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Increased resistance of tumor cells to daunorubicin after transfection of cDNAs coding for anthracycline inactivating enzymes

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Abstract

Carbonyl reduction is a main but undesired metabolic pathway of the anti-cancer drug daunorubicin (DRC). The resulting alcohol metabolite daunorubicinol has a far less anti-tumor potency and, in addition, is responsible for the life-threatening cardiac toxicity that limits the clinical use of DRC. Elevated levels of carbonyl-reducing enzymes in cancer cells may therefore contribute to the development of DRC chemoresistance and affect the clinical outcome. In the present investigation, human pancreas carcinoma cells were transfected with three important DRC reductases, namely carbonyl reductase (CBR1), aldehyde reductase (AKR1A1) and aldose reductase (AKR1B1), and levels of resistance towards DCR determined. Overexpression of all three reductases lead to a higher DRC inactivation and to an elevation of chemoresistance (7-fold for CBR1, 4.5-fold for AKR1A1 and 3.7-fold for AKR1B1), when IC_{50}-values were considered. Coadministration of DRC reductase inhibitors in DRC chemotherapy may be desirable since this would reduce the formation of the cardiotoxic alcohol metabolite and prevent drug resistance.

Keywords: Daunorubicin; Daunorubicinol; Drug resistance; Cardiotoxicity; Carbonyl reductase; Aldo–keto reductase

1. Introduction

Resistance towards anticancer drugs is a major problem in the chemotherapy of malignant tumors. The development of classical multidrug-resistance (MDR) is generally associated with a decrease of intracellular drug concentrations through an

Abbreviations: AKR1A1, human aldehyde reductase; AKR1B1, human aldose reductase; CBR1, human carbonyl reductase; DRC, daunorubicin; DRCOL, daunorubicinol.

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ATP-dependent efflux of the unmodified drug from the cell [1]. This form of MDR is the result of, at least in part, the activity of efflux transporters of the ATP-binding cassette (ABC) family such as ABCB1 (P-glycoprotein 170, P-gp), the ABCC (multidrug resistance-related protein, MRP) family and ABCG2 (breast cancer resistance protein, BCRP) [2–5]. Another mechanism of resistance towards chemotherapeutic drugs is the induced expression of drug metabolizing enzymes, like aldehyde dehydrogenase [6] or the phase II conjugating enzymes glucuronyl transferase and glutathione transferase [7–10].

Anthracycline antibiotics such as daunorubicin (DRC) and doxorubicin are among the most effective anti-cancer drugs ever developed [11]. Their main antineoplastic effect is topoisomerase II inhibition, DNA intercalation, and RNA synthesis inhibition [12,13]. Other proposed mechanisms of anthracycline action include generation of free radicals and induction of apoptosis [13]. As with any other anticancer agent, however, the clinical use of both doxorubicin and DRC soon proved to be hampered by such serious problems as the development of resistance in tumor cells or toxicity in healthy tissues, most notably in the form of chronic cardiomyopathy and congestive heart failure [13,14].

In addition to ATP-driven effluxes, metabolic inactivation by carbonyl reduction contributes to an acquired resistance of potent anthracycline chemotherapeutics like doxorubicin and DRC [15–20]. Carbonyl reduction is a phase I biotransformation reaction and takes place on the side chain C-13 carbonyl moiety of doxorubicin and DRC, resulting in the formation of the secondary alcohol metabolites doxorubicinol and daunorubicinol (DRCOL), respectively [21–23].

Since the 13-hydroxy metabolites doxorubicinol and DRCOL are significantly less potent than the parent drug in terms of inhibiting tumor cell growth *in vitro* [18–23], carbonyl-reducing enzymes are considered to constitute an important mechanism in the development of resistance towards these drugs. Moreover, doxorubicinol and DRCOL, but not doxorubicin and DRC, are thought to be responsible for the cardiotoxicity observed upon chemotherapy [19,24–26]. As a consequence, the pharmacologic inhibition of anthracycline carbonyl reduction may be cardioprotective without a loss of antineoplastic potency.

Responses to pharmacological agents in the human population are highly variable, owing in part to variations in gene expression. These differences require identification and characterization of the candidate protein. An important group of enzymes has been described, acting as carbonyl reductases towards the carbonyl group of doxorubicin and DRC. These enzymes belong to two protein superfamilies, the short-chain dehydrogenase/reductases (SDR) [27] and the aldo–keto reductases (AKR) [28]. SDR and AKR enzymes have overlapping substrate specificities including many endogenous and xenobiotic compounds [29–32]. Enzymes that are important for DRC carbonyl reduction to DRCOL have previously been identified in *in vitro* studies as being carbonyl reductase (CBR1; EC 1.1.1.184), aldehyde reductase (AKR1A1; EC 1.1.1.2) [33] and aldose reductase (AKR1B1; EC 1.1.1.21) [34]. Whereas human CBR1 is a member of the SDRs [29,30], AKR1A1 and AKR1B1 are important human AKRs [31,32].

For the present investigation, CBR1, AKR1A1 and AKR1B1 were cloned from a human liver genomic library and transiently expressed in pancreas carcinoma cells. The overexpression of these DRC reductases resulted in a higher DRC carbonyl reducing activity, which was paralleled by a several fold increase in resistance of the pancreas carcinoma cells towards DRC.

2. Materials and methods

2.1. Chemicals

DRC was supplied by Rhône-Poulenc Pharma GmbH and DRCOL was donated by Farmitalia Carlo Erba GmbH. All other chemicals were of highest commercially available grade.

2.2. Cell lines and culture conditions

The DRC-sensitive human pancreas adenocarcinoma cell line EPP85-181P was kindly provided by Prof. M. Dietel. The tumor cell line was grown as monolayer culture in Leibovitz L15 medium (PAA Laboratories) completed with the following supplements: 10% fetal bovine serum, 1 mM L-glutamine, 6.25 mg/l fetuin, 80 IE/l insulin, 2.5 mg/l transferrin, 1 g/l glucose, 1.1 g/l $NaHCO_3$, 1% minimal essential vitamins, 20,000 KIU (Kallikrein-Inhibitor-Units)/l Trasylol, 5 mg/l gentamicin and 200 mg/l piperacillin (Wyeth-Pharma) in a humidified atmosphere of 5% CO_2 at 37°. Mean population doubling times were approximately 10 h. The cells were free of mycoplasma as judged by staining with 4,6-diamino-2-phenylindole-dihydrochloride.