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Human carbonyl reductase 1 participating in intestinal first-pass drug metabolism is inhibited by fatty acids and acyl-CoAs

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ABSTRACT

Human carbonyl reductase 1 (CBR1), a member of the short-chain dehydrogenase/reductase (SDR) superfamily, reduces a variety of carbonyl compounds including endogenous isatin, prostaglandin E_2 and 4-oxo-2-nonenal. It is also a major non-cytochrome P450 enzyme in the phase I metabolism of carbonyl-containing drugs, and is highly expressed in the intestine. In this study, we found that long-chain fatty acids and their CoA ester derivatives inhibit CBR1. Among saturated fatty acids, myristic, palmitic and stearic acids were inhibitory, and stearic acid was the most potent (IC_{50} 9 μM). Unsaturated fatty acids (oleic, elaidic, γ-linolenic and docosahexaenoic acids) and acyl-CoAs (palmitoyl-, stearoyl- and oleoyl-CoAs) were more potent inhibitors (IC_{50} 1.0–2.5 μM), and showed high inhibitory selectivity to CBR1 over its isozyme CBR3 and other SDR superfamily enzymes (DCXR and DHRS4) with CBR activity. The inhibition by these fatty acids and acyl-CoAs was competitive with respect to the substrate, showing the K_i values of 0.49–1.2 μM. Site-directed mutagenesis of the substrate-binding residues of CBR1 suggested that the interactions between the fatty acyl chain and the enzyme's Met141 and Trp229 are important for the inhibitory selectivity. We also examined CBR1 inhibition by oleic acid in cellular levels: The fatty acid effectively inhibited CBR1-mediated 4-oxo-2-nonenal metabolism in colon cancer DLD1 cells and increased sensitivity to doxorubicin in the drug-resistant gastric cancer MKN45 cells that highly express CBR1. The results suggest a possible new food-drug interaction through inhibition of CBR1-mediated intestinal first-pass drug metabolism by dietary fatty acids.

1. Introduction

Carbonyl reductase (CBR, EC 1.1.1.184) catalyzes the NADPH-linked reduction of the carbonyl groups in various aldehydes, ketones and quinones to their corresponding alcohols [1,2]. The enzyme exists in two forms in both the cytosol (CBR1 and CBR3) and the mitochondria (CBR2 and CBR4) of mammalian cells, and the four CBRs belong to the short-chain dehydrogenase/reductase (SDR) superfamily. Among them, the gene for CBR2 is not present in the human genome and human CBR4 is reported to act as a quinone reductase and ketoacyl reductase [3,4]. Human CBR1 and CBR3 are monomeric proteins sharing 72% amino acid sequence identity, but they differ in their enzymatic properties [5,6]. CBR1 reduces a wide range of biologically active carbonyl compounds, while CBR3 is active toward limited carbonyl compounds. Since homozygous null (*Cbr1* −/−) mice are fetal lethal [7], CBR1 has been suggested to play pivotal roles in the metabolism of endogenous carbonyl compounds. Such endogenous substrates of the enzyme have been reported to be isatin [8], prostaglandin E_2 [9],

Abbreviations: CBR, carbonyl reductase; SDR, short-chain dehydrogenase/reductase; CYP, cytochrome P450; AKR, aldo-keto reductase; DOX, doxorubicin; FA, fatty acid; ONE, 4-oxo-2-nonenal; 13h, 8-hydroxy-2-imino-2*H*-chromene-3-carboxylic acid (2-chlorophenyl)amide; DHRS4, dehydrogenase/reductase SDR family member 4; DCXR, dicarbonyl/L-xylulose reductase; PQ, 9,10-phenanthrenequinone; *S*-tetralol, (*S*)-(+)-1,2,3,4-tetrahydro-1-naphthol; RT, reverse transcription; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GSH, glutathione; UGT, UDP-glucuronosyltransferase; CES1, carboxylesterase 1.

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6-pyruvoyltetrahydropterin [10], *S*-nitrosoglutathione [11] and lipid peroxidation-derived aldehydes [12,13].

CBR1 also reduces a wide variety of xenobiotic carbonyl compounds to their corresponding alcohols, which are easily conjugated and eliminated [1,2]. Thus, the enzyme plays a major role in the phase I metabolism of xenobiotic compounds including drugs. In drug metabolism, CBR1 is one of predominant non-cytochrome P450 (CYP) enzymes [14]. Although several aldo-keto reductases (AKRs) and enzymes in the SDR superfamily are involved in the reduction of carbonyl-containing drugs [1,2,15], CBR1 is a primary reductase of haloperidol [16,17], mebendazole [17], fenofibric acid [18], loxoprofen [16,19], daunorubicin and doxorubicin (DOX) [20], and is also involved in the metabolism of warfarin [21], exemestane [22] and other ten drugs [15,23]. In an immunochemical study, CBR1 was detected in all human tissues, with the highest concentration in human liver and the epithelial cells of stomach and small intestine [24]. An *in vitro* study on the metabolism of haloperidol and mebendazole in human liver and intestine has suggested significance of intestinal first-pass metabolism by CBR1 [17].

Food-drug interactions occur in various systems in the body, in which the inhibition of intestinal metabolism and transport of oral drugs by dietary substances is an important biochemical mechanism [25,26]. In phase I drug metabolism, several CYPs are known to be inhibited by phytochemicals [26] and fatty acids (FAs) [27,28] contained in foods. However, little is known about inhibition of the drug metabolizing enzyme CBR1 by food constituents, except for plant polyphenols such as flavonoids, stilbenes and curcuminoids [29]. In rats, n-3 polyunsaturated FAs have been shown to possess protective effects on DOX-induced cardiotoxic side-effect [30,31], in which the reduction of the anticancer drug into its cardiotoxic 13-alcohol metabolite by CBR1 is one of the underlying mechanisms [7,32]. In case reports, co-administration of warfarin, a CBR1 substrate, with n-3 polyunsaturated FAs leads to additional anticoagulation [33,34]. Therefore, we examined the effects of FAs, food ingredients in daily meals, on activity of CBR1, and found that the activity is potently inhibited by long-chain FAs and acyl-CoAs. We report herein an *in vitro* investigation on structure-activity relationship of FAs as CBR1 inhibitors, their inhibitory selectivity and binding site in the enzyme. The inhibitory efficacy of an inhibitory FA, oleic acid, was also evaluated in cellular levels.

2. Materials and methods

2.1. Chemicals

α-Linolenic acid, acetyl-CoA, acetoacetyl-CoA and daunorubicin were obtained from Sigma-Aldrich (St. Louis, MO), and other unsaturated FAs and 4-oxo-2-nonenal (ONE) were from Cayman Chemical (Ann Arbor, MI). Saturated FAs and fatty alcohols were purchased from Wako Pure Chemical Industries (Osaka, Japan), Nacalai Tesque (Kyoto, Japan) and Tokyo Chemical Industry (Tokyo, Japan). Parmitoyl-, stearoyl- and oleoyl-CoAs were kindly donated by Dr. Koji Yashiro (Fujita Health University School of Medicine, Japan). DOX was obtained from Kyowa Hakko Kogyo (Tokyo, Japan). A CBR1 inhibitor, 8-hydroxy-2-imino-2*H*-chromene-3-carboxylic acid (2-chlorophenyl)amide (13 h) was synthesized and its purity (>99%) was confirmed by high-resolution mass spectroscopy analysis, as described previously [35]. All other chemicals were of the highest grade that could be obtained commercially.

2.2. Preparation of recombinant enzymes

The recombinant CBR1 [5], its mutant enzymes, Met141Gln and Trp229Leu [35], CBR3 [5], dehydrogenase/reductase SDR family member 4 (DHRS4) [36] and dicarbonyl/L-xylulose reductase (DCXR) [37] were prepared and purified to homogeneity, as described previously.

2.3. Assay of enzyme activity

The reductase and dehydrogenase activities of CBR1 were assayed at 37 °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 [38]. The IC_{50} values for inhibitors were determined in the reaction mixture that consisted of 0.1 M potassium phosphate buffer, pH 7.4, 0.1 mM NADPH, substrate, inhibitor and enzyme, in a total volume of 2.0 mL. The reaction was initiated by the addition of the enzyme. Although the substrate in most experiments was 10 μM isatin, 10 μM 9,10-phenanthrenequinone (PQ), 20 μM menadione and 60 μM daunorubicin were also used as alternative substrates. The substrates of CBR3, DCXR and DHRS4 were 70 μM menadione, 120 μM diacetyl and 4 μM PQ, respectively. The concentrations of these substrates corresponded to their approximate K_m values, which were determined for the four enzymes in the above reaction mixture by fitting the initial velocities to the Michaelis-Menten equation,

$$v = V_{max}[S]/(K_m + [S])$$

where v is the initial velocity; [S] is the substrate concentration; and V_{max} is the maximum velocity. The dehydrogenase activity of CBR1 was assayed using 0.25 mM $NADP^+$ and an appropriate amount of (*S*)-(+)-1,2,3,4-tetrahydro-1-naphthol (*S*-tetralol) as the coenzyme and substrate, respectively, in the above reaction mixture. FAs and their derivatives were dissolved in methanol and added to the reaction mixture, not to exceed 2% of the methanol concentration. Acyl-CoAs were dissolved in 10 mM potassium phosphate buffer, pH 7.4, and their concentrations were estimated using their absorbance at 260 nm with ϵ = 15,400 M^{-1}. One unit (U) of enzyme activity was defined as the amount of enzyme that catalyzes the formation or oxidation of 1 μmol NADPH per min.

The kinetic studies in the presence of inhibitors were carried out in both isatin reduction and *S*-tetralol oxidation over a range of five or six substrate concentrations at the above saturating concentration of NADPH or $NADP^+$ [38]. The inhibition pattern was judged from Lineweaver-Burk double reciprocal plots of initial velocities versus substrate concentrations using the following equation,

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left[1 + \frac{[I]}{K_i}\right]\frac{1}{[S]} + \frac{1}{V_{max}}$$

where [I] is the inhibitor concentration and K_i is the inhibition constant. The K_i for the competitive inhibitor in the *S*-tetralol oxidation was determined from replots of the slopes (K_m/V_{max}) of the reciprocal plots versus inhibitor concentrations ([I]), where a straight line of the replots was obtained and its [I]-axis intercept was equal to $-K_i$. In addition, the estimates of the K_i were obtained from Dixon plots using the following equation,

$$\frac{1}{v} = \frac{K_m}{V_{max}K_i[S]}[I] + \frac{1}{V_{max}}\left[1 + \frac{K_m}{[S]}\right]$$

2.4. Cell-based experiments

Human colon cancer DLD1 cells were obtained from American Type Culture Collection (Manassas, VA). Human gastric cancer DOX-resistant MKN45 cells previously established and their parental cells (Health Science Research Resources Bank, Osaka, Japan) [39] were used in the present experiments. The cells were grown