PHOSPHONATE O-DEETHYLATION OF [4-(4-BROMO-2-CYANO-PHENYL CARBAMOYL) BENZYL]-PHOSPHONIC ACID DIETHYL ESTER, A LIPOPROTEIN LIPESE-PROMOTING AGENT, CATALYZED BY CYTOCHROME P450 2C8 AND 3A4 IN HUMAN LIVER MICROSONES

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ABSTRACT:

NO-1886 ([4-(4-bromo-2-cyano-phenylcarbamoyl) benzyl]-phosphonic acid diethyl ester) increases lipoprotein lipase activity, resulting in a reduction in plasma triglycerides and an increase in high-density lipoprotein cholesterol. The metabolism of NO-1886 in human liver was investigated in the present study. Ester cleavage of NO-1886 from diethyl phosphonate to monoethyl phosphonate was the major metabolic pathway catalyzed by cytochrome P450. In addition, the minor metabolic pathway in human liver was the hydrolysis of the amide bond of NO-1886 by a specific cytosolic esterase. Eadie-Hofstee plots of phosphonate O-deethylation of NO-1886 in human liver microsomes showed a biphasic curve, indicating low- and high-\( K_m \) components. Inhibition experiments with chemical inhibitors and antibodies against various cytochrome P450 isoforms suggested the involvement of CYP2C8 and CYP3A in the phosphonate O-deethylation. Recombinant CYP3A4 and CYP2C8 expressed in baculovirus-infected insect cells and human lymphoblastoid cells exhibited a high activity for phosphonate O-deethylation of NO-1886. The recombinant cytochrome P450 enzymes indicated that CYP2C8 and CYP3A4 were responsible for the low- and high-\( K_m \) components in human liver microsomes, respectively. The selectivity of CYP2C8 in catalyzing phosphonate O-deethylation indicates that coadministration of drugs that are metabolized by the same enzyme requires careful consideration.

It has been reported that the novel compound NO-1886\(^1\) ([4-(4-bromo-2-cyano-phenylcarbamoyl) benzyl]-phosphonic acid diethyl ester; Fig. 1) increases lipoprotein lipase (LPL) activity, resulting in a reduction in plasma triglycerides and a concomitant increase in high-density lipoprotein cholesterol in experimental animals, including rats, hamsters, and rabbits (Tsutsumi et al., 1993, 1995, 1997). It was also demonstrated that long-term administration of NO-1886 significantly prevented the development of atherosclerosis in cholesterol-fed rats (Tsutsumi et al., 1993) and rabbits (Chiba et al., 1997). As shown in Fig. 1, the metabolic pathways of NO-1886 in rats have been identified as 1) O-deethylation of the phosphoric acid ester, 2) hydrolysis of the amide bond, 3) hydroxylation of the amino compound (M-1) produced by hydrolysis of the amide bond, and 4) sulfation following hydroxylation of M-1 (Morioka et al., 1996). NO-1886 was almost completely excreted in the urine (28%) and feces (64%) as metabolites within 24 h of postdosing in rats that were maintained in metabolic cages. The major metabolite was monoethyl phosphate (M-2), which accounted for 70% of all metabolites in rats (Morioka et al., 1996). None of these metabolites, including the major metabolite (the monooester form), increases the activity of LPL (Morioka et al., 1996). Therefore, the metabolic activity in humans is an important factor in determining the duration of administration in clinical use. In the present study, the metabolic disposition of NO-1886 in human...
liver S9 and microsomes was investigated and the major enzyme involved in metabolism was identified.

**Materials and Methods**

**Chemicals and Biochemicals.** NO-1886 and its metabolite (M-2) were synthesized at Otsuka Pharmaceutical Factory (Tokushima, Japan). Triacetyloleandomycin, 13-cis-retinoic acid, quinidine, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical (St. Louis, MO). β-NADPH was obtained from Oriental Yeast (Tokyo, Japan). 4-Methylnitrosourea and coumarin were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Furafylline, ketoconazole, rabbit anti-human CYP1A1/1A2, CYP2D6, and CYP3A4 sera, and goat anti-human CYP2C serum were purchased from Daiichi Pure Chemicals (Tokyo, Japan). Other chemicals were of the highest grade commercially available. A mixed pool of human liver S9 and microsomes from 15 donors was obtained from In Vitro Technologies, Inc. (lot 1001; Baltimore, MD). Recombinant P450 enzymes expressed in microsomes of baculovirus-infected insect cells and human lymphoblastoid cells were obtained from GENTEST (Woburn, MA).

**Incubation Conditions.** All incubations related to the liver microsomes were carried out at a protein concentration of 0.5 mg/ml in 100 mM potassium phosphate buffer, pH 7.4, including 0.1 mM EDTA and an NADPH-generating system (1.25 mM NADP⁺, 2.5 mM glucose 6-phosphate, 6 mM MgCl₂, and 0.75-unit/ml glucose 6-phosphate dehydrogenase) at 37°C. In enzyme kinetic experiments, 0.2 to 110 μM NO-1886 was incubated at a final volume of 0.5 ml for 60 min. The reaction was initiated by the addition of the sample solution after 5-min preincubation at 37°C. After incubation, the reaction was terminated with the addition of 1 ml of ice-cold acetonitrile containing 1-μg/ml p-hydroxybenzoic acid butyl ester as an internal standard. After the reaction mixture was centrifuged at 3000 rpm for 10 min at 4°C, the supernatant was evaporated to dryness, and the residue was dissolved in 25 μl of a solution consisting of acetonitrile and sodium phosphate buffer (10 mM, pH 6.4) in a ratio of 1:4 (v/v). A 100-μl aliquot of the sample was subjected to high-performance liquid chromatography (HPLC). Incubation with the liver S9 was carried out for 120 min at a protein concentration of 12 mg/ml under the same conditions as those for liver microsomes. The linearity of reaction with protein concentration and incubation time was confirmed under these experimental conditions.

**Chemical Inhibition.** Inhibition experiments were carried out with 4.4 and 110 μM NO-1886 in a final volume of 0.5 ml for 60 min. The stock solutions of the inhibitors were added immediately before the addition of NO-1886. For the mechanism-based inhibitors furafylline and triacetyloleandomycin, a mixture of the inhibitor, microsomes, and the NADPH-generating system was preincubated for 10 min at 37°C before the addition of NO-1886. M-2 formation in the presence of inhibitor was compared against appropriate controls and the results were calculated as a percentage of the uninhibited rate.

**Immunoinhibition by Anti-P450 Antiserum.** Various concentrations of anti-P450 antiserum and microsomes (0.5 mg/ml) were incubated for 30 min at room temperature. The NADPH-generating system was then added, and the mixture was maintained at 37°C for 5 min. The reaction (60 min) was initiated by the addition of NO-1886 (4.4 μM final concentration). The results were calculated as a percentage of the duplicate control measurements.

**Metabolism of NO-1886 by Recombinant P450 Enzymes.** This experiment involved the use of microsomes from two kinds of cells (baculovirus-infected insect cells and human lymphoblastoid cells), which expressed CYP1A2, 2A6, 2B6, 2C8, 2C9-Arg144, 2C9-Cys144, 2C18, 2C19, 2D6, 2E1, 3A4, and 3A5. Control microsomes were obtained from cells of the same type that had not been transfected. All P450s were coexpressed (from cDNA) with NADPH-P450 reductase. In addition, CYP3A4 and CYP2E1 were also coexpressed with cytochrome b₅. The final concentrations of microsomes and NO-1886 were 50 to 150 pmol of P450/ml and 0.2 to 440 μM, respectively. After 5-min preincubination of microsomes containing the NADPH-generating system, a 60-min reaction at 37°C was initiated by adding NO-1886.

**HPLC Analysis.** NO-1886 and M-2 levels were determined using a Shimadzu (Tokyo, Japan) HPLC system comprising an LC-6A pump, SPD-6A UV detector and CR-4A Chromato-integrator, SIL-6A autosampler, CTO-6A column oven, and SCL-6AV system controller. An aliquot of the sample was injected onto a TSKgel ODS-120A column (5 μm, 4.6 × 250-mm i.d.; Tosoh, Tokyo, Japan) and eluted at a flow rate of 1.2 ml/min with 50 mM phosphate buffer, pH 2.2/acetonitrile according to the following gradient schedule: 20% acetonitrile for the first 20 min; a linear gradient from 20 to 24% over the next 20 min; a linear gradient from 24 to 35% over 10 min; 35% for 20 min; and a linear gradient from 35 to 70% over 10 min, which was then maintained at 70% for 10 min. The temperature of the column was maintained at 45°C. UV detection was performed at 260 nm and the detection limit for M-2 was 5 nM in incubation samples.

**Data Analysis.** Experimental reaction velocity measurements were combined to obtain mean ± S.D. values. The parameters Kₘ and Vₘₐₓ were calculated by fitting the Michaelis-Menten equation to the data by nonlinear regression analysis (MULTI; Yamaoka et al., 1981) with weighted data (1/y).

**Results**

**Metabolism of NO-1886 in Human Liver.** The metabolism of NO-1886 was investigated in pooled human liver S9 fractions and microsomes. Figure 2 shows the HPLC chromatogram of the human liver S9 fraction after reaction with NO-1886 for 60 min. The formation rates of metabolites in the S9 fraction are listed in Table 1. It is clear that the cleavage of diethyl phosphonate is the major metabolic pathway of NO-1886 in human liver as well as in the rat and that the amide bond is scarcely hydrolyzed in human liver. The M-2 formation rate per protein content was 15 times greater in microsomes than in the S9 fraction. The lower activity of S9 than microsomes might be explained by the microsomal protein content in the S9 fraction (Duve et al., 1955). M-2 formation was detected at 9.77 pmol/min/mg of protein in human liver microsomes without the NADPH-generating system (Table 1), suggesting that A-esterase and other esterases were involved in the formation of M-2 from NO-1886. In contrast, hydrolysis of the amide bond of NO-1886 occurred in human liver S9 with and without the NADPH-generating system but not in microsomes. These data suggest that the amide bond of NO-1886 is hydrolyzed by cytosolic esterases.

**Phosphonate O-deethylation of NO-1886 to M-2 in Human Liver Microsomes.** Figure 3 shows an Edgie-Hofstet plot of the formation of M-2 from NO-1886 in pooled human liver microsomes. The biphasic nature of the plot indicates that multiple enzymes contribute to the phosphate O-deethylation. The calculated Kₘ and Vₘₐₓ.
TABLE 1
Metabolism of NO-1886 in human liver S9 and microsomes in the presence and absence of NADPH

<table>
<thead>
<tr>
<th></th>
<th>Metabolism Rate</th>
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<tbody>
<tr>
<td></td>
<td>M-2</td>
<td>M-3</td>
</tr>
<tr>
<td><strong>Microsomes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADPH(+)</td>
<td>118.31</td>
<td></td>
</tr>
<tr>
<td>NADPH(−)</td>
<td>9.77</td>
<td></td>
</tr>
<tr>
<td>S9</td>
<td>7.91</td>
<td>0.45</td>
</tr>
<tr>
<td>NADPH(+)</td>
<td>0.02</td>
<td>0.59</td>
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</tbody>
</table>

Fig. 3. Eadie-Hofstee plot for NADPH-dependent M-2 formation from NO-1886 by pooled human liver microsomes.

Human liver microsomes (0.5 mg/ml) were incubated with NO-1886 (0.4–110 μM) for 60 min, and the rates of formation of M-2 were determined by HPLC. Data are expressed as the mean of three determinations.

values for the low-\( K_m \) component (\( K_{m1} \) and \( V_{max1} \)) were 3.56 ± 0.01 μM and 14.2 ± 1.2 pmol/min/mg, respectively. For the high-\( K_m \) component, the \( K_{m2} \) was 172.5 ± 37.1 μM and the \( V_{max2} \) was 334.2 ± 56.3 pmol/min/mg.

Inhibition Analysis. To identify the specific P450 isozyme(s) involved in the biotransformation of NO-1886 to M-2, incubation was performed using anti-human P450 antibodies. As shown in Fig. 4, anti-human CYP3A4 antibody and anti-human CYP2C antibody inhibited 40 to 50% of M-2 formation from 4.4 μM NO-1886 with the addition of 160 μl of antisera. In contrast, anti-human CYP1A1/1A2, CYP2A6, CYP2D6, and CYP2E1 antibodies showed no effect on the hydrolysis of NO-1886.

To further confirm the specific P450 isozyme(s) involved in NO-1886 metabolism, a chemical inhibition study was conducted using specific chemical inhibitors of various P450 isozymes (Fig. 5). Triacetyloleandomycin (an inhibitor of CYP3A4/5; Chang et al., 1994) and 13-cis-retinoic acid (an inhibitor of CYP2C8; Rahman et al., 1994; Baldwin et al., 1999) significantly inhibited the phosphonate O-deethylation of 4.4 μM NO-1886 to approximately 50% of the control values. Ketoconazole, a selective CYP3A4/5 inhibitor at low concentrations and a relatively nonselective P450 inhibitor at high concentrations (Masimirembwa et al., 1999), inhibited 90% of the human microsomal M-2 formation. Coumarin (an inhibitor of CYP2A6; Yamano et al., 1990), sulfaphenazole (an inhibitor of CYP2C9; Mancy et al., 1996), quinidine (an inhibitor of CYP2D6; Otton et al., 1988), and orphenadrine (an inhibitor of CYP2B6; Reidy et al., 1989) showed about 30% inhibition of phosphonate O-deethylation of NO-1886. Furafylline (an inhibitor of CYP1A2; Sesardic et al., 1990), S-(+)-mephenytoin (an inhibitor of CYP2C19; Wright et al., 1995), and 4-methylpyrazole (an inhibitor of CYP2E1; Newton et al., 1995) had no effect on the metabolism of NO-1886.

Furthermore, the dose-dependent inhibition of the phosphonate O-deethylation of NO-1886 by ketoconazole, triacetyloleandomycin, 13-cis-retinoic acid, and sulfaphenazole was investigated. As shown in Fig. 6, 13-cis-retinoic acid, a selective inhibitor of CYP2C8, inhibited the phosphonate-O-deethylation of NO-1886 more strongly than sulfaphenazole, which is very potent inhibitor of CYP2C9 (\( K_i = 0.3 \) μM) and a modest inhibitor of CYP2C8 and CYP2C18 (\( K_i = 63 \) and 29 μM, respectively) (Mancy et al., 1996). Sulfaphenazole inhibited the reaction of NO-1886 in a dose-dependent manner and showed...
Although the $K_m$ value for recombinant CYP3A4 was the same in the presence and absence of $b_5$, the $V_{max}$ was significantly increased with the coexpression of $b_5$. There are two possible explanations for the difference in activity between recombinant CYP3A4 with and without the coexpression of $b_5$. One explanation is the high expression of reductase in rCYP3A4 with $b_5$ (reductase/P450 ratio = 0.38 for rCYP3A4 without $b_5$ and 3.14 for rCYP3A4 with $b_5$). The other explanation is that the coexpressed cytochrome $b_5$ might play an important role in electron transfer when CYP3A catalyzes the cleavage of the phosphonate moiety of NO-1886 in the human liver. Cytochrome $b_5$ has been found to be required for optimal CYP3A activity, but its effect appears to be dependent upon the particular CYP3A substrate (Gillam et al., 1995; Yamazaki et al., 1996a,b).

### Discussion

A number of studies have shown a significant inverse relationship between high-density lipoprotein (HDL) cholesterol and coronary heart disease, and HDL cholesterol is now known to be a strong protector against coronary artery sclerosis (Gorden and Rifkind, 1989). Plasma HDL originates from three sources: the liver, small intestine, and lipoprotein lipid-mediated lipolysis of chylomicrons and very low-density lipoproteins (Eisenberg, 1984). Because manipulation of physiological processes in the organs will undoubtedly involve the perturbation of complicated biological systems, up-regulation of lipoprotein lipase activity appears to be a more promising strategy in the development of new therapeutic agents. The novel compound NO-1886 was shown to increase lipoprotein lipase activity.
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and to induce an elevation of HDL cholesterol in a few studies (Tsutsuini et al., 1993, 1995, 1997). The O-deethylation of the phosphoric acid ester of diethyl phosphonate and the hydrolysis of amide bonds have been reported to be the metabolic pathways of NO-1886, and the metabolites of NO-1886 do not increase lipoprotein lipase activity (Morioka et al., 1996). Because the disposition of NO-1886 has been investigated only in the rat (Morioka et al., 1996), it is necessary to clarify the metabolism of this new drug in humans. The enzymes involved in the metabolism of NO-1886 were therefore identified to obtain information to avoid drug-drug interactions in the clinical use of NO-1886.

Incubation of NO-1886 in the human liver S9 fraction and microsomes demonstrated the major metabolic pathway of NO-1886 to be conversion to monoethyl phosphonate (M-2), with the hydrolysis of the amide bond as the minor pathway (Fig. 2; Table 1). Most esterases are capable of catalyzing hydrolytic reactions of several types of bonds such as those of carboxylester, carboxyamide, carboxythioester, and phosphoric acid esters. Among these esterases, carboxylesterase has been found to be frequently involved in the detoxification process of several types of ester compounds. In the case that some organophosphates are poor substrates for carboxylesterase, A-esterase is normally the enzyme that catalyzes the hydrolytic reactions (Walker and Mackness, 1983; Tang and Chambers, 1999). Therefore, it has been thought that A-esterase and/or carboxylesterase catalyzes the biotransformation from diethyl phosphonate to the monoethyl phosphonate of NO-1886. The esterases in S9 and microsomes generally catalyze hydrolysis in the absence of NADPH. However, M-2 formation without NADPH was only 9.77 pmol/min/mg of protein in human liver microsomes. The results of the present study show that more than 90% of the biotransformation of NO-1886 to M-2 requires NADPH. Meanwhile, there have been several reports concerning the NADPH- and oxygen-dependent hydrolysis of phosphoric acid ester (Søderlund et al., 1979; Dunkov et al., 1997). For example, tris-(2,3-dibromopropyl)phosphate is converted to bis(2,3-dibromopropyl)phosphate after biotransformation from diethyl phosphonate to the monoethyl phosphonate of NO-1886. The esterases S9 and microsomes generally catalyze hydrolysis in the absence of NADPH. However, M-2 formation without NADPH was only 9.77 pmol/min/mg of protein in human liver microsomes. The results of the present study show that more than 90% of the biotransformation of NO-1886 to M-2 requires NADPH. Meanwhile, there have been several reports concerning the NADPH- and oxygen-dependent hydrolysis of phosphoric acid ester (Søderlund et al., 1979; Dunkov et al., 1997). For example, tris-(2,3-dibromopropyl)phosphate is converted to bis(2,3-dibromopropyl)phosphate after oxidation of the 2,3-dibromopropyl group by cytochrome P450 (Søderlund et al., 1984). Therefore, the phosphonate bond of NO-1886 is probably cleaved after oxidation of the ethyl group by P450.

In contrast, the hydrolysis of the amide bond of NO-1886 might be catalyzed by cytosolic esterases. The hydrolysis of the amide bond of NO-1886 is the second major metabolic pathway in rats (Morioka et al., 1996), and it mainly proceeds in rat plasma (data not shown). However, the amide cleavage might occur at very low levels in humans, because the amide hydrolysis of NO-1886 was not observed in 30-min incubation in human plasma (data not shown). The existence of this phenomenon is suggested by the fact that esterase such as carboxylesterase is hardly expressed in human plasma as opposed to abundant expression of esterase in rat plasma (Satoh and Hosokawa, 1998).

To identify the cytochrome P450 isoform involved in the phosphonate O-deethylation of NO-1886, further experiments were performed in human liver microsomes. Eadie-Hofstee plots indicated that at least two cytochrome P450 enzymes in human liver microsomes might catalyze M-2 formation, as shown in Fig. 3. The results of inhibition studies with highly specific anti-P450 antibodies and chemical inhibitors suggested that the phosphonate O-deethylation of NO-1886 was catalyzed by CYP3A4 and CYP2C8. At the concentration of NO-1886 (4.4 μM) used in chemical and immuno-inhibition studies, the metabolic rates of human liver microsomal formation of M-2 are calculated to be 8.34 and 7.74 pmol/min/mg of protein for the high- and low-Km components, respectively, by using the observed Km and Vmax values. Therefore, it is expected that chemical inhibitors and anti-P450 antibody incompletely inhibit the formation of M-2 from NO-1886 in human liver microsomes. The maximum inhibition of the human liver microsomal formation of M-2 by triacetyloleandomycin, a selective CYP3A inhibitor, was only 50%. Anti-CYP3A inhibited the human liver microsomal M-2 formation by a maximum of about 40% (Fig. 4). The close similarity between the inhibition of human liver microsomal M-2 formation by anti-CYP2C (a maximum of about 55%; Fig. 4) and 13-cis-retinoic acid, a selective inhibitor of CYP 2C8, suggests that 50 to 55% of the human liver microsomal M-2 formation from 4.4 μM NO-1886 is catalyzed by CYP2C8 isoforms. Thus, the inhibition data for the formation of M-2 from 4.4 μM NO-1886 by human liver microsomes suggest that the contribution of CYP2C8 and CYP3A isoforms is similar.

In addition, the M-2 formation by recombinant P450s suggested that CYP3A4 might be responsible for the high-Km component, whereas CYP2C8 might be responsible for the low-Km component for phosphonate O-deethylation of NO-1886 in human liver microsomes. This assumption is supported by the chemical inhibition of the human liver microsomal M-2 formation at a high concentration of NO-1886 (110 μM; Fig. 5). However, the observed Km value for M-2 formation by recombinant P450 isoforms is different from the Km value in human liver microsomes, possibly due to several reasons, e.g., binding of NO-1886 to intracellular and/or microsomal proteins, or a conformational change of the P450 molecule by binding with endogenous compounds.

The human liver microsomal CYP2C subfamily is known to comprise at least four members: CYP2C8, CYP2C9, CYP2C18, and CYP2C19. CYP2C19 is considered to be the polymorphically expressed (S)-mephentyn 4′-hydroxylase that is involved in the metabolism of omeprazole, diazepam, imipramine, propanolol, and progesterone (Goldstein and de Moraes, 1994; Goldstein et al., 1994). On the other hand, CYP2C8 exhibits selectivity for retinol (hydroxylation), Taxol (6α-hydroxylation), and arachidonic acid (epoxidation) (Leo et al., 1989; Daikó et al., 1994; Rahman et al., 1994). CYP2C8 has been reported to constitute approximately 25% of the P450 isoforms expressed in human liver microsomes (Shimada et al., 1994). In addition, 60% of the CYP2C cDNA clones isolated from a human liver library were CYP2C9. Only 1% was CYP2C19, whereas CYP2C8 and CYP2C18 content was much lower than that of CYP2C19 (Inoue et al., 1997). Among the recombinant CYP2C enzymes, only CYP2C8 showed activity for the phosphonate O-deethylation of NO-1886 with a low Km value. Considering the relative quantities of CYP2C8 and CYP3A4 in the human liver, it is suggested that CYP2C8 may be saturated in the early stage of metabolism of NO-1886 in the liver. The fact that the phosphonate O-deethylation of NO-1886 is partly mediated by CYP2C8 in a high-affinity manner suggests that drug-drug interactions may occur with CYP2C8 substrates. Because NO-1886 may be administered for prolonged periods in patients with hyperglycemia, drugs that are selectively metabolized by CYP2C8 should be carefully monitored or should be avoided as much as possible.

CYP3A in the human liver has been reported to be approximately 29% of the total P450 content in Japanese and Caucasian people (Shimada et al., 1994). The CYP3A4 level in some individuals is more than 60% of the total P450 content, probably due to induction by various chemical agents (Guengerich, 1995). Because NO-1886 might be administered at relatively high doses in clinical use according to its pharmacological activity in phase I trials, CYP3A4 will become an important isozyme for the elimination of NO-1886. The O-deethylation of NO-1886 mediated by CYP3A4 may not occur only in the liver but also in other CYP3A4-rich sites such as the intestine. Therefore, individual differences in the plasma concentration of NO-1886, which
is influenced by the CYP3A content in the liver and intestine, may be observed in the clinical setting. In addition, the hepatic clearance for the NADPH-dependent M-2 formation from NO-1886 was estimated using the $K_{m}$ and $V_{max}$ values for the low- and high-$K_{m}$ components of human liver microsomes and the average protein content of microsomes (51 mg/g of liver) in the human liver (21 g of liver/kg of body weight). The intrinsic hepatic clearance was calculated to be 6.34 ml/min/kg, and hepatic clearance was then roughly calculated to be 0.42 ml/min/kg by using 21 ml/min/kg of hepatic blood flow rate and 0.067 of the free fraction of NO-1886 in human plasma (Y. Morioka, M. Harada, S. Naito, T. Imai, unpublished data). The estimated hepatic clearance is much lower than the hepatic blood flow rate, suggesting that NO-1886 is slowly eliminated from the body, with almost no first-pass metabolism in the liver.

In conclusion, the results of the present study suggest that both CYP2C8 and CYP3A4 are major P450 enzymes involved in the phosphorylase-$O$-deethylolation of NO-1886 in the human liver. The roles of these two enzymes vary depending on the amounts available in the liver. CYP3A is the major P450 subfamily in the human liver and intestine and is involved in the metabolism of a variety of pharmaceutical agents that are metabolized by P450. CYP3A enzymes have been reported to be involved in interactions with several drugs such as macrolides, ketoconazole, and cyclosporin (Pichard et al., 1990; Periti et al., 1992). In addition, CYP2C8 that catalyzes the oxidative reaction of selective substrates plays an important role as the low-$K_{m}$ component in the human liver. To further confirm the clinical safety of NO-1886, enzymatic inhibition studies focusing on drug-drug interactions related to metabolism by P450 enzymes are currently underway.

References


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