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

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Running title: Contribution of pulmonary metabolism to the drug elimination

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Number of text pages: 31

Figures: 3

Tables: 4

References: 39

Number of Words: 5424

Abstract: 222

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Abbreviations:

i.a., intra-arterial administration; i.v., intravenous administration; i.p., intra-portal administration; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; f_{u,microsome}, protein unbound ratio in microsomes; k_e, terminal elimination rate constant; AUC, area under the time-plasma concentration curve; C_{last}, last data point; E_{lung}, pulmonary extraction; E_{liver}, hepatic extraction; AUC_{i,a}, area under the time-plasma concentration curve after intra-arterial
DMD #32227

administration; AUC\textsubscript{ip}, area under the time-plasma concentration curve after intra-portal administration; AUC\textsubscript{iv}, area under the time-plasma concentration curve after intravenous administration; CL\textsubscript{tot, observed}, observed total clearance; CL\textsubscript{tot, calculated}, calculated total clearance; CL\textsubscript{lung}, pulmonary clearance; CL\textsubscript{iver}, hepatic clearance; Q\textsubscript{lung}, pulmonary blood flow; Q\textsubscript{iver}, hepatic blood flow; K\textsubscript{ms}, Michaelis Menten constant; V\textsubscript{max}, maximum velocity; f\textsubscript{b}, protein unbound ratio in blood.
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 intra-portal routes. The pulmonary extraction ratios of lidocaine, midazolam, and nifedipine, calculated from the 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 intra-portal 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.
Introduction

The lung has a variety of drug metabolizing enzymes, such as CYP1A, 2A, 2B, 2E, 2F, 2J, 3A, and 4B families (de Waziers et al., 1990; Domin et al., 1986; Ueno and Gonzalez, 1990; Nhamburo et al., 1990; Zeldin et al., 1996; Dees et al., 1982, Debri et al., 1995). The lung is an efficient organ for extracting drugs from the blood circulation because all cardiac output goes through it (Perreault et al., 1993). Additionally, 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., 1998), 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 (Raoof et al., 1996), phenol (Cassidy and Houston, 1980), and 1-naphthol (Mistry and Houston, 1998). In humans, a high percentage of propranolol (75%; Geddes et al., 1979) and lidocaine (60%; Jorfeldt et al., 1979) are 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, (Anttila et al., 1997; Debri et al., 1995; Krishna et al., 1994; 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 the intra-arterial and intravenous administration. However, it
is difficult to carry out 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 (Tokyo, Japan). As internal standards, nitrazepam and trimethoprim were purchased from Sigma Chemical Co. (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 NIH Guide for the Care and Use of Laboratory Animals 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 (i.a.), intravenous administration (i.v.), or intra-portal administration (i.p.), 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 600, respectively, and administered at a dose of 10 mg/kg. At designated times, blood samples were collected from the femoral artery, and centrifuged at 7,800 × g, 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 (PMSF), 1 mM dithiothreitol (DTT), and 150 mM potassium chloride, and cut into segments with surgical blades. The lung segments were homogenized in 100 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM PMSF, 1 mM DTT, and 150 mM potassium chloride (approximately 1 g lung/10 mL) using a Polytron PT-MR 3000 (Kinematica AC, Lucerne, Switzerland) and 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 microsomal protein/mL for lidocaine and nifedipine; 0.3 mg microsomal protein/mL for midazolam) and pulmonary microsomes (1 mg microsomal protein/mL for lidocaine; 3.87 mg microsomal protein/mL for midazolam; 2.95 mg microsomal protein/mL for nifedipine), substrates (liver: 10-100 μM for lidocaine, 1-100 μM for midazolam, 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, 1 U/mL
glucose-6-phosphate dehydrogenase) in a total volume of 1.0 mL 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, 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) trimethoprim 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 of 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 (fₜₐₜ, microsome)**

The lidocaine, midazolam, and nifedipine concentrations in incubation mixtures were
5-100, 6-100, and 6-100 μM, respectively, for rat liver, 5-100, 6-60, and 4-30 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 ultrafiltration tube (Microcon
YM-30, Millipore Corp., Bedford, MA). These tubes were centrifuged (7,740 × g) 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 400 $\times$ g for 10 min, a portion (850 $\mu$L) 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 $\mu$L). A portion (100 $\mu$L) of each sample was injected into the HPLC.

For midazolam and nifedipine, samples were mixed well using a vortex mixer and centrifuged at 7,740 $\times$ g, 4°C for 10 min. A portion (100 $\mu$L) of each sample was injected into the HPLC.

The concentrations of lidocaine, midazolam, and nifedipine in samples were determined using a Jasco HPLC system (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. $\times$ 250 mm, 5 $\mu$m, Tosoh, Tokyo, Japan) was used with a mobile phase of 93% 100 mM phosphate buffer (pH3.0) 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. $\times$ 250 mm, 5 $\mu$m, Tosoh, Tokyo, Japan) was used with a mobile phase of 45% 10 mM phosphate buffer (pH7.4), 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 Pack C18 SG120 S-5mm column (4.6 mm I.D. $\times$ 250 mm, 5 $\mu$m, Shiseido, Tokyo, Japan) was used with a mobile phase of 50% 50 mM phosphate buffer (pH7.0), 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 curve (AUC) was calculated in accordance with the trapezoidal rule up to the last data point (C_last) and adding the extrapolated terminal area, calculated as C_last/ke. More than 90% of the total AUC were accounted by AUC values which were calculated without the extrapolated portion.

The pulmonary and hepatic extractions of lidocaine, midazolam, and nifedipine were calculated using the following equations (Cassidy and Houston, 1980):

\[
E_{\text{lung}} (\%) = \frac{\text{AUC}_{i.a.}}{\text{AUC}_{i.v.}} \times 100
\]

\[
E_{\text{liver}} (\%) = \frac{\text{AUC}_{i.p.}}{\text{AUC}_{i.v.}} \times 100
\]

where \( E_{\text{lung}} \) and \( E_{\text{liver}} \) represent the pulmonary and hepatic extraction ratio, respectively, and the AUC_{i.a.}, AUC_{i.p.}, and AUC_{i.v.} values are the AUC of the compounds after intra-arterial, intra-portal, 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 the following equation (Gibaldi and Perrier, 1982):

\[
\text{CL}_{\text{tot, observed}} = \frac{\text{Dose}_{i.v.}}{\text{AUC}_{i.v.}}
\]

where the Dose_{i.v.} is the dose which was administered intravenously.

In general, total clearance is expressed as the sum of organ clearances except pulmonary clearance. The pulmonary arterial blood (\( C_{a, \text{pulmonary}} \)) flows in the lung, and is pumped as the pulmonary venous blood. Drug concentrations in the pulmonary venous blood are same as that in the systemic arterial blood (\( C_{a, \text{systemic}} \)). Therefore, the \( C_{a, \text{systemic}} \) is expressed as
When the compounds were eliminated by only the liver and lung, the total clearances ($CL_{tot, \text{calculated}}$) were also calculated using the following equation:

$$CL_{tot, \text{calculated}} = \frac{1}{(1-E_{\text{lung}})} \times CL_{\text{lung}} + CL_{\text{liver}}$$

(4)

where $CL_{\text{lung}}$ and $CL_{\text{liver}}$ are the pulmonary and hepatic clearance, respectively.

The $CL_{\text{lung}}$ and $CL_{\text{liver}}$ were calculated using the following equations (Grundy et al., 1997):

$$CL_{\text{lung}} = Q_{\text{lung}} \times E_{\text{lung}}$$

(5)

$$CL_{\text{liver}} = Q_{\text{liver}} \times E_{\text{liver}}$$

(6)

where $Q_{\text{lung}}$ is the pulmonary blood flow (178 mL/min/kg, Lin et al., 1984), and $Q_{\text{liver}}$ is the hepatic blood flow (58.8 mL/min/kg, Murata et al., 1998).
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 i.v. and i.a or i.p. administration to rats. The increases in AUC_{i.a.}, AUC_{i.v.} and AUC_{i.p.} of lidocaine, midazolam, and nifedipine were linear up to a dose of 10 mg/kg (data not shown). The $E_{\text{lung}}$ and $E_{\text{liver}}$ values calculated using eq. 1 and 2 showed that all the compounds were eliminated in the rat lung and liver. The $E_{\text{lung}}$ and $E_{\text{liver}}$ values of lidocaine were the largest among these compounds.

Calculation of in vivo total clearance

Fig. 1 shows the correlation between the observed total clearance ($CL_{\text{tot, observed}}$, eq. 3) and the calculated total clearance ($CL_{\text{tot, calculated}}$, eq. 4) or hepatic clearance ($CL_{\text{liver}}$, eq. 6). The $CL_{\text{tot, observed}}$ correlated with $CL_{\text{tot, calculated}}$ and $CL_{\text{liver}}$. The slope of the line between $CL_{\text{tot, observed}}$ and $CL_{\text{tot, calculated}}$ was approximately 1, although that between $CL_{\text{tot, observed}}$ and $CL_{\text{liver}}$ 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 the compounds were metabolized in the rat pulmonary and hepatic microsomes. Lidocaine showed the largest $V_{\text{max}}/K_m/f_{u, \text{microsome}}$ values in the pulmonary microsomes among these compounds as estimated by the $E_{\text{lung}}$ values (Table 1). The $V_{\text{max}}/K_m/f_{u, \text{microsome}}$ values were corrected using the protein unbound ratio in blood ($f_{b}$) to correspond to the in vivo organ clearance. A good correlation was observed between $E_{\text{lung}}$ and $f_{b} \times V_{\text{max}}/K_m/f_{u, \text{microsome}}$ (Fig.
On the other hand, midazolam showed the largest $V_{\text{max}}/K_m/f_u, \text{microsome}$ value in the hepatic microsomes among these compounds (Table 4) contrary to expectation from the $E_{\text{liver}}$ (Table 2). The correlation with the liver was lower than that with the lung (Fig. 3).

**Comparison of intrinsic clearance between the lung and liver of rat microsomes**

The pulmonary intrinsic clearance, $V_{\text{max}}/K_m/f_u, \text{microsome}$ values, of lidocaine, midazolam, and nifedipine was 11.3, 0.672, and 0.150% of their hepatic value, respectively, showing that the pulmonary intrinsic clearance in rat microsomes was lower than the corresponding hepatic clearance for these three drugs.
Discussion

In this paper, 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 (Jacobsen et al., 1999; Hall et al., 1999). Our studies demonstrated that the lung also contributed to the elimination of lidocaine, midazolam, and nifedipine in rats. Sulbutamol and lidocaine are reported to be metabolized by rabbit lung (Perreault et al., 1993; Lê et al., 1996). However, in the case of drugs mainly metabolized by CYP, such as midazolam, the contribution of pulmonary metabolism has not been reported yet.

In this study, all 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 i.v. or p.o. administration. The reported values of the bioavailability of lidocaine, midazolam, and nifedipine in rats are 16%, 12%, and 61%, respectively (de Leede et al., 1983; Higashikawa et al., 1999; Grundy et al., 1997). The present results support the importance of pulmonary metabolism for drugs that are CYP3A substrates and their 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 CL\textsubscript{tot, observed} determined from AUC and CL\textsubscript{tot, 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 vivo 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. Especially, the prediction of hepatic clearance of midazolam was succeeded in a previous study (Higashikawa et al., 1999). In 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 $V_{\text{max}}/K_{\text{m}}$ values in rat pulmonary microsomes were midazolam = lidocaine > nifedipine (Table 3), while 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 $V_{\text{max}}/K_{\text{m}}$ values of these drugs in the lung should be same as that in the liver. These results showed that there is an organ difference in the metabolism of these drugs between the liver and lung. 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 (Anttila et al., 1997; Debri et al., 1995; Krishna et al., 1994; 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). Hashimoto 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 liver and lung. 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 (Austin et al., 2002; De Buck et al., 2007; Naritomi et al., 2001; Nestorov et al., 2002). Our study showed the importance of the liver and lung as metabolic organs for the drugs examined. As shown in Fig 3, \( E_{\text{lung}} \) values correlated with \( V_{\text{max}}/K_m/f_u, \text{microsome} \) values, suggesting the possibility of quantitative prediction of the pulmonary extraction 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 multi-organ 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 accounts. On the other hand, enzyme activities decrease rather rapidly compared with microsomes during the storages. In this study, $E_{\text{lung}}$ values correlated with $V_{\text{max}}/K_m/f_{u, \text{microsome}}$ values determined by pulmonary microsomes. These results support that a good correlation between in vitro intrinsic clearance and in vivo $E_{\text{lung}}$ is reasonable, and that the elimination of the compounds in the lung involves their metabolism. Each ratio of standard error value to $f_b \times V_{\text{max}}/K_m/f_{u, \text{microsome}}$ value was calculated in order to estimate the suitability of the data. There was no little difference of the value among three compounds, suggesting that the extent of error bar value depend on the extent of $f_b \times V_{\text{max}}/K_m/f_{u, \text{microsome}}$ value. Our study showed the possibility of quantitative prediction of rat $E_{\text{lung}}$ 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 the pulmonary extraction in humans. We believe that our experiment 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 $E_{\text{lung}}$ values correlated with $f_b \times V_{\text{max}}/K_m/f_{u, \text{microsome}}$ values, suggesting that the $E_{\text{lung}}$ 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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Zeldin DC, Foley J, Ma J, Boyle JE, Pascual JM, Moomaw CR, Tomer KB, Steenbergen C
Footnotes

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Legends for Figures

Fig. 1  Correlation between observed total clearance and calculated total clearance or hepatic clearance. A dotted line represents a 1:1 correlation.

$CL_{tot, observed}$, observed total clearance after intravenous administration; $CL_{tot, calculated}$, total clearance calculated by eq. 3 and 4; $CL_{liver}$, hepatic clearance calculated by eq. 5 and 6.

Fig. 2  Correlation of pulmonary metabolism between in vitro and in vivo experiments for lidocaine, midazolam, and nifedipine

$E_{lung}$, pulmonary extraction calculated by eq. 1; $f_b$, protein unbound ratio in blood; $V_{max}$, maximum velocity; $K_m$, Michaelis Menten constant; $f_u$, microsomes, protein unbound ratio in microsomes. Values are expressed as the mean ± S.E., n=6 (lidocaine), 3 (midazolam), or 4 (nifedipine).

Fig. 3  Correlation of hepatic metabolism between in vitro and in vivo experiments for lidocaine, midazolam, and nifedipine

$E_{liver}$, hepatic extraction calculated by eq. 1; $f_b$, protein unbound ratio in blood; $V_{max}$, maximum velocity; $K_m$, Michaelis Menten constant; $f_u$, microsomes, protein unbound ratio in microsomes. Values are expressed as the mean ± S.E., n=3.
Table 1

Pharmacokinetic parameters of lidocaine, midazolam, and nifedipine after intravenous and intra-arterial administration to rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Administration route</th>
<th>AUC (\mu\text{M} \cdot \text{min})</th>
<th>CL \text{mL/min/kg}</th>
<th>(E_{\text{lung}}) %</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>
</tr>
<tr>
<td></td>
<td>i.a.</td>
<td>0.419 ± 0.063</td>
<td>88.3 ± 10.0</td>
<td></td>
</tr>
<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>
<td></td>
</tr>
</tbody>
</table>

i.v., intravenous administration; i.a., intra-arterial administration; AUC, area under the plasma concentration curve; CL, total plasma clearance; \(E_{\text{lung}}\), hepatic extraction. Each CL was calculated as dose/AUC. Values are expressed as the mean ± S.E., n=3.
Table 2

Pharmacokinetic parameters of lidocaine, midazolam, and nifedipine after intra-portal and intravenous administration to rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Administration route</th>
<th>AUC μM・min</th>
<th>CL mL/min/kg</th>
<th>E_{liver} %</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.v.</td>
<td>3.65 ± 0.31</td>
<td>7.90 ± 0.72</td>
<td></td>
</tr>
</tbody>
</table>

i.p., intra-portal administration; i.v., intravenous administration; AUC, area under the plasma concentration curve; CL, total plasma clearance; E_{liver}, pulmonary extraction. Each CL was calculated as dose/AUC. Values are expressed as the mean ± S.E., n=3.
Table 3

Kinetic parameters for the metabolism of lidocaine, midazolam, and nifedipine in pulmonary microsomes of rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>( V_{\text{max}} ) pmol/min/mg protein</th>
<th>( K_m ) ( \mu )M</th>
<th>( V_{\text{max}}/K_m ) ( \mu )L/min/mg protein</th>
<th>( f_u, \text{microsome} ) %</th>
<th>( V_{\text{max}}/K_m/f_u, \text{microsome} ) ( \mu )L/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>338 ± 11</td>
<td>20.2 ± 0.9</td>
<td>16.8 ± 0.1</td>
<td>39.7 ± 14.2</td>
<td>42.2 ± 15.1</td>
</tr>
<tr>
<td>Midazolam</td>
<td>86.7 ± 9.1</td>
<td>5.04 ± 1.21</td>
<td>17.2 ± 4.5</td>
<td>62.5 ± 7.1</td>
<td>27.5 ± 7.8</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>139 ± 34</td>
<td>26.4 ± 11.0</td>
<td>5.27 ± 2.53</td>
<td>47.9 ± 11.3</td>
<td>11.0 ± 5.9</td>
</tr>
</tbody>
</table>

\( V_{\text{max}}, \) maximum velocity; \( K_m, \) Michaelis Menten constant; \( f_u, \text{microsome}, \) protein unbound ratio in microsomes. Values are expressed as the mean ± S.E., n=6 (lidocaine), 3 (midazolam), or 4 (nifedipine).
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Table 4

Kinetic parameters for the metabolism of lidocaine, midazolam, and nifedipine in hepatic microsomes of rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>V_max nmol/min/mg protein</th>
<th>K_m μmol/L</th>
<th>V_max/K_m mL/min/mg protein</th>
<th>f_u, microsome %</th>
<th>V_max/K_m/f_u, microsome mL/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>5.52 ± 0.33</td>
<td>23.9 ± 3.0</td>
<td>0.231 ± 0.03</td>
<td>61.6 ± 10.3</td>
<td>0.375 ± 0.082</td>
</tr>
<tr>
<td>Midazolam</td>
<td>6.97 ± 0.62</td>
<td>5.42 ± 0.91</td>
<td>1.29 ± 0.25</td>
<td>71.4 ± 5.3</td>
<td>1.80 ± 0.37</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>8.36 ± 0.66</td>
<td>20.8 ± 2.5</td>
<td>0.402 ± 0.058</td>
<td>61.6 ± 8.8</td>
<td>0.652 ± 0.133</td>
</tr>
</tbody>
</table>

V_max, maximum velocity; K_m, Michaelis Menten constant; f_u, microsome, protein unbound ratio in microsomes. Values are expressed as the mean ± S.E., n=3.
Fig. 1

**Left Panel:**
- Y-axis: \( \text{CL}_{\text{tot, calculated}} \) (mL/min/kg)
- X-axis: \( \text{CL}_{\text{tot, observed}} \) (mL/min/kg)
- Points: Nifedipine, Lidocaine, Midazolam
- Line: \( y = x \)
- \( R = 0.968 \)

**Right Panel:**
- Y-axis: \( \text{CL}_{\text{liver}} \) (mL/min/kg)
- X-axis: \( \text{CL}_{\text{tot, observed}} \) (mL/min/kg)
- Points: Nifedipine, Midazolam, Lidocaine
- Line: \( y = x \)
- \( R = 0.979 \)
Fig. 2

\[
E_{\text{lung}} \, \% = f_b \times \frac{V_{\text{max}}}{K_m} / f_u, \text{microsome} (\mu \text{L/min/mg microsomal protein})
\]

R = 0.979

- Lidocaine
- Midazolam
- Nifedipine
Fig. 3

\[ f_b \times \frac{V_{max}}{K_m}f_u, \text{microsome} \] (µL/min/mg microsomal protein)