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Microdetermination of cimetidine in rat plasma by high-performance liquid chromatography

Y. Gomita [1], M. Nanba [1], K. Furuno [1], K. Eto [2] and Y. Araki [1]

[1] *Department of Hospital Pharmacy, Okayama University Medical School and* [2] *Okayama University Dental School, Okayama (Japan)*

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Summary

A rapid and simple method for the microdetermination of cimetidine in plasma from a single animal was investigated using high-performance liquid chromatography (HPLC). A microquantity (20 μl) of plasma from the rat tail vein was used without solvent extraction, and pharmacokinetic studies did not require the sacrifice of many animals. The recovery of added cimetidine averaged 97%. Coefficients of within-day variation ranged between 2.6 and 8.6%. The coefficient of correlation for the calibration curve in the concentration range of 0 to 10.0 μg/ml was 0.998. The detection limit was 0.1 μg/ml in the plasma. An excellent correlation was found between the concentration of cimetidine in the tail vein and that in the inferior vena cava. In addition, the plasma cimetidine concentration–time data for small animals could be obtained by this method.

Introduction

Cimetidine, an H_2-blocker, has been widely used clinically as an antiulcer drug. This drug is used often in combination with other drugs. Attention has, therefore, been focussed on the pharmacokinetic interactions among these drugs. In order to evaluate such interactions in experimental animals, a simple method for the measurement of the drug concentrations in the plasma is desired.

The methods for measurement of the cimetidine concentration in the blood so far employ high-performance liquid chromatography (HPLC) (Fleitman et al., 1982; Bartlett and Segelman, 1983; Nitsche and Mascher, 1983; Lloyd and Martin, 1985; Chiou et al., 1986; Kaneniwa et al., 1986). These methods, however, require large plasma samples. In addition, solvent extraction is required at the pretreatment stage, which makes the procedure more complicated. Therefore, these methods are inadequate for pharmacokinetic studies in small animals. In this study a method is established for determining cimetidine by HPLC in a microamount of plasma and the chronological changes in the plasma concentration of cimetidine in small animals are studied.

Materials and Methods

Drugs

Cimetidine, pure powder and injection, was donated by Smith Kline and French-Fujisawa. For oral administration, the pure powder was

Correspondence: Y. Gonita, Department of Hospital Pharmacy, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan.

suspended in 0.5% carboxymethyl-cellulose (CMC). The injection was dissolved in physiological saline for s.c. and i.v. administrations. Methanol and reagent grade $(NH_4)_2HPO_4$ were obtained from Wako, and methyl-*p*-hydroxybenzoate was obtained from Tokyo Kasei.

HPLC apparatus and conditions for drug determination

A Waters Assoc. HPLC apparatus (Type 510 pump, Type 481 UV detector, Type 710 B automatic sample processor and Type 730 Data Module) was used for the determination of the plasma concentration of cimetidine. The columns were packed with μ-Bondapak C-18 (Waters Assoc.; 3.9 mm diam. × 300 mm length). The mobile phase, methanol/0.05 M $(NH_4)_2HPO_4$, pH 8.4 (35/65 in volume), was pumped at a flow speed of 1.5 ml/min. The wavelength of maximal absorption by cimetidine, 228 nm (0.005 AUFS), was employed for spectroscopy. The standard curve was constructed using cimetidine solution (20 mg/20 ml of 50% methanol) diluted in plasma to concentrations of 0.5, 1, 2.5, 5, 10 and 20 μg/ml. Methyl-*p*-hydroxybenzoate solution (25 μg/ml) in 50% methanol was used as the internal standard.

Experimental animals and method of blood sampling

Male Wistar rats weighing 250–300 g were used. Throughout the experiment, animals were housed in plastic cages (26 × 36 × 25 cm) in groups of 4–5 under a 12-h light–dark cycle (light: 8.00–20.00 h). Water and food were withheld for 16 h before the drug administration and 8 h after the administration.

The upper part of the tail was cut slightly with a knife, and blood was sampled in a 60-μl capillary (Ames) for plasma separation. The sample was centrifuged for 3 min at 12,000 rpm in an ultramicrohematocrit centrifuge (Compur M1100, Ames). A 20-μl sample of plasma was used for each determination.

To determine the respective plasma concentrations of cimetidine between samples obtained from the tail vein and from the inferior vena cava, rats were laparotomized under ether anesthesia immediately after collecting from the tail vein, and approximately 5 ml of blood were collected from the inferior vena cava. Blood was sampled from the inferior vena cava 1–2 h after drug administration. The sample was centrifuged for 10 min at 3,000 rpm, and 20 μl of plasma was used for each determination.

Pretreatment of plasma

A Bond Elut C-18 minicolumn (1 ml volume, No. 607101 Analytichem Int.), washed twice with 1 ml methanol and twice with 1 ml distilled water, was loaded with 750 μl 0.05M $(NH_4)_2HPO_4$ solution, pH 8.4. Twenty μl of sample and 20 μl of internal standard solution were added separately to the minicolumn and stored in the column. After washing once with 1 ml 0.5 M $(NH_4)_2HPO_4$ solution (pH 8.4), elution was carried out with 250 μl of methanol to obtain the sample for HPLC.

Plasma drug concentration–time curve in rats

Cimetidine was administered p.o., s.c. or i.v. in rats to obtain the plasma drug concentration data. Cimetidine, at a dose of 50 mg/kg in 0.1 ml/100 g b.wt., was administered p.o. or s.c., followed by blood sampling from the tail vein 0.25, 0.5, 1, 2, 4 and 8 h after administration. For i.v. administration, cimetidine was administered at doses of 5 and 10 mg/kg, followed by blood sampling 5, 10, 15, 30 min, 1, 2 and 4 h after administration. The cimetidine concentration in the plasma was then measured. Cimetidine was administered at 09.00 h, taking into consideration the circadian rhythm of the animals.

Results

Conditions for measurement by HPLC

Blank rat plasma, blank plasma to which cimetidine and the internal standard solution were added, and rat plasma obtained after oral administration of cimetidine were treated as described above, and analyzed by HPLC. Typical chromatograms for plasma are shown in Fig. 1. The retention times for cimetidine and the internal standard were 4.7 and 7.1 min, respectively, with a good separation. No peaks corresponding to these retention times were noted in the blank plasma. No

interfering peaks due to cimetidine metabolites and other substances were noted.

Calibration curve, accuracy of measurement and recovery rate

The standard curve was prepared by adding cimetidine plasma solution at the concentrations of 0.5, 1, 2.5, 5, 10 and 20 μg/ml. An excellent linear correlation was noted in the range of 0–20 μg/ml between the concentration of cimetidine in the blood and the area ratio in the chromatogram between cimetidine and the internal standard ($r = 0.9999$, $y = 0.08x + 0.01$). The limit of detection calculated with an S/N (signal/noise) ratio of 2 was about 0.1 μg/ml. Coefficients of variation (CV) with reference to the within-day reproducibility in unknown samples I, II and III (obtained from the vena cava 2 h after oral administration of 100, 50 and 10 mg/kg cimetidine, respectively), were 5.2% (6.5 ± 0.34 μg/ml, $n = 10$), 2.6% (3.6 ± 0.09 μg/ml, $n = 10$) and 8.8% (0.8 ± 0.07 μg/ml, $n = 10$), respectively. The rate of recovery calculated by adding standard solutions of known concentrations of cimetidine to plasma samples averaged 97.0% (92.0–104.0%) (Table 1).

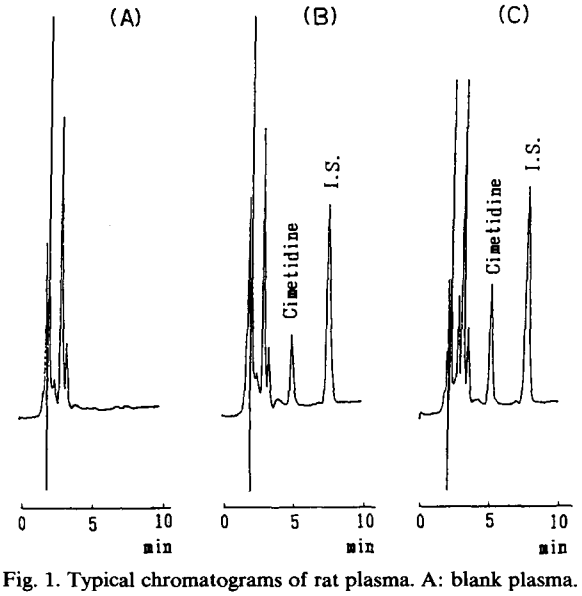

Fig. 1. Typical chromatograms of rat plasma. A: blank plasma. B: plasma with 2.5 μg/ml cimetidine. C: plasma after oral administration of cimetidine at a dose of 50 mg/kg (cimetidine concentration, 4.7 μg/ml). I.S., internal standard.

TABLE 1

Recovery of cimetidine in HPLC determination

Cimetidine in plasma sample (μg/ml) (A)	Cimetidine (μg/ml)			Recovery (%) (C − A)/ B × 100
	Added (B)	Measured (C)	Recovered (C − A)	
1.8	2.5	4.2	2.4	96.0
2.6	2.5	5.2	2.6	104.0
5.1	2.5	7.5	2.4	96.0
10.1	2.5	12.4	2.3	92.0
				Mean: 97.0

Correlation of plasma concentration between blood samples obtained from the tail vein and from the inferior vena cava

Cimetidine, at doses of 20–75 mg/kg, was administered p.o. to 16 rats, followed by sampling from the tail vein 1–2 h after the administration. Immediately after sampling from the tail, a laparotomy was performed, and a blood sample was obtained also from the inferior vena cava. The plasma cimetidine concentration was measured in both plasma samples, and the correlation coefficient was $r = 0.968$ (regression equation of $y = 1.06x + 0.02$), indicating a high correlation between these two concentrations.

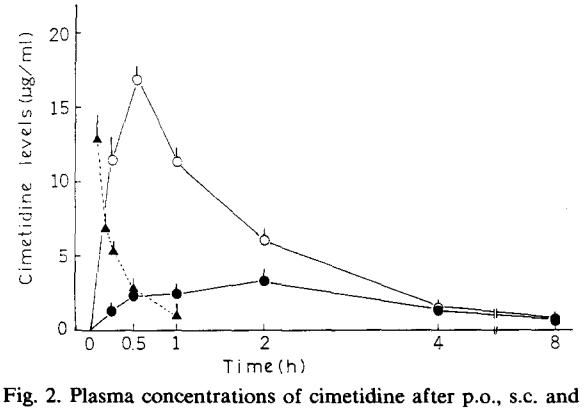

Fig. 2. Plasma concentrations of cimetidine after p.o., s.c. and i.v. administration of cimetidine in rats. Values represent the mean ± S.E.M. ●———●, p.o. administration of 50 mg/kg; ○———○, s.c. administration of 50 mg/kg; ▲- - - - -▲, i.v. administration of 10 mg/kg.

Chronological changes in the plasma concentrations

Chronological changes in the plasma concentration of cimetidine after p.o., s.c. and i.v. administrations are shown in Fig. 2. In the group given cimetidine s.c. at a dose of 50 mg/kg, the drug was rapidly transferred to the blood, reaching the highest level 0.5 h after administration. In the group given the same dose, the transfer was delayed, and the maximum level was reached only after 2 h. The terminal phase disposition rate constant was approximately the same in the s.c. and p.o. administration groups. In the group given cimetidine i.v. at a dose of 10 mg/kg, the blood level rapidly fell. Thus, the plasma cimetidine concentration–time data for small animals could be obtained by this method.

Discussion

Methods for measurement of cimetidine in the blood (Fleitman et al., 1982; Bartlet and Segelman, 1983; Nitsche and Mascher, 1983; Lloyd and Martin, 1985; Chiou et al., 1986; Kaneniwa et al., 1986) so far have relied on solvent extraction of the plasma before determination by HPLC. To study the pharmacokinetics in small animals, a large number of animals are required for each sampling, which is done from the vena cava after decapitation. In the present method, a microquantity of plasma is used without solvent extraction, and many animals need not be sacrificed to obtain blood drug concentration–time data. An excellent correlation was found between the concentration of the drug in the inferior vena cava, the site of blood sampling so far, and that in the tail vein. This would justify the use of 20 μl of plasma obtained from blood sampled from the tail vein to measure the concentration of cimetidine in the blood. The whole amount (0.36 ml) of blood sampled from one rat in one experiment corresponds to less than the ordinary amount (approximately 20 ml) of blood taken from one human blood donor. The feasibility of observing plasma cimetidine dynamics in chronological sequence represents a great advantage. For the pretreatment of the sample, a Bond Elut C-18 minicolumn was used, without mixing with the extracting solvent, centrifugation or condensation of the extract. Simple addition of plasma and an internal standard to the column was sufficient to prepare the sample.

The present method has made it possible to obtain the plasma cimetidine concentration–time data in a single rat and, thus, the same data in parallel in several rats, following simple and rapid pretreatment of microquantities of plasma. In the future, the method will be useful to study the interactions of cimetidine with other drugs and the changes of plasma concentration of cimetidine produced by other influencing factors.

It is said that the plasma concentration of cimetidine needed to cause an inhibitory secretion of gastric acid in clinical therapy is approximately 0.1–1.0 μg/ml (Burland et al, 1975; Larsson et al, 1979; Sonne et al, 1981). The limit of detection in the present method using 20 μl plasma sample was about 0.1 μg/ml. Accordingly, it is possible that the plasma concentration of cimetidine near C_{max} is detected by this method, but the lower concentrations may not be accurately detected with this sample volume. However, if a plasma volume 2–5 times the above volume is prepared, the detection of lower plasma concentrations is also possible.

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