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Determination of unbound ceftriaxone in rat blood by on-line microdialysis and microbore liquid chromatography

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Abstract

In vivo microdialysis was used to determine unbound ceftriaxone in rat blood. A microdialysis probe was inserted into the jugular vein/right atrium of Sprague-Dawley rats, and dose of 10 mg/kg ceftriaxone was then administered via the femoral vein. Dialysates were automatically collected and injected into a liquid chromatographic system via an on-line injector. Isocratic elution of ceftriaxone within 10 min was achieved using a microbore liquid chromatographic system. The chromatographic mobile phase consisted of methanol-100 mM monosodium phosphoric acid (15:85, v/v, pH 7.0). The wavelength of the UV detector was set at 280 nm. Intra- and inter-assay accuracy and precision of the assay were less than 15%. The detection limit of ceftriaxone was 20 ng/ml. The results suggest that unbound ceftriaxone in rat blood is best fit to a biexponential decay model.

Keywords: Microdialysis; Ceftriaxone; Biexponential decay model

1. Introduction

Ceftriaxone is a third-generation cephalosporin that has a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria. A single dose of ceftriaxone is effective in the treatment of urethral, cervical rectal, and pharyngeal gonorrhea (Rajan et al., 1982; Handsfield and Murphy, 1983). Determination of ceftriaxone in biological fluids has been carried out by microbiological assays (Chichmanian et al., 1984; de Barbeyrac et al., 1987; Nakanomyo et al., 1990) and by liquid chromatographic methods with protein precipitation procedures (Ascalone and Dal Bo, 1983; Bowman et al., 1984; Ti et al., 1984; Granich and Krogstad, 1987; Jungbluth and Jusko, 1989). However, these procedures have the disadvantages of co-precipitation and the need for time consuming procedures. Another shortcoming is that these methods provide only total (free form and bounded form) drug concentrations.

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Microdialysis is a well-established sampling method to collect chemicals from extracellular fluids in brain and blood. To sample the protein-free fraction of a drug or/and its metabolites, a passive diffusion is applied to exclude macromolecules from the membrane (Fettweis and Borlak, 1996; Johansen et al., 1997). To further minimize the degradation of ceftriaxone, in vivo on-line microdialysis was employed for this determination of ceftriaxone in rat blood.

2. Experimental

2.1. Chemicals and reagents

Ceftriaxone (Fig. 1) 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 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 at least 5 days before the experiments. On the day of experiments, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and supplements of sodium pentobarbital were given as needed throughout the experimental period.

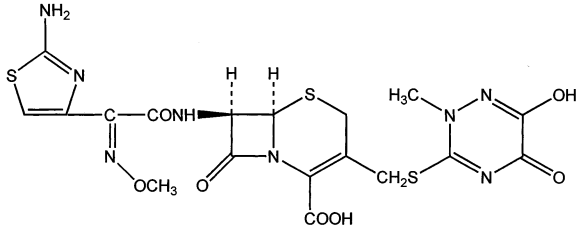

Fig. 1. Chemical structure of ceftriaxone.

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 280 nm, Varian, CA, USA). Ceftriaxone was eluted using a microbore column (BAS, reversed phase C18, 150×1 mm I.D.; particle size 5 μm) maintained at room temperature (24 ± 1°C). The mobile phase was comprised of methanol-100 mM monosodium phosphoric acid (15:85, v/v, pH 7.0), and the flow rate of the mobile phase was 0.05 ml/min. The buffer was filtered through a Millipore 0.22 μm filter and degassed prior to use. Output signals from the LC-UV were recorded via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Method validation

In general, calibration curves of ceftriaxone (external standards) were made prior to the experiments with correlation values (R^2) of at least 0.995. The intra-day and inter-day variabilities of ceftriaxone were assayed ($n = 6$) at concentrations of 0.05, 0.1, 0.5, 1 and 5 μg/ml on the same day and on 6 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 (%) = $[(C_{obs} - C_{nom})/(C_{nom})] \times 100$. The precision coefficient of variation (CV) was calculated from the observed concentrations as follows:% CV = [standard deviation (S.D.)/C_{obs}] $\times 100$. Accuracy (% Bias) and precision (% CV) values of within ± 15% covering the range of actual experimental concentrations were considered acceptable (Causon, 1997).

2.5. Microdialysis experiment

The on-line microdialysis system consisted of a microinjection pump (CMA/100) and an on-line injector (CMA/160) (Tsai et al., 1998, 1999). Concentric microdialysis probes were made of silica

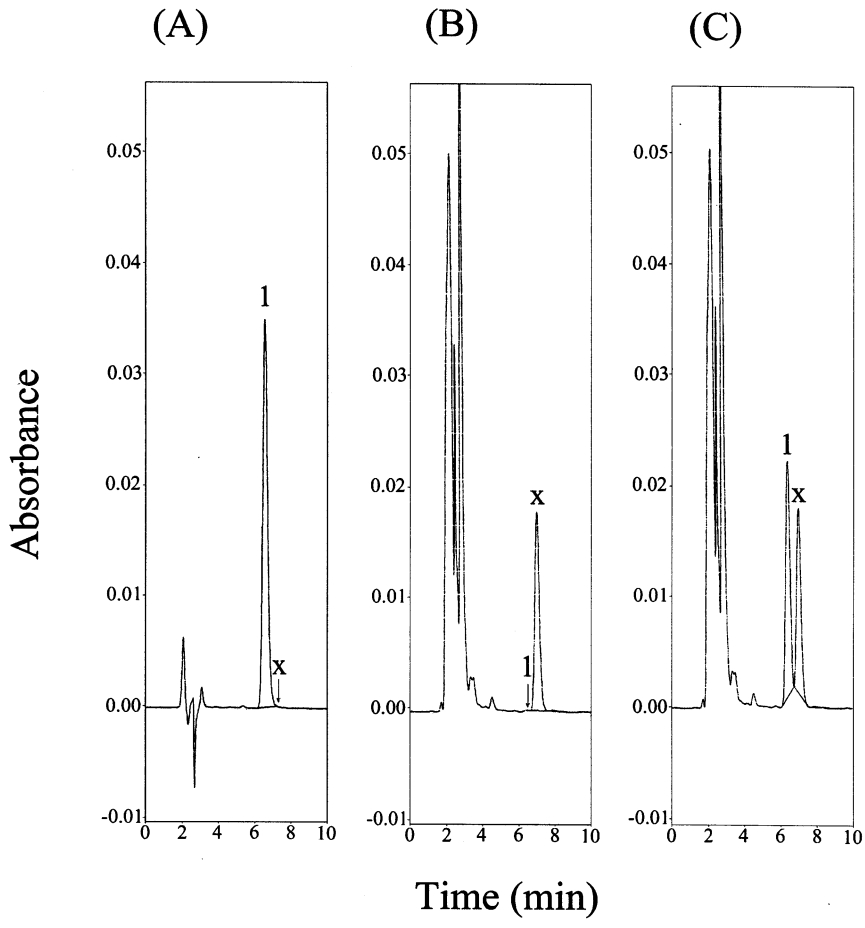

Fig. 2. Typical chromatograms of (A) standard ceftriaxone (5 μg/ml); (B) a blank blood dialysate; and (C) blood dialysate sample containing ceftriaxone (2.8 μg/ml) collected from jugular vein at 10 min after ceftriaxone administration (10 mg/kg, i.v.). 1: ceftriaxone; x: unidentified peak.

capillary and 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, Ringer's solution (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$, 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 (10 μl dialysate) into an on-line injector (CMA/160) and assayed by the microbore LC system (Tsai et al., 1999).

2.6. Recovery of microdialysate

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A blood microdialysis probe was inserted into the jugular vein of the rat. ACD solution containing ceftriaxone (1 μg/ml) was perfused at a flow rate of 1 μl/min using a microinjection pump (CMA/100). A retrograde calibration technique was used to determine in vivo recovery of each probe (Sato et al., 1996). After a 2-h stabilization period, the inlet (C_{in}) and

Table 1
Intra-assay and inter-assay accuracy and precision of ceftriaxone measurement by the microbore LC-UV system

Nominal concentration (μg/ml)	Observed concentration (μg/ml)[a]	CV (%)	Accuracy (% Bias)
Intra-assay (n = 6)			
0.05	0.051 ± 0.007	13.7	2
0.1	0.097 ± 0.007	7.2	−3
0.5	0.501 ± 0.016	3.2	0.2
1	1.005 ± 0.022	2.2	0.5
5	5.01 ± 0.004	0.1	0.2
Inter-assay (n = 6)			
0.05	0.048 ± 0.005	10.4	−4
0.1	0.097 ± 0.006	6.5	−3
0.5	0.49 ± 0.01	2.0	−2
1	1.01 ± 0.03	3.0	1
5	5.03 ± 0.08	1.5	0.6

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

outlet (C_{out}) concentrations of ceftriaxone were determined by the microbore LC. The in vivo recovery rates were then calculated by the following equation: $\text{Recovery}_{\text{in vivo}} = 1 - (C_{out}/C_{in})$

2.7. Pharmacokinetic study

Calibration curves were constructed based on LC analyses of a standard mixture prior to each experiment. Following a 2 h stabilization period, ceftriaxone was administered (10 mg/kg, i.v.). Dialysates were collected and injected every 10 min by an on-line injector (CMA/160) for an additional 3 h after ceftriaxone administration. Ceftriaxone concentrations in rat blood dialysates were determined from calibration curves. Absolute ceftriaxone concentration in extracellular fluid was calculated from its concentration in the dialysate by the following equation: $\text{Conc}_{abs} = \text{Conc}_{dialysate}/\text{Recovery}_{\text{in vivo}}$.

Pharmacokinetic parameters were calculated on each set of data. These data were fitted to a biexponential decay 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 extrapolating the formation slope determined by the method of residuals. The areas under the concentration curves (AUCs) were calculated by the trapezoid method. Half-lives ($t_{1/2}$) 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 (ver. 1.1, SCI Software Inc., Lexington, KY, USA).

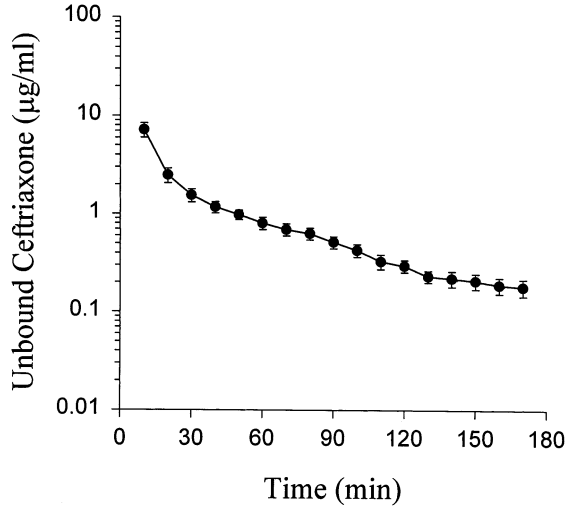

Fig. 3. Unbound ceftriaxone concentration-time profile in the jugular vein after ceftriaxone administration (10 mg/kg, i.v., $n = 6$).

Table 2
Estimated pharmacokinetic parameters in rat blood after ceftriaxone administration (10 mg/kg, i.v.)[a]

Parameters	Estimated
A (μg/ml)	37.75 ± 11.09
B (μg/ml)	2.37 ± 0.36
α (1/min)	0.19 ± 0.014
β (1/min)	0.017 ± 0.002
$t_{1/2,\alpha}$ (min)	3.74 ± 0.27
$t_{1/2,\beta}$ (min)	44.13 ± 6.47
AUC (μg/min/ml)	329 ± 54
VOL (ml)	335 ± 71
Cl (ml/min/kg)	35 ± 6
MRT (min)[b]	31.67 ± 5.22

[a] Data are expressed as means $\pm$ SEM ($n = 6$).
[b] MRT: mean residence time.

3. Results and discussion

Typical chromatograms of standard mixtures containing ceftriaxone are shown in Fig. 2. Isocratic separation of ceftriaxone from some endogenous chemicals in blood dialysate was achieved in an optimal mobile phase containing 85% of 100 mM monosodium phosphate (pH 5.0) and 15% of methanol.

Fig. 2A shows a typical chromatogram of a standard mixture containing ceftriaxone (5 μg/ml). Retention time of ceftriaxone was 6.9 min. Peak-areas of ceftriaxone were linear ($r^2 > 0.995$) over a concentration range of 0.05–10 μg/ml. A typical chromatogram of a blood dialysate shows that the chromatographic conditions revealed no observed peaks that would significantly interfere with the determination of ceftriaxone (Fig. 2B). Fig. 2C depicts a chromatogram of ceftriaxone (2.8 μg/ml) obtained from a blood dialysate 10 min after ceftriaxone administration (10 mg/kg, i.v.).

Intra-assay and inter-assay (Table 1) precision and accuracy values for ceftriaxone fell well within predefined limits of acceptability ($< 15\%$), with a detection limit for ceftriaxone of 20 ng/ml. In vivo recovery of ceftriaxone was $37 \pm 4\%$ ($n = 6$, a stock solution containing 1 μg/ml ceftriaxone).

Dialysate samples collected over the first 2 h were discarded to allow recovery from acute effects of the surgical and the insertion procedures of a microdialysis probe. Then microdialysis–microbore LC was applied to determine ceftriaxone in rat blood microdialysates. Dialysis samples were collected at 10-min intervals over the entire experimental period. Fig. 3 shows the concentration-time profile of unbound ceftriaxone in the rat blood (corrected by in vivo recovery) after ceftriaxone (10 mg/kg, i.v.) administration. Two pharmacokinetic models (one- and two-compartment) were compared according to Akaike's information criterion (AIC) (Yamoaka et al., 1978) and the Schwartz criterion (SC) (Schwartz, 1978). The best representative model of the drug concentration-time course data resulted from minimal AIC and SC values. A two-compartment model was proposed and validated through the program to explain a bi-phasic disposition of blood ceftriaxone after an intravenous bolus injection (Fig. 3). The pharmacokinetic parameters are shown in Table 2, suggesting the pharmacokinetics of unbound ceftriaxone in rat blood best fit the kinetics of a two-compartment model. This is in agreement with previous reports (Borner et al., 1985; Hakim et al., 1989). The volume of distribution (VOL) and clearance (Cl) were 335 ± 71 ml and 35 ± 6 (ml/min/kg), respectively.

In summary, an on-line and sensitive microdialysis-liquid chromatographic method for the determination of ceftriaxone 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 ceftriaxone in rat blood appears to follow a two-compartment pharmacokinetic model. The present analytical tool is suitable to study the pharmacokinetics of other drugs.

Acknowledgements

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