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Note

Sensitive detection and determination of cimetidine in human tissues with high-performance liquid chromatography

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There have been many reports [1–17] of high-performance liquid chromatographic (HPLC) methods for the analysis of the potent H_2 receptor antagonist, cimetidine, in biological fluids, but only Berg *et al.* [18] have described an analysis of this drug in human solid tissues. The pharmacokinetics were reported, but the sensitivity or reproducibility of the method was given little or no attention.

In forensic toxicological examinations, fresh body fluids are not always available because putrefaction, degradation and contamination have often occurred. Even whole blood cannot always be obtained owing to haemorrhage. We, therefore, have developed a sensitive, reliable and reproducible method to detect cimetidine in human tissues, the objective being to procure a precise toxicological evaluation.

EXPERIMENTAL

Apparatus

The liquid chromatographic system we used was a Yokogawa Model LC100 (Tokyo, Japan) consisting of a Model LC100P pump and a Model LC100U UV detector. The system was connected via a GP-IB data bus to a computer for system control and data integration.

Reagents

Cimetidine was provided by Fujisawa Pharmaceutical (Osaka, Japan) and metiamide used as the internal standard (I.S.) was from Smith Kline Fujisawa (Tokyo, Japan). Two buffer solutions were prepared: one of pH 9.0 was prepared by adding 0.1 *M* potassium dihydrogenphosphate solution to 0.1 *M* disodium hydrogenphosphate solution, and the other of pH 4.9 by mixing 1/30 *M* potassium dihydrogenphosphate solution and 1/30 *M* disodium hydrogenphosphate so-

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lution. 1-Octanol, 1-butanol and acetonitrile were purchased from Ishizu Seiyaku (Osaka, Japan). Acetonitrile was of HPLC grade, and the other reagents used were of analytical grade.

Biological samples

Human solid tissues including whole blood, liver and skeletal muscle were collected at the time of autopsy and were kept at −20°C until analysis.

Standard solutions of cimetidine and the I.S.

Cimetidine (10 mg) was dissolved in distilled water and the volume was adjusted to 100 ml to give a concentration of 100 ng/μl. This standard solution was diluted to give concentrations of 10 and 1 ng/μl. A standard solution of the I.S. was prepared to give a concentration of 50 ng/μl.

Extraction procedure

Each 1.0 g of whole blood or tissues was weighed and homogenized using a tissue homogenizer in a mixture of 5 ml of phosphate buffer (pH 9.0) and 100 μl of the I.S. solution (5 μg of metiamide) in a 30-ml centrifuge tube. Then 10 ml of 1-octanol were added and the preparation was shaken for 15 min After centrifugation at 850 *g* for 20 min, 8 ml of the organic layer were transferred to a 30-ml centrifuge tube containing 5.0 ml of 0.05 *M* hydrochloric acid. The mixture was then shaken for 15 min and centrifuged at 850 *g* for 20 min. A 4-ml volume of the aqueous layer was transferred to a 15-ml centrifuge tube, and neutralized with 0.2 ml of 1 *M* sodium hydroxide solution followed by the addition of 5 ml of phosphate buffer (pH 9.0). To the solution was added 2.5 ml of 1-butanol, and the preparation was shaken for 15 min. After 20-min centrifugation at 1800 *g*, 1.5 ml of the organic layer were evaporated to dryness in a water-bath at 70°C, under a stream of nitrogen. The residue was dissolved in 100 μl of distilled water, and a 10-μl aliquot of the solution was injected into the liquid chromatograph. The extraction procedure is summarized in Fig. 1.

Chromatography

The analytical column was a LiChrosorb RP-8 (5 μm, 250 mm × 4.0 mm I.D.) commercially pre-packed by E. Merck (Darmstadt, F R.G.). The mobile phase, water–phosphate buffer (pH 4.9)–acetonitrile (39:1.40, v/v), was pumped at a flow-rate of 1.0 ml/min through the column maintained at 40°C. The absorbance of the effluent was monitored at 228 nm. To prevent deterioration in column performance, the column was washed with 0.1% acetic acid solution and then with methanol for *ca.* 1 h after each experimental day.

Preparation of calibration curves

Samples of whole blood were prepared to contain cimitidine at concentrations of 0.01, 0.05, 0.10, 0.50, 1.00, 5.00 and 10.00 μg/g. The samples of liver and

Sample (*ca* 1 g)
- 1 Add metiamide solution (5.0 μg of metiamide)
- 2 Add 0 1 *M* phosphate buffer (5 ml, pH 9.0)
- 3 Homogenize
- 4 Add 1-octanol (10.0 ml)
- 5 Shake (15 min) and centrifuge (850 *g*, 20 min)

Organic layer (9 0 ml)
- 1 Add 0.05 *M* hydrochloric acid (5.0 ml)
- 2 Shake (15 min) and centrifuge (850 *g*, 20 min)

Aqueous layer (4 0 ml)
- 1 Neutralize with 1 0 *M* sodium hydroxide (0 2 ml)
- 2 Add 0 1 *M* phosphate buffer (5 ml, pH 9 0)
- 3 Add 1-butanol (2.5 ml)
- 4 Shake (15 min) and centrifuge (1800 *g*, 20 min)

Organic layer (1.5 ml)
- 1 Evaporate to dryness
- 2 Dissolve in distilled water (100 μl)

Solution (10 μl)

HPLC

Fig 1. Extraction procedure for cimetidine.

skeletal muscle were prepared to contain the same series of concentrations as the blood sample by spiking of the standard solution into their homogenates. These samples were extracted, as described above. Calibration curves were obtained by plotting the peak-area ratio of cimetidine to the I.S. for the cimetidine concentrations in each tissue sample.

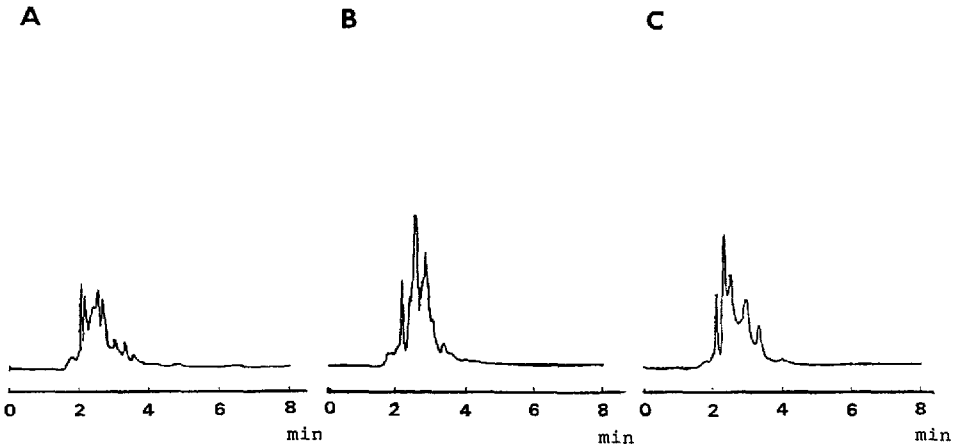

Fig. 2. Chromatograms of the extracts from drug-free tissues (A) whole blood, (B) liver; (C) skeletal muscle

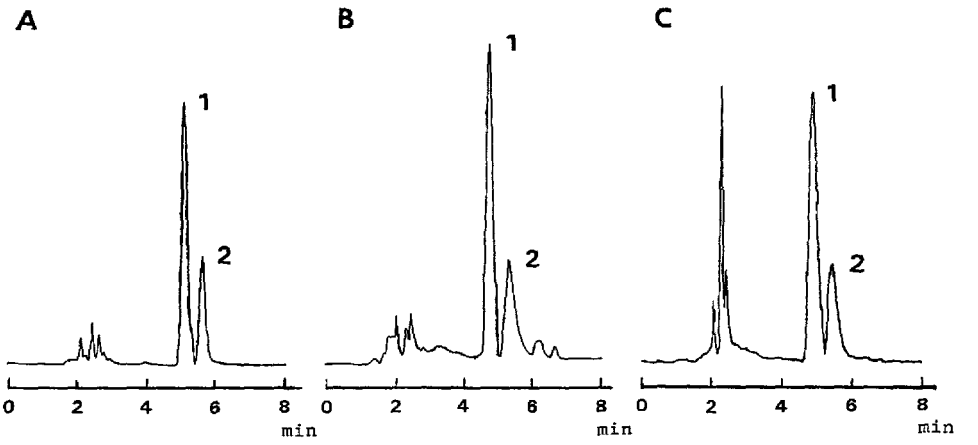

Fig 3 Chromatograms of the extracts from tissues containing 10 μg/g cimetidine and 5 μg/g I.S., (A) whole blood; (B) liver; (C) skeletal muscle Peaks 1 = cimetidine, 2 = I S

RESULTS AND DISCUSSION

There were no interfering peaks of endogenous substances in the chromatograms of the extract from blank tissues, as shown in Fig. 2, but a single extraction yielded interfering peaks, particularly in case of the solid tissues.

Typical chromatograms of the extracts from samples are shown in Fig. 3. Cimetidine and the I.S. were clearly separated as sharp and symmetrical peaks, and the retention times were 5.07 and 5.61 min, respectively. Each sample could be analysed within 8 min.

We successfully stabilized the retention times using phosphate buffer (pH 9.0) as an alkalizing reagent after neutralization with sodium hydroxide solution, and 1-butanol as a re-extraction solvent in the third step. Thus, better results were obtained compared with the method used by Kaneniwa *et al.* [10]. We attribute the stability to the smaller volume of sodium hydroxide injected into the eluent.

The calibration curves for cimetidine extracted from whole blood, liver and skeletal muscle were linear in the concentration range from 100 ng/g to at least 10 μg/g, with correlation coefficients of 0.999, 0.996 and 0.994, for the respective tissues. The lower detection limit, at a signal-to-noise ratio of 10, was 50 ng/g for each tissue, but it could be reduced to *ca.* 10 ng/g with an increase in the sample weight or in the volume injected. The calculated recoveries of cimetidine were 66–81% over the concentration range tested (Table I).

The reproducibility of this assay was checked by determining the intra- and inter-day variation, using three different concentrations (0.5, 1.0 and 5.0 μg/g), by adding cimetidine to blank whole blood, liver and skeletal muscle. The coefficients of variation (C.V.) for each tissue ranged from 1.7 to 8.6% for the intra-day assay and from 3.1 to 9.1% for the inter-day assay (Table II).

TABLE I

DATA ON RECOVERY OF VARIOUS CONCENTRATIONS OF CIMETIDINE IN HUMAN TISSUES

For each concentration, $n = 5$, theoretical recovery = 100%

Tissue	Concentration (μg/g)	Recovery (mean ± S.D.) (%)
Whole blood	0.1	66 95 ± 3.06
	0.5	77.61 ± 4 96
	1 0	81 30 ± 5.21
	5.0	78.67 ± 6.30
	10 0	73.24 ± 3 14
Liver	0 1	78 27 ± 6 11
	0 5	70.29 ± 3 86
	1.0	73 16 ± 1 70
	5.0	80 82 ± 5 89
	10.0	75.34 ± 2.42
Skeletal muscle	0.1	71.73 ± 8 46
	0 5	67 14 ± 4 02
	1 0	69 47 ± 6 95
	5.0	72 61 ± 2 08
	10.0	71.36 ± 4 74

TABLE II

PRECISION AND ACCURACY OF THE CIMETIDINE ASSAY

Tissue	Spiked concentration (μg/g)	Intra-day ($n = 5$)		Inter-day ($n = 5$)	
		Concentration (mean ± S D) (μg/g)	C V (%)	Concentration (mean ± S.D) (μg/g)	C.V (%)
Whole blood	0 5	0 51 ± 0 02	4.2	0.52 ± 0.03	5 7
	1.0	0 99 ± 0 08	7.6	1.00 ± 0 08	8 1
	5.0	5 02 ± 0.29	5.7	5 09 ± 0 41	8 0
Liver	0.5	0 51 ± 0.02	4.5	0.52 ± 0.03	5 8
	1 0	1 00 ± 0.09	8 7	1.08 ± 0 10	8.8
	5.0	5.02 ± 0 23	4.6	5 13 ± 0 47	9.1
Skeletal muscle	0 5	0.52 ± 0 02	4 8	0.52 ± 0.03	6 0
	1.0	1 04 ± 0 07	6.7	1.05 ± 0.09	8 1
	5 0	4 95 ± 0 09	1.7	4 91 ± 0 15	3 1

TABLE III

CONCENTRATIONS OF CIMETIDINE IN TISSUES OBTAINED AT AUTOPSY ON A CIMETIDINE-MEDICATED INDIVIDUAL

Autopsy was performed 6 h after death

Tissue	Concentration (μg/g)
Whole blood	5.6
Brain	0.9
Lung	8.2
Liver	0.8
Kidney	0.6
Spleen	1.6
Skeletal muscle	4.7
Adipose	1.8

These values are considered to be sensitive and reliable enough to measure levels of the drug prescribed in therapeutic doses.

Application

A 56-year-old man was found lying severely wounded on a street following a traffic accident. He was brought to the hospital, where cimetidine was reportedly administered to prevent Cushing ulcer. He died of excessive blood loss. In this particular case, a toxicological examination was ordered to determine whether or not the clinical treatment had been appropriate. A systematic toxicological examination revealed no evidence of any toxic substance The method described in this paper was used to determine the concentration of cimetidine in the tissue, as shown in Table III.

The values revealed the concentration of cimetidine to be at therapeutic levels. Cimetidine concentrations in biological fluids have been determined [1–17], but our HPLC assessments made feasible the determination of the level of this drug in human solid tissues.

CONCLUSION

A sensitive and reliable HPLC assay was developed to determine the concentration of cimetidine in human fluids and solid tissues. The lower limit of detection was *ca.* 10 ng/g.

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REFERENCES

1 N. E. Larsen, P. Hesselfeldt, S. J. Rune and E F Hvidberg, *J Chromatogr*, 163 (1979) 57
2 M. G. Kunitani, D. A. Johnson, R A Upton and S. Riegelman, *J. Chromatogr.*, 224 (1981) 156.
3 D. R P. Guay, H. N. Bockbrader and G R Matzke, *J. Chromatogr.*, 228 (1982) 398
4 J. Fleitman, G. Torosian and J. H. Perrin, *J Chromatogr*, 229 (1982) 255
5 J M Bartlett and A. B. Segelman, *J Chromatogr*, 255 (1983) 239
6 M Kozma and L. Vereczkey, *J Chromatogr.*, 273 (1983) 223
7 V Nitsche and H. Mascher, *J Chromatogr*, 273 (1983) 449
8 C W Lloyd, W J Martin, J. Nagle and A. R Hauser, *J Chromatogr*, 339 (1985) 139
9 A Adedoyin, L. Aarons and J B Houston, *J. Chromatogr*, 345 (1985) 192.
10 N Kaneniwa, T Funaki, S. Furuta and N Watari, *J. Chromatogr.*, 374 (1986) 430
11 R Chiou, R J. Stubbs and W F Bayne, *J Chromatogr*, 377 (1986) 441
12 H A Strong and M. Spino, *J Chromatogr.*, 422 (1987) 301.
13 W C Randolph, V L. Osborne, S S. Walkenstein and A P Intoccia, *J Pharm Sci*, 66 (1977) 1148
14 S J. Soldin, D. R. Fingold, P C Fenje and W. A Mahon, *Ther Drug Monit*, 1 (1979) 371
15 J. A Ziemniak, D A. Chiarmonte and J J. Schentag, *Clin Chem.*, 27 (1981) 272.
16 M Abdel-Rahim, D Ezra, C Peck and J Lazer, *Clin. Chem.*, 31 (1985) 621
17 G. Sticht, H. Kaferstein, M Oehmichen and M. Staak, *Beitr. Gerichtl Med*, 44 (1986) 263
18 M J. Berg, R K Lantz, J J Schentag and B. A Vern, *J Forensic Sci*, 29 (1984) 147