

Drug metabolism by flavin-containing monooxygenases of human and mouse

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1. Introduction

Flavin-containing monooxygenases (FMOs; EC 1.14.13.8) are present in a wide range of organisms, including bacteria, fungi, plants, invertebrates and vertebrates. In eukaryotes, the enzymes are located in the membranes of the endoplasmic reticulum and catalyze the oxidative metabolism of a broad range of structurally diverse chemicals, including therapeutic drugs, dietary-derived compounds and pesticides [1,2]. Substrates contain, as the site of oxygenation, a nucleophilic heteroatom, typically nitrogen, sulphur, phosphorus or selenium [3]. FMOs are one of the most important of the non-cytochrome P450 (CYP) enzymes involved in the phase-1 metabolism of drugs. Of 860 drugs surveyed, FMOs contribute to the metabolism of about 5%, catalyzing about 2% of the more than 4,000 oxidoreduction reactions undergone by these drugs [4]. However, as outlined below, this might be an underestimate.

In this review, we focus on the role of FMOs in the metabolism of drugs in human and mouse. We describe the FMO genes and proteins of human and mouse, the catalytic mechanism of FMOs and its significance for the metabolism of drugs. We outline differences between FMOs and CYPs; discuss factors contributing to potential underestimation of the contribution of FMOs to drug metabolism; summarise the developmental stage- and tissue-specific expression of *FMO* genes and how this differs between human and mouse; and describe factors that induce or inhibit FMOs. We describe the contribution of FMOs of human and mouse to the metabolism of drugs *in vitro* and *in vivo* and how genetic variation of FMOs affects drug metabolism *in vitro* and *in vivo*. Finally, we discuss the utility of animal models for FMO-mediated drug metabolism in humans.

2. FMOs of human and mouse

Humans possess five functional *FMO* genes, designated *FMO1* to *FMO5* [5,6]. *FMO1* to *FMO4* are clustered on chromosome 1, in the region q24.3 [6] (Figure 1). The cluster contains an additional *FMO* gene, *FMO6P*, classified as a pseudogene because of its inability to produce a correctly spliced mRNA [7]. *FMO5* is located ~26 Mb closer to the centromere, at 1q21.1 [6]. A second *FMO* gene cluster, composed entirely of pseudogenes, *FMO7P* to *FMO11P*, is present at 1q24.2 [6]. The human genome, therefore, contains 11 *FMO* genes, five of which are functional. In the mouse, five *Fmo* genes, designated *Fmo1* to *Fmo4* and *Fmo6*, which are orthologous to the corresponding genes of human, are clustered on Chromosome 1 (Figure 1) [6]. In mouse, as is the case in humans, the gene encoding FMO5 is located outside the main *Fmo* gene cluster, in this case on mouse Chromosome 3 [6]. A second *Fmo* gene cluster, which contains three genes, designated *Fmo9*, *Fmo12* and *Fmo13*, is located on mouse Chromosome 1, ~3.5 Mb from the main *Fmo* gene cluster [6]. *Fmo6*, *Fmo9*, *Fmo12* and *Fmo13* encode full-length open-reading frames and possess no obvious features that would categorize them as pseudogenes. The mouse genome, therefore, contains orthologues of the five functional *FMO* genes of human and four additional, potentially functional *Fmo* genes.

3. Catalytic mechanism

Much of our knowledge of the catalytic mechanism and substrate preferences of FMOs derives from the pioneering work of Dan Ziegler and colleagues (reviewed in [8-10]). For catalysis, FMOs require flavin adenine dinucleotide (FAD) as a prosthetic group, NADPH as a cofactor and molecular oxygen as a cosubstrate. The catalytic mechanism (Figure 2) is unusual because FMOs activate oxygen, in the form

of the C4a hydroperoxide derivative of FAD, in the absence of a bound oxygenatable substrate. Steps 1 and 2 are fast and the enzyme is present as the active C4a-hydroperoxyflavin form, capable of oxygenating any soft nucleophile able to gain access to the active site.

The unusual mechanism of FMOs accounts for their broad substrate range [8-10]. The rate-limiting step is considered to be either the breakdown of FADH-OH to release water or the release of NADP⁺, both of which occur after the oxygenation of substrate and the release of oxygenated product. Consequently, in almost all cases, the catalytic constant (k_{cat}) is independent of the structure of the substrate and the specificity constant (k_{cat}/K_M) is determined largely by the K_M for the substrate. Because FMOs do not form classical Michaelis-Menten enzyme-substrate complexes, K_M is a measure of the ease with which a substrate can gain access to the active site. Size and shape are important factors that restrict access to the active site [8-11]. Charge is also important [8-10]: the best substrates are uncharged or have a single positive charge. In contrast, zwitterions or compounds with more than one positive charge are excluded, as are compounds with a single negative charge, except in cases where the charge is located at an appropriate distance from the site of oxygenation.

4. Differences between FMOs and CYPs

There are distinct differences in the catalytic mechanisms of FMOs and CYPs. In contrast to FMOs, which accept electrons directly from NADPH, CYPs receive electrons from NADPH via an accessory protein, NADPH-cytochrome P450 reductase, and bind and activate oxygen only after binding oxygenatable substrate. Whereas FMOs stabilize the active hydroperoxy flavin intermediate, CYPs form a relatively unstable ferrous-O₂ complex. FMOs have a preference for nucleophilic

compounds, whereas CYPs accept less nucleophilic ones. FMOs and CYPs share some substrates in common, but often produce different metabolites: although CYPs, in common with FMOs, can effect heteroatom oxygenation of nitrogen or sulfur, they more often catalyze carbon hydroxylation, heteroatom release (dealkylation) or epoxidation [12].

CYPs oxidize chemicals via sequential one-electron processes and, thus, are more capable of producing reactive, potentially toxic products, some of which can inactivate or inhibit CYPs. In contrast, FMOs oxygenate substrates, at a nucleophilic heteroatom, via a two-electron mechanism that generally produces polar, readily excretable detoxification products. Products of FMO-catalyzed reactions, even those that are relatively reactive, do not inhibit the enzyme, but can sometimes inhibit or inactivate nearby proteins, including CYPs [13,14].

5. Factors leading to possible underestimation of the contribution of FMOs to drug metabolism

Methods by which microsomes are prepared and incubated may have lead to underestimation of the contribution of FMOs to drug metabolism. In the absence of NADPH, FMOs are thermally labile [15]. Consequently, liver perfusion *in situ*, to remove blood before preparation of microsomes, could reduce FMO activity. To avoid autooxidative reactions, to which CYPs are prone, microsomes are often incubated at 37 °C in the presence of substrate and the reaction initiated by the addition of NADPH. Preincubation in the absence of NADPH would diminish FMO activity. Optimal FMO activity can be maintained by preparing microsomes at 4 °C, adding an NADPH-generating system to the microsomes before preincubation and initiating reactions by addition of substrate [15].

To distinguish between the contributions of FMOs and CYPs to the metabolism of a drug, incubations can be carried out on microsomes that have been pre-heated at 55 °C in the absence of NADPH, which knocks out FMO activity [15]. To selectively knock out CYP activities microsomes can be incubated at pH 10, or in the presence of carbon monoxide, high concentrations of non-ionic detergents, an inhibitory antibody to NADPH CYP reductase, or chemical inhibitors of CYPs [15]. However, some CYP inhibitors, such as SKF525A, are substrates for FMO and, therefore, could act as an alternative substrate competitive inhibitor of FMO. The ability of an FMO to catalyze oxygenation of a particular drug can be confirmed by the use of recombinantly expressed FMO.

6. Differences in expression of *FMO* genes between human and mouse

There are distinct differences between human and mouse in the tissue-specific expression of FMOs and in mouse marked gender-specific differences. In humans, expression of the *FMO1* gene in liver is switched off at birth [16,17]. This is in contrast to mouse, in which *FMO1* constitutes a major form of the enzyme present in adult liver [18,19]. Silencing of the *FMO1* gene in adult human liver may be due to the presence, upstream of the hepatic promoter P0, of a LINE-1 element that acts as a powerful transcriptional repressor [20]. In extra-hepatic tissues, alternative promoters, P1 and P2, are used [6,20]. In adult human, *FMO1* is present in kidney, its main site of expression [21-23], and in small intestine [22]. In adult mouse, *Fmo1* is expressed in liver, kidney, lung [19], white adipose tissue [24] and, to a small extent, in whole brain, in which it is the most highly expressed FMO [19]. In mouse, expression of *Fmo1* in liver and lung is greater in females than in males, but in kidney is greater in females [19].

In both human and mouse, the main site of expression of *FMO2* is lung [1,19,25]. *FMO2* is also present in kidney [1,19].

In humans, expression of *FMO3* in liver is switched on after birth [17,21] and continues to increase throughout childhood and adolescence, reaching a maximum in adulthood [17]. *FMO3* declines during menstruation [26] but otherwise there is little difference in expression between males and females. In mouse, however, there is a profound gender difference in the expression of *Fmo3* [19,27]. In females, *Fmo3* expression is switched on after birth, increases to reach a peak at five weeks of age and is maintained, albeit at a slightly lower level, throughout adulthood; in males, it increases from birth to three weeks of age and is subsequently switched off, being undetectable by five weeks of age.

The *FMO4* gene is expressed in several tissues in human and mouse but, with the exception of human pancreas, in all cases the expression is very low [1,6,19].

The gene for *FMO5* is expressed in the embryo and in many tissues of fetal and adult human and mouse [6]. In both organisms it is most highly expressed in adult liver and in this organ its abundance is similar to or greater than that of *FMO3* [1,19]. In humans, *FMO5* is highly expressed also in stomach and pancreas [6] and in the small intestine, in which it is the most highly expressed *FMO* gene [1]. In mouse, it is expressed also in kidney [19] and in small and large intestine, where it is the most highly expressed isoform [28]. In mouse liver and kidney, *Fmo5* expression is higher in males than in females [19].

In mouse liver, mRNAs for *FMO1*, *FMO2*, *FMO4* and *FMO5* are present at birth. Those encoding *FMO2* and *FMO4* exhibit no significant age-related changes. In contrast, expression of *Fmo1* and *Fmo5* changes with age, increasing after birth, to reach a peak at five weeks post-partum [19]. As described above, *Fmo3* expression

exhibits gender-specific age-related changes. With the exception of FMO3 (see above), no equivalent information is available on the effect of age on *FMO* expression in humans.

7. Complement of FMOs in major organs involved in drug metabolism

The complement of FMOs present in major sites of drug metabolism, such as liver, kidney, lung and small intestine, differs qualitatively and quantitatively in both human and mouse and there are marked species differences [1,19] (Table 1). In adult human liver, the main FMOs are FMO3 and FMO5, with FMO1 being absent. FMO3 and FMO5 are abundant in female mouse liver, but FMO1 is also present. In adult male mouse liver, FMO1 and FMO5 are present, but FMO3 is absent. In human kidney, the most abundant FMO is FMO1, followed by FMO2. In female mouse kidney, FMO1 is the most abundant isoform, but in male mouse kidney, the abundance of FMO5 is similar to that of FMO1. In human lung, by far the most highly expressed FMO mRNA is that for FMO2. However, as discussed in Section 12, most humans do not produce functional FMO2 from the mRNA. In mouse lung, both FMO1 and FMO2 are expressed [19,29]. FMO5 is the main isoform present in the small intestine in both human and mouse.

Interindividual variations have been reported in the abundance in adult human kidney of FMO1 (up to 5-fold) [30], and in adult human liver of FMO3 (10- to 20-fold) [17,31,32] and FMO5 (10-fold) [31]. With the exception of FMO5, FMOs are not readily inducible by foreign chemicals (see Section 9). Consequently, individual variations in the abundance of FMO1 and FMO3 are likely due to genetic or physiological factors, but in the case of FMO5 differential exposure to foreign chemicals might contribute.

The presence of FMO1 and FMO5 in fetal human liver [6,23,33] suggests that drug substrates for these enzymes that can cross the placenta will be metabolized in fetal liver. The amount of FMO1 in adult human kidney [22] is more than that of CYPs [34]. Thus, in adult human, FMO1 is likely to contribute substantially to the renal metabolism of drugs. The amount of FMO3 in adult human liver [31] is ~ 60% of that of CYP3A4 [35], the most abundant hepatic CYP, which catalyzes almost 30% of all oxidoreduction reactions undergone by drugs [4]. Given the relative abundance of FMO3 in liver and FMO1 in kidney, it is perhaps surprising that only about 2% of oxidoreduction reactions undergone by drugs has been attributed to FMOs and suggests that their contribution to drug metabolism might be underestimated.

8. Regional-specific expression of FMOs

In adult female mouse liver, mRNAs encoding FMO2, FMO3 and FMO4 are expressed in the periportal region, the expression of FMO3 mRNA being far greater than that of FMO2 or FMO4. In contrast, mRNAs encoding FMO1 and FMO5 are expressed primarily in the perivenous region, with a gradient across the acini [19]. In the kidney, expression of all five FMO mRNAs is localized to the distal and proximal tubules and the collecting ducts [19]. FMO1 mRNA was detected also in the glomerulus [19]. In lung, FMO1, FMO2 and FMO5 mRNAs are localized to the endothelial lining of the alveoli and terminal bronchiole [19]. FMO3 mRNA is present only in the terminal bronchiole, and, unlike the situation in liver, in lung this isoform is present in both adult male and female mice [19].

Little information is available on the regional localization of FMOs in human tissues such as liver and kidney. However, in human skin, an organ that is exposed to numerous chemicals, FMO1, FMO3, FMO4 and FMO5 mRNAs are localized in the

epidermis, sebaceous glands and hair follicles [36].

9. Induction and inhibition of FMOs

In contrast to CYPs, FMOs are not readily inducible by foreign chemicals. An exception is FMO5, which is inducible in human hepatocytes by rifampicin [37], in HepG2 cells by hyperforin [38] and in a breast cancer cell line by the synthetic progestin R5020 [39]. In primary hepatocytes isolated from mice there are gender differences in *Fmo5* expression in response to some chemicals [40]. In response to progesterone, the abundance of FMO5 mRNA was increased in hepatocytes isolated from female mice, but reduced in cells isolated from males, and rifampicin induced *Fmo5* expression to a much greater extent in hepatocytes isolated from female than from male mice. In mouse, the abundance of FMO mRNAs can be influenced by diet [41].

The repression of *Fmo3* gene expression in adult male mouse liver can be reversed: FMO3 mRNA is induced ~ 6000-fold by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), but this is not accompanied by a corresponding increase in FMO catalytic activity [42]. The response of *Fmo3* expression in females to TCDD was modest. The increase in the abundance of FMO3 mRNA represents a reversal of the repression of *Fmo3* expression in the liver of adult male mice rather than an induction of gene expression. The results indicate that if mice are the selected species for investigation of FMO3 induction, studies should include both male and female animals.

In contrast to CYPs, FMOs are not readily irreversibly inhibited by foreign chemicals. Exceptions are the inhibition of the activity of porcine FMO1 *in vitro* by (*N,N*-dimethylamino) stilbene carboxylates [43] and of human FMO3 *in vitro* and *in*

vivo by dietary indoles, present in brassicas [44]. FMOs are, however, subject to competitive inhibition by alternative substrates. One such competitive inhibitor, methimazole, has been used to distinguish FMO- from CYP-mediated metabolism of drugs. However, this approach is problematic because the product of FMO-mediated *S*-oxygenation of methimazole, a sulfenic acid, results in a decrease in CYP content [13].

10. Drug substrates of FMOs

Of the FMOs, FMO1 has the broadest substrate range, followed by FMO3. FMO1 and FMO3 share some substrates in common, e.g., benzydamine, itopride, *N*-deacetyl ketoconazole, voriconazole and tamoxifen, but are relatively selective towards others, e.g., FMO1 for chlorpromazine, imipramine and quazepan, FMO3 for procainamide. FMO2 and FMO5 have a more restricted range of substrates. In comparison with other FMOs, FMO4 is extended at its C-terminus by 23 to 26 amino-acid residues [5,45]. Expression of full-length FMO4 has proved problematic. Consequently, almost nothing is known of its substrate range and, as FMO4 is expressed in very low amounts, it is thought not to play a significant role in drug metabolism.

Therapeutic drugs that are substrates for human FMO1, FMO2, FMO3 and FMO5 are listed in Tables 2, 3, 4 and 5, respectively. Most drug substrates of FMOs are tertiary amines, which are *N*-oxygenated to form the *N*-oxide, or sulfides, which are *S*-oxygenated to the sulfoxide. Others include primary amines, such as amphetamine [46], which are converted to an *N*-hydroxylamine, then, via a second oxygenation and loss of water, to an oxime; secondary amines, such as *N*-deacetyl ketoconazole [47], which are converted initially to an *N*-hydroxyamine, then, via a second oxygenation, to a nitron; and thiocarbamides, such as ethionamide [29], which are converted to a sulfoxide. *N*-oxides and sulfoxides are polar and readily

excreted and, thus, FMO-mediated metabolism of tertiary amines and sulfides represents a detoxification. In contrast, some of the *N*-hydroxylated products of FMO-mediated metabolism of primary and secondary amines can inhibit CYPs and, in some cases, e.g., the hydroxylated product of *N*-deacetyl ketoconazole, have toxic effects [48].

FMOs can be stereoselective in the production of product. Examples include nicotine [49], pargyline [5], amphetamine [50], sulindac sulfide [30] and cimetidine [51]. In some cases, the stereoselectivity of FMOs differs. In the case of pargyline *N*-oxygenation, human FMO1 produces only the (+)-enantiomer, whereas human FMO3 produces predominantly the (-)-enantiomer [5]. For cimetidine *S*-oxygenation, human FMO1 produces more (-)- than (+)-enantiomer, but for human FMO3 the opposite is the case [51]. Rettie et al. showed that prochiral sulfoxidation of the non-drug substrate methyl *p*-tolyl sulfide was stereoselective for FMO1, which produces exclusively the (*R*)-sulfoxide, whereas FMO3 produces equal amounts of both the (*R*)- and (*S*)-sulfoxides [52]. Thus, this compound can be used to distinguish the activities of FMO1 and FMO3 in preparations of microsomes in which both of these enzymes are present [52].

FMO5 displays no or poor reactivity towards classical FMO substrates and, consequently, was thought not to play a significant role in drug metabolism. FMO5 is known to catalyze the *N*- or *S*-oxygenation of only a small number of drugs *in vitro* (Table 5). A comparison of mouse FMO1, FMO3 and FMO5 catalytic activity towards a range of substrates identified unique properties of FMO5 [53]. Recently, human FMO5 has been identified as a Baeyer-Villiger monooxygenase [54] able to catalyze oxidation of a wide range of carbonyl compounds, via insertion of an oxygen atom into a carbon-carbon bond adjacent to the carbonyl group (aldehyde or ketone),

to form an ester. Drugs that have been identified as being metabolized by FMO5-mediated Baeyer-Villiger oxidation include the anticancer compound E7016 [55] and the antibacterial MRX-1 [56]. Now that FMO5 has been shown to catalyze a Baeyer-Villiger reaction, more substrates for this enzyme are likely to be identified. Earlier work showed that porcine FMO1 is able to catalyze the Baeyer-Villiger oxidation of several carbonyl compounds [57]. However, it is not known whether human or mouse FMO1 has this ability.

In the majority of cases, drug substrates of FMOs are metabolized also by other enzymes, particularly CYPs, and the FMO-catalyzed reaction does not represent the major route of metabolism. However, for individuals who possess polymorphic variants that decrease catalytic activity of CYPs the contribution of FMOs to drug metabolism is likely to be greater. One such example is that of nicotine, which is metabolized predominantly by CYP2A6 [58], but also by FMO3 [49]. The oxidation of a substrate by CYPs and FMOs usually results in formation of distinct products. This is certainly the case for aliphatic tertiary amines, FMOs producing exclusively the *N*-oxide, whereas CYP-mediated oxidation results in *N*-dealkylation [59].

Drugs that are likely to be metabolized exclusively or predominantly by FMOs include benzydamine [60], itopride [61], olopatadine [62], pargyline [5], ranitidine [31,63] and xanomeline [64], via *N*-oxygenation, and albendazole [65], cimetidine [31,51,66], ethionamide [29] and sulindac sulfide [30], via *S*-oxygenation.

Another factor contributing to the potential underestimation of the contribution of FMOs to the *N*-oxygenation of drugs containing tertiary amines is that the *N*-oxides can be retro-reduced to the parent compound by CYPs or other reductases [67], such as quinone reductase [68] and aldehyde oxidase [69]. The

contribution of FMOs to the metabolism of drugs containing secondary amines may also be underestimated. In this case, the initial product of the FMO-catalyzed reaction, an *N*-hydroxylamine, is converted, via a second oxygenation, to a nitron, which is hydrolyzed to produce an aldehyde and a hydroxylated primary amine [70], the latter being enzymatically reduced to a primary amine, the same product as would be produced from the initial secondary amine substrate by a CYP-mediated *N*-dealkylation [71].

In addition to drugs, FMOs catalyze the oxygenation of several other foreign chemicals, including the pro-neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (FMO1) [72], insecticides, such as phorate and disulfoton (FMO2) [73], and dietary-derived compounds, such as trimethylamine (FMO3) [74,75]. However, discussion of the involvement of FMOs in the metabolism of non-drug xenobiotics is beyond the scope of this review.

11. Role of FMOs in endogenous metabolism

Because of charge restrictions, discussed in Section 3, almost all small endogenous nucleophiles are excluded from the active site of FMOs. Exceptions include the biogenic amines tyramine [76] and phenethylamine [77], which are converted by human FMO3 to the *N*-oxide, then via a second, stereoselective, *N*-oxygenation to the *trans*-oxime. Human FMO3 can also catalyze *S*-oxygenation of methionine, but, as the K_M for the reaction is in the mM range, its physiological relevance is questionable. Porcine FMO1 can *S*-oxygenate cysteamine and cysteine *S*-conjugates, but again, in the latter case the K_M is high, casting doubt on its metabolic relevance.

The use of *Fmo*-knockout mouse lines has revealed that, in addition to contributing to the metabolism of drugs, FMO1 and FMO5 have roles in endogenous

metabolism, FMO1 as a regulator of energy balance [24] and FMO5 as a regulator of metabolic aging, acting via pleiotropic effects, including influencing weight gain and plasma concentrations of glucose and cholesterol [78].

12. Genetic variants of FMOs and their effect on drug metabolism

Most humans are homozygous for a nonsense mutation of *FMO2*, c.1414C>T (p.(Gln472*)) [79]. The resultant allele, *FMO2*2A*, encodes a truncated polypeptide, FMO2.2A, that lacks 64 amino-acid residues from its carboxy terminus and is catalytically inactive [79]. Thus, in contrast to mouse, most humans do not express functional FMO2. The ancestral allele, *FMO2*1*, which encodes a full-length polypeptide, FMO2.1, is present in populations of recent African descent, and in some regions of sub-Saharan Africa almost 50% of individuals possess at least one *FMO2*1* allele [80]. Other deleterious variants have been identified, but these occur on the *FMO2*2A* allele [81]. Individuals who possess an *FMO2*1* allele, thus, are likely to express a functional protein. The presence of full-length active FMO2 in lung microsomes isolated from an *FMO2*1* individual has been confirmed [82]. The distribution of *FMO2*1* and *FMO2*2A* alleles among world populations has implications for interethnic and, in African populations, interindividual variation in response to drugs, particularly those for which the lung is a target organ or route of entry, such as the antitubercular prodrugs ethionamide and thiacetazone.

Rare genetic variants that abolish or severely impair FMO3 activity cause primary trimethylaminuria [2,74,83-85]. Affected individuals are unable to metabolize dietary-derived trimethylamine, a substrate that is specific for FMO3 [75], to its non-odorous *N*-oxide and, consequently, excrete the smelly free amine in breath, sweat and urine [86]. It is expected that sufferers of trimethylaminuria will have

impaired ability to metabolize drugs that are substrates for FMO3, and this has been shown to be the case for benzydamine [87].

In addition to rare, loss-of-function variants, at least 15 nonsynonymous single-nucleotide polymorphic variants (SNPs) of *FMO3* have been identified [2,84,88]. A catalogue of genetic variants of *FMO3* and their effect on drug metabolism [89] can be accessed at the *FMO3* locus-specific database (<http://databases.lovd.nl/shared/genes/FMO3>). The effect of many of the SNPs on the metabolism of drugs has been tested *in vitro*. Individually, the most common SNPs, c.472G>A(p.(Glu158Lys)) and c.923A>G(p.(Glu308Gly)), have little or no effect on enzyme activity [1,2,90,91]. In Europeans and Asians, however, the two SNPs are often linked, occurring on the same chromosome [92-95]. The effect of the compound variant, (p.(Glu158Lys;Glu308Gly)), on enzyme activity is greater than that of either variant alone, and the extent to which it reduces activity depends on the substrate [1,2,90,91,94,96,97].

The influence of c.769G>A(p.(Val257Met)), a SNP that is common in Asian populations, also is substrate dependent. This is illustrated by its effect on the ability of FMO3 to catalyze the *N*-oxygenation of the anti-cancer aurora kinase inhibitors danusertib and tozasertib [98]. The variant significantly reduces the *N*-oxygenation of danusertib, but has no effect on production of tozasertib *N*-oxide. The consequences for an individual undergoing treatment with danusertib of possession of a c.769G>A(p.(Val257Met)) variant are not known.

Three other SNPs affect FMO3 activity: c.183C>A(p.(Asn61Lys)) causes a marked reduction in enzyme activity towards four different substrates [99]; c.613C>T(p.(Arg205Cys)) has a moderate effect on activity [97]; and c.1079T>C(p.(Leu360Pro)) increases activity by 2- to 5-fold [96]. However, each is

present at low frequency and/or occurs in a single population group [2] and, consequently, has limited significance for the general population.

Several SNPs have been identified in the promoter region of *FMO3* [100]. These segregate into 15 haplotypes. Of these, two markedly decrease transcription *in vitro*, whereas one increases transcription 8-fold [100]. The effect of the promoter-region SNPs on transcription of the *FMO3* gene *in vivo* is not known.

Drug-drug interactions are thought to be rare for substrates of FMO3 [59]. However, a study *in vitro* demonstrated that FMO3-catalyzed *N*-oxygenation of benzydamine was inhibited by several substrates, including itopride, toizatertib, methimazole and trimethylamine, and *S*-oxygenation of sulindac sulfide was inhibited by methimazole [101], but, as discussed in Section 9, the use of methimazole as an inhibitor of FMO activity can be problematic. It is suggested that individuals with reduced FMO3 activity, such as those with the (p.(Glu158Lys;Glu308Gly)) compound variant, might experience unexpected consequences in drug response if exposed to more than one FMO3 substrate.

FMO3 activity might be influenced by factors other than polymorphic variation. For instance, in the presence of excess nitric oxide, FMO3 undergoes *S*-nitrosylation, which reduces the ability of the enzyme to catalyze ranitidine *N*-oxygenation [102], and the availability of the transcription factors HNF-4 and NF-Y may influence *FMO3* expression [103].

Few nonsynonymous SNPs have been identified in *FMO1* and each occurs at low frequency [2,91,104]. Of those tested, only one had an effect on enzyme activity *in vitro*: c.1504C>T(p.(Arg502*)), which abolished activity towards methimazole, but had no effect on activity towards three other substrates [105]. A relatively common SNP, g.-9536C>A (the *FMO1**6 allele), located in a YY1 element of the fetal liver-

specific promoter of *FMO1*, prevented binding of the transcription factor and decreased promoter activity *in vitro*, but did not correlate with lower expression of *FMO1* in fetal liver *in vivo* [106].

Very few nonsynonymous SNPs have been identified in the genes encoding FMO4 and FMO5 [2,104]. Of these, most occur at very low frequency and none is known to affect enzyme activity [2,104].

Although *FMO* genetic variants, especially those affecting the activity of FMO3, are known to influence drug metabolism *in vitro*, an understanding of the impact of *FMO* variants *in vivo* is limited to a small number of studies in humans. In mouse, studies *in vivo* using *Fmo*-knockout lines have provided additional information (see Section 13).

Sulindac sulfoxide is a prodrug converted by gut bacteria to sulindac sulfide, the active form of the drug, which is metabolized by FMO3 back to the inactive sulfoxide and, then, to sulindac sulfone [30]. The FMO3 variants (p.(Glu158Lys)) and (p.(Glu308Gly)) decrease FMO3 activity towards sulindac *in vitro* [30]. In patients with familial adenomatous polyposis undergoing treatment with sulindac, the variants are associated with regression of existing polyps and protection against adenoma formation, particularly in individuals homozygous for the double variant (p.(Glu158Lys;Glu308Gly)) [107,108]. It is thought that reduction of FMO3 activity, as a consequence of these polymorphic variants, would result in higher circulating concentrations of the active sulindac sulfide.

Conversion of ranitidine to its *N*-oxide by FMO3 was demonstrated *in vitro* using human liver microsomes [31]. Subsequently, the double variant (p.(Glu158Lys;Glu308Gly)) was found to have a reduced capacity for ranitidine *N*-oxygenation [94]. *N*-oxygenation of ranitidine by FMO3 has been confirmed *in vivo*

[93]; individuals heterozygous or homozygous for the (p.(Glu158Lys;Glu308Gly)) double variant excreted lower amounts of ranitidine *N*-oxide in their urine than did individuals homozygous for the ancestral (p.(Glu158;Glu308)) allele [93,94].

Although benzydamine is effectively converted *in vitro* to benzydamine *N*-oxide by FMO1 and FMO3 [60], the lack of expression of FMO1 in adult human liver enables benzydamine to be used as a probe for FMO3 activity *in vivo*. The role of FMO3 in benzydamine metabolism *in vivo* was confirmed in a study of patients suffering from trimethylaminuria, caused by mutations that severely impair FMO3 activity, in whom plasma and urinary concentrations of benzydamine *N*-oxide were markedly reduced [87].

The antipsychotic olanzapine is converted *in vitro* to its *N*-oxide by FMO3 [109] and, through the action of CYPs, can be hydroxylated or *N*-demethylated [109,110]. The *N*-oxide can be formed also by CYP2D6 *in vitro*, but olanzapine metabolism *in vivo* is not influenced by *CYP2D6* genotype [111,112]. Olanzapine *N*-oxide formation *in vitro* by the FMO3 variant (p.(Glu158Lys;Glu308Gly)) was lower than by the ancestral form, (p.(Glu158;Glu308)) [113]. When the influence of this compound variant was examined *in vivo* the C/D (median dose-adjusted steady-state serum concentration) of olanzapine *N*-oxide in patients homozygous for (p.(Glu158Lys;Glu308Gly)) was ~50% lower than in individuals heterozygous or homozygous for the ancestral form, but the variant allele had no effect on the plasma concentration of olanzapine [114]. In a study of Japanese, several genotypes of *FMO3* and *CYPs* were found not to be associated with changes in olanzapine clearance, the conclusion being that the drug is subject to multi-pathway metabolism and if one pathway is less effective others compensate [112]. Variants of *FMO1* have been shown to alter olanzapine metabolism. In this case, the non-coding variant *FMO1**6,

which decreases promoter activity *in vitro* (see above), was found to increase serum C/Ds in patients [114].

Itopride, a gastroprokinetic agent, undergoes *N*-oxygenation through the action of FMO3 *in vitro* [61] and *in vivo* [115] and this is considered the major route of metabolism. Chinese individuals homozygous for (p.(Glu158Lys;Glu308Gly)) had higher plasma concentrations of itopride and lower concentrations of the *N*-oxide than those homozygous for the ancestral form (p.(Glu158;Glu308)) [115].

The involvement of FMO3 in clozapine *N*-oxygenation was demonstrated *in vitro* [116]. A study *in vivo* found no correlation between clozapine *N*-oxide production and the (p.(Glu158Lys)), (p.(Val257Met)) or (p.(Glu308Gly)) variants [92].

FMO3 converts nicotine to its *N*-oxide [49] and, in humans, 4-7% of nicotine equivalents are excreted as the *N*-oxide. CYP2A6, however, is the most important contributor to nicotine metabolism and nicotine *N*-oxide excretion is increased to ~30% of the absorbed nicotine in individuals homozygous for a *CYP2A6* deletion [117]. Polymorphisms that influence FMO3 activity would, therefore, be expected to be of greater importance in individuals with compromised CYP2A6 activity.

13. Mouse models for FMO-mediated drug metabolism

A consequence of the marked differences between human and mouse in expression of FMO1 and FMO3 in adult liver and the lack of functional FMO2 in most humans is that there are distinct differences between the species in the metabolism of drugs *in vivo* by FMOs. For instance, drug substrates of FMO1 will be metabolized in mouse, but not human, liver; substrates of FMO2 will be metabolized in mouse, but not in the majority of humans; and substrates for FMO3 will be metabolized in liver of humans and female mice, but not of male mice. Therefore, wild-type mouse is not a good

animal model for FMO-mediated drug metabolism in humans. However, female mice, because they express FMO3 in adult liver, may be useful as a model for FMO3-mediated metabolism, and, if used in combination with adult male mice, which lack hepatic FMO3, for confirming the participation of FMO3 in metabolism of a drug.

Few studies have directly compared the contribution of FMOs to drug metabolism *in vivo* in humans and mice. A study of procainamide, using a urinary metabolite profiling approach, concluded that in humans *N*-oxidation represents a major metabolic route, producing procainamide *N*-oxide and *N*-acetyl procainamide *N*-oxide, whereas in mice this was a minor route [118]. Experiments *in vitro* confirmed that both FMO3 and FMO1 catalyze oxygenation of procainamide and *N*-acetyl procainamide, the rates of formation being higher for FMO3. However, the mouse study used adult males and the differences found between human and mouse likely result from the lack of FMO3 in adult male mouse liver.

Female mice in which the genes for FMO1, FMO2 and FMO4 have been knocked out (*Fmo1*^{-/-}, *Fmo2*^{-/-}, *Fmo4*^{-/-}) [119] express in adult liver FMO3 and FMO5, but no other FMOs, and, thus, for most humans, would be a good animal model for FMO-mediated drug metabolism. The use of *Fmo*-knockout mouse lines can also help assess the contribution of a FMO protein to the metabolism of a drug that undergoes multi-pathway metabolism *in vivo* [120]. For instance, the antidepressant imipramine is oxygenated only by FMO1, to produce the *N*-oxide [119]. Imipramine *N*-oxide undergoes retroreduction *in vivo*, thus, quantification of the oxygenated product in plasma or urine can miss or underestimate the contribution of FMO1 to overall metabolism and clearance of the drug. Using an *Fmo1*-null mouse model, imipramine was shown to cause sedation only in wild-type animals, i.e., those that produced imipramine *N*-oxide in brain [119]. Adverse behavioural changes, such

as tremor, were observed only in mice that lacked FMO1 (the knockout mice), which produced higher amounts in brain of desipramine, a product of CYP-mediated metabolism.

FMO2 catalyzes the *S*-oxygenation of the antitubercular prodrugs ethionamide and thiacetazone *in vitro* [29,121]. The triple knockout mouse (*Fmo1*^(-/-), *Fmo2*^(-/-), *Fmo4*^(-/-)) was used to better mimic the situation in human, with respect to expression of FMOs in lung, and to establish whether a lack of FMO2 influences the metabolism of ethionamide *in vivo* [122]. The plasma concentration of ethionamide was found to be higher in knockout than in wild-type mice and that of ethionamide *S*-oxide to be higher in wild-type than in knockout animals, confirming the involvement of FMO2 in the metabolism of ethionamide. To be effective ethionamide must be taken up by mycobacteria in the lungs and subsequently metabolized, by the bacterial monooxygenase EtaA, to the sulfenic acid, which acts as the therapeutic agent [123]. Metabolism of ethionamide in the lung by FMO2 to the sulfoxide and, subsequently, to a potentially toxic sulfenic acid, would be expected to reduce drug efficacy, by diminishing the amount of prodrug available for uptake by mycobacteria, and increase harmful side effects.

14. Conclusions

Five functional FMOs, FMOs 1-5, are present in humans. The catalytic mechanism of FMOs, in which oxygen is activated in the absence of bound substrate, is unusual. It enables FMOs to oxygenate any nucleophile able to gain access to the active site, thus accounting for their broad substrate range. Human, FMO5 has been identified as a Baeyer-Villiger enzyme that can oxygenate a range of electrophilic substrates. Baeyer-Villiger oxidations can also be catalyzed by porcine FMO1.

Whether this is the case for FMO1 of human or mouse remains to be established.

FMO-catalyzed reactions are generally detoxifications, producing polar, readily excretable metabolites. Most drug substrates of FMOs are metabolized also by other enzymes, particularly CYPs, but some are metabolized predominantly or exclusively by FMOs. The contribution of FMOs to drug metabolism might be underestimated, owing to the use of methods for preparation and incubation of microsomes that do not preserve FMO activity. In contrast to CYPs, FMOs are not readily induced or inhibited, but genetic variation, particularly of FMO2 and FMO3, affects the metabolism of drug substrates of these enzymes.

There are marked differences between human and mouse in developmental stage- and tissue-specific expression of FMOs. Consequently, human and mouse differ in the complement of FMOs present in liver, kidney and lung, the main sites of drug metabolism. The species differences are most striking in adult liver: in humans, the most abundant FMOs are FMO3 and FMO5, with FMO1 being absent; in mouse FMO1, along with FMO5, is present and, in females, but not in males, FMO3 also is present. Thus, mouse is not a good animal model for FMO-mediated drug metabolism in humans. However, female mice in which the *Fmo1*, *Fmo2* and *Fmo4* genes have been disrupted (*Fmo1*^(-/-), *Fmo2*^(-/-), *Fmo4*^(-/-)) express in adult liver FMO3 and FMO5, but no other FMOs, and, thus, for most humans are a good model for FMO-mediated drug metabolism.

15. Expert opinion

A number of drugs have been identified as substrates for FMOs, some being metabolized predominantly or exclusively by these enzymes. Research on Phase-1 drug metabolism has concentrated on CYPs, and methods commonly used for the

preparation and incubation of microsomes can result in selective loss of FMO activity, leading to potential underestimation of the contribution of FMOs to drug metabolism. Thus, despite the broad substrate range of FMOs and their relative abundance in liver, kidney and lung, the major sites of drug metabolism, the number of drugs identified as being metabolized by FMOs is relatively small. The adoption of methods for microsome preparation and incubation that favour retention of FMO activity, combined with selective ablation of CYP activity, would help achieve a more complete appreciation of the contribution of FMOs to drug metabolism. The identification of FMO5 as a Baeyer-Villiger monooxygenase indicates that FMO5 has a more important role in drug metabolism than hitherto suspected and that additional drug substrates of FMO5 are likely to be identified.

The substrate preferences and reactions catalyzed by FMO5 differ from those of other mammalian FMOs, suggesting that FMO5 may have a distinct mechanism of action. It will be important to establish whether this is the case. A detailed understanding of the mechanism of action of mammalian FMOs has been hindered by the lack of 3D structures of these proteins.

FMO-catalyzed reactions generally form polar, readily excretable detoxification products, whereas CYPs are more able to produce reactive, potentially toxic products. In contrast to CYPs, FMOs, with the exception of FMO5, are not readily induced or inhibited by foreign chemicals. These differences between FMOs and CYPs indicate that drugs that are metabolized predominantly by FMOs would be less likely to elicit drug-drug interactions and potentially harmful side effects, and that the design of such drugs would offer clinical advantages.

The human gut microbiome makes important contributions to the metabolism of orally administered drugs, for instance, in production of sulindac sulfide, the active

form of the prodrug sulindac, which is subsequently inactivated by metabolism by FMO3. There are considerable inter- and intra-individual differences in the composition of gut flora and the effect of these on drug metabolism is not well understood.

The general lack of induction or inhibition of FMOs indicates that the interindividual variation in abundance or activity of FMOs is largely the result of genetic or physiological factors. This offers the possibility that genotyping individuals for *FMO* variants would identify those who might experience problems when treated with drugs metabolized by FMOs.

Investigation of FMO-mediated drug metabolism would benefit greatly from the availability of an animal model that accurately reflects the metabolic capacity of human with respect to FMOs. The functional capabilities *in vitro* of FMOs of mouse are very similar to those of their human orthologues. However, owing to marked differences between human and mouse in the abundance of FMOs in the major organs of drug metabolism, particularly liver, mouse is not a good model for investigation of FMO-mediated metabolism *in vivo*. This problem can be overcome by the development and use of knockout-mouse lines in which selected *Fmo* genes have been disrupted. For instance, female *Fmo1*^(-/-), *Fmo2*^(-/-), *Fmo4*^(-/-) mice express in adult liver FMO3 and FMO5, but not FMO1, a complement of FMOs similar to that in the liver of Europeans, Asians and most Africans, and, thus, are a good model for most humans. Wild-type adult male mice are natural liver-specific knockouts for *Fmo3* and, thus, in conjunction with wild-type females, which do express *Fmo3*, can be used to investigate the role of FMO3 in metabolism of a drug. It is predicted that the use of knockout-mouse lines will facilitate advances in understanding the contribution of FMOs to the metabolism of therapeutic drugs.

The discovery that FMO1 and FMO5 function in endogenous metabolism initiates an exciting new area of research and has important implications for drug therapy. Drugs that are substrates for these enzymes may compete with endogenous substrates and, thus, affect energy homeostasis, in the case of FMO1, or metabolic aging, in the case of FMO5, with potential effects on therapeutic response and endogenous metabolic functions. Induction of FMO5 by some therapeutics may also have adverse effects on the metabolic health of patients.

Article highlights box

- FMOs are involved in the metabolism of a number of therapeutic drugs; in most cases the drugs are metabolized also by other enzymes, particularly CYPs, but some are metabolized exclusively or predominantly by FMOs
- The contribution of FMOs to drug metabolism may be underestimated, in particular, the ability of FMO1 and FMO5 to catalyze Baeyer-Villiger reactions
- Human and mouse differ with respect to the complement of FMOs present in major organs of drug metabolism, consequently, wild-type mouse is not a good model for FMO-mediated metabolism in human
- Genetic variants of FMOs, particularly of FMO2 and FMO3, affect metabolism of substrates of these enzymes
- FMO1 and FMO5 function in endogenous metabolism, which has implications for drug therapy
- Female knockout mice in which the *Fmo1*, *Fmo2* and *Fmo4* genes have been disrupted are a good model for FMO-mediated drug metabolism in human

Figure Legends

Figure 1. Chromosomal localization and arrangement of *FMO* genes of human and mouse. Arrows represent direction of transcription. Diagram not to scale.

Figure 2. Catalytic cycle of mammalian FMOs. Evidence for the cycle is based on spectrophotometric and kinetic studies (reviewed in [8,10]). NADPH binds and reduces the prosthetic group FAD to FADH₂ (step 1). Molecular oxygen then binds and is reduced, forming C4a-hydroperoxyflavin, which is stabilized by NADP⁺ (step 2). Substrate (S) is oxygenated via nucleophilic attack on the distal oxygen of the C4a-hydroperoxyflavin, leaving the prosthetic group in the form of C4a-hydroxyflavin (step 3). Water is then released, reforming FAD (step 4) and the final step is the release of NADP⁺ (step 5). Adapted from [10].

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Table 1. Major FMO forms expressed in adult human and mouse tissues

| | Human | Mouse male | Mouse female |
|--------|------------|-------------|------------------|
| Liver | FMO3, FMO5 | FMO1, FMO5 | FMO1, FMO3, FMO5 |
| Lung | FMO2 | FMO1, FMO2, | FMO1, FMO2 |
| Kidney | FMO1 | FMO1, FMO5 | FMO1 |

Table 2. Drug substrates of human FMO1

| Substrate | Type of drug | Reaction | Analytical system | Reference |
|---|--|--------------------------------------|---|-----------|
| <i>N</i> -(3 <i>R</i>)-1-azabicyclo[2.2.2]oct-3-ylfuro[2,3- <i>c</i>]pyridine-5-carboxamide | α_7 nicotinic acetylcholine receptor agonist | <i>N</i> -oxygenation | in vivo; kidney microsomes; recombinant protein | [124] |
| Benzydamine | nonsteroidal antiinflammatory | <i>N</i> -oxygenation | recombinant protein | [60] |
| C-1311 | antitumour agent | <i>N</i> -oxygenation | recombinant protein | [125] |
| Cimetidine | antiulcerative, histamine H2-receptor antagonist | <i>S</i> -oxygenation | recombinant protein | [51] |
| Deprenyl | monoamine oxidase type B inhibitor | <i>N</i> -oxygenation | recombinant protein | [126] |
| Disulfiram metabolite, <i>s</i> -methyl <i>n,n</i> -diethylthiocarbamate ^a | antialcoholic | <i>S</i> -oxygenation | kidney microsomes; recombinant protein | [127] |
| Ethionamide | antitubercular | <i>S</i> -oxygenation | recombinant protein | [29] |
| GSK5182 | antidiabetic, estrogen-related receptor γ modulator | <i>N</i> -oxygenation | recombinant protein | [128] |
| Imipramine | antidepressant, 5HT/noradrenalin re-uptake inhibitor | <i>N</i> -oxygenation | recombinant protein | [105] |
| Itopride | dopamine D2 blocker and acetylcholinesterase inhibitor | <i>N</i> -oxygenation | recombinant protein | [61] |
| <i>N</i> -deacetyl ketoconazole ^b | antifungal | <i>N</i> -hydroxylation | recombinant protein | [47] |
| Lorcaserin | selective human 5-hydroxytryptamine 2C agonist | <i>N</i> -hydroxylation | kidney microsomes; recombinant protein | [129] |
| <i>S</i> - and <i>R</i> -Metamphetamine | psychostimulant | <i>N</i> -hydroxylation ^c | recombinant protein | [126] |
| Methimazole | thyroperoxidase inhibitor | <i>S</i> -oxygenation | recombinant protein | [11] |
| <i>S</i> -methyl esonarimod ^d | cytokine production inhibitor | <i>S</i> -oxygenation | recombinant protein | [130] |
| MK-0457 (Tozasertib) | aurora kinase inhibitor | <i>N</i> -oxygenation | recombinant protein | [131] |
| MK-0767 methyl sulphide | peroxisome proliferator receptor activator | <i>S</i> -oxygenation | recombinant protein | [132] |
| Nomifensine | antidepressant | <i>N</i> -oxygenation | recombinant protein | [133] |
| Olopatadine | antihistamine, | <i>N</i> -oxygenation | recombinant | [62] |

| | | | | |
|-------------------------------|--|----------------------------|---|-----------|
| | histamine H1 receptor-selective antagonist | | protein | |
| Pargyline | monoamine oxidase type B inhibitor | <i>N</i> -oxygenation | recombinant protein | [5] |
| Phospho-sulindac | nonsteroidal anti-inflammatory | <i>S</i> -oxygenation | recombinant protein | [134] |
| Procainamide | type I antiarrhythmic agent | <i>N</i> -oxygenation | recombinant protein | [118] |
| Quazepam | benzodiazepine | Desulfuration (2-oxo form) | small intestine microsomes; recombinant protein | [135] |
| Selenomethionine | anticancer agent | <i>Se</i> -oxygenation | recombinant protein | [136] |
| SNI-2011 | muscarinic agonist | <i>N</i> -oxygenation | kidney microsomes; recombinant protein | [137] |
| Sulindac sulfide ^e | nonsteroidal anti-inflammatory (colorectal cancer) | <i>S</i> -oxygenation | kidney microsomes | [30] |
| Tamoxifen | antiestrogen, estrogen receptor modulator | <i>N</i> -oxygenation | recombinant protein | [67] |
| Tazarotenic acid ^f | retinoic acid receptor modulator | <i>S</i> -oxygenation | recombinant protein | [138] |
| TG100435 | src kinase Inhibitor | <i>N</i> -oxygenation | recombinant protein | [139] |
| Thiacetazone | antitubercular | <i>S</i> -oxygenation | recombinant protein | [121,140] |
| Voriconazole | antifungal | <i>N</i> -oxygenation | recombinant protein | [141] |
| Xanomeline | M1 muscarinic agonist | <i>N</i> -oxygenation | kidney microsomes; recombinant protein | [64] |

^a metabolite of disulfiram, product is a sulfine; ^b major metabolite of ketoconazole; ^c metamphetamine hydroxylamine is further converted by FMO action to a mixture of nitrones, which are subsequently hydrolysed to yield an aldehyde and a hydroxylated primary amine, the latter then being reduced to a primary amine; ^d active metabolite of esonarimod; ^e the active metabolite of the prodrug sulindac; ^f major metabolite of tazarotene.

Table 3. Drug substrates of human FMO2.1

| Substrate | Type of drug | Reaction | Analytical system | Reference |
|------------------|---------------------------|-----------------------|--------------------------|------------------|
| Ethionamide | antitubercular | <i>S</i> -oxygenation | Recombinant protein | [29] |
| Methimazole | thyroperoxidase inhibitor | <i>S</i> -oxygenation | Recombinant protein | [82] |
| Thiacetazone | antitubercular | <i>S</i> -oxygenation | Recombinant protein | [121] |

Table 4. Drug substrates of human FMO3

| Drug | Substrate | Reaction | Analytical system | Reference |
|---|---|-----------------------|--|-------------|
| Albendazole | anthelmintic | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [65] |
| <i>N,N</i> -diallyltryptamine | psychostimulant | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [142] |
| Almotriptan | antimigraine 5-HT1B, 1D receptor agonist | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [143] |
| Amphetamine ^a | dopamine transporter ligand (antipsychotic) | <i>N</i> -oxygenation | recombinant protein | [50] |
| <i>N</i> -(3 <i>R</i>)-1-azabicyclo[2.2.2]oct-3-ylfuro[2,3- <i>c</i>]pyridine-5-carboxamide | α_7 nicotinic acetylcholine receptor agonist | <i>N</i> -oxygenation | in vivo; liver microsomes; recombinant protein | [124] |
| Benzylamine | nonsteroidal antiinflammatory | <i>N</i> -oxygenation | in vivo; liver microsomes; recombinant protein | [60,87,144] |
| Cimetidine | histamine H2-receptor antagonist | <i>S</i> -oxygenation | in vivo; liver microsomes; recombinant protein | [31,66] |
| Clozapine | antipsychotic dopamine D2, 5-HT2 and 5-HT1C receptor antagonist | <i>N</i> -oxygenation | liver microsomes; purified protein | [116] |
| Danuserib | aurora kinase inhibitor | <i>N</i> -oxygenation | recombinant protein | [98] |
| Dasatinib | BCR-ABL and SRC family kinase inhibitor | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [145] |
| Deprenyl | monoamine oxidase type B inhibitor | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [126] |
| C-1311 | antitumour agent | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [125] |
| 5,6-dimethylxanthenone-4-acetic acid (vadimezan) | anticancer agent, cytokine inducer | methyl hydroxylation | liver microsomes; recombinant protein | [146] |
| Disulfiram metabolite, <i>s</i> -methyl <i>n,n</i> - | antialcoholic | <i>S</i> -oxygenation | liver microsomes; | [147] |

| | | | | |
|--|--|-------------------------|--|-----------|
| diethyldithiocarbamate ^b | | | recombinant protein | |
| Ethionamide | antitubercular | <i>S</i> -oxygenation | recombinant protein | [29] |
| GSK5182 | antidiabetic, estrogen-related receptor γ modulator | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [128] |
| Itopride | dopamine D2 blocker and acetylcholinesterase inhibitor | <i>N</i> -oxygenation | in vivo; microsomes; recombinant protein | [61,115] |
| K11777 | peptidomimetic, cysteine protease inhibitor | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [148] |
| <i>N</i> -deacetyl ketoconazole ^c | antifungal | <i>N</i> -hydroxylation | recombinant protein | [47] |
| L-775,606 | 5-HT(1D) receptor agonist | <i>N</i> -oxygenation | liver microsomes | [149] |
| Loxapine | tricyclic antipsychotic | <i>N</i> -oxygenation | liver microsomes | [150] |
| <i>S</i> -Methamphetamine ^d | psychostimulant | <i>N</i> -hydroxylation | recombinant protein | [50] |
| Methimazole | thyroperoxidase inhibitor | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [31] |
| <i>S</i> -methyl esonarimod ^e | cytokine production inhibitor | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [130] |
| MK-0457 (Tozasertib) | aurora kinase inhibitor | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [98,131] |
| MK-0767 methyl sulphide | peroxisome proliferator receptor activator | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [132] |
| Moclobemide | monoamine oxidase type A inhibitor | <i>N</i> -oxygenation | recombinant protein | [151] |
| Nicotine | adenosine receptor ligand, stimulant | <i>N</i> -oxygenation | in vivo; recombinant protein | [49,152] |
| Nomifensine | antidepressant | <i>N</i> -oxygenation | recombinant protein | [133] |
| Olanzapine | multi receptor antagonist, antipsychotic | <i>N</i> -oxygenation | liver microsomes; in vivo | [109,114] |
| Olopatadine | antihistamine, histamine H1 receptor-selective antagonist | <i>N</i> -oxygenation | recombinant protein | [62] |
| Pargyline | monoamine oxidase type B inhibitor | <i>N</i> -oxygenation | recombinant protein | [5] |
| Phospho-sulindac | nonsteroidal anti- | <i>S</i> -oxygenation | liver | [134] |

| | | | | |
|-------------------------------|---|--|--|-----------|
| | inflammatory | | microsomes; recombinant protein | |
| Primaquine | antimalarial | not known | recombinant protein; primary hepatocytes | [153] |
| Procainamide | type I antiarrhythmic agent | <i>N</i> -oxygenation | in vivo; liver microsomes; recombinant protein | [118] |
| Pyrazolacridine | antitumour | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [154] |
| Ranitidine | histamine H2- receptor antagonist, antiulcerative | <i>N</i> - and <i>S</i> - oxygenation | liver microsomes; recombinant protein | [31,63] |
| S 16020 | topoisomerase II inhibitor, antitumour | <i>N</i> -oxygenation | primary hepatocytes; recombinant protein | [155] |
| Selenomethionine | anticancer agent | <i>Se</i> -oxygenation | recombinant protein | [136] |
| Sulindac sulfide ^f | nonsteroidal anti- inflammatory | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [30] |
| Tamoxifen | antiestrogen, estrogen receptor modulator | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [67] |
| Tazarotenic Acid ^g | retinoic acid receptor modulator | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [138] |
| TG100435 | Src kinase inhibitor | <i>N</i> - oxygenation | liver microsomes; recombinant protein | [139] |
| Thiacetazone | antitubercular | <i>S</i> -oxygenation | recombinant protein | [121,140] |
| Trifluoperazine | calmodulin antagonist | <i>N</i> -oxygenation | liver microsomes; recombinant protein | [156] |
| Voriconazole | antifungal | <i>N</i> - oxygenation | liver microsomes; recombinant protein | [141] |
| Xanomeline | M1 muscarinic agonist | <i>N</i> - oxygenation | liver microsomes; recombinant protein | [64] |

^athe initial product, an *N*-hydroxylamine, is converted, via a second oxygenation and loss of water, to an oxime; ^bmetabolite of disulfiram, product is a sulfine; ^cmajor metabolite of ketoconazole; ^dmetamphetamine hydroxylamine is further converted by FMO action to a mixture of nitrones, which are subsequently hydrolysed to yield an aldehyde and a hydroxylated primary amine, the latter then

being reduced to a primary amine; ^eactive metabolite of esonarimod; ^fthe active metabolite of the prodrug sulindac; ^gmajor metabolite of tazarotene.

Table 5. Drug substrates of human FMO5

| Substrate | Type | Reaction | Analytical system | Reference |
|--|---|-----------------------|---|-----------|
| <i>S</i> -methyl esonarimod ^a | cytokine production inhibitor | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [130] |
| E7016 | anticancer agent, inhibitor of poly(ADP-ribose) polymerase | BV oxidation | recombinant protein, fortified with liver cytosol | [55] |
| MRX-I | antibacterial | BV oxidation | recombinant protein, fortified with liver cytosol | [56] |
| Nomifensine | antidepressant | <i>N</i> -oxygenation | recombinant protein | [133] |
| Phospho-sulindac | nonsteroidal anti-inflammatory | <i>S</i> -oxygenation | liver microsomes; recombinant protein | [134] |
| Ranitidine | histamine H ₂ -receptor antagonist, antiulcerative | <i>S</i> -oxygenation | purified protein | [63] |

^aactive metabolite of esonarimod; BV, Baeyer-Villiger.



