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Cancer biomarker AKR1B10 and carbonyl metabolism

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Abstract

A member of the aldo-keto reductase (AKR) protein superfamily, AKR1B10, is overexpressed in human liver cancers as well as in many adenocarcinoma cases due to smoking. AKR1B10 is also detected in instances of cervical and endometrial cancer in uterine cancer patients. In addition, AKR1B10 has been identified as a biomarker for non-small-cell lung cancer by a combined bioinformatics and clinical analysis. Furthermore, in breast cancer cells, fatty acid biosynthesis is regulated by AKR1B10. AKR1B10 contains 316 residues, shares 70% sequence identity with aldose reductase (AKR1B1) and has the conserved Cys residue at position 299. Carbonyl groups in some anticancer drugs and dl-glyceraldehyde are converted by AKR1B10 to their corresponding alcohols. The anticancer drug daunorubicin, which is currently used in the clinical treatment of various forms of cancer, is converted by AKR1B10 to daunorubicinol with a K_m and k_{cat} of 1.1 ± 0.18 mM and $1.4 \pm 0.16 \text{ min}^{-1}$, respectively. This carbonyl reducing activity of AKR1B10 decreases the anticancer effectiveness of daunorubicin. Similarly, kinetic parameters K_m and k_{cat} (NADPH, DL-glyceraldehyde) for the reduction of dl-glyceraldehyde by wild-type AKR1B10 are 2.2 ± 0.2 mM and $0.71 \pm 0.05 \text{ sec}^{-1}$, respectively. Mutation of residue 299 from Cys to Ser in AKR1B10 reduces the protein affinity for dl-glyceraldehyde and enhances AKR1B10's catalytic activity but overall catalytic efficiency is reduced. For dl-glyceraldehyde reduction that is catalyzed by the Cys299Ser mutant AKR1B10, K_m is 15.8 ± 1.0 mM and k_{cat} (NADPH, DL-glyceraldehyde) is $2.8 \pm 0.2 \text{ sec}^{-1}$. This implies that the substrate specificity of AKR1B10 is drastically affected by mutation of residue 299 from Cys to Ser. In the present paper, we use this mutation in AKR1B10 to characterize a library of compounds regarding their different inhibitory potency on the carbonyl reducing activity of wild-type and the Cys299Ser mutant AKR1B10.

Keywords

Aldo-keto reductase; AKR1B10; Cancer; Chemotherapy; Inhibitor

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1. Introduction

Aldose reductase (AKR1B1) subfamily member AKR1B10 was first discovered as an enzyme overexpressed in human liver cancers [1–4]. Also, in smoking-associated cancers such as squamous cell carcinoma and adenocarcinoma AKR1B10 is overexpressed and considered as a potential diagnostic biomarker of smokers's nons-mall cell lung carcinomas [5].

One of the first identified anthracyclines, daunorubicin, was isolated in the early 1960s and then developed as an anticancer drug with widespread clinical use [6]. Today, daunorubicin is a key component in chemotherapy regimens for acute leukemia [7], and used in the treatment of lung cancer [6,8]. However, human myocardial tissue metabolizes daunorubicin to its secondary alcohol metabo-lite daunorubicinol which contributes to Fe(II) delocalization and drug-induced cardiac damage [9]. Moreover, daunorubicinol [10] has a reduced chemotherapeutic potential such that C-13 carbonyl reduction of daunorubicin can be regarded as drug inactivation [11,12]. Since AKR1B10 has been identified as a major daunorubicin reductase [10] and is overexpressed in tumor tissues, we aimed at identifying compounds that inhibit the AKR1B10 catalyzed reduction of daunorubicin.

AKR1B10 shares 70% amino acid sequence similarity with AKR1B1 [2], and carbonyl reduction activity of AKR1B1 is modulated by several fibrates [13–15]. However, sorbinil, an AKR1B1 inhibitor, was withdrawn from human clinical trials due to adverse side effects [16,17]. These adverse effects are believed to be caused by a closely-related enzyme of the AKR1B subfamily, namely aldehyde reductase (AKR1A1, EC 1.1.1.2) [18,19]. A critical amino acid residue found in AKR1B1 is Cys298 which, upon mutation and chemical modification, caused functional changes in the enzyme properties [20,21]. Replacement of residue Cys298 to Ser in AKR1B1 converted the enzyme from unactivated (low $V_{\max}$ /low K_m) to its activated form (high $V_{\max}$ /high K_m) which showed lowered sensitivity to sorbinil because the Cys298 residue is located in the active site[20].Hence this article concentrates on the role of residue Cys299 though there are other residues that may not be conserved in the AKR1B subfamily.

Bioinformatic and structural analyses have shown that in the AKR1B10 primary structure Cys299 represents the Cys298 homolog of AKR1B1 [22] which may therefore play a significant role in carbonyl reducing activity of AKR1B10. Moreover, due to this conserved Cys299 residue, AKR1B10 may be equivalently inhibited by fibrates. On the other hand, application of AKR1B10 inhibitors may result in the same side effects as have been observed e.g. upon inhibition of AKR1B1 with sorbinil. As a consequence, we felt necessary to seek for potent compounds that are capable of inhibiting AKR1B10 with less or no side effects. Since Cys298 in AKR1B1 has been postulated as being responsible for the side effects observed upon sorbinil inhibition, our strategy is to use, as a first step, the Cys299Ser mutant of AKR1B10 to identify and characterize potent AKR1B10 inhibitors that might be used in chemotherapy without causing side effects. In the present paper, we review the potential of selected fibrate derivatives to inhibit the carbonyl reducing activity of wild-type AKR1B10 and the Cys299Ser mutant thereof by using dl-glyceraldehyde and the anticancer drug daunorubicin as substrates.

2. Enzyme kinetic role of residue 299 in AKR1B10

The wild-type AKR1B10 reduces dl-glyceraldehyde with K_m , DL-glyceraldehyde, k_{cat} (NADPH, DL-glyceraldehyde) and k_{cat}/K_m values of $2.2 \pm 0.2 \text{ mM}$, $0.71 \pm 0.05 \text{ s}^{-1}$ and $0.32 \pm 0.03 \text{ s}^{-1} \text{ mM}^{-1}$, respectively (Fig. 1). The corresponding K_m , DL-glyceraldehyde, k_{cat} (NADPH, DL-glyceraldehyde) and k_{cat}/K_m values for the reduction of DL-glyceraldehyde catalyzed by the Cys299Ser mutant AKR1B10 (Fig. 1) are $15.8 \pm 1.0 \text{ mM}$, $2.8 \pm 0.2 \text{ s}^{-1}$ and $0.18 \pm 0.01 \text{ s}^{-1} \text{ mM}^{-1}$, respectively. The comparison of kinetic parameters for wild-type and the Cys299Ser mutant AKR1B10 indicates that substitution of serine for cysteine at position 299 reduces the enzyme affinity for DL-glyceraldehyde by about 7-fold, enhances its catalytic activity by about 3.9-fold and reduces the catalytic efficiency by about 1.8-fold. Substrate specificity as well as catalysis of AKR1B10 is all affected by the mutation of the residue 299 from Cys to Ser. Therefore, binding of dl-glyceraldehyde as well as its catalytic rate of reduction depend on the residue 299 in AKR1B10.

3. AKR1B10 inhibitor selectivity

Aldose reductase inhibitors, zopolrestat, Wy 14,643, sorbinil, gemfibrozil, fenofibrate, fenofibric acid, EBPC and ciprofibrate (Fig. 2) were tested for their inhibition potential against AKR1B10 activity of DL-glyceraldehyde reduction. As shown in Table 1, zopolrestat, sorbinil and EBPC follow pure non-competitive mode of inhibition, whereas Wy 14,643, fenofibrate, fenofibric acid and ciprofibrate track mixed non-competitive pattern of inhibition for wild-type AKR1B10 catalyzed reduction of DL-glyceraldehyde. Inhibition parameters shown in Table 2 reveal that compounds zopolrestat, Wy 14,643, fenofibrate, gemfibrozil and ciprofibrate have mixed non-competitive characteristics, whereas only fenofibric acid demonstrated purely non-competitive mode of inhibition for Cys299Ser mutant AKR1B10 catalyzed reduction of DL-glyceraldehyde. Overall, mutation of residue Cys299Ser does not affect the inhibition potential of zopolrestat, but negatively influences EBPC, Wy 14,643, sorbinil, ciprofibrate and positively impacts fenofibrate and gemfibrozil. The prodrug fenofibrate is more potent than its hydrolyzed fibrate derivative, fenofibric acid in the reduction of DL-glyceraldehyde that is catalyzed by wild-type (Table 1) as well as the Cys298Ser mutant (Table 2) AKR1B10. Similarly, fenofibrate is 25-fold more effective than fenofibric acid in inhibiting the AKR1B10 catalyzed reduction of daunorubicin as well (Table 3). Fifty percent inhibition activity of AKR1B10 catalyzed reduction of daunorubicin is achieved by zopolrestat and EBPC, 35–40% inhibition was observed with the other compounds, which can be grouped in terms of their inhibition potency as follows: fenofibrate, Wy 14,643 and sorbinil are comparable and more potent than ciprofibrate and fenofibric acid.

4. Preference for fenofibrate over fenofibric acid

Fenofibrate shows more inhibition potential than fenofibric acid in the reduction of dl-glyceraldehyde that is catalyzed by wild-type as well as the Cys299Ser mutant AKR1B10. This trend is preserved even in the AKR1B10 catalyzed reduction of daunorubicin. The superimposed structures of fenofibrate and fenofibric acid (Fig. 3) indicate that the carboxyl group in fenofibrate occupies a different orientation than in fenofibric acid [23]. Therefore,

the difference in the direction and the position of the carboxyl group with respect to the ketone carbonyl group found in the inhibitors might play a role in the molecular recognition that is required for the modulation of carbonyl metabolism.

Mutation of residue 299 in AKR1B10 from Cys to Ser did not alter the IC_{50} value for zopolrestat but affected sorbinil, Wy 14,643 and EBP Cnegatively and fenofibrate, fenofibric acid and gemfibrozil positively in their potency to inhibit carbonyl reduction of DL-glyceraldehyde. If indeed the side effects seen in the clinical trials of sorbinil are due to aldehyde reductase and the non-conservative replacement of residue Cys298 (aldose reductase residue numbering), then the different trends seen with fenofibrate, fenofibric acid and gemfibrozil will make these derivatives as critical lead compounds in the design of very specific AKR1B10 inhibitors that are not affected by the role of conserved residue 299.

5. Consequence of AKR polymorphism

Analysis of histological grade samples by differential twodimensional gel electrophoresis (2D-DIGE) along with mass spectrometry from 18 liver cancer patients revealed that AKR1B10 is differentially expressed in well differentiated hepatocellular carcinoma (HCC) [24]. HCC is one of the most common and aggressive human malignant tumors with especially high prevalence [25–27]. In addition, aldose reductase like proteins and their polymorphic members have been discovered in various circumstances such as colorectal [28] and uterine cancer [29], to mention a few. With the number of enzyme variants belonging to the AKR1B subfamily on the increase there will be a need to find specific inhibitors in order to modulate the activities of the alleles overexpressed under some deleterious conditions.

During ischemia and reperfusion aldose reductase is activated in the heart [30–32] leading to oxidation of Cys298 [33]. This observation implies that residue 298 may have a special role in the biology of the AKR1B subfamily members. On the other hand, the development of clinical cardiotoxicity in some patients hampers the use of anthracyclines (daunorubicin, doxorubicin) for cancer treatment [34]. The pathogenesis of anthracycline-related cardiotoxicity is implicated to be mediated by a combination of oxidative stress and intracardiac metabolic perturbations induced by the respective C-13 anthracycline alcohol metabolites (daunorubicinol, doxorubicinol) [35]. Besides AKR1B10, overexpression of carbonyl reductase (CBR1), aldehyde reductase (AKR1A1) and aldose reductase (AKR1B1) leads to a higher daunorubicin inactivation and to an elevation of chemoresistance (7-fold for CBR1, 4.5-fold for AKR1A1 and 3.7-fold for AKR1B1) [36]. Therefore, in any health condition that requires the clinical use of daunorubicin, overexpression of polymorphic variant enzymes that inactivate this chemotherapeutic anthracycline by carbonyl reduction may benefit from some of the fibrate inhibitors already available. Otherwise, if existing fibrate derivatives do not inhibit carbonyl reduction of daunorubicin, these fibrates could at least serve as lead compounds for the design of specific inhibitors for allelic variants of AKRs.

Mechanisms of fibrate action are anticipated to operate through aldose reductase, aldo-keto reductase and aldose reductase like proteins in addition to peroxisome proliferator-activated

receptors (PPARs) [15]. Also the extent of peroxisome proliferation induced by discrete fibrate derivatives varies. Furthermore fibrate treatment results in favor compared to adverse consequences when different species are considered [15]. Therefore the structural divergence present in the class of fibrates and the mode of their action in dissimilar tissues and species may become powerful criteria in the design of specific modulators for a target. Alternatively incorporating emerging relationship between the biomarkers of AKR polymorphic enzymes and chemically diverged fibrates would be one of the guiding steps in the selectivity and toxicity.

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Fig. 1.

A 15% SDS-PAGE gel corresponding to the purification of recombinant wildtype (lanes 2–4) and C299S mutant (lanes 5–8) AKR1B10. Lane assignments are as follows: protein marker (1 and 7), soluble proteins after cell lysis (2 and 6), extract of whole cells (3 and 5) and purified enzyme (4 and 8).

Fig. 2. Chemical structures of compounds studied. Ciprofibrate for 2-[p-(2,2-dichlorocyclopropyl)-phenoxy]-2-methylpropanoic acid, EBPC for Ethyl-1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate, Fenofibrate for the isopropyl ester of 2-[4-(4-chlorobenzoyl)-phenoxy]-2-methylpropanoic acid, Fenofibric acid for 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid, Gemfibrozil for 2,2-dimethyl-5-[2,5-dimethylphenoxy]-pentanoic acid, Sorbinil for 2,4-dihydro-6-fluorospiro[4H-1-benzopyran-4,4imidazolidine]-2-dione, Wy 14,643 for 4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid and Zopolrestat for 3,4-dihydro-4-oxo-3-{[5-(trifluoromethyl)-2benzothiazolyl]methyl}-1-phthalazineacetic acid

Fig. 3. View down the plane of the phenoxy ring along the C=O double bond direction of the ketone moiety. Fenofibric acid and fenofibrate are superimposed with green and cyan color, respectively.

Table 1

Inhibition kinetics parameters for dl-glyceraldehyde reduction activity of wild-type AKR1B10 by various inhibitors.

Compound	Inhibition constant (μM)			Mode of inhibition
	IC_{50}	K_{ii}	K_{is}	
Ciprofibrate	210	190	240	mNC
EBPC	12	13	13	pNC
Fenofibrate	25	30	27	mNC
Fenofibric Acid	300	220	250	mNC
Sorbinil	100	80	90	pNC
Wy 14,643	60	60	45	mNC
Zopolrestat	8	10	10	pNC

mNC—mixed non-competitive; pNC—pure non-competitive; K_{is} —is the slope inhibition constant and K_{ii} —is the intercept inhibition constant.

Table 2

Inhibition kinetic parameters for dl-glyceraldehyde reduction activity of Cys299Ser mutant AKR1B10 by various inhibitors.

Compound	Inhibition constant (μM)			Mode of inhibition
	IC_{50}	K_{ii}	K_{is}	
Ciprofibrate	ND	815	705	mNC
Fenofibrate	10	11	16	mNC
Fenofibric acid	220	250	240	pNC
Gemfibrozil	380	415	370	mNC
Wy 14,346	350	360	240	mNC
Zopolrestat	9	11	12	mNC

mNC–mixed non-competitive; pNC–pure non-competitive; ND–not detectable; EBPC and sorbinil did not show measurable levels of inhibiting Cys299Ser mutant AKR1B10.

Table 3

Inhibition potency (percentage inhibition) of various inhibitors for the reduction of daunorubicin by AKR1B10 wild-type protein.

Inhibitors	Concentration (M)	Inhibition (%)
Zopolrestat	20	43
	50	47
EBPC	100	54
	250	57
Fenofibrate	20	35
Wy 14,643	250	37
	500	50
	100	31
Sorbinil	250	37
	250	33
	500	39
Fenofibric acid	250	25
	500	35