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AGE ASSOCIATED ALTERATION OF LIDOCAINE METABOLISM IS POSITION SELECTIVE

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SUMMARY: Patterns of associated alterations in N-deethylation, 3-hydroxylation, and aromatic methylhydroxylation of a single substrate, lidocaine in liver microsomes from 0.7 - 28 months old Fischer 344 rats were examined. These three patterns were all different from one another. In addition to this position selectiveness, a clear sex difference was observed in the pattern of alteration in N-deethylation. These results are consistent with the hypothesis that age associated alterations in drug metabolism are caused by age associated alterations in relative abundance of multiple species of cytochrome P-450.

INTRODUCTION: Very little research on liver drug metabolism using senescent animals has been carried out, despite the importance of the subject. Studies on age associated alterations in microsomal drug metabolizing enzyme activities have been performed mostly in male rats and an age-associated decline is the generally observed pattern. Several hypotheses have been proposed to explain the decrease. Schmucker et al. (1) suggested that alteration in NADPH cytochrome c reductase activity was the cause of the decrease in metabolism. Stier et al. (2) and Armbecht et al. (3) proposed that the cause was altered membrane quality, while others (4-6) suggested alterations in the quality of cytochrome P-450 with age.

In our previous study, we have shown that the patterns of age associated alterations in drug metabolism in rat liver microsomes are different depending on the substrate used and the sex of the animals (5,6). These phenomena could not be explained by the observed alterations in NADPH-cytochrome c reductase activity nor in the total microsomal content of cytochromes P-450 and/or b5 (5). These observations, together with the fact that the peak-height ratio of

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ethylisocyanide difference spectra of reduced microsomes from male rat liver approached the female values with age (5,6), supported the hypothesis that the quality of cytochrome P-450 alters with age. In light of the recent advances in the study of multiple species of cytochrome P-450, we interpret the alteration in the "quality" of P-450 as the result of changes in the relative abundance of multiple species of cytochrome P-450. If indeed this be the cause for the age associated alteration in drug metabolism, position selective alterations should be expected in the metabolism of a single substrate.

In this study, the age associated alterations in lidocaine metabolism at the three different sites were investigated to test this possibility.

MATERIALS AND METHODS

Animals -- SPF Fischer-344 rats of both sexes were purchased from Japan Charles River Co. (Atsugi). They were raised in the SPF aging farm of our laboratory.

Chemicals -- Lidocaine and its metabolites were used as their HCl-salts throughout this study. Lidocaine was purchased from Fujisawa Pharmaceutical Co. (Japan), lidocaine metabolites, 3-hydroxylidocaine (3-OH LID), monoethylglycinexylidide (MEGX) and aromatic methylhydroxylated lidocaine (Me-OH LID)[2] were synthesized in our laboratory. The purity of the products were checked by elementary analysis, mass spectroscopy, and NMR. NADP, glucose-6-phosphate (G-6-P) and G-6-P dehydrogenase were purchased from Sigma (USA).

Methods -- Male and female rats of ages ranging from 3 weeks to 28 months were killed by decapitation and liver microsomes were prepared by the method of Omura and Sato (7). The rate of oxidation of lidocaine was measured in a system containing 100 μM lidocaine, microsomes (1 mg of protein), 50 mM Tris-HCl buffer (pH 7.4), 10 mM $MgCl_2$, 10 μM $MnCl_2$, 10 mM G-6-P, and 2 units of G-6-P dehydrogenase per 1 ml incubation mixture. Incubations were performed for 2.5 min at 37° in air after preincubation for 5 min. The reaction was started by addition of 20 μl 50 mM NADP and stopped by addition of ice-cold 1 ml of 1 M carbonated buffer (pH 10.5).

Lidocaine and its metabolites were extracted in ethylacetate and then back extracted into an acidic aqueous layer containing an internal standard, procaine, followed by neutralization of this layer. The residual ethylacetate was evaporated in vacuo. The aqueous layer was then applied to the HPLC system. The method for simultaneous quantitation of lidocaine and its metabolites will be reported elsewhere, but briefly, the HPLC column used was a reversed-phase C18 μBondapak (Waters Associates Inc., Millford, MA). The mobile phase was 0.1 M phosphate buffer (pH 3.0) containing 10 % acetonitrile. The solvent flow rate was 2.0 ml/min.

RESULTS

Fig. 1A shows the age associated alterations in lidocaine N-deethylase activity in male and female Fischer-344 rats with age ranging from 3 weeks to

[2] ω-diethylamino 2-hydroxymehthyl 6-methylacetanilide identified as a new lidocain metabolite in our laboratory (to be published elsewhere)

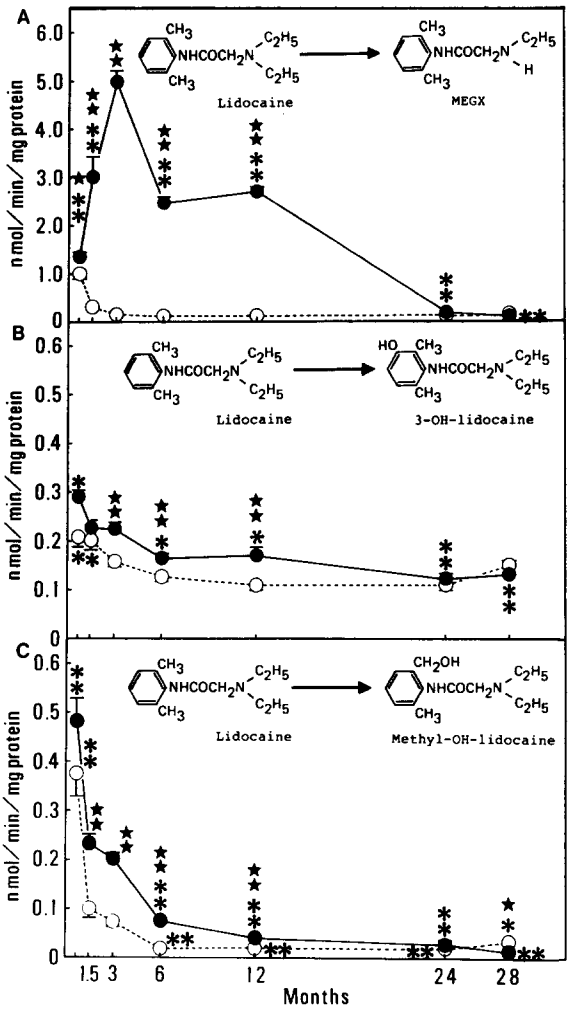

Fig. 1 Age-associated alterations in activities on lidocaine N-deethylase (upper), 3-hydroxylase (middle) and aromatic methylhydeoxylase (lower panel) in liver microsomes from male (●) and female (○) rats.

*,** significantly different from values of 3 months old rats ($p<0.05$ and $P<0.01$ respectively).

★,★★ significantly different from corresponding female values ($p<0.05$ and $p<0.01$ respectively).

28 months. Activity in males was slightly higher than in females at the age of 3 weeks, and rapidly increased till 3 months of age, when the activity was more than 4 times the 3-week value, and about 20 times as large as that of female rats of the same age. The activity in males nearly halved by the age of 6 months, but was then maintained at that level until 12 months. It then decreased rapidly and by the age of 24 months the activity in males was

reduced to the female levels, one-fifth of the activity of 3-month-old male rats. In contrast, activity in females was highest at 3 weeks. It then decreased quickly up to 1.5 month after which it remained level throughout life.

Lidocaine 3-hydroxylase activity (Fig. 1B) in male rats was slightly higher than in females up to the age of 12 months. The activity then gradually decreased and by the age of 24 and 28 months, the values became significantly lower than the 3-month-old value and there was no longer any sex difference. The activity in females remained almost constant after 3 months, while the 3-week and 1.5-month-old values were slightly higher.

Fig. 1C shows the age associated alterations in lidocaine aromatic methyl-hydroxylase activity. In both male and female rats, the activities showed the highest values at the youngest age, 3 weeks, followed by a logarithmic decrease. Twelve-month-old values were about one tenth the 3-week-old values. Two-fold sex differences (male > female) were observed at the ages of 1.5 and 3 months.

DISCUSSION

Attempts have been made to explain the age associated decline in drug oxidation in terms of altered NADPH cytochrome c reductase activity (1), or altered membrane quality (2,3), or altered quality of cytochrome P-450 (4,5). Although there is sufficient evidence that these parameters are altered with age (1-3, 5), there is not enough evidence to indicate which mechanism plays the major role in this process. If NADPH cytochrome c reductase activity plays the major role, monooxygenations of all the substrates should be similarly affected, and the activities should parallel the activity of NADPH cytochrome c reductase. However, our previous study (5) as well as others (4) showed that the alterations of monooxygease activities were substrate selective and are no way congruent with the alterations in NADPH cytochrome c reductase activity.

The observed substrate and sex selective alterations in drug monooxygenations are most likely caused by age associated alterations in the quality of cytochrome p-450, but altered membrane quality could not be ruled out.

Affinity of the membrane toward substrates may change with age in a substrate selective manner, thereby limiting the accessibility of the selected substrates to cytochrome P-450, the active site of which is embedded in the microsomal membrane. Therefore, in this study, we used a single substrate, lidocaine, which possesses three different metabolic pathways. The alteration in membrane quality should equally affect the activities of enzymes engaging in lidocaine metabolism at the three different positions. However, the patterns of age associated alterations in the metabolism of lidocaine at these three different positions were all different (Fig. 1), suggesting that the alteration of membrane quality is not the likely explanation.

On the other hand, these results are well explained by alterations in the quality of cytochrome P-450 or alterations in the relative abundance of multiple species of cytochrome P-450. The relative abundance of cytochrome P-450 species engaging in one pathway of lidocaine metabolism (e.g. lidocaine N-deethylation) appears to be changed with age in a pattern different from the other two (e.g. lidocaine 3-hydroxylation or aromatic methylhydroxylation). The appearance and disappearance of sex differences in the metabolism of lidocaine can also be explained by the difference and alterations in the relative abundance of cytochrome P-450 species in male and female rats.

In conclusion, by determining the enzyme activities catalyzing three different metabolic pathways of lidocaine we have suggested that alterations in the quality of cyto-chrome P-450 quality may play a major role in age-associated alterations in hepatic drug monooxygenation. In addition, this observation has important clinical and toxicological implications, because it is likely that not only the rate of metabolism of lidocaine, but also relative quantity of the three metabolites alters with age.

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