The antithyroid drug methimazole (MMZ) can cause severe, tissue-specific toxicity in mouse olfactory mucosa (OM), presumably through a sequential metabolic activation of MMZ by cytochrome P450 (P450) and flavin monoxygenases (FMO). The aims of this study were to determine whether CYP2A5, one of the most abundant P450 enzymes in the mouse OM, is involved in MMZ metabolic activation, by comparing Cyp2a5-null with wild-type (WT) mice, and whether hepatic microsomal P450 enzymes, including CYP2A5, are essential for MMZ-induced OM toxicity, by comparing liver-Cpr-null (LCN) mice, which have little P450 activity in hepatocytes, with WT mice. We showed that the loss of CYP2A5 expression did not alter systemic clearance of MMZ (at 50 mg/kg, i.p.); but it did significantly decrease the rates of MMZ metabolism in the OM, whereas FMO expression in the OM was not reduced. MMZ induced depletion of nonprotein thiols, as well as pathological changes, in the OM of WT mice; the extent of these changes was much reduced in the Cyp2a5-null mice. Thus, CYP2A5 plays an important role in mediating MMZ toxicity in the OM. In contrast, the rate of systemic clearance of MMZ was significantly reduced in the LCN mice, compared to WT mice, whereas the MMZ-induced OM toxicity was not prevented. Therefore, hepatic P450 enzymes are essential for systemic MMZ clearance, but they are not required for MMZ-induced OM toxicity. We conclude that the tissue-specific toxicity of MMZ is mediated by target tissue metabolic activation, and the reaction is partly catalyzed by CYP2A5 in the OM.

Introduction

Methimazole (1-methyl-2-mercaptoimidazole; Tapazole) (MMZ), a drug commonly used for treating hyperthyroidism, and also often used as a model substrate, as well as an inhibitor, for the flavin-containing monoxygenases (FMO), is known to cause tissue-selective toxicity in rodent olfactory mucosa (OM) (Brittebo, 1995; Genter et al., 1995; Bergman and Brittebo, 1999; Bergström et al., 2003; Casallo Blanco et al., 2007). MMZ-induced olfactory deficits have also been reported in human patients (Bandyopadhyay et al., 2002; Woebber, 2002). In mice, cytochrome P450 (P450)-catalyzed MMZ metabolic activation is believed to be involved in MMZ’s tissue toxicity, given the observation that pretreatment of mice with P450 inhibitors can suppress MMZ toxicity in both liver and OM (Brittebo, 1995; Mizutani et al., 1999). Therefore, both the olfactory toxicity and the hepatotoxicity of MMZ depend on the in vivo formation of its electrophilic intermediates. It has been proposed that the bioactivation of MMZ is catalyzed sequentially by P450 and FMO, with P450 enzymes catalyzing the epoxidation of the C-4,5 double bond of MMZ, forming N-methylthioureia, which is further metabolized by FMO to the reactive sulfenic and sulfinic acid (Mizutani et al., 1999). The sulfenic/sulfinic acid is most likely responsible for the hepatotoxicity (Mizutani et al., 2000), and presumably also OM toxicity, of MMZ. However, the specific P450 enzymes responsible for MMZ metabolic activation have not been identified, and it is unclear whether target-tissue metabolic activation is essential for the OM toxicity.

In the present study, we have determined whether CYP2A5, one of the most abundant P450 enzymes in the mouse OM, is involved in MMZ metabolic activation, and whether hepatic microsomal P450 enzymes, including CYP2A5, are essential for MMZ-induced OM toxicity. We studied MMZ metabolism and toxicity in two knockout mouse models: 1) a Cyp2a5-null mouse (Zhou et al., 2010), which had been used recently for demonstration of the role of CYP2A5 in the metabolic activation and OM-specific toxicity of the herbicide 2,6-

**ABBREVIATIONS:** MMZ, methimazole; FMO, flavin-containing monoxygenases; OM, olfactory mucosa; P450, cytochrome P450; NPSH, nonprotein sulfhydryl; DCBN, 2,6-dichlorobenzonitrile; LCN, liver-Cpr-null; WT, wild type; B6, C57BL/6; HPLC, high-performance liquid chromatography; AUC, area under the concentration-time curve; CPR, NADPH-cytochrome P450 reductase.

Received December 21, 2010; accepted March 15, 2011

**ABSTRACT:**

The antithyroid drug methimazole (MMZ) can cause severe, tissue-specific toxicity in mouse olfactory mucosa (OM), presumably through a sequential metabolic activation of MMZ by cytochrome P450 (P450) and flavin monoxygenases (FMO). The aims of this study were to determine whether CYP2A5, one of the most abundant P450 enzymes in the mouse OM, is involved in MMZ metabolic activation, by comparing Cyp2a5-null with wild-type (WT) mice, and whether hepatic microsomal P450 enzymes, including CYP2A5, are essential for MMZ-induced OM toxicity, by comparing liver-Cpr-null (LCN) mice, which have little P450 activity in hepatocytes, with WT mice. We showed that the loss of CYP2A5 expression did not alter systemic clearance of MMZ (at 50 mg/kg, i.p.); but it did significantly decrease the rates of MMZ metabolism in the OM, whereas FMO expression in the OM was not reduced. MMZ induced depletion of nonprotein thiols, as well as pathological changes, in the OM of WT mice; the extent of these changes was much reduced in the Cyp2a5-null mice. Thus, CYP2A5 plays an important role in mediating MMZ toxicity in the OM. In contrast, the rate of systemic clearance of MMZ was significantly reduced in the LCN mice, compared to WT mice, whereas the MMZ-induced OM toxicity was not prevented. Therefore, hepatic P450 enzymes are essential for systemic MMZ clearance, but they are not required for MMZ-induced OM toxicity. We conclude that the tissue-specific toxicity of MMZ is mediated by target tissue metabolic activation, and the reaction is partly catalyzed by CYP2A5 in the OM.
dichlorobenzonitrile (DCBN) (Xie et al., 2010, and 2) the liver-Crp-
null (LCN) mouse (Gu et al., 2003), which has been found valuable for
differentiating between hepatic and extrahepatic P450 contributions
in studies of systemic drug clearance and target tissue toxicities (e.g., Gu
et al., 2005; Weng et al., 2007; Fang et al., 2008; Xie et al., 2010).
We first compared wild-type (WT) and Cyp2a5-null mice, for rates of
MMZ metabolism by OM microsomes, and extents of MMZ-induced
depletion of nonprotein thiols, as well as histopathological changes.

As controls, we examined whether the loss of CYP2A5 expression
altered systemic clearance of MMZ, or was associated with a decrease
in the expression of FMO proteins in the OM. We then compared WT
and LCN mice, for rates of systemic MMZ clearance and extents of
MMZ-induced toxicity, to further determine whether hepatic P450
enzymes (including CYP2A5) are required for MMZ-induced OM
toxicity. Our findings indicate that the tissue-specific OM toxicity of
MMZ is mediated by target-tissue metabolic activation, and the reac-
tion is partly catalyzed by CYP2A5 in the OM.

Materials and Methods

Chemicals and Animal Treatments. MMZ (>99% pure) and other chem-
icals were purchased from Sigma-Aldrich (St. Louis, MO), unless stated
otherwise. Procedures involving animals were approved by the Institutional
Animal Care and Use Committee of the Wadsworth Center (Albany, NY).

LCN [nearly congenic strain after backcrossing to the C57BL/6 (B6) strain for
10 generations], their corresponding WT littermates, Cyp2a5-null on B6 back-
ground, and WT B6 mice were from breeding stocks maintained at the
Wadsworth Center. Animals were normally maintained at 22°C with a 12-h
on/12-h off light cycle and were allowed free access to water and a standard
laboratory diet. Mice were treated with a single intraperitoneal dose of MMZ
at 50 mg/kg. MMZ was dissolved in pure, sterile water at 10 mg/ml. Control
mice received injections of vehicle only. Animals were dosed at 10:00 to 11:00
AM, local time. Tail blood samples (10 or 20 μl each) were collected from
individual mice at various times after dosing (5, 30 min, and 1, 2, 4, 8, and
24 h) using heparin-coated capillaries. Each blood sample was diluted with one
volume of saline and then centrifuged at 4°C and 9000g for 10 min. The
plasma was stored at −80°C until use. At 24 h after MMZ injection, the mice
were euthanized by CO₂ overdose, and noses were dissected for histopatho-
logical examination. For determination of tissue levels of NPSH, mice were
fasted overnight, and liver and OM were dissected at 2 h after MMZ injection;
tissue samples were stored frozen at −80°C before analysis.

Determination of Tissue NPSH Levels. NPSH was determined using a
reported method (Tonge et al., 1998), with modifications in the homogeniza-
tion step as reported (Xie et al., 2010), to accommodate the small amount
of nasal tissues available from a single mouse. Liver was homogenized on ice
with a Polytron (model GT 10-35; Kinematica Inc., Bohemia, NY), in 100 mM
Tris-acetate buffer, pH 7.4, containing 1.0 mM EDTA and 150 mM potassium
chloride (buffer H), at a tissue-to-buffer ratio of ~1:10. OM (~20 mg wet
weight) from individual mice was homogenized with use of a Bullet Blender
(Next Advance, Averill Park, NY) in an 1.5-ml Eppendorf centrifuge tube
containing 400 μl of buffer H and two stainless steel beads (0.5-mm diameter).
The samples were homogenized in the blender at a speed setting of 4,
continuously for 4 min, at 4°C. Reduced GSH was used as the standard.

Histopathological Examination. Tissue blocks containing the nasal pas-
sages were dissected and soaked in Bouin’s solution for fixation and decali-
fication for 2 weeks, as described previously (Gu et al., 2005). Each tissue
block was cut into smaller blocks at four anterior-rostral levels, as described
by Young (1981). Paraffin sections (4 μm) from levels 1 to 4 were stained with
hematoxylin and eosin for pathological examination. For semiquantitative
assessment of the extent of tissue toxicity, the severity of lesions in the OM
was graded, as described previously (Gu et al., 2005); for each treatment
group, 4 to 5 mice were examined (4 blocks/mouse; 1 section/block). For
quantitative analysis of the extent of epithelial injury, the total length of intact
olfactory epithelium in the dorsal medial meatus was measured (1 section/
mouse). Level-3 sections of the dorsal medial meatus of each nasal cavity
(Young, 1981) were photographed at 4× magnification and printed (8 × 10
inch prints). Length measurements were made with a MapWheel (Scalex,
Carlsbad, CA), calibrated to a stage micrometer photographed at the same
magnification. Tissue sectioning and staining were performed at the Wad-
sworth Center Pathology core facility. Images were obtained using a Nikon
model 50i light microscope (Nikon Instruments, Melville, NY), fitted with a
digital camera, at the Wadsworth Center Light Microscopy core.

Determination of MMZ in Blood. A HPLC-UV protocol was established,
based on a method described previously (Hoffman et al., 2002), for the
determination of plasma concentrations of MMZ. An Agilent model 1100
(Agilent Technologies, Santa Clara, CA) HPLC system with a diode array UV
detector was used. Two volumes of methanol were added to the plasma
samples to precipitate proteins. Aliquots of the supernatant fraction (5 μl) were
analyzed using a 4-μm Nova-Pak C18 column (3.9 × 150 mm; Waters,
Milford, MA) and an isotropic mobile phase consisting of 10 mM ammonium
acetate, pH 4.0 (95.5%, v/v), and acetonitrile (3.5%), at a flow rate of 0.8
ml/min. MMZ was detected by UV at the wavelength of 254 nm. Standard
curves were prepared by spiking authentic MMZ into blank mouse plasma at
various concentrations (400–4000 ppb), before precipitation with methanol.
The recovery of MMZ from plasma samples was ~50%.

Assay for MMZ In Vitro Metabolism. OM microsomes were prepared as
reported (Gu et al., 1998), from pooled tissues from 6 to 8 mice per sample.
Activity was determined by measuring rates of MMZ disappearance in micro-
some reaction mixtures containing 50 mM phosphate buffer, pH 7.4, 5 μM
MMZ, 0.2 mg/ml microsomal protein from 2- to 3-month-old male WT or
Cyp2a5-null mice, and 1 mM NADPH, in a final volume of 0.1 ml. Reactions
without NADPH addition were used as negative controls. The reactions
were carried out at 37°C and were stopped by the addition of an equal volume
of methanol. After centrifugation at 9000g for 10 min, the supernatant
was analyzed using HPLC-UV for levels of residual MMZ, as described under
Determination of MMZ in Blood. A calibration curve was prepared by spiking
authentic MMZ into complete incubation mixtures containing boiled micro-
somes, to give a series of standard calibrating samples with final concentra-
tions ranging from 0 to 40 μM.

Other Methods. Protein concentration was determined by the bichoninic
acid method (Pierce Chemical, Rockford, IL). Immuno blot analysis of FMO
expression was carried out using goat anti-FMO1, FMO2, and FMO5 antibod-
ies (Genter and Ali, 1998). The levels of calnexin were also determined, as
a loading control, using rabbit anti-human calnexin (GenScript, Piscataway, NJ).
Pharmacokinetic parameters, including area under concentration-time curve
(AUC), maximum plasma concentration (Cmax), the time when Cmax is reached
(Tmax), elimination half-time (t1/2), and clearance, were calculated using the
WinNonlin software (Pharsight, Mountain View, CA). Statistical significance
of differences between two groups in various parameters was examined using
the Student’s t test, with use of the SigmaStat software (SPSS, Chicago, IL).

Results

CYP2A5 Plays an Important Role in Mediating MMZ Toxicity
in the OM. WT and Cyp2a5-null mice were treated with MMZ at 50
mg/kg i.p., a dose known to induce observable tissue toxicity in the
OM of WT mice (Brittebo, 1995). The MMZ-induced histopathological
changes in the OM of WT mice were mainly characterized as diffuse
degeneration of the olfactory epithelium, at 24 h after the treatment;
the epithelium was often absent, with only a denuded basement
membrane remaining. In some areas of the nasal cavity (dorsal,
caudal, and lateral), epithelium was still present, though markedly
abnormal. The olfactory epithelium, when still present, was often
degraded from the basement membrane, fractured and disorganized
(Fig. 1). In the Cyp2a5-null mice, there was less congestion in the
submucosa as well as less glandular necrosis, compared to the WT
mice; in addition, considerably more reserve cells remained attached
to the basement membrane. The total length of intact olfactory epi-
thelium in the dorsal medial meatus, an area that can be reproducibly
sampled in the nasal cavity, was also measured, to provide a quanti-
tative assessment of the extent of epithelial injury. The results
showed that the fraction of intact olfactory epithelium (mean ± S.D.)
in Cyp2a5-null (60.9 ± 15.9%) was significantly larger than that in
WT (14.6 ± 17.4%; P < 0.01; n = 5).
MMZ treatment (at 50 mg/kg i.p.) induced NPSH depletion in the OM of WT mice, at 2 h after dosing (by 51.8 ± 3.4%, compared to vehicle-treated mice; n = 3–6, P < 0.01, Student’s t test). MMZ also induced NPSH depletion in the OM of the Cyp2a5-null mice (by 41.5 ± 6.9%; n = 3, P < 0.01). The extent of NPSH depletion was significantly lower for the Cyp2a5-null mice than for the WT mice (P < 0.05, Student’s t test).

The results from both the histopathological analysis and the NPSH determination indicated that the Cyp2a5 gene deletion led to a reduction in the extent of MMZ-induced cytotoxicity in the OM. This result suggested that CYP2A5 is partly responsible for the metabolic activation of MMZ in vivo. Consistent with this idea, the rates of MMZ metabolism by OM microsomal P450 were decreased in the Cyp2a5-null mice, compared to WT mice. To assay the in vitro metabolism of MMZ, we measured the rates of MMZ disappearance in microsomal incubations. With MMZ at 5 μM, 58.7 ± 5.6% (n = 3) of added MMZ was metabolized after a 15-min incubation with OM microsomes from WT mice; however, only 28.7 ± 1.7% (n = 3; P < 0.01, significantly lower than the WT value) of the added MMZ was metabolized after a 15-min incubation with OM microsomes from Cyp2a5-null mice.

The pharmacokinetic profile (Fig. 2A) and pharmacokinetic parameters (Table 1) for plasma MMZ were not significantly different between the Cyp2a5-null and the WT B6 mice, indicating that the loss of CYP2A5 expression did not alter systemic clearance of MMZ. As an additional control, we confirmed by immunoblot analysis that the expression of FMO1 and FMO2 proteins (the major FMOs detected) in OM microsomes was not changed in the Cyp2a5-null mice, compared to WT mice (Supplemental Fig. 1). Thus, the observed decrease in microsomal activity toward MMZ did not result from a decrease (which is unexpected) in FMO expression.

Hepatic P450 Enzymes Are Essential for MMZ Clearance, but Not for MMZ-Induced OM Toxicity. To further determine whether hepatic P450 enzymes (including CYP2A5) are required for MMZ-induced OM toxicity, we compared WT and LCN mice, for extents of MMZ-induced OM toxicity. As shown in Fig. 1, the nasal lesion caused by MMZ treatment in the LCN mice was not less (rather, it was actually slightly more) severe than the lesion found in MMZ-treated WT mice, as evidenced by a more noticeable denudation of the basement membranes. The fraction of intact olfactory epithelium in the dorsal medial meatus (mean ± S.D.) in WT littermates (39.8 ± 22.5%) was actually greater than that in LCN mice (0%; P < 0.05; n = 4). Likewise, significant decreases in NPSH levels were detected in both WT and LCN mice at 2 h after the MMZ injection, compared to the corresponding vehicle-treated mice (by 51.8 ± 3.4 and 48.2 ± 3.7%, respectively; n = 3–6, P < 0.01, Student’s t test). Thus, hepatic P450 enzymes (including CYP2A5) are not required for MMZ-induced OM toxicity.

To confirm that hepatic MMZ metabolism was indeed suppressed in the LCN mice, compared with the WT mice, we further determined the impact of hepatic Cpr loss on MMZ clearance in vivo. In contrast to the consequence of Cyp2a5 gene deletion, the loss of hepatic Cpr expression led to substantial decreases in the rates of systemic clearance of MMZ (Fig. 2B; Table 1). The AUC values, which represent total exposure amounts, as well as the maximal plasma MMZ concentrations, were significantly higher in the LCN mice than those in the WT mice. Thus, tissues of the LCN mice were exposed to greater amounts of the unmetabolized MMZ (and lesser amounts, if any, of hepatic P450-produced MMZ metabolites) than did tissues of the WT mice.

Discussion

Our results of the current study with the LCN mouse model indicated that hepatic P450s play a large role in MMZ clearance, because loss of hepatic microsomal P450 activity significantly reduced the systemic clearance rate for MMZ. However, 24 h after the single intraperitoneal injection at 50 mg/kg, plasma MMZ was decreased to an undetectable level in the LCN as well as WT mice, indicating that pathways other than hepatic microsomal P450s (e.g., FMO, extrahaematic P450s) are capable of clearing MMZ, although less efficiently. The reduced clearing rate of MMZ in LCN mice led to an increased total exposure to MMZ (by ~4-fold) in these mice than in the WT mice, which explains the increased (rather than comparable) OM toxicity in the LCN mice.
In summary, the present study demonstrated that hepatic P450s are important in systemically clearing MMZ, a model FMO substrate. The olfactory toxicity of MMZ does not require hepatic P450-catalyzed activation but partly depends on olfactory CYP2A5-catalyzed activation. This study also exemplified the use of the LCN mouse model for delineating the relative contributions of hepatic microsomal P450 enzymes (other than CYP2A5) to MMZ toxicity in the OM. In addition, it remains to be determined whether human orthologs of CYP2A5, CYP2A6 and CYP2A13, are also active toward MMZ, which may have important implications for understanding potential MMZ nasal toxicity in human patients.

In summary, the present study demonstrated that hepatic P450s are important in systemically clearing MMZ, a model FMO substrate. The olfactory toxicity of MMZ does not require hepatic P450-catalyzed activation but partly depends on olfactory CYP2A5-catalyzed activation. This study also exemplified the use of the LCN mouse model for delineating the relative contributions of hepatic microsomal P450 versus other biotransformation enzymes, such as FMO, to systemic clearance of xenobiotics.

Acknowledgments

We gratefully acknowledge the use of the services of the Pathology Core and the Advanced Light Microscopy and Image Analysis Core Facilities of the Wadsworth Center. We thank Dr. David Williams (Oregon State University, Corvallis, OR) for helpful discussions and Weizhu Yang (Wadsworth Center) for assistance with mouse breeding.

Loss of hepatic microsomal P450 did not protect the LCN mice from MMZ-induced olfactory toxicity, suggesting that locally produced toxic MMZ intermediates are responsible for causing OM toxicity. Thus, MMZ is similar to two other olfactory toxicants, acetaminophen and DCBN, which have been found to cause olfactory toxicities through extrahepatic (mostly likely in situ) bioactivation (Gu et al., 2005; Xie et al., 2010). However, MMZ differ from DCBN toxicities through extrahepatic (mostly likely in situ) bioactivation acetaminophen and DCBN, which have been found to cause olfactory toxicity. Thus, MMZ is similar to two other olfactory toxicants, produed toxic MMZ intermediates are responsible for causing OM from MMZ-induced olfactory toxicity, suggesting that locally pro-

Materials and Methods

Plasma levels of MMZ (see Fig. 2, A and B) were used to calculate various pharmacokinetic parameters. Values represent means ± S.D. (n = 4 for each strain).

<table>
<thead>
<tr>
<th>Strains</th>
<th>T_{1/2}</th>
<th>T_{max}</th>
<th>C_{max}</th>
<th>AUC_{INF}</th>
<th>CL</th>
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<tr>
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<td>µg/ml</td>
<td>µg · h/ml</td>
<td>ml/h</td>
</tr>
<tr>
<td>WT</td>
<td>1.5 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>48.4 ± 8.5</td>
<td>149 ± 38</td>
<td>8.9 ± 2.8</td>
</tr>
<tr>
<td>LCN</td>
<td>4.3 ± 1.7</td>
<td>0.6 ± 0.3</td>
<td>69.5 ± 13.1</td>
<td>585 ± 169</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>WT B6</td>
<td>1.3 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>67.2 ± 12.7</td>
<td>128 ± 26</td>
<td>10.7 ± 2.8</td>
</tr>
<tr>
<td>Cyp2a5-null</td>
<td>1.4 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>76.8 ± 7.5</td>
<td>154 ± 15</td>
<td>8.7 ± 0.6</td>
</tr>
</tbody>
</table>

* P < 0.05 and * P < 0.01, compared to WT in the same treatment group; Student’s t test.

Loss of hepatic microsomal P450 did not protect the LCN mice from MMZ-induced olfactory toxicity, suggesting that locally produced toxic MMZ intermediates are responsible for causing OM toxicity. Thus, MMZ is similar to two other olfactory toxicants, acetaminophen and DCBN, which have been found to cause olfactory toxicities through extrahepatic (mostly likely in situ) bioactivation (Gu et al., 2005; Xie et al., 2010). However, MMZ differ from DCBN in that whereas CYP2A5 was largely responsible for DCBN’s OM toxicity, it was only partially responsible for the OM toxicity of MMZ. Thus, this is the first study to show that CYP2A5, which is one of the most abundant P450 isoforms in the mouse OM (Gu et al., 1998) and is known to be active toward a number of nasal toxicants and procarcinogens (Jalas and Hecht, 2003; Xie et al., 2010), is able to metabolize MMZ, as indicated by the decreased MMZ metabolism in Cyp2a5-null OM microsomes. Previous studies have shown that several isoforms of FMO are active in metabolizing MMZ (Siddens et al., 2008). Furthermore, NADPH-supported formation of N-methylimidazole from MMZ by rat hepatic microsomes was largely dependent on the P450 system (Lee and Neal, 1978). However, the specific P450 enzymes responsible for microsomal MMZ metabolism in either liver or OM have not been identified. In that connection, FMO1 and FMO2, as well as many P450s, are known to be expressed in the OM (Genter et al., 1995; Shehin-Johnson et al., 1995; Ling et al., 2004). Further studies are needed to determine the contributions of these FMOs and P450 enzymes (other than CYP2A5) to MMZ toxicity in the OM. In addition, it remains to be determined whether human orthologs of CYP2A5, CYP2A6 and CYP2A13, are also active toward MMZ, which may have important implications for understanding potential MMZ nasal toxicity in human patients.

In summary, the present study demonstrated that hepatic P450s are important in systemically clearing MMZ, a model FMO substrate. The olfactory toxicity of MMZ does not require hepatic P450-catalyzed activation but partly depends on olfactory CYP2A5-catalyzed activation. This study also exemplified the use of the LCN mouse model for delineating the relative contributions of hepatic microsomal P450 versus other biotransformation enzymes, such as FMO, to systemic clearance of xenobiotics.
Authorship Contributions

Participant in research design: Xie, Genter, Gu, and Ding.

Performed experiments: Xie, Zhou, Genter, and Gu.

Performed data analysis: Xie, Behr, and Ding.

Wrote or contributed to the writing of the manuscript: Xie, Genter, and Ding.

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