the release constant but determined the duration of fluoride release.

According to this it may be assumed that by an increase in the thickness of the coating applied to the plates, a prolonged continuous increase of F in saliva will be possible. The increase in the thickness of the coating may be easily achieved by adding additional layers of the coating solution.

The continuous presence of fluoride in saliva, achieved in this study, may favor the remineralization phase occurring between periods of enamel dissolution (2) and significantly increase the value of fluoride in caries prevention.

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*D. Harary**
M. Friedman ˣ ‡
* Departments of Orthodontic and ‡Pharmacy
Schools of Dental Medicine and Pharmacy
The Hebrew University of Jerusalem
Jerusalem 91120, Israel

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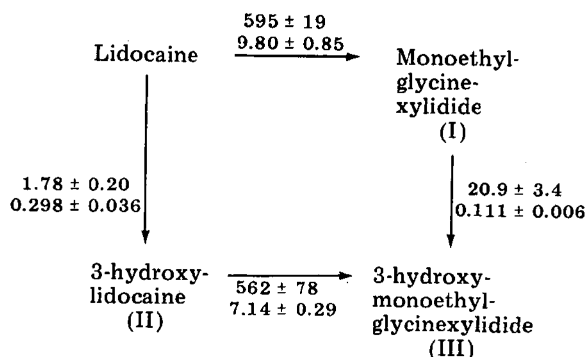
Precursor–Metabolite Interaction in the Metabolism of Lidocaine

Keyphrases ☐ Lidocaine—precursor–metabolite interaction, metabolism ☐ metabolites—interaction with precursor in metabolism of lidocaine ☐ metabolism—lidocaine in the liver

To the Editor:

Lidocaine is a local anesthetic and antiarrhythmic drug that has been known to be extracted exclusively by the liver with a high extraction ratio after oral administration (1–4). As shown in Scheme I (5), lidocaine is extensively metabolized in rats to monoethylglycinexylidide (I) and 3-hydroxylidocaine (II) by P_{450}-dependent N-deethylase and 3-hydroxylase activities. These primary metabolites are further 3-hydroxylated and N-deethylated, respectively, to form a common secondary metabolite, 3-hydroxymonoethylglycinexylidide (III). In common with other drugs highly cleared by the liver, the areas under the blood concentration *versus* time curve (AUC) obtained after portal vein infusion of lidocaine at a dose range of



Scheme I—Parallel metabolic pathways of lidocaine and the kinetic parameters. The figures are expressed as means ±SE (n = 3) μM for apparent Michaelis constants (top numbers) and nmol/min·mg microsomal protein for maximum velocities (lower numbers). Substrate concentration range: 200–2000 μM for N*-deethylation of lidocaine and 3-hydroxylidocaine (II); 1–100 μM for 3-hydroxylation of lidocaine and monoethylglycinexylidide (I).*

1–10 mg/kg were smaller (approximately one half) than those after peripheral vein infusion of equal doses in rats (6). However, of special interest is our preliminary finding that the AUC of I, a pharmacologically active lidocaine metabolite, obtained after portal vein infusion of lidocaine was ~10 times as large as that obtained after peripheral vein infusion (6). The AUC of metabolite II, on the other hand, did not differ significantly depending on the route of administration of lidocaine. If a drug and its metabolites are eliminated linearly and exclusively by hepatic metabolism, the AUC of the metabolites, or the ratios of AUC of any two metabolites, are independent of the route of administration (4). The unusually high AUC of I and also the difference in the AUC ratios of the two metabolites of lidocaine depending on the route of administration can be explained by the following possibilities: (*a*) the elimination of I itself is nonlinear; (*b*) 3-hydroxylation of lidocaine is saturable at lidocaine concentrations attained by portal vein infusion but N-deethylation is not, resulting in the formation of I in higher concentrations; (*c*) lidocaine inhibits the metabolism of I in the liver and the elimination of I decreases in the presence of high concentrations of liodcaine. The AUC of I (1.5–7.5 mg/kg) was found to increase in an apparently linear fashion with increasing intraportal dosage (6). Therefore, either the second or the third possibility or both of them may be operative in these phenomena.

Studies by von Bahr *et al.* (7) and from our laboratory (8) have indicated that the 3-hydroxylation and N-deethylation pathways of lidocaine are catalyzed by different P_{450} species. It is then of interest to test if the parallel hydroxylation pathways (3-hydroxylations of lidocaine and I) are catalyzed by the same P_{450} species or different. If they are catalyzed by the same P_{450} species, lidocaine and I would be mutually competitive inhibitors of their metabolism. Then this would partly explain the unusually high AUC of I obtained after portal vein infusion of lidocaine as described above. By the same token, it is also of interest to examine if the N-deethylation pathways of lidocaine and II share the same species of P_{450}. In this communication, we report *in vitro* metabolic kinetics of lidocaine and its metabolites in order to characterize the nature of lidocaine metabolism and to investigate the

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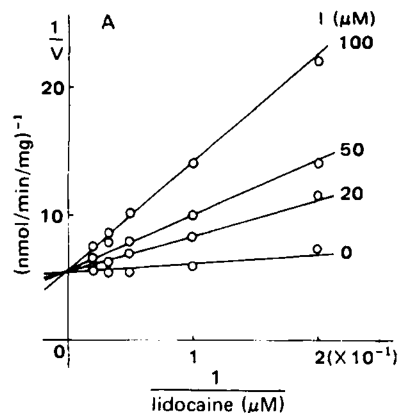

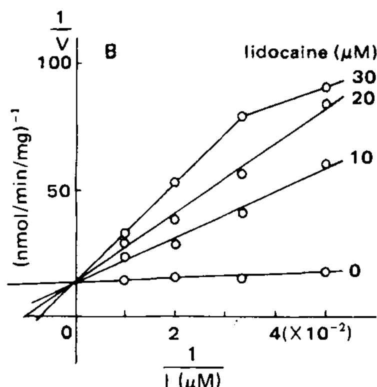

Figure 1—*Lineweaver–Burk plots of lidocaine and monoethylglycinexylidide (I) 3-hydroxylations in the presence of inhibitors, monoethylglycinexylidide(I) and lidocaine, respectively.*

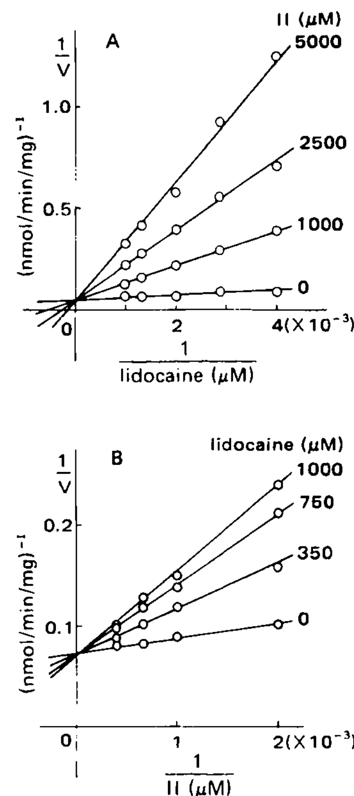

Figure 2—*Lineweaver–Burk plots of lidocaine and 3-hydroxylidocaine (II) (N)-deethylations in the presence of inhibitors, 3-hydroxylidocaine (II), and lidocaine, respectively.*

possible precursor–metabolite interactions between the parallel metabolic pathways.

N-deethylations and 3-hydroxylations of lidocaine and its metabolites were studied at 37°C using a hepatic microsomal suspension obtained from male Wistar rats weighing from 280 to 310 g. The incubation mixture contained the substrate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP in 50 mM Tris-HCl buffer, pH 7.4. The concentrations of I, II, and III after incubation for 2.5 min were determined using a reverse-phase[1] high-performance liquid chromatographic assay[2]. The rate of formation of I and II from lidocaine was linear up to 3 min at the substrate concentration range used in this study. Formation of III was not detected within this incubation period. The linearities of these reactions were lost, thereafter, due to the conversion of I and II into III. The formation rate of III from I or II was also linear, at least during the first 3 min.

Scheme I shows kinetic parameters for the metabolism of lidocaine and its metabolites. The metabolism of lidocaine consisted of *N*-deethylation with a large apparent Michaelis constant (K_m) and a high maximum velocity (V_{max}) and 3-hydroxylation with a small K_m and a low V_{max}. These data reflect the effects of substrate concentration on these reactions; that is, at low concentrations (<5 μM) larger fractions of lidocaine were 3-hydroxylated than *N*-deethylated, but at high concentrations larger fractions were *N*-deethylated than 3-hydroxylated. These results indicate that the second possibility stated above seems to be at least one of the reasons for the unusually high AUC of I after portal vein infusion of lidocaine. This also seems to be one of the reasons for the large difference in the AUC of I and II. The K_m of 3-hydroxylation of I was ~10-fold that of lidocaine 3-hydroxylation and V_{max}, about a third of that of lidocaine 3-hydroxylation. The *N*-deethylation of II possessed similar K_m and V_{max} values to those of lidocaine *N*-deethylation.

The results of the studies on the effects of lidocaine metabolites on lidocaine metabolism or *vice versa* are shown in Figs. 1 and 2.

Figure 1A shows the Lineweaver–Burk plots of the rate of formation of II from lidocaine (lidocaine 3-hydroxylase activities) in the presence of various concentrations of I. A clear competitive inhibition was observed. Figure 1B shows the Lineweaver–Burk plots of the rate of formation of III from I in the presence of various concentrations of lidocaine. Since III is formed from lidocaine added as an inhibitor *via* its metabolites (I and II) as well as from the substrate (I), the apparent rate of formation of III in this assay system is not the accurate rate of 3-hydroxylation of I, but is the sum of the rates of formation of III from I and lidocaine *via* its two metabolites. Therefore, the linearity of the plots in Fig. 1B obtained with this assay system, especially in the presence of a high lidocaine concentration, was not as good as the plots in Fig. 1A. However, the general pattern of these plots indicates the competitive nature, and since the inhibition of lidocaine 3-hydroxylation by I is competitive, it is naturally assumed

[1] μBondapak C-18; Waters, Associates, Milford, Mass.
[2] Unpublished data.

that the 3-hydroxylation of I is also competitively inhibited by lidocaine.

Figure 2A shows the Lineweaver–Burk plots of the rate of formation of I from lidocaine (lidocaine *N*-deethylase activities) in the presence of various concentrations of II. As in the case of the inhibition of lidocaine 3-hydroxylase activity by I, lidocaine *N*-deethylase activity was competitively inhibited by II. Figure 2B shows the Lineweaver–Burk plots of the rate of formation of III from II in the presence of various concentrations of lidocaine. Since III is formed from lidocaine as well as I, as stated above, the rate of formation of III does not accurately measure the rate of *N*-deethylation of II as in the case of the assay for 3-hydroxylation of I. However, these plots clearly show that the inhibition is of a competitive nature, as in the case of the inhibition of 3-hydroxylation of I by lidocaine.

The 3-hydroxylations of lidocaine and I and the *N*-deethylations of lidocaine and II are each mutually competitive, indicating that those two 3-hydroxylation pathways and *N*-deethylation pathways are each sharing the same species of P_{450}. The inhibition of 3-hydroxylation of I by lidocaine was more effective than that of lidocaine 3-hydroxylation by I. The inhibitions of *N*-deethylations of II and lidocaine by lidocaine and II, respectively, were of the same magnitude as expected from the K_m values of the corresponding inhibitors. The observed precursor inhibition, the inhibition of the further metabolism of a primary metabolite (I) by the precursor (lidocaine) may be operative in an *in vivo* system (the third possibility). The findings that 3-hydroxylation of lidocaine is saturable at a low concentration but *N*-deethylation is not, even at a high concentration, and that the precursor inhibition for II is much less effective than that for I may explain the difference in the ratios of the AUC of the two primary metabolites. Therefore, it appears that both the second and third possibilities stated above may cause the unusually high AUC of I only after portal vein infusion of lidocaine.

These types of precursor–metabolite interactions (precursor inhibition and product inhibition) between the metabolic pathways that are parallel to each other, as shown in Scheme I, is not an isolated phenomenon for lidocaine metabolism alone, but was observed in the metabolism of imipramine[3] and amitriptyline (9). These "parallel pathway interactions" may be observed in drugs possessing similar metabolic pathways, such as methamphetamine and diazepam.

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Tokuji Suzuki[x]
Shoichi Fujita
Ryosei Kawai
Department of Biopharmaceutics
Faculty of Pharmaceutical Sciences
Chiba University
1-33, Yayoicho, Chiba, Japan

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[3] Unpublished data.