**Laboratory Note**

**Determination of Lidocaine in Blood and Tissues**

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LIDOCAINE has been investigated widely as a local anesthetic and a cardiac antiarrhythmic agent. Several gas chromatographic assays of blood and plasma have been reported, each requiring an evaporation step which has limited sensitivity due to either high background activity or loss of lidocaine in submicrogram amounts. Keenaghan and Boyes measured unchanged lidocaine in rat tissues after wet oxidation with perchloric acid and hydrogen peroxide, but encountered interfering peaks in feces and carcass so that lidocaine could not be determined in these samples. Ahmad and Medzhiradska reported another tissue method which required deproteinization, ether wash, and methylene chloride extraction, followed by an evaporation step. This paper reports a modification of the method of Rowland et al., using multiple partitioning steps. The method is rapid, sensitive to a 0.01 µg/ml concentration of lidocaine in blood, and adaptable to analysis of tissue samples with minimal background interference.

**Materials and Methods**

**Apparatus**

A Varian 1200 gas chromatograph with a 6-foot, ½-inch outer-diameter glass column is used. The packing is 3 per cent OV-17 coated onto 100-120-mesh Chromosorb W DMCS/113 AW, conditioned for 24 hours at 250°C without gas flow and 24 hours with gas flow. Operating conditions are: oven temperature, 170°C; detector and injector port, 260°C; nitrogen and hydrogen flow, 20 ml/min; air flow, 300 ml/min.

**Procedure**

Heparinized human or rhesus monkey blood or plasma, 0.5 to 1.5 ml, was placed in a 15-ml screw-capped centrifuge tube. The volume was adjusted to 2.0 ml with an internal standard solution of a mixture of 0.4 µg/ml 2-diethylamino-acetanilide (DEA) and 1.5 µg/ml 2-diethylamino-3'-bromacetanilide (Bromo DEA) made in 0.1 M phosphate buffer, pH 7.4, and with phosphate buffer when necessary. Sample size and volume of internal standard were chosen on the basis of expected lidocaine content to give comparable peak heights of lidocaine and internal standards. The solution was shaken with 7 ml distilled ether for one minute and then centrifuged. The ether layer was then transferred to a nipple-bottomed centrifuge tube containing 0.2 ml 0.1 N HCl. This tube was specially made from a screw-capped centrifuge tube to which 18 mm of a 7-mm outer-diameter tubing was fused at the bottom. Total volume of the nipple was about 100 µl. A similarly designed tube has been diagrammed by Beckett. The ether-acid mixture was shaken manually by repetitive inversion for 60 seconds, care being taken to mix the liquid in the nipple with the bulk of the solution. After centrifugation at 2,000 rpm for 10 minutes, the ether layer was aspirated off and discarded. The acid layer was air-dried for 10 min to remove residual ether, then alkalinized with 0.1 ml 0.5 N NaOH.

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ratio of lidocaine to each of the internal standards was measured and the concentration of lidocaine determined with reference to standard calibration curves.

Tissue samples were obtained from rhesus monkeys undergoing long-term lidocaine infusions. Approximately 5-g samples of fresh tissue obtained immediately after sacrifice were added to preweighed vials each containing 20 ml 10 N NaOH. After exact tissue solution ratios had been obtained, the samples were homogenized in a Waring blender and allowed to solubilize. One-gram amounts of these homogenates were processed in a manner similar to blood samples. Caution was taken to check the pH of the aqueous layers in the various extraction steps with lung, brain and fatty tissues because of the extraction of ether-soluble basic substances. Appropriate amounts of HCl or NaOH were added whenever necessary to readjust the pH of the acidic phase to <2 and that of the basic phase to >10. Most tissues extracted cleanly, but in a few cases with lung and brain samples the acid-base extraction steps were repeated before addition of CS2 in order to reduce background peaks.

Results and Discussion

Gas chromatographic patterns from blank samples of monkey and human blood revealed only the internal standard peaks. A sample lidocaine chromatograph is shown in Figure 1. The flame ionization detector yielded a minimal response to CS2, so that the solvent front was of short duration. Repeated injections of samples dissolved in the CS2 phase over several days revealed no significant change in peak-height ratios. The relative retention times of DEA, lidocaine, and Bromo DEA were .53, 1, and 1.71, respectively. No peaks were seen after the Bromo DEA internal standard peak, which appeared at 11 min, 48 sec. Repeat injections may be performed at 15-min intervals.

Standard curves were constructed by addition of 0.2, 0.5, 1.0, and 2.0 μg lidocaine in phosphate buffer to blank blood. Calibration curves were constructed with respect to each internal marker. Coefficients of variation of the peak-height ratios at 0.2, 0.5, 1.0, and 2.0 μg lidocaine/ml blood (n = 4 for each
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point) were: lidocaine/DEA: 1.81, 5.45, 2.42, and 7.07 per cent; lidocaine/Bromo DEA: 2.91, 7.61, 2.31, and 3.19 per cent. The extraction was performed within 30 min of the CS$_2$ step. Delays in aqueous phases may lead to loss of DEA, reflected by lowering of DEA/Bromo DEA ratios and of the standard curve. Samples were stable in the CS$_2$ phase for at least seven days. When extraction times were consistent, the ratio of the peak heights of the internal standards to one another remained fairly constant and was used as an index of satisfactory extraction conditions. Estimates of sample lidocaine content were computed as the arithmetic mean of the results based on each of the internal standards. No difference between assaying blood and assaying aqueous solution was noted. The chromatograms obtained with this partition method are exceptionally clean because there is no concentration of undesired substances in an evaporation step. With long-term infusions of lidocaine in both man and monkey, there appeared a double peak just before the lidocaine peak at a relative retention time of 0.83, which was not seen in monkey or man not receiving lidocaine and which probably represents metabolic products of lidocaine. Using 1.5-ml blood samples with 0.5 ml internal standard solution, a lidocaine concentration as low as 0.01 µg/ml can be estimated.

Tissue sample blanks of liver, lung, muscle, brain, and fat were extracted and yielded no peaks with the same retention time as lidocaine or internal standards. Standard curves for various tissues with addition of lidocaine in vitro were linear with identical calibration factors to blood. A brain tissue chromatograph, which represents the most difficult tissue extraction, is illustrated in figure 2. Repeat assays of tissue samples after 250 days in 10 N NaOH reveal no significant change in lidocaine content of liver, brain, kidney and muscle. Coefficients of variation for assay of skeletal muscle, liver, lung, and brain (n = 4 for each tissue) were 2.5, 5.0, 2.6, and 8.2 per cent, respectively. This method has been used to study partition of lidocaine into all body tissues of the monkey.

The method allows direct extraction from homogenized tissue by the same procedure used for blood. In addition, it is simpler and more rapid than that of Ahmad and Medzhiradskiy. It provides remarkably clean chromatographic patterns even when determining low concentrations of lidocaine in blood and plasma. Background peaks which interfere with tissue determinations are eliminated in most tissues and reduced to assayable levels in brain, lung, and fatty tissues.

References

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