DIFFERENCES IN CYTOCHROME P450 FORMS INVOLVED IN THE METABOLISM OF N,N-DIPROPYL-2-[4-METHOXY-3-(2-PHENYLETHOXY)PHENYL]ETHYLAMINE MONOHYDROCHLORIDE (NE-100), A NOVEL SIGMA LIGAND, IN HUMAN LIVER AND INTESTINE

TAKAHITO YAMAMOTO, NAOKO HAGIMA, MASATO NAKAMURA, YOSHIRO KOHNO, KIYOSHI NAGATA, AND YASUSHI YAMAZOE

Department of Drug Metabolism, Medicinal Research Laboratory, Taisho Pharmaceutical Co., Ltd., Saitama, Japan (T.Y., N.H., M.N., Y.K.); and Division of Drug Metabolism and Molecular Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University, Miyagi, Japan (K.N., Y.Y.)

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ABSTRACT:

N,N-Dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]ethylamine monohydrochloride (NE-100) has been developed to treat subjects with schizophrenia. This drug is mainly excreted in the form of oxidative metabolites. In the present study, identification of P450 forms involved in the metabolism was carried out using human livers and intestinal microsomes (HLM and HIM). Eadie-Hofstee plots for NE-100 disappearance in HLM were biphasic, thus indicating the involvement of at least two P450 forms. The metabolism of NE-100 was mediated with recombinant CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. A significant correlation was observed between activities of NE-100 metabolism and dextromethorphan O-demethylation (a specific activity for CYP2D6) or testosterone 6β-hydroxylation (a specific activity for CYP3A4) in HLM. The activity of NE-100 metabolism was inhibited by approximately 80% by an anti-CYP2D6 antibody and only by quinine among the P450-selective inhibitors at a low substrate concentration (0.1 μM). In contrast, with a high substrate concentration (10 μM), the activity was inhibited by an anti-CYP3A4 antibody and by ketoconazole. On the other hand, in HIM, the Eadie-Hofstee plots for NE-100 disappearance were monophasic, and the metabolism was strongly inhibited by an anti-CYP3A4 antibody and by ketoconazole but not by other inhibitors used. These results strongly suggest that NE-100 has different profiles regarding metabolism between liver and intestine. During absorption, NE-100 is mainly metabolized by CYP3A4 in the intestine and thereafter by CYP2D6 in the liver in the presence of therapeutic doses.
practice. Particularly, interactions through drug metabolism might influence pharmacokinetic properties, therapeutic efficacy, and the frequency of side effects. To predict drug-drug interactions, therefore, identification of drug-metabolizing enzymes involved in the metabolism of new compounds being developed is most important. In this study, we have identified P450 forms involved in the primary metabolism of NE-100, using human livers, intestine, and recombinant P450 forms.

Materials and Methods

Chemicals and Reagents. NE-100 hydrochloride was synthesized in Taisho Pharmaceutical Co. Ltd. (Saitama, Japan). \[^{14}C\]\(NE-100\) labeled at the phenoxoy ring (97.5%, radiochemical purity, 8.33 MBq/mg), was synthesized at Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). The chemical structure and the labeled portion are shown in Fig. 1. Arachidonic acid, phenacetin, coumarin, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and NADP\(^+\) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Furafylline, sulfaphenazole, 5-mephenytoin, quinidine and ketononazole, and 6-hydroxychlorzoxazone were purchased from Ultrafine Chemicals Co. (Manchester, UK). Acetaminophen, 7-hydroxycoumarin, 7-ethoxy-4-trifluoro-methylcoumarin, dextromethorphan, dextrorphan, chlorzoxazone, testosterone, lauric acid, 12-hydroxydodecanoic acid, troleandomycin, 7-ethoxy-4-trifluoro-methylcoumarin, dextromethorphan, and NADP\(^+\) were purchased from Sigma-Aldrich (St. Louis, MO). 5-Hydroxymephenrazole and SKF-525A were from Fujisawa Astra Co. (Osaka, Japan). Hydroxytestosterone was purchased from Sumika Chemical Analysis Service (Osaka, Japan). Furafylline, sulfaphenazole, and ketononazole were purchased from Taisho Pharmaceutical Co. Ltd. (Saitama, Japan). \[^{14}C\]\(NE-100\), labeled at the phenoxy ring (97.5%, radiochemical purity, 8.53 MBq/mg), was synthesized at Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). The chemical structure and the labeled portion are shown in Fig. 1. Arachidonic acid, phenacetin, coumarin, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and NADP\(^+\) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Furafylline, sulfaphenazole, 5-mephenytoin, quinidine and ketononazole, and 6-hydroxychlorzoxazone were purchased from Ultrafine Chemicals Co. (Manchester, UK). Acetaminophen, 7-hydroxycoumarin, 7-ethoxy-4-trifluoro-methylcoumarin, dextromethorphan, dextrorphan, chlorzoxazone, testosterone, lauric acid, 12-hydroxydodecanoic acid, troleandomycin, 7-ethoxy-4-trifluoro-methylcoumarin, dextromethorphan, and NADP\(^+\) were purchased from Sigma-Aldrich (St. Louis, MO). 5-Hydroxymephenrazole and SKF-525A were from Fujisawa Astra Co. (Osaka, Japan).

Kinetic Study of NE-100 Metabolism by Human Liver and Intestinal Microsomes. Incubation medium contained 0.20 mg/ml HLM or 0.25 mg/ml HIM and 50 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (0.4 mM NADP\(^+\), 8 mM glucose 6-phosphate, 8 mM MgCl\(_2\), 0.5 IU/ml glucose 6-phosphate hydrogenase) and NE-100, in a final volume of 500 \(\mu l\) (for HLM) or 250 \(\mu l\) (for HIM). For HLM (Batch 2), after preincubation for 5 min at 37°C, the reaction was initiated by adding NE-100 at concentrations ranging from 0.05 to 5 \(\mu M\) NE-100 for 0.25 to 60 min then was stopped by adding 1 ml of methanol. For HIM, NE-100 metabolism was assessed using concentrations ranging from 0.2 to 200 \(\mu M\) NE-100 for 2 to 30 min, in a final volume of 250 \(\mu l\). Other reaction conditions were as described above. All reactions were carried out in a linear range with a protein concentration and disappearance velocity of NE-100 at the substrate concentration range was calculated from a linear range of the reaction in incubation time.

Correlation Study. The disappearance rate of NE-100 in HLM was compared with phenacetin \(O\)-deethylation (CYP1A1/2; Tassaneeyakul et al., 1993), coumarin 7-hydroxylation (CYP2A6; Koenigs et al., 1997), 7-ethoxy 4-trifluomethyl coumarin \(O\)-deethylation (CYP2B6; Ekins et al., 1997), omeprazole 5-hydroxylation (CYP2C19; Andersson et al., 1993), dextromethorphan 6-hydroxylation (CYP2D6; Ducharme et al., 1996), chlorzoxazone 6-hydroxylation (CYP2E1; Lucas et al., 1996), testosterone 6β-hydroxylation

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<th>Enzyme</th>
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<tr>
<td>(K_{m1})</td>
<td>(K_{m2})</td>
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<td>(\mu M)</td>
<td>nmol/min/mg protein</td>
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<tr>
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<tr>
<td>Intestine</td>
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For NE-100 metabolism in the liver, \(K_{m1}\) and \(V_{max1}\) represent apparent affinity constant and apparent maximum metabolic activity for high-affinity components, and \(K_{m2}\) and \(V_{max2}\) represent apparent affinity constant and apparent maximum metabolic activity for low-affinity components. \(CL_{int}\) in liver was calculated as the sum of the ratio of \(V_{max}\) to \(K_{m}\) in high- and low affinity components based on two enzyme model. For NE-100 metabolism in the intestine, \(K_{m}\) and \(V_{max}\) represent apparent affinity component and apparent maximal metabolic activity. \(CL_{int}\) in intestine was calculated as the ratio of \(V_{max}\) to \(K_{m}\).

**TABLE 1**

**Kinetic parameters for NE-100 metabolic activity in human liver and intestinal microsomes.**

**Fig. 1.** Chemical structure of \[^{14}C\]NE-100.

**Fig. 2.** Eadie-Hofstee plots for the disappearance of NE-100 in human liver (A) and intestinal microsomes (B). Ranges of substrate concentration used are 0.05 to 5 \(\mu M\) for human liver and 0.2 to 200 \(\mu M\) for human intestine. \(V\); NE-100 disappearance velocity; \(S\); NE-100 concentration.
(CYP3A4; Sanwald et al., 1995), and lauric acid H9275-hydroxylation activities (CYP4A11; Yamada et al., 1991), using microsomes obtained from 28 human livers. NE-100 metabolism was assessed in incubation mixtures containing 0.1 mg/ml microsomes, the NADPH-generating system and 1 M NE-100 in 50 mM potassium phosphate buffer (pH 7.4) for 5 min.

Immunoinhibition Study. The immunoinhibition of NE-100 metabolism was assessed using HLM (50 pmol/P450) preincubated with rabbit anti-CYP2C11, anti-CYP2D6, or anti-CYP3A2 sera at room temperature for 30 min prior to the addition of NE-100 and the NADPH-generating system. Rabbit anti-CYP2C11, anti-CYP2D6, and anti-CYP3A2 sera used for immunoinhibition studies strongly inhibited omeprazole 5-hydroxylation (CYP2C19), dextromethorphan O-demethylation (CYP2D6), and testosterone 6β-hydroxylation (CYP3A4) activities, respectively (not shown data). In B and C, initial substrate concentrations used were 0.1, 1.0, and 10 μM. Human liver microsomes were incubated with various amounts of antibodies for CYP2D6 and CYP3A4 on ice for 15 min prior to the addition of NE-100 and the NADPH-generating system. The antiserum raised against CYP2C13 cross-reacts with CYP2C8, 2C9, and 2C19 (the data sheet provided by the manufacturers).

The inhibitory effects of monoclonal anti-CYP2D6 and anti-CYP3A4 antibodies on NE-100 metabolism were also assessed using HLM. After preincubation with HLM (0.2 mg/ml) and an antibody on ice for 15 min, the reaction was carried out by adding NE-100 (0.1, 1, and 10 μM) at 37°C for 5, 10, and 15 min, respectively. For HIM, a monoclonal anti-CYP3A4 antibody or anti-CYP2C13 serum were preincubated with HIM (0.25 mg/ml) on ice for 15 min or at room temperature for 30 min, respectively. The reaction was carried out by adding NE-100 (10 and 200 μM) at 37°C for 20 and 30 min, respectively. All reaction was stopped using 1 ml of methanol.

In A, initial concentration of substrate was 1.0 μM. Human liver microsomes were incubated with various amounts of anti-CYP2C11, 2D6, or 3A2 sera at room temperature for 30 min prior to the addition of NE-100 and the NADPH-generating system. Rabbit anti-CYP2C11, anti-CYP2D6, and anti-CYP3A2 sera used for immunoinhibition studies strongly inhibited omeprazole 5-hydroxylation (CYP2C19), dextromethorphan O-demethylation (CYP2D6), and testosterone 6β-hydroxylation (CYP3A4) activities, respectively (not shown data). In B and C, initial substrate concentrations used were 0.1, 1.0, and 10 μM. Human liver microsomes were incubated with various amounts of antibodies for CYP2D6 and CYP3A4 on ice for 15 min prior to the addition of NE-100 and the NADPH-generating system. The antiserum raised against CYP2C13 cross-reacts with CYP2C8, 2C9, and 2C19 (the data sheet provided by the manufacturers).

**Fig. 3.** Effect of anti-P450 serum (A), antibody for CYP2D6 (B) and CYP3A4 (C) on NE-100 metabolism in human liver microsomes.
Chemical Inhibition Study. Studies of selective inhibitors for the metabolism of NE-100 in the HLM and in the HIM were undertaken with 0.1, 10 \mu M (for HLM) and 10, 200 \mu M (for HIM) NE-100. The following selective inhibitors were used: furafylline (CYP1A2), sulfaphenazole (CYP2C9), S-mephenytoin (CYP2C19), lanosoprazole (CYP2C19), quinidine (CYP2D6), ketoconazole (CYP3A4), arachidonic acid (CYP2J2), and SKF-525A (a non-selective-inhibitor). The concentration of inhibitors used in this experiment was based on that described by other investigators (Beischlag et al., 1992; Maurice et al., 1992; Kunze and Trager, 1993; Chang et al., 1994; Rodrigues et al., 1994; Baldwin et al., 1995). Each inhibitor was dissolved in either methanol or acetonitrile. The inhibition in HLM (0.200 mg/ml) was done with the coaddition of NE-100 and inhibitors at 37°C for 3 or 15 min for 0.1 or 10 \mu M NE-100, respectively. The mechanism-based inhibitors, furafylline and troleandomycin, were preincubated with microsomes and cofactor for 30 min prior to the addition of NE-100. The inhibition in HIM (0.250 mg/ml) was assessed by the coaddition of NE-100 and inhibitors at 37°C for 20 or 30 min for 10 or 200 \mu M NE-100, respectively. The reaction was stopped as described above. Final methanol and acetonitrile concentrations in the incubation medium were less than 0.5%. Control experiments were done under the same conditions without an inhibitor, as described above.

NE-100 Metabolism by Human Recombinant P450 Forms. Incubations of NE-100 with various recombinant P450 microsomes were carried out at 37°C for 30 min. The incubation mixture (final volume of 0.5 ml) consisted of 50 mM potassium phosphate buffer (pH 7.4), NADPH-generating system, 0.25 mg/ml microsomal protein, and 0.1 or 20.0 \mu M NE-100. Other methods were the same as described for human liver microsomes.

Assay of the Unchanged Drug. The reaction mixtures containing added methanol were shaken and centrifuged at 3,000 rpm for 10 min, then the supernatant was evaporated to dryness and re-dissolved with methanol. To detect NE-100 in the sample, thin-layer chromatography (TLC) was carried out using the TLC plates and the solvent system described as follows: NH2 HPTLC plates (Merck Co. Ltd, Whitehouse Station, NJ) and hexane/chloroform/methanol = 7:2:1. Radioluminography, BAS2000 system (Fuji-film, Tokyo, Japan), was used for quantification of NE-100.

Data Analysis. Linearity of the disappearance of NE-100 with regard to the concentration of microsomes and the incubation time was assessed by least-squares linear regression, using Microsoft Excel (Microsoft, Redmond, WA). Enzyme kinetic data, apparent \( K_m \) and \( V_{max} \) values (\( K_{m1} \) and \( V_{max1} \) for high affinity component, \( K_{m2} \) and \( V_{max2} \) for low affinity component) for NE-100 disappearance, were estimated using Eadie-Hofstee plots in the substrate concentration ranging from 0.05 to 5.0 \mu M for HLM and from 0.2 to 200 \mu M for HIM, based on visual inspection. Intrinsic clearance (CL\(_{int}\)) was calculated as the ratio of \( V_{max} \) value to \( K_m \) value. Correlation between the disappearance velocity of NE-100 and the metabolite formation rates of the respective P450 forms-specific substrates was examined by the least-squares linear regression analysis. The statistical significance of differences between control and inhibitor treatments was determined using Dunnett’s test (SAS system for windows, version 6.1; SAS Institute Inc., Cary, NC). A p-value <0.05 was considered to be statistically significant.

Results

Kinetic Study of NE-100 Metabolism by Human Liver and Intestinal Microsomes. Eadie-Hofstee plots for NE-100 disappearance in HLM and HIM are shown in Fig. 2. The plots in HLM showed biphasic curves (high- and low-affinity component), suggesting that NE-100 metabolism in HLM is catalyzed by multiple P450 forms. On the other hand, the plots in HIM showed a monophasic curve. As shown in Table 1, the \( K_m \) value in HLM was very small compared with the \( K_m \) value in HIM and CL\(_{int}\) in HIM was approximately 25 times greater than that in HIM. This result means that the rate of metabolism of NE-100 in HIM is faster than that in HIM.

Correlation Study. We determined the correlation between activities of NE-100 metabolism and a specific substrate toward the re-
spective P450 forms in 28 different human liver microsomes. Among the specific substrate activities examined, NE-100 metabolic activities significantly correlated with the activities of dextromethorphan O-demethylation and testosterone 6β-hydroxylation ($r^2 = 0.868$ and 0.763, respectively) at 1 μM NE-100 concentration.

**Immunoinhibition Study on Human Liver Microsomes.** Inhibitory effects on NE-100 metabolism by anti-sera raised against CYP2C11, CYP2D6, and CYP3A2 are shown in Fig. 3A. Anti-CYP2C11 and anti-CYP2D6 sera inhibited the metabolism of NE-100 in a dose-dependent manner in HLM. At the highest amount of antiserum, anti-2D6 and anti-CYP2C11 sera inhibited the activity of NE-100 metabolism by 87.2 and 18%, respectively. On the other hand, anti-CYP3A2 serum had no significant effect on NE-100 metabolism.

Figure 3, B and C, shows inhibitory effects of anti-CYP2D6 and anti-CYP3A4 antibodies on NE-100 metabolism of various concentrations of the substrate (0.1, 1 and 10 μM) in HLM. Depending on the increase in substrate concentration, the inhibitory effect on the CYP2D6 activity decreased, but the effect on the CYP3A4 activity increased. This finding suggests that P450 forms such as CYP3A4 other than CYP2D6 are involved in NE-100 metabolism under conditions of the high substrate concentrations.

**Chemical Inhibition Study with Human Liver Microsomes.** Effects of selective P450 inhibitors on NE-100 metabolism determined using HLM (H161, lot.1; BD Gentest Co.) were examined at two substrate concentrations (0.1 and 10 μM). As shown in Fig. 4, at 0.1 μM substrate concentration, SKF-525A (a nonselective P450 inhibitor) and quinidine (a typical inhibitor for CYP2D6) inhibited activities of NE-100 metabolism by 100 and 88.7% at 100 and 1 μM, respectively. Ketoconazole (for CYP3A4, 0.5 μM) and sulfaphenazole (for CYP2C9, 10 μM) slightly inhibited the activity, and the rates were 18.3 and 7.5%, respectively. On the contrary, furafylline (for CYP1A2) and S-mephenytoin (for CYP2C19) showed no inhibitory effects on the metabolism of NE-100, at a low substrate concentration. At 10 μM substrate concentration, SKF-525A strongly inhibited the activity of NE-100 metabolism, but the effect of quinidine was slight. On the other hand, ketoconazole strongly inhibited the activity of NE-100 metabolism, and the rate of inhibition was approximately 75%. Other inhibitors, sulfaphenazole and S-mephenytoin also slightly inhibited the activity, but furafylline did not do so.

**NE-100 Metabolism with Human Recombinant P450 Forms.** As shown in Fig. 5, NE-100 metabolism in human recombinant P450 forms (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9-Arg, 2C9-Cys, 2C19, 2D6-Met, 2D6-Val, 2E1, 3A4, and 4A11) were determined using two substrate concentrations (0.1 and 10 μM). Among all recombinant P450 forms examined, CYP2D6-Met and CYP2D6-Val showed the highest activity of NE-100 metabolism (0.347 and 0.329 nmol/30 min/mg of protein, respectively), followed by CYP1A1, 2C19, 3A4, 2C9-Arg, and 1A2 (0.120, 0.071, 0.049, 0.036, and 0.036 nmol/30 min/mg of protein, respectively) at a low NE-100 concentration (0.1 μM). Other P450 forms showed little or negligible activity of NE-100 metabolism under the same conditions. On the other hand, at a high concentration of NE-100 (20 μM), the activity of NE-100 metabolism was mostly the same among CYP2D6, 2C19, 2C9-Arg, and 1A1 (8.24, 8.44, 6.48, and 6.54 nmol/30 min/mg of protein, respectively). CYP3A4 and CYP1A2 also exhibited the activity of NE-100 metabolism at a high concentration of NE-100. Other P450 forms did not mediate the activity.

**Inhibition Studies with Human Intestinal Microsomes.** As shown in Fig. 6, an immunoinhibition study using anti-CYP3A4 antibody and anti-CYP2C13 serum was done, because it has been reported that CYP3A4 is the most abundant P450 form expressed in HIM followed by CYP2C (Zhang et al., 1999). At the highest amount of the antibody and serum, anti-CYP3A4 antibody inhibited the activity of NE-100 metabolism at substrate concentrations of 10 and 200
μM by approximately 70 and 45%, respectively. Anti-CYP2C13 sera only inhibited it at both substrate concentrations by 17 and 20%, respectively. Effects of selective P450 inhibitors on NE-100 metabolism in HIM were also evaluated at substrate concentrations of 10 and 200 μM. For 10 μM NE-100, ketoconazole strongly inhibited the activity of NE-100 metabolism by 85 and 97%, at 0.5 and 5 μM, respectively. In addition, inhibitory effects of lansoprazole (for CYP2C19) and arachidonic acid (for CYP2J2) were slight, but other inhibitors did not affect NE-100 metabolism. For the 200 μM NE-100, only ketoconazole inhibited the metabolic activity, and the rates were approximately 70 and 90% at 0.5 and 5 μM, respectively.

**Discussion**

We considered that a large first-pass effect of NE-100 after oral administration to rats might contribute to low bioavailability. The cause seems to be based on an extensive metabolism rather than an incomplete gastrointestinal absorption. Although the liver has been considered to be the major site of first-pass metabolism, recent studies indicated that the small intestine contributes significantly to the overall first-pass metabolism of many drugs (Lampen et al., 1995; Wu et al., 1995; Paine et al., 1996). Therefore, it seemed important to identify the enzyme responsible for the metabolism of NE-100 not only in the human liver but also in the intestine. We identified the principal P450 forms catalyzing the NE-100 metabolism by measuring the disappearance of NE-100 instead of the velocity of formation of each metabolite, because separation of all metabolites using the TLC method was not feasible.

In a previous study, we found that NE-100 metabolism was a NADPH requirement and was strongly inhibited by SKF-525A (unpublished data). Eadie-Hofstee plots for the NE-100 disappearance in HLM showed biphasic curves (consist of high- and low-affinity components), which suggests that NE-100 metabolism is catalyzed by P450 forms of more than two enzymes (Fig. 2A). In the correlation study using HLM, the activity of NE-100 metabolism showed a good correlation with activities of dextromethorphan O-demethylation and testosterone 6β-hydroxylation at a 1.0 μM substrate concentration. Furthermore, the inhibitory effect of the anti-CYP2D6 and anti-CYP3A4 antibodies on NE-100 metabolism in HLM differed among the substrate concentrations used (0.1, 1 and 10 μM). Therefore, the chemical inhibition for NE-100 metabolism were studied at two concentrations of substrate that are close to the K_{m1} and K_{m2} values observed in HLM. The activity of NE-100 metabolism at the low concentration of substrate was strongly suppressed by quinidine, but the activity at the high concentration of substrate was inhibited by multiple inhibitors. Among them, ketoconazole was the strongest inhibitor. These results suggest that high- and low-affinity enzymes involved in NE-100 metabolism in liver are mainly CYP2D6 and CYP3A4, respectively. Especially, the K_{m1} value (0.059 μM) of the high-affinity component was very low compared with that of drugs predominantly metabolized by CYP2D6 such as bufuralol (3.4 μM; Mankowski, 1999), propranolol (3 μM; Obach, 1997), imipramine (2.15 μM; Obach, 1997), nortriptyline (2.08 μM; Venkatakrishnan et al., 1999), and perphenazine (1–2 μM; Olesen and Linnet, 2000). In case of NE-100 metabolism with recombinant P450 forms, CYP2D6-Met and CYP2D6-Val showed the highest activity at a low concentration (0.1 μM). On the other hand, multiple P450 forms such as CYP1A1, 1A2, 2C9, 2C19, CYP2D6, and 3A4 also catalyzed NE-100 metabolism at a high concentration (20 μM). These findings strongly support evidence that NE-100 may be predominantly metabolized by CYP2D6 at a low concentration of substrate and be metabolized by CYP1A2, 2C9, 2C19, and 3A4 in addition to CYP2D6 at a high concentration of substrate. However, among these P450 forms, CYP1A1 is not likely to be involved in the metabolism of NE-100 because of the very low amount expressed in the human liver (Wrigh-

![Graph showing the effect of antibody of CYP3A4 and anti-CYP2C13 serum on metabolism of NE-100 in human intestinal microsomes.](image-url)
In pharmacokinetic studies on rats, when [14C]NE-100 of the therapeutic dose (0.5 mg/kg, 255 μM) was orally administered, radioactivity concentrations in plasma liver, and intestinal tissues at t_max (maximal concentration of NE-100 in plasma) were 0.15 μM, 3.73 μM (0.06 μM as concentration of NE-100) and 7.21 μM, respectively. After oral administration of NE-100, when it is assumed that the distribution of NE-100 to the intestinal tissue of human is similar to that in rats and that the concentration in intestinal tissue is almost owing to an unchanged drug, the concentrations of NE-100 in the liver and the intestinal tissue might be estimated to be 0.18 and 22 μM, respectively. These data were predicted from data on rats. The estimated micromolar concentrations are close to the K_m value of CYP2D6 in HLM and the K_m value of CYP3A4 in HIM.

In conclusion, at a low substrate concentration (therapeutic dose), the metabolism of NE-100 is mainly catalyzed by CYP2D6 in the liver, and the K_m value is lower than that of drugs metabolized by CYP2D6 reported previously. At high substrate concentration, however, several P450 forms mediate the NE-100 metabolism in human livers. The metabolism of NE-100 is also observed in the human small intestine. In this case, the metabolism is apparently catalyzed only by single enzyme, CYP3A4. Together with these data, this large first-pass effect on NE-100 after oral administration occurs not only in human livers but also in the small intestine with different P450 forms. Therefore, NE-100 might interact with drugs metabolized by CYP2D6 in the liver, by CYP3A4 in the intestine, or inhibitors of those P450s, in schizophrenia patients taking multiple drugs such as fluboxamine, flutamide, erythromycin, and itraconazole, under in vivo conditions.


