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METABOLISM OF LIDOCAINE BY RAT PULMONARY CYTOCHROME P450

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Abstract—The metabolism of lidocaine was studied using microsomes from extrahepatic tissues of rats, including lung, kidney and brain, or using a reconstituted system with purified CYP2B1 and CYP4B1. Rat pulmonary microsomes metabolized lidocaine to an N-deethylated metabolite, monoethylglycinexylidide (MEGX). Renal microsomes produced MEGX and 3-hydroxylidocaine (3-OH LID), although the rate of MEGX formation was much lower in renal than in pulmonary microsomes. Other metabolites were not detected. Lidocaine was not metabolized by brain microsomes. In extrahepatic tissues, pulmonary microsomes had the highest activity. Hence, two major forms of cytochrome P450 isozymes, CYP2B1 and CYP4B1, in rat pulmonary microsomes were used for further study. The study with a reconstituted system using purified cytochrome P450 isozymes revealed that only CYP2B1 showed lidocaine deethylation activity; the other form of cytochrome P450 in the lung, CYP4B1, did not. The Michaelis–Menten constant for lidocaine N-deethylation by rat pulmonary microsomes was 0.27 mM. Antibody against CYP2B1 completely inhibited the formation of MEGX by pulmonary microsomes. These results suggest that lidocaine is metabolized by rat lung, including CYP2B1.

Key words: cytochrome P450; rat lung; lidocaine

Lidocaine is a local anesthetic and antiarrhythmic drug that is used frequently in the clinic, and it is administered intravenously to patients with cardiac dysfunction. The metabolic fate of lidocaine has been studied extensively in experimental animals [1–5] and humans [4–6] (Fig. 1). Most of the lidocaine administered intravenously is taken up by the lung, transported to the liver, and metabolized by microsomal cytochrome P450 [7]. Intrinsic clearance of lidocaine in the liver is very high, and its metabolism is regulated mainly by hepatic blood flow [8]. Cytochrome P450 in extrahepatic tissues, including lung, kidney and brain, has been studied [9], and extrahepatic metabolism of lidocaine and other xenobiotics has been reported [10–15]. Of the organs mentioned, the lung receives the entire cardiac output and contributes to blood gas exchange, as well as being exposed to airborne organic pollutants. Additionally, pulmonary toxicants and carcinogens are also bioactivated by the monooxygenase system in the lung [16, 17]. These findings suggest the possibility that the lung also contributes to the metabolism of lidocaine.

Results of some studies led to the belief that lidocaine is not metabolized in the lung [18, 19], and some experimental methodologies in regard to the pharmacokinetics of lidocaine in the lung have been based on this hypothesis [7]. In these studies, however, lidocaine metabolism was investigated by thin-layer chromatography, and lidocaine metabolite standards were not utilized. In addition, the content of cytochrome P450 in the lung is very low, and high sensitivity is needed to detect the metabolites [20]. Sensitive experimental techniques could have enabled the authors to detect the small amount of metabolites formed in the lung. Also, experiments regarding the metabolism of lidocaine using the pulmonary microsomal monooxygenase system have not been performed before.

In this paper, we demonstrate the metabolism of lidocaine by rat pulmonary microsomes and compared it with that in other extrahepatic microsomes; finally, we elucidate the cytochrome P450 isozymes involved in the reaction.

MATERIALS AND METHODS

Materials. Lidocaine, MEGX‡ and GX were supplied by the Fujisawa Pharmaceuticals Co., Ltd. (Osaka, Japan). 3-OH LID and Me-OH LID were gifts from S. Fujita of Hokkaido University. DLPC was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.), and NADPH from the Oriental Yeast Co. (Tokyo, Japan). A reverse-phase octadecasilyl column (TSKgel ODS-120T) was purchased from the Tosoh Corp. (Tokyo, Japan). Other reagents and organic solvents were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Preparation of microsomes and purification of cytochrome P450. Male Sprague–Dawley rats

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‡ Abbreviations: MEGX, monoethylglycinexylidide; GX, glycinexylidide; 3-OH LID, 3-hydroxylidocaine; Me-OH LID, methylhydroxylidocaine; DLPC, dilauroylphosphatidylcholine; PB, phenobarbital and IgG, immunoglobulin G.

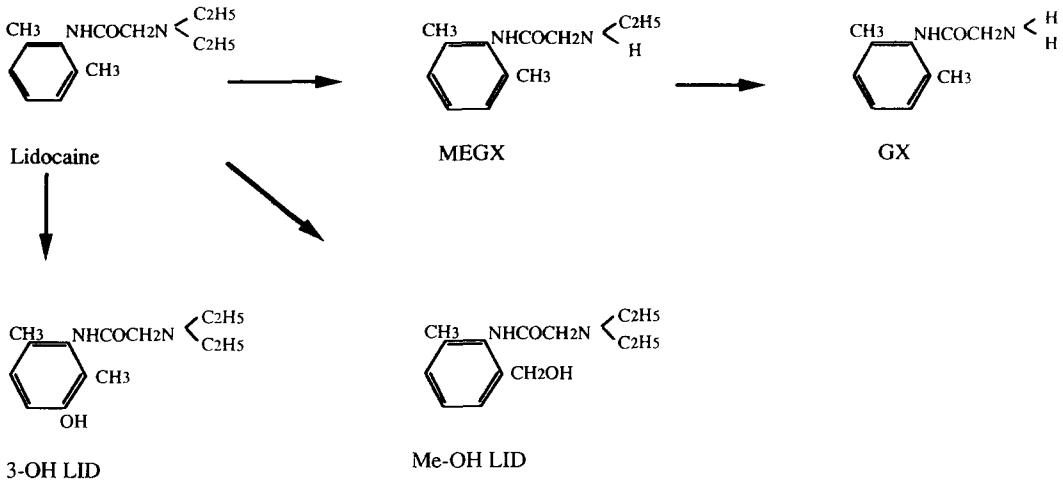

Fig. 1. Lidocaine metabolic pathways by cytochrome P450. Abbreviations: MEGX, monoethylglycinexylidide; GX, glycinexylidide; 3-OH LID, 3-hydroxylidocaine; and Me-OH LID, methylhydroxylidocaine.

weighing 200–250 g were obtained from Nippon Clea (Tokyo, Japan). PB (80 mg/kg, dissolved in saline) was given intraperitoneally daily for 4 days. Microsomes from rat liver, lung, kidney and brain were prepared as reported elsewhere [21, 22]. CYP2B1 and CYP4B1 were purified as described previously [20, 21]. NADPH–cytochrome P450 reductase was purified following the method reported by Yasukochi and Masters [23]. Protein content was measured by the method of Lowry *et al.* [24]. The concentration of pulmonary cytochrome P450 was determined by the method of Matsubara *et al.* [25].

Assay of lidocaine metabolism. The metabolic activity of lidocaine was measured according to a method described previously [3]. The reaction was started by the addition of 20 μL of 10 mM NADPH and carried out in air at 37° for 10 min. Brain microsomes were incubated for 60 min. The reaction was stopped by the addition of 50 μL of 1 N NaOH and the mixture was immediately placed on ice. The metabolites were extracted with 1.5 mL of ethyl acetate. The organic phase was evaporated *in vacuo* at 40°, and the residue was dissolved in 200 μL of 10 mM potassium phosphate buffer (pH 3.0). Then 100 μL of the solution was injected onto a high-performance liquid chromatography apparatus with an ODS-120T column (4.6 × 250 mm). The column was developed by isocratic elution with 10 mM potassium phosphate buffer (pH 3.0) and acetonitrile (9:1). The chromatography was done at a flow rate of 1.2 mL/min at 60°, and the metabolites were monitored at 214 nm. The amount of metabolites was calculated from the peak area with a data processor (C-R4A, Shimadzu, Kyoto, Japan). Catalytic activities of all enzyme preparations were assayed under conditions in which the metabolism was proportional to the cytochrome P450 concentrations and time of incubation. The amounts of NADPH-cytochrome P450 reductase and DLPC were optimum for the purified hemoproteins, and the NADPH concentration was saturable.

Other methods. Antibody against purified CYP2B1 was raised in a rabbit, and IgG was prepared as reported previously [26]. The raised antibody reacts specifically with CYP2B1 and CYP2B2, but not other cytochrome P450 isozymes. CYP2B2 has not been detected in rat lung [27, 28]. The immunoblotting study was carried out using 7.5% polyacrylamide gel, and protein was transferred to a nitrocellulose sheet with a horizontal blotting system (AE-8350, Atto Co., Tokyo, Japan) [29, 30]. The nitrocellulose sheet was stained with antibody against CYP2B1 using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, U.S.A.) [27].

The effect of antibody against CYP2B1 on MEGX formation in microsomes from PB-treated rat liver and from untreated rat lung was studied following a method described previously [27].

For statistical analysis, an unpaired *t*-test was used, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Metabolism of lidocaine by microsomes. The specific content of cytochrome P450 in microsomes from rat lung, kidney, and liver tissues, and the lidocaine metabolic activity of those microsomes, are shown in Table 1. Cytochrome P450 content in pulmonary microsomes from rats treated with PB did not differ from that in untreated rats. MEGX was the only metabolite detected when lidocaine was incubated with pulmonary microsomes; GX (not shown), 3-OH LID and Me-OH LID were not formed. Lidocaine N-deethylation activities in pulmonary microsomes from untreated and PB-treated rats were 0.87 ± 0.11 and 0.98 ± 0.21 nmol/min/mg protein, respectively. There was no significant difference in turnover rates of MEGX between these two groups of microsomes; they were 18 and 20% of the value obtained by microsomes from untreated rat liver. These results suggest that cytochrome P450 isozymes, which catalyze the formation of 3-OH LID and Me-OH LID, are not involved in the pulmonary microsomes. Also, cytochrome P450 isozymes that catalyze conversion of lidocaine to MEGX were not induced by PB.

Table 1. Specific content of cytochrome P450 and lidocaine metabolic activity in rat microsomes

Microsomes	Content of P450 (pmol/mg protein)	Lidocaine metabolites (nmol/min/mg)		
		MEGX	3-OH LID	Me-OH LID
Untreated lung	30 ± 12	0.87 ± 0.11	—	—
PB-treated lung	20 ± 6	0.98 ± 0.21	—	—
Kidney	118 ± 15	0.024 ± 0.004*	0.022 ± 0.001	—
Untreated liver	485 ± 92	4.84 ± 1.31	0.64 ± 0.19	0.24 ± 0.09
PB-treated liver	1612 ± 145	11.38 ± 1.54	0.50 ± 0.13	2.13 ± 0.24

The content of cytochrome P450 is expressed as picomoles per milligram of microsomal protein (mean ± SD of 5–10 experiments). The reaction mixture, containing 500 μg of microsomal protein from liver, lung and kidney and 0.2 μmol NADPH, in a final volume of 0.5 mL was incubated for 10 min at 37° in 0.1 M potassium phosphate buffer, pH 7.4. Lidocaine metabolites were extracted with ethyl acetate and analyzed by HPLC; values are expressed as nanomoles of product per minute per milligram of microsomal protein (mean ± SD of 5–10 experiments). Catalytic activities of less than 0.01 nmol of product/min/mg protein are expressed as "—".

Abbreviations: MEGX, monoethylglycinexylidide; 3-OH LID, 3-hydroxylidocaine; Me-OH LID, methylhydroxylidocaine; and PB, phenobarbital.

* $P < 0.01$, compared with values obtained from untreated and PB-treated rat lung.

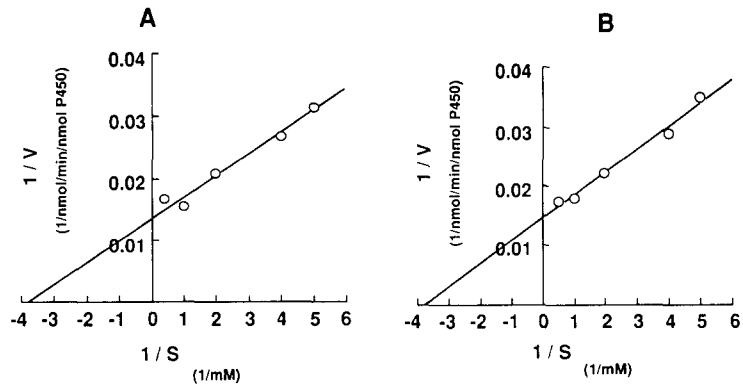

Fig. 2. Lineweaver–Burk plots of the rates of lidocaine N-deethylation by microsomes from untreated rat lung (A), and PB-treated rat liver (B). The reaction mixture contained 500 μg of microsomal protein and 0.2 μmol of NADPH, in a final volume of 0.5 mL. Substrate concentration varied from 0.2 to 2.0 mM. The reaction mixture was incubated for 10 min at 37° in 0.1 M potassium phosphate buffer, pH 7.4. For microsomes from untreated rat lung, K_m and V_{max} values were 0.27 mM and 75 nmol/min/nmol of cytochrome P450, respectively. For microsomes from PB-treated rat liver, K_m and V_{max} values were 0.27 mM and 68 nmol/min/nmol of cytochrome P450, respectively. The linear regression correlation coefficients were 0.983 for microsomes from untreated rat lung and 0.984 for microsomes from PB-treated rat liver.

Table 2. Lidocaine metabolic activity of purified cytochrome P450

Purified cytochrome P450	Lidocaine metabolites (nmol/min/nmol P450)		
	MEGX	3-OH LID	Me-OH LID
CYP2C11	73.2	—	—
CYP2B1	74.1	—	—
CYP4B1	—	—	—

For lidocaine metabolism, the reaction mixture was the same as in the footnote of Table 1, except for a reconstituted system containing 30 pmol of purified cytochrome P450, 0.3 units of NADPH–cytochrome P450 reductase, and 5 μg of dilauroylphosphatidylcholine being used in place of the microsomes. Values are expressed as nanomoles of product per minute per nanomole of cytochrome P450. Metabolites were assayed by a comparison of peak areas monitored at 214 nm with those of authentic samples. Catalytic activities of less than 0.1 nmol of product/min/nmol of cytochrome P450 are expressed as "—".

MEGX and 3-OH LID were formed by kidney microsomes. However, these microsomes formed MEGX at a rate much lower than lung microsomes. The kidney-microsome rate was 0.5% of the value obtained from untreated rat liver microsomes, suggesting that the kidney was minimally involved in the metabolism of lidocaine in the body. Microsomes from rat brain did not form any metabolites. These results indicate that lidocaine is metabolized to MEGX predominantly by *pulmonary* microsomes among extrahepatic organs in the rat, although metabolic activity is lower in pulmonary than in hepatic microsomes.

Analysis by Lineweaver–Burk plots of pulmonary microsome metabolism. The major constitutive forms of cytochrome P450 isozymes in rat pulmonary microsomes are CYP2B1 and CYP4B1 [20, 27]. CYP2B1 is also the major PB-inducible cytochrome P450 in rat liver [31]. We performed kinetic studies using microsomes from untreated rat lung, and compared the kinetic parameters with those obtained from PB-treated rat liver microsomes. The maximum rate of metabolism (V_{max}) and the Michaelis–Menten constant (K_m) for the formation of MEGX from lidocaine were determined by linear regression from Lineweaver–Burk double-reciprocal plots. Single-phase straight curves were obtained with pulmonary microsomes from untreated rats, suggesting that a single cytochrome P450 isozyme is involved in this reaction (Fig. 2A). The K_m value for microsomes from untreated rat lung was 0.27 mM, which was comparable with the K_m value obtained simultaneously for hepatic microsomes from PB-treated rat liver (Fig. 2B). These results suggest that common forms of cytochrome P450 isozymes in microsomes from untreated rat lung and PB-treated rat liver are involved in lidocaine N-deethylation.

Metabolism of lidocaine by purified CYP2B1 and CYP4B1. We studied the metabolism of lidocaine by the two constitutive forms of cytochrome P450 isozymes in pulmonary microsomes, CYP2B1 and CYP4B1, in a reconstituted system. MEGX was the only metabolite when lidocaine was metabolized by CYP2B1. The turnover rate of the formation of MEGX by CYP2B1 was 74.1 nmol/min/nmol of cytochrome P450 when 1 mM was used as the substrate concentration. Lidocaine was not metabolized by CYP4B1 (Table 2). In the kinetic study, the K_m value of CYP2B1 for lidocaine N-deethylation was 0.52 mM (Fig. 3). These results were consistent with those obtained from pulmonary microsomes, suggesting that CYP2B1 is the isozyme that catalyzes lidocaine to MEGX in rat pulmonary microsomes.

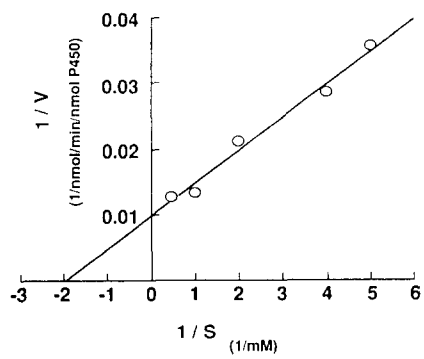

Fig. 3. Lineweaver–Burk plots of the rates of lidocaine N-deethylation by CYP2B1. The reconstituted system contained 30 pmol purified cytochrome P450, 0.3 units NADPH–cytochrome P450 reductase, 5 μg DLPC, 0.2 μmol NADPH and 0.2 to 2.0 mM lidocaine. The reaction mixture (0.5 mL) was incubated for 10 min at 37° in 0.1 M potassium phosphate buffer, pH 7.4. K_m and V_{max} values for lidocaine N-deethylation were 0.52 mM and 103 nmol/min/nmol of cytochrome P450, respectively. The linear regression correlation coefficients was 0.954.

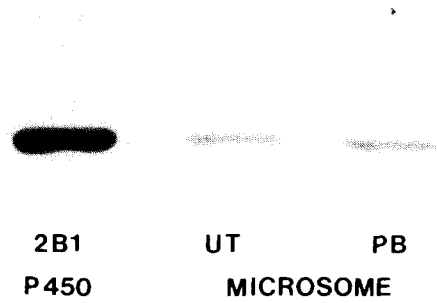

Fig. 4. Immunoblots of microsomes from untreated rat lung and of purified CYP2B1 stained with antibody against CYP2B1. Lane 1 contained purified CYP2B1 (0.6 pmol), and lanes 2 and 3 contained microsomes from untreated (UT) rat lung (30 μg protein) and PB-treated rat liver (30 μg protein), respectively. Microsomes and purified cytochrome P450 were separated by electrophoresis with 7.5% polyacrylamide gels and transferred electrophoretically to a nitrocellulose membrane. The nitrocellulose was stained with antibody against CYP2B1.

Immunoblotting analysis and immunoinhibition study on pulmonary microsomes. Immunoblots of purified CYP2B1 and pulmonary microsomes stained with antibody against CYP2B1 are shown in Fig. 4. Antibody against CYP2B1 gave a single band with pulmonary microsomes, and this protein showed the same mobility as that of CYP2B1 on SDS–PAGE. The content of CYP2B1, assayed by densitometry of the nitrocellulose after blotting from the polyacrylamide gel and immunochemical staining, was 13 ± 5 pmol/mg protein. It represented 43% of the whole amount of cytochrome P450 measured spectrally in the pulmonary microsomes (Table 1).

The antibody against CYP2B1 inhibited the formation of MEGX by hepatic microsomes from PB-treated rats by approximately 70%, and completely inhibited the formation of MEGX by pulmonary microsomes from untreated rats, suggesting that the N-deethylation of lidocaine by rat pulmonary microsomes is catalyzed exclusively by CYP2B1 (Fig. 5). These results are consistent with those obtained by pulmonary microsomes from untreated rats, by hepatic microsomes from rats treated with PB, and by the reconstituted system with CYP2B1 and CYP4B1.

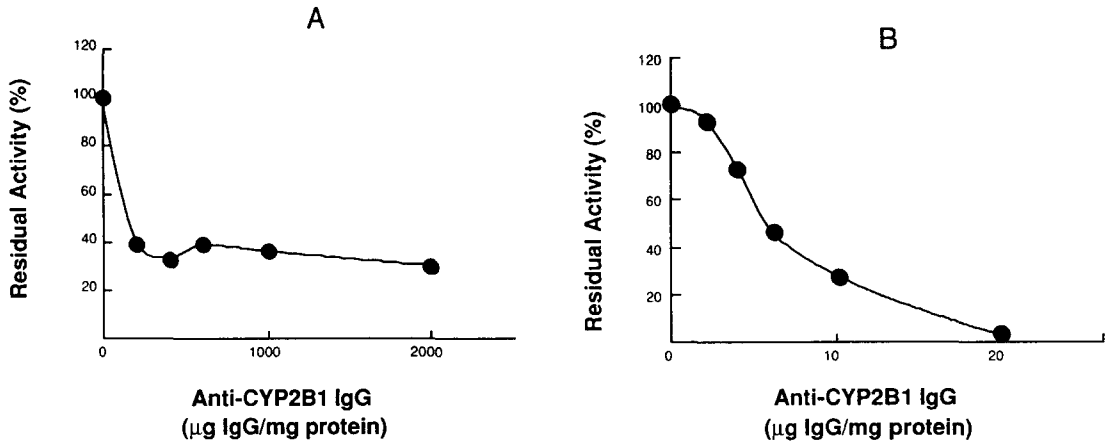

Fig. 5. Effect of antibody against CYP2B1 on the formation of MEGX. (A) MEGX formation rate in microsomes from PB-treated rat liver. (B) MEGX formation rate in microsomes from untreated rat lung. The designated amount of IgG was preincubated with microsomes from PB-treated rat liver (containing 30 pmol cytochrome P450) and from untreated rat lung (containing 30 pmol cytochrome P450) for 30 min at room temperature. Residual activity was expressed as a percentage of the value measured with control rabbit IgG. Turnover rates of MEGX in microsomes from PB-treated rat liver and from untreated rat lung were 11.0 and 0.90 nmol/min/mg of protein, respectively.

DISCUSSION

In daily practice, lidocaine is administered intravenously to prevent arrhythmias. Sometimes it is administered intratracheally to depress coughing during anesthesia and for resuscitation [32]. Recently, significant changes of lidocaine pharmacokinetics have been reported in animals with right-to-left cardiac shunt [33], and MEGX has also been detected after intratracheal administration of lidocaine [34]. In addition, cytochrome P450 in the lung has also been observed to have catalytic activities towards various xenobiotics [9, 17]. These reports led to our speculation that lidocaine could be metabolized by the lung.

In this study, we prepared microsomes and purified cytochrome P450 isozymes from the lung and other tissues and studied their metabolism of lidocaine. Rat pulmonary microsomes had high activity, but renal and brain microsomes had much lower activity. In a reconstituted system with a major pulmonary cytochrome P450, CYP2B1 metabolized lidocaine to MEGX, whereas CYP4B1 did not. The K_m value of CYP2B1 for lidocaine N-deethylation was 0.52 mM, which was higher than the value obtained with rat pulmonary microsomes. This disparity could have been due to the experimental conditions, such as the lipid content of the reconstituted systems, because the composition of lipids influences the metabolism of lidocaine by purified cytochrome P450 isozymes [5]. Microsomes from rat lung mainly contain CYP2B1 and CYP4B1 [20]. CYP4B1 is the major constitutive form of cytochrome P450 that shows catalytic activity toward lauric acid ω-hydroxylation [20]. CYP2B1 content is very low in microsomes from untreated rat liver, and CYP4B1 has not been found in those microsomes [35]. This suggests that cytochrome P450 isozymes show an organ specificity as well as a species specificity.

MEGX formation in rat pulmonary microsomes was inhibited completely by antibody against CYP2B1, suggesting that lidocaine is N-deethylated by CYP2B1 in the rat lung to form MEGX.

In the human liver, lidocaine is metabolized to MEGX by CYP3A4, which belongs to the same gene family as CYP3A2, a major form of cytochrome P450 in rat liver. CYP3A2 also forms MEGX from lidocaine in rats [5, 6]. In this study, we found CYP2B1 to be a major enzyme that metabolizes lidocaine in rat lung. These results suggest that the lung contributes to the metabolism of lidocaine in humans as well as in rats because the whole volume of lidocaine passes through the lung immediately after intravenous administration, although microsomes from the lung show less lidocaine catalytic activity than microsomes from the liver.

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