Drug Metabolism and Pharmacokinetics 30 (2015) 70–74

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Note

Potential for drug interactions mediated by polymorphic flavin-containing monooxygenase 3 in human livers

Makiko Shimizu, Arisa Shiraishi, Ayumi Sato, Satomi Nagashima, Hiroshi Yamazaki*

Laboratory of DMPK, Showa Pharmaceutical University, 3-3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan

ARTICLE INFO

Article history:
Received 23 August 2014
Received in revised form
17 September 2014
Accepted 21 September 2014
Available online 5 October 2014

Keywords:
FMO3
FMO3 polymorphism
FMO3 activity
Drug oxygenation
Drug interaction

ABSTRACT

Human flavin-containing monooxygenase 3 (FMO3) in the liver catalyzes a variety of oxygenations of nitrogen- and sulfur-containing medicines and xenobiotic substances. Because of growing interest in drug interactions mediated by polymorphic FMO3, benzydamine *N*-oxygenation by human FMO3 was investigated as a model reaction. Among the 41 compounds tested, trimethylamine, methimazole, itopride, and tozasertib (50 μM) suppressed benzydamine *N*-oxygenation at a substrate concentration of 50 μM by approximately 50% after co-incubation. Suppression of *N*-oxygenation of benzydamine, trimethylamine, itopride, and tozasertib and *S*-oxygenation of methimazole and sulindac sulfide after co-incubation with the other five of these six substrates was compared using FMO3 proteins recombinantly expressed in bacterial membranes. Apparent competitive inhibition by methimazole (0–50 μM) of sulindac sulfide *S*-oxygenation was observed with FMO3 proteins. Sulindac sulfide *S*-oxygenation activity of Arg205Cys variant FMO3 protein was likely to be suppressed more by methimazole than wild-type or Val257Met variant FMO3 protein was. These results suggest that genetic polymorphism in the human *FMO3* gene may lead to changes of drug interactions for *N*- or *S*-oxygenations of xenobiotics and endogenous substances and that a probe battery system of benzydamine *N*-oxygenation and sulindac sulfide *S*-oxygenation activities is recommended to clarify the drug interactions mediated by FMO3.

1. Introduction

The flavin-containing monooxygenases (FMOs, EC 1.14.13.8) are a family of NADPH-dependent enzymes that catalyze the oxygenation of a wide variety of nucleophilic compounds containing a nitrogen, sulfur, phosphorous, or selenium atom [1,2]; such compounds include the anti-inflammatory drug benzydamine, antithyroid drug methimazole, gastroprokinetic agent itopride, hereditary polyposis drug sulindac sulfide, anti-cancer agent tozasertib, and diet-derived trimethylamine (Fig. 1) [3–5]. Histamine H_2-receptor antagonist ranitidine [6], dipeptidyl peptidase IV inhibitor teneligliptin [7], α7 neuronal nicotinic receptor agonist AZD0328 [8], and peroxisome proliferator-activated receptor dual agonist MK-0767 [9] are listed as medicinal and new drug candidate substrates of FMO.

FMO3 is considered the prominent functional FMO form expressed in adult human liver [10,11]. Human protein-coding gene *FMO3* and its mRNA expression levels are given in http://www.ncbi.nlm.nih.gov/UniGene/under [UniGene 240387 – Hs.445350]. Genetic polymorphism of *FMO3* [12–14] and/or post-translational modification by environmental factors such as nitric oxide could cause interindividual differences in FMO3 levels or FMO3 catalytic function [15,16]. Loss-of-function mutations, nonsense mutations, and missense mutations of FMO3 [17–19] produce phenotypes associated with the inherited disorder trimethylaminuria (also known as fish odor syndrome). In the literature, reported mutations in the *FMO3* gene are given using systematic and trivial names [18]. In the course of identification of novel mutations of *FMO3* and functional analysis in Japanese individuals suffering from trimethylaminuria, we found common and unique FMO3 variants [19]. We previously reported that Val257Met FMO3 protein had almost the same activity as wild-type FMO3, but [Glu158Lys; Glu308Gly] and Arg205Cys FMO3 proteins exhibited slightly and moderately decreased activities, respectively, with respect to *N*-oxygenation of trimethylamine [19].

FMOs form a complementary enzyme system to the cytochrome P450 enzymes and have been found to oxygenate several soft, highly polarizable nucleophilic heteroatom-containing chemicals and several drugs analyzed [20]. It has previously been suggested

Abbreviations: FMO, flavin-containing monooxygenase (FMO, EC 1.14.13.8).

* Corresponding author.
E-mail address: hyamazak@ac.shoyaku.ac.jp (H. Yamazaki).

http://dx.doi.org/10.1016/j.dmpk.2014.09.008

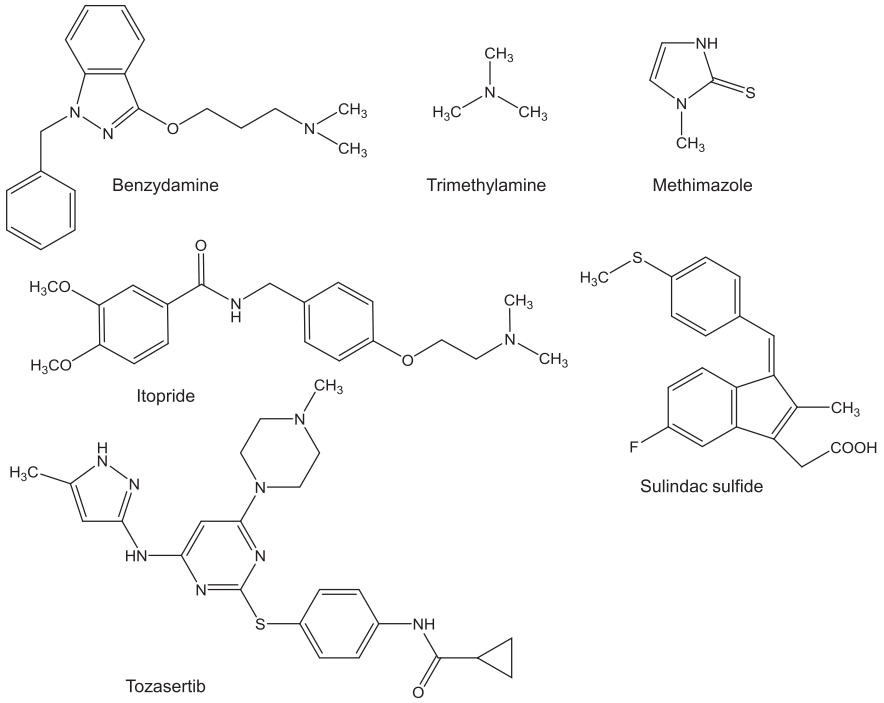

Fig. 1. Chemical structures of typical FMO3 substrates.

that the products of FMO3-mediated metabolism are generally benign, highly polar, and readily excreted [20]. The absence of induction of FMO3 by xenobiotics is strikingly different to the case for cytochrome P450 enzymes, which are induced by a number of xenobiotics [1]. FMO has been shown to exhibit a stable 4a-flavin hydroperoxide intermediate capable of oxygenating both nucleophiles and electrophiles in its catalytic cycle, even in the absence of oxygenatable substrate [21]. In this context, little information regarding apparent drug interactions has been reported so far for FMO3 [22]. Some of the preclinical research and development areas related to FMOs are not yet fully mature. Therefore, in the present study, we investigated the effects of a variety of *N*- and *S*-containing chemicals on benzydamine *N*-oxygenation to test its suitability as an index reaction for the enzymatic activity of recombinant human FMO3. Further investigations were carried out on FMO3-mediated oxidations of trimethylamine, methimazole, itopride, and tozasertib (selected as strong suppressors of benzydamine *N*-oxygenation) and sulindac sulfide (selected as typical *S*-containing clinical substrate). Strong suppression by *S*-containing methimazole on sulindac sulfide *S*-oxygenation by polymorphic FMO3 was observed. The present results suggest that a battery system of benzydamine *N*-oxygenation and sulindac sulfide *S*-oxygenation activities would be useful as probe substrate reactions to clarify drug interactions mediated via polymorphic human FMO3.

2. Materials and methods

2.1. Chemicals and enzymes

Benzydamine hydrochloride, itopride, methimazole, sulindac sulfoxide, and sulindac sulfide were purchased from Sigma–Aldrich (St. Louis, MO, USA) and trimethylamine from Wako Pure Chemicals (Osaka, Japan). Tozasertib was purchased from Cayman Chemical (Ann Arbor, MI, USA). Benzydamine *N*-oxide was a generous gift from Prof. Allan E. Rettie (University of Washington). Recombinant human FMO3 proteins were prepared as reported previously [23]. In the preliminary trials, the use of human livers for this study (to prepare liver microsomes as enzymes sources and DNA samples for genotyping) [19] was approved by the Ethics Committees of Showa Pharmaceutical University. The other chemicals shown in Fig. 2 and the reagents used were obtained in the highest grade commercially available.

2.2. Analysis of drug oxygenation activities

Rates of *N*-oxygenation of benzydamine [24], itopride [25], and tozasertib [5] and *S*-oxygenation of methimazole [26] and sulindac sulfide [27] were determined using liquid chromatography in fluorescence mode or in UV detection mode, as described previously [23,28]. A typical incubation mixture consisted of 100 mM potassium phosphate buffer (pH 8.4, the optimal pH condition for human FMO3), an NADPH-generating system (0.25 mM $NADP^+$, 2.5 mM glucose 6-phosphate, and 0.25 units ml^{-1} glucose 6-phosphate dehydrogenase), substrate (50 μM), and *Escherichia coli* membranes expressing FMO3 protein (1.0–50 pmol FMO equivalent) in a final volume of 0.20 ml, unless otherwise stated. The substrate concentration of 50 μM was chosen because it was similar to the reported K_m values for these reactions in our studies [23,28]. For FMO3 functional activity, the reactions were initiated in the presence of an NADPH-generating system by substrate addition because of the known instability of FMO enzymes; reaction mixtures were incubated at 37 °C for 10–30 min. Incubations were terminated by adding 0.20 ml ice-cold acetonitrile or methanol. The aqueous supernatant was centrifuged at 2000 g for 10 min and subjected to liquid chromatography using an octadecylsilane (C_{18}) column (4.6 mm × 150 mm, 5 μm, Capcell Pak, Shiseido, Tokyo, Japan) [23,28].

The rates of *N*-oxygenation of trimethylamine mediated by recombinant variants of FMO3 were determined by gas chromatography as described previously [29]. The kinetic analyses of *N*- or *S*-oxygenations were performed using non-linear regression analysis with the Michaelis–Menten model (Prism, GraphPad Software, La