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# Kinetic analysis of mutual metabolic inhibition of lidocaine and propranolol in rat liver microsomes

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**Abstract**—The metabolic interaction between lidocaine (LD) and propranolol (PL) was analysed kinetically in rat liver microsomes. Employing a very short incubation time of 30 sec, we demonstrated that PL competitively inhibited liver microsomal 3-hydroxylation of LD, but did not affect either the formation of monoethylglycinexylidide or methylhydroxylidocaine from LD in PL concentrations up to 1 μM. On the other hand, LD competitively inhibited PL 4-, 5- and 7-hydroxylations, but the inhibition type of LD for PL N-desisopropylation could not be clarified. Comparison of the kinetic data for liver microsomes from Wistar and Dark Agouti rats indicated that among the primary metabolic pathways of LD, the $V_{max}$ value for 3-hydroxylation was markedly less in female Dark Agouti rats. The results suggest that LD 3-hydroxylation and PL ring hydroxylations are mediated by the same isozyme(s) belonging to the CYP2D subfamily.

The pharmacokinetic interaction between lidocaine (LD*) and propranolol (PL) was treated as a simple interaction induced by haemodynamic changes in cardiac output and hepatic blood flow in dogs [1] and in human subjects [2], since LD is avidly metabolized by the liver and its clearance is limited by the degree of hepatic blood flow. However, Conrad *et al.* [3] suggested that a large part of the effect of PL on LD elimination is a direct effect of the drug or its metabolites on hepatic uptake of LD or on inhibition of hepatic oxidative enzymes.

Otton *et al.* [4] observed that the rate of sparteine oxidation by 9000 *g* supernatant from human liver was inhibited competitively by LD and PL. This finding suggests that LD and PL may be oxidized by the same cytochrome P450 (P450) isozyme(s), which catalyse sparteine oxidation in human livers. Al-Asady *et al.* [5] showed that oxidative metabolism of LD in rat liver microsomes was inhibited by various β-blockers including PL. In the present study, we performed enzyme kinetic experiments in more detail using rat liver microsomes and demonstrated that the metabolic interaction of LD for PL ring-hydroxylations and of PL for LD 3-hydroxylation was competitive. The kinetic parameters for LD metabolism in liver microsomes from Wister and Dark Agouti (DA) rats of both sexes were also determined.

## *Materials and Methods*

Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADPH were obtained from the Oriental Yeast Co. Ltd (Tokyo, Japan); PL HCl from the Sigma Chemical Co. (St Louis, MO, U.S.A.); 4-hydroxy-PL (4-OH-PL) from the Sumitomo Chemical Ind. Ltd (Osaka, Japan); desisopropyl-PL from the ICI Pharmaceutical Co. (Macclesfield, U.K.); 5-OH- and 7-OH-PLs [6], monoethylglycinexylidide [7], 3-hydroxylidocaine (3-OH-LD) and methylhydroxylidocaine [8] were synthesized by the reported methods. LD HCl was JPXII grade.

Preparation of liver microsomes from Wistar and DA rats of both sexes (7 weeks old) and measurement of their protein concentrations and LD oxidation activities were performed as reported previously [9] using LD concentrations from 1 μM to 3 mM at an incubation time of 2.5 min. To examine the inhibitory effect of PL on oxidative metabolism of LD, PL (final concentrations of 0, 0.5 and 1.0 μM) was added to the reaction mixture [9] containing LD (1.25, 2, 5 and 10 μM), and incubated for 30 sec. The inhibitory effect of LD (0, 2.0, 5.0 and 10.0 μM) on oxidative metabolism of PL (0.25, 0.5, 0.75 and 1.5 μM) was assessed by the reported method [10] in the reaction mixture [9] using an incubation time of 30 sec. The inhibition studies were performed using liver microsomes from male Wistar rats.

## *Results and Discussion*

We have examined the effect of PL on microsomal LD oxidation in liver microsomes from male Wistar rats using an incubation time of 30 sec. LD 3-hydroxylation was suppressed by PL in a concentration-dependent manner. The Lineweaver–Burk plot of these data clearly showed that the inhibition of PL for LD 3-hydroxylation was competitive (Fig. 1A). The value of the inhibition constant ($K_i$) of PL for LD 3-hydroxylation was calculated to be 0.096 ± 0.013 μM (N = 3, mean ± SE), and was similar to the values (around 0.1 μM) of the Michaelis constants for PL ring-hydroxylations [11]. However, the addition of PL to the reaction mixture did not affect significantly either monoethylglycinexylidide or methylhydroxylidocaine formation from LD in the PL concentration range used.

The inhibitory effect of LD on microsomal PL oxidation forming 4-OH-PL, 5-OH-PL and 7-OH-PL and *N*-desisopropylpropranolol was examined in microsomes from male Wistar rats. The formation rates of all the metabolites were suppressed by LD in a concentration-dependent manner. As shown in Fig. 1B, the Lineweaver–Burk plot of the data for PL 7-hydroxylation revealed that the inhibition was competitive. The data for PL 4- and 5-hydroxylations was also found to be competitive (data not shown), and the $K_i$ values of PL were calculated to be 2.48 ± 0.78, 1.93 ± 0.27 and 2.03 ± 0.22 μM (N = 3, mean ± SE) for PL 4-, 5- and 7-hydroxylations, respectively. On the other hand, the rate of PL N-desisopropylation was lower at the substrate concentrations used compared with the rates of the ring-hydroxylation, and the type of inhibition could not be established.

Incubation time is important for the kinetic analysis of LD and PL. Initial formation rates of the primary metabolites may be underestimated, because these compounds are rapidly metabolized and simultaneously biotransformed to secondary metabolites. Most of PL, if added to the incubation mixture at low concentrations as an inhibitor, is metabolized during an incubation time of more than 2 min [12]. A reason why Al-Asady *et al.* [5] did not identify the mechanism of the metabolic inhibition of PL for LD metabolite formation may have been the use of an inappropriate incubation time. We thus employed a

* Abbreviations: LD, lidocaine; 3-OH-LD, 3-hydroxylidocaine; PL, propranolol; X-OH-PL, X-hydroxypropranolol; P450, cytochrome P450; DA, Dark Agouti.

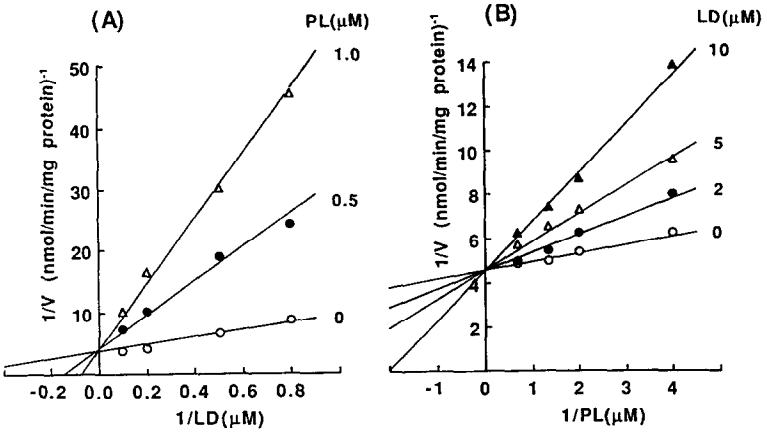


Fig. 1. Lineweaver–Burk plots showing inhibition of LD 3-hydroxylation activities by PL (A) and that of PL 7-hydroxylation activities by LD (B) in liver microsomes from male Wistar rats. Each plot shows a typical result of three determinations.

Table 1. Kinetic parameters for 3-hydroxylation, N-deethylation and 2-methylhydroxylation of LD in liver microsomes from DA and Wistar rats

| | 3-Hydroxylation | | N-Deethylation | | 2-Methylhydroxylation | | | |
|---|---|---|---|---|---|---|---|---|
| Strain/sex | $K_m$ (μM) | $V_{max}$ (nmol/mg/min) | $K_m$ (μM) | $V_{max}$ (nmol/mg/min) | $K_{m}1$ (μM) | $V_{max}1$ (nmol/mg/min) | $K_{m}2$ (μM) | $V_{max}2$ (nmol/mg/min) |
| DA/male | 2.34 ± 0.07 | 0.060 ± 0.006† | 245 ± 40 | 21.74 ± 3.17 | 18.7 ± 6.1 | 0.107 ± 0.019 | 575 ± 162 | 0.140 ± 0.017 |
| | (2.11 ± 0.26) | (0.413 ± 0.096) | (378 ± 75) | (24.81 ± 4.20) | (22.1 ± 6.5) | (0.170 ± 0.065) | (542 ± 155) | (0.160 ± 0.066) |
| DA/female | 1.98 ± 0.17 | 0.018 ± 0.003*‡ | 468 ± 80 | 1.42 ± 0.28‡ | 15.6 ± 2.5 | 0.068 ± 0.016 | 210 ± 6 | 0.038 ± 0.014§ |
| | (2.10 ± 0.36) | (0.364 ± 0.022) | (554 ± 103) | (1.70 ± 0.12‡) | (15.5 ± 3.8) | (0.058 ± 0.025) | (337 ± 115) | (0.040 ± 0.008) |

The formation rates of LD metabolites were determined at 15 different LD concentrations over the range of 1 to 3000 μM in liver microsomes from DA and Wistar rats of both sexes.

The kinetic parameters were analysed by the nonlinear least squares regression analysis program based on a simplex method [14]. Each value represents the mean ± SE of four determinations.

Values in parentheses represent values of Wistar rats for the corresponding sex.

* and †: significantly different from Wistar rats for the corresponding sex ($P < 0.01$ and $P < 0.05$, respectively).

‡ and §: significantly different from male rats for the corresponding strain ($P < 0.01$ and $P < 0.05$, respectively).

very short incubation time of 30 sec in the reaction mixture containing 1 mg/mL microsomal protein to minimize the influence of the formation of secondary metabolites and of a reactive metabolic intermediate [12] formed from PL during incubation. It is thought that this modification has made possible the elucidation of the mechanism of inhibition for the interaction between LD and PL in the present study.

Female DA rats are known as an animal model for CYP2D isozyme deficiency [13]. We reported selective 3-hydroxylation deficiency at a LD concentration of 2 mM in liver microsomes from male and female DA rats [9]. Therefore we examined kinetic parameters for the three metabolic pathways of LD in liver microsomes from DA rats of both sexes and compared the data with those from Wistar rats of both sexes (Table 1). The $V_{max}$ value of LD 3-hydroxylation in female DA rats was significantly less than that of male DA or female Wistar rats without any difference in $K_m$ value, while the kinetic parameters for N-deethylation and 2-methylhydroxylation were not significantly different between DA and Wistar rats for the corresponding sex. LD suppressed PL ring-hydroxylations competitively at the positions 4, 5 and 7, and the $K_i$ values for these ring-hydroxylations by LD were similar to the values (around 2 μM) of the Michaelis constant for LD 3-hydroxylation (Table 1). These findings and the mutual competitive inhibition found between LD and PL in the present study suggest that LD 3-hydroxylation and PL ring-hydroxylations at the 4-, 5- and 7-positions are mediated by the same P450 isozyme(s) belonging to the CYP2D subfamily.

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