BIOCHEMICAL PHARMACOLOGY 75 (2008) 752–760

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Oxidation of 5-methoxy-N,N-diisopropyltryptamine in rat liver microsomes and recombinant cytochrome P450 enzymes

Shizuo Narimatsu [a,*], Rei Yonemoto [a], Kazufumi Masuda [b], Takashi Katsu [b], Masato Asanuma [c], Tooru Kamata [d], Munehiro Katagi [d], Hitoshi Tsuchihashi [d], Takuya Kumamoto [e], Tsutomu Ishikawa [e], Shinsaku Naito [f], Shigeru Yamano [g], Nobumitsu Hanioka [a]

[a] Laboratory of Health Chemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan
[b] Laboratory of Pharmaceutical Physical Chemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan
[c] Department of Brain Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata, Okayama 700-8558, Japan
[d] Forensic Science Laboratory, Osaka Prefectural Police Headquarters, 1-3-18 Hommachi, Chuo-ku, Osaka 541-0053, Japan
[e] Laboratory of Medicinal Organic Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan
[f] Division of Pharmacology, Drug Safety and Metabolism, Otsuka Pharmaceutical Factory Inc., Naruto, Tokushima 772-8601, Japan
[g] Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Fukuoka University, Jonan-ku, Fukuoka 814-0180, Japan

ARTICLE INFO

Article history:
Received 27 August 2007
Accepted 21 September 2007

Keywords:
5-Methoxy-N,N-diisopropyltryptamine
Rat
O-Demethylation
N-Deisopropylation
6-Hydroxylation
CYP2C11
CYP2D2
CYP3A2
CYP1A1

ABSTRACT

The oxidative metabolism of 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT), a tryptamine-type designer drug, was studied using rat liver microsomal fractions and recombinant cytochrome P450 (CYP) enzymes. 5-MeO-DIPT was biotransformed mainly into a side-chain N-deisopropylated metabolite and partially into an aromatic ring O-demethylated metabolite in liver microsomal fractions from untreated rats of both sexes. This metabolic profile is different from our previous findings in human liver microsomal fractions, in which the aromatic ring O-demethylation was the major pathway whereas the side-chain N-deisopropylation was minor [Narimatsu S, Yonemoto R, Saito K, Takaya K, Kumamoto T, Ishikawa T, et al. Oxidative metabolism of 5-methoxy-N,N-diisopropyltryptamine (Foxy) by human liver microsomes and recombinant cytochrome P450 enzymes. Biochem Pharmacol 2006;71:1377–85]. Kinetic and inhibition studies indicated that the side-chain N-dealkylation is mediated by CYP2C11 and CYP3A2, whereas the aromatic ring O-demethylation is mediated by CYP2D2 and CYP2C6 in untreated male rats. Pretreatment of male rats with β-naphthoflavone (BNF) produced an aromatic ring 6-hydroxylated metabolite. Recombinant rat and human CYP1A1 efficiently catalyzed 5-MeO-DIPT 6-hydroxylation under the conditions used. These results provide valuable information on the metabolic fate of 5-MeO-DIPT in rats that can be used in the toxicological study of this designer drug.

* *Corresponding author*. Tel.: +81 86 251 7942; fax: +81 86 251 7942.
E-mail address: shizuo@pharm.okayama-u.ac.jp (S. Narimatsu).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.09.019

1. Introduction

5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) is a tryptamine-type designer drug that is used world-wide including Japan, particularly by young people [1–5]. Its toxicity has not been examined systematically, although some information has been obtained using experimental animals [6–9]. Furthermore, the *in vivo* and *in vitro* metabolic profiles of 5-MeO-DIPT in humans and experimental animals have been reported [10–12], but the relationship between its metabolic fate and toxicity remains obscure.

Kamata et al. [10] analyzed the urine samples of 5-MeO-DIPT users, and found 5-hydroxy-*N*,*N*-diisopropyltryptamine (5-OH-DIPT), 5-methoxy-*N*-isopropyl-tryptamine (5-MeO-IPT) and 6-hydroxy-5-methoxy-*N*,*N*-diisopropyltryptamine (6-OH-5MeO-DIPT) and their conjugation metabolites (sulfate and glucuronide). We studied the oxidative metabolism of 5-MeO-DIPT, and revealed that in pooled human liver microsomal fractions, aromatic ring *O*-demethylation forming 5-OH-DIPT was the major pathway and side-chain *N*-deisopropylation forming 5-MeO-IPT was a minor pathway [11]. Further detailed studies using recombinant human cytochrome P450 (CYP) enzymes and CYP inhibitors indicated that the aromatic ring *O*-demethylation was catalyzed predominantly by CYP2D6, whereas the side-chain *N*-deisopropylation was catalyzed by CYP1A2, CYP3A4 and CYP2C19 [11].

Kanamori et al. [12] administered 5-MeO-DIPT orally to rats, and identified 5-hydroxy-*N*-isopropyltryptamine and 5-methoxyindole acetic acid in addition to 5-OH-DIPT and 5-MeO-IPT as urinary metabolites by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry. Considering the illegal usage of 5-MeO-DIPT, systematic toxicological studies using experimental animals are necessary. The rat is appropriate for such studies because its genetic background is relatively well understood. Information on the kinds of CYP enzymes involved in the oxidative metabolism of 5-MeO-DIPT will be important for the analysis of toxicokinetic data obtained in rats.

We thus conducted experiments *in vitro* on the oxidative metabolism of 5-MeO-DIPT using liver microsomal fractions from untreated and CYP inducer-pretreated rats as well as recombinant rat CYP enzymes. The results obtained were then compared with those of *in vitro* studies using human liver microsomal fractions and recombinant enzymes reported previously [11].

2. Materials and methods

2.1. Materials

5-MeO-DIPT was supplied by Dr. Funada, National Institute of Mental Health, National Center of Neurology and Psychiatry (Kodaira, Japan). It was used without further purification because it had the purity of more than 99% in HPLC. 5-OH-DIPT [11], 5-MeO-IPT [11] and 6-OH-5-MeO-DIPT [10] were synthesized as described previously. Furafylline (a CYP1A2 inhibitor [13]) and cimetidine (a CYP2C11 inhibitor [14,15]) were obtained from Sigma–Aldrich Co. (St. Louis, MO); quinine (a CYP2D1 and CYP2D2 inhibitor [16]) was from Nacalai Tesque (Kyoto, Japan); β-naphthoflavone (BNF), glucose-6-phosphate (G-6-P), G-6-P dehydrogenase and NADPH were from Wako Pure Chemical Ind. (Osaka, Japan). Ketoconazole (a CYP3A1/2 inhibitor [13]) was kindly supplied by Dr. Y. Yoshida, Mukogawa Women's University (Nishinomiya, Japan). Recombinant rat CYP enzymes (CYP1A1, -1A2, -2A1, -2B1, -2C6, -2C11, -2C12, -2E1 and -3A2) or human CYP1A1 expressed in insect cells (Supersomes) were purchased from BD Biosciences Discovery Labware (Bedford, MA). Recombinant rat CYP2D1 and CYP2D2 expressed in yeast cell microsomes were obtained by a method reported previously [17]. Adult male and female Wistar rats (SPF, 7–8 weeks old, 150–220 g in body weight) were obtained from Charles River Japan (Yokohama, Japan). The animals were kept in stainless steel cages ($n = 3$ each) in an air-conditioned room at an ambient temperature of 22–24 °C with a light-dark cycle of 12 h. They were allowed to access food (CRF-1, Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. The food was removed 12 h before sacrifice.

2.2. Animal treatment

The male rats were intraperitoneally administered BNF dissolved in corn oil (20 mg/kg/2 ml, once a day for 3 days). The control rats were given the same volume of the vehicle only. The animals were killed 24 h after the last administration, their livers were excised, and microsomal fractions were prepared according to a published method [17]. Liver microsomal fractions from untreated male and female rats were also prepared in the same manner. Protein concentrations of the microsomal fractions were measured by the method of Lowry et al. [18].

2.3. Assay of oxidizing activity for 5-MeO-DIPT

The activity to oxidize 5-MeO-DIPT was measured according to a published method [11]. Briefly, a typical reaction mixture consisted of G-6-P (10 mM, final concentration), NADPH (1 mM), $MgCl_2$ (10 mM), EDTA (0.2 mM), rat liver microsomes (0.01 mg protein) or recombinant CYP enzymes (1–25 pmol) and the substrate (0.1–2000 μM) in 50 mM potassium phosphate buffer (pH 7.4) in a 1.5 ml Eppendorf-type tube (a final volume of 200 μl). Following preincubation at 37 °C for 5 min, the reaction was started by adding the enzyme source (rat liver microsomes, Supersomes expressing various rat CYP enzymes, or yeast cell microsomes expressing CYP2D1 or CYP2D2), continued for 5–10 min, and stopped by adding aqueous 2 M phosphoric acid (10 μl) and 20 mM ascorbic acid (20 μl), vigorously mixing with a Vortex mixer, and chilling in an ice bath for 10 min. The tube was then centrifuged at 14,000 × *g* for 10 min at 4 °C, and the supernatant was passed through a 0.45 μm membrane filter (Millipore, Billerica, MA). An aliquot (20 μl) was subjected to HPLC under the conditions described below. Calibration curves for 5-OH-DIPT and 5-MeO-IPT were made by spiking ice-cold reaction medium with known amounts of the synthetic compounds, followed by the addition of aqueous 2 M phosphoric acid and 20 mM ascorbic acid and treatment as described above. The detection limits for 5-OH-DIPT and 5-MeO-IPT were 0.5 and 1.0 pmol/ml, with a signal-to-noise ratio of three in both cases. The intra- and inter-day variation coefficients did not exceed 10% in any

assay. For the assay of 6-OH-5-MeO-DIPT formation, the calibration curve for 5-MeO-IPT was used because 6-OH-5-MeO-DIPT was labile and its peak was quickly attenuated on the chromatogram.

2.4. Inhibition study

Furafylline was dissolved in dimethylsulfoxide (DMSO). Cimetidine, quinine and ketoconazole were dissolved in a mixture of DMSO/methanol (1:1 by volume). Each inhibitor (furafylline, 5, 10 or 50 μM as a final concentration; cimetidine, 10, 50 or 200 μM; quinine, 1, 5 or 20 μM; ketoconazole, 1, 5, 20 or 50 μM) was added to the incubation medium containing the substrate (1, 250 or 500 μM as a final concentration) and preincubated at 37 °C for 5 min as described above. The control contained the vehicle only. The concentration of the organic solvent was 1% in the incubation mixture.

2.5. HPLC conditions

The HPLC apparatus consisted of a Hitachi L-2130 pump, an L-2480 fluorescence detector, an L-2300 column oven, a D-2000 system manager (version 1.1) and a Rheodyne type 7725i injector. Other conditions were as follows: column, Inertsil C8 (150 mm × 4.6 mm i.d., GL Sciences Co. Ltd., Tokyo, Japan); column temperature, 40 °C; detection, excitation/emission wavelength 280/340 nm. The mobile phase used was a linear gradient system consisting of (A) 20 mM ammonium acetate (pH 4.0)/acetonitrile (92:8, by volume) and (B) 20 mM ammonium acetate (pH 4.0)/acetonitrile (80:20) as follows: 0–3 min, (A) 100%; 3–15 min, from (A) 100% to (B) 100%; 15–25 min, (B) 100%; 25–30 min, from (B) 100% to (A) 100%; 30–40 min, (A) 100% at a flow rate of 0.9 ml/min.

2.6. Others

Total holo-CYP2D1 and -CYP2D2 levels in yeast cell microsomal fractions were measured spectrophotometrically by assessing reduced-carbon monoxide (CO) spectra according to the method of Omura and Sato [19] using 91 mM^{-1} cm^{-1} as the absorption coefficient. Kinetic parameters (apparent K_m and V_{max} values) were estimated by analyzing Michaelis–Menten plots or Eadie–Hofstee plots using the computer program Prism ver. 4.0 software (GraphPad Software, San Diego, CA). Statistical comparisons were made with Student's *t*-test, and a difference was considered statistically significant when the *p*-value was <0.05.

3. Results

Rat liver microsomal fractions efficiently mediated the oxidation of 5-MeO-DIPT at a substrate concentration of 1 mM. Fig. 1 shows typical HPLC chromatograms of 5-MeO-DIPT oxidation by microsomes from untreated (Fig. 1A) and BNF-pretreated (Fig. 1B) male rats. Retention times of 5-OH-DIPT, 6-OH-5-MeO-DIPT, 5-MeO-IPT and the parent compound (5-MeO-DIPT) were 10.3, 12.1, 15.1 and 20.4 min, respectively. Interestingly, the peak of 6-OH-5-MeO-DIPT was observed only in the microsomal fraction from the BNF-pretreated male rats, not in that from the untreated male rats. In the HPLC chromatogram for BNF-pretreated male rats (Fig. 1B), there appeared five unknown peaks having retention times of 2.2, 15.7, 17.8, 21.8 and 23.0 min. In the LC/MS analysis, only the peak of 17.8 min showed fragment ions that could be of a 5-MeO-DIPT metabolite, i.e., 305 $[M + H]^+$, 290, 204, 191 and 114, whereas the other four peaks did not show reliable fragment ions as metabolites of 5-MeO-DIPT under the LC/MS conditions used.

Fig. 2 summarizes the oxidizing activities for 5-MeO-DIPT in liver microsomal fractions from untreated and BNF-pretreated males, and untreated female rats at a substrate concentration of 1 mM. The data for pooled human liver microsomes [11] are also shown as a comparison. 5-MeO-DIPT *O*-demethylase activities were similar among untreated male, BNF-pretreated male and untreated female rats, while the activity of human liver microsomes was significantly lower than that of untreated male or female rats. In contrast, in terms of *N*-deisopropylase activity, the ranking was untreated male rats > BNF-pretreated male rats > untreated female rats. In this index also, the activity of pooled human liver microsomes was significantly lower than that in untreated rats of either sex. 5-MeO-DIPT 6-hydroxylase activity was observed only in BNF-pretreated male rats and not in untreated male or female rats, while pooled human liver microsomes produced 6-OH-5-MeO-DIPT, although in a trace amount, which had been reported as an unidentified metabolite (M-2) in our previous study [11].

Eadie–Hofstee plots are shown for the three oxidation reactions of 5-MeO-DIPT in liver microsomes from BNF-pretreated male rats in Fig. 3. 5-MeO-DIPT *O*-demethylation exhibited biphasic kinetics, whereas both *N*-deisopropylation and 6-hydroxylation yielded monophasic kinetics. Similar profiles were observed in the plots for the *O*-demethylation and *N*-deisopropylation of 5-MeO-DIPT in microsomal fractions from untreated rats of both sexes. Table 1 lists the kinetic parameters for these reactions by rat liver microsomes together with those of human liver microsomes [11]. In untreated male and female rats, the aromatic ring *O*-demethylation gave biphasic kinetics, and kinetic parameters in both low-K_m and high-K_m phases were similar between male and female rats, although the K_m value (0.21 μM) of female rats was significantly higher than that of male rats (0.13 μM). In BNF-pretreated rats, the intrinsic clearance (V_{max}/K_m) value (5.8 μl/(min mg protein)) of the high-K_m phase was significantly higher than that in untreated male rats (2.0 μl/(min mg protein)), which may be due to the decrease in the K_m value in the BNF-pretreated group.

The side-chain *N*-dealkylation showed monophasic kinetics in untreated and BNF-pretreated rats, whereas it exhibited triphasic kinetics in pooled human liver microsomes in our previous study [11] (Table 1). It should be noted that a clear sex difference (male > female) was found in both V_{max} and intrinsic clearance values between untreated male and female rats. The pretreatment of male rats with BNF did not significantly change the kinetic profiles.

To clarify to what extent each CYP enzyme contributes to rat liver microsomal 5-MeO-DIPT, we examined the capacities of various rat recombinant CYP enzymes. In this experiment, we employed two substrate concentrations, 20 μM for the low-K_m phase and 500 μM for high-K_m phases. As shown in Fig. 4A,

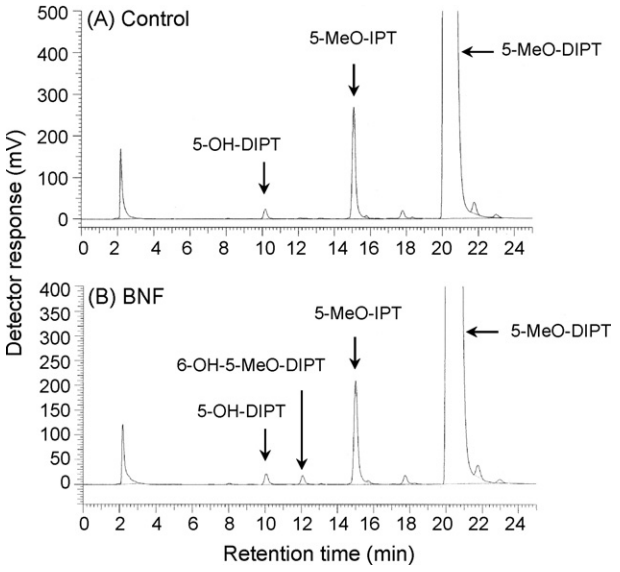

Fig. 1 – Comparison of typical HPLC chromatograms of the metabolism of 5-MeO-DIPT by liver microsomal fractions between untreated and BNF-pretreated male rats. The substrate concentration used was 200 μM. Other conditions employed were given in Section 2. (A) Untreated male rat, (B) BNF-pretreated male rat.

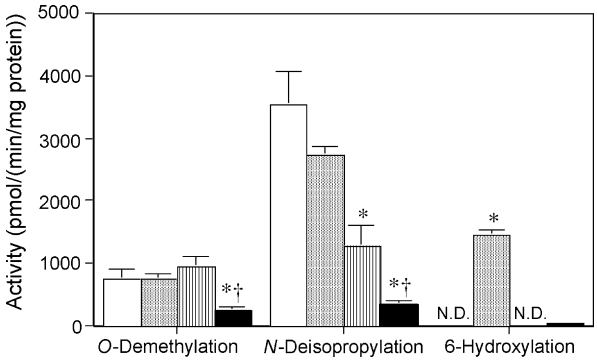

Fig. 2 – Comparison of 5-MeO-DIPT-oxidizing activities among liver microsomal fractions from untreated rats of both sexes, BNF-pretreated male rats and humans. Open column, untreated male rat; hatched column, BNF-pretreated male rat; striped column, untreated female rat; closed column, human. Each value represents the mean ± S.D. ($n = 3$). The data for pooled human liver microsomes are from our previous study [11]. *Significantly different from the values for untreated male rats ($p < 0.05$). †Significantly different from the values of untreated female rats ($p < 0.05$).

three rat CYP enzymes, CYP2D2, CYP2C6 and CYP1A1, exhibited considerable activity under the conditions used. In contrast, four rat CYP enzymes, CYP2C11, CYP1A2, CYP2C6 and CYP3A2, were shown to catalyze the side-chain *N*-deisopropylation (Fig. 4B). To our interest, among 11 rat CYP enzymes examined, only CYP1A1 exhibited some 5-MeO-DIPT 6-hydroxylase activity under the conditions employed. We then examined the capacity of human CYP1A1 for the oxidation of 5-MeO-DIPT under the similar conditions. Similarly to the results for the rat enzyme, human CYP1A1 also catalyzed 5-MeO-DIPT 6-hydroxylation.

Table 2 summarizes the kinetic parameters. For 5-MeO-DIPT *O*-demethylation, CYP2D2 had the lowest K_m (0.1 μM) and the highest V_{max} (10 pmol/(min pmol CYP)) values, giving the

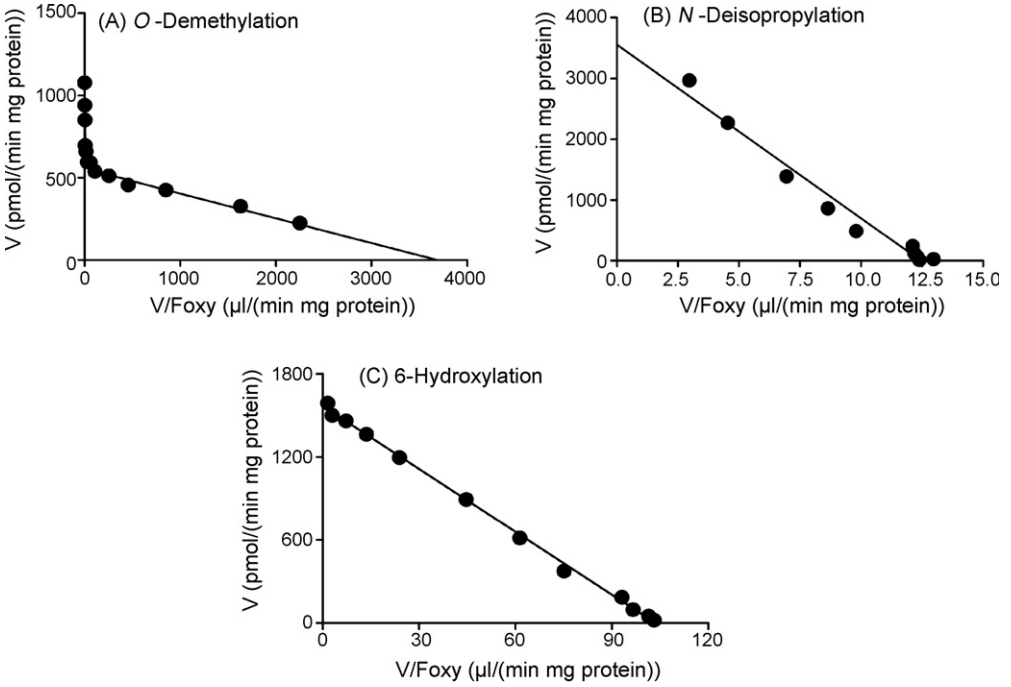

Fig. 3 – Typical Eadie–Hofste plots showing the oxidation of 5-MeO-DIPT by liver microsomes from BNF-pretreated male rats. (A) 5-MeO-DIPT *O*-demethylation; (B) 5-MeO-DIPT *N*-deisopropylation; (C) 5-MeO-DIPT 6-hydroxylation.

Table 1 – Kinetic parameters for 5-MeO-DIPT oxidation by rat liver microsomes

	K_m (μM)	V_{max} (pmol/(min mg protein))	V_{max}/K_m (μl/(min mg protein))
O-Demethylation			
Untreated male rats			
Low-K_m phase	0.13 ± 0.02	408 ± 167	2150 ± 960
High-K_m phase	273 ± 193	390 ± 233	1.99 ± 1.20
BNF-pretreated male rats			
Low-K_m phase	0.13 ± 0.02	404 ± 82	3170 ± 960
High-K_m phase	70.8 ± 11.9	406 ± 33	5.80 ± 0.61**
Untreated female rats			
Low-K_m phase	0.21 ± 0.04*	475 ± 110	2180 ± 110
High-K_m phase	230 ± 186	536 ± 386	2.47 ± 0.40
Human[a]	5.02	140	27.9
N-Deisopropylation			
Untreated male rats	445 ± 72	5050 ± 1450	11.3 ± 2.3
BNF-pretreated male rats	288 ± 8	3340 ± 220	11.5 ± 1.0
Untreated female rats	403 ± 143	1780 ± 890*	4.28 ± 0.68**
Human[a]			
Low-K_m phase	24	25	1.03
Medium-K_m phase	257	178	0.69
High-K_m phase	1200	409	0.34
6-Hydroxylation			
BNF-pretreated male rats	14.6 ± 0.6	1400 ± 150	95.6 ± 6.5

The values for rats are the mean ± S.D. ($n = 3$). Significantly different from the values for untreated male rats (*$p < 0.05$ and **$p < 0.01$).
[a] The values for humans (the mean value of two independent determinations) are quoted from Narimatsu et al. [11].

highest intrinsic clearance value (112 μl/(min pmol CYP)) among the four recombinant rat CYP enzymes. For 5-MeO-DIPT *N*-deisopropylation, the male-specific CYP2C11 had the highest K_m (450 μM) and V_{max} (153 pmol/(min pmol CYP)) values among six rat recombinant enzymes. As compared with other three CYP enzymes (CYP1A1, CYP2D1 and CYP3A2), CYP1A2, CYP2C6 and CYP2C11 showed relatively high intrinsic clearance values. For 5-MeO-DIPT 6-hydroxylation, rat and human CYP1A1 yielded similar kinetic parameters, and their intrinsic clearance values (3–4 μl/(min pmol CYP)) were secondly high, which follows the value of CYP2C11, among the recombinant CYP enzymes tested.

Among four CYP inhibitors examined, quinine, the inhibitor of rat CYP2D enzymes, exhibited the greatest inhibitory

Table 2 – Kinetic parameters for 5-MeO-DIPT oxidation by rat recombinant CYP enzymes

	K_m (μM)	V_{max} (pmol/(min pmol CYP))	V_{max}/K_m (μl/(min pmol CYP))
O-Demethylation			
Rat CYP1A1	10.1	2.02	0.20
Rat CYP2C6	124	6.83	0.06
Rat CYP2D1	68.8	0.18	0.003
Rat CYP2D2	0.09	10.1	112
Human CYP1A1	2.67	0.33	0.13
N-Deisopropylation			
Rat CYP1A1	7.98	0.42	0.06
Rat CYP1A2	53.6	25.6	0.49
Rat CYP2C6	161	20.8	0.14
Rat CYP2C11	450	153	0.35
Rat CYP2D1	59.6	0.76	0.013
Rat CYP3A2	420	13.5	0.03
Human CYP1A1	4.24	1.03	0.25
6-Hydroxylation			
Rat CYP1A1	9.65	27.2	2.82
Human CYP1A1	2.57	10.7	4.21

Each value represents the mean of two independent experiments.

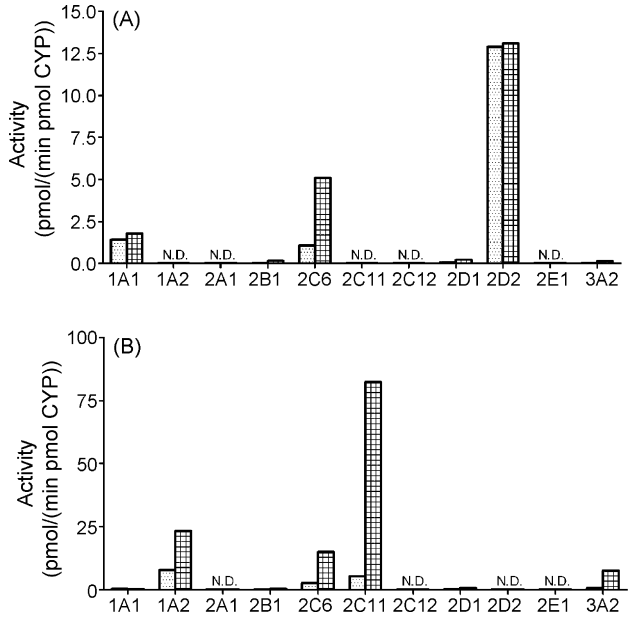

Fig. 4 – Comparison of 5-MeO-DIPT-oxidizing activities of rat recombinant CYP enzymes. (A) 5-MeO-DIPT *O*-demethylation; (B) 5-MeO-DIPT *N*-deisopropylation. The substrate concentrations used were 20 μM (dotted column) and 500 μM (hatched column). Each value represents the mean of two independent determinations. N.D., not detectable.

effect on the *O*-demethylation of 5-MeO-DIPT by untreated male rat liver microsomes (Fig. 5). At a substrate concentration of 1 μM, qinine (5 μM) suppressed the activity almost completely, whereas cimetidine (200 μM), the inhibitor of CYP2C11, suppressed only 50% of the activity. At the highest substrate concentration, 500 μM, ketoconazole (20 μM), the inhibitor of rat CYP3A1/2, decreased the activity by 32%, which is similar to the effect of quinine, while the other two inhibitors (furafylline and cimetidine) had almost no effect. For the *N*-deisopropylation by untreated male rat liver microsomes, ketoconazole was found to be the most potent inhibitor: at 20 μM it abolished the activity almost completely at a substrate concentration of 500 μM (Fig. 6). The potency of the inhibitory effects of cimetidine and quinine was found to follow that of ketoconazole, but furafylline showed almost no inhibitory effect on the activity under the conditions used.

4. Discussion

We previously reported that 5-MeO-DIPT was mainly oxidized to the aromatic ring *O*-demethylated metabolite, 5-OH-DIPT, and the side-chain *N*-deisoproylated metabolite, 5-MeO-IPT, was a minor metabolite in human liver microsomes [11]. In kinetic studies, human liver microsomal *O*-demethylation was monophasic, whereas the *N*-deisopropylation was triphasic. Some lines of experimental evidence obtained using enzyme inhibitors and recombinant enzymes indicated that CYP2D6 is the major and preferred CYP enzyme for the *O*-demethylation

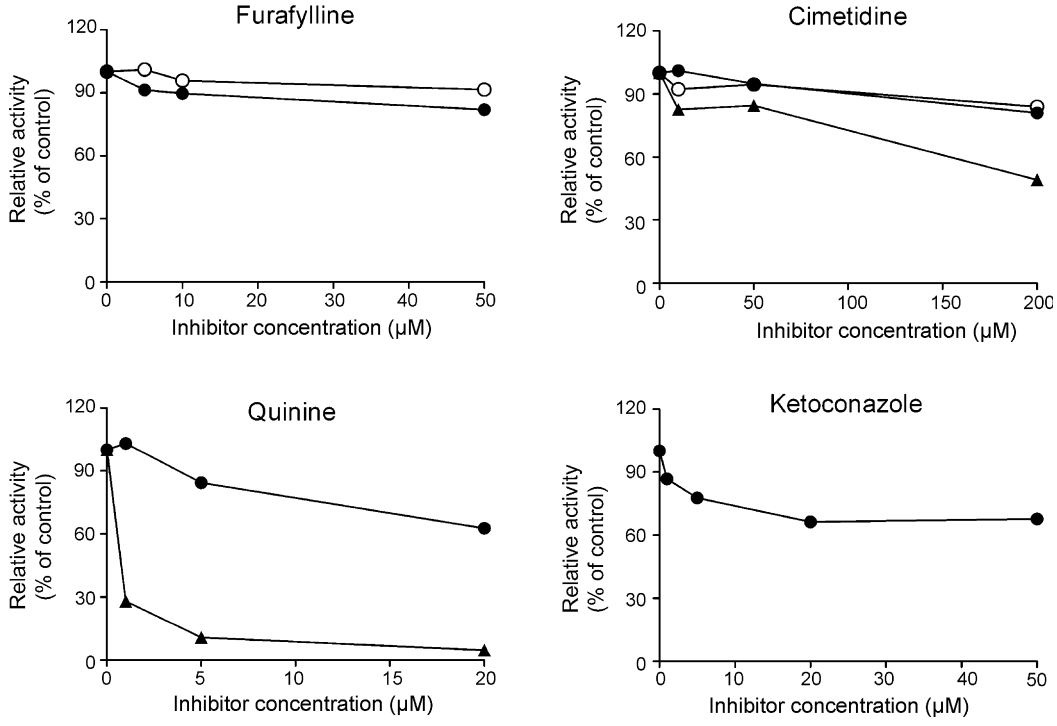

Fig. 5 – Effects of CYP inhibitors on 5-MeO-DIPT *O*-demethylation by untreated male rat liver microsomes. The substrate concentrations used were 1 μM (closed triangle), 250 μM (open circle) and 500 μM (closed circle). The results are expressed as a percentage of the control activity. The control levels of activity at 1, 250 and 500 μM were 226, 464 and 513 pmol/(min mg protein), respectively.

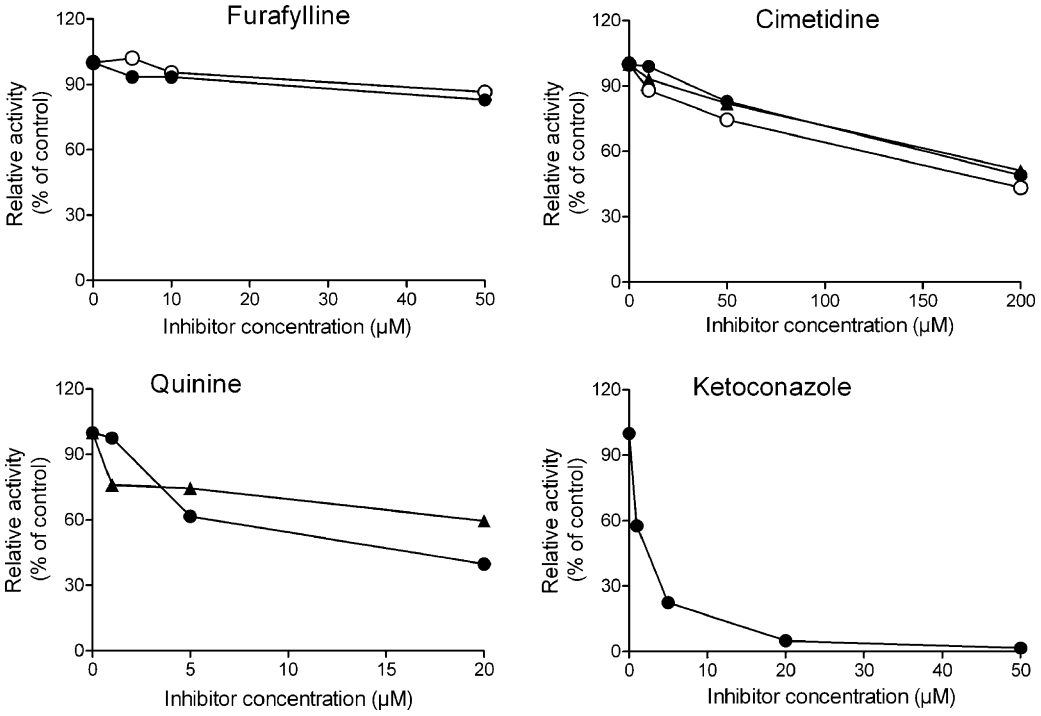

Fig. 6 – Effects of CYP inhibitors on 5-MeO-DIPT *N*-deisopropylation by untreated male rat liver microsomes. The substrate concentrations used were 1 μM (closed triangle), 250 μM (open circle) and 500 μM (closed circle). The results are expressed as a percentage of the control activity. The control levels of activity at 1, 250 and 500 μM were 17.7, 1670 and 3420 pmol/(min mg protein), respectively.

of 5-MeO-DIPT, while several CYP enzymes such as CYP1A2, CYP2C9, CYP2C19 and CYP3A4 are involved in *N*-deisopropylation [11] (Fig. 7).

In the present study, we demonstrated that the aromatic ring *O*-demethylation is the major pathway for the metabolism of 5-MeO-DIPT in a low substrate concentration range, and the side-chain *N*-deisopropylation becomes predominant in a high substrate concentration range in liver microsomes from untreated rats of both sexes, which is a similar situation in human liver microsomes. Moreover, in the same rat liver microsomal fractions, the *O*-demethylation was analyzed to be biphasic, whereas the *N*-deisopropylation gave monophasic kinetics, which is different from the human profile.

The results of the present experiments using rat CYP inhibitors and recombinant enzymes indicated that CYP2D2 is responsible for the low-K_m phase *O*-demethylation, and CYP1A1 and/or CYP2C6 is for the high-K_m phase reaction in rat liver microsomes. Although *N*-deisopropylation in

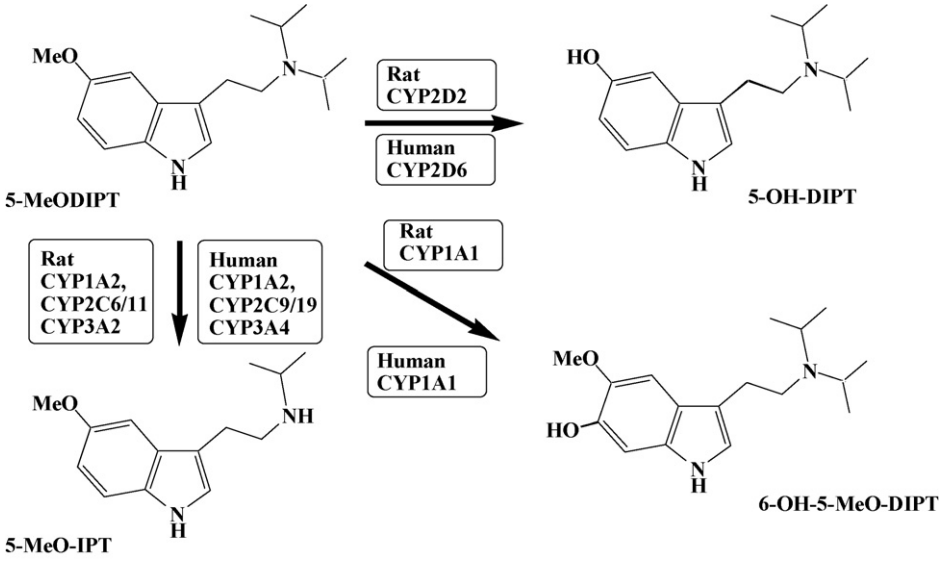

Fig. 7 – Involvement of CYP enzymes in 5-MeO-DIPT oxidation pathways in rats and humans.

untreated male rat liver microsomes yielded monophasic kinetics, CYP2C11 and CYP3A2, both of which have similar K_m values of around 400 μM, seem to be involved in this reaction. These enzymes are known to be male-specific or much more abundant in male rats than in females [20–22], causing the sex difference (male > female) in the V_{max} values for this reaction. However, it remains obscure at present what kinds of CYP enzymes are involved in the *N*-dealkylation of 5-MeO-DIPT in female rat liver microsomes.

5-OH-DIPT was identified as a major metabolite in urine samples from 5-MeO-DIPT users [10]. Moreover, 6-OH-5-MeO-DIPT was also found as another 5-MeO-DIPT metabolite in the same samples [10]. In our previous *in vitro* study using pooled human liver microsomes [11], we did not confirm the presence of 6-OH-5-MeO-DIPT because of the lack of an authentic standard. This time, the research group of Dr. Tsuchihashi of Osaka Prefectural Police Headquarters supplied with an authentic sample, so we identified the metabolite. Comparison of the HPLC chromatograms between rats and humans, suggested the unidentified metabolite, M-2, in our previous study [11] to be 6-OH-5-MeO-DIPT. It is thus thought that a trace amount of 6-OH-5-MeO-DIPT can be formed from 5-MeO-DIPT in human liver microsomes.

In the present study, the pretreatment with BNF caused the formation of 6-OH-5-MeO-DIPT in male rat liver microsomes, while this metabolite was undetectable in liver microsomal fractions from untreated rats of either sex. Because the fluorescence intensity of the metabolite peak corresponding to 6-OH-5-MeO-DIPT on the chromatogram was quickly attenuated, we added some ascorbic acid to the reaction mixture just after incubation according to a previous *in vivo* study [10], which was effective of protecting the peak, but not complete. We thus employed the calibration curve for 5-MeO-IPT to estimate the activity to form 6-OH-5-MeO-DIPT on the assumption that the fluorescence intensity was similar between 6-OH-5-MeO-DIPT and 5-MeO-IPT.

The K_m value for the formation of 6-OH-5-MeO-DIPT in liver microsomes from BNF-pretreated male rats was about 15 μM, which is close to that of recombinant rat CYP1A1, the only enzyme mediating the 6-hydroxylation of 5-MeO-DIPT under the conditions employed. In addition, the intrinsic clearance value (96 μl/(min mg protein)) for 6-hydroxylation was much higher than that for *N*-deisopropylation (4–11 μl/(min mg protein)) in rat liver microsomal fractions, indicating that the 6-hydroxylation, which is catalyzed by CYP1A1, is one of the major pathways by which 5-MeO-DIPT is oxidized in BNF-pretreated rats, particularly in a low substrate concentration range.

On the basis of the results, we examined the capacity of human CYP1A1 as 5-MeO-DIPT 6-hydroxyase in the present study. As a result, human CYP1A1 was found to efficiently catalyze 5-MeO-DIPT 6-hydroxylation. As described above, 6-OH-5-MeO-DIPT was also identified as human urinary metabolite as well as an *in vitro* metabolite under the conditions using human liver microsomal fraction. It is well known that human CYP1A1 expresses not in the liver but in the lung [23]. Therefore, it is reasonable to think that CYP enzyme(s) other than CYP1A1 is responsible for the formation of 6-OH-5-MeO-DIPT in human livers. Fig. 7 summarizes the CYP enzymes that may be involved in 5-MeO-DIPT oxidation pathways in rats and humans. The present study indicates that *O*-demethylation is the major oxidation pathway of 5-MeO-DIPT while *N*-deisopropylation is the minor pathway in both human and rat liver microsomal fractions, which is in agreement with the previous *in vivo* results [10,12].

In toxicological studies of methylenedioxymethamphetamine (MDMA), another drug of abuse, the research group of Dr. Cho at UCLA suggested that demethylenation of MDMA *via* hydroxylation of the methylene bridge by CYP2D enzymes produces a chemically reactive *o*-quinone, which may cause neurotoxicity in MDMA abusers [24]. Similarly, 6-OH-5-MeO-DIPT can be converted to *o*-quinone *via* further *O*-demethylation of the aromatic ring. The possibility of the formation of the quinone metabolite and its potential neurotoxicity should be evaluated next.

In summary, 5-MeO-DIPT was biotransformed mainly into 5-MeO-IPT and partially into 5-OH-DIPT in liver microsomal fractions from untreated rats of both sexes. Kinetic and inhibition experiments indicated that the side-chain *N*-dealkylation is mediated by CYP2C11 and CYP3A2, whereas the aromatic ring *O*-demethylation is mediated by CYP2D2 and CYP2C6 in untreated male rats. Pretreatment of male rats with BNF produced 6-OH-5-MeO-DIPT, the formation of which is thought to be mediated by CYP1A1 induced to express by BNF. These results provide valuable information on the metabolic fate of 5-MeO-DIPT in rats that can be used in the toxicological study of this designer drug.

Acknowledgments

We would like to express our gratitude to Dr. M. Funada, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kodaira, Japan. This study was supported in part by Grants-in-Aid for Scientific Research (17390035 and 18590116) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

[1] Shulgin AT, Carter MF. *N*,*N*-Diisopropyltryptamine (DIPT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT), two orally active tryptamine analogs with CNS activity. Commun Psychopharmacol 1980;4:363–9.
[2] Meatherall R, Sharma P. Foxy, a designer tryptamine hallucinogen. J Anal Toxicol 2003;27:313–7.
[3] Wilson JM, McGeorge F, Smalinske S, Meatherall R. A foxy intoxication. Forensic Sci Int 2005;148:31–6.
[4] Kikura-Hanajiri R, Hayashi M, Saisho K, Goda Y. Simultaneous determination of nineteen hallucinogenic tryptamines/β-calbolines and phenethylamines using gas chromatography-mass spectrometry and liquid chromatography-electrospray ionization-mass spectrometry. J Chromatogr B 2005;825:29–37.
[5] Ishida T, Kudo K, Kiyoshima A, Inoue H, Tsuji A, Ikeda N. Sensitive determination of alfa-methyltryptamine (AMT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine using gas chromatography-mass spectrometry. J Chromatogr B 2005;823:47–52.
[6] Fantegrossi WE, Harrington AW, Kiessel CL, Eckler JR, Rabin RA, Winter JC, et al. Hallucinogen-like actions of

5-methoxy-*N*,*N*-diisopropyltryptamine in mice and rats. Pharmacol Biochem Behav 2006;83:122–9.
[7] Compton DM, Selinger MC, Testa EK, Larkins KD. An examination of the effects of 5-Methoxy-*n*,*n*-di(ISO)propyltryptamine hydrochloride (Foxy) on cognitive development in rats. Psychol Rep 2006;98:651–61.
[8] Nagai F, Nonaka R, Satoh H, Kamimura K. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 2007;559:132–7.
[9] Sogawa C, Sogawa N, Tagawa J, Fujino A, Ohyama K, Asanuma M, et al. 5-Methoxy-*N*,*N*-diisopropyltryptamine (Foxy), a selective and high affinity inhibitor of serotonin transporter. Toxicol Lett 2007;170:75–82.
[10] Kamata T, Katagi M, Kamata HT, Miki A, Shima N, Zaitsu K, et al. Metabolism of the psychotomimetic tryptamine derivative 5-methoxy-*N*,*N*-diisopropyltryptamine in humans: identification and quantification of its urinary metabolites. Drug Metab Dispos 2006;34:281–7.
[11] Narimatsu S, Yonemoto R, Saito K, Takaya K, Kumamoto T, Ishikawa T, et al. Oxidative metabolism of 5-methoxy-*N*,*N*-diisopropyltryptamine (Foxy) by human liver microsomes and recombinant cytochrome P450 enzymes. Biochem Pharmacol 2006;71:1377–85.
[12] Kanamori T, Kuwayama K, Tsujikawa K, Miyaguchi H, Iwata Y, Inoue H, et al. *In vivo* metabolism of 5-methoxy-*N*,*N*-diisopropyltryptamine in rat. J Health Sci 2006;52:425–30.
[13] Kobayashi K, Urashima K, Shimada N, Chiba K. Selectivities of human cytochrome P450 inhibitors toward rat P450 isoforms: study with cDNA-expressed systems of the rat. Drug Metab Dispos 2003;31:33–6.
[14] Chang T, Levine M, Bellward GD. Selective inhibition of rat hepatic microsomal cytochrome P-450. II. Effect of the in vitro administration of cimetidine. J Pharmacol Exp Ther 1992;260:1450–5.
[15] Hanioka N, Jinno H, Kitazawa K, Tanaka-Kagawa T, Nishimura T, Ando M, et al. *In vitro* biotransformation of atrazine by rat liver microsomal cytochrome P450 enzymes. Chem Biol Interact 1998;116:181–98.
[16] Kobayashi S, Murray S, Watson D, Sesardic D, Davies DS, Boobis AR. The specificity of inhibition of debrisoquine 4-hydroxylase activity by quinidine and quinine in the rat is the inverse of that in man. Biochem Pharmacol 1989;38:2795–9.
[17] Isobe T, Hichiya H, Hanioka N, Yamamoto S, Shinoda S, Funae F, et al. Different effects of desipramine on bufuralol 1″-hydroxylation by rat and human CYP2D enzymes. Biol Pharm Bull 2005;28:634–40.
[18] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[19] Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 1964;239:2370–8.
[20] Waxman DJ. Rat hepatic cytochrome P-450 isozenzyme 2c: identification as a male-specific, developmentally induced steroid 16α-hydroxylase and comparison to a female-specific cytochrome P-450 isoenzyme. J Biol Chem 1984;259:15481–90.
[21] Gonzalez FJ, Sing B-J, Hardwick JP. Pregnenolone 16α-carbonitrile-inducible P-450 gene family: gene conversion and differential regulation. Mol Cell Biol 1986;6:2969–76.
[22] Hanioka N, Jinno H, Nishimura T, Ando M. Changes in hepatic cytochrome P450 enzymes by *cis*- and *trans*-1,2-chloroethylenes in rat. Xenobiotica 1998;28:41–51.
[23] Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers and inhibitors. Drug Metab Rev 1997;28:413–580.
[24] Cho AK, Narimatsu S, Kumagai Y. Metabolism of drugs of abuse by cytochrome P450. Addict Biol 1999;4:283–301.