2) and attenuation of survivin expression. Taken together, our results indicate that proadifen is a promising compound for further *in vivo* experiments related to overcoming multidrug resistance and sensitization of resistant ovarian carcinoma to CDDP.

1. Introduction

Ovarian cancer is the seventh most common cancer and eighth known cause of death from cancers in women (Ferlay et al., 2014). Therapy includes surgical resection followed by chemotherapy with platinum derivatives, either alone or in combination with paclitaxel (McGuire et al., 1996). Cisplatin (CDDP) is a platinum-containing compound, which belongs to the first-line chemotherapeutic agents for the treatment of human ovarian cancer. However, successful treatment of patients is often limited by chemoresistance and developed recurrence (Parmar et al., 2003; Singer et al., 2005). Mechanisms responsible for clinical CDDP resistance are complicated and have not yet been well defined. Multiple factors associated with resistance to CDDP include decreased uptake of cisplatin into the cells, increased efflux by transport proteins or thiol-mediated detoxification, increased repair of DNA damage or increased tolerance to DNA damage and alterations in cell death pathways (reviewed in: Borst et al., 2008; Galluzzi et al., 2014).

Proadifen (SKF-525A), a well-known cytochrome P450 monooxygenase (CYP450) inhibitor, is a drug approved by the US Food and Drug Administration (Kretschy et al., 2013). It has

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been reported that proadifen is able to inhibit several CYP450s, including CYP2B6, CYP2D6, CYP2C9, CYP2C19, CYP3A4/5 and to a lesser extent, CYP1A2, CYP2A6 and CYP2E1 (Emoto et al., 2005; Franklin and Hathaway, 2008; Jones et al., 2007; Ono et al., 1996). For its inhibitory potential, proadifen should be included in the large class of non-steroidal anti-inflammatory drugs (NSAIDs) together with inhibitors of cyclooxygenases and lipoxygenases. Proadifen has been used to study the effects of P450 monooxygenase inhibition on xenobiotic biotransformation and hepatotoxicity induced by various drugs. Some studies documented the anti-proliferative and pro-apoptotic potential of proadifen in cancer cells of different tissue origin (Hoferova et al., 2004; Hofmanova et al., 2000; Jendzelovsky et al., 2012; Kleban et al., 2007). Moreover, we have previously demonstrated that proadifen affected the function of the MRP1 and BCRP transport proteins, increased intracellular hypericin content and potentiated the effect of hypericin-mediated photodynamic therapy (HY-PDT) in HT-29 colon adenocarcinoma cells (Jendzelovsky et al., 2009). Additionally, downregulation of BCRP and anti-apoptotic proteins by proadifen enhanced the toxicity of mitoxantrone in mitoxantrone-resistant promyelocytic leukaemia cells (Hilovska et al., 2015).

It is known that increased expression of some ABC transporters and anti-apoptotic proteins reduces the sensitivity of ovarian cancer cells to CDDP (Ma et al., 2009; Wang et al., 2015; Xing et al., 2012). Considering the above-mentioned results and assumptions, in the present study we investigated whether proadifen could modulate some of the molecular mechanisms responsible for the reduced CDDP toxicity and/or development of CDDP resistance. The impact of proadifen pre-treatment on CDDP action was analysed in CDDP-sensitive and CDDP-resistant ovarian adenocarcinoma cells.

2. Materials and methods

2.1. Cell culture and reagents

The human ovarian carcinoma cell line A2780 was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA), and its CDDP-resistant subline A2780cis was kindly provided by prof. Alois Kozubík (Institute of Biophysics, Brno, Czech Republic). For maintaining the resistance of A2780cis cells, we applied CDDP to the culture medium once a week at a 1 μmol/dm^3 final concentration. Cells were grown in complete RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS; Gibco) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml and amphotericin 25 μg/ml; Gibco) at 37 °C, 95% humidity and in an atmosphere of 5% CO_2.

Proadifen (PRO; SKF-525A; α-phenyl-α-propylbenzeneacetic acid 2-(diethylamino)ethyl ester; CAS No.: 62-68-0; Sigma–Aldrich, St. Louis, MO, USA) stock solution (10 mmol/dm^3) was prepared in distilled water and stored at −20 °C. Cisplatin (CDDP; *cis*-diamminedichloroplatinum; CAS No.: 15663-27-1) aqueous solution (0.5 mg/ml) was manufactured by EBEWE Pharma GmbH Nfg KG (Unterach, Austria). Indomethacin (INDO; CAS No.: 53-86-1; Sigma–Aldrich), MK-571 (CAS No.: 115103-85-0; Sigma–Aldrich) and Ko143 (CAS No.: 461054-93-3; Santa Cruz Biotechnology, Santa Cruz, CA, USA) stock solutions (10 mmol/dm^3) were prepared in dimethyl sulphoxide (DMSO) and stored at −20 °C. Working solutions of each of the above-mentioned reagents were always freshly prepared immediately before addition to the cell culture. The final concentrations of DMSO and distilled water did not influence the cytokinetic parameters. Because no significant differences in the response to diluents were observed, these data are considered the control.

2.2. Experimental design

For the experiments, cells were seeded in 96-well plates (MTT assay), in 6-well plates (flow cytometry analyses, quantification of cell number, analysis of relative glutathione levels) or in 60-mm Petri dishes (Western blot analysis) (all TPP, Trasadingen, Switzerland). Subsequently, cells were allowed to settle for 24 h before treatment (Fig. 1a,b).

2.2.1. Experimental scheme (a)

For the determination of the IC20 values (20% inhibitory concentration) of PRO and CDDP, MTT assays were performed 24, 48 and 72 h after PRO and CDDP addition (0 in the time schedule). Changes in relative glutathione levels were analysed 16 h after PRO treatment.

2.2.2. Experimental scheme (b)

Cells were pre-treated with PRO for 24 h (−24 h in the time schedule) prior to CDDP addition (0 in the time schedule). Changes in the metabolic activity, cell number, cell cycle distribution, mitochondrial membrane potential, cell death and phosphorylation of the H2AX histone were analysed 24 and 48 h after CDDP addition. The relative glutathione levels were examined 1 and 24 h after CDDP treatment. The expression of selected proteins was detected 1, 6 and 24 h after CDDP addition.

2.3. MTT assay

The MTT assays were performed as previously reported (Kleban et al., 2007) to evaluate changes in the metabolic activity of cells that occurred as the consequence of single- and combined-drug treatment. The results were evaluated as percentages of the absorbance (λ = 584 nm) of the untreated control. Proadifen and CDDP IC20 values were extrapolated from an exponential fit to the metabolic activity data using OriginPro 8.5.0 SR1 (OriginLab Corp., Northampton, MA, USA). According to changes in metabolic activity, the effect of proadifen on CDDP action was evaluated by CalcuSyn software (Biosoft, Ferguson, MO, USA) (Chou and Talalay, 1984).

2.4. Quantification of cell number

For the assessment of total cell numbers within individual experimental groups, cells were harvested at scheduled time points (Fig. 1b) and counted using a Coulter Counter (Model ZF, Coulter Electronics Ltd., Luton, Bedfordshire, UK). The total cell number was expressed as a percentage of the untreated control of the total cell number.

2.5. Cell cycle analysis

Flow cytometry analysis of the cell cycle was performed as previously reported (Kleban et al., 2007). The DNA content was analysed using a BD FACSCalibur flow cytometer (Becton

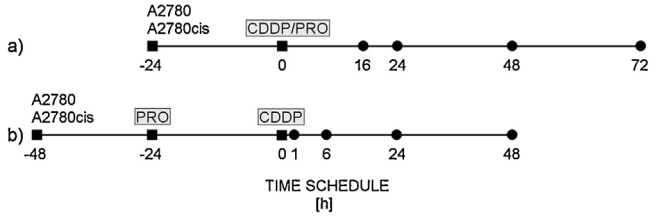

Fig. 1. Experimental design. (a) Analyses of single-drug treatment; (b) Analyses of combined-drug treatment.