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Potent and selective inhibition of the tumor marker AKR1B10 by bisdemethoxycurcumin: Probing the active site of the enzyme with molecular modeling and site-directed mutagenesis

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ABSTRACT

A human member of the aldo–keto reductase (AKR) superfamily, AKR1B10, shares high sequence identity with aldose reductase (AR), and was recently identified as a therapeutic target in the treatment of several types of cancer. We have compared the inhibitory effects of plant components on recombinant AKR1B10 and AR. AKR1B10 was inhibited by curcuminoids, magnolol, honokiol and resveratrol, with IC_{50} values of 0.06–5 μM, which were lower than their values for AR. Among them, bisdemethoxycurcumin was the most potent competitive inhibitor (K_i = 22 nM) with the highest selectivity (85-fold versus AR), and acted as an effective inhibitor in cellular level. In contrast, demethoxycurcumin and curcumin showed >3-fold less potency and selectivity. Molecular docking studies of the curcuminoids in the AKR1B10–$NADP^+$ complex and site-directed mutagenesis of the putative binding residues suggest that Gln114, Val301 and Gln303 are important for determining the inhibitory potency and selectivity of the curcuminoids.

AKR1B10 is a human NADPH-dependent aldo–keto reductase (AKR), which was recently identified as an aldose reductase (AR)-like 1 and human small intestinal AR [1,2]. The overall amino acid sequence of AKR1B10 shows 71% identity with that of human AR, which is named AKR1B1 in the AKR superfamily (http://www.med.upenn.edu/akr/). AKR1B10 reduces a variety of aldehydes and ketones, including endogenous substrates such as methylglyoxal [1], acrolein [3], 4-hydroxynonenal [4], phospholipid aldehydes [5], retinals [6], farnesal and geranylgeranial [7], that are also the substrates of AR. Among these substrates, retinals, farnesal and geranylgeranial are excellent substrates for AKR1B10, indicating much higher catalytic efficiency compared to human AR. Thus, AKR1B10 is suggested to play roles in the metabolism of retinoids and isoprenoids.

AKR1B10 was reported to be up-regulated in lung and hepatic carcinomas (squamous cell carcinoma and adenocarcinoma) [1,8,9], as well as in esophageal and uterine cancers [10,11], suggesting its potential role as a tumor marker. In addition, AKR1B10-gene silencing results in the inhibition of colorectal cancer cell growth, suggesting that AKR1B10 regulates cell proliferation [3]. Furthermore, current studies suggest that AKR1B10 participates in the cell carcinogenesis and tumor development by detoxifying cytotoxic carbonyls [4], mediating retinoic acid homeostasis [6,12], and regulating cellular fatty acid synthesis and lipid metabolism [13]. Thus, this enzyme is a target for the prevention and treatment of the above types of cancer. However, there are three reports on the inhibitors of AKR1B10, which include some AR inhibitors, fibrate derivatives, anti-inflammatory agents and phenolphthalein [6,14,15]. Among them, an AR inhibitor tolrestat is the most potent inhibitor with an IC_{50} value in the low nM range [6,16] for both enzymes. The other inhibitors show IC_{50} values of 10–300 μM for AKR1B10 [14,15], and have not been studied for their selectivity, which is ideally required for development of drugs targeting to this enzyme.

In the present work, we have examined the inhibitory effects of dietary plant polyphenols on the tumor marker AKR1B10 and human AR. The components found to be potent and selective inhibitors of AKR1B10 were curcumin (Cur), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), honokiol, magnolol and resveratrol, which have been reported to show anti-cancer properties [17–20]. Among them, BDMC was the most potent and selective, showing a K_i value of 22 nM for AKR1B10 and 85-fold less inhibition against human AR. The structural base responsible for the high affinity of BDMC for AKR1B10 was investigated by com-

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paring the docked models of the curcuminoid–AKR1B10–NADP$^+$ ternary complexes and site-directed mutagenesis studies of the active site residues of the enzyme.

Materials and methods

Purification of recombinant enzymes. Recombinant AKR1B10 with the N-terminal 6-His tag, human AR and aldehyde reductase without any additional amino acid were expressed in *Escherichia coli* BL21 (DE3) pLysS cells transformed with the expression plasmids harboring their cDNAs, and purified to homogeneity as described previously [7,21] .

Site-directed mutagenesis. Mutagenesis was performed using a QuickChange site-directed mutagenesis kit (Stratagene) and the pCold I expression plasmid harboring the cDNA for AKR1B10 [7] as the template according to the protocol described by the manufacturer. The primer pair used for the mutagenesis was composed of sense and antisense oligonucleotides to alter one or two codons of AKR1B10 cDNA. The 29- to 35-mer primers were synthesized to give the Q114T, W220Y, V301L, Q303S, S304A and V301L/Q303S mutant enzymes. The coding regions of the cDNAs in the expression plasmids were sequenced in order to confirm the presence of the desired mutation and ensure that no other mutation had occurred. The mutant enzymes were expressed in the *E. coli* cells, and purified to homogeneity as described above for the wild-type enzyme [7].

Assay of enzyme activity. The reductase and dehydrogenase activities of AKRs 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 [7]. The IC_{50} values of inhibitors for AKR1B10 and AR were determined in the reaction mixture consisted of 0.1 M potassium phosphate, pH 7.4, 0.1 mM NADPH, 0.2 mM pyridine-3-aldehyde and enzyme in a total volume of 2.0 ml. In the assay for human aldehyde reductase 10 mM pyridine-3-aldehyde was used as the substrate. Kinetic studies in the presence of inhibitors were carried out in both pyridine-3-aldehyde reduction and NADP$^+$-linked geraniol oxidation over a range of five substrate concentrations ($0.2–5 \times K_m$) at a saturating concentration of coenzyme, and vice versa. The IC_{50} and K_i values are expressed as the means ± standard errors of at least three determinations.

Molecular modeling and energy minimization. The coordinates for AKR1B10 were obtained from the RCSB Protein Data Bank (PDB code 1ZUA). The structure was prepared using the Maestro (Schrödinger, LLC) software package Version 8.5 as described previously [7]. In order to eliminate any bond length and bond angle biases from the crystal structure, the ligand, BMDC or Cur, was subjected to a full minimization prior to the docking. The docking calculations were performed using Glide 5.0 [22] on a Linux workstation under the conditions described previously [7]. Figures were generated using *PyMOL* (DeLano Scientific).

Cell culture experiments. The culture of HeLa cells, transfection of the pGW1 plasmids harboring the cDNA for AKR1B10, activity assay of the expressed enzyme and analysis of the metabolism of [1-^{14}C] *trans,trans*-farnesol in the cells were carried out as described previously [7].

Results and discussion

Inhibition of AKR1B10 by plant polyphenols

The inhibitory plant components found in this study were polyphenols (Table 1), and their structures are illustrated in Fig. 1. Among them, BDMC exhibited the lowest IC_{50} value and highest selectivity to AKR1B10 (85-fold versus AR). The inhibitory potency and selectivity were decreased in DMC and Cur that have one or two methoxy groups on the phenolic rings of BDMC. Other polyphenols, resveratrol, honokiol and magnolol showed less inhibition for both AKR1B10 and AR, while their selectivities were comparable to that of DMC. Human aldehyde reductase, a functionally related enzyme to human AR and AKR1B10, was not inhibited by honokiol, resveratrol (each 50 μM), BDMC, DMC, Cur and magnolol (each 20 μM).

Table 1
Effects of polyphenols on reductase activities of AKR1B10 and human AR.

Inhibitor	IC_{50} (μM)		Ratio AR/1B10
	AKR1B10	AR	
BDMC	0.060 ± 0.009	5.1 ± 0.2	85
DMC	0.18 ± 0.01	1.2 ± 0.1	7
Cur	0.38 ± 0.05	7.3 ± 0.2	20
Magnolol	3.6 ± 0.2	28 ± 3	8
Honokiol	3.8 ± 0.1	36 ± 3	9
Resveratrol	5.0 ± 0.6	73 ± 5	12

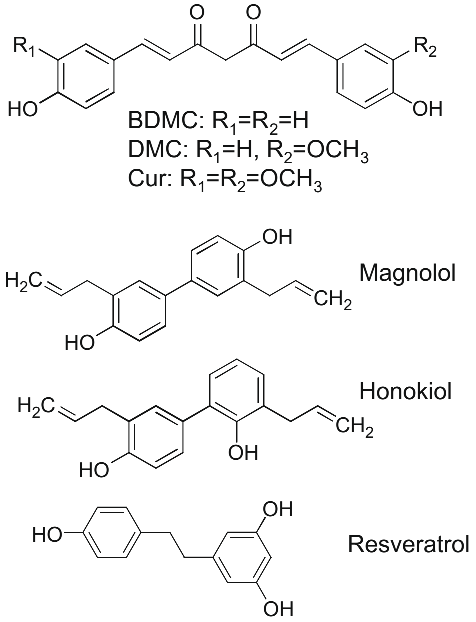

Fig. 1. Structures of plant polyphenols.

AKR1B10 catalyzes both reduction of carbonyl compounds and oxidation of several alcohols such as geraniol and farnesol, and the inhibition patterns of tolrestat and inhibitory steroids for the reaction directions catalyzed by the enzyme are different [7]. BDMC inhibited the enzyme noncompetitively with respect to the pyridine-3-aldehyde and NADPH in the reduction direction, whereas the inhibition patterns in the oxidation direction are uncompetitive and competitive with respect to NADP$^+$ and geraniol, respectively. The inhibition patterns were the same as those reported previously for tolrestat and the inhibitory steroids [7], and the K_i value for BDMC estimated from the competitive inhibition with respect to geraniol was 22 ± 4 nM. The inhibition patterns of DMC, Cur and honokiol with respect to geraniol were also competitive, showing the K_i values of 60 ± 5 nM, 170 ± 30 nM and 1.9 ± 0.1 μM, respectively. Thus, the affinity for BDMC was the highest among the inhibitors tested, and is comparable to those for previously known potent inhibitors, tolrestat and isolithocholic acid [6,7].

Inhibitory effect on cellular metabolism by AKR1B10

The efficacies of AKR1B10 inhibition by BDMC, Cur and honokiol were examined in the cellular metabolism of farnesal using the HeLa