Activation and Deactivation of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine by Cytochrome P450 Enzymes and Flavin-Containing Monoxygenases in Common Marmosets (Callithrix jacchus)

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ABSTRACT

The potential proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces Parkinson-like syndromes in common marmosets, other primates, and humans. MPTP is metabolically activated to 1-methyl-4-phenyl-2,3-dihydropyridinium and 1-methyl-4-phenylpyridinium ions (MPDP+ and MPP+, respectively) by desaturation reactions. MPTP is deactivated to 4-phenyl-1,2,3,6-tetrahydropyridine (PTP) by N-demethylation and is also deactivated to MPTP N-oxide. The roles of cytochrome P450 (P450) enzymes and flavin-containing monoxygenases (FMOs) in the oxidative metabolism of MPTP-treated marmosets are not yet fully clarified. This study aimed to elucidate P450- and FMO-dependent MPTP metabolism in marmoset liver and brain. Rates of MPTP N-oxygenation in liver microsomes were similar to those in brain microsomes from 11 individual marmosets (substrate concentration, 50 μM) and were correlated with rates of benzydamine N-oxygenation (r = 0.75, P < 0.05); the reactions were inhibited by methimazole (10 μM). MPTP N-oxygenation was efficiently mediated by recombinantly expressed marmoset FMO3. Rates of PTP formation by MPTP N-demethylation in marmoset liver microsomes were correlated with bufuralol 1'-hydroxylation rates (r = 0.77, P < 0.01) and were suppressed by quinidine (1 μM), thereby indicating the importance of marmoset CYP2D6 in PTP formation. MPTP transformations to MPDP+ and MPP+ were efficiently catalyzed by recombinant marmoset CYP2D6 and human CYP1A2. These results indicated the contributions of multiple drug-metabolizing enzymes to MPTP oxidation, especially marmoset FMO3 in deactivation (N-oxygenation) and marmoset CYP2D6 for both MPTP deactivation and MPTP activation to MPDP+ and MPP+. These findings provide a foundation for understanding MPTP metabolism and for the successful production of preclinical marmoset models.

Introduction

Cytochrome P450 (P450; EC 1.14.14.1) enzymes have been characterized with respect to drug metabolism and disposition (Johansson and Ingelman-Sundberg, 2011). Research has focused on the P450s as well as on another monoxygenase family, the flavin-containing monoxygenases (FMOs; EC 1.14.13.8) (Yamazaki and Shimizu, 2013), which are involved in the oxidation of a variety of compounds associated with pharmacological and/or toxicological effects in humans. The potential proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) degenerates dopaminergic neurons and causes parkinsonism in primates, including common marmosets, cynomolgus monkeys, and humans (Langston and Ballard, 1983; Ballard et al., 1985; Davis et al., 1997). Several metabolic pathways of MPTP have been reported and are summarized in Fig. 1. The 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPDP+) (Fig. 1) is reportedly an unstable compound that undergoes further oxidation to form the active toxic 1-methyl-4-phenylpyridinium ion (MPP+) (Chiba et al., 1985). The metabolic transformation of MPTP to its toxic metabolite MPP+ via intermediate MPDP+ is mediated by monoamine oxidase or CYP2D6 in human brains (Langston and Ballard, 1983; Ballard et al., 1985; Davis et al., 1997). On the other hand, MPTP is also metabolized to non-neurotoxic 4-phenyl-1,2,3,6-tetrahydropyrididine (PTP) and MPTP N-oxide (Fig. 1) by CYP2D6 and FMO, respectively, in human livers (Baijai et al., 2013; Herrera et al., 2013). The common marmoset (Callithrix jacchus) is a member of the New World nonhuman primate family Callitrichidae (Abbott et al., 2003; Mansfield, 2003; Carrion and Patterson, 2012; Okano et al., 2012; Tokuno et al., 2012). The species has attracted considerable attention as a potentially useful animal model in fields such as neuroscience and drug toxicology (Mansfield, 2003) because of its size, availability, unique biologic characteristics (Abbott et al., 2003), and evidence of crossreactivity with human cytokines and hormones.

ABBRVIATIONS: FMO, flavin-containing monoxygenase; HPLC, high-performance liquid chromatography; MPDP+, 1-methyl-4-phenyl-2,3-dihydropyridinium ion; MPP+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; P450, cytochrome P450; PCR, polymerase chain reaction; PTP, 4-phenyl-1,2,3,6-tetrahydropyridine; RT, reverse transcription.
Deactivation

PTP

MPTP

N-oxide

MPDP^* 

MPP^*

Activation

Neurotoxicity

Materials and Methods

Animals. Adult common marmosets (aged >2 years) were purchased from CLEA Japan (Tokyo, Japan). The animals were maintained in cages (400 x 610 x 1578 mm) at 24°C–27°C and 40%–60% relative air humidity with a 12-hour/12-hour light/dark cycle and had free access to a balanced diet (CMS-1M; CLEA Japan) with added vitamins and water. This study was approved by the animal ethics committees and gene recombination experiment safety management committees of the Central Institute for Experimental Animals and was performed in accordance with the 2006 Science Council of Japan Guidelines for Proper Conduct of Animal Experiments. Animal care was conducted in accordance with the recommendations of the 2011 Institute for Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals. Brain and liver samples were collected from 11 common marmosets (five males and six females, aged 2–6 years) after euthanasia by exsanguination under ketamine (60 mg/kg) and isoflurane deep anesthesia as previously described (Shimizu et al., 2011). No FMOs in the marmoset have been investigated in terms of their drug-metabolizing activities thus far.

This study combined analyses to identify novel common marmoset FMO1/FMO3 and P450 enzymes that have been unreported until now and to investigate P450 oxidative metabolism. We report herein that marmoset FMO3 mainly contributed to deactivation of MPTP and that marmoset CYP2D6 was responsible for both deactivation and metabolic activation of MPTP to MPP^+. These findings should prove an important resource for future biomedical research and will facilitate use of the common marmoset as an animal preclinical parkinsonism model.

Molecular Cloning of cDNAs for Marmoset P450s and FMOs. To obtain cDNAs of marmoset P450s and FMOs, reverse transcription (RT)–polymerase chain reaction (PCR) was performed using brain and liver total RNAs of marmosets. Total RNA was isolated from each tissue using RNeasy Mini Kits (Qiagen, Valencia, CA) according to the protocols in the manufacturer’s instructions. Then, a first-strand cDNA was prepared by RT reaction at 50°C for 1 h in a mixture containing 1 μg total RNA, oligo(dT), and SuperScript III RT reverse transcriptase (Invitrogen, Carlsbad, CA). PCR was performed using KOD-Plus-Neo DNA polymerase (Toyobo, Osaka, Japan) with the RT product as described by the protocols in the manufacturer’s instructions. Full-length cDNAs encoding marmoset P450s and FMOs were amplified by PCR with the following oligonucleotide primers: 5'-CATGATGCAAATGCGAATCTCTTGAC-3' and 5'-CCGTATTGATCAGTAGAC-3' for CYP1A2; 5'-ATGGAGCTCACCGTCTTCCTCT-3' and 5'-CTGGATGACCCGGAATCTCTTCCT-3' for CYP2B6; 5'-CTGGATGACCCGGAATCTCTTCCT-3' and 5'-CTGGATGACCCGGAATCTCTTCCT-3' for CYP2B6; 5'-GAGGAGTTCAGT-3' and 5'-CTAGATGCAAATGCGAATCTCTTGAC-3' for CYP1A2; 5'-ATGGAGCTCACCGTCTTCCTCT-3' and 5'-CTGGATGACCCGGAATCTCTTCCT-3' for CYP2B6; 5'-GAGGAGTTCAGT-3' and 5'-CTAGATGCAAATGCGAATCTCTTGAC-3' for CYP1A2; and 5'-ATGGAGCTCACCGTCTTCCTCT-3' and 5'-CTGGATGACCCGGAATCTCTTCCT-3' for CYP2B6.
Recombinant Marmoset P450s and FMOs. Recombinant marmoset P450s (CYP1A2, CYP2B6, CYP2C8, CYP2D6, CYP2E1, CYP3A4, CYP3A5, and CYP3A90) and FMOs (FMO1 and FMO3) were heterologously expressed in *Escherichia coli* using expression plasmids. For P450s, the N terminus modification was conducted by PCR using the following forward and reverse primers: 5′-GGAATTCCATATGGCTCTGTTATTATGAGCT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for CYP1A2, 5′-GGAATTCCATATGGCTCTGTTATTATGAGCT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for CYP2C8, 5′-GGAATTCCATATGGCTCTGTTATTATGAGCT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for CYP2D6, 5′-GGAATTCCATATGGCTCTGTTATTATGAGCT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for CYP2E1, 5′-GGAATTCCATATGGCTCTGTTATTATGAGCT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for CYP3A4, and 5′-GGAATTCCATATGGCTCTGTTATTATGAGCT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for CYP3A5, respectively. The Ndel and XbaI sites (underlined) in the forward and reverse primers, respectively, were used for subcloning of the product into pCW vectors that contained human NADPH-P450 reductase cDNA (Uno et al., 2010). Membrane preparation and measurement of P450 protein and reductase contents in each sample were performed as previously described (Uehara et al., 2010). Expected drug oxidation activities of marmoset P450 enzymes were confirmed with typical human P450 probe substrates (Yamazaki et al., 2002; Uehara et al., 2011).

For FMOs, PCR was carried out with the following primers: 5′-ATGGGCAAGC-CAGGTGCACTGAAATCCACTAGTTAGGAATATCACGT-3′ and 5′-GCCTCAGATCAGACAGGAATGAAGCAGATCTGGTA-3′ for FMO1, and 5′-ATGGGCAAGC-CAGGTGCACTGAAATCCACTAGTTAGGAATATCACGT-3′ and 5′-ATGGGCAAGC-CAGGTGCACTGAAATCCACTAGTTAGGAATATCACGT-3′ for FMO3. After restriction enzyme digestion using XhoI (the restriction site is underlined), the PCR products were subcloned to pET30 vectors (Novagen, Madison, WI) to provide a 6× His-tag at the N terminus (Uno et al., 2013). Protein expression and *E. coli* membrane preparations were performed as previously described (Yamazaki et al., 2014) in a similar manner to that used for human FMO1 and FMO3 preparations. The final amount of recombinant P450 proteins in bacterial membranes was normalized to the flavin adenine dinucleotide contents (Yamazaki et al., 2014) in a similar manner to that used for human FMO1 and FMO3 metabolites.

For MPTP oxidation catalyzed by marmoset P450s and FMOs, the four metabolites of MPTP, namely PTP, MPTP-oxidation catalyzed by marmoset liver and brain microsomes. Representative HPLC chromatograms of MPTP metabolites in marmoset liver microsomes are shown in Fig. 2. A and B, after 10-minute incubation of MPTP (50 μM) in the presence of an NADPH-generating system. The four metabolites of MPTP shown in Fig. 1 could be detected with UV (at 290 nm) and fluorescence detection (excitation at 332 nm and emission at 44 nm) in an analytical reverse-phase HPLC system. To elucidate the metabolic pathways of MPTP in the marmoset, the formation rates of PTP, MPTP-oxidation catalyzed by marmoset liver and brain microsomes from 11 individual marmosets were determined (Fig. 3). Although rates of MPTP-oxidation catalyzed by marmoset liver microsomes were similar to those in liver microsomes from marmosets at a substrate concentration of 50 μM, formation rates of PTP, MPP+, and MPDP+ in brain microsomes were lower than those in liver microsomes. MPDP+ and MPP+ formation rates in liver microsomes (Fig. 3, B and D) were approximately 2- and 4-fold faster, respectively, than PTP formation rates (Fig. 3A). The formation rates of four MPTP metabolites in individual marmosets varied less than 5-fold within the liver and brain microsomes analyzed in this study (Fig. 3); larger interindividual variations in the marmoset were observed for MPP+ formation in the brain (4.7-fold). Interestingly, PTP formation rates (Fig. 3A) correlated with MPDP+ formation rates (Fig. 3B) in marmoset liver microsomes (r = 0.77, n = 11, P < 0.05).

Correlation analyses revealed that MPTP N-demethylation (PTP formation) activities were correlated with activities of midazolam 1'-hydroxylation (r = 0.92, n = 10, P < 0.001), bufuralol 1'-hydroxylation (r = 0.77, n = 10, P < 0.01), and 7-ethoxresorufin O-deethylation (r = 0.73, n = 10, P < 0.05) (Table 1). Significant correlation coefficients (P < 0.05) were also observed between MPDP+ and MPP+ formations and warfarin 7-hydroxylation activities, between MPP+ formation and midazolam 1'-hydroxylation activities, and between MPTP N-oxygenation and benzoylamine N-oxygenation (Table 1). No correlations were seen between MPTP metabolite formation and coumarin 7-hydroxylation, paclitaxel 6a-hydroxylation, S-mephenytoin 4'-hydroxylation, or chlorozoxazone hydroxylation activities under these conditions.

The effects of P450 and FMO inhibitors (α-naphthoflavone, sulfaphenazole, ticlopidine, quinidine, ketocnazole, and methimazole) on MPTP oxidation in liver microsomes were investigated at substrate concentrations of 50 μM MPTP (Fig. 4). Quinidine (1–10 μM), a CYP2D2 inhibitor, strongly inhibited MPTP N-demethylation (PTP formation) (Fig. 4A) and moderately suppressed MPP+ formation (Fig. 4D) in marmoset liver microsomes. Ticlopidine (2–20 μM), a CYP2C2 inhibitor, moderately suppressed MPTP N-demethylation (Fig. 4A) and MPP+ formation (Fig. 4D) in liver microsomes. α-Naphthoflavone (0.2–5 μM), a CYP1A inhibitor, partly suppressed MPDP+ formation (Fig. 4B) in liver microsomes. Methimazole (10–100 μM), a FMO substrate and inhibitor, strongly suppressed MPTP N-oxygenation in liver microsomes (Fig. 4C). By contrast, inhibitory effects of ketoconazole (0.02–0.2 μM) or sulfaphenazole (1–20 μM), inhibitors of CYP3A4 and CYP2C9, respectively, were not seen on MPTP oxidation under these conditions.
MPTP Metabolism by Recombinantly Expressed P450s and FMOs. MPTP (50 μM) was incubated with recombinant marmoset FMO1 and FMO3 and marmoset CYP1A2, CYP2B6, CYP2C8, CYP2D6, CYP3A4, CYP3A5, and CYP3A90, along with human FMO1 and FMO3 and human CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. Marmoset FMO3 (Fig. 2C) catalyzed MPTP N-oxygenation (14.5 nmol/min per nmol FMO, Table 2) more efficiently than FMO1 did; a similar pattern was evident for human FMO3 (78.0 nmol/min per nmol FMO) versus FMO1. Marmoset CYP2D6 (Fig. 2D) efficiently catalyzed PTP

Fig. 2. Representative HPLC chromatograms of MPTP oxidized by marmoset liver microsomes (A and B) and recombinant FMO3 (C) and CYP2D6 (D). MPTP and its metabolites were analyzed with a reverse-phase liquid chromatography system using fluorescence and UV detection as described in Materials and Methods.

Fig. 3. Variation in formation rates of PTP (A), MPDP⁺ (B), MPTP N-oxide (C), and MPP⁺ (D) from MPTP by liver and brain microsomes of 11 individual marmosets. MPTP (50 μM) was incubated with liver and brain microsomes (0.50 mg/ml; black and white bars, respectively) from 11 individual marmosets for 10 minutes in the presence of an NADPH-generating system. The individual marmosets were numbered in the increasing order of MPP formation rates in liver microsomes.
formation (6.6 nmol/min per nmol P450) in a similar manner to human CYP2D6 (3.5 nmol/min per nmol P450) (Fig. 5). Marmoset CYP2D6 also mediated MPDP⁺ and MPP⁺ formation (2.0, and 5.7 nmol/min per nmol P450, respectively), but the rates of MPDP⁺ and MPP⁺ formation mediated by human CYP2D6 were much lower (Fig. 5). Marmoset CYP1A2 and CYP2C8 also mediated the formation of PTP, MPDP⁺, and MPP⁺, but to a lesser extent than CYP2D6. In addition, human CYP1A2 showed high activities for the formation of MPDP⁺ and MPP⁺, whereas human CYP2D6 showed much lower activities under these conditions.

Kinetic parameters for MPTP oxidation by marmoset and human liver microsomes and recombinant CYP2D6 and FMO3 were determined. Apparent $K_m$ values for PTP formation by liver microsomes from marmosets and humans were 451 and 577 μM, respectively; for recombinant marmoset and human CYP2D6, $K_m$ values were 71 and 125 μM, respectively (Table 3). The calculated $V_{max}/K_m$ values for PTP formation were 0.95 and 0.23 μl/min per mg protein and 220 and 92 μl/min per nmol P450, respectively. Marmoset CYP2D6 showed a roughly 2-fold $V_{max}/K_m$ value for PTP formation compared with that of human CYP2D6. Apparently consistent $K_m$ values (162 μM and 137 μM) for MPTP N-oxygenations by marmoset liver microsomes and marmoset FMO3, respectively, were obtained in a similar manner to those in humans. Apparently consistent $K_m$ values (66 μM and 72 μM) for MPP⁺ formation by marmoset liver microsomes and

<table>
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<tr>
<th>Probe Reaction Activity</th>
<th>P450 Isoform or FMO</th>
<th>Correlation Coefficient for Rates of Formation of MPTP Metabolites</th>
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<tbody>
<tr>
<td>7-Ethoxyresorufin O-deethylation</td>
<td>CYP1A</td>
<td>0.73*</td>
</tr>
<tr>
<td>Coumarin 7-hydroxylation</td>
<td>CYP2A</td>
<td>−0.16</td>
</tr>
<tr>
<td>Paclitaxel 6a-hydroxylation</td>
<td>CYP2C</td>
<td>0.29</td>
</tr>
<tr>
<td>Warfarin 7-hydroxylation</td>
<td>CYP2C</td>
<td>0.60</td>
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<tr>
<td>S-Mephenytoin 4'-hydroxylation</td>
<td>CYP2C</td>
<td>0.30</td>
</tr>
<tr>
<td>Bufuralol 1'-hydroxylation</td>
<td>CYP2D</td>
<td>0.77***</td>
</tr>
<tr>
<td>Chlorzoxazone hydroxylation</td>
<td>CYP2E</td>
<td>−0.27</td>
</tr>
<tr>
<td>Midazolam 1'-hydroxylation</td>
<td>CYP3A</td>
<td>0.92***</td>
</tr>
<tr>
<td>Benzydamine N-oxygenation</td>
<td>FMO</td>
<td>0.63</td>
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*The P450 isoform or FMO mainly responsible for catalyzing the probe reactions.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 

![Fig. 4](https://example.com/fig4.png) Effects of chemical inhibitors on the formation rates of PTP (A), MPDP⁺ (B), MPTP N-oxide (C), and MPP⁺ (D) from MPTP in marmoset liver microsomes. Percentages of the control (without inhibitors) are shown: α-Naphthoflavone, sulfaphenazole, ticlopidine, quinidine, ketoconazole, and methimazole, respectively, inhibit CYP1A, CYP2C9, CYP2C, CYP2D, CYP3A4, and FMO.
marmoset CYP2D6 were observed with a high $V_{\text{max}}/K_m$ value (150 $\mu$M/min per nmol CYP2D6), but the $K_m$ value for MPP+ formation in human liver microsomes was much lower than that of human CYP2D6, resulting in a low $V_{\text{max}}/K_m$ value (8.5 $\mu$M/min per nmol) for human CYP2D6. In terms of kinetic parameters for MPDP+ formation, a similar pattern of differences between marmosets and humans was seen to that for MPP+ formation.

**Discussion**

Oxidation of potential proneurotoxin MPTP has been reported in various animal species, including mice, rats, and humans (Chiba et al., 1990; Coleman et al., 1996; Yoshihara et al., 2000). In vitro studies using rat liver microsomes have shown approximately 20 times faster MPTP N-oxygenation rates than MPTP N-demethylation rates (Cashman and Ziegler, 1986). In this study, fast MPTP N-oxygenation reactions in vitro were confirmed in marmoset liver and brain microsomes (Fig. 3). These facts suggest that MPTP N-oxygenation might be the major detoxification pathway in most animal species, including common marmosets. MPTP N-oxygenation in marmoset liver microsomes was likely mediated by FMO3 because of suppression of the reaction by methimazole (an inhibitor for FMO, Fig. 4) and the high metabolic capacity of marmoset FMO3 compared with FMO1 (Table 3). In terms of FMO-mediated reactions, apparent $K_m$ values for NADPH-dependent drug oxidations were generally in the range of approximately 50–100 $\mu$M (Yamazaki et al., 2014), presumably because FMO exhibits a stable 4a-flavin hydroperoxide intermediate capable of oxygenating both nucleophiles and electrophiles in its catalytic cycle, even in the absence of an oxygenatable substrate (Jones and Ballou, 1986).

In this study, the main roles of liver microsomal CYP2D6 and FMO3 in MPTP oxidation in common marmosets were demonstrated (Fig. 5; Table 3). Marmoset liver microsomes were the main focus of this study to investigate P450-dependent metabolic activation of MPTP, because efficient catalytic activities of liver microsomes with respect to MPTP oxidation were seen compared with the generally lower activities for marmoset brain microsomes. It could be noted that our correlation analysis using 10 marmoset liver microsomes with relatively fewer interindividual variations might not be as predictive as the chemical inhibitor and recombinant enzyme experiments. Several lines of evidence in this study using marmoset liver microsomes with correlation or chemical inhibition studies and experiments with recombinantly expressed P450 enzymes and FMO3 suggested that the MPTP N-demethylation, MPTP N-oxygenation, and MPDP+ and MPP+ formation reactions shown in Fig. 1 were mediated by multiple drug-metabolizing enzymes, such as CYP1A, CYP2C, CYP2D, and FMO in marmoset liver microsomes, but these reactions were mainly catalyzed by marmoset CYP2D6 or FMO3. Kinetic analysis of MPP+ formation revealed that the apparent $K_m$ values for marmoset liver microsomes and recombinant marmoset CYP2D6 were consistent (Table 3), although minor roles of other P450 isoforms could not be ruled out. It should be noted that occasional species differences were seen with regard to the roles of P450 enzymes between marmosets and humans (Fig. 5); human CYP1A2 seemed to have high capacity for MPDP+ and MPP+ formation under these conditions. Although monoamine oxidase exists in another cellular fraction that was not

**TABLE 2**

<table>
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<tr>
<th>MPTP N-oxygenation catalyzed by marmoset and human FMO1 and FMO3</th>
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<tr>
<td>Marmoset and human recombinant FMOs (20 pmol/ml) were incubated with 200 $\mu$M MPTP at 37°C for 10 minutes in the presence of an NADPH-generating system. Formations of PTP, MPDP+, and MPP+ by FMO were below the detection limits (for MPDP+ and MPP+ &lt;0.1 nmol/min per nmol FMO, for PTP &lt;0.01 nmol/min per nmol FMO).</td>
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<tr>
<td>FMO</td>
<td>MPTP N-Oxygenation</td>
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<td>mmol/min per nmol FMO</td>
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<td>Marmoset</td>
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<tr>
<td>FMO1</td>
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<tr>
<td>FMO3</td>
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<td>4.2</td>
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<tr>
<td>FMO3</td>
<td>78.0</td>
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**Fig. 5.** Formation of PTP (A and D), MPDP+ (B and E), and MPP+ (C and F) from MPTP mediated by marmoset (A–C) and human (D–F) recombinant P450 enzymes. Marmoset and human recombinant P450 enzymes (20 pmol/ml) were incubated with 50 $\mu$M MPTP at 37°C for 10 minutes in the presence of an NADPH-generating system.
tested in this study, these results collectively suggested that in the marmoset, CYP2D6 is mainly responsible for both deactivation of MPTP to PTP and metabolic activation of MPTP to MPP⁺ to produce the preclinical marmoset model for Parkinson-like syndromes. In our preliminary study, we were unable to isolate CYP2D30 cDNA from marmoset livers by RT-PCR, and we thus excluded CYP2D30 from further analysis. This was consistent with a reported finding that CYP2D30 was not isolated from another source of marmosets using the same CYP2D30 primer sets (Hichiya et al., 2004).

Our findings should provide a foundation for understanding MTPP metabolism and the successful production of preclinical marmoset parkinsonism models. Although some differences were seen in this study in terms of major roles of drug-metabolizing enzymes involved in the MTPP activation and deactivation pathways in humans and marmosets, marmosets are again recognized as a good preclinical model for Parkinson disease, as previously reported (Ando et al., 2012, 2014). A variety of genetic polymorphisms of CYP2D6 causing gene duplication, reduced function, or loss of function in humans are well known (Kiyotani et al., 2010). Genetic polymorphisms of CYP2D enzymes in cynomolgus and rhesus macaques (Uno et al., 2014) were also recently demonstrated. In this study, the interindividual variations for MTPP oxidations in marmoset liver microsomes (Fig. 3) were not as high as expected, presumably because no genetic variations for marmoset CYP2D6 were confirmed in our preliminary study using these 11 marmosets. To effectively produce the Parkinson preclinical animal model, it will be of interest to find more efficient individual marmosets (i.e., rapid metabolizers of CYP2D6 substrates) in further studies.

In conclusion, these results indicated the contributions of multiple drug-metabolizing enzymes in MTPP oxidation, especially marmoset FM03 in MTPP deactivation (N-demethylation) and metabolic activation of MTPP (by desaturation reactions), leading to active neurotoxic compound MPP⁺.

### Authorship Contributions

**Participated in research design:** Uehara, Uno, Yamazaki.  
**Conducted experiments:** Uehara, Uno, Murayama, Shimizu.  
**Contributed new reagents or analytic tools:** Inoue, Sasaki.  
**Performed data analysis:** Uehara, Uno, Yamazaki.  
**Wrote or contributed to the writing of the manuscript:** Uehara, Uno, Yamazaki.

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