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A Simple Method for Gas Chromatographic Determination of Lidocaine in Tissues

Emiko Naito, Ph.D.,* Michiko Matsuki, M.D.,† Koki Shimoji, M.D.‡

A simple gas chromatographic method for the determination of lidocaine in tissues is described. Lidocaine is extracted from the tissue samples using liquid nitrogen. The recoveries of lidocaine from brain, liver, and muscle were 98.6, 99.8 and 89.1 per cent, respectively. Results were reproducible to within 1.0 per cent of lidocaine administered. (Key words: Anesthetics, local, lidocaine; Measurement techniques, gas chromatography.)

LIDOCAINE has been investigated widely as a local anesthetic, cardiac antiarrhythmic agent, and an adjuvant for general anesthesia. The quantitative determination of lidocaine in tissue is obviously important for assessment of the distribution, metabolism, and toxicity of the drug following various routes of administration. Although several chromatographic assays of lidocaine have been reported, most of them are analyses of only whole blood or plasma.1–4 Benowitz and Roland2 described a rapid, sensitive method for measuring lidocaine in tissues, but it requires a large amount of tissue in one sample, thereby precluding serial measurement, especially in small animals. Keenan and Boyes5 measured unchanged lidocaine in rat tissue by a wet oxidative technique but interfering gas chromatographic peaks in carcass extracts made it impossible to determine the quantity of lidocaine. Ahmad and Medzhirdsky6 reported a complex method for analysis of lidocaine in tissues. This method requires several steps, including deproteinization, ether wash, methylene chloride extraction, and evaporation. In this paper we report a method for extracting lidocaine from tissue samples using liquid nitrogen and determining its concentration by a modification of the method of Ishikawa.4 Our method is simpler, more rapid, and enables quantification of lidocaine concentrations as low as 0.5 μg/g in approximately 0.3-g samples of tissue.

Methods

The gas chromatographic unit used was a Shimazu GC-3BF with a hydrogen-flame ionization detector.

The chromatographic column, a coiled glass tube 300 cm long and 3.0 mm ID, was packed with Infusorial Earth 80/100 mesh coated with OV-17, 3 per cent by weight. The flow rates of nitrogen and hydrogen gases were 68 and 45 ml/min, respectively. Column and injection temperatures were 235 and 285 C, respectively. The internal standard used was mepivacaine, 25–50 μg.

Samples of fresh tissue (brain, liver, muscle) of approximately 0.3 g each were obtained from mongrel dogs undergoing lidocaine infusion. The samples were frozen immediately in liquid nitrogen. After crushing the frozen tissue with a precooled piston, the powdered samples were weighed accurately in a 10-ml preweighed homogenizer tube. A 3-ml volume of perchloric acid, 0.6 N, and mepivacaine were added. The amount of mepivacaine was adjusted in accordance with the expected tissue lidocaine concentration. After homogenization and centrifugation at 4,500 rpm for 10 minutes, the supernatant was transferred to a glass centrifuge tube. A 2-ml volume of perchloric acid, 0.2 N, was added to the residue and homogenization and centrifugation were repeated. The combined supernatants were adjusted to pH 6.5 with potassium hydroxide, 5 N. Then crystallized potassium perchlorate was removed by centrif-

* Instructor.
† Assistant Professor.
‡ Professor and Chairman.

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Address reprint requests to Dr. Naito.
§ Shimazu Seisakusho Ltd., Kyoto, Japan.

Fig. 1. Specimen record of a gas chromatogram, showing peaks of lidocaine (80.0 μg/g) and mepivacaine (50.0 μg/g). Tissue sample was 0.5 g of canine brain.
Fig. 2. Calibration curves of lidocaine concentration. Two straight lines were obtained in relation to the weight ratio of lidocaine to mepivacaine: calibration curve 1 when the weight ratio was less than 0.2, and calibration curve 2 when the weight ratio was more than 0.2 (mean ± SD, n = 7 at each point).

\[ y = 1.5337x - 0.0119 \]
\[ r = 1.000 \]
\[ P < 0.001 \]

\[ y = 1.8110x - 0.1344 \]
\[ r = 0.9991 \]
\[ P < 0.001 \]

Results

A specimen record of a gas chromatogram of a brain tissue sample is shown in figure 1. The retention time for lidocaine was 6 minutes and that for mepivacaine, 12 minutes. No interference with the assay was detected. Repeated injection could be performed at 15-minute intervals. The standard calibration curves are shown in figure 2. When the weight ratio was less than 0.2, calibration curve 1 was obtained, and when the ratio was more than 0.2, calibration curve 2 was obtained. In both, good correlation between the weight ratio and the peak height ratio was obtained. The determination of lidocaine was made in concentrations ranging from 0.5 to 200 \( \mu \)g/g tissue. Recoveries of lidocaine from brain, liver, and muscle were 98.6, 99.8, and 89.1 per cent, respectively. The results were reproducible to within 1.0 per cent of administered lidocaine. Thus, this method is simple and reliable, and can be used for repeated determinations of lidocaine concentrations in various tissues.

References