Importance of Blood-Collection Tubes in Plasma Lidocaine Determinations

W. W. Stargel,1 Charles R. Roe,2 P. A. Routledge,3 and D. G. Shand3

In 25 clinical samples serum lidocaine concentrations fell from a mean of 6.5 ± 2.1 mg/L (mean ± SD) to 4.9 ± 1.8 mg/L (p < 0.001) when the blood sample was allowed to make contact with the stopper of the Vacutainer® collection tube. In vitro experiments showed that this effect of the stopper occurred only with whole blood and was dependent on sample concentration. The plasma binding of lidocaine decreased from a normal value of 56% ± 2.2 (mean ± SD) to 28% ± 2.2 (p < 0.001) when exposed to the Vacutainer stopper. We conclude that a chemical leached from such stoppers displaces lidocaine from its plasma-binding sites and that the drug is then redistributed into the erythrocytes, producing spuriously low lidocaine concentrations in plasma or serum. Such artifacts are important in therapeutic drug monitoring and can lead to erroneous clinical decisions.

Additional Keyphrases: drug assay • blood-collection tubes • variation, source of

Therapeutic monitoring of drug concentrations in plasma has now become an accepted part of clinical practice, and many new assay procedures are being developed. Although it is known that spurious values can result from the use of certain tubes for blood collection (1), this information has not been very widely disseminated. It was first shown that lower plasma propranolol concentrations resulted when Vacutainer Tubes (Becton-Dickinson, Rutherford, NJ 07070) rather than an all-glass system were used for blood collection. Since then, the same artifact has been noted with the other basic drugs: alpenolol, imipramine, chlorimipramine, and nortriptyline (2), as well as meperidine (3). Diminished plasma binding of quinidine from blood collected in Vacutainer Tubes has also been noted, but the resulting effect on plasma concentration was small (4, 5).

We recently noted erratic concentrations of the antiarrhythmic drug lidocaine in plasma, and have investigated whether this involves binding displacement and redistribution because of the use of the blood-collection tubes.

Materials and Methods

Patients’ Samples

Blood samples were collected into plastic syringes from patients and transferred to Vacutainer Tubes from which the red stopper had been removed. One aliquot was allowed to clot, while another aliquot was placed in a red-stoppered tube, allowed to contact the stopper, and clot. The two serum samples were assayed for lidocaine with one lot of EMIT®-cad (Syva Corp., Palo Alto, CA) reagents.

In Vitro Experiments

One hundred milliliters of whole blood from a normal volunteer receiving no medication was collected by direct venipuncture in a plastic syringe and immediately transferred to a glass tube containing 500 US units of heparin. Lidocaine hydrochloride was added to give a final concentration of 3 µg of lidocaine per milliliter of blood. Two-milliliter aliquots were then transferred to five glass-stoppered tubes, five green-stoppered Vacutainer Tubes, and five red-stoppered Monoject® tubes (Sherwood Medical Industries, St. Louis, MO). The remaining blood was then centrifuged, and 1-mL aliquots of the plasma layer were added to various tubes, as described for whole blood. All tubes were then inverted 10 times and plasma obtained from the blood samples. All plasma samples were assayed for lidocaine with one lot of EMIT reagent.

The effect of increased concentrations of lidocaine (up to 12 µg/mL) added to blood collected in Vacutainer Tubes or in an all-glass system was also investigated in quadruplicate.

Plasma Binding

Plasma was obtained after blood collection into Vacutainer Tubes or glass-stoppered tubes, and lidocaine was added to a final concentration of 2 µg/mL. Five 1-mL samples of plasma from each collection system were dialyzed against 1 mL of Sorensen’s phosphate buffer (pH 7.38) at 37 °C for 6 h by means of Teflon dialysis cells and Spectrapor® membranes (Spectrum Medical Industries, Los Angeles, CA). Lidocaine concentrations in both plasma and buffer were measured and calculated from appropriate standard curves, and the per cent plasma binding was calculated.

Results

In the 25 clinical samples, exposure to the rubber stopper lowered lidocaine concentration in serum from 6.5 ± 2.1 µg/mL (mean ± SD) to 4.9 ± 1.8 µg/mL (p < 0.001 by a paired t-test).

In the in vitro experiment, there was a significant reduction in the lidocaine concentration of plasma obtained from blood collected in the commercial tubes (Table 1). This was not seen when plasma was added to the tubes, which shows that er-

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Discussion

Cotham and Shand (1) postulated that there was in some rubber stoppers a chemical that could leach into blood and reduce the plasma binding of propranolol. The increased free drug could then partition with the drug bound to erythrocytes, which would lead to spuriously low plasma concentrations. The plasticizer tria(2-butoxyethyl) phosphate (TBEP) has been reported to be a contaminant in plasma collected in Vacutainer Tubes (6) and identified as an inhibitor of drug binding to protein (7). Borga and coworkers subsequently have shown that TBEP-inhibited protein binding of alpranolol and imipramine is due to a selective effect on α1-acid glycoprotein, a probable major binding site for these basic ligands (2). In our studies we have found a similar phenomenon with lidocaine, with reduced binding in plasma confirmed. It is therefore likely that 'TBEP' is the compound that displaces lidocaine from its binding sites in plasma, although the relevant binding protein for lidocaine has not yet been identified (8). It is interesting that the magnitude of the effect of this phenomenon on lidocaine concentrations in plasma is very similar to the mean percentage effect on plasma propranolol concentration (28%) in plasma, calculated from the data of Cotham and Shand (1), despite the fact that propranolol is 90–95% bound at therapeutic concentrations whereas lidocaine is only about 60% bound at a plasma concentration of 3 μg/mL (8). Presumably, the relative affinity of TBEP for the binding sites in plasma is much greater than the affinity of lidocaine.

Tucker and coworkers also showed that the binding of lidocaine varied from approximately 70% at 1 μg/mL of plasma to around 40% at 12 μg/mL (8). Theoretically, therefore, the effect of Vacutainer Tubes on the lidocaine concentration in plasma measured from a whole blood sample should fall with increasing lidocaine concentration, and we found this to be the case (Figure 1). However, the percentage change of lidocaine concentration in plasma was still marked throughout the "therapeutic" range (1.5 to 5.0 μg/mL of plasma). The figure also shows that the concentration ratio of blood-to-plasma increases with increasing lidocaine concentration in whole blood.

The enzyme immunoassay (EMIT) of lidocaine in serum or plasma has been shown to be rapid, specific, and sensitive and therefore particularly suitable for therapeutic monitoring (9). We feel it is important to emphasize that these advantages may be nullified and patients exposed to risk if clinical decisions are made on the basis of spuriously low concentrations of lidocaine in plasma or serum because of the use of certain types of commercially available collection tubes.

We are grateful to Jan Admiraal and Peggy Rogers for their excellent technical assistance and to Syva Corporation for the reagents.

References


Table 1. Effect of Various Blood-Collection Tubes on Measured Lidocaine Concentration (μg/mL) in Plasma

<table>
<thead>
<tr>
<th></th>
<th>Control (all glass)</th>
<th>Green Vacutainer Tube</th>
<th>Red Vacutainer Tube</th>
<th>Red Monopject</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean a</td>
<td>SD b</td>
<td>Mean a</td>
<td>SD b</td>
</tr>
<tr>
<td>Whole blood</td>
<td>3.12 ± 0.13</td>
<td>2.42c ± 0.08</td>
<td>2.22c ± 0.15</td>
<td>2.28c ± 0.08</td>
</tr>
<tr>
<td>Plasma</td>
<td>3.16 ± 0.06</td>
<td>3.02 ± 0.19</td>
<td>3.08 ± 0.13</td>
<td>3.18 ± 0.28</td>
</tr>
</tbody>
</table>

a mean of five separate aliquots of the same blood sample or plasma derived from the blood sample. Lidocaine was added to freshly withdrawn blood to make a concentration of about 3 μg of lidocaine per milliliter of whole blood.

b SD = standard deviation.

c p < 0.001 by analysis of variance.

Fig. 1. Effects of increasing lidocaine concentrations in whole blood collected in Vacutainer Tubes or in an all-glass system on the subsequent lidocaine concentration in plasma

Each dot represents the mean and range of four samples; line of identity, ...
Improved Automated Kinetic Determination of Uric Acid in Serum by Use of Uricase/Catalase/Aldehyde Dehydrogenase

Knut Bartl, Max Brandhuber, and Joachim Ziegenhorn

The enzymatic determination of serum uric acid by use of uricase, catalase, and aldehyde dehydrogenase according to Haeckel [J. Clin. Chem. Clin. Biochem. 14, 101 (1976)] showed interferences from ethanol-converting enzymes, which are present in some patients’ sera. We have identified these enzymes as alcohol dehydrogenase isoenzymes. Among other substances, a mixture of pyrazole and oxalate can be used to eliminate these interferences. This inhibitor system gives good results when used in the automated kinetic uric acid determination, as is shown by a comparison with the manual assay for uric acid according to Kageyama [Clin. Chim. Acta 31, 421 (1971)].

Additional Keyphrases: centrifugal analyzer • variation, source of • uremia • liver disease • kidney disease

A new kinetic method for the automated determination of uric acid by use of uricase (EC 1.7.3.3), catalase (EC 1.11.1.6), and aldehyde dehydrogenase (EC 1.2.1.5) according to Haeckel (1) has been developed in our laboratory (2). The reaction sequence is as follows:

\[
\text{Uric acid} + 2 \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{uricase}} \text{allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2 \\
\text{Ethanol} + \text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} \text{acetaldehyde} + 2 \text{H}_2\text{O} \\
\text{Acetaldehyde} + \text{NADP}^+ \xrightarrow{\text{aldehyde dehydrogenase}} \text{acetate} + \text{NADPH} + \text{H}^+ \\
\]

The amount of acetaldehyde formed from ethanol by means of H\textsubscript{2}O\textsubscript{2}/catalase equals the amount of uric acid present in the sample. If, e.g., ethanol-converting enzymes such as alcohol dehydrogenases are present in the sample, acetaldehyde is continuously formed from ethanol, thus giving falsely high values for uric acid:

\[
\text{Ethanol} + \text{NADP}^+ \xrightarrow{\text{ADH}} \text{acetaldehyde} \\
(\text{EC 1.1.1.1}) + \text{NADPH} + \text{H}^+ \\
\text{Acetaldehyde} + \text{NADP}^+ \xrightarrow{\text{aldehyde dehydrogenase}} \text{acetate} + \text{NADPH} + \text{H}^+ \\
\]

During our evaluation of the method, a few serum samples from patients suffering from acute liver or renal disease caused interferences, which led to an overestimate of uric acid. A similar effect has already been reported by Haeckel (1), who, to increase the accuracy of the assay system, proposed use of NADP\textsuperscript{+} instead of NAD\textsuperscript{+} as coenzyme and determination of a sample blank. However, using NADP\textsuperscript{+} diminished but did not eliminate the interference; furthermore, determination of a sample blank is inconvenient in an automated assay. To improve the specificity and practicability of the assay system, we investigated the molecular basis of the interference described above. The results allowed us to modify the kinetic assay so that it yields precise results in each case without determination of sample blanks (3).

**Materials and Methods**

**Apparatus**

We used an ENI GEMSAEC centrifugal analyzer, including DEC-PDP 8 and DEC tape (Electro-Nucleonics, Fairfield, NJ 07006), an Abbott ABA-100 analyzer (Abbott Scientific Products Division, S. Pasadena, CA 91030), and a LKB 8600 reaction rate analyzer (LKB-Produkter, Bromma, Sweden) for the automated experiments. Manual assays were done with

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