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Determination of unbound cefmetazole in rat blood by on-line microdialysis and microbore liquid chromatography: a pharmacokinetic study

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Abstract

A specific and sensitive microbore liquid chromatographic method for the determination of unbound cefmetazole in rat blood was developed. A microdialysis probe was inserted into the jugular vein/right atrium of a Sprague–Dawley rat. Cefmetazole (10 mg/kg, i.v.) was then administered via the femoral vein. Dialysates were automatically injected into a liquid chromatographic system via an on-line injector. Isocratic elution of cefmetazole was achieved by LC–UV within 10 min. Intra- and inter-assay accuracy and precision of the assay were ≤10%. The detection limit of cefmetazole was 20 ng/ml. Pharmacokinetic analysis of results indicated that unbound cefmetazole levels in rats best fit a biexponential decay model.

Keywords: Pharmacokinetics; Cefmetazole

1. Introduction

Cefmetazole (Fig. 1), 7-β-cyanomethylthio-acetamide-7-α-methoxy-3-{[(1-methyl-1H-tetrazol-5yl)-thio]methyl}-3-cephem-4-carboxylate, is a derivative of cephamycin [1]. Cefmetazole has been shown to have a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria [2–4]. Because it is highly resistant to β-lactamases, it has proven to be an effective antibiotic. Cefmetazole is also effective for the treatment of a number of infections and for prophylactic use prior to surgery [1,5,6].

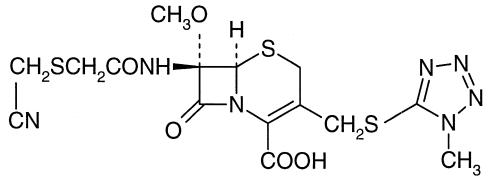

Fig. 1. Chemical structure of cefmetazole.

Cefmetazole levels in biological fluids have previously been determined using microbiological assay [7] or liquid chromatography (LC) [8–11]. These procedures have provided measurements of protein-

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bounded drug concentrations. To obtain unbound drug levels to construct pharmacokinetic data, these procedures have to be modified. Microdialysis membrane excluding macromolecules for sampling of unbound drugs and/or their metabolites was developed in the previous reports [12,13]. To minimize the degradation of cefmetazole, an automatic sampling system was found to be beneficial. An in vivo on-line microdialysis sampling method coupled with microbore LC was used for the measurement of cefmetazole in rat blood in the present study.

2. Experimental

2.1. Chemicals and reagents

Cefmetazole was purchased from Sigma (St. Louis, MO, 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.

2.2. Animals

Adult male Sprague–Dawley rats (280–350 g) were obtained from the Laboratory Animal Center of 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 at least 5 days before the 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.

2.3. Chromatographic conditions

The microbore LC system consisted of a chromatographic pump (BAS PM-80, Bioanalytical Systems Inc., West Lafayette, IN, USA), an on-line injector (CMA/160, Stockholm, Sweden) equipped with a 10 μl sample loop and a Dynamax UV detector (set at 254 nm, Walnut Creek, CA, USA). Cefmetazole was eluted using a microbore column (BAS, reversed-phase C_{18}, 150×1 mm I.D.; particle size 5 μm) maintained at room temperature (24±1°C). The mobile phase was comprised of methanol-100 m*M* monosodium phosphoric acid (20:80, v/v, pH 5.0), and the flow-rate of the mobile phase was 0.05 ml/min. The buffer was filtered through a Millipore filter (0.22 μm, type GVWP Durapore membrane) and degassed prior to use. Output signal from the LC–UV was recorded via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Method validation

All calibration curves of cefmetazole (external standards) were constructed prior to the experiments with correlation values of at least 0.995. The intra-day and inter-day variabilities of cefmetazole 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})]×100. The precision coefficient of variation (C.V.) was calculated from the observed concentrations as follows: % C.V.=[standard deviation (SD)/C_{obs}]×100. Accuracy (% Bias) and precision (% C.V.) values of within ±15% covering the range of actual experimental concentrations were considered acceptable [14].

2.5. Microdialysis experiment

The on-line microdialysis system consisted of a microinjection pump (CMA/100) and an on-line injector (CMA/160) [15,16]. 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 13 000, Laguna Hills, CA). Prior to the experiment, perfusate solution (147 m*M* NaCl, 4 m*M* KCl, and 2.3 m*M* $CaCl_2$ of Ringer's solution, pH 7.4) was degassed. Each microdialysis probe was perfused with degassed Ringer's 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 Ringer's solution at a

flow-rate of 1 μl/min. The body temperature of the rat was maintained at 37°C with a heating pad. Dialysates were collected at 10-min intervals (each with 10 μl of dialysate) into the on-line injector (CMA/160) and assayed with the microbore LC system [15].

2.6. Recovery of microdialysate

For in vivo recovery, a retrograde calibration technique was used. The blood microdialysis probe was inserted into the jugular vein of a rat under anesthesia with sodium pentobarbital. ACD solution containing cefmetazole (0.5, 1, or 2 μg/ml) was perfused through the probe at a flow-rate of 1 μl/min using a microinjection pump (CMA/100). After a stabilization period of 2 h, the inlet (C_{in}) and outlet (C_{out}) concentrations of cefmetazole were determined by LC. The in vivo recovery ratios were then calculated by the following equation [17]: Recovery $_{in\ vivo} = 1 - (C_{out}/C_{in})$.

2.7. Pharmacokinetic study

Calibration curves were constructed based on LC analyses of standard mixture prior to experiments. An equilibration period of 2-h was allowed to obtain baseline condition. Cefmetazole was then administered (10 mg/kg, i.v.). Dialysates were injected every 10-min by an on-line injector (CMA/160) for an additional 150 min following cefmetazole administration. Concentrations of cefmetazole in rat blood dialysates were determined from calibration curves. Absolute cefmetazole concentration in extracellular fluid was calculated from its concentration in the dialysate by the following equation: Concentration=dialysate/recovery.

Pharmacokinetic calculations were performed for each individual set of data. Blood data were fitted to a biexponential model given by the following formula: $C = Ae^{-\alpha t} + Be^{-\beta t}$. The distribution and elimination rate constants α and β were calculated using the equation: α or $\beta = (\ln C_2 - \ln C_1)/(t_2 - t_1)$; where C_1 is the value of C at time t_1, and C_2 is 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: $t_{1/2,\alpha} = 0.693/\alpha$ and $t_{1/2,\beta} = 0.693/\beta$ for distribution and elimination half-life, respectively. Pharmacokinetic parameters were calculated by the WinNonlin software program (version 1.1, Scientific Consulting Inc., Apex, NC, USA).

3. Results and discussion

The microdialysis–microbore liquid chromatographic method proposed in this study was applied to determine cefmetazole levels in jugular vein blood in rats. Typical chromatograms of standard mixtures containing cefmetazole are shown in Fig. 2. The separation of cefmetazole from endogenous chemicals in the blood dialysate was achieved by an optimal mobile phase. The mobile phase contained 80% 100 m*M* monosodium phosphate (pH 5.0) and 20% methanol. Retention time of cefmetazole was 6.9 min. Peak-areas of cefmetazole were linear ($r^2 > 0.995$) over a concentration range of 0.05–10 μg/ml.

Fig. 2(A) shows a typical chromatogram of a standard mixture containing cefmetazole (5 μg/ml). The blank sample (Fig. 2(B)) shows that under the chromatographic conditions in this study, there were no observed peaks that would significantly interfere with the determination of cefmetazole. Fig. 2(C) depicts a chromatogram of unbound cefmetazole (1.91 μg/ml) obtained from a blood dialysate 20 min after cefmetazole administration (10 mg/kg, i.v.).

Intra-assay and inter-assay (Table 1) precision and accuracy of the microbore LC–UV system for the determination of cefmetazole fell well within predefined limits of acceptability. All % bias and % C.V. values were <10%. The detection limit of cefmetazole was 20 ng/ml (at signal-to-noise ratio=3). In vivo recoveries of cefmetazole are shown in Table 2.

The two pharmacokinetic models (one- and two-compartment) were compared according to Akaike's information criterion (AIC) [18] and the Schwartz criterion (SC) [19], 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

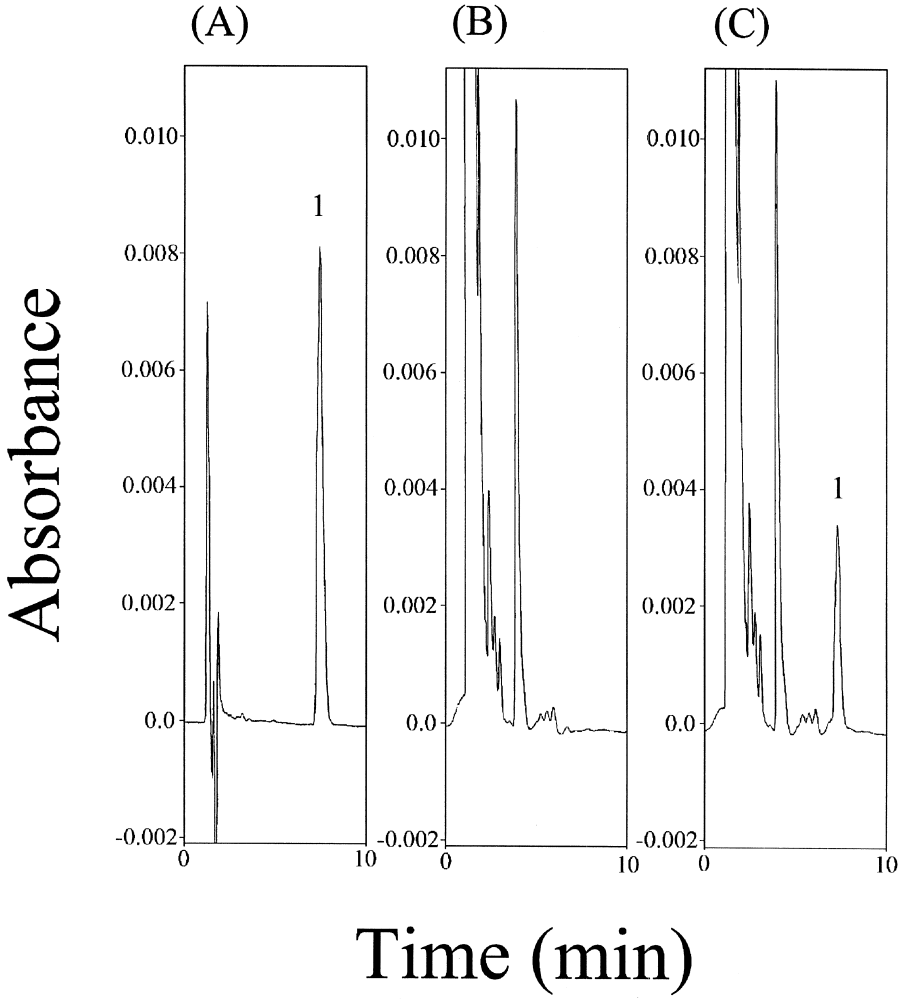

Fig. 2. Typical chromatograms of (A) standard cefmetazole (5 μg/ml), (B) a blank blood dialysate, and (C) a blood dialysate sample containing cefmetazole (1.91 μg/ml) collected from jugular vein at 20 min after cefmetazole administration (10 mg/kg, i.v.). 1: cefmetazole.

the program to explain the apparent bi-phasic disposition of blood cefmetazole after an intravenous bolus injection (Fig. 3). The pharmacokinetic parameters, derived from these data and calculated by the 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 procedures. Then, microdialysis–microbore LC was applied to determine the pharmacokinetic characterization of cefmetazole in rats. Fig. 3 shows the concentration profile of unbound cefmetazole in rat blood corrected by in vivo recovery, after cefmetazole (10 mg/kg, i.v.) administration. Dialysis samples were collected at 10 min intervals over the entire experimental period. These data suggest that the pharmacokinetics of unbound cefmetazole in rat blood best fit a two-compartment model, which was in agreement with previous reports [20,21]. The volume of distribution (Vol), clearance (Cl) and mean residence time (MRT) were 0.79 ± 0.14 l/kg, 0.042 ± 0.0045 l/min/kg and 26.9 ± 7.5 min respectively. Other pharmacokinetic parameters are shown in Table 3.

The microdialysis technique provides protein-free

Table 1
Intra-assay and inter-assay accuracy and precision of the microbore LC–UV system for the determination of cefmetazole

Nominal concentration (μg/ml)	Observed concentration (μg/ml)[a]	C.V. (%)	Accuracy (% Bias)
Intra-assay (n=6)			
0.10	0.10 ±0.01	9.7	3.0
0.50	0.50 ±0.01	1.6	−0.4
1.00	1.01 ±0.01	0.6	0.5
5.00	4.99 ±0.03	0.6	−0.3
10.00	10.02 ±0.03	0.3	0.2
Inter-assay (n=6)			
0.10	0.11 ±0.01	9.1	8.0
0.50	0.48 ±0.02	3.3	−3.6
1.00	1.00 ±0.02	1.9	0.3
5.00	4.98±0.05	0.9	−0.4
10.00	10.01±0.02	0.2	0.1

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

Table 2
In vivo microdialysis recoveries (%) of cefmetazole in rat blood[a]

Concentration (μg/ml)	Recovery (%)
0.50	45±3
1.00	43±2
2.00	40±2

[a] Data are expressed as means±S.E.M. ($n=6$).

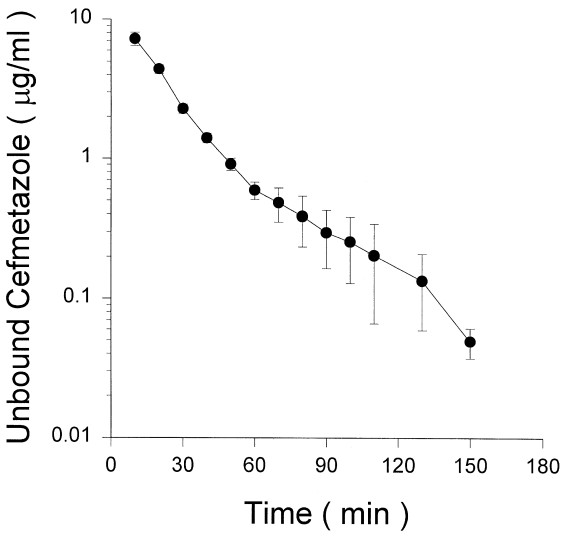

Fig. 3. Mean unbound levels of cefmetazole in jugular vein blood after cefmetazole administration (10 mg/kg, i.v., $n=6$).

Table 3
Estimated pharmacokinetic parameters in rats after cefmetazole administration (10 mg/kg, i.v.)[a]

Parameters	Estimate
A (μg/ml)	10.4±3.2
B (μg/ml)	4.4±1.6
α (1/min)	0.07±0.01
β (1/min)	0.04±0.01
$t_{1/2,\alpha}$ (min)	11.8±2.0
$t_{1/2,\beta}$ (min)	29.0±15.1
AUC (μg min/ml)	259.1±38.3
Vol (l/kg)	0.8±0.1
Cl (l/min/kg)	0.04±0.005
MRT (min)	26.9±7.5

[a] Data are expressed as means±S.E.M. ($n=6$).

samples that can be directly injected into a liquid chromatographic system for continuous in vivo monitoring of unbound drugs in blood [22]. Furthermore, sampling by microdialysis is based on the theory that the microdialysis probe acts similarly to a blood vessel, with dialytic exchange with the surrounding tissues primarily of small molecular substances. However, this method may be limited by the dialytic efficiency of the equipment. Extraction drugs from biological samples have been routinely assayed by liquid–liquid extraction methods [23] or protein precipitation with organic solvent [24]. Microdialysis technique offers many advantages over these conventional assays. It continuously samples and monitors unbound drug concentrations in the brain of the same animal, causes less biological fluid losses, and minimizes stress on hemodynamics [12,13,25,26].

In summary, a rapid and sensitive microdialysis-liquid chromatographic method for the determination of cefmetazole in rat blood vessels was demonstrated in the present study. This method exhibits no endogenous interference with sufficient sensitivity in blood dialysates. The disposition of cefmetazole in rat blood appears to follow a two-compartment pharmacokinetic model. Finally, this method is applicable to further pharmacokinetic studies of drugs in rats.

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