Biochemical Pharmacology, Vol. 46, No. 10, pp. 1867–1869, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

Participation of the CYP2D subfamily in lidocaine 3-hydroxylation and formation of a reactive metabolite covalently bound to liver microsomal protein in rats

(*Received* 10 *June* 1993; *accepted* 3 *August* 1993)

Abstract—Lidocaine metabolism was investigated in rat liver microsomes and in a reconstituted system containing P450BTL, a cytochrome (P450) isozyme belonging to the CYP2D subfamily (Suzuki *et al.*, *Drug Metab Dispos* **20**: 367–373, 1992). P450BTL biotransformed lidocaine into 3-hydroxylidocaine (3-OH-LID) but not monoethylglycinexylidide and 2-methylhydroxylidocaine, in the reconstituted system including NADPH-P450 reductase and dilauroylphosphatidylcholine. An antibody against P450BTL inhibited microsomal lidocaine 3-hydroxylase activity by 97%. Thus, P450BTL and/or its immunorelated P450 isozyme(s) belonging to the CYP2D subfamily appear to be involved in lidocaine 3-hydroxylation. Furthermore, the antibody also suppressed the amounts of a lidocaine metabolite(s) bound to microsomal protein. These results suggest that the CYP2D subfamily biotransformed lidocaine into 3-OH-LID via an epoxy intermediate, which binds to microsomal macromolecules.

Lidocaine is a local anesthetic and antiarrhythmic drug widely used clinically. It is primarily metabolized via N-deethylation, aromatic 3-hydroxylation and 2-methylhydroxylation to form monoethylglycinexylidide (MEGX*), 3-hydroxylidocaine (3-OH-LID) and methylhydroxylidocaine (Me-OH-LID), respectively, in rat liver microsomes [1–4]. Multiple species of cytochrome P450 (P450) isozyme were shown to be involved in lidocaine N-deethylation, i.e. eight forms of P450 purified from rat liver microsomes showed dealkylation activity in the reconstituted system, and higher activities were noted for CYP1A2, CYP2B1, CYP2C11 and CYP3A2, while 3-hydroxylase and methylhydroxylase activities were observed only for CYP1A2 and CYP2B2, respectively [5, 6]. It was also observed that antibodies against CYP2C11 and CYP3A2 partially suppressed lidocaine N-deethylation in liver microsomes from untreated rats [5, 6].

We reported that lidocaine 3-hydroxylation was selectively deficient in the Dark Agouti rat known as a poor-metabolizer animal model for debrisoquine 4-hydroxylation, suggesting that the CYP2D subfamily is involved in lidocaine 3-hydroxylation [7]. In addition, we found that a chemically reactive species which bound to liver microsomal protein was formed through lidocaine metabolism, and that a CYP2D isozyme(s) might be involved in the metabolic activation [8]. However no direct evidence with any CYP2D isozyme or antibody has yet been available for the involvement of the isozyme(s). Recently, we have purified a CYP2D isozyme, named P450BTL, which was very active in 4-hydroxylations of debrisoquine and bunitrolol [9]. In the present study, we investigated the catalytic activity of P450BTL toward lidocaine in a reconstituted system containing NADPH-P450 reductase and dilauroylphosphatidylcholine (DLPC), and the effects of the antibody raised against P450BTL on metabolism and activation of lidocaine in rat liver microsomes.

Materials and Methods

Chemicals. Lidocaine HCl and DLPC were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). MEGX, 3-OH-LID and Me-OH-LID were synthesized as hydrochlorides by the methods described previously [3, 4]. [^{14}C]Lidocaine HCl labelled at the carbonyl position (sp. act. 2 GBq/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). The radiochemical purity of the compound was at least 99.0%. Glucose 6-phosphate (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PDH) and NADPH were purchased from the Oriental Yeast Co. (Tokyo, Japan). Other chemicals used were of analytical grade.

Preparation of hepatic microsomes and purification of P450BTL. Male Wistar rats (2 months old) were obtained from the Takasugi Experimental Animals (Kasukabe, Japan). Hepatic microsomal fractions were prepared according to the method of Omura and Sato [10]. Protein concentrations were assayed by the method of Lowry *et al.* [11]. P450BTL was purified from hepatic microsomes of male Sprague–Dawley rats (2 months old, the Shizuoka Laboratory Co., Shizuoka, Japan) as described elsewhere [9]. It had a high debrisoquine 4-hydroxylase activity (2.22 and 5.63 nmol/min/nmol P450 at substrate concentrations of 50 μM and 2 mM, respectively) and its N-terminal amino acid sequence (GLLIGXDLMAVVXFXAIXLL) was similar to CYP2D2 [9]. An antibody against P450BTL was raised in female Japanese white rabbits (3 months old, the Takasugi Experimental Animals) and its immunoglobulin G (IgG) fraction was prepared according to the reported method [9]. The anti-P450BTL IgG inhibited microsomal debrisoquine 4-hydroxylase [9] and imipramine 2-hydroxylase activities almost completely but not imipramine *N*-demethylase or phenacetin *O*-deethylase activity (unpublished observation). NADPH-P450 reductase was purified from liver microsomes of phenobarbital-treated male Wistar rats by the published method [12].

Incubation of lidocaine in a reconstituted system or a microsomal reaction mixture. A reconstituted system consisted of 50 pmol P450BTL, 0.5 U NADPH-P450 reductase, 0.1 mg sodium cholate, 5 μg DLPC, 5 mM G-6-P, 1 U/mL G-6-PDH, 1 mM NADPH, 5 mM $MgCl_2$, 5 μM $MnCl_2$ and 10 μM or 2 mM lidocaine in 50 mM Tris–HCl buffer (pH 7.4). After preincubation of the mixture without NADPH for 5 min at 37°, incubation was started by adding NADPH, and was carried out in air for 5 min. A 1 mL microsomal reaction mixture contained 1 mg microsomal protein, 5 mM G-6-P, 1 U/mL G-6-PDH, 1 mM NADPH, 5 mM $MgCl_2$, 5 μM $MnCl_2$, 0–5 mg preimmune IgG or anti-P450BTL IgG and 15 μM ^{14}C-labelled or unlabelled lidocaine in 50 mM Tris–HCl buffer (pH 7.4). The IgG fractions were preincubated with microsomes at 25° for 30 min, followed by adding other components of the

* Abbreviations: MEGX, monoethylglycinexylidine; 3-OH-LID, 3-hydroxylidocaine; Me-OH-LID, methylhydroxylidocaine; P450, cytochrome P450; DLPC, dilauroylphosphatidylcholine; G-6-P, glucose 6-phosphate; G-6-PDH, glucose 6-phosphate dehydrogenase; IgG, immunoglobulin G.

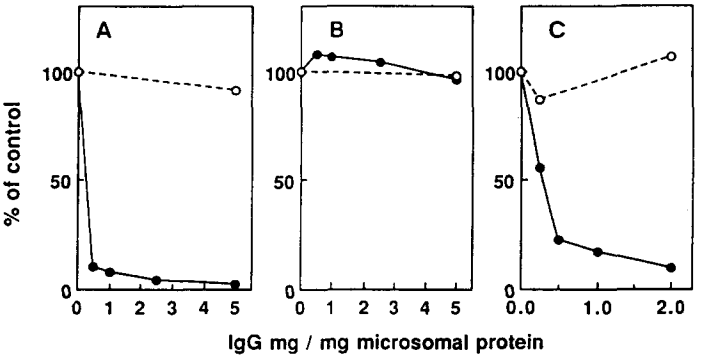

Fig. 1. Effects of anti-P450BTL IgG on lidocaine metabolism and covalent binding of a ^{14}C-metabolite to rat liver microsomes. Hepatic microsomes of male Wistar rats were preincubated with anti-P450BTL IgG (●) or preimmune IgG (○) at 25° for 30 min, and followed by incubation with ^{14}C-labelled or unlabelled lidocaine under the conditions described in Materials and Methods. A, B and C show lidocaine 3-hydroxylase, *N*-deethylase activities and covalent binding of a [^{14}C]lidocaine metabolite to microsomal protein, respectively. Results are expressed as per cent of the values in the absence of the IgG fraction. The values of A, B and C in the absence of the IgG fraction were 0.295, 0.908 nmol/min/mg protein and 3.46 pmol bound/min/mg protein, respectively.

reaction mixture. The incubation was carried out at 37° for 2.5 min when metabolic activities were determined, and for 15 min when covalent binding was determined.

Assays of lidocaine metabolites and determination of covalent binding of radioactivity. MEGX, 3-OH-LID and Me-OH-LID in the reaction mixture were measured by the HPLC method as described previously [8]. The covalent binding of the radioactivity to hepatic microsomal protein after incubation of [^{14}C]lidocaine was measured according to the reported method [8].

Results and Discussion

P450BTL exhibited lidocaine 3-hydroxylase activity in a reconstituted system (1.45 and 2.69 nmol/min/nmol P450 at substrate concentrations of 10 μM and 2 mM, respectively), and the latter activity was close to the reported value of CYP1A2 at a substrate concentration of 1 mM [5]. On the other hand, lidocaine *N*-deethylase or methylhydroxylase activity was less than detection limits under the conditions used (<0.1 nmol/min/nmol P450).

Effects of the antibody against P450BTL on lidocaine 3-hydroxylase and *N*-deethylase activities in liver microsomes from male Wistar rats are shown in Fig. 1A and B, respectively. The anti-P450BTL IgG fraction inhibited the microsomal lidocaine 3-hydroxylase activity by 97% at a protein ratio (IgG to microsomes) of 5.0 (Fig. 1A), whereas it did not affect lidocaine *N*-deethylase (Fig. 1B) or methylhydroxylase activity (data not shown). The 3-hydroxylase activity of P450BTL in the reconstituted system and the immunoinhibition by anti-P450BTL-IgG fraction indicate that P450BTL and/or its immunorelated P450 isozyme(s) belonging to the CYP2D subfamily are involved in 3-hydroxylation of lidocaine in rat liver microsomes. The present observation is compatible with our previous results [7] demonstrating selective deficiency of lidocaine 3-hydroxylase activity in the male and female Dark Agouti rats, an animal model for human debrisoquine poor-metabolizer.

We reported previously that incubation of [^{14}C]lidocaine with rat liver microsomes in the presence of an NADPH-generating system resulted in covalent binding of a ^{14}C-labelled material to microsomal protein [8]. A chemically reactive metabolite(s) covalently bound to microsomal protein was found to be formed by P450-dependent monooxygenation [8]. As shown in Fig. 1C, the anti-P450BTL IgG fraction suppressed the metabolism-dependent covalent binding of lidocaine to microsomes by 90% at a protein ratio of 2.0. These results showed that a P450 isozyme(s) belonging to the CYP2D subfamily participated in the metabolic activation of lidocaine as well as in 3-hydroxylation of lidocaine. Although direct evidence is not available at present, it seems reasonable to assume that a CYP2D isozyme(s) biotransforms lidocaine into a chemically reactive epoxide(s), some of which bind to microsomal protein, and the rest are converted to 3-OH-LID. A few studies have reported that the CYP2D subfamily is involved in bioactivation of drugs or chemicals [13, 14], but their toxicological significance remains to be elucidated. Further studies are required to pursue this possibility.

Laboratory of Biopharmaceutics
Faculty of Pharmaceutical Sciences
Chiba University
1-33, Yayoi-cho
Inage-Ku
Chiba 263
Japan

YASUHIRO MASUBUCHI
SHIN UMEDA
SHIGEKI IGARASHI
SHOICHI FUJITA*
SHIZUO NARIMATSU†
TOKUJI SUZUKI

REFERENCES

1. von Bahr C, Hedlund I, Karlen B, Backstrom D and Grasdalen H, Evidence for two catalytically different binding sites of liver microsomal cytochrome P-450: importance for species differences in oxidation pattern of lidocaine. *Acta Pharmacol Toxicol* **41**: 39–48, 1977.
2. Fujita S, Tatsuno J, Kawai R, Kitagawa H, Suzuki T and Kitani K, Age associated alteration of lidocaine metabolism is position selective. *Biochem Biophys Res Commun* **126**: 117–122, 1985.
3. Kawai R, Fujita S and Suzuki T, Simultaneous quantitation of lidocaine and its four metabolites by

* Current address: Department of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, North 18, West 9, North Ward, Sapporo 060, Japan.
† Corresponding author. Tel. (81) 43-290-2936; FAX (81) 43-255-1574.

high-performance liquid chromatography: application to studies on *in vitro* and *in vivo* metabolism of lidocaine in rats. *J Pharm Sci* **74**: 1219–1224, 1985.
4. Kawai R, Fujita S and Suzuki T, A new lidocaine metabolite, ω-diethylamino-2-hydroxymethyl-6-methylacetanilide. *Drug Metab Dispos* **14**: 277–279, 1986.
5. Oda Y, Imaoka S, Nakahira Y, Asada A, Fujimori M, Fujita S and Funae Y, Metabolism of lidocaine by purified rat liver cytochrome P-450 isozymes. *Biochem Pharmacol* **38**: 4439–4444, 1989.
6. Imaoka S, Enomoto K, Oda Y, Asada A, Fujimori M, Shimada T, Fujita S, Guengerich FP and Funae Y, Lidocaine metabolism by human liver cytochrome P-450s purified from hepatic microsomes: comparison of those with rat hepatic cytochrome P-450s. *J Pharmacol Exp Ther* **255**: 1385–1391, 1990.
7. Masubuchi Y, Umeda S, Chiba M, Fujita S and Suzuki T, Selective 3-hydroxylation deficiency of lidocaine and its metabolite in Dark Agouti rats. *Biochem Pharmacol* **42**: 693–695, 1991.
8. Masubuchi Y, Araki J, Narimatsu S and Suzuki T, Metabolic activation of lidocaine and covalent binding to rat liver microsomal protein. *Biochem Pharmacol* **43**: 2551–2557, 1992.
9. Suzuki T, Narimatsu S, Fujita S, Masubuchi Y, Umeda S, Imaoka S and Funae Y, Purification and characterization of a cytochrome P-450 isozyme catalyzing bunitrolol 4-hydroxylation in liver microsomes of male rats. *Drug Metab Dispos* **20**: 367–373, 1992.
10. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
12. Yasukouchi Y and Masters BSS, Some properties of detergent solubilized NADPH-cytochrome *c* (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344, 1976.
13. Hietanen E, Malaveille C, Camus AM, Bereziat JC, Brun G, Castegnaro M, Michelon J, Idle JR and Bartsch H, Interstrain comparison of hepatic and renal microsomal carcinogen metabolism and liver S9-mediated mutagenicity in DA and Lewis rats phenotyped as poor and extensive metabolizers of debrisoquine. *Drug Metab Dispos* **14**: 118–126, 1986.
14. Masubuchi Y, Narimatsu S and Suzuki T, Activation of propranolol and irreversible binding to rat liver microsomes: strain differences and effects of inhibitors. *Biochem Pharmacol* **43**: 635–637, 1992.