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Fibrates in the Chemical Action of Daunorubicin

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Abstract

Anthracyclines are an important reagent in many chemotherapy regimes for treating a wide range of tumors. One of the primary mechanisms of anthracycline action involves DNA damage caused by inhibition of topoisomerase II. Enzymatic detoxification of anthracycline is a major critical factor that determines anthracycline resistance. Natural product, daunorubicin a toxic analogue of anthracycline is reduced to less toxic daunorubicinol by the AKR1B10, enzyme, which is overexpressed in most cases of smoking associate squamous cell carcinoma (SCC) and adenocarcinoma. In addition, AKR1B10 was discovered as an enzyme overexpressed in human liver, cervical and endometrial cancer cases in samples from uterine cancer patients. Also, the expression of AKR1B10 was associated with tumor recurrence after surgery and keratinization of squamous cell carcinoma in cervical cancer and estimated to have the potential as a tumor intervention target colorectal cancer cells (HCT-8) and diagnostic marker for non-small-cell lung cancer. This article presents the mechanism of daunorubicin action and a method to improve the effectiveness of daunorubicin by modulating the activity of AKR1B10.

Keywords

Fibrate; AKR1B10; cancer; AKR; daunorubicin

INTRODUCTION

Mechanism of chemical action of a target plays an important role in the efficacy of a drug that is in therapeutic use as well as obtaining clinical approval for a potential reagent. Aldose reductase [1] and aldose reductase like protein, AKR1B10 [2, 3] are targets of action for antilipidemic drugs, fibrates which have demonstrated clinical use. Expression of these proteins reduces the drug resistance due to their catalytic power for daunorubicin [4]. This article focuses on an approach to increase the chemotherapeutic benefits of daunorubicin with the combined application of fibrates.

ANTHRACYCLINES AND THEIR THERAPEUTIC APPLICATION

Natural products like daunorubicin is traditionally produced from microorganism *Streptomyces peucetius* and *Streptomyces coeruleorubidus* by fermentation process [5, 6].

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Daunorubicin is an antitumor antibiotic with proven clinical use as a powerful agent in leukemias, lymphomas, sarcomas, and solid breast, ovary, and lung tumors [7]. This is a compound with a glycoside bond linking a tetracyclic aglycone possessing a substituted anthraquinone chromophore and the aminosugar [5]. Anthracyclines are routinely employed in combination regimes with other groups of medications in which each drug generally exhibits a different mechanism of action to increase tumor cell killing and to minimize induced resistance to these drugs.

MECHANISM OF ANTHRACYCLINE ACTION

The mechanism of action and the cytotoxicity caused by daunorubicin analogues are not completely understood. However multiple mechanisms are beginning to be recognized for anthracyclines derivatives. Some of them are; (1) induction of topoisomerase II mediated DNA strand breaks, (2) generation of reactive oxygen species (ROS) mediated lipid peroxidation and (3) topoisomerase II independent doxazolidine mediated apoptosis.

The nucleic acid topology is modified through nuclear processes replication, transcription, chromatin remodeling, chromosome condensation/decondensation, recombination and repair by DNA topoisomerases [8–10]. Though DNA topoisomerases can introduce breaks into DNA segments topoisomerase II transport a double helix through a double-stranded cut of another duplex. However topoisomerase activity can be harmful to cells due to the action of small compounds [9, 11–13], known as topoisomerase poisons. These compounds transform topoisomerase II into lethal DNA-damaging agents by stabilizing an enzyme-DNA complex [9, 11, 14].

UPREGULATION OF AKR1B10 IN TUMOR

Biomarker, AKR1B10, Aldo-Keto Reductase (AKR) protein family member, is overexpressed in most instances of squamous cell carcinoma (SCC) and adenocarcinoma. Both SCC and adenocarcinoma are associated with metabolism of tobacco carcinogens [15]. The AKR1B10 belongs to aldose reductase (AR) subfamily (AKR1B), was discovered as an enzyme upregulated in human liver cancers [16–19] and detected in 20.0% cervical cancer cases and 15.8% endometrial cancer cases in samples from uterine cancer patients [20]. In addition, statistical analysis indicated that AKR1B10 expression was associated with tumor recurrence after surgery and keratinization of squamous cell carcinoma in cervical cancer [20]. AKR1B10 is upregulated during tumorigenic transformation of human mammary epithelial cells [21]. Also, bioinformatics analysis of the public gene expression data and validation of clinical specimens reveal AKR1B10 as a potential diagnostic marker for non-small-cell lung cancer [22]. Moreover, studies on the effect of small interfering RNA (siRNA)-mediated downregulation of AKR1B10 on proliferation of colorectal cancer cells (HCT-8) estimated its potential as a tumor intervention target [23]. Besides AKR1B10 is a novel regulator of the biosynthesis of fatty acid which is an essential component of the cell membrane in breast cancer cells [21].

AKR1B10 ACTIVITY

Daunorubicin, utilized in the treatment of lung cancer, is reduced by AKR1B10 in the presence of NADPH with the catalytic efficiencies of $k_{cat}/K_m = 1.3 \text{ mM}^{-1}$. The AKR1B10 catalyzed reduction of the carbonyl group in daunorubicin results in the formation of the corresponding alcohol at atom position 13 (Fig. 1). The alcohol that is formed, daunorubicinol, is inactive towards interacting with topoisomerase II. The reduction of the activity of anthracyclines leads to drug resistance. This is largely because of the alcohol metabolites of anthracyclines have been shown to exhibit significantly reduced anticancer properties [24, 25]. Furthermore the toxic effects on the heart associated with anthracycline-based cancer treatment are largely attributable to anthracycline alcohol metabolite(s) that form and accumulate in cardiac cells. These metabolites are known to disrupt several key processes in heart muscle and thus impair heart function [26].

SIDE EFFECTS OF ANTHRACYCLINES

Serious side effects of anthracyclines include acute cardiac injury and chronic congestive heart failure [27]. Acute toxicity is known to develop immediately after anthracycline treatment begins and consists of brief and usually manageable arrhythmias and hypotension [28]. Other cardiac effects are irreversible and limit the dosage of anthracycline, restricting lifetime cumulative dose [29]. Also decreases in the left ventricular ejection fraction (LVEF) seen in studies involving treatment of childhood cancer have been correlated with high dosage of anthracycline. Furthermore a clinical study indicated that an estimated cumulative 26% of patients would experience anthracycline-related Congestive Heart Failure at a cumulative dose of 550 mg/m^2 [30]. Anthracyclines induce acute cardiac lesions through effects on the sarcoplasmic reticulum (SR) in adults. Anthracyclines exhibited effects similar to those of caffeine, an agent known to render the SR nonfunctional by the depletion of the releasable SR calcium pool [31]. The most severe damage is cardiomyopathy, which has permanent effects and is related to high peak plasma anthracycline [32]. Cardiomyopathy develops from defense against free radical damage to the myocytes and repeated damage to the mitochondria [33, 34].

ACTIVE SITE AND SUBSTRATE SPECIFICITY OF AKR1B10

Aromatic interactions play a role in the binding of the tetracyclic moiety of daunorubicin in the AKR1B10 active site (Fig. 2). The cavity made by residues Gly19, Thr20, Trp21, Lys22, Asp44, Tyr49, Lys78, Trp80, His111, Trp112, Gln114, Phe116, Phe123, Lys125, Gly129, Ser160, Asn161, Gln184, Tyr210, Ser211, Pro212, Leu213, Ser215, Pro216, Asp217, Pro219, Leu229, Trp220, Ala246, Ile261, Pro262, Lys263, Ser264, Thr266, Arg269, Glu272, Cys299, Val301, Gln303 and Ser304 create space for the binding of cofactor and substrate in AKR1B10. The substrate binding pocket is encompassed by the nicotinamide ring of NADP^+ and residues Trp21, Val48, Tyr49, Lys78, Trp80, Pro81, Thr82, His111, Trp112, Gln114, Phe116, Phe123, Lys125, Ala131, Asn161, Tyr210, Cys299, Val301, Gln303 and Ser304. In addition to the polar and van der Waals interactions, aromatic interactions play a critical role in the binding of daunorubicin to AKR1B10.

CATALYTIC MECHANISM OF AKR1B10

The catalytic mechanism of AKR1B10 is anticipated to involve a stereo specific transfer of the pro-R hydride from C4 position of the nicotinamide. The regiospecific transfer of the hydride to the re-face of the substrate carbonyl carbon atom of the carbonyl double bond leads to the formation of alcoholate anion. This hydride transfer is followed by protonation of the substrate carbonyl oxygen anion by an enzyme functional group. Based on the site-directed mutagenesis, kinetic, structural, quantum mechanical and molecular mechanics studies performed on aldose reductase it is proposed that the proton is donated from Tyr48. The Tyr48 mediated proton donation is facilitated by an extended network of hydrogen bonding interactions involving residues Asp43 and Lys77 in aldose reductase. Corresponding residues in AKR1B10 are Asp44, Tyr49 and Lys78 if similar mechanism is in operation. Also in AKR1B10, NZ atom of Lys78 is at 2.7Å and 3.1Å from OD2 atom of Asp44 and OH atom of Tyr49, respectively. Therefore it is conceivable that the proton transfer to the substrates to take place through Tyr49 in AKR1B10.

IMPROVING THE EFFECTIVENESS OF ANTHRACYCLINES BY VIRTUE OF INHIBITORS

Fibrates, ciprofibrate, fenofibrate, fenofibric acid and Wy 14,643 inhibit the reduction of daunorubicin by 35–40% that is catalyzed by AKR1B10 (Fig. 3 and Table 1) [1–3]. Although fenofibrate and Wy 14,643 have a comparable inhibition potential ciprofibrate and fenofibric acid show lower potential. Importantly the prodrug, fenofibrate is 25-fold more effective than its hydrolyzed fibrate derivative, fenofibric acid, in inhibiting the AKR1B10 catalyzed reduction of daunorubicin. Both fenofibrate and fenofibric acid demonstrated same level of inhibiting AKR1B10 catalyzed reduction of daunorubicin but 20 and 500 µM concentrations of respective fibrates are required to achieve the same level of inhibition. This indicates that the prodrug, fenofibrate, which is the isopropyl ester of fenofibric acid, has higher potency than the hydrolyzed acid form. Several structurally divergent class of compounds used in this study such as ciprofibrate, fenofibric acid and fenofibrate are antilipidemic drugs that are in wide clinical use for the treatment of hyperglycemia [35, 36]. Fibrates show several attractive pharmacological properties [37–39]. Therefore, the undesired activity of AKR1B10 in the chemical action of anthracycline could be modulated by the exploitation of ciprofibrate, fenofibrate, fenofibric acid, gemfibrozil and Wy 14,643.

CONCLUSIONS AND FUTURE PROSPECTIVE

Fibrates are a class of synthetic compounds with diverged chemical structures and known pharmacological properties. They could be used alone or in Statin-fibrate combination therapy to reduce incidences of cardiovascular disease in patients with Type 2 diabetes, decrease serum triglycerides, lower insulin resistance and fasting blood glucose levels in non-obese Japanese Type 2 diabetic patients, decrease concentrations of circulating, small, low density lipoproteins (LDL), increase high density lipoproteins (HDL) and improve glucose tolerance, resulting in favorable effects on blood coagulation and global fibrinolytic function as well as to reduce the lipid content of intermediate density lipoprotein in Type 1 diabetes. The fact that the metabolite, fenofibric acid is less potent than the pro-drug

fenofibrate in modulating the AKR1B10 catalyzed reduction of anthracyclines it is necessary to design and generate a derivative that is less hydrolysable and more stable in the biophase.

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Fig. (1).

AKR1B10 catalyzed reduction of daunorubicin. Ketone moiety in the tetracyclic ring is converted to alcohol in the presence of NADPH.

Fig. (2).
Binding of daunorubicin in the active site cavity of AKR1B10. Active site of AKR1B10 is shown in the surface representation and color coded in white, red and blue to reflect the neutral, negative and positive residues. Nicotinamide ring of the cofactor in yellow and the daunorubicin in white are presented with N and O atoms in blue and red respectively.

Fig. (3). Chemical structures of the inhibitors capable of modulating AKR1B10 catalyzed inactivation of daunorubicin reaction. Ciprofibrate for 2-[p-(2,2-dichlorocyclopropyl)-phenoxy]-2-methylpropanoic acid, 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 and Wy 14,643 for 4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid.

Table 1.

Percentage Inhibition of Fibrates in the AKR1B10 Catalyzed Reduction of Daunorubicin

Inhibitors	Concentration (μM)	Inhibition (%)
Ciprofibrate	250	33
	500	39
Fenofibrate	20	35
Fenofibric Acid	250	25
	500	35
Wy 14,643	250	37
	500	50