Chemico-Biological Interactions 258 (2016) 153–158

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

In vitro metabolism of fenofibric acid by carbonyl reducing enzymes

Petra Malátková [a], Matthildi Kanavi [a], Milan Nobilis [b], Vladimír Wsól [a, *]

[a] Department of Biochemical Sciences, Faculty of Pharmacy, Charles University, Heyrovského 1203, CZ-50005 Hradec Králové, Czech Republic
[b] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, Charles University, Heyrovského 1203, CZ-50005 Hradec Králové, Czech Republic

ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form
31 August 2016
Accepted 2 September 2016
Available online 4 September 2016

Keywords:
AKR
Carbonyl reducing enzymes
Carbonyl reductase
Fenofibric acid
Reductive drug metabolism
Short-chain dehydrogenase/reductase

ABSTRACT

Fenofibric acid is a hypolipidemic drug that is used as an active ingredient per se or is administered in the form of fenofibrate that releases fenofibric acid after absorption. The metabolism of fenofibric acid is mediated primarily by glucuronidation. However, the other part of fenofibric acid is excreted as reduced fenofibric acid. Enzymes responsible for the formation of reduced fenofibric acid as well as their subcellular localization have remained unknown until now. We have found that the predominant site of fenofibric acid reduction is the human liver cytosol, whereas liver microsomes reduced fenofibric acid to a lower extent and exhibited a lower affinity for this drug (K_m > 1000 μM). Of nine carbonyl-reducing enzymes (CREs) tested, CBR1 exhibited the greatest activity for fenofibric acid reduction (CL_{int} = 85.975 μl/mg protein/min). CBR1 predominantly formed (−)-enantiomers of reduced fenofibric acid similar to liver cytosol and in accordance with the *in vivo* data. AKR1C1, AKR1C2, AKR1C3 and AKR1B1 were also identified as reductases of fenofibric acid but are expected to play only a minor role in fenofibric acid metabolism.

1. Introduction

Fenofibric acid (2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid) is a hypolipidaemic drug indicated for the treatment of severe hypertriglyceridaemia, primary hypercholesterolaemia and mixed dyslipidaemia. Fenofibric acid is used directly as the active ingredient of drugs or administered as the prodrug fenofibrate, the isopropyl ester of fenofibric acid that is rapidly hydrolysed *in vivo* to fenofibric acid. Fenofibric acid works by activating the peroxisome proliferator activated receptor alpha (PPARalpha) that regulates the metabolism of lipoproteins and fatty acids. Fenofibric acid and fenofibrate play an important role in the therapy of dyslipidaemia due to their great tolerability and safety profile when used alone or in combination with statin therapy [1].

The predominant route of fenofibric acid elimination is the conjugation with glucuronic acid and its excretion via urine. A smaller amount of fenofibric acid undergoes a carbonyl reduction to a benzhydrol metabolite called reduced fenofibric acid (Fig. 1) that is expected to exhibit pharmacological activity [2]. The amount of fenofibric acid excreted in the reduced form in the urine, either in unconjugated or conjugated form, has been reported to be between 10 and 20% [3]. Reduced fenofibric acid exists in the form of two enantiomers, (+)- and (−)-reduced fenofibric acid, but the absolute configuration of the enantiomers has remained unknown. In humans, the reduction of fenofibric acid is slightly stereoselective to (−)-reduced fenofibric acid. Specifically, the enantiomeric ratio of (−)/(+)-reduced fenofibric acid excreted via urine has been reported to be 52:48 [4].

Although the drug has been clinically used for a long time, the carbonyl reduction of fenofibric acid has been totally unexplored until now, despite its significance. The reduction of fenofibric acid has been detected in rat liver [5,6] and can be expected to also take place in human liver, the principal site of drug metabolism. Several CREs that participate in drug metabolism are expressed in liver. These CREs belong to the aldo-ketoreductase (AKR) and short-chain dehydrogenase/reductase (SDR) superfamilies [7–9]. Our aim was to investigate the carbonyl reduction of fenofibric acid in human liver cytosol and microsomes and to identify the enzyme(s) responsible for the formation of reduced fenofibric acid, thus expanding the understanding of fenofibric acid metabolism.

Abbreviations: AKR, aldo-keto reductase; CL_{int}, internal clearance; CREs, carbonyl reducing enzymes; SDR, short-chain dehydrogenase/reductase.

* Corresponding author.
E-mail addresses: petra.malatkova@faf.cuni.cz (P. Malátková), kanmatild@yahoo.gr (M. Kanavi), nobilis@faf.cuni.cz (M. Nobilis), vladimir.wsol@faf.cuni.cz (V. Wsól).

http://dx.doi.org/10.1016/j.cbi.2016.09.001
0009-2797/

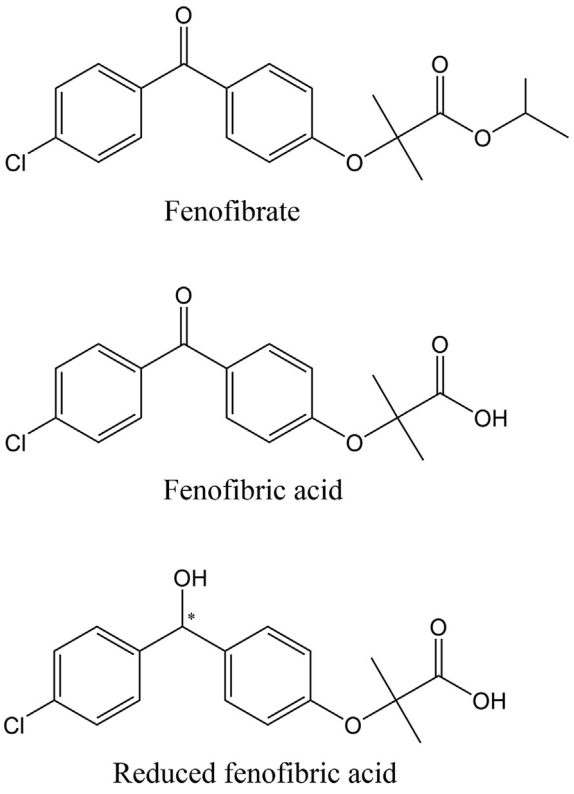

Fig. 1. Structure of fenofibrate, fenofibric acid and reduced fenofibric acid.

2. Materials and methods

2.1. Chemicals and materials

Fenofibric acid was purchased from Sigma-Aldrich (Prague, Czech Republic). An NADPH-generating system consisted of $NADP^+$, glucose-6-phosphate, magnesium chloride (Sigma-Aldrich, Czech Republic) and glucose-6-phosphate dehydrogenase (Roche, Germany). HPLC-grade methanol was obtained from Sigma-Aldrich (Prague, Czech Republic) and HPLC-grade acetonitrile from Fisher Chemical. HPLC-grade water was prepared using a Millipore Milli-Q reverse osmosis Millipore system (Millipore, Bedford, MA, USA). All other chemicals were of the highest purity commercially available. The internal standard (its structure will be described in submitted article by Vybíralová et al.) and reduced fenofibric acid (2-{4-[(4-chlorophenyl)hydroxymethyl]phenoxy}-2-methylpropanoic acid) were synthesized as previously described (Achiral and chiral HPLC-PDA analyses of fenofibrate metabolites in various biomatrices, Vybíralová et al., submitted).

2.2. Preparation of human liver subcellular fractions

Human liver samples were obtained from the cadaver donor program of the Transplant Centre of the Faculty of Medicine (Hradec Kralove, Czech Republic) in accordance with Czech legislation. Cytosol and microsomes were prepared as previously described [10]. Pooled cytosol and microsomes prepared from 5 individuals were used in these experiments.

2.3. Preparation of the recombinant forms of carbonyl reducing enzymes

A human liver cDNA library was used to generate the recombinant forms of AKR1A1, AKR1B1, AKR1B10, AKR1C1, AKR1C2, AKR1C3, AKR1C4, CBR1 and CBR3. The recombinant proteins were prepared in an *E. coli* expression system as was described earlier [11].

2.4. Incubation with fenofibric acid

The carbonyl reduction of fenofibric acid was assessed using pooled human liver cytosolic and microsomal fractions (0.5 mg/mL final protein concentration) or using recombinant enzymes (0.2 mg/mL final protein concentration). The incubation mixture consisted of 20 μL of enzyme source, 20 μL of an NADPH-regenerating system (final concentrations: 0.8 mM $NADP^+$, 6 mM glucose-6-phosphate, 0.35 units glucose-6-phosphate dehydrogenase, 3 mM $MgCl_2$), 50 μL of 0.1 M sodium phosphate buffer (pH 7.4) and 10 μL of fenofibric acid. The reaction was initiated by the addition of fenofibric acid and performed for 30 min at 37 °C. Controls without added enzyme source (i.e., without the liver fraction or the recombinant enzyme) were included in each incubation. The controls showed no or negligible formation of reduced fenofibric acid.

A stock solution of 50 mM fenofibric acid was prepared in 100% methanol and was further diluted with 0.1 M sodium phosphate buffer (pH 7.4) to yield the working concentrations. The final concentration of methanol in the incubation mixture ranged from 0.2% to 2.0% (v/v) depending on the concentration of fenofibric acid used (100–1500 μM). A higher concentration of fenofibric acid (1500 μM fenofibric acid) and consequently a higher amount of methanol (3.0% (v/v) of methanol) were used in the incubations with AKR1B1 and AKR1C2.

The reaction was terminated by the addition of 40 μL of 1 M HCl and cooling to 0 °C in an ice bath for 15 min. Next, 10 μg of internal standard was added. Samples were extracted using ethyl acetate. Specifically, after the addition of 1 mL of ethyl acetate, samples were shaken for 15 min and then centrifuged for 2 min at 12,000×*g*. The organic layer was taken and evaporated to dryness in a vacuum. The residues were reconstituted in 200 μL of mobile phase, and an aliquot of 100 μL was injected into the HPLC system. Reduced fenofibric acid was quantified by an achiral and chiral HPLC as reported previously (Achiral and chiral HPLC-PDA analyses of fenofibrate metabolites in various biomatrices, Vybíralová et al., submitted).

2.5. Determination of enzyme kinetics

The liver fractions and the CREs that exhibited reductase activity toward fenofibric acid were used to determine the kinetic parameters. Incubations were performed as described in the previous section (Section 2.4) with a final concentration of fenofibric acid in the range of 100–1000 μM, or, in the case of AKR1B1 and AKR1C2, in the range of 100–1500 μM. The apparent kinetic parameters were calculated using GraphPad Prism version 6.05 for Windows (GraphPad Software, La Jolla California, USA). The formation of reduced fenofibric acid was fitted to the Michaelis-Menten hyperbola and allosteric sigmoidal kinetic models. The best-fitting model was determined using an extra sum-of-squares F test. The enzymatic activity was expressed as the specific activity (pmol of reduced fenofibric acid formed per mg of protein in 1 min). The internal clearance (CL_{int}) represents the enzymatic efficiency and is defined as V_{max}/K_m. The values are expressed as the mean ± SD from $n = 3$ or more experiments.