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Inhibition of aldo-keto reductase family 1 member B10 by unsaturated fatty acids

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ABSTRACT

A human member of the aldo-keto reductase (AKR) superfamily, AKR1B10, is a cytosolic NADPH-dependent reductase toward various carbonyl compounds including reactive aldehydes, and is normally expressed in intestines. The enzyme is overexpressed in several extraintestinal cancers, and suggested as a potential target for cancer treatment. We found that saturated and *cis*-unsaturated fatty acids inhibit AKR1B10. Among the saturated fatty acids, myristic acid was the most potent, showing the IC_{50} value of 4.2 μM *cis*-Unsaturated fatty acids inhibited AKR1B10 more potently, and linoleic, arachidonic, and docosahexaenoic acids showed the lowest IC_{50} values of 1.1 μM. The inhibition by these fatty acids was reversible and kinetically competitive with respect to the substrate, showing the K_i values of 0.24 −1.1 μM. These fatty acids, except for α-linoleic acid, were much less inhibitory to structurally similar aldose reductase. Site-directed mutagenesis study suggested that the fatty acids interact with several active site residues of AKR1B10, of which Gln114, Val301 and Gln303 are responsible for the inhibitory selectivity. Linoleic and arachidonic acids also effectively inhibited AKR1B10-mediated 4-oxo-2-nonenal metabolism in HCT-15 cells. Thus, the *cis*-unsaturated fatty acids may be used as an adjuvant therapy for treatment of cancers that up-regulate AKR1B10.

1. Introduction

Cytosolic NADPH-dependent reductase AKR1B10 is a human member of the aldo-keto reductase (AKR) superfamily [1]. AKR1B10 is a 36-kDa reductase that shares similarities in amino acid sequence (71% identity) and tertiary structure with aldose reductase (AKR1B1) [1–4]. This enzyme is highly expressed in small intestine and colon [2,3], where it is specifically present in the epithelial cells [5]. On the other hand, AKR1B10 is overexpressed in extraintestinal tumors, such as hepatocarcinoma [2,6], lung carcinomas [7], breast cancer [8], pancreatic carcinoma [9] and uterine carcinomas [10]. AKR1B10 has been thought to be involved in the formation and development of carcinomas through multiple mechanisms, including detoxification of cytotoxic carbonyls [2,11–14], modulation of retinoic acid level [4,15], regulation of cellular fatty acid synthesis and lipid metabolism [14,16,17], and elevation of sphingosine-1-phosphate [18] and integrin α5/δ-catenin mediated FAK/Src/Rac1 signaling pathways [19]. Furthermore, AKR1B10 is suggested to be implicated in developing cancer cell resistance to anticancer drugs such as mitomycin C, oxaliplatin, cisplatin and doxorubicin [20–23]. Thus, this enzyme has been recognized not only as a potential diagnostic and/or prognostic marker, but also as a potential therapeutic target for the treatment of the above types of cancer and the chemoresistance. During the past five years, many synthetic inhibitors of AKR1B10 have been reported, as described in recent reviews [21,24].

Recently, AKR1B10 expression has been reported to be markedly decreased in ulcerative colitis and colorectal cancers [25,26], in contrast to its overexpression in the above many cancers. In

Abbreviations: AKR, aldo-keto reductase; ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; ONE, 4-oxo-2-nonenal; PG, prostaglandin; WT, wild type AKR1B10.

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intestines, AKR1B10 was suggested to exert a protective role against oxidative and carbonyl stresses through eliminating reactive aldehydes, such as 4-hydroxy-2-nonenal and 4-oxo-2-nonenal (ONE) [27,28], as well as its role in reduction of all-*trans*-retinal derived from cleavage of dietary provitamin β-carotene [15]. Due to the pivotal roles of intestinal AKR1B10, we were interested in modulation of its activity by dietary and endogenous components, and examined the effects of fatty acids and their metabolites, because the enzyme promotes de novo fatty acid synthesis by stabilizing acetyl-CoA carboxylase-α [16] and is inhibited by prostaglandin (PG) A_1 [29]. We report here an *in vitro* investigation showing that fatty acids, particularly *cis*-unsaturated fatty acids, potently inhibit AKR1B10, but have low or no inhibitory potency toward structurally and catalytically similar AKR1B1 and human aldehyde reductase (AKR1A1). The structural reason for the inhibitory selectivity of the *cis*-unsaturated fatty acids was investigated by site-directed mutagenesis of the residues in the binding site of AKR1B10, and their inhibitory efficacy was also evaluated in a cellular level.

2. Materials and methods

2.1. Materials

α-Linolenic acid (ALA) and oleoyl-CoA were obtained from Sigma-Aldrich, and other unsaturated fatty acids, PGA_1 and ONE were from Cayman Chemical (Ann Arbor, MI). Saturated fatty acids, fatty alcohols and linoleic acid (LA) methyl ester were obtained from Wako Pure Chemical Industries (Osaka, Japan), Nacalai Tesque (Kyoto, Japan) and Tokyo Chemical Industry (Tokyo, Japan). All other chemicals were of the highest grade that could be obtained commercially.

2.2. Preparation of recombinant enzymes

AKR1B10 with N-terminal 6-His tag [30], AKR1B1 and AKR1A1 without any additional amino acids [31] were expressed in *Escherichia coli* BL21 (DE3) pLysS cells transformed with the expression plasmids harboring their cDNAs, and purified to homogeneity as described previously. Mutant forms of AKR1B10 (W112F, Q114T, F123A, K125L, W220Y, V301L, Q303S, S304A and V301L/Q303S) [32–34] were expressed in the *E. coli* cells transformed with expression plasmids harboring their cDNAs, and purified to homogeneity, as described previously. Site-directed mutagenesis of Asn300Ala in AKR1B10 was performed using a KOD site-directed mutagenesis kit (Toyobo, Osaka, Japan), a sense primer (5′- GGAGGGCCTGTGCCGTGTTGCAAT-3′) and the corresponding antisense primer, according to the protocol described by the manufacturer. The coding regions of the cDNAs in the expression plasmids were sequenced by using a Beckman CEQ8000XL DNA sequencer in order to confirm the presence of the desired mutation and ensure that no other mutation had occurred. The expression constructs were transformed into the *E. coli* cells. The mutant enzyme (N300A) was expressed in the *E. coli* cells, and purified to homogeneity as described above for wild type AKR1B10 (WT) [30].

2.3. Assay of enzyme activity

The reductase and dehydrogenase activities of the enzymes were determined at 25 °C by measuring the rate of change in NADPH absorbance (at 340 nm) and fluorescence (at 455 nm with an excitation wavelength of 340 nm), respectively, in reaction mixtures (total volume of 2.0 mL) containing 0.1 M potassium phosphate, pH 7.4, coenzyme, substrate and enzyme [30]. In the IC_{50} determination of AKR1B10 inhibitors, the substrate and coenzyme were 20 μM pyridine-3-aldehyde and 0.1 mM NADPH, respectively, in the reductase activity, and were 25 μM geraniol (*trans*-3,7-dimethyl-2,6-octadien-1-ol) and 0.25 mM $NADP^+$, respectively, in the dehydrogenase activity. The reductase activities of AKR1B1 and AKR1A1 were assayed with 20 μM and 2 mM pyridine-3-aldehyde, respectively, as the substrates. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the oxidation or formation of 1 μmol NADPH per minute at 25 °C.

Fatty acids and their derivatives were dissolved in methanol and added to the reaction mixture, not to exceed 2% of the methanol concentration. Kinetic studies in the presence of inhibitors were carried out in both NADPH-linked pyridine-3-aldehyde reduction and $NADP^+$-linked geraniol oxidation over a range of five or six substrate concentrations at a saturating concentration of coenzyme. The inhibition patterns were judged from the Lineweaver-Burk plots, and inhibition constant, K_i, of the competitive inhibitor was determined from replots of the slopes versus inhibitor concentration. Inhibition patterns of arachidonic acid (ARA) with respect to $NADP^+$ were examined in the geraniol oxidation over a range of six coenzyme concentrations at a saturating substrate concentration (0.4 mM geraniol). In the experiments with the mutant enzymes, the K_i values were determined in the oxidation of geraniol using five substrate concentrations ($0.3–10 \times K_m$). The K_m values for geraniol of WT, W112F, Q114T, F123A, K125L, W220Y, N300A, V301L, Q303S, S304A and V301L/Q303S were 25, 73, 130, 38, 27, 138, 40, 47, 38, 43 and 113 μM, respectively, and the k_{cat} values of the mutant enzymes were ranged from 0.6 to 2.0-fold of that of WT. The IC_{50} and K_i values are expressed as the means ± SD of at least three determinations.

2.4. Molecular docking

The atomic coordinates for AKR1B10-$NADP^+$-tolrestat complex (PDB code: 1ZUA) [4] were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/). Fatty acid docking stimulation was performed using the program CDOCKER algorithm with a CHARMm force field in Discovery Studio 3.1 (BIOVIA, San Diego, CA) as described by Nakashima et al. [35]. Briefly, the structures of AKR1B10-$NADP^+$ complex and ARA were prepared using the CHARMm force field, and ARA was docked to the fatty acid-binding site. During the docking stimulation, all the parameters were the default setting, although the orientations to refine were changed from 10 to 30 and the number of random conformations was increased from 10 to 30. The CDOCKER interaction energy of ARA was - 49.7. Figures were generated using PyMOL (DeLano Scientific, San Carlos, CA).

2.5. Cell culture experiments

Human colon cancer HCT-15 cells were obtained from American Type Culture Collection (Manassas, VA), and grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator containing 5% CO_2. For establishment of AKR1B10-overexpressing and the control cells, HCT-15 cells were transfected with mammalian pGW1 expression vectors harboring AKR1B10 cDNA and the empty vectors, respectively [30]. After 48 h-culture, the cells were washed twice with serum-free growth medium, and then subjected to treatments with the agents that were dissolved in dimethyl sulfoxide as a vehicle, whose concentration for the treatment was less than 0.5%. The expression level of AKR1B10 in the cells was analyzed by Western blotting using anti-AKR1B10 antibodies [22]. Sensitivity to ONE toxicity was estimated by monitoring the cell viability, which was