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GAS—LIQUID CHROMATOGRAPHIC DETERMINATION OF LIDOCAINE IN CAT PLASMA USING MEPIVACAINE AS INTERNAL STANDARD

H. BREUER

Zentrum Pharmakologie und Toxikologie, Abteilung Pharmakologie und Toxikologie II, Universität Göttingen, Robert-Koch-Strasse 40, D-3400 Göttingen (G.F.R.)

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SUMMARY

A method for the gas—liquid chromatographic determination of lidocaine in cat plasma with mepivacaine as internal standard is described. The investigations demonstrated a high reliability in the method, although the precautions required are relatively few. Under the cited conditions the plasma concentrations determined with the method after lidocaine treatment of cats were proportional to the infusion rates and obeyed a logarithmic normal distribution.

INTRODUCTION

Lidocaine is used systemically in the treatment of cardiac arrhythmias. In order to optimize therapy it is important to know its plasma concentration.

In a recent experimental investigation by Vogt et al. [1] on cats (see Table I), lidocaine plasma concentrations were to be determined by gas—liquid chromatography (GLC) (see also ref. 2)*.

Numerous GLC determinations of lidocaine have already been carried out (see, for example, refs. 3—19 and papers cited therein) using internal and external standards. Because of the expected higher precision an internal standard was used in the present paper.

In most of the works where an internal standard was used, substances with retention times similar to that of lidocaine or in the solvent "tail" region were chosen, both possibly leading to inaccuracies in the results. As mepivacaine does not have these disadvantages, it was used as an internal standard in this

*The cited authors kindly supplied the plasma samples and encouraged work on the present paper.

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paper, as in refs. 15—19, the latter being work using GLC—mass spectrometry. The retention time of mepivacaine was about twice that of lidocaine under the conditions described under Methods (3.35 min for lidocaine).

EXPERIMENTAL

Materials

Lidocaine—HCl (Xylocain®), 20%, and mepivacaine—HCL (Scandicain®), 3%, were from Astra Chemicals (Stockholm, Sweden). Dichloromethane, analytical grade, was from Merck (Darmstadt, G.F.R.).

Methods

Heparinized plasma (0.9 ml) of lidocaine-treated cats (see Table I) was either used at once or stored at —20°C. To the sample, either fresh or thawed out, were added 50 μl of 0.1% mepivacaine—HCl as an internal standard and 50 μl of 10 *M* sodium hydroxide. Then it was agitated twice with 3 ml of dichloromethane for 1 min, each 3-ml fraction being removed as completely as possible after centrifugation. The two dichloromethane fractions were collected in a conical centrifuge tube. The dichloromethane was removed by blowing nitrogen on its surface, occasionally inclining the tube in order to avoid too much deposition of the residue on the wall of the tube. After complete evaporation of the solvent 20 μl of dichloromethane were dispensed onto the bottom of the tube and the latter was agitated carefully for a few seconds. Then 1 μl of the solution was injected directly onto the GLC column. Similar extraction procedures are described in refs. 4 and 15.

The gas chomatograph HP 5730 A was equipped with a flame ionisation detector (FID) and a glass column, 1.83 m × 6.35 mm I.D., containing 3% OV-17 on 80—100 mesh Chromosorb W HP. Both the detector and injector temperatures were 250°C, the column temperature was held constant at 190°C.

The carrier gas was nitrogen at a flow-rate of 30 ml/min; the flame for the FID was generated with air and hydrogen at 240 ml/min and 60 ml/min, respectively.

The lidocaine concentrations were calculated from the ratio of lidocaine/mepivacaine according to a previously established standard curve, as described below.

RESULTS

GLC method

The following points were investigated in order to check the reliability of the method.

Linearity and reproducibility on different days. For the establishment of a standard curve lidocaine—HCl was dissolved in pooled human plasma and the samples were subsequently prepared as described previously. The linearity between peak height and lidocaine—HCl concentration is good (correlation coefficient $r = 0.9998$) and the standard deviation between repetitive experiments on different days is small (corresponding to ± 0.2 μg/ml lidocaine—HCl up to a concentration of ca. 50 μg/ml, $n = 6$ duplicate experiments).

Another standard curve was established in the same way but with half the amount of mepivacaine standard for an investigation on cats with lower infusion rates [2] than in ref. 1.

Recovery and effectivity of extraction. It does not make any difference if cat plasma, human plasma or simply distilled water is used for the preparation of the standard curve samples, as the recovery in terms of the lidocaine/mepivacaine ratio is equal in each case ($p > 0.3$, $n = 4$, lidocaine—HCl = 10 μg/ml). No lidocaine or mepivacaine could be detected in the aqueous phase after extraction.

Precision. Upon examination of the precision for five consecutive determinations in pooled human plasma at lidocaine—HCl concentrations of 5 μg/ml and 10 μg/ml, a coefficient of variation of 1.9% and 1.8%, respectively, was found.

Background peaks. Occasionally small background peaks were found in the lidocaine peak region without any lidocaine treatment of the cats (corresponding to 0.3 ± 0.2 μg/ml lidocaine—HCl in three cats, see Fig. 1). Although the influence on the results was small (compare Fig. 2) a corresponding "blank" value was subtracted. The background did not disappear if additional extraction steps between the organic phase and acid as well as basic media were carried out as described in ref. 5.

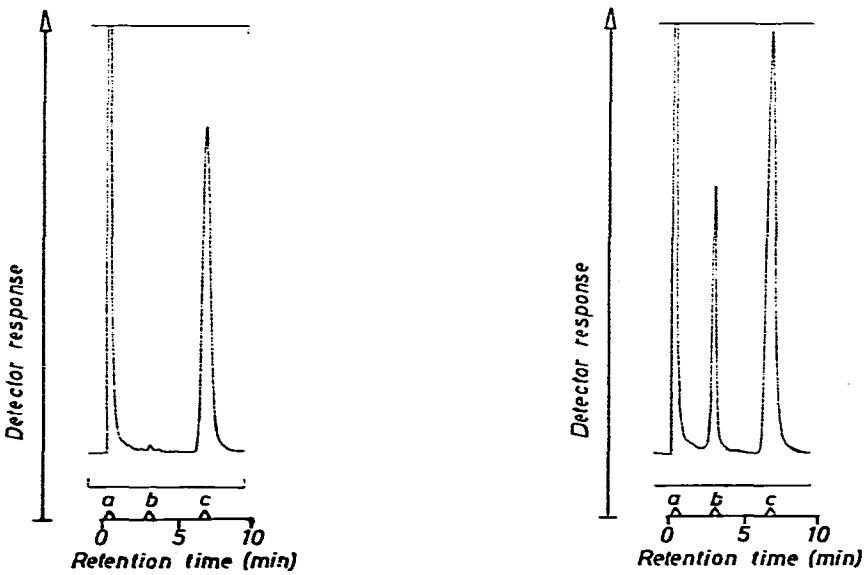

Fig. 1. Typical gas chromatogram of a sample from an untreated cat. a = Solvent peak (methylene chloride); b = endogenous peak with retention time equal to that of lidocaine; c = mepivacaine peak (internal standard).

Fig. 2. Typical gas chromatogram of a sample from a cat at the end of lidocaine—HCl infusion (0.70 mg/kg·min). Same animal as in Fig. 1. a = Solvent peak (methylene chloride); b = lidocaine peak; c = mepivacaine peak (internal standard).

Influence of gas flow-rates. The peak height ratio of lidocaine/mepivacaine was practically independent of the gas flow-rates within ± 50% limits.

Sample stability. Incubation of samples at 25°C and 60°C before and after the alkalinisation with sodium hydroxide showed stability of both lidocaine

and mepivacaine against enzymic and spontaneous hydrolysis. Freezing for one week at —20° C did not alter the results obtained in fresh samples.

Detection limit. If a blank value before lidocaine treatment is available, the detection limit of the method is approximately 0.1 μg/ml, otherwise it is approximately 0.5 μg/ml lidocaine—HCl.

Lidocaine concentrations in cat plasma during and after infusion

The results of the lidocaine determination in cat plasma are summarized in Table I. Apparently a doubling of the infusion rate results in a doubling of the lidocaine concentration. Occasionally small metabolite peaks with retention times less than that of lidocaine partially overlapped with the lidocaine peak obtained 1 h after infusion stopped. The contribution of these peaks to the lidocaine peak height could be neglected.

TABLE I

LIDOCAINE PLASMA CONCENTRATION DURING AND AFTER INTRAVENOUS INFUSION IN CATS

Treatment (see Vogt et al. [1])	Number (n)	Lidocaine—HCl concentration (μg/ml) (mean ± S.D.)	Log (lidocaine—HCl concentration)
Bolus intravenous injection of 2 mg/kg lidocaine—HCl, then 20 min infusion of 0.35 mg/kg-min, finally collection of arterial blood sample	24	10.1 ± 2.8	0.991 ± 0.117
Doubling of the infusion rate: 0.70 mg/kg-min; second sample after further 20 min	20	21.4 ± 6.4	1.312 ± 0.127
Infusion stop and last sample after further 60 min*	11	3.2 ± 1.4**	0.468 ± 0.171

*The peak identification by gas chromatography—mass spectrometry was kindly carried out by Dr. H. Luthe, Medizinische Universitätsklinik Göttingen, Abteilung Klinische Chemie.
**One "outlier" replaced by a new measuring value (see ref. 1).

An arterial blood sample was taken from a cat and then the animal was treated as outlined in Table I (two further samples collected) with the exception that after the second infusion period a third 20-min period with three times the original infusion rate (1.05 mg/kg-min) was included, at the end of which another sample was taken. The dependence of the lidocaine concentration from the infusion rate in this experiment shows a high linear correlation of the two parameters (r = 0.9997).

In another animal several samples were taken at different times during the two infusion periods mentioned in Table I. The lidocaine concentrations during this time are shown in Fig. 3.

Fig. 4 shows the lidocaine concentrations in the elimination period of a third animal on a semi-logarithmic scale. Obviously the elimination occurs in two phases; therefore, the calculations of the pharmacokinetic parameters have

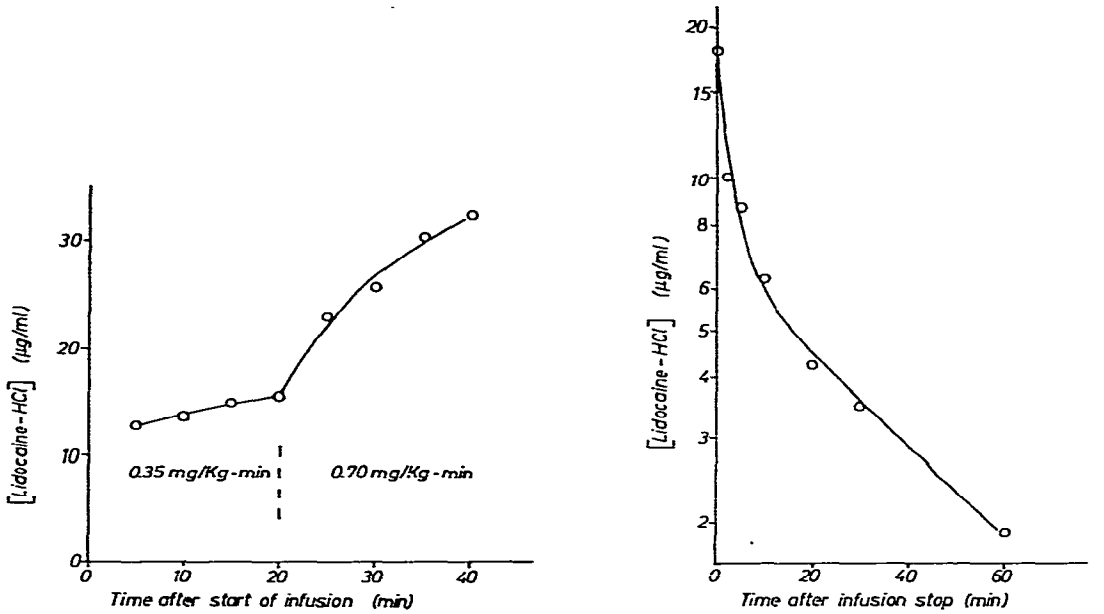

Fig. 3. Lidocaine plasma concentrations during infusion.

Fig. 4. Lidocaine elimination after infusion stop.

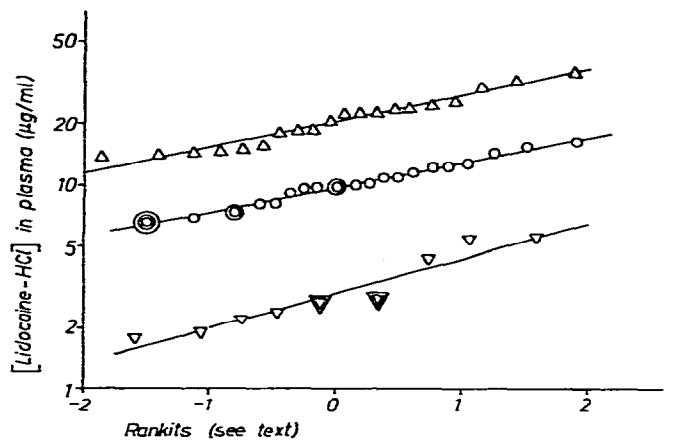

Fig. 5. Test for logarithmic normal distribution of lidocaine concentrations in cats during and after intravenous influsion (see text). △, Infusion of 0.70 mg/kg·min; ○, infusion of 0.35 mg/kg·min; ▽, 1 h after infusion stopped.

been carried out according to a two-compartment model by Gauss—Newton iteration (see refs. 20 and 21).

For the infusion period a one-compartment model gave satisfactory results. The lines drawn in Figs. 3 and 4 were obtained using the calculated pharmacokinetic parameters.

In Fig. 5 the ranked lidocaine concentrations are plotted on a logarithmic scale against the "rankits", i.e. the values theoretically to be expected for a

normal distribution. The rankits [22] were taken from tables [23]. If concentration values were equal, the rankit for the mean of their rank numbers was used.

DISCUSSION

As the peak height of the lidocaine/mepivacaine ratio is proportional to the lidocaine concentration, integration of the peak area is not necessary for the calculation of the concentrations; simple measurement of the height is sufficient.

The high precision and accuracy of the described method follows from the stability of the samples against freezing and storage, completeness of lidocaine and mepivacaine extraction, high linear correlation of the standard curve, low coefficient of variation in consecutive measurements and good reproducibility of the measurements at different days with mepivacaine as an internal standard.

Cyclizine, which has been used, for example, by Zylber-Katz et al. [6] as an internal standard, has a retention time near to that of lidocaine. Although a "to and fro" extraction* between acid and basic media, automatic peak integration and temperature programming was performed by these authors, they found a higher coefficient of variation in consecutive measurements (3.1%) than in the present paper at a similar lidocaine concentration. The possible reasons for this difference are mentioned in the Introduction.

Pape et al. [18] used mepivacaine as internal standard but only a single initial extraction. Their coefficient of variation was about twice that of this paper, suggesting the importance of a double extraction (see below).

Other papers reporting methods with a mepivacaine standard can not be compared with the present one because they used other instrumentation (nitrogen–phosphorus detector [17], gas chromatography–mass spectrometry [19], did not give details concerning the reliability of the method [15], or used material other than plasma [16].

As lidocaine is directly extracted from the plasma without previous protein precipitation, and because of the simplicity of the calculations, the method is relatively easy to carry out.

Due to the small influence of the gas flow-rates on the measured values, the measurements require few precautions and the method can therefore be carried out routinely. The same is true for the extraction. As it is difficult to remove the organic phase completely from the aqueous phase in one step, a double extraction was carried out, which reduced the error possibilities to a minimum. Furthermore, a single extraction may be incomplete and different for lidocaine and mepivacaine [15, 18]. The pipetting of the 50-μl volume of internal standard is essentially the only step to be done with a higher degree of care.

With an appropriate variation the described method should be suitable for mepivacaine determinations with lidocaine as an internal standard as well.

Limitations of the method arise from the low concentrations found during

*The plasma sample is made alkaline and extracted into *n*-hexane, re-extracted into a small volume of an aqueousacid phase, and finally extracted into 50 μl of methylene chloride after alkalinization.

dental anaesthesia. The lidocaine and mepivacaine concentrations in this case are 0.3 μg/ml and 0.4 μg/ml, respectively [13], thus being near to the detection limit of the described method. In lumbar and other forms of regional anaesthesia the maximum lidocaine concentrations were between 1 μg/ml and 4 μg/ml [11, 12], which could still be detected with sufficient reliability by this method. In any case the method is more suitable to analysis after systemic therapeutic treatment or intoxication.

Clearly, the patients must not be treated with mepivacaine at the same time if a high accuracy of the results is desired.

The lidocaine concentrations in the cat fit a logarithmic normal distribution better than a linear one. The elimination half-life of the "slow" phase determined in one animal (32 min) is of the same order of magnitude as found by others [14].

Especially in the high level infusion period (0.70 mg/kg·min) the steady state was not completely reached after 20 min, as at the end of the infusion the plasma concentration curve was not yet parallel to the abscissa and a "fast" elimination compartment could subsequently be observed (Figs. 3 and 4).

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