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SHORT COMMUNICATIONS

Selective 3-hydroxylation deficiency of lidocaine and its metabolite in Dark Agouti rats

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Lidocaine is a local anesthetic and antiarrhythmic drug widely used clinically. The metabolic fate of lidocaine has been studied extensively in experimental animals [1–7] and humans [1, 8]. As shown in Scheme 1, lidocaine is N-deethylated, ring- and methyl-hydroxylated to form monoethylglycinexylidide (MEGX), 3-hydroxylidocaine (3-OH LID) and methyl-hydroxylidocaine (Me-OH LID), respectively, in rat liver microsomes [2–7]. MEGX and 3-OH LID are subsequently ring-hydroxylated and N-deethylated, respectively, forming a common secondary metabolite 3-hydroxymonoethylglycinexylidide (3-OH MEGX), and MEGX is also N-deethylated further to form glycinexylidide (GX). These reactions are catalysed by microsomal cytochrome P450. A marked regioselective sex difference in the rat is known in lidocaine metabolism [2, 5]. Age-associated alteration of lidocaine metabolism in male rats also is regioselective [5]. These findings suggest that multiple isozymes of cytochrome P450 are involved in the oxidation of lidocaine. Reconstituted studies using purified cytochrome P450 isozymes showed directly that various cytochrome P450 isozymes have different metabolic abilities in lidocaine N-deethylation, 3-hydroxylation and methyl-hydroxylation [7].

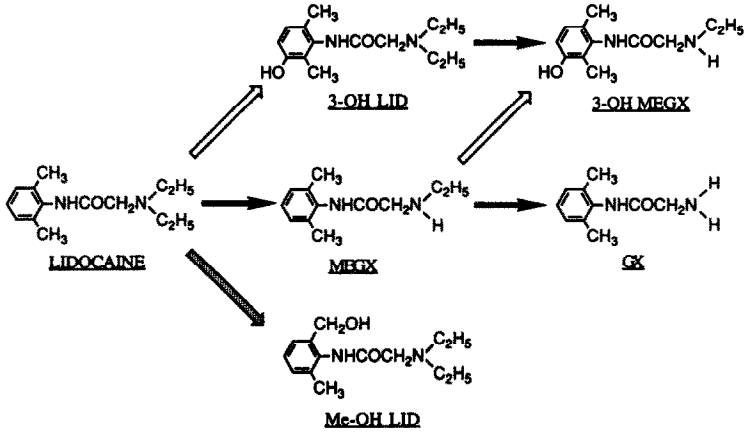
Genetic polymorphism in the oxidation of debrisoquine and sparteine in man has been recognized [9, 10]. The same form of cytochrome P450 has been reported to be responsible for debrisoquine and sparteine metabolism [11]. Lidocaine competitively inhibited sparteine oxidation as well as propranolol in human liver microsomes [12]. This indicates that lidocaine is capable of occupying the same enzymatic site as sparteine. These facts suggest that cytochrome P450 isozymes that metabolize debrisoquine are involved in lidocaine metabolism. To examine this, we investigated the metabolism of lidocaine and its metabolites in liver microsomes obtained from Wistar rats and Dark Agouti (DA) rats, since the female DA rat is known as an animal model for human debrisoquine poor metabolizers [13, 14].

Materials and Methods

Chemicals. Lidocaine, MEGX, GX, 3-OH LID, 3-OH MEGX and Me-OH LID were used as hydrochloride derivatives. These were synthesized as described previously [4, 6]. Glucose 6-phosphate (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PDH) and NADPH were obtained from Oriental Chemicals (Tokyo, Japan). Other chemicals were of analytical grade.

Preparation of hepatic microsomes. Male and female Wistar rats were obtained from Takasugi Exp. Animal (Kasukabe, Japan). Male and female DA rats were obtained from Sizuoka Laboratory Co. (Sizuoka, Japan). They were all 2 months old. Hepatic microsomal fractions were prepared according to the method of Omura and Sato [15]. Protein concentrations were assayed by the method of Lowry *et al.* [16].

In vitro *metabolism of lidocaine and its primary metabolites.* The rates of oxidation of lidocaine and its primary metabolites were measured in a 1-mL microsomal reaction mixture containing 1 mg microsomal protein, 5 mM G-6-P, 1 unit/mL G-6-PDH, 1 mM NADPH, 5 mM $MgCl_2$, 5 μM $MnCl_2$ and 2 mM lidocaine, MEGX or 3-OH LID in 50 mM Tris–HCl buffer (pH 7.4). After preincubation of the reaction mixture without NADPH for 5 min at 37°, reaction was started by the addition of NADPH. The reaction was carried out in air for 2.5 min when lidocaine and 3-OH LID were used as substrates and



Scheme 1. Metabolic pathways of lidocaine in rat liver microsomes. Arrows show (⇨) 3-hydroxylation, (➡) N-deethylation and (⇨) methyl-hydroxylation, respectively.

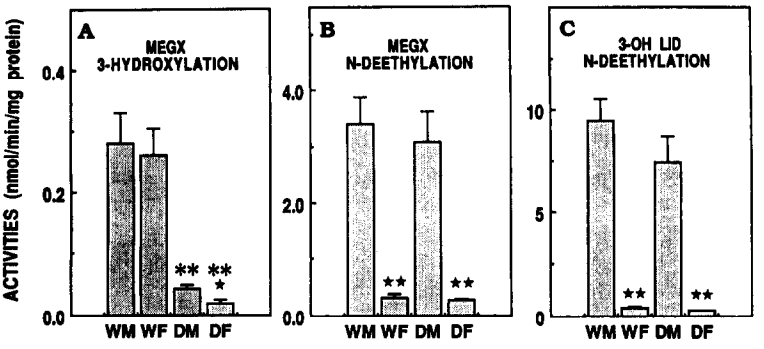


Fig. 1. Strain and sex differences in lidocaine metabolism in rat liver microsomes. WM, WF, DM and DF indicate the enzyme sources which were obtained from Wistar male, Wistar female, DA male and DA female rats, respectively. Values represent mean ± SEM from four determinations. ** Significantly different from mean values of Wistar rats for the respective sexes ($P < 0.01$). ★ and ★★ Significantly different from mean values of male rats for the respective strains ($P < 0.05$ and $P < 0.01$, respectively) by Student's *t*-test.

for 10 min when MEGX was used as a substrate. The reaction was stopped by the addition of 1 mL of 1 N NaOH. The extraction of lidocaine metabolites from the reaction mixture and their high-performance liquid chromatographic assay were performed as describd previously [4].

Results and Discussion

Lidocaine 3-hydroxylase activities for the corresponding sexes were much lower in DA rats than in Wistar rats ($P < 0.01$, Fig. 1A). Sex difference was not observed in Wistar rats, but male DA rats had two-fold higher activity than female DA rats ($P < 0.01$, Fig. 1A). These hypoactivities in male and female DA rats and particularly the lower activity in female DA rats coincided with the strain and sex difference of the amount of debrisoquine 4-hydroxylase named cytochrome P450 UT-H [17, 18] or P450 db1 (IID1) [19, 20]. Therefore, lidocaine 3-hydroxylase may be related to debrisoquine 4-hydroxylase in rat liver microsomes.

On the other hand, strain differences in lidocaine *N*-deethylase and methyl-hydroxylase activities were not observed in either sex (Fig. 1B and C), and *N*-deethylase activities of male rats were much higher than those of female rats in both strains ($P < 0.01$, Fig. 1B). This suggests that there is no contribution of debrisoquine 4-hydroxylase to N-deethylation and methyl-hydroxylation of lidocaine.

When MEGX was used as a substrate, a much lower 3-hydroxylase activity was demonstrated in DA rats than in Wistar rats, as in the case of lidocaine 3-hydroxylation ($P < 0.01$, Fig. 2A). In N-deethylation of MEGX and 3-OH LID, marked sex differences (male > female, $P < 0.01$) in both strains and no strain difference in either sex were shown (Fig. 2B and C), similar to lidocaine N-deethylation. These results and our previous findings [3], the competitive metabolic inhibition between the N-deethylations of lidocaine and 3-OH LID and between the 3-hydroxylations of lidocaine and MEGX, suggest that the metabolic reactions indicated by the same arrows in Scheme 1 are mainly catalysed by the same cytochrome P450 species.

Genetic deficiency in lidocaine metabolism has not been clinically reported. Lidocaine N-deethylation and 3-hydroxylation have been known in lidocaine metabolism in human liver microsomes, but the latter is a minor metabolic pathway [8]. Lidocaine N-deethylation rates always exceeded markedly 3-hydroxylation rates at all substrate concentrations ranging from 1 to 100 μM [8]. In Wistar rats, however, lidocaine 3-hydroxylation was much more important than N-deethylation in low substrate concentrations less than 10 μM [4]. This species difference of lidocaine 3-hydroxylation might reflect the species difference of the effect of enzyme deficiency on lidocaine metabolism.

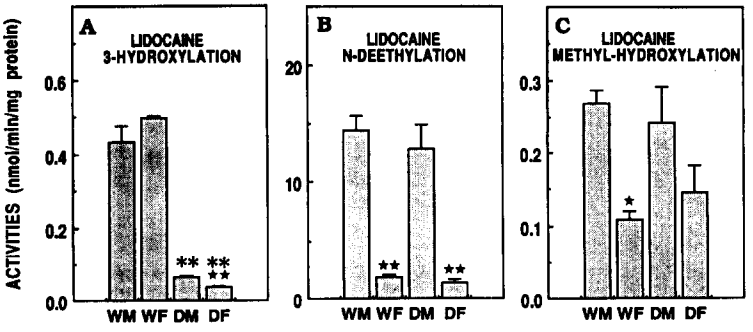


Fig. 2. Strain and sex differences in metabolism of primary metabolites of lidocaine in rat liver microsomes. Abbreviations and symbols correspond to those of Fig. 1. Values represent mean ± SEM from four determinations.

In summary, selective and marked 3-hydroxylase deficiency of lidocaine and its N-deethylated metabolite, MEGX, were observed in male and female DA rats. These findings suggest that cytochrome P450 isozymes metabolizing debrisoquine may be involved in the 3-hydroxylations of lidocaine and MEGX in rats and humans.

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