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Lidocaine Metabolism in Normal and Phenobarbital-pretreated Dogs

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The metabolism of lidocaine was investigated in four dogs before and after pretreatment with phenobarbital. The extraction of lidocaine and production of monoethylglycinexylidide (MEGX) and glycinexylidide (GX) were calculated from gradients across the liver. The lidocaine fraction removed was 25 per cent in the controls and 50 per cent following phenobarbital pretreatment. The principal metabolite found was MEGX. Phenobarbital pretreatment may have increased the rate at which MEGX, formed from lidocaine, is metabolized further. Contrary to results of previous studies, GX was discovered to be an additional metabolite of lidocaine. The formation and excretion of GX were accelerated by phenobarbital pretreatment. (Key words: Lidocaine; Metabolism; Enzyme induction.)

DESPITE WIDE USE of lidocaine, knowledge of its metabolism remains incomplete. Hollunger [1-3] described the metabolism of lidocaine by nonspecific enzyme systems in rabbit liver fractions. Heinonin [4] observed that the efficiency of this nonspecific system could be increased by pretreatment with drugs which produced enzyme induction. Dynamic measurements of lidocaine metabolism *in vivo* have not been made because methods for the simultaneous measurement of the drug and its metabolites were lacking. A method developed recently [5] was used in this study to examine lidocaine metabolism *in vivo* and to quantitate the effects of pretreatment with phenobarbital on lidocaine metabolism.

Method

Four mongrel dogs weighing 15–20 kg were anesthetized with halothane, and anesthesia was maintained with 1 per cent inspired halothane in oxygen during laparotomy. The femoral artery (considered equivalent to blood in the hepatic artery), portal vein, and hepatic vein were cannulated, and blood was drawn for control chromatographic examination. Hepatic-vein samples were drawn slowly to avoid admixture with inferior vena caval blood. Urine samples were collected from an indwelling catheter. Chromatographic quantitation of lidocaine, monoethylglycinexylidide ‡ and glycinexylidide § in blood and urine was achieved with a Hewlett Packard 5750 gas chromatograph equipped with a flame ionization detector and a 2-mm, 2-foot glass column containing 10 per cent UCW 98 on Chromosorb W AWHP.[5] Column temperature was 150 C. The injection port and the detector were kept at 250 C. The carrier gas was nitrogen, and the flame for maximal response was achieved by adjusting the hydrogen–air mixture. Three milliliters of blood or urine were placed in a wide-mouthed, glass-stoppered bottle. One milliliter of concentrated ammonium hydroxide and 10 ml of chloroform were added. Mepivicaine was added as the internal standard. The mixture was shaken mechanically for 10 minutes, and after centrifugation, the chloroform layer was collected. The water phase was re-extracted with 10 ml of fresh chloroform. The chloroform layers were combined and evaporated to dryness. The residue was redissolved in 25 μl of chloroform and, after evaporation to 2 to 5 μl, was injected on the column into the chromatograph.

After administration of 500 ml of 5 per cent dextrose in Ringer's lactate solution to the dog over 30–45 minutes, the concentration of inspired halothane was decreased to 0.25–0.5 per cent and lidocaine, 2 mg (8.5 μM)/kg/min, was administered intravenously over 20

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‡ Monoethylglycinexylidide = MEGX.
§ Glycinexylidide = GX.

minutes. Blood drawn from the three vessels after 30, 60 and 90 minutes and urine produced during the same intervals were analyzed chromatographically. Blood pressure was transduced and recorded on a Grass polygraph. Upon completion of this study, the dog was returned to the vivarium; after a two-day rest, phenobarbital, 16 mg/kg/day sc, was administered for 20–30 days. Phenobarbital was discontinued two days prior to the day of the study. The experimental procedure was repeated using the same dose of lidocaine in each dog.

Extraction of lidocaine by the liver was calculated from the concentration gradient of the drug across the liver and is expressed as the fraction of drug removed.¶ It was assumed that all blood enters the liver by way of the hepatic artery and portal vein and that the portal vein-to-hepatic artery blood-flow ratio was 2:1.[6] Assuming the ratio was 3:1 or 1:1, or that it changed during halothane anesthesia, would have little effect on the extraction calculated, since concentrations in the hepatic artery and portal vein were similar.

The concentration gradient for metabolites of lidocaine across the liver was calculated and expressed as a function of the concentration gradient of lidocaine. For example, if a 2-μM/l ** concentration decrement of lidocaine was associated with a 1-μM/l concentration increment in MEGX, then half of the lidocaine removed was accountable as MEGX. Urinary excretion of lidocaine and its metabolites was calculated as a fraction of the dose of lidocaine administered.

Means and standard deviations for the extraction ratios of lidocaine before and after pretreatment with phenobarbital were calculated and subjected to statistical analysis.

Results

Blood concentrations of lidocaine, MEGX, and GX, expressed as micromoles per liter, are shown in table 1 for both control and pretreated dogs. The calculated fractions of lidocaine removed before and after pretreatment are shown in figure 1. In the control group, 25 per cent of the lidocaine entering the liver was removed. After pretreatment with phenobarbital, this fraction increased to 50 per cent ($P < 0.01$). Both fractions remained constant during the two hour experiment.

The concentration decrements of lidocaine that could be accounted for by the appearance of MEGX and GX are shown in table 2. MEGX appeared as the principal metabolite of lidocaine in dogs before and after pretreatment. The lidocaine removed that could be accounted for as MEGX achieved a high of 76 per cent in the control group.

This study has provided the first evidence that GX is also a metabolite of lidocaine. This metabolite appeared in the first samples of blood and urine after phenobarbital pretreatment, while it appeared after an hour in the control study. Once GX has appeared, it remains as a constant fraction (12–16 per cent) of the lidocaine removed by the liver.

The total amount of lidocaine present in urine in the two-hour period of measurement was small. In the control study 4 per cent of the dose administered appeared in the urine; a third of this amount was metabolites and the remainder was free lidocaine. A great increase in urinary excretion of GX occurred in pretreated dogs, while the ratio of free drug to metabolite in the urine remained constant (table 3).

¶ Fraction removed $= 1 - \dfrac{(C_{hv})\dot{Q}_{H(out)}}{(C_{ha})(\frac{1}{3}\dot{Q}_{H(in)}) + (C_{pv})(\frac{2}{3}\dot{Q}_{H(in)})}$

$= 1 - \dfrac{C_{hv}}{\frac{1}{3}C_{ha} + \frac{2}{3}C_{pv}}$

Q_H = hepatic blood flow = $Q_{H(in)} = \dot{Q}_{H(out)}$
C = lidocaine concentration in hepatic vein (C_{hv}), portal vein (C_{pv}), hepatic artery (C_{ha})

** Lidocaine, 1 μM/l = 0.234 μg/ml. MEGX, 1 μM/l = 0.206 μg/ml. GX, 1 μM/l = 0.193 μg/ml.

TABLE 1. Gas Chromatographic Data: (Concentrations as μM/l)*

	30 Minutes			60 Minutes			120 Minutes		
	Hepatic Vein	Portal Vein	Artery	Hepatic Vein	Portal Vein	Artery	Hepatic Vein	Portal Vein	Artery
Control values, four dogs									
Lidocaine	29±7.2	34.2±7.2	40.8±4.2	22.1±3.5	28.3±9.4	25.8±4.3	14.0±5.6	19.5±8.1	19.5±8.3
MEGX	17.8±3.0	14.4±3.3	16.2±2.0	20.5±7.1	16.4±8.0	16.5±3.4	17.2±8.7	16.2±6.5	16.7±8.0
GX	0	0	0	1.8±0.6	1.2±0.1	1.1±0.1	3.1±0.9	2.0±0.6	2.5±0.7
Values in same dogs after pretreatment with phenobarbital									
Lidocaine	24.9±10.2	44.6±9.6	40.2±7.1	18.2±5.1	32.4±10.1	29.3±6.9	12.8±3.7	22.4±5.1	19.4±4.0
MEGX	28.7±16.4	22.3±11.7	21.7±12.7	23.9±11.8	24.0±13.7	21.8±11.3	12.8±2.8	15.9±4.2	13.8±2.6
GX	3.6±1.5	1.4±0.9	1.6±0.4	4.2±2.3	2.3±1.3	2.7±1.6	4.8±2.3	3.5±1.5	3.5±1.5

* Means ± SD. Lidocaine, 1 μM/l = 0.234 μg/ml; MEGX, 1μM/l = 0.206 μg/ml; GX, 1 μM/l = 0.193 μg/ml.

Although pretreatment with phenobarbital produced lethargy in the first few days of treatment, the dogs were judged comparable for the two studies. Anesthetic requirements for halothane, blood pressures under anesthesia, and blood gases appeared similar in the two studies. We did not attempt to define the effects of halothane on the ability of the liver to metabolize various concentrations of lidocaine or to define the effect of halothane on the metabolic rate of lidocaine due to changing hepatic blood flow.

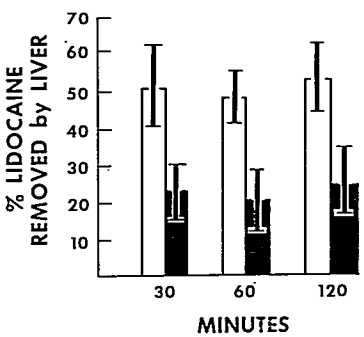

FIG. 1. Means ±SD of lidocaine fractions removed by the liver, calculated from the data for individual dogs. Open bars represent the control values, and solid bars the values obtained after phenobarbital pretreatment. ($P < 0.05$ at 30 min; $P < 0.01$ at 60 and 120 minutes.)

Discussion

The disappearance of a drug from blood depends on several factors: 1) distribution; 2) biotransformation; 3) urinary excretion; 4) biliary excretion. Immediately after intravenous administration of lidocaine, the concentration in the blood is influenced primarily by distribution of the drug into organs of the vessel-rich group.[7] With time, redistribution occurs, and the stores of lidocaine in various compartments, especially muscle, re-enter the circulation as the concentration in blood decreases. Of the other factors leading to decreases of lidocaine concentration in the blood, only biotransformation has a dominant role. Excretion of lidocaine in urine is a minor factor. Eriksson[8] observed the excretion of the drug to be *p*H-dependent, but in normal subjects, total urinary excretion is reported to be 3–11 per cent of the dose administered.[9, 10] Biliary excretion is also a minor factor. Katz[7] found less than 3 per cent of ^{14}C-labelled lidocaine or its metabolites in bile after five hours.

The biotransformation of lidocaine has been observed only in the liver.[1, 9] In the following studies *in vitro*, the principal metabolic pathway was established; it is illustrated in figure 2. Hollunger,[2] using rabbit liver microsomes, defined the oxidative de-ethylation reaction forming MEGX and acetaldehyde (reaction 1, figure 2) as the first major step in lidocaine biotransformation. The MEGX formed was found to be actively hydrolyzed by a rabbit liver fraction amidase to 2,6-xylidine and N-ethylglycine (reaction 2). Metabolism of 2,6-

TABLE 2. Mean Changes in Blood Concentrations after Passage of Lidocaine Through the Liver

	30 Minutes		60 Minutes		120 Minutes	
	μM/l	Per Cent*	μM/l	Per Cent *	μM/l	Per Cent*
Controls						
Lidocaine removed	7.4	—	5.4	—	5.5	—
MEGX produced	2.8	38	4.1	76	0.8	15
GX produced	0	0	0.6	11	0.9	16
After phenobarbital						
Lidocaine removed	18.2	—	13.2	—	8.6	—
MEGX produced	5.6	31	0.6	5	−2.6†	—
GX produced	2.1	12	1.8	14	1.3	15

* Fraction of lidocaine disappearance that can be attributed to the appearance of each metabolite.
† The negative value for MEGX produced indicates that less MEGX came out of the liver than went in.

xylidine proceeds prinicipally to 4-hydroxy-2, 6-xylidine and to a small extent to 2-aminobenzoic acid (reaction 3).[11] The amidase active in the hydrolysis of MEGX has little activity in the hydrolysis of lidocaine or GX. The hydrolysis of lidocaine, once thought to be the major pathway for biotransformation of the drug,[12] is now included only as a minor, slow, questionable reaction[3] (reaction 4).

This study of the metabolism of lidocaine *in vivo* has quantitated the removal of the drug by the dog's liver. In the control state, the fraction of lidocaine removed by passage through the liver was approximately 25 per cent throughout the two-hour period of observation (fig. 1). The principal route for the metabolism of lidocaine *in vivo* is through the formation of MEGX. At one point in the control experiment, 76 per cent of the lidocaine which disappeared could be accounted for as MEGX formed; MEGX, however, is only an intermediary metabolite, and it also undergoes biotransformation by the liver.[2] The liver outflow concentration of MEGX reflects a balance between MEGX formation from lidocaine biotransformation and MEGX uptake, biotransformation, and biliary excretion by the liver. In this study (table 2), a variable relationship between lidocaine decrement and MEGX increment (blood concentration) was found, suggesting different rates of removal of lidocaine and MEGX by the liver.

Previous drug administration that produced enzyme induction was observed by Heinonin[4] to alter the rate of lidocaine metabolism *in vitro*. Burns *et al.*[13] found that enzyme induction was produced in dogs by administration of 16 mg/kg/day of phenobarbital, and this dose was used in this study. The amount of lidocaine removed by the liver after phenobarbital pretreatment increased twofold ($P < 0.01$), and corresponds with the twofold increase *in vitro* observed by Heinonin. Phenobarbital pretreatment also altered the balance between MEGX formation and removal by the liver. The fraction of lidocaine removed that could be accounted for by an increment in MEGX was markedly smaller after phenobarbital pretreatment. This fraction actually became negative after two hours, indicating that more MEGX is removed by the liver than is formed by lidocaine biotransformation. This effect of phenobarbital pretreatment could be the result of an increased rate of MEGX biotransformation, increased utilization of alternate lidocaine metabolic pathways (ring hy-

TABLE 3. Urinary Excretion of Lidocaine and Its Metabolites in Two Hours (Per Cent of Lidocaine Administered*)

	Lidocaine	MEGC	GX
Control values, four dogs	2.63 ± 1.16 (66.2 per cent)†	1.25 ± 0.69 (31.4 per cent)	0.095 ± 0.07 (2.4 per cent)
Values in same dogs after pretreatment with phenobarbital	4.53 ± 0.78 (59.6 per cent)	1.80 ± 0.79 (23.7 per cent)	1.27 ± 0.97 (16.7 per cent)

* Mean and SD calculated as per cent of total dose of lidocaine. The figures represent the lidocaine, MEGX, and GX which appeared in the urine in the two-hour period of study.
† Percentage of total urinary excretion of the substance.

FIG. 2. Summary of all known pathways of the metabolism of lidocaine. The relative importance of each pathway is indicated by the type of arrow.

droxylation) or increased biliary excretion of lidocaine and/or MEGX. Further studies should help to define this effect.

Despite evidence to the contrary,[2, 10] this study has demonstrated that GX is formed as a metabolite of lidocaine (reaction 5) in dogs both before and after pretreatment with phenobarbital. Phenobarbital pretreatment appears to accelerate formation and urinary excretion of GX. This increase in urinary excretion is probably related to the earlier appearance of this metabolite in the blood after pretreatment and to the resistance of this substance to further degradation, *i.e.*, hydrolysis. Once this metabolite begins to appear, the amount of GX formed appears to be a constant fraction of the lidocaine removed. In his recent study, Boyes[14] has also verified that GX appears in the urine of man and several animal species along with small amounts of meta-hydroxy lidocaine and meta-hydroxy MEGX. Hydroxylation of the aromatic ring to phenol derivatives has been postulated from indirect evidence,[9, 15] however, direct measurements have been lacking. All possible pathways for the biotransformation of lidocaine known at this time are included in figure 2.

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