EFFECT OF PHENOBARBITAL ON THE PHARMACOKINETICS OF LIDOCAINE, MONOETHYLGLYCINEXYLIDIDE AND 3-HYDROXYLIDOCAINE IN THE RAT: CORRELATION WITH P450 ISOFORM LEVELS

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ABSTRACT:

To elucidate the effect of cytochrome P450 levels in hepatic microsomes on the metabolism of lidocaine in vivo, we investigated the metabolism of lidocaine in untreated (UT group) and phenobarbital-treated rats (PB group) in vivo and compared the results with those obtained by immunoblotting of rat hepatic microsomes. There were no differences in pharmacokinetic parameters for lidocaine between the UT and PB groups. The plasma concentrations of the N-deethylated metabolite of lidocaine, monoethyl-glycinexylidide (MEGX), in the PB group were significantly higher than those in the UT group. On the other hand, the plasma concentrations of the aromatic ring hydroxylated metabolite of lidocaine, 3-hydroxy-lidocaine (3-OH LID), were significantly lower in the PB group than in the UT group. When lidocaine metabolism was studied with hepatic microsomes prepared from rats in the UT and PB groups, the rates of formation of MEGX were higher in the microsomes of the PB group than in those of the UT group. The contents of CYP2B1 and 3A2 in rat hepatic microsomes of the PB group measured by immunoblotting were significantly higher than those of the UT group. Strong correlations were found between the area under the plasma concentration vs. time curve for MEGX and specific contents of CYP2B1 and 3A2. These findings suggest that formation of MEGX in vivo is dependent on the levels of CYP2B1 or 3A2 in rat liver.

Lidocaine is a widely used local anesthetic and antiarrhythmic drug. Lidocaine is metabolized by P450, a hepatic microsomal enzyme, to the primary metabolites MEGX and 3-OH LID (1, 2). In previous studies we have elucidated the metabolism of lidocaine by purified P450 and by microsomes and have found that CYP3A is the major isoform involved in the metabolism of lidocaine in both rats and humans (1, 2). Although many reports describe the metabolism of lidocaine (1–6), most have been performed following either of two different experimental procedures: biochemical reaction with microsomal P450 in vitro, or pharmacokinetic analysis in vivo in animals. P450 isozymes involved in the specific metabolic pathways of lidocaine have been elucidated in vitro studies, although contribution of each P450 isoform to the metabolism of lidocaine in the body has not been proved (1, 2, 5, 6). Metabolism of lidocaine has also studied in vivo (3, 4), while relationships between pharmacokinetics of lidocaine and its metabolites, and levels of each P450 isozone have been remained unclear.

CYP3A is a major P450 isoform in humans as well as rats (7, 8) and is involved in the metabolism of lidocaine to its major metabolite, MEGX, which has the same degree of central nervous and cardiovascular toxicity as lidocaine (1, 2, 6, 9). CYP3A is inducible in human liver by various drugs including barbiturates, steroids, and macloride antibiotics (8), suggesting that metabolism of lidocaine is also influenced by these agents. Moreover, MEGX may be used as a reliable indicator of liver function in patients with chronic liver disease and following hepatic transplantation (10, 11). However, the pharmacokinetics of lidocaine are also influenced by many factors such as age, protein binding, and hepatic function, and do not always correlate with the results obtained with microsomal P450 (12, 13). Therefore, investigation of the relationship between the metabolism of lidocaine in vivo and the levels of P450 isoforms in the liver microsomes is of clinical importance.

In this study, we used phenobarbital, a P450 inducer, to investigate the relationships between P450 levels and plasma concentrations of lidocaine and its primary metabolites and then evaluated the contribution of P450 isoforms to the metabolism of lidocaine both in vivo and in vitro.

Materials and Methods

Chemicals. Lidocaine, MEGX, and 2’6’-pinecoloxylidide were supplied by Fujisawa Pharmaceuticals Co., Ltd. (Osaka, Japan). 3-OH LID was a kind gift from Dr. Fujita, Hokkaido University. Ethyl acetate and acetonitrile were purchased from Wako Pure Chemicals (Tokyo, Japan). Other reagents and organic solvents were obtained from Wako Pure Chemicals.

Animals. Male Sprague-Dawley rats aged 8–9 weeks (Nihon Clea, Tokyo, Japan) were weighed 250–300 g and maintained on a 12-hr light/dark cycle with food and water available ad libitum. Rats were fasted before the experiments for 24 hr. Phenobarbital was dissolved in saline (80 mg/ml) and administered intraperitoneally (80 mg/kg) for 4 days consecutively before experiments (PB group, N = 8). Other rats were administered the same volume of saline as those in the PB group (UT group, N = 8).
Lidocaine was infused at 1 mg/kg body weight per minute for 10 min. There were no differences in the concentration of lidocaine between the untreated and phenobarbital-treated groups. The concentration of monoethylglycinexylidide was significantly higher and the concentration of 3-hydroxylidocaine significantly lower in the phenobarbital-treated group than in the untreated group (p < 0.05, respectively).

Values are presented as mean ± SE.

In vivo study. All rats were anesthetized with pentobarbital (25 mg/kg, intraperitoneally), and tracheostomy was performed. They were mechanically ventilated with an animal ventilator (SN-480–7: Shimon, Tokyo, Japan) to maintain arterial oxygen and carbon dioxide tensions within the normal range. Base deficits in the blood were corrected with sodium bicarbonate and the pH of arterial blood was adjusted to 7.4. Polyelectrolyte catheters were inserted into the carotid artery and jugular vein for monitoring of arterial blood pressure and central venous pressure, respectively. Catheters were inserted into the femoral artery and vein for blood sampling and drug infusion, respectively. Rectal temperature was maintained at 37°C with an infrared ray lamp.

Infusion of lidocaine was started more than 20 min after completion of animal preparation and stabilization of hemodynamics. Lidocaine (3 mg/ml in saline) was administered at 1 mg/kg/min for 10 min intravenously with a syringe pump (CVF-3100: Nihon Koden, Tokyo, Japan). Blood samples (0.3 ml) were collected via the femoral artery and placed in heparinized microcentrifuge tubes. The blood volume removed from the animals was replaced with an equal volume of blood containing heparin to avoid the circulatory disturbances induced by blood loss. Plasma obtained by centrifuging blood at 3,000 rpm for 10 min was stored at −30°C until analysis. The blood sampling times were 0, 5, 10, 11, 12, 15, 17, 20, 25, 35, 45, 60, 75, 90, 120, 150, and 180 min after initiation of lidocaine infusion.

In vitro study. After the experiments described above, the liver was perfused with 1.15% KCl buffer (pH 7.4), and microsomes were prepared by differential centrifugation as described by Funae and Imaoka (7). Microsomal protein were assayed using the method of Lowry et al (14). Hepatic P450 contents were assayed using the method described by Omura and Sato (15). Rates of formation of MEGX and 3-OH LID by rat hepatic microsomes were measured using a method described previously (1). The concentrations of lidocaine in the microsomal reaction mixtures were 10 and 100 μM, and were comparable with those in plasma within the therapeutic range. Hepatic microsomes were also reacted with lidocaine (100 μM) at 37°C for 30 min with or without UDP-glucuronic acid (1.5 mM) by the condition described by Mulder (16).

HPLC analysis of lidocaine and its metabolites in blood samples. Concentrations of lidocaine and its metabolites, MEGX and 3-OH LID, in the blood samples were measured using the method described by Kawai et al. (3) with small modifications. Briefly, 150 μl of serum was used in place of whole blood, and HPLC was performed with a mobile phase consisting of acetonitrile : potassium phosphate buffer (10 mM, pH 3.0), 1:9 (v/v), at a flow rate of 1.0 ml/min and column temperature at 45°C. 2’6’-dipropoxyliidide was used as the internal standard. The retention times of lidocaine, MEGX, and 3-OH LID were 24.4, 15.3 and 7.4 min, respectively. Analytical recoveries of lidocaine and metabolites were above 90%, and the limit of detection was 0.01 μg/ml. Calibration curves for lidocaine and metabolites were linear throughout the range studied. The coefficients of variation were 8.3, 8.5 and 7.0% at 0.02 μg/ml, and 4.0, 4.5, and 3.8% at 2 μg/ml for lidocaine, MEGX and 3-OH LID, respectively.

Other methods. Antibodies against rat CYP1A2, 2B1, 2C11, 2D1, and 3A2 were raised in a rabbit and were characterized as previously described (17). Levels of P450 isoforms were measured by immunoblotting as previously described (18, 19).

Data analysis. All values given are mean values ± SE. The pharmacokinetics of lidocaine were analyzed with a two-compartment model, as described in a previous study (20). The AUC0-t values were calculated using linear trapezoidal approximation. Clearance was defined as administered dose divided by AUC. The significance of differences in plasma concentrations of lidocaine and metabolites were determined by repeated-measures ANOVA. The significance of correlations between the AUC of MEGX and contents of P450s were examined by linear least-squares correlation analysis. Other data were analyzed by ANOVA, followed by Bonferroni corrected t-tests when appropriate. Differences were considered statistically significant when p values were less than 0.05.

RESULTS

Plasma concentrations of lidocaine and its metabolites. During experiments, blood pressure and central venous pressure were stable in all rats, and there were no differences in these hemodynamic parameters between the UT and PB groups. In our preliminary study, a decrease in arterial oxygen tension and increase in arterial carbon dioxide tension each induced significant changes in blood pressure and heart rate, and there was a tendency for hypotension to be induced by withdrawing 0.3 ml of blood from the femoral artery under general anesthesia with pentobarbital. We maintained arterial oxygen and carbon dioxide tensions within the normal range, and blood loss was replaced by the same volume of whole blood to avoid circulatory disturbances possibly affecting hepatic blood flow. There were no differences in the plasma concentrations of lidocaine, the pharmacokinetic parameters including distribution and elimination half-times, or the clearance of lidocaine between the UT and PB groups (fig. 1, table 1). Plasma concentrations of MEGX were significantly higher in the PB group than in the UT group (p < 0.05, fig. 1). The AUC of MEGX was also significantly larger in the PB group than in the UT group (p < 0.01, table 1). The plasma concentration and AUC of 3-OH LID were significantly lower in the PB group than in the UT group (p < 0.05 and 0.01, respectively, fig. 1 and table 1).
Metabolism of lidocaine by rat hepatic microsomes. The specific contents of P450 in hepatic microsomes prepared from rats in the UT and PB groups were 0.43 ± 0.06 and 0.95 ± 0.10 nmol/mg of protein, respectively. The rates of formation of MEGX were significantly higher in microsomes from rats in the PB group than in microsomes from the UT group at substrate concentrations of 10 and 100 μM (p < 0.001, table 2). The plasma concentrations of MEGX were significantly higher in the PB group than in the UT group (fig. 1). These findings suggest that P450 isoforms involved in the metabolism of lidocaine to MEGX were induced in the hepatic microsomes in the PB group.

On the contrary, there were no differences in the rates of formation of 3-OH LID between hepatic microsomes from rats of the UT and PB groups, although plasma concentrations of 3-OH LID in the PB group were significantly lower than those in the UT group (p < 0.01, table 1). These results suggest that 3-OH LID is formed predominantly by CYP3A2 and 2B1 in hepatic microsomes from untreated rats (fig. 2). This hypothesis is further supported by the finding that the formation of 3-OH LID between hepatic microsomes from rats of the PB group was significantly lower than that in the UT group (fig. 1). These results suggest that P450 isoforms involved in the metabolism of lidocaine to MEGX were induced in the hepatic microsomes in the PB group.

Correlations between plasma concentration of MEGX and levels of P450 isoforms in hepatic microsomes. Levels of P450 isoforms in rat hepatic microsomes are presented in table 3. Levels of CYP2B1 and 3A2 were significantly increased by treatment with phenobarbital (p < 0.001). On the other hand, there were no differences in the levels of CYP2C11 in hepatic microsomes between the UT and PB groups. Levels of CYP1A2 and 2D1 were slightly lower in the PB group than in the UT group, although the difference between groups was not statistically significant. There were significant correlations between the AUC for MEGX and levels of CYP2B1 and 3A2 (r = 0.741 and r = 0.737, p < 0.01, respectively; figs. 3A and B), whereas there were no correlations between the AUC for MEGX and levels of CYP1A2, 2C11, and 2D1 (figs. 3C, D, and E).

Discussion
Numerous studies of the metabolism of lidocaine have been performed (1–6). We have found that MEGX is the major metabolite of lidocaine and that it is formed predominantly by CYP3A2 and 2B1 in hepatic microsomes from untreated and phenobarbital-treated rats, respectively (1). Although DiFazio and Brown (4) reported increased plasma concentrations of MEGX in dogs chronically treated with...
phenobarbital, the relationships between MEGX concentration and levels of P450 isoforms have not been investigated. In our study strong correlations were found between the AUC for MEGX and levels of CYP3A2 and 2B1 in rats, suggesting that the rate of formation of MEGX in vivo is reflected by levels of P450 isoforms. In untreated rats lidocaine would be metabolized to MEGX by CYP3A2 since contents of CYP2B1 are very low in the liver of the UT group, as shown in the present study and in our previous study (7). Content of CYP2B1 increased remarkably following treatment with phenobarbital and was comparable with that of CYP3A2, suggesting that CYP2B1, as well as CYP3A2, is responsible for the formation of MEGX in the PB group.

In the present study, regression lines describing the relationships between specific contents of CYP2B1, 3A2, and the AUC for MEGX did not pass near the origin, suggesting that other enzymes in microsomes are involved in the formation of MEGX from lidocaine in rats. These findings suggest that CYP2B1 and 3A2 are involved in the metabolism of lidocaine to MEGX in rat liver and are induced by phenobarbital, resulting in the remarkably increased plasma concentrations of MEGX in the PB group.

Recently, the rates of formation of MEGX following intravenous administration of lidocaine has been used as a reliable indicator of liver function in patients with chronic liver disease and following hepatic transplantation (10, 11). As in rats, the CYP3A form is involved in the metabolism of lidocaine to MEGX in the human liver (2, 6), suggesting that the rates of formation of MEGX in humans would be expected to be influenced by levels of CYP3A.

The hepatic extraction ratio of lidocaine is high, the blood concentration of lidocaine depends primarily on hepatic blood flow (12), and hepatic blood flow is not influenced by phenobarbital pretreatment in rats anesthetized with intraperitoneally administered pentobarbital (21). Intraperitoneally administered pentobarbital does not affect lidocaine metabolism either (22). Correspondingly, in the present study there were no differences in the concentrations of lidocaine between the UT and PB groups, indicating that the pharmacokinetics of lidocaine are not affected by P450 levels.

The plasma concentrations of 3-OH LID were lower in the PB group than in the UT group, but the rates of formation of 3-OH LID from lidocaine with microsomes from rats in the PB group did not differ from that with microsomes from the UT group. The decreased plasma concentrations of 3-OH LID in the PB group would have resulted from increased UDP-glucuronyl transferase activity since 3-OH LID is conjugated immediately after its formation in the plasma and UDP-glucuronyl transferase activity is remarkably increased by phenobarbital (3, 16). In our present study formation rates of 3-OH LID were significantly decreased by adding UDP-glucuronic acid to the reaction mixture containing microsomes from rats in the PB group although they were not influenced in the UT group. These results support our hypothesis.

In conclusion, we have shown that the rates of formation of the major metabolite of lidocaine, MEGX, in vivo are reflected by the levels of specific hepatic P450 isoforms. These findings may contribute to speculations of lidocaine metabolism in vivo from hepatic levels of P450 isoforms.

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References


