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Simultaneous Determination of Unbound Cefoperazone in Rat Blood and Brain Using Microdialysis

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Abstract

A sensitive microbore HPLC method was developed for the simultaneous determination of unbound cefoperazone in rat blood and brain using microdialysis.

Two microdialysis probes were inserted into the jugular vein/right atrium and brain striatum of Sprague–Dawley rats. Cefoperazone (50 mg kg^{-1}, i.v.) was then administered via the femoral vein. Blood and brain dialysates were collected and eluted with a mobile phase containing methanol–100 mM monosodium phosphoric acid (30 : 70, v/v, pH 5·5). The wavelength of the UV detector was set at 254 nm. The detection limit of cefoperazone was 20 ng mL^{-1}. Isocratic separation of cefoperazone was achieved within 10 min. The intra- and inter-assay accuracy and precision of the analyses were $\leq$10% in the range of 0·05–10 μg mL^{-1}.

The ratio of the area under the concentration curve of cefoperazone in rat brain and blood was estimated to be about 7·8%. It is concluded that cefoperazone is capable of penetrating the blood–brain barrier.

Cefoperazone (Figure 1) is a semi-synthetic cephalosporin and is classified as one of the third-generation cephalosporin antibiotics. It has a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa* (Alestig et al 1983; Barriere & Flaherty 1984). It is also resistant to inactivation by β-lactamase and has proved to be an effective antibiotic. Indeed, cefoperazone is effective in the treatment of liver and bile-duct infections (Craig & Gerber 1981; Gerber & Craig 1981), and some postoperative infections that may occur after brain surgery.

Cefoperazone levels in biological fluids have been determined by HPLC. Before carrying out analysis by HPLC, deproteinization of biological samples by methanol (Muder et al 1982; Dokladalova et al 1983; Signs et al 1984) or acetonitrile (La Follette et al 1988) was applied. These conventional procedures contributed to the measurement of total drug concentration, including protein-bound and -unbound drug. In this study, a microdialysis method was carried out to sample the protein-unbound fraction of the drug (Johansen et al 1997; Davies 1999). An on-line and automatic-analysis HPLC system was developed to minimize the degradation of cefoperazone during the processes of sample preparation.

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Materials and Methods

Chemicals

Cefoperazone was purchased from Sigma (St Louis, MO). Liquid-chromatographic-grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triply de-ionized water (Millipore, Bedford, MA) was used for all preparations.

Animals

Adult, male Sprague–Dawley rats (280–350 g) were obtained from the Laboratory Animal Science

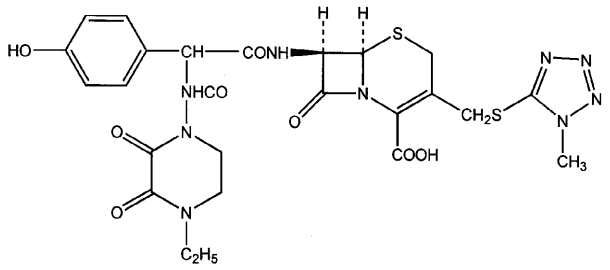

Figure 1. Chemical structure of cefoperazone.

Center of the National Yang-Ming University (Taipei, Taiwan). These rats were specifically pathogen-free and were allowed to acclimatize to their environmentally controlled quarters (24 ± 1°C and 12-h light–dark cycle) for at least 5 days before the experiments. Rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.). Supplements of sodium pentobarbital were given as needed throughout the experimental period.

HPLC analysis of cefoperazone

The microbore HPLC system consisted of a chromatographic pump (BAS PM-80, Bioanalytical System, West Lafayette, IN), an on-line injector (CMA/160, Stockholm, Sweden) equipped with a 10-μL sample loop and a Dynamax UV/Vis absorbance detector (Walnut Creek, CA). Cefoperazone was eluted using a microbore column (BAS, reversed-phase C18, 150 × 1 mm i.d.; particle size 5 μm) maintained at ambient temperature. The mobile phase was methanol–100 mM monosodium phosphoric acid (30 : 70, v/v, pH 5·5) and the flow rate was 0·05 mL min^{-1}. The buffer was filtered through a Millipore 0·22-μm filter and degassed before use. The UV wavelength for detection was set at 254 nm. The output signal from the HPLC-UV was recorded via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA).

Method validation

All calibration curves of cefoperazone (external standards) were constructed before experiments, achieving correlation values of at least 0·995. The intra-day and inter-day variabilities for cefoperazone were assayed (six replicates) at concentrations of 0·05, 0·1, 0·5, 1, 5, and 10 μg mL^{-1} 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 the observed concentration (C_{obs}) by equation 1.

$$\text{Bias (\%)} = [(C_{obs} - C_{nom})/C_{nom}] \times 100 \quad (1)$$

The precision coefficient of variation (CV) was calculated from the observed concentrations by equation 2.

$$\text{\% CV} = [\text{Standard deviation (s.d.)}/C_{obs}] \times 100 \quad (2)$$

Accuracy (% Bias) and precision (% CV) values within $\pm$ 15% covering the actual range of experimental concentrations were considered acceptable (Causon 1997).

Microdialysis experiment

Blood and brain microdialysis systems consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes. The dialysis probes for blood (10 mm in length) and brain (3 mm in length) were made of silica capillary in a concentric design with their tips covered by dialysis membrane (150 μm o.d., with a cut-off at nominal MW 13 000; Spectrum, Laguna Hills, CA).

The blood microdialysis probe was positioned within the jugular vein/right atrium (toward the heart) and then perfused with ACD solution (pH 4·5; citric acid 3·5 mM, sodium citrate 7·5 mM, dextrose 13·6 mM) at a flow rate of 1 μL min^{-1}. Blood dialysates were then automatically injected onto the microbore HPLC system by an on-line injector (CMA/160) at 10-min intervals.

After the implantation of the blood microdialysis probe, the rat was mounted on a Kopf stereotaxic frame for the implantation of the brain microdialysis probe in the striatum (coordinates: AP 0·2 mm; ML −3·2 mm; DV −7·0 mm) according to the Paxinos & Watson (1982) atlas. The body-temperature of the rat was maintained at 37°C with a heating pad. The brain microdialysis probe was perfused with Ringer's solution (147 mM Na^+, 2·2 mM Ca^{2+}, 4 mM K^+; pH 7·0) at a flow rate of 1 μL min^{-1}. Following a 2-h baseline collection, cefoperazone (50 mg kg^{-1}) was intravenously administered via the femoral vein. Brain dialysates were collected by a fraction collector (CMA/140) at 10-min intervals. The position of each brain microdialysis probe was verified at the end of the experiments (Hsiao et al 1990; Tsai & Chen 1996; Tsai et al 1998). Samples (10 μL) of brain dialysates were also assayed by microbore HPLC.

Recovery of microdialysate

For in-vivo recovery determinations, a retrograde calibration technique was used, in which the blood and brain microdialysis probes were inserted into the rat jugular vein and brain striatum, respectively,

under anaesthesia with sodium pentobarbital. ACD solution (for blood microdialysis) containing cefoperazone (0·5 or 1 $\mu g\,mL^{-1}$) or Ringer's solution (for brain microdialysis) containing cefoperazone (0·1 or 0·5 $\mu g\,mL^{-1}$) was perfused through a probe at a constant flow rate (1 $\mu L\,min^{-1}$) using the infusion pump. After a 2-h stabilization period, the inlet (C_{in}) and outlet (C_{out}) concentrations of cefoperazone were determined by HPLC. The in-vivo recovery ($Recovery_{in\text{-}vivo}$) of cefoperazone across the microdialysis probe was calculated by equation 3 (Sato et al 1996).

$$Recovery_{in\text{-}vivo} = [(C_{in} - C_{out})/C_{in}] \times 100 \quad (3)$$

Pharmacokinetic analysis

Calibration curves were constructed based on HPLC analyses of various concentrations of cefoperazone. The concentrations of cefoperazone in rat-blood dialysates were determined from the calibration curves. Following a 2-h period of stabilization, cefoperazone (50 $mg\,kg^{-1}$, i.v.) was administered. Blood and brain dialysates were assayed every 10 min for an additional 210 min following cefoperazone administration. Cefoperazone concentrations in blood and brain were corrected by the estimated in-vivo recovery from the respective microdialysis probes.

Pharmacokinetic calculations were obtained by the WinNonlin software program (version 1.1, Scientific Consulting Inc., Apex, NC) following the non-compartmental model (Benet & Galeazzi 1979; Watari & Benet 1989). The incremental areas under the concentration curves (AUC) and area under the moment vs time curve (AUMC) were calculated using the linear trapezoid method, according to equations 4 and 5.

$$AUC = AUC_{last} + C_{last}/\lambda_z \quad (4)$$

$$AUMC = AUMC_{last} + (t_{last} \times (C_{last}/\lambda_z)) + C_{last}/(\lambda_z)^2 \quad (5)$$

where C_{last} and t_{last} are the last observed concentration and time, respectively and λ_z is the terminal slope which is estimated by linear regression of the logarithmic value of the last observe data. The clearance (CL) and the mean residence time (MRT) were estimated by equations 6 and 7, respectively.

$$CL = Dose/AUC \quad (6)$$

$$MRT = AUMC/AUC \quad (7)$$

The volume of distribution at steady state (V_{ss}) was estimated by means of equation 8 (Gabrielsson & Weiner 1994).

$$V_{ss} = CL \times MRT \quad (8)$$

Results and Discussion

This microbore liquid chromatographic method was applied to determine cefoperazone from rat brain and blood. Typical chromatograms of standards and dialysates containing cefoperazone are shown in Figure 2. Isocratic separation of cefoperazone from some endogenous chemicals in the blood or brain dialysate was achieved in an optimal mobile phase containing 70% 100 mM monosodium phosphate (pH 5·5) and 30% methanol. Cefoperazone in both blood and brain dialysates was adequately resolved using the validated microbore HPLC conditions. The retention time of cefoperazone was 7·6 min (approx.). Peak-areas of cefoperazone were linear ($r^2 > 0{\cdot}995$) over a concentration range of 0·05–10·00 $\mu g\,mL^{-1}$.

Figure 2A shows a standard of cefoperazone (5 $\mu g\,mL^{-1}$). None of the observed peaks in a blank blood dialysate interfered with cefoperazone (Figure 2B). Figure 2C shows a typical chromatogram of a blood dialysate containing cefoperazone (2·75 $\mu g\,mL^{-1}$), collected 30 min after cefoperazone administration (50 $mg\,kg^{-1}$, i.v.). A typical chromatogram of a brain dialysate containing cefoperazone (0·30 $\mu g\,mL^{-1}$), collected 30 min after cefoperazone administration is shown in Figure 2D.

The validation of the microbore HPLC assay was also performed before experiments. Intra-assay and inter-assay precision and accuracy values for cefoperazone were well within the predefined limits of acceptability (Table 1). All % bias and % CV values were within $\pm 10\%$. In addition, this microbore HPLC method provides an ultimate quantitation limit (50 $ng\,ml^{-1}$) of cefoperazone.

The in-vivo recovery (or dialysis efficiency) can be affected by certain factors, mostly physical in nature, such as temperature and perfusion rate, as well as materials and dimensions of the probe. Therefore, each microdialysis probe has to be calibrated before experiments at the same experimental conditions. Generally, in-vivo recovery of microdialysis probes in blood were higher than those in brain (Table 2).

Microdialysis coupled with microbore HPLC is sufficiently sensitive to allow the measurement of unbound cefoperazone in rat brain and blood for pharmacokinetic studies. Figure 3 shows the

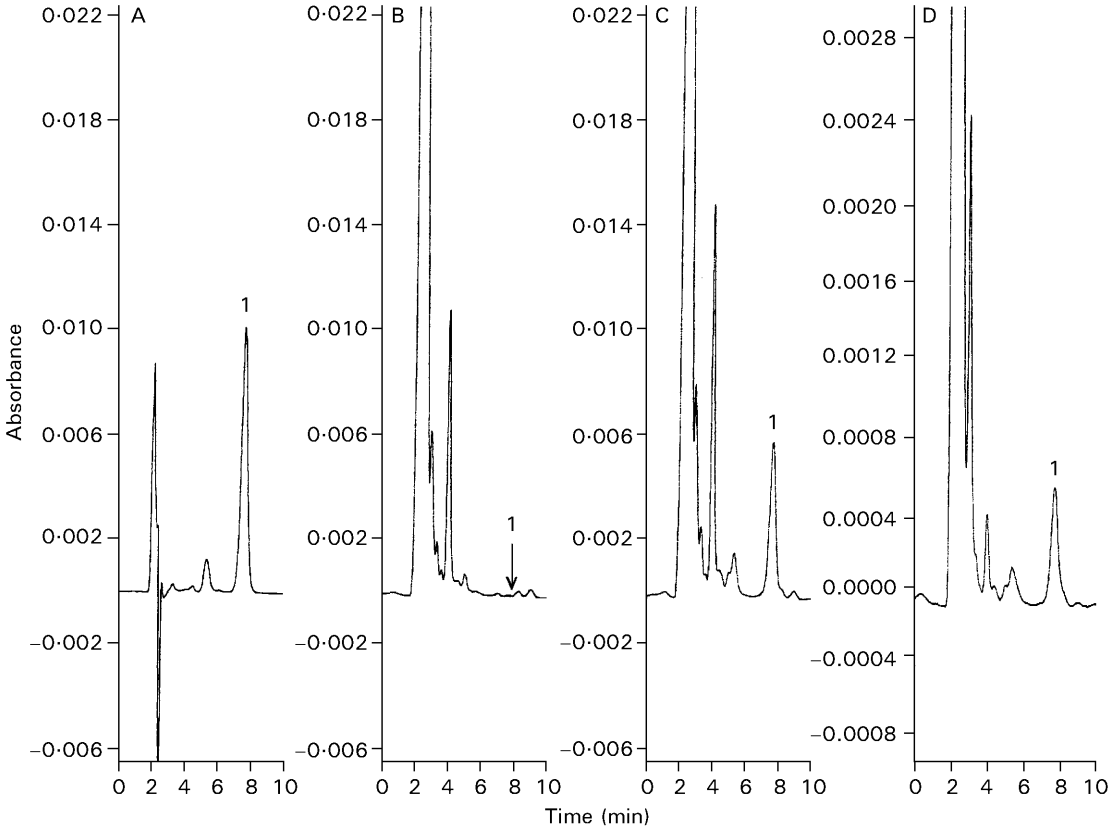

Figure 2. Typical chromatograms. A. Standard cefoperazone (5 μg mL^{-1}); B. Blank blood dialysate from the microdialysis probe before drug administration; C. blood dialysate sample containing cefoperazone (2·75 μg mL^{-1}) collected from the rat blood microdialysate 30 min post cefoperazone administration (50 mg kg^{-1}, i.v.). D. Brain dialysate sample containing cefoperazone (0·30 μg mL^{-1}) collected from a rat brain microdialysate 30 min post cefoperazone administration (50 mg kg^{-1}, i.v.). 1 = cefoperazone.

Table 1. Intra-assay and inter-assay of accuracy and precision values for cefoperazone.

Nominal concn (μg mL^{-1})	Observed concn (μg mL^{-1})[a]	CV (%)	Accuracy (% bias)
Intra-assay (n = 6)			
0·05	0·0520 ± 0·0020	4·10	5·10
0·10	0·1100 ± 0·0061	5·70	6·70
0·50	0·4900 ± 0·0053	1·00	−0·04
1·00	0·9900 ± 0·0096	1·00	−0·06
5·00	4·9400 ± 0·0330	0·70	−1·20
10·00	10·0300 ± 0·014	0·14	0·30
Inter-assay (n = 6)			
0·05	0·0490 ± 0·0040	8·2	−1·0
0·10	0·1100 ± 0·0085	7·6	10·0
0·50	0·4900 ± 0·0060	1·2	−0·3
1·00	0·9900 ± 0·0090	1·0	−0·1
5·00	4·9400 ± 0·0630	1·3	−1·1
10·00	10·0300 ± 0·0300	0·3	0·3

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

unbound cefoperazone concentration profile over time in rat blood and brain during cefoperazone administration (50 mg kg^{-1}, i.v.). Cefoperazone concentrations in brain (0·5 μg mL^{-1}) increased during the first 20-min interval. It reached a peak concentration (2 μg mL^{-1}) at 20 min after drug administration. It gradually decreased to 0·5 μg mL^{-1} (approx.) at 60 min after drug administration. The brain dialysate levels were approaching the borderline of the quantitation limit after 90 min of drug administration. Therefore, the data points were measured up to 90 min in brain

Table 2. In-vivo microdialysis recovery (%) of cefoperazone in rat blood and brain.

Concn (μg mL^{-1})	Recovery (%)
In rat blood	
0·5	33·5 ± 7·3
1·0	36·4 ± 5·5
In rat brain	
0·1	13·8 ± 5·7
0·5	12·3 ± 4·4

Data are expressed as mean ± s.d., n = 6.

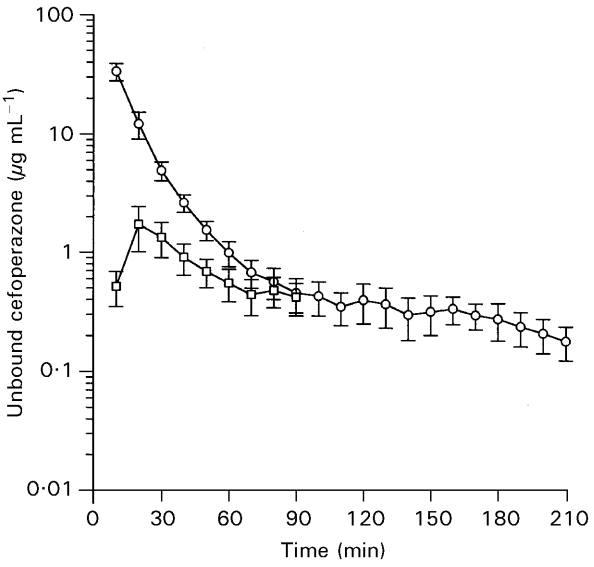

Figure 3. Mean unbound levels of cefoperazone in rat jugular vein blood (○) and brain (□) after cefoperazone administration (50 mg kg^{-1}, i.v., n = 6).

dialysates. The cefoperazone concentrations in blood dialysates decreased exponentially immediately after drug administration. These data were corrected by in-vivo recovery of corresponding brain or blood microdialysis probes.

Pharmacokinetic parameters of cefoperazone in blood and brain are shown in Table 3. Based on the pharmacokinetic parameters, the ratio of AUC of cefoperazone in rat brain and blood was about 7·8%. The observed results show that unbound concentrations of cefoperazone in rat blood are much higher than the unbound cefoperazone concentrations in the extracellular space of the brain. The results also suggest that cefoperazone is capable of penetrating the blood–brain barrier. This observation is in agreement with reports in the literature (Honda et al 1988; Meulemans 1992). These data also provide pharmacological evidence for the prevention of postoperative infections after brain surgery by cefoperazone (Honda et al 1988).

Table 3. Estimated pharmacokinetic parameters following cefoperazone administration (50 mg kg^{-1}, i.v.) to rats.

Parameters	Estimated
Blood	
AUC (min mg mL^{-1})	1·18 ± 0·21
CL (L kg^{-1} min^{-1})	0·059 ± 0·021
MRT (min)	17·17 ± 3·84
V_{ss} (L kg^{-1})	0·91 ± 0·26
Brain	
AUC (min mg mL^{-1})	0·092 ± 0·031
MRT (min)	66·81 ± 7·75

Data are expressed as means ± s.e.m., n = 6.

Compared with conventional extraction or precipitation methods (Brisson & Fourtillan 1981; Muder et al 1982; Dokladalova et al 1983; Signs et al 1984; La Follette et al 1988), the microdialysis sampling technique offers at least three advantages. Firstly, it continuously monitors unbound drugs in the same animals. Secondly, it minimizes biological-fluid loss during sampling. Finally, it also minimizes stress on haemodynamics (Fettweis & Borlak 1996). Therefore, the microdialysis technique permits multiple sampling with minimal disturbance on the homoeostasis and physiological processes. In addition, microdialysates can be directly injected onto a liquid chromatographic system without analytical interference. It is widely applied in the monitoring of unbound drugs in organs, blood vessels and brain tissues (de Lange et al 1997).

In summary, a rapid and sensitive microbore chromatographic method for the determination of cefoperazone in rat brain and blood was developed. This method exhibits no endogenous interference and the sensitivity is sufficient for the measurement of cefoperazone in biological dialysates. The intravenous injection of 50 mg kg^{-1} of cefoperazone to the anaesthetized rat was applicable to pharmacokinetic studies on protein-unbound cefoperazone in rat blood and brain. It is possible to apply this validated method to other drugs which are permeable to the blood–brain barrier for pharmacokinetic or clinical applications.

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