The Effects Of Hemodilution, pH, and Protamine on Lidocaine Plasma Protein Binding and Red Blood-Cell Uptake *in Vitro*[1]

Rory P. Remmel,[2] Alan K. Copa,[3-5] and David M. Angaran[3,4]

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INTRODUCTION

In the cardiopulmonary bypass (CPB) patient, lidocaine protein binding may be highly variable because of the dynamic physiology associated with bypass. Hemodilution and hypothermia disrupt the fluid balance during CPB (1). Postoperatively, hypoalbuminemia is common and plasma concentrations of alpha-1-acid-glycoprotein (AGP) have been reported to increase twofold by the third post-bypass day (2). The changing acid–base balance during CPB may also influence the free fraction of lidocaine. As free concentrations of lidocaine may correlate better with a pharmacologic effect than total concentrations, accurate estimates of free concentrations in blood are essential.

In the general population, lidocaine is 60–80% bound to plasma proteins (3,4). Of the fraction bound, approximately 70% is associated with AGP and 30% with albumin (4). Lidocaine binding is influenced by the lidocaine concentration (3,5), pH (4,6), ionic buffers (6), AGP levels (3,4) smoking (7), and certain drugs (4,8). Lidocaine plasma protein binding varies considerably between patients and normal volunteers (3–5). This variability is related in part to the plasma concentrations of AGP (3,4). AGP, an acute phase reactant, displays both inter- and intrapatient variability (3,9–11).

In vitro factors that can affect the measured degree of lidocaine binding have been reported (12,13). Bypass patients receive very large doses of heparin (300 U/kg). Heparin releases lipoprotein lipase from tissues, which in turn increases free fatty acid concentrations in plasma. The lipase continues to produce free fatty acids *ex vivo*, resulting in a "heparin artifact" for the binding of lidocaine and several other drugs (12–14). In order to offset the heparin artifact, Brown *et al.* evaluated lipoprotein lipase inhibitors and suggested that protamine (14 mg/ml) was the agent of choice for lidocaine (13).

The purpose of this study was to examine methods for the proper handling of blood samples for the analysis of lidocaine in CPB patients. Specifically, the effects of hemodilution, protamine, and pH on lidocaine plasma protein binding and red blood-cell (RBC) uptake *in vitro* are described.

MATERIALS AND METHODS

Materials

A unit of whole blood was collected from a normal volunteer and anticoagulated with 1.5 mg/ml of EDTA. Hemoglobin, hematocrit, cholesterol, triglycerides, serum albumin, total protein, free fatty acids, and AGP were all within normal limits. Plasmalyte A (Baxter-Travenol, Deerfield, IL) was used as a diluent.

^{14}C-Lidocaine (sp act, 55.0 mCi/mmol) was obtained from DuPont/New England Nuclear (Waltham, MA). After purification by reversed-phase HPLC, it was >99% radiochemically pure. A methanolic solution of radiolabeled lidocaine (final concentration of 3.36 μg/ml of unlabeled lidocaine and approximately 100,000 dpm of ^{14}C-lidocaine) was added to silanized glass screw-capped tubes and evaporated to dryness. Three milliliters of whole blood were added and the tubes were shaken for 30 min on a reciprocal shaker (LabIndustries, Berkeley, CA) to equilibrate the lidocaine between plasma and erythrocytes. All glassware was treated with Surfasil (Pierce Chemical Co., Rockford, IL) to prevent adsorption of lidocaine to the tubes.

Membrane Ultrafiltration

The Amicon Centrifree micropartition system (Danvers, MA) was employed for protein binding determinations. Ha *et al.* (6) previously compared equilibrium dialysis and membrane ultrafiltration and reported no significant difference between the two methods for lidocaine. In preliminary experiments, we determined that trace concentrations of ^{14}C-lidocaine were not bound by the ultrafiltration membrane.

Protamine vs. EDTA

To determine the effect of protamine, blood (4 ml) containing 1.5 mg/ml EDTA or 1.5 mg/ml EDTA plus 14 mg/ml of protamine sulfate (Grade X, Sigma Chemical Co., St. Louis, MO) was adjusted to pH 7.4 by bubbling with 95% O_2/5% CO_2. After equilibration for 30 min with ^{14}C-lidocaine, three 50-μl aliquots of whole blood were removed and counted. The pH remained at 7.4 in the capped tubes during equilibration. Red blood cells were digested and counted by a wet oxidation method with perchloric acid (15). All samples were counted in Eco-Lite (WestChem, San Diego, CA) on a Beckman LS-6800 liquid scintillation counter. Typical efficiencies were 90–94% for ^{14}C, and the counts

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[2] To whom correspondence should be addressed at Department of Medicinal Chemistry, 8-174 Health Sciences Unit F, College of Pharmacy, University of Minnesota, 308 Harvard St. S.E., Minneapolis, Minnesota 55455.

[3] Department of Pharmacy Practice, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.

[4] Department of Biomedical Research, United Hospitals, Saint Paul, Minnesota 55102.

[5] Present address: Department of Pharmacy, Methodist Hospital, Indianapolis, Indiana 46202.

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were corrected for quench. The blood was then centrifuged at 1500*g* for 5 min, and three 50-μl aliquots of plasma were counted directly. Unless otherwise stated, the pH was adjusted to 7.4 with 95% O_2/5% CO_2 and 300 μl of plasma were placed in the ultrafiltration device. The ultrafiltration device was centrifuged at 2000*g* for 30 min in a fixed-angle centrifuge at room temperature. Then 50 μl of the ultrafiltrate were counted. The free fraction (ff) was calculated by the following equation.

$$\text{ff} = \text{dpm 50 μl ultrafiltrate/dpm 50 μl plasma}$$

and the fraction bound as

$$\text{fraction bound} = 1 - \text{ff}$$

Hemodilution

One to two liters of Plasmalyte A, a crystalloid diluent, is used to prime the CPB pump at our institution. Whole blood samples were diluted by 25, 50, or 75% with Plasmalyte A and compared to undiluted whole blood. The lidocaine concentration was 3.36 μg/ml for all samples to eliminate the influence of concentration on lidocaine binding (3,6). The samples were processed in quadruplicate as described above. The following values were calculated for each dilution: plasma concentration [P], RBC concentration [RBC], blood-to-plasma ratio (B/P); and red blood-cell:plasma ratio (RBC/P). These values were calculated by the following equations:

$$\text{B/P} = \text{dpm 50 μl blood/dpm 50 μl plasma}$$

$$\text{RBC/P} = \frac{\text{dpm 50 μl blood} - [\text{dpm 50 μl plasma} \times (1 - \text{Hct})]}{\text{dpm 50 μl plasma} \times \text{Hct}}$$

Effect of pH

The influence of pH was evaluated over a stepped range of 7.20–7.65. The pH of plasma was adjusted by bubbling with 95% O_2/5% CO_2 to the following values: 7.20–7.24, 7.25–7.29, 7.30–7.34, 7.35–7.45, 7.50–7.55, and 7.60–7.65. To simulate the situation in bypass patients, the effects of 50% hemodilution and pH on lidocaine plasma protein binding were also investigated. Stepped ranges with the following values were studied: 7.15–7.19, 7.25–7.29, 7.30–7.34, 7.35–7.45, and 7.50–7.54. All samples were processed as described above.

Statistical Analysis

Analysis of variance (ANOVA) was used to compare the sample means. The Scheffé procedure was employed to make individual comparisons. For the protamine/EDTA vs EDTA data, results were compared with Student's *t* test. Comparisons with a *P* value of <0.05 were considered significantly different.

RESULTS

For the EDTA group, a free fraction in plasma of 0.291 ± 0.007 was found ($n = 20$). For blood collected on EDTA plus 14 mg/ml protamine, the free fraction was 0.290 ± 0.009 ($n = 22$). There was no significant difference between the two treatments. In addition, the ultrafiltration method was found to be highly reproducible with a coefficient of variation of less than 5%.

Table I. The Effects of Hemodilution on Lidocaine Plasma Protein Binding[a]

Parameter	Percentage dilution			
	Control	25	50	75
Free fraction	0.291	0.451*	0.604*	0.787*
SD	±0.007	±0.009	±0.011	±0.017
B/P	0.839	0.936*	1.03*	1.07*
SD	±0.022	±0.020	±0.021	±0.015
RBC/P	0.617	0.796*	1.15*	1.67*
SD	±0.049	±0.064	±0.099	±0.139
RBC/free	2.11	1.77*	1.92	2.13
SD	0.208	0.140	0.171	0.179
Plasma conc.[b]	4.01	3.59*	3.26*	3.14*
SD	±0.103	±0.076	±0.067	±0.045
Free conc.[b]	1.15	1.62*	1.96*	2.46*
SD	±0.032	±0.044	±0.061	±0.051
RBC conc.[b]	2.47	2.86*	3.75*	5.24*
SD	±0.128	±0.170	±0.243	±0.364
n	12	21	20	11

[a] B/P, concentration in blood:concentration in plasma ratio; RBC/P, concentration in red blood cells:concentration in plasma ratio; RBC/free, concentration in red blood cells:free concentration in plasma ratio; *n*, number of replicates.
[b] Mean concentration as μg/ml.
* Significantly different from control, $P < 0.01$, ANOVA, Scheffé's procedure.

Table I shows the effects of hemodilution on lidocaine

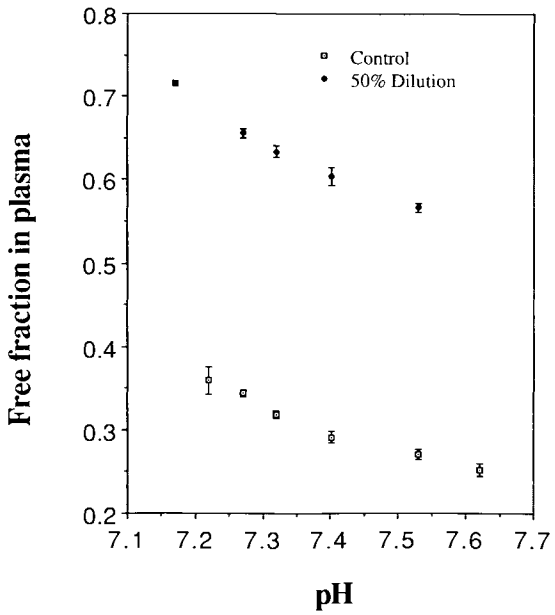

Fig. 1. The effect of pH on the free fraction of lidocaine in control plasma (open squares) and plasma diluted with an equal volume (50% dilution) of Plasmalyte A (filled diamonds). The pH is the mean value of the following pH ranges: 7.12–7.18, 7.20–7.24, 7.25–7.29, 7.30–7.34, 7.35–7.45, 7.50–7.55, and 7.60–7.64.

Table II. Effect of pH on Lidocaine Plasma Protein Binding

	Mean plasma pH (pH range)					
	7.22 (7.20–7.24)	7.27 (7.25–7.29)	7.32 (7.30–7.34)	7.4 (7.35–7.45)	7.53 (7.50–7.55)	7.62 (7.60–7.64)
Mean ff	0.359	0.344	0.318	0.291	0.271	0.252
SD	0.016	0.004	0.005	0.007	0.006	0.008
n	5	9	6	20	7	8

plasma protein binding, partition coefficients (B/P and RBC/P), and drug concentrations in various blood components in whole or diluted blood spiked with 3.36 μg/ml of lidocaine (therapeutic range, 1.5–5.0 μg/ml). Hemodilution resulted in significant increases in the ff, the B/P ratio, RBC/P ratio, the free concentration, and the concentration in the RBCs (all P's < 0.01, ANOVA). This was accompanied by a decrease in the total plasma concentration ($P < 0.01$). The intrinsic partition coefficient represented by the RBC/free ratio was relatively constant at all dilutions, although the ratio for the 25% dilution was significantly lower than the control. Figure 1 shows the effects of pH on the free fraction of lidocaine in plasma from undiluted blood and in 50% diluted blood. The two curves were parallel but significantly different at all pH values studied.

DISCUSSION

We found that protamine (14 mg/ml) did not alter protein binding. Our results concur with those of Brown *et al.* (13), who found that protamine effectively inhibited the heparin artifact. Dubé *et al.* (16) had reported protamine to be ineffective in preventing the heparin artifact, and that concentrations >10 mg/ml resulted in severe hemolysis. The difference may be due to the source of protamine or plasma pH, which was not corrected. Recently, Dubé *et al.* reexamined the effect of addition of protamine base on plasma pH and plasma protein binding of lidocaine (17). Addition of 10 mg/ml of protamine base increased plasma pH from 7.54 to 8.20, resulting in a decreased free fraction of lidocaine, but no alterations were observed in lidocaine binding if the pH was adjusted back to 7.4 (17). We observed no hemolysis with protamine sulfate concentrations of 14 mg/ml; however, the blood pH was reduced by 0.2–0.3 pH unit with the addition of protamine sulfate. Thus protamine appears to prevent heparin-induced artifacts, if the plasma pH is corrected in all samples.

Hemodilution occurs with the initiation of CPB because of the necessity of priming the pump oxygenator. This results in a 40–50% dilution of blood volume to a hematocrit of approximately 25% (18). The increased free fraction of lidocaine upon hemodilution correlated well with the degree of dilution ($r^2 > 0.99$), presumably because of a smaller concentration of plasma protein binding sites. For drugs like lidocaine that enter and bind to RBCs, the increased free fraction may have significant implications, because of the uptake into the erythrocytes.

The clinical ramifications of this process in CPB are difficult to discern. With hemodilution, total blood concentrations should drop proportionately. However, free fraction increases with hemodilution and this would be important, especially if one assumes that the free concentrations correspond more closely to the pharmacological effect.

An additional consideration of hemodilution involves the determination of pharmacokinetic parameters. Lidocaine is a high-clearance drug, such that its total-body clearance is close to that of hepatic blood flow. Since blood is presented to the eliminating organ(s) rather than plasma, total blood concentrations may be more appropriate for the determination of clearance. As total plasma concentrations decrease with hemodilution, estimates of clearance based on plasma concentrations would be falsely high.

A second important variable in lidocaine binding is pH (Table II), because the pK_a of lidocaine, a weak base, is close to that of physiological pH (7.61 at 36°C) (19). The pK_a of lidocaine changes with temperature and ionic strength, two factors that also change during hypothermic CPB (19). It has been previously reported that the choice of buffers, especially bicarbonate based buffers (20), or the ionic strength of phosphate buffers (6) significantly changes lidocaine free fraction in equilibrium dialysis. Thus as suggested by Ha *et al.* (6), we adjusted the pH with 95% O_2/5% CO_2 and used an ultrafiltration technique to minimize the potential changes in pH.

During CPB, respiratory alkalosis is often observed, and anesthesiologists at our institution maintain blood pH near 7.4 during CPB. Upon rewarming, it would be expected that the blood would become more acidotic. For accurate determinations of lidocaine binding during CPB, the pH should be adjusted to the physiological blood pH at the time of collection. When large amounts of heparin are given prior to bypass, blood should be collected on protamine. These methods should provide more accurate estimates of free lidocaine concentrations.

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