Toxicology and Applied Pharmacology 278 (2014) 238–248

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Anthracycline resistance mediated by reductive metabolism in cancer cells: The role of aldo-keto reductase 1C3

Jakub Hofman, Beata Malcekova, Adam Skarka, Eva Novotna, Vladimir Wsol *

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic

ARTICLE INFO

Article history:
Received 20 January 2014
Revised 29 April 2014
Accepted 30 April 2014
Available online 14 May 2014

Keywords:
Drug resistance
Metabolism
Aldo-keto reductase 1C3
Anthracyclines
Enzyme induction

ABSTRACT

Pharmacokinetic drug resistance is a serious obstacle that emerges during cancer chemotherapy. In this study, we investigated the possible role of aldo-keto reductase 1C3 (AKR1C3) in the resistance of cancer cells to anthracyclines. First, the reducing activity of AKR1C3 toward anthracyclines was tested using incubations with a purified recombinant enzyme. Furthermore, the intracellular reduction of daunorubicin and idarubicin was examined by employing the transfection of A549, HeLa, MCF7 and HCT 116 cancer cells with an AKR1C3 encoding vector. To investigate the participation of AKR1C3 in anthracycline resistance, we conducted MTT cytotoxicity assays with these cells, and observed that AKR1C3 significantly contributes to the resistance of cancer cells to daunorubicin and idarubicin, whereas this resistance was reversible by the simultaneous administration of 2′-hydroxyflavanone, a specific AKR1C3 inhibitor. In the final part of our work, we tracked the changes in AKR1C3 expression after anthracycline exposure. Interestingly, a reciprocal correlation between the extent of induction and endogenous levels of AKR1C3 was recorded in particular cell lines. Therefore, we suggest that the induction of AKR1C3 following exposure to daunorubicin and idarubicin, which seems to be dependent on endogenous AKR1C3 expression, eventually might potentiate an intrinsic resistance given by the normal expression of AKR1C3. In conclusion, our data suggest a substantial impact of AKR1C3 on the metabolism of daunorubicin and idarubicin, which affects their pharmacokinetic and pharmacodynamic behavior. In addition, we demonstrate that the reduction of daunorubicin and idarubicin, which is catalyzed by AKR1C3, contributes to the resistance of cancer cells to anthracycline treatment.

Introduction

Aldo-keto reductases represent an important superfamily of NAD(P)H oxidoreductase enzymes that metabolize a broad spectrum of carbonyl-bearing endogenous compounds and xenobiotics, which include steroid hormones, prostaglandins, retinaldehydes, monosaccharides, drugs, aflatoxins, polycyclic aromatic hydrocarbons and other toxicants (Hoffmann and Maser, 2007; Jin and Penning, 2007; Matsunaga et al., 2006; Penning and Drury, 2007). From a pharmacokinetic viewpoint, aldo-keto reductases belong to phase I biotransformation enzymes because the conversion of reactive aldehydic or ketonic drugs into corresponding hydroxy metabolites increase their water solubility and facilitate the elimination process.

A growing body of evidence indicates an eminent role of aldo-keto reductases in the development of tumorous diseases (Jin et al., 2006; Penning and Byrns, 2009). Aldo-keto reductase 1C3 (AKR1C3) is overexpressed in numerous cancers, which includes those cancers of the prostate, breast, uterine, blood, lung, brain and kidney, whereas its upregulation frequently correlates with tumor invasiveness and aggressiveness (Azzarello et al., 2009; Birtwistle et al., 2009; Jansson et al., 2006; Lin et al., 2004; Mahadevan et al., 2006; Miller et al., 2012; Nakamura et al., 2005; Park et al., 2010; Rizner et al., 2006; Stanbrough et al., 2006). In hormone-dependent prostate cancer, AKR1C3 converts androstenedione to testosterone, which, in turn, excessively activates androgen receptors and promotes tumor growth (Adeniji et al., 2013; Penning et al., 2006). Analogously, AKR1C3, which catalyzes the overproduction of testosterone followed by aromatization to 17β-estradiol, stimulates the proliferation of breast and endometrial cancer cells (Byrns et al., 2010; Rizner, 2012; Smuc and Rizner, 2009). In addition, AKR1C3 is able to directly reduce estrone and progesterone to 17β-estradiol and 20α-hydroxyprogesterone, respectively, thereby potentiating this pro-proliferative signal (Smuc and Rizner, 2009). By increasing proliferative PGF2 isomers and decreasing antiproliferative PGJ2 products, the prostaglandin F synthase activities of AKR1C3 have the potential to impact both hormone-dependent and hormone-independent cancers (Desmond et al., 2003; Khanim et al., 2009; Rizner, 2012). Considering the important role of

Abbreviations: AKR1A1, aldo-keto reductase 1A1; AKR1B1, aldo-keto reductase 1B1; AKR1B10, aldo-keto reductase 1B10; AKR1C3, aldo-keto reductase 1C3; CBR1, carbonyl reductase 1; qRT-PCR, quantitative real-time reverse transcription PCR; UHPLC, ultra-high performance liquid chromatography.

* Corresponding author. Fax: +420 495067168.
E-mail address: wsol@faf.cuni.cz (V. Wsol).

http://dx.doi.org/10.1016/j.taap.2014.04.027
0041-008X/

AKR1C3 in carcinogenesis, the modulation of this enzyme is of great clinical interest; considerable effort has been devoted to the development of potent and specific AKR1C3 inhibitors because such molecules could attenuate the pro-proliferative signaling that is induced by the activity of AKR1C3 in cancer cells (Brozic et al., 2011; Byrns et al., 2011). In addition to affecting intracellular pathways, AKR1C3 was recently shown to be implicated in the metabolism of clinically administered anthracyclines, doxorubicin and daunorubicin (Bains et al., 2010; Kassner et al., 2008; Novotna et al., 2008).

Since their first discovery over six decades ago (Brockmann and Bauer, 1950), anthracyclines have been recognized as efficient anticancer drugs. Over time, doxorubicin, daunorubicin and idarubicin have become a "gold standard" for the treatment of various cancers, such as hematological (leukemia, lymphoma) and solid breast, ovarian, lung and liver tumors (Hortobagyi, 1997; Minotti et al., 2004). Nevertheless, the clinical success of these agents is overshadowed by serious side effects, such as systemic toxicity, cardiotoxicity or drug resistance (Den Boer et al., 1998; Mordente et al., 2009). Anthracycline resistance can be elicited by various factors, such as increased drug efflux by ABC transporters, enhanced enzymatic detoxification, reduced availability of intracellular drug targets and defects in pathways that activate apoptosis (Den Boer et al., 1998). It has become widely accepted that the elevated enzymatic reduction of anthracyclines to their less potent secondary C13-hydroxy metabolites constitutes one of the mechanisms that cause pharmacokinetic anthracycline resistance in tumors (Ax et al., 2000; Gavelova et al., 2008; Heibein et al., 2012; Kuffel et al., 1992; Schott and Robert, 1989; Soldan et al., 1996). Several carbonyl-reducing enzymes (carbonyl reductase 1, CBR1; aldo-keto reductase 1A1, AKR1A1; aldo-keto reductase 1B1, AKR1B1; aldo-keto reductase 1B10, AKR1B10) have been demonstrated to participate in the resistance of cancer cells to doxorubicin, daunorubicin or idarubicin (Gavelova et al., 2008; Plebuch et al., 2007; Zhong et al., 2011). Although the role of AKR1C3 in doxorubicin resistance has previously been suggested (Heibein et al., 2012; Veitch et al., 2009), the possible contribution of AKR1C3 to the cell resistance to other anthracyclines has not been addressed to date and remains to be elucidated.

In the present paper, we aimed to investigate AKR1C3 reducing activity toward doxorubicin, daunorubicin and idarubicin by employing incubations with a purified recombinant AKR1C3 enzyme. Our investigation continued on the cellular level when we examined the reduction of daunorubicin and idarubicin by AKR1C3 inside transiently transfected HCT 116, MCF7, HeLa and A549 cancer cells. Consequently, we determined whether AKR1C3 activity can induce anthracycline resistance in cancer cells by employing an MTT cytotoxicity test. Finally, we focused on the changes in AKR1C3 expression after anthracycline treatment to determine whether intrinsic cell resistance, which is given by the normal expression of AKR1C3, might be potentiated by enzyme induction.

Materials and methods

Reagents and chemicals

Daunorubicin, idarubicin and the selective AKR1C3 inhibitor 2′-hydroxyflavanone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Doxorubicin, doxorubicinol and daunorubicinol were obtained from Toronto Research Chemicals (North York, ON, Canada). Idarubicinol was prepared as described previously (Skarka et al., 2011). BugBuster Protein Extraction Reagent was purchased from Merck Millipore (Darmstadt, Germany). $NADP^+$ and glucose-6-phosphate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glucose-6-phosphate dehydrogenase was supplied by Roche Diagnostics (Mannheim, Germany). Full-length AKR1C3 cDNA (accession no. BC001479) in the pOTB7 vector was purchased from ImaGenes (Berlin, Germany). All restriction enzymes were obtained from New England Biolabs (Ipswich, MA, USA). Shrimp alkaline phosphatase was purchased from Takara Bio (Shiga, Japan). jetPRIME was supplied by Polyplus Transfection (Illkirch, France). All oligonucleotide primers, a reverse transcription master mix, a qRT-PCR master mix and a TaqMan qRT-PCR system for the analysis of AKR1C3 mRNA expression were from Generi Biotech (Hradec Kralove, Czech Republic). TRI Reagent RT and 4-bromoanisole were purchased from Molecular Research Center (Cincinnati, OH, USA). Bradford reagent, MTT and a protease inhibitor cocktail were from Sigma-Aldrich (St. Louis, MO, USA). An anti-AKR1C3 antibody (ab84327), an anti-β-actin antibody (ab8226) and a secondary anti-mouse antibody (ab6789) were obtained from Abcam (Cambridge, MA, USA). A secondary anti-rabbit antibody was from Dako (Glostrup, Denmark). Amersham ECL Prime Reagent was purchased from GE Healthcare Life Sciences (Uppsala, Sweden). Cell culture reagents were supplied by Lonza (Walkersville, MD, USA) and by PAA Laboratories (Pasching, Austria). All other chemicals and reagents were of the highest purity that was commercially available.

Cell cultures

Human breast adenocarcinoma MCF7 cells were purchased from the European Collection of Cell Cultures (Salisbury, UK). HCT 116 (human colorectal carcinoma), HeLa (human cervical adenocarcinoma) and A549 (human lung carcinoma) cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in complete Dulbecco's modified Eagle's medium, which was supplemented with 10% fetal bovine serum, at standard conditions (37 °C, 5% CO_2). Routine cultivation and all experiments were performed in antibiotic-free medium. Cell lines were periodically tested for mycoplasma contamination. Cells from passages 10 to 20 were used in all experiments. Dimethyl sulfoxide was applied as a 2′-hydroxyflavanone solvent in concentrations that did not exceed 0.1%.

Cloning of AKR1C3-coding plasmid for transient transfections

The cDNA encoding the human AKR1C3 enzyme was amplified from commercially available AKR1C3 cDNA using primers that annealed with the 5′- and 3′-ends of the coding region and that had the following sequences: 5′-primer = 5′-CTC AGC CTC GAG ATG GAT TCC AAA-3′ (containing a XhoI restriction site) and 3′-primer = 5′-GTC AGC GGC CGC TTA ATA TTC ATC-3′ (containing a NotI restriction site). The PCR amplicon and the pCI mammalian vector were cleaved by XhoI and NotI-HF restriction enzymes. The pCI_AKR1C3 was generated by the ligation of linearized pCI vector and an insert, which contained the AKR1C3 gene. pCI_AKR1C3 was isolated from the competent HB101 strain of *Escherichia coli* after heat shock transformation and selection. The correct insertion of the AKR1C3 gene was confirmed by restriction analysis and sequencing.

pCI_AKR1C3 was applied in transient transfections and was further employed as a standard in qRT-PCR experiments. For the latter purpose, the pCI_AKR1C3 vector was linearized by BamHI, dephosphorylated by shrimp alkaline phosphatase and purified. Successful linearization was verified by agarose gel electrophoresis.

Cloning, overexpression and purification of recombinant AKR1C3

The cDNA encoding the human AKR1C3 enzyme was amplified from commercially available AKR1C3 cDNA using primers that annealed with the 5′- and 3′-ends of the coding region and that had the following sequences: 5′-primer = 5′-GGA ATT CCA TAT GGA TTC CAA ACA CCA-3′ (containing an NdeI restriction site) and 3′-primer = 5′-CGC GGA TCC TTA ATA TTC ATC TGA ATA TG-3′ (containing a BamHI restriction site). The PCR product was cloned into the bacterial expression vector pET15b employing the same procedure as described above with NdeI and BamHI restriction enzymes. The BL21(DE3) strain of *E. coli* was transformed with the prepared pET15b_AKR1C3 vector, and