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Note

Determination of dibucaine in biological samples by gas chromatography with a nitrogen–phosphorus detector

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Dibucaine, 2-butoxy-N-(2-diethylaminoethyl)cinchoninamide, has been used as a potent local anesthetic for relief of pain or for spinal anesthesia since the 1930s. Therapeutic deaths associated with this drug have occurred following spinal anesthesia during appendectomy and caesarean birth [1,2]. Therefore, it is important from the viewpoint of forensic toxicology to detect small amounts of the drug in the biological fluids and tissues.

Several methods for the determination of dibucaine have been reported [3–6]: spectrophotometry, paper chromatography, thin-layer chromatography and gas chromatography (GC). These methods are useful, but are relatively non-specific and insensitive. Because of the low dose used in spinal anesthesia, the development of a simpler, specific and sensitive assay for the detection of dibucaine is required. Recently, a method involving assay by gas chromatography–mass spectrometry (GC–MS) has been reported [7,8], but it is neither economically nor technically feasible for routine use in most forensic toxicological laboratories. In addition to the GC–MS assay, a GC assay using a nitrogen–phosphorus detector (NPD), which is very sensitive and specific for the compounds containing nitrogen and phosphorus, has been reported.

We describe here a procedure for the determination of dibucaine in the biological samples using GC–NPD. This procedure is sufficiently sensitive to permit measurement of biological fluids and tissues.

EXPERIMENTAL

Apparatus

We used a Hewlett-Packard Model 5710A gas–liquid chromatograph equipped with a flame-ionization detector and a nitrogen–phosphorus detector. The coiled

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glass column (1.2 m×2 mm I.D.) was silanized and then packed with 2% OV-101 on 100–120 mesh Chromosorb W HP (Hewlett-Packard, Avondale, PA, U.S.A.). The temperatures of the column, injector and detector were maintained at 240°C, 250°C and 300°C, respectively, but during simultaneous analysis of some local anesthetics, the temperature of the column was programmed from 160°C to 240°C at a rate of 8°C/min. The gas flow-rates were: helium carrier gas, 34 ml/min; hydrogen, 3.4 ml/min; air, 80 ml/min.

Reagents

Dibucaine hydrochloride and T-caine (*p*-butylaminobenzoyldiethylaminoethanol hydrochloride) were purchased from Teikoku Chemical Industry (Osaka, Japan). Tetracaine hydrochloride and procaine hydrochloride were obtained from Hoei Pharmaceutical (Osaka, Japan). Lidocaine hydrochloride was obtained from Fujisawa Pharmaceutical (Osaka, Japan). 2-Butoxy-N-(dimethylaminoethyl)cinchoninamide, the internal standard (I.S.), was synthesized according to the procedure described by Aeschlimann [9] and Miescher [10]. All other chemicals were of reagent grade available commercially.

Procedure for sample preparation

The urine sample (1 ml) or plasma sample (0.5 ml) was alkalinized with 1 *M* sodium hydroxide, and 0.1 ml of the I.S. solution (2 μg/ml) was added. The contents of the tube were shaken with 10 ml of hexane for 1 min. After centrifugation at 700 *g* for 5 min, 8–9 ml of the solvent were evaporated to dryness in vacuo. The residue was dissolved in 0.1 ml of methanol, and 2 μl were injected into the gas chromatograph.

The tissue sample (1 g) was homogenized in 5 ml of 0.1 *M* hydrochloric acid and then centrifuged at 10 000 *g* for 15 min. The supernatant (2 ml) was alkalinized with 0.1 ml of 4 *M* sodium hydroxide, and 0.1 ml of the I.S. solution was added. The rest of the procedure was as described above.

Applicaton of the method

Male Wistar strain rats (200–250 g) were injected intraperitoneally with 10 mg/kg dibucaine hydrochloride dissolved in saline. Urine was collected for 0–24 h and 24–48 h after administration. Rats were killed by decapitation under light diethyl ether anesthesia at various time intervals, and the blood and the tissues were then removed. The plasma was immediately separated from whole blood by centrifugation.

Five healthy volunteers (22–35 years old, 55–65 kg) were orally administered 10 mg of dibucaine hydrochloride in 5% sucrose solution. Urine was collected five times at 2-h intervals after administration.

All the samples were frozen at −20°C until analysed.

RESULTS AND DISCUSSION

The response of local anesthetics, including dibucaine to NPD was examined. Fig. 1 shows the simultaneous temperature-programmed GC analyses of some

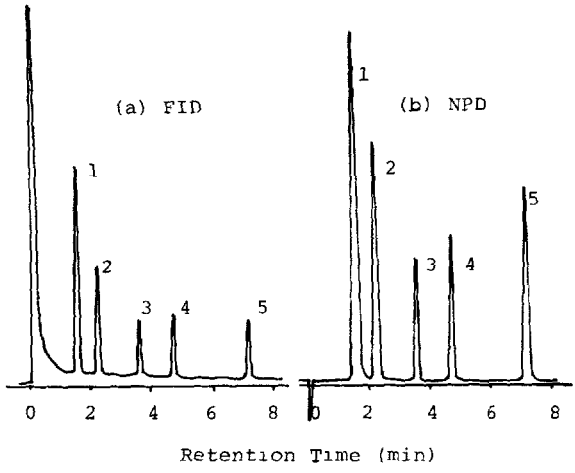

Fig. 1. GC of some local anesthetics using (a) FID and (b) NPD. Peaks: 1 = lidocaine; 2 = procaine; 3 = tetracaine; 4 = T-caine; 5 = dibucaine. The GC analysis with FID was carried out on 100 ng of each compound and NPD was carried out on 10 ng of each compound. The column temperature was programmed from 160 °C to 240 °C at 8 °C/min.

local anesthetics. As can be seen, the NPD response to each compound was 20–30 times more sensitive than that of a flame-ionization detector (FID). Additionally the amounts of ca. 50 pg were readily detectable by NPD. These findings suggest that it is possible to use GC–NPD for a sensitive and specific analysis for these local anesthetics.

We developed the GC–NPD assay to detect small amounts of dibucaine in biological samples. Hexane was chosen as the organic solvent to extract dibucaine from the biological samples, because it has a higher efficiency for extraction of dibucaine from the alkalinized samples than other solvents and because it extracts smaller amounts of endogenous substances from biological samples than other solvents.

Typical gas chromatograms of extracts from plasma, urine and tissue (lung) samples are shown in Fig. 2. There are no endogenous interfering peaks at retention times corresponding to those of dibucaine and I.S. on the chromatograms to those of dibucaine and I.S. on the chromatograms of blank samples. Furthermore, dibucaine and I.S. are well separated.

The calibration curves were prepared by adding known amounts of dibucaine to the biological samples, and were obtained by plotting the peak-height ratio of dibucaine to I.S. against the concentrations of dibucaine. They were linear in the concentration range 20–2000 ng/ml. The correlation coefficients were greater than 0.996 over the concentration range studied. Moreover, the lower limit for the detection of dibucaine in each biological sample was ca. 10 ng/ml or ng/g with a signal-to-noise ratio of ca. 10:1.

The extraction recoveries of dibucaine from the biological samples were determined at three different concentrations. As shown in Table I, the mean extraction recovery was ca. 98% for urine, ca. 99% for plasma, ca. 100% for lung and kidney,

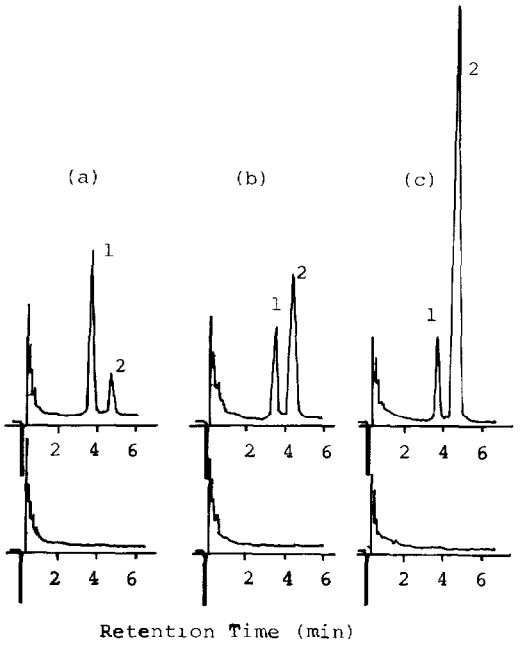

Fig. 2. Upper chromatograms obtained from (a) plasma (92 ng/ml), (b) urine (310 ng/ml) and (c) lung (940 ng/ml) samples of rats receiving dibucaine intraperitoneally (10 mg/kg). Lower chromatograms obtained from drug-free (a) plasma, (b) urine and (c) lung samples. Peaks: 1 = I.S., 2 = dibucaine.

and ca. 95% for liver, respectively. The coefficients of variation (C.V.) were less than 5%.

The inter-assay precision of this method was calculated from biological samples, which were analysed as replicates on different days using a new calibration curve each day. For plasma, urine and liver samples, the mean coefficients of variation were 5.0%, 2.5% and 5.6%, respectively (Table II).

The method was applied to the determination of dibucaine in plasma, tissue and urine samples of rats after intraperitoneal administration of dibucaine (10

TABLE I

EXTRACTION RECOVERIES OF DIBUCAINE FROM SEVERAL BIOLOGICAL SAMPLES

Each value represents mean ± S.D. of three experiments.

Sample	Extraction recovery (%)			C.V. (%)
	50 ng/ml	100 ng/ml	500 ng/ml	
Urine	99.1 ± 2.7	95.6 ± 2.1	98.0 ± 1.8	2.2
Plasma	96.4 ± 4.0	99.8 ± 4.6	102.4 ± 3.9	4.2
Lung	98.8 ± 4.5	100.4 ± 4.1	101.1 ± 3.0	3.9
Kidney	102.7 ± 3.9	100.7 ± 3.3	99.2 ± 4.1	3.7
Liver	95.4 ± 4.7	93.4 ± 2.9	95.0 ± 3.2	3.8

TABLE II

INTER-ASSAY PRECISION OF THE DETERMINATION OF DIBUCAINE IN BIOLOGICAL SAMPLES

Experiments were performed with biological samples from rats after intraperitoneal administration of dibucaine (10 mg/kg).

Sample	Mean concentration (ng/ml)	C.V. (%)	*n*
Plasma	89	6.3	5
	530	4.2	5
	748	4.5	3
Urine	105	1.8	5
	355	3.2	5
Liver	185	7.0	5
	638	5.6	5
	1480	4.2	5

mg/kg). The time courses of mean concentrations in plasma and tissue (liver, lung and kidney) are shown in Fig. 3. The plasma concentration of dibucaine peaked (ca. 1020 ng/ml) 30 min after administration, and then decreased gradually to approach ca. 90 ng/ml at 2 h. The tissue concentration showed similar behaviour and was highest in lung (ca. 19.5 μg/g at 30 min) than other tissues. The cumulative urinary excretion (0–48 h) in rats was less than 1% of the dose, and most of this were excreted in the 24-h urine (Table III).

Urine samples from healthy volunteers receiving oral administration of dibucaine hydrochloride (10 mg) were also analysed by this method. As shown in Table III, small amounts of dibucaine (31–410 ng/ml) were detected in the urine.

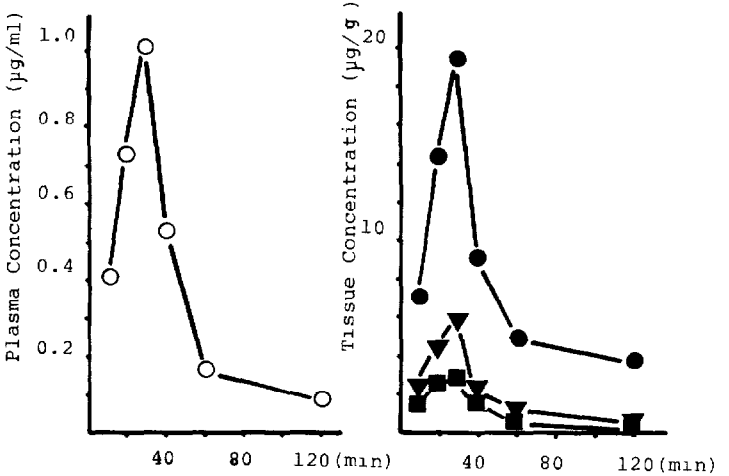

Fig. 3. Plasma concentration and tissue concentration of dibucaine in rats receiving dibucaine intraperitoneally (10 mg/kg). The values are the mean of three animals. Data points: ○ = plasma; ● = lung, ▼ = kidney; ■ = liver.

TABLE III

URINARY EXCRETION OF DIBUCAINE AFTER INTRAPERITONEAL ADMINISTRATION TO RATS (10 mg/kg) AND ORAL ADMINISTRATION TO HEALTHY VOLUNTEERS (10 mg)

Each value represents mean ± S.D. of five animals or five volunteers.

Rat			Human		
Time (h)	Concentration (ng/ml)	Percentage of dose	Time (h)	Concentration (ng/ml)	Percentage of dose
0–24	334 ± 81	0.66 ± 0.25	0– 2	104 ± 46	0.06 ± 0.05
24–48	77 ± 31	0.08 ± 0.04	2– 4	146 ± 127	0.13 ± 0.10
			4– 6	76 ± 76	0.09 ± 0.06
			6– 8	103 ± 86	0.11 ± 0.09
			8–10	90 ± 77	0.09 ± 0.08
Total		0.77 ± 0.26			0.47 ± 0.40

Moreover, the cumulative urinary excretion (0–10 h) of dibucaine in humans was less than 1% of the dose.

Kageura et al. [7] and Alkalay et al. [8] have reported a method for determining nanogram amounts of dibucaine from biological materials by GC–MS. Kageura et al. developed a method for determining dibucaine in biological materials and demonstrated that the detection limit was 0.8 ng per sample used (g or ml). Alkalay et al. also demonstrated that the assaying limit was ca. 2 ng/ml in serum using chemical ionization GC–MS. Our GC–NPD method has a sensitivity comparable with those of the procedures described by Kageura et al. and Alkalay et al., and is a quicker, simpler and more economical process than one using GC–MS.

In conclusion, the present method is a specific and sensitive method for the determination of dibucaine in biological samples, and is suitable for routine and rapid analysis in forensic toxicological and clinical toxicological laboratories.

REFERENCES

1 C. Wakasugi and I. Shikata, Jpn. J. Legal Med., 30 (1976) 391.
2 Investigation Committee of the Medico-Legal Society of Japan, Jpn. J. Legal Med., 37 (1983) 434.
3 N. Kinoshita, Fukuoka Acta Med., 48 (1957) 814.
4 M. Fukui, Jpn. J. Legal Med., 24 (1970) 136.
5 E.C.G. Clarke, Isolation and Identification of Drugs, Pharmaceutical Press, London, 1974, p. 262.
6 A. Hamada, Y. Takeda and K. Goukon, J. Hyg. Chem., 14 (1968) 308.
7 M. Kageura, K. Totoki and T. Nagata, Jpn. J. Legal Med., 32 (1978) 188.
8 D. Alkalay, S. Carlsen and W.E. Wagner, Anal. Lett., 14 (1981) 1745.
9 J.A. Aeschlimann, J. Chem. Soc., Part II, (1926) 2902.
10 K. Miescher, Helv. Chim. Acta, 15 (1932) 163.