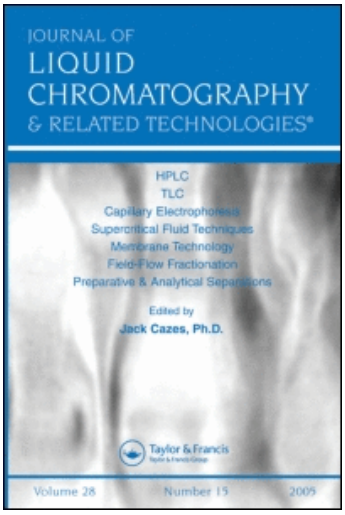

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DETERMINATION OF UNBOUND CIMETIDINE IN RAT BLOOD BY MICRODIALYSIS AND LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple and sensitive method for the determination of protein-unbound cimetidine in rat blood was developed, based on the sampling method of microdialysis. The microdialysis probe was inserted into the jugular vein/right atrium of male Sprague-Dawley rats. Cimetidine (10 mg/kg, i.v.) was then administered via the femoral vein. Separation was achieved on a LiChrosorb RP-18 column with a mobile phase consisting of acetonitrile-100 mM monosodium phosphate, pH 3.0 (22:78, v/v). The UV detector wavelength was set at 228 nm. The detection limit of cimetidine was 50 ng/mL. The in vivo recovery of microdialysate for cimetidine at 0.5 and 1 μg/mL were 73 ± 8% and 74 ± 8%, respectively (n=5). Intra- and inter-assay accuracy and precision of the analyses were ≤ 10% in the range of 0.05 through 10 μg/mL. Pharmacokinetic analysis of results obtained using such a micro-

dialysis-chromatographic method indicated unbound cimetidine in the rat fitted best to a biexponential decay model.

INTRODUCTION

Cimetidine is an H_2 receptor antagonist which competitively inhibits the interaction of histamine with H_2 receptors causes a significant reduction in gastric acid secretion and its H^+ concentration for the treatment of gastric and duodenal ulcers.[1] In general, H_2 receptor antagonists are rapidly absorbed after oral administration. The first-pass hepatic metabolism and excreted in large part in the urine without metabolized limits the oral bioavailability of cimetidine is approximately 50%. Cimetidine levels in biological fluids have previously been determined using a liquid chromatographic method with liquid-liquid extraction,[2-6] solid-phase extraction.[7-10] To reduce the complex clean-up procedures, as well as obtain protein-unbound sample, a microdialysis technique was applied to sample the drug from biological fluids. In recent years the microdialysis technique is also being applied in pharmacokinetic studies.[11] Because microdialysis technique provides no biological fluid, the change in the physiological situation of the animal could be minimized.

In this study, we constructed blood microdialysis probes, then inserted them into the rat jugular veins for sampling of cimetidine from biological fluids after cimetidine was administered intravenously. The quantitative analysis was carried out using a liquid chromatographic method with ultraviolet detection. Microdialysis, therefore, appears to be a suitable technique for pharmacokinetic studies of protein-unbound drug.

EXPERIMENTAL

Chemicals and Reagents

Cimetidine (Figure 1) was purchased from RBI (Research Biochemicals International, Natick, MA, USA). Liquid chromatographic grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triple de-ionized water (Millipore, Bedford, MA, USA) was used for all preparations.

Animals

Adult, male Sprague-Dawley rats (280-350 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). These animals were specifically pathogen-free and allowed to acclimate to their environmentally controlled quarters (24 ±1°C and 12:12 h light-dark cycle) for

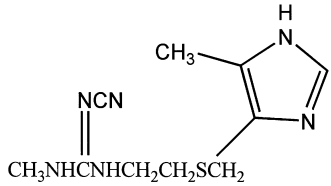

Figure 1. Chemical structure of cimetidine.

at least 5 days before the experiments. At the start of experiments, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Supplements of sodium pentobarbital were given as needed throughout the experimental period.

Chromatography

The liquid chromatographic system consisted of a chromatographic pump (BAS PM-80, Bioanalytical System, West Lafayette, IN, USA), a CMA/140 fraction collector (CMA/Microdialysis, Stockholm, Sweden) equipped with a 20 µL sample loop and a Dynamax UV/Vis absorbance detector (Varian, Walnut Creek, CA, USA). Cimetidine dialysate was separated using a LiChrosorb RP-18 column (Merck, 250 x 4 mm i.d.; particle size 5 µm; catalog no. 1.50995) maintained at ambient temperature. The mobile phase was comprised of acetonitrile-100 mM monosodium phosphate, pH 3.0 (22:78, v/v), and the flow rate of mobile phase was 1 mL/min. The mobile phase was filtered through a Millipore 0.22 µm filter and degassed prior to use. Detecting UV wavelength was set at 228 nm. Output signal from the HPLC-UV was recorded via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).[12-13]

Method Validation

All calibration curves of cimetidine (external standards) were made prior to the experiments with correlation values of at least 0.995. The intra-day and inter-day variabilities for cimetidine were assayed (six replicates) at concentrations of 0.1, 0.5, 1, 5, and 10 µg/mL on the same day and on six sequential days, respectively. The accuracy (% Bias) was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentration (C_{obs}) as follows: Bias (%) = [(C_{obs}-C_{nom})/(C_{nom})] x 100. The precision coefficient of variation (CV) was calculated from the observed concentrations as follows: % CV = [standard deviation (SD)/C_{obs}] x 100. Accuracy (% Bias) and precision (% CV) values of within ± 15% covering the range of actual experimental concentrations were considered acceptable.[14]

Microdialysis Experiment

The microdialysis system consisted of a microinjection pump (CMA/100) and a fraction collector (CMA/140). Microdialysis probes were made of silica capillary and concentrically designed dialysis membrane (Spectrum, 10-mm length, 150 μm outer diameter with a cut-off at nominal molecular weight of 13000, Laguna Hills, CA, USA). Prior to the experiment, perfusate ACD solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) was degassed. Following a 2 h baseline collection of dialysates, cimetidine (10 mg/kg) was intravenously administered via the femoral vein. Each microdialysis probe was washed with degassed ACD solution for at least 40 min prior to use. A microdialysis probe was inserted into the jugular vein/right atrium (toward the heart) of an anesthetized rat and perfused with ACD solution at a flow-rate of 2 μL/min using the microinjection pump. The body temperature of the rat was maintained at 37°C with a heating pad.

Recovery of Microdialysate

For in vivo recovery, a retrograde calibration technique was used. The blood microdialysis probe was inserted into the rat jugular vein under anesthesia with sodium pentobarbital. ACD solution containing cimetidine (0.5 or 1 μg/mL) was passed through the probe at a constant flow rate (2 μL/min) using the infusion pump (CMA/100). After a stabilization period of 2 h, the inlet (C_{in}) and outlet (C_{out}) concentrations of cimetidine were determined by HPLC. The in vivo recovery ratios were then calculated by the following equation:[15]

$$\text{Recovery}_{\text{in vivo}} = 1 - (C_{out}/C_{in})$$

Pharmacokinetic Study

Calibration curves were constructed based on HPLC analyses of various concentrations of cimetidine. The concentrations of cimetidine in rat blood dialysates were determined from the calibration curves. Following a 2 h period of stabilization, cimetidine (10 mg/kg, i.v.) was administered. Dialysates were collected every 12 min for an additional 72 min following cimetidine administration. Absolute concentrations in extracellular fluid were calculated from the concentrations in dialysates by the following equation: Concentration = dialysate / recovery.

Pharmacokinetic calculations were performed on each individual set of data. Blood data were fitted to a biexponential decay given by the following formula: $C = Ae^{-t} + Be^{-t}$. The distribution and elimination rate constants, α and

β were calculated using the equation: α or β = (ln C_2 - ln C_1) / (t_2 - t_1); where C_1: the value of C at time t_1; C_2: the value of C at time t_2. Formation rate constants were calculated by extrapolation of the formation slope determined by the method of residuals. The areas under the concentration curves (AUCs) were calculated by the trapezoid method. Half-life ($t_{1/2}$) values were calculated using the equations: $t_{1/2,a}$ = 0.693 / α and $t_{1/2,b}$ = 0.693 / β for distribution and elimination of half-life, respectively. All data were subsequently processed by the computer program WinNonlin (version 1.1, SCI Software Inc., Lexington, KY, USA) for the calculation of pharmacokinetic parameters.

RESULTS AND DISCUSSION

The present liquid chromatographic method was applied to determine cimetidine from rat blood of jugular veins. Typical chromatograms of standard containing cimetidine are shown in Figure 2. Separation of cimetidine from some endogenous chemicals in blood dialysate was achieved in an optimal mobile phase containing 78% of 100 mM monosodium phosphate (pH 3.0) and 22% of acetonitrile. Retention time of cimetidine was 6.2 min. Peak-areas of cimetidine was linear ($r^2 > 0.995$) over a concentration range of 0.05-10 μg/mL.

Figure 2A shows a typical chromatogram of a standard solution containing cimetidine (5 μg/mL). The blank sample (Figure 2B) shows that the chromatographic conditions revealed no biological substances that would significantly interfere with the accurate determination of the drug. Figure 2C depicts a chromatogram of actual unbound cimetidine in rat blood. The dialysate sample contains cimetidine (4.52 μg/mL) collected from jugular vein at 12 min following cimetidine administration (10 mg/kg, i.v.).

Intra-assay and inter-assay (Table 1) precision and accuracy values for cimetidine fell well within predefined limits of acceptability. All % bias and % CV values were within ± 10%. This method has quantitative limits of 50 ng/mL for cimetidine. The in vivo recovery of cimetidine is shown in Table 2.

The pharmacokinetic models (one- and two-compartment) were compared according to the Akaike's information criterion (AIC)[16] and Schwartz criterion (SC),[17] with minimum AIC and SC values regarded as the best representation of the blood concentration-time course data. A two-compartment model was proposed and validated through the program to explain the apparent bi-phasic disposition of cimetidine in blood after an intravenous bolus injection (Figure 3). The pharmacokinetic parameters, as derived from these data and calculated by WinNonlin program, are shown in Table 3. The dialysate samples collected over the first 2 h were discarded to allow recovery from the acute effects of the surgical procedure. The microdialysis sampling system and liquid chromatographic system were then applied to the pharmacokinetic characterization of

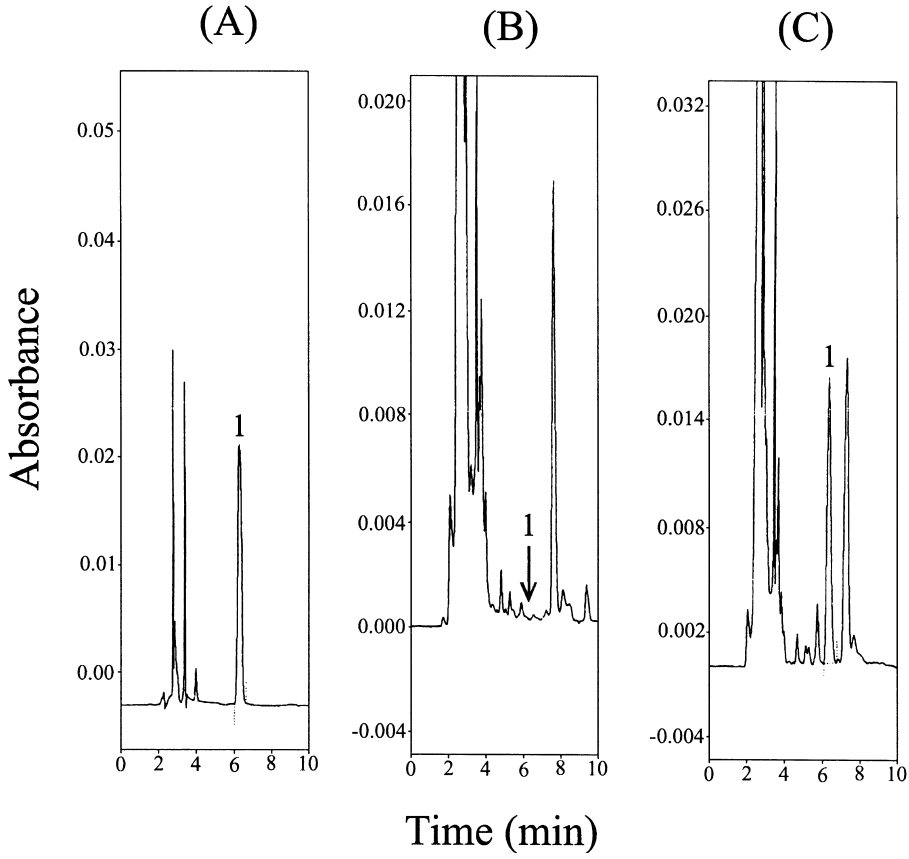

Figure 2. Typical chromatogram of injection of (A) standard cimetidine (5 μg/mL), (B) a blank blood dialysate, and (C) a blood dialysate sample containing cimetidine (4.52 μg/mL) collected from jugular vein at 12 min after cimetidine administration (10 mg/kg, i.v.). 1: cimetidine.

cimetidine in rats. The concentration of unbound cimetidine in the rat blood corrected by in vivo recovery, after cimetidine (10 mg/kg, i.v.) administration, is shown in Figure 3. The samples were collected at 12 min intervals during the entire experimental period. The results, as derived from the microdialytic studies and corrected for in vivo recoveries, suggested that the pharmacokinetics of unbound cimetidine appeared to best fit the kinetics of a two-compartment model in rat blood. The volume of distribution (VOL) and clearance (Cl) were

Table 1

Precision and Accuracy of Cimetidine

Nominal Concentration	Measured Concentration[a]	C.V. (%)	Bias (%)
Intra-assay (n=6)			
0.05	0.053 ± 0.005	10	6
0.1	0.101 ± 0.008	8	1
0.5	0.501 ± 0.005	1	0.2
1	1.01 ± 0.02	2	1
5	4.98 ± 0.04	1	-0.4
10	10.01 ± 0.02	0.2	0.1
Inter-assay (n=6)			
0.05	0.051 ± 0.004	8	2
0.1	0.096 ± 0.006	6	6
0.5	0.501 ± 0.017	3	0.2
1	0.99 ± 0.04	4	-1
5	5.01 ± 0.07	1	0.2
10	10.02 ± 0.08	1	0.2

[a] Observed concentration data are expressed as rounded means ± S.D.

Table 2

In Vivo Recovery of Cimetidine for Blood Microdialysis Probe*

Cimetidine Concentration (μg/mL)	In Vivo (%)
0.5	73.2 ± 8.2
1	74.1 ± 8.1

* Data are expressed as means ± S.E.M. (n = 6).

Table 3

Estimated Pharmacokinetic Parameters Following Cimetidine Administration

Parameters	Estimated
A (μg/mL)	52.69 ± 25.19
B (μg/mL)	1.22 ± 0.28
α (1/min)	0.20 ± 0.0073
β (1/min)	0.024 ± 0.044
$t_{1/2,\alpha}$ (min)	3.47 ± 0.13
$t_{1/2,\beta}$ (min)	30.61 ± 5.38
AUC (μg min/mL)	301.64 ± 117.28
VOL (L)	1.01 ± 0.78
Cl (L/min/kg)	0.081 ± 0.048
MRT (min)	19.44 ± 8.17

* Cimetidine Administration: (10 mg/kg, i.v.). Data are expressed as means ± S.E.M. (n = 6). MRT: Mean residence time.

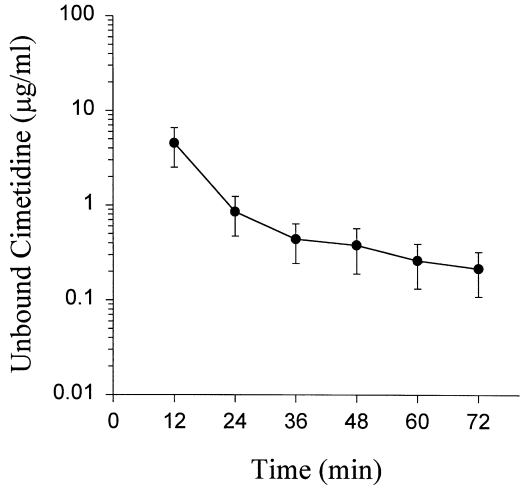

Figure 3. Mean unbound levels of cimetidine in blood of the jugular vein after cimetidine administration (10 mg/kg, i.v., n=6).

1.01 ± 0.78 (L) and 0.081 ± 0.048 (L/min/kg), respectively. Other pharmacokinetic parameters are shown in Table 3.

A microdialysis technique provides protein-free samples that can be directly injected into a liquid chromatographic system for continuous in vivo monitoring of unbound drugs in blood, other biological fluids and tissue.[18] Further, sampling by microdialysis was based on the theory that the microdialysis probe acts as a blood vessel with dialytic exchange of mainly small molecular substances with the surrounding tissues, but this method may be limited by its dialytic efficiency. Compared to other in vivo methods which extracted drugs from the biological sample by liquid-liquid extraction[2-6] or protein precipitated by organic solvent,[19] microdialysis offers many advantages such as continuous monitoring of analyte concentrations in the extracellular compartment in the same animals, less biological fluid loss, and, therefore, minimal stress on hemodynamics.[20]

In summary, we introduced the rapid chromatographic method for the determination of cimetidine in the rat blood vessel using in vivo microdialysis with HPLC-UV. This method exhibits no endogenous interference with sufficient sensitivity in blood dialysates. This method appears to be applicable to further pharmacokinetic studies on cimetidine in rats. The disposition of cimetidine in rat blood appears as a two-compartment pharmacokinetic model.

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