Contribution of Rat Pulmonary Metabolism to the Elimination of Lidocaine, Midazolam, and Nifedipine

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Abstract:
The contribution of the lung to drug metabolism was investigated in rats and the possibility of prediction of in vivo metabolism from in vitro studies using rat pulmonary microsomes was assessed. Lidocaine, midazolam, or nifedipine was administered to rats at a dose of 10 mg/kg by the intra-arterial, intravenous, and intraportal routes. The pulmonary extraction ratios of lidocaine, midazolam, and nifedipine, calculated from the area under the time-plasma concentration curve (AUC) after the intra-arterial and intravenous administrations, were 39.0 ± 0.5, 18.3 ± 0.7, and 12.3 ± 0.3%, respectively. The hepatic extraction ratios of lidocaine, midazolam, and nifedipine, calculated from the AUC after the intraportal and intravenous administrations, were 68.0 ± 3.3, 52.6 ± 0.4, and 13.5 ± 0.2%, respectively. These results showed that both the liver and the lung contributed to the metabolism of these drugs. The above in vivo pulmonary extraction ratios correlated with the in vitro intrinsic clearance values, which were corrected with the protein unbound ratio in microsomes and plasma, suggesting that pulmonary extraction ratios can be predicted quantitatively from in vitro data. The pulmonary intrinsic clearance values of lidocaine, midazolam, and nifedipine in rat microsomes were lower than their hepatic intrinsic clearance, showing that there was an organ difference in metabolism between the liver and lung. Our results support the importance of the estimation of pulmonary metabolism to predict the total clearance more accurately.

The lung has a variety of drug-metabolizing enzymes, such as the CYP1A, 2A, 2B, 2E, 2F, 3A, and 4B families (Dees et al., 1982; Domin et al., 1986; de Waziers et al., 1990; Nhamburo et al., 1990; Ueno and Gonzalez, 1990; Debru et al., 1995; Zeldin et al., 1996). The lung is an efficient organ for extracting drugs from the blood circulation because all cardiac output goes through it (Perreault et al., 1993). In addition, drugs can undergo first-pass metabolism in the lung after not only oral administration but also intravenous administration. These characteristics of the lung suggest that the lung plays an important role in the elimination of a variety of compounds.

Lidocaine (Tanaka et al., 1994), testosterone (Imaoka et al., 1989), aminopyrine (Funae et al., 1985), and p-nitroanisole (Funae et al., 1985) are metabolized in pulmonary microsomes of rats. In rats, the pharmacokinetic parameters after intravenous and intra-arterial administration showed that the lung contributed to the in vivo elimination of drugs such as propofol (Roof et al., 1996), phenol (Cassidy and Houston, 1980), and 1-naphthol (Mistry and Houston, 1985). In humans, a high percentage of propranolol (75%) (Geddes et al., 1979) and lidocaine (60%) (Jorfeldt et al., 1979) is taken up by the lung during the first passage of a drug through the pulmonary circulation. These reports support the importance of the lung for the elimination of drugs.

The lung has CYP3A families (Krishna and Klotz, 1994; Debru et al., 1995; Anttila et al., 1997; Macé et al., 1998), suggesting the possibility that CYP3A plays an important role in the elimination of a variety of substrates in the lung. However, the actual importance of the lung in the metabolism of CYP3A substrates has not been clarified yet.

Pulmonary extraction can be obtained by the comparison of the pharmacokinetic parameters of compounds after intra-arterial and intravenous administration. However, it is difficult to perform intra-arterial administration in humans. Accordingly, there is a need for a method to predict pulmonary clearance using pulmonary specimens.

In this study, we estimated the importance of the lung for the elimination of lidocaine, midazolam, and nifedipine, which are mainly metabolized by CYP3A in rats, and examined the usefulness of pulmonary microsomes for the prediction of pulmonary clearance. We also aimed to clarify the difference in pulmonary and hepatic metabolism in rat microsomes.

Materials and Methods
Chemicals. Midazolam was kindly supplied by Nippon Roche (Tokyo, Japan). Lidocaine and nifedipine were purchased from Sigma-Aldrich Japan.

ABBREVIATIONS: $k_{	ext{e}}$, terminal elimination rate constant; AUC, area under the time-plasma concentration curve; $C_{\text{lmax}}$, last data point; $E_{\text{lung}}$, pulmonary extraction; $E_{\text{liver}}$, hepatic extraction; AUC_{i.a.}, area under the time-plasma concentration curve after intra-arterial administration; AUC_{i.v.}, area under the time-plasma concentration curve after intravenous administration; CL_{tot, observed}, observed total clearance; CL_{tot, calculated}, calculated total clearance; CL_{lung}, pulmonary clearance; CL_{liver}, hepatic clearance; Q_{lung}, pulmonary blood flow; Q_{liver}, hepatic blood flow; $f_u$, microsome, protein unbound ratio in microsomes; $f_p$, protein unbound ratio in blood.
(Tokyo, Japan). As internal standards, nizatidine and trimethoprim were purchased from Sigma-Aldrich (St. Louis, MO). NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). All the other chemicals and reagents were commercial products and of analytical grade.

Animals. Male Wistar rats aged 8 to 9 weeks were supplied by Tokyo Experimental Animals (Tokyo, Japan). All procedures using animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and were approved by the animal ethics committees of the Faculty of Pharmaceutical Sciences, Tokyo University of Science.

In Vivo Experiment: Estimation of Hepatic and Pulmonary Extraction Ratios. Under ether anesthesia, the jugular artery, femoral vein, or portal vein was cannulated with polyethylene tubing for intra-arterial administration, intravenous administration, or intraportal administration, respectively. The femoral artery of the each rat was also cannulated with polyethylene tubing for blood sampling. Lidocaine, midazolam, and nifedipine were dissolved in saline, a mixed solution of ethanol-propylene glycol-saline (1:1:2) or polyethylene glycol 6000, respectively, and administered at a dose of 10 mg/kg. At designated times, blood samples were collected from the femoral artery and centrifuged at 7800g and 4°C for 10 min. A portion of the supernatant was used as plasma in the assay. As internal standards, 10 μg/ml (final concentration) trimethoprim in 1 M sodium hydroxide, 10 μg/ml (final concentration) nitrazepam in acetonitrile, and 10 μg/ml (final concentration) midazolam in acetonitrile were added to the samples containing lidocaine, midazolam, and nifedipine, respectively.

In Vitro Experiment. Preparation of hepatic and pulmonary microsomes. Liver specimens from rats were rinsed with 100 mM potassium phosphate buffer (pH 7.4) and homogenized in 100 mM potassium phosphate buffer (pH 7.4). Microsomes were prepared by differential centrifugation, and 105,000 g pellets were rinsed and resuspended in 100 mM potassium phosphate buffer (pH 7.4) using a Teflon-glass homogenizer. Lung specimens from rats were rinsed with 100 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 150 mM potassium chloride and cut into segments with a Teflon-glass homogenizer. Microsomes were prepared by differential centrifugation, and 105,000 g pellets were rinsed and resuspended in 100 mM potassium phosphate buffer (pH 7.4) using a Teflon-glass homogenizer.

Microsomal incubation. For rats, incubation mixtures consisted of hepatic microsomes (1 mg of microsomal protein/ml for lidocaine and nifedipine and 0.3 mg of microsomal protein/ml for midazolam) and pulmonary microsomes (1 mg of microsomal protein/ml for lidocaine, 3.87 mg of microsomal protein/ml for midazolam, and 7.05 mg of microsomal protein/ml for nifedipine), substrates (liver: 10–100 μM for lidocaine, 1–100 μM for midazolam, and 1–30 μM for nifedipine; lung: 5–100 μM for lidocaine, 2–60 μM for midazolam, and 2–60 μM for nifedipine), MgCl₂ (4 mM), and an NADPH-generating system (0.5 mM NADP⁺, 2.0 mM glucose-6-phosphate, and 1 U/ml glucose-6-phosphate dehydrogenase) in a total volume of 1.0 ml of potassium phosphate buffer (100 mM, pH 7.4). Reactions were commenced by the addition of the NADPH-generating system, and the incubation mixtures were shaken in a thermostated water bath at 37°C. At time 0 and at two points up to 10 min, incubation samples (150 μl) were removed and transferred to another tube, and 150 μl of termination solutions containing the internal standards were added to the tubes. As internal standards, 10 μg/ml (final concentration) trimethoprin in 1 M sodium hydroxide, 10 μg/ml (final concentration) nitrazepam in acetonitrile, and 10 μg/ml (final concentration) midazolam in acetonitrile were used for lidocaine, midazolam, and nifedipine, respectively. The linearity between the formation rate and incubation time or protein concentration was confirmed in our preliminary studies. The R² values were >0.9. The concentrations of lidocaine, midazolam, and nifedipine were determined individually. Therefore, we could use midazolam as the internal standard for the determination of the concentrations of nifedipine.

Protein unbound ratio in microsomes. The lidocaine, midazolam, and nifedipine concentrations in incubation mixtures were 5 to 100, 6 to 100, and 6 to 100 μM, respectively, for rat liver, and 5 to 100, 6 to 100, and 4 to 30 μM, respectively, for rat lung. After incubation at 37°C for 5 min without the NADPH-generating system, a portion (50 μl) of the incubation mixture was taken to determine the total drug concentration, and the remainder was transferred to an ultracentrifuge tube (Microcon YM-30; Millipore Corporation, Billerica, MA). These tubes were centrifuged (7740g) for 10 min (liver) or 20 min (lung) at 37°C, and then a portion (50 μl) of the filtrate was removed to determine the unbound concentration.

Assay. For lidocaine, ether (1.2 ml) was added to each sample and mixed well using a vortex mixer. After centrifugation at 400g for 10 min, a portion (850 μl) of the supernatant was transferred to another tube. The supernatant was evaporated to dryness under reduced pressure, and the residue was dissolved in mobile phase (200 μl). A portion (100 μl) of each sample was injected into the high-performance liquid chromatograph.

For midazolam and nifedipine, samples were mixed well using a vortex mixer and centrifuged at 7740g and 4°C for 10 min. A portion (100 μl) of each sample was injected into the high-performance liquid chromatograph.

The concentrations of lidocaine, midazolam, and nifedipine in samples were determined using a high-performance liquid chromatography system (Jasco, Tokyo, Japan), consisting of a pump (PU-980), an automatic sampler (AS-950-10), and a UV detector (UV-1570). For lidocaine, a TSKgel ODS-80Tm column (4.6 mm i.d. × 250 mm, 5 μm; Tosoh, Tokyo, Japan) was used with a mobile phase of 93% 100 mM phosphate buffer (pH 3.0) and 7% acetonitrile, eluted at a flow rate of 1.0 ml/min. The detection wavelength was 214 nm. For midazolam, a TSKgel ODS-80Tm column (4.6 mm i.d. × 250 mm, 5 μm) was used with a mobile phase of 45% 10 mM phosphate buffer (pH 7.4), 23% methanol, and 32% acetonitrile, eluted at a flow rate of 1.0 ml/min. The detection wavelength was 254 nm. For nifedipine, a Capcell Pak C18 SI210S column (4.6 mm i.d. × 250 mm, 5 μm; Shiseido, Tokyo, Japan) was used with a mobile phase of 50% 50 mM phosphate buffer (pH 7.0), 15% methanol, and 35% acetonitrile, eluted at a flow rate of 1.0 ml/min. The detection wavelength was 254 nm. Peak area ratios of the substrates to the internal standards were used to obtain the substrate concentrations in samples.

Pharmacokinetic Analysis. Calculation of pulmonary and hepatic extractions. The terminal elimination rate constant (kₑ) was calculated by linear regression of the last n (n = 3–4) plasma concentrations. The R² values of the linear regression were <0.9. The area under the concentration-time curve (AUC) was calculated in accordance with the trapezoidal rule up to the last data point (Cₙₐₜ) and addition of the extrapolated terminal area, calculated as AUC₅₀₋₉₅. More than 90% of the total AUC was accounted for by AUC values, which were calculated without the extrapolated portion.

The pulmonary and hepatic extractions of lidocaine, midazolam, and nifedipine were calculated using eqs. 1 and 2 (Cassidy and Houston, 1980):

\[ E_{pulm} (%) = \frac{\text{AUC}_{\text{pulm}}}{\text{AUC}_{\text{sys}}} \times 100 \]  
\[ E_{hep} (%) = \frac{\text{AUC}_{\text{hep}}}{\text{AUC}_{\text{sys}}} \times 100 \]  

where \( E_{pulm} \) and \( E_{hep} \) represent the pulmonary and hepatic extraction ratios, respectively, and the \( \text{AUC}_{\text{pulm}} \), \( \text{AUC}_{\text{hep}} \), and \( \text{AUC}_{\text{sys}} \) values are the AUCs of the compounds after intra-arterial, intraportal, and intravenous administration, respectively.

Calculation of total, pulmonary, and hepatic clearance. The observed total clearances (CL_{tot, observed}) of lidocaine, midazolam, and nifedipine were calculated using eq. 3 (Gibaldi and Perrier, 1982):

\[ \text{CL}_{\text{tot, observed}} = \text{dose}_{i.v.}/\text{AUC}_{\text{sys}} \]  

where \( \text{dose}_{i.v.} \) is the dose that was administered intravenously.

In general, total clearance is expressed as the sum of organ clearances except pulmonary clearance. The pulmonary arterial blood \( (C_{a,pulmonary}) \) flows in the lung and is pumped as the pulmonary venous blood. Drug concentrations in the pulmonary venous blood are same as those in the systemic arterial blood \( (C_{a,sys}) \). Therefore, the \( C_{a,sys} \) is expressed as \( (1 - k_{hep}) \times C_{a,pulmonary} \). When the compounds were eliminated by only the liver and lung, the total clearances (CL_{tot, calculated}) were also calculated using eq. 4:
In this article, both in vivo and in vitro results showed that lidocaine, midazolam, and nifedipine were metabolized in rat lung. Although the metabolism in the lung was lower than that in the liver, our study demonstrated that the lung is also responsible for the elimination of these drugs. In general, the liver is considered to be the primary organ for drug metabolism, and several studies have shown that the intestine contributes to the first-pass metabolism of CYP3A4 substrates (Hall et al., 1999; Jacobsen et al., 1999). Our studies demonstrated that the lung also contributed to the elimination of lidocaine, midazolam, and nifedipine in rats. Salbutamol and lidocaine are reported to be metabolized by rabbit lung (Perreault et al., 1993; Lé et al., 1996). However, in the case of drugs mainly metabolized by cytochrome P450, such as midazolam, the contribution of pulmonary metabolism has not been reported yet.

In this study, all of the tested compounds were extracted in the lung, and lidocaine and midazolam were particularly highly extracted by rat lung (68 and 53%, respectively). The compounds were therefore metabolized in both liver and lung, leading to lower amounts of drugs being able to reach the systemic circulation after intravenous or oral administration. The reported values for the bioavailability of lidocaine, midazolam, and nifedipine in rats are 16, 12, and 61%, respectively (de Lee et al., 1983; Grundy et al., 1997; Higashikawa et al., 1999). The present results support the importance of pulmonary metabolism for drugs that are CYP3A substrates and have low migration into the systemic circulation. As observed in our studies, the total clearances of lidocaine and midazolam were higher than the hepatic blood flow in rats (Shand et al., 1975; Kotegawa et al., 2002), suggesting that extrahepatic organs contribute to the elimination of these compounds. In addition, the slope of the correlation line between CLtot, observed determined from AUC and CLtot, calculated determined from the extraction ratio was approximately 1 (Fig. 1). These results indicate that lidocaine, midazolam, and nifedipine are mainly metabolized in the lung and liver. For accurate prediction of the total clearance of these drugs, pulmonary metabolism should be taken into consideration. The high hepatic extraction ratio of midazolam in the in vitro study (Table 2), the lower correlation of the liver compared with that of the lung (Fig. 3), and the high intrinsic clearance of midazolam in the in vitro study (Table 4) indicate that the hepatic clearance of midazolam in rats is hepatic blood flow-limited. In particular, the hepatic clearance of midazolam was successfully predicted in a pre-

### Results

#### Estimation of In Vivo Pulmonary and Hepatic Metabolism.

Tables 1 and 2 show the pharmacokinetic parameters calculated from the plasma concentration profiles of lidocaine, midazolam, and nifedipine after intravenous and intrarterial or intraportal administration to rats. The increases in AUC, AUCi.v., and AUCi.p. of lidocaine, midazolam, and nifedipine were linear up to a dose of 10 mg/kg (data not shown). The Ehang and Eiver values calculated using eqs. 1 and 2 showed that all of the compounds were eliminated in the rat lung and liver. The Ehung and Eiver values of lidocaine were the largest among these compounds.

#### Calculation of In Vivo Total Clearance.

Figure 1 shows the correlation between the observed total clearance (CLtot, observed) (eq. 3) and the calculated total clearance (CLtot, calculated) (eq. 4) or hepatic clearance (CLliver) (eq. 6). The CLtot, observed correlated with CLtot, calculated and CLliver. The slope of the line between CLtot, observed and CLtot, calculated was approximately 1, although that between CLtot, observed and CLliver was 0.23, indicating that the lung contributed to the total clearance of these compounds.

#### Calculation of In Vitro Pulmonary Intrinsic Clearance.

Tables 3 and 4 show the kinetic parameters for the metabolism of lidocaine, midazolam, and nifedipine in pulmonary and hepatic microsomes, respectively, of the rats. All of the compounds were metabolized in the rat pulmonary and hepatic microsomes. Lidocaine showed the largest Vmax/Km, microsome values in the pulmonary microsomes among these compounds as estimated by the Ehang values (Table 1). The Vmax/Km, microsome values were corrected using the protein unbound ratio in blood (fub) to correspond to the in vivo organ clearance. A good correlation was observed between Ehang and fub × Vmax/Km, microsome (Fig. 2). On the other hand, midazolam showed the largest Vmax/Km, microsome value in the hepatic microsomes among these compounds (Table 4) contrary to expectation from Eiver (Table 2). The correlation with the liver was lower than that with the lung (Fig. 3).

### Tables

#### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Administration Route</th>
<th>AUC μM · min</th>
<th>CL m/min/kg</th>
<th>Ehang %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>i.v.</td>
<td>0.255 ± 0.058</td>
<td>145 ± 32</td>
<td>39.0 ± 0.5</td>
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<tr>
<td></td>
<td>i.a.</td>
<td>0.419 ± 0.063</td>
<td>88.3 ± 10.0</td>
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<tr>
<td>Midazolam</td>
<td>i.v.</td>
<td>0.353 ± 0.029</td>
<td>87.0 ± 6.7</td>
<td>18.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>i.a.</td>
<td>0.432 ± 0.100</td>
<td>71.1 ± 20.2</td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>i.v.</td>
<td>3.65 ± 0.31</td>
<td>7.90 ± 0.72</td>
<td>12.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>i.a.</td>
<td>4.17 ± 0.63</td>
<td>6.93 ± 1.24</td>
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</table>

#### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Administration Route</th>
<th>AUC μM · min</th>
<th>CL m/min/kg</th>
<th>Ehang %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>i.p.</td>
<td>0.0817 ± 0.0118</td>
<td>457 ± 72</td>
<td>68.0 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>0.255 ± 0.058</td>
<td>145 ± 32</td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>i.p.</td>
<td>0.167 ± 0.044</td>
<td>184 ± 65</td>
<td>52.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>0.353 ± 0.029</td>
<td>87.0 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>i.p.</td>
<td>3.16 ± 0.34</td>
<td>9.13 ± 1.34</td>
<td>13.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>i.a.</td>
<td>3.65 ± 0.31</td>
<td>7.90 ± 0.72</td>
<td></td>
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</table>
Consistent with the previous studies, our study showed the usefulness of hepatic microsomes for quantitatively predicting the hepatic clearances of lidocaine, midazolam, and nifedipine. Our study also showed the importance of the liver and lung as metabolic organs for the drugs examined.

As shown in Table 3, lidocaine, midazolam, and nifedipine were metabolized in pulmonary microsomes of rats. The $V_{\text{max}}/K_m$ values in rat pulmonary microsomes were midazolam > lidocaine > nifedipine (Table 3), whereas those in rat hepatic microsomes were midazolam > nifedipine > lidocaine (Table 4). If same enzyme contributes the metabolism of these drugs both in the liver and lung, the order of $V_{\text{max}}/K_m$ values of these drugs in the lung should be same as that in the liver. These results showed that there is an organ difference between the liver and lung in the metabolism of these drugs. The organ difference suggests that more than two enzymes are involved in
the metabolism and the compositions of the enzymes are different between the liver and lung. The lung (Krishna and Klotz, 1994; Debru et al., 1995; Anttila et al., 1997; Macé et al., 1998) and the liver (Mahnke et al., 1997) have CYP3A subfamilies, which mainly metabolize midazolam and nifedipine. However, the enzymes involved in the lidocaine metabolism in rats have not been completely identified. The N-deethylation of lidocaine by rat pulmonary microsomes is catalyzed exclusively by CYP2B1 (Tanaka et al., 1994). Aiba et al. (2003) suggested that the CYP2D subfamily is also involved in the metabolism of lidocaine. These results suggest that CYP2B1 and/or other enzymes contribute to the metabolism of lidocaine in rat lung. Thus, the difference in enzyme activity between the lung and liver produces a difference in the extent of drug-drug interactions between the lung and liver. O,O,O-Trimethylphosphorothioate and O,O,S-trimethylphosphorodithioate are very selective inhibitors of pulmonary CYP2B1 activity (Verschoyle et al., 1993). Single administration of sodium arsenite caused a 5-fold increase in pulmonary 7-ethoxyresorufin O-deethylation activity, whereas 7-pentoxyresorufin O-depentylation activity was inhibited by 35% in the liver (Albores et al., 1995). Thus, there are metabolism-mediated drug-drug interactions in not only the liver but also the lung. In addition to hepatic microsomes, the use of pulmonary microsomes allows more accurate prediction of drug-drug interactions. Hepatic metabolism is the most important factor for drug disposition, and the quantitative prediction of hepatic clearance of various compounds from in vitro data has been reported (Naritomi et al., 2001; Austin et al., 2002; Nestorov et al., 2002; De Buck et al., 2007). We studied the enzyme activity of the liver and lung as metabolic organs for the drugs examined. As shown in Fig. 3, $E_{150}$ values correlated with $V_{max}/K_{m, microsome}$ values, suggesting the possibility of quantitative prediction of the metabolism of the compounds in the lung and clearance from in vitro data. The usefulness of precision-cut rat tissue slices to predict metabolic drug clearance in vivo was investigated by De Kanter et al. (2004), and they concluded that the model of multiorgan precision-cut slices including the lung is a useful in vitro tool for the prediction of in vivo metabolic clearance. Organ slices are useful in evaluating drug metabolism but have the added advantage of taking membrane transport effects into account. On the other hand, enzyme activities decrease rather rapidly compared with microsomes during the storage. In this study, $E_{150}$ values correlated with $V_{max}/K_{m, microsome}$ values determined by pulmonary microsomes. These results support the fact that a good correlation between in vitro intrinsic clearance and in vivo $E_{150}$ is reasonable and that the elimination of the compounds in the lung involves their metabolism. Each ratio of S.E. value to $f_{150} \times V_{max}/K_{m, microsome}$ values was calculated to estimate the suitability of the data. There was no difference in the value among the three compounds, suggesting that the extent of error bar value depended on the extent of the $f_{150} \times V_{max}/K_{m, microsome}$ values. We showed the possibility of quantitative prediction of rat $E_{150}$ from in vitro data. Future studies should include experiments for estimating the intrinsic clearances of lidocaine, midazolam, and nifedipine using human pulmonary microsomes to predict pulmonary extraction in humans. We believe that our experimental results will help in further studies of pulmonary metabolism in humans.

In conclusion, we demonstrated that the lung contributes to the elimination of lidocaine, midazolam, and nifedipine in rats as observed in the liver. The $E_{150}$ values correlated with $f_{150} \times V_{max}/K_{m, microsome}$ values, suggesting that the $E_{150}$ value can be predicted quantitatively from in vitro data. Our results support the importance of the estimation of drug metabolism in the lung to predict the total clearance more accurately.


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