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Simultaneous measurement of cefuroxime in rat blood and brain by microdialysis and microbore liquid chromatography Application to pharmacokinetics

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Abstract

To characterize the pharmacokinetics of cefuroxime in rat blood and brain, microdialysis probes were inserted into the jugular vein and brain striatum, respectively. Cefuroxime (20 mg/kg, i.v.) was administered via the femoral vein. Blood microdialysates were automatic injected onto microbore liquid chromatography via an on-line injectors. The mobile phase consisted of methanol–100 m*M* monosodium phosphoric acid (25:75, v/v, pH 5.0) with a flow-rate of 0.05 ml/min. Ultraviolet detector was set at a wavelength of 280 nm for cefuroxime. The present assay enhanced the detection sensitivity and enabled the determination of cefuroxime down to 5 ng/ml. The pharmacokinetic data demonstrated that the area under the concentration curve (AUC) ratio of unbound cefuroxime in rat brain and blood was about 4.2% after cefuroxime (20 mg/kg, i.v.) administration. These results provided further evidence that cefuroxime could penetrate the blood–brain barrier.
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1. Introduction

Pharmacokinetic studies provide valuable information regarding drug disposition in the determination of dosage regimens. In general, most drugs exert their therapeutic effects not in the protein-bound form but on the protein-unbound form. The total concentration of a drug (protein bound and unbound drug) in the blood does not immediately reflect its concentration at the cellular level, particularly in the case of central acting drugs that must penetrate the blood–brain barrier. Monitoring the concentration of a drug in the interstitial space is crucial to understand its time course of biological activity.

Cefuroxime is a second-generation cephalosporin that may be able to permeate the blood–brain barrier for the treatment of meningitis due to *H. influenzae*, *N. meningitidis*, or *Strep. pneumoniae* [1]. A literature survey from 1984 to 1999 identified 23 articles

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with the key words "cefuroxime" and "brain". Many articles described studies on cefuroxime penetration into brain under brain trauma, meningitis, neurosurgery, or infant with impaired blood–brain barrier. To evaluate its pharmacological role or clinical application, it is necessary to detect cefuroxime in blood and brain. The previous reports found cefuroxime levels were from 1.8 μg/ml to 66.9 μg/ml in serum [2] and from 0.15 μg/ml to 5.36 μg/ml in cerebrospinal fluid (CSF) [2,3]. Conventional liquid chromatography (LC) was applied in the determination of these levels without difficulty. However, cefuroxime in brain dialysates ranging from 0.01 to 0.30 μg/ml were estimated in our preliminary studies. There is a need to develop a sensitive assay in the study of cefuroxime levels in brain dialysates.

Various methods have been developed to determine cefuroxime levels in blood, CSF, and brain tissues, all of which involve various pretreatment procedures prior to reversed-phase liquid chromatography [4–8]. These pretreatment procedures include liquid-phase [6] or solid-phase [8–10] extraction, and deproteination by organic solvent [11] or diluted acidic solution [12]. These techniques suffer from the need for intensive sampling of body fluids, and time consuming clean-up procedures for small samples. In addition, these conventional techniques suffer from their detection sensitivity. In general, their sensitivities are between 0.1 and 0.4 μg/ml which are also related to the volumes of sample injected. Some extraction or pre-concentrated procedures may push the limit of detection down to 50–100 ng/ml. To simplify the sample clean-up steps and improve the detection sensitivity, an on-line microdialysis was coupled to a microbore LC for the simultaneous determination of unbound cefuroxime in rat blood and brain.

2. Experimental

2.1. Reagents

Cefuroxime (Fig. 1) and chemical reagents were purchased from Sigma (St. Louis, MO, USA). The chromatographic solvents were obtained from BDH (Poole, UK). Triple de-ionized water from Millipore (Bedford, MA, USA) was used for all preparations.

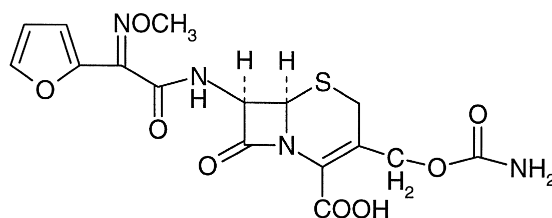

Fig. 1. Chemical structure of cefuroxime.

2.2. Liquid chromatography

The liquid chromatographic system consisted of a chromatographic pump (BAS PM-80, Bioanalytical System, West Lafayette, IN, USA), an on-line injector (CMA 160, Stockholm, Sweden) equipped with a 10-μl sample loop and a Dynamax UV–Vis detector (Varian, Walnut Creek, CA, USA). Dialysates were separated using a reversed-phase C_{18} microbore column (150×1 mm I.D.; particle size 5 μm, Bioanalytical System) maintained at an ambient temperature. The mobile phase consisted of methanol–100 m*M* monosodium phosphoric acid (25:75, v/v, pH 5.0) with a flow-rate of 0.05 ml/min. The mobile phase mixture was filtered through a 0.22-μm Millipore membrane, then degassed prior to use. The optimal UV detection wavelength for cefuroxime was 280 nm [8]. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.3. Animals

Adult male Sprague–Dawley rats (280–320 g) were obtained from the Laboratory Animal Science Center of the National Yang-Ming University (Taipei, Taiwan). These animals were specifically pathogen-free and were allowed to acclimate in our environmentally controlled quarters (24±1°C and 12:12 h light–dark cycle) for at least five days before experimentation. The rats were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and supplements of pentobarbital were given as needed throughout the experimental period. The femoral

vein was exposed for further drug administration. The rat's body temperature was maintained at 37°C with a heating pad.

2.4. Blood and brain microdialysis

Blood and brain microdialysis systems consisted of a CMA/100 microinjection pump (CMA) and microdialysis probes. Blood (10 mm in length) and brain (2 mm in length) dialysis probes were made of silica capillary in a concentric design and covered at the tips by dialysis membranes (Spectrum, 150 μm outer diameter with a cut-off at nominal molecular mass of 13 000, Laguna Hills, CA, USA). A blood microdialysis probe was positioned within the jugular vein/right atrium (toward the heart). The probe was perfused with ACD solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 m*M*) at a flow-rate of 1 μl/min.

After a blood microdialysis probe was implanted, the rat was mounted on a Kopf stereotaxic frame for the implantation of a brain microdialysis probe in the right striatum (coordinates: AP 0.2 mm; ML 3.2 mm; DV 7.0 mm) according to the Paxinos and Watson atlas [13]. The brain microdialysis probe was perfused with Ringer's solution (147 m*M* Na^+; 2.2 m*M* Ca^{2+}; 4 m*M* K^+; pH 7.0) at a flow-rate of 1 μl/min. After a 2-h baseline collection, cefuroxime (20 mg/kg) was intravenously administered via the femoral vein. Blood dialysates were injected onto the microbore chromatographic system by an on-line injector (CMA/160) at 10-min intervals. In the mean time, 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 [14,15]. Aliquots of 10 μl of blood or brain dialysates were assayed by microbore LC.

2.5. Recovery of microdialysis probe

For in vivo recovery determinations, the blood and brain microdialysis probes were inserted into the rat jugular vein and brain striatum, respectively, under anesthesia with sodium pentobarbital. ACD solution (for blood microdialysis) containing cefuroxime (1 or 5 μg/ml) or Ringer's solution (for brain microdialysis) containing cefuroxime (50 or 100 ng/ml) was perfused through a probe at a constant flow-rate (1 μl/min) using the infusion pump. After a 2-h stabilization period, the inlet (C_{in}) and outlet (C_{out}) concentrations of cefuroxime were determined by LC. The in vivo recovery ($R_{in\ vivo}$) of cefuroxime across a microdialysis probe was calculated by the following equation [16]: $R_{in\ vivo}=([C_{in}-C_{out}]/C_{in})$.

2.6. Precision and accuracy

The linearity of calibration curve was performed by the standard cefuroxime in the concentration range of 0.05, 0.1, 0.5, 1, 5 and 10 μg/ml. Intra- and inter-assay precision and accuracy of the examine were obtained from these samples was used to establish the limit of detection and limit of quantitation, according to the procedure described by Armbruster et al. [17]. The intra-assay variability was assessed with replicates ($n=6$) and the inter-assay was assessed during six consecutive days in the concentration range from 0.05 to 10 μg/ml. This analytical method was determined by a method demonstrated in previous report [18] and was shown as the mean deviation of all concentrations from the theoretical value.

2.7. Pharmacokinetics

Cefuroxime concentrations were corrected by in vivo recoveries of the respective microdialysis probes. Pharmacokinetic calculations were obtained by treatment of observed data. The computer program WinNonlin (version 1.1, Science Consulting, Apex, NC, USA), subsequently processed all data for the calculation of pharmacokinetic parameters following non-compartmental model [19]. The areas under the concentration curves (AUCs) and area under the moment versus time curve (AUMC) were calculated using the trapezoid method. The clearance (Cl) and the mean residence time (MRT) were calculated as follows: Cl=dose/AUC and MRT=AUMC/AUC.

3. Results and discussion

Isocratic separation of cefuroxime in blood and brain dialysates was adequately resolved using mi-

crobore LC. The retention time of cefuroxime was 7.1 min (Fig. 2). Fig. 2A shows a typical chromatogram of cefuroxime (5 μg/ml). Fig. 2B shows a chromatogram of a blank blood dialysate. No interfering peaks are present in the blank blood dialysate. Fig. 2C shows a chromatogram of a blood dialysate sample containing cefuroxime (3.7 μg/ml) collected from a rat blood vessel 20 min after cefuroxime administration (20 mg/kg, i.v.). Blood cefuroxime levels were between 0.1 and 30 μg/ml after correction by in vivo recovery.

Fig. 3A shows a typical chromatogram of standard cefuroxime (100 ng/ml). Fig. 3B shows a chromatogram of a blank brain dialysate. No interfering peaks are present in the blank brain dialysate. Fig. 3C shows a chromatogram of a brain dialysate sample containing cefuroxime (75 ng/ml) collected from a rat brain 30 min following cefuroxime administration (20 mg/kg, i.v.). Brain cefuroxime levels were between 80 and 400 ng/ml after correction by in vivo recovery.

The calibration curve of cefuroxime was obtained prior to LC analysis of dialysates over a concentration range of 0.05–10 μg/ml. The amounts of cefuroxime concentration were linearly related to peak areas of the chromatogram (r^2>0.995). The

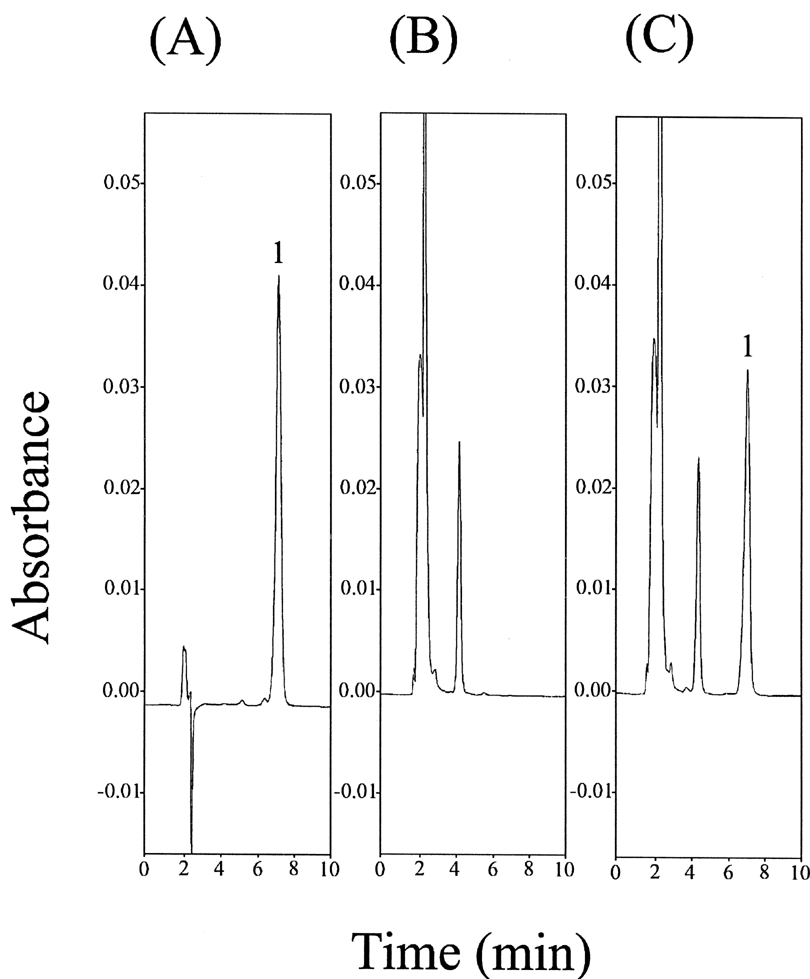

Fig. 2. Typical chromatograms of (A) standard cefuroxime (5 μg/ml), (B) a blank blood dialysate from the microdialysis probe prior to drug administration, and (C) a blood dialysate sample containing cefuroxime (3.7 μg/ml) collected from a rat blood microdialysate 20 min post cefuroxime administration (20 mg/kg, i.v.). 1=Cefuroxime.

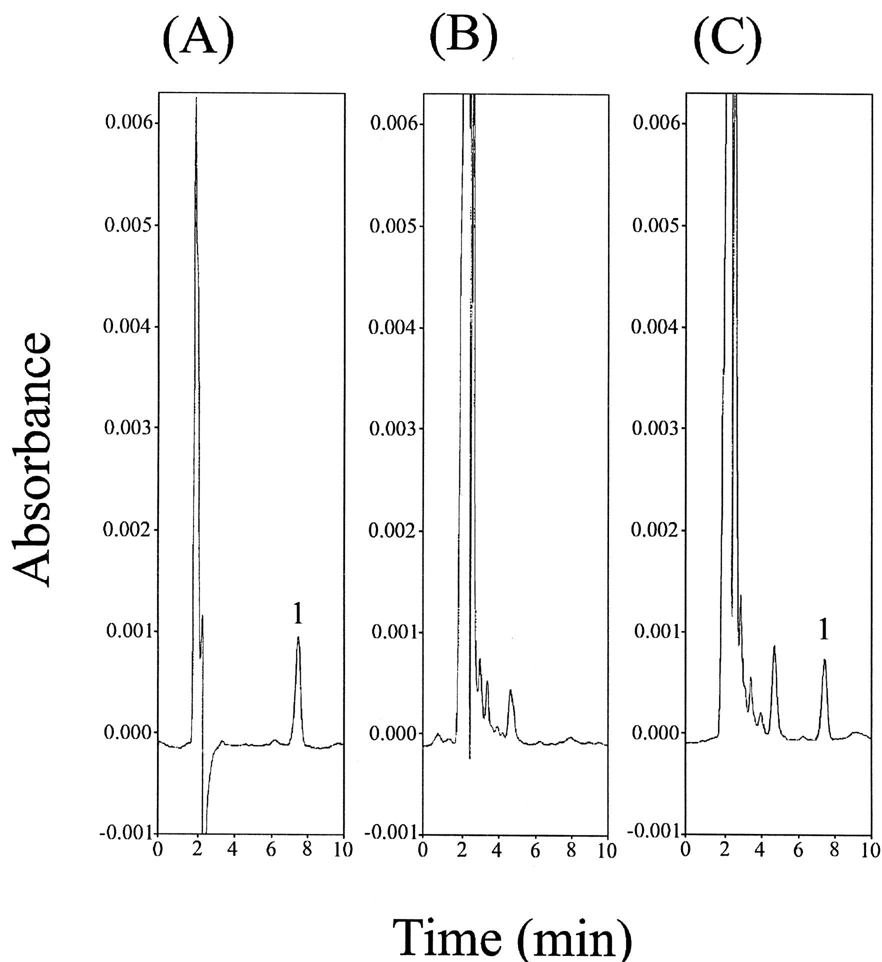

Fig. 3. Typical chromatograms of (A) standard cefuroxime (100 ng/ml), (B) a blank brain dialysate from the microdialysis probe prior to drug administration, and (C) a brain dialysate sample containing cefuroxime (75 ng/ml) collected from a rat brain microdialysate 30 min post cefuroxime administration (20 mg/kg, i.v.). 1=Cefuroxime.

detection limit of cefuroxime, at a signal-to-noise ratio of 3, was 5 ng/ml, which was 1/10 less than that described in the literature [4–8]. As shown in Table 1, overall mean precision, as defined by the relative standard deviation (RSD) range from 1.0 to 8.8%. Analytical accuracy, expressed as the percent difference of the mean observed values compared with known concentration varied from −1.6% to 4%. The accuracy and precision of the assay was thus found to be acceptable for the analysis of dialysis sample, in support of pharmacokinetic studies.

In vivo recoveries of cefuroxime in rat blood were 26.5 and 34.0% in 1 and 5 μg/ml, respectively (Table 2). In vivo recoveries of cefuroxime in rat brain were 9.2 and 9.3% in 50 and 100 ng/ml, respectively (Table 2). All physical conditions have to be kept constantly during experiments. In general, in vivo recoveries in blood were higher than in vivo recoveries in brain. 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. Thus, each probe has to be calibrated prior to experiments. On-line microdialysis coupled with microbore LC is sufficiently sensitive to allow simultaneous measurement of unbound cefuroxime

Table 1
Intra-assay and inter-assay accuracy and precision of the HPLC method for the determination of cefuroxime

Nominal concentration	Observed concentration[a]	RSD (%)	Accuracy (% Bias)
Intra-assay (n=6)			
50 (ng/ml)	51±4	7.8	2
100 (ng/ml)	102±9	8.8	2
500 (ng/ml)	499±6	1.2	−0.2
1 (μg/ml)	1.02±0.01	1.0	0.2
5 (μg/ml)	4.92±0.1	2.0	−1.6
10 (μg/ml)	10.04±0.1	1.0	0.4
Inter-assay (n=6)			
50 (ng/ml)	52±4	7.7	4
100 (ng/ml)	102±5	5.3	2
500 (ng/ml)	507±14	2.8	1.4
1 (μg/ml)	1.00±0.01	1.0	0.3
50 (μg/ml)	5.00±0.06	1.2	−0.1
10 (μg/ml)	10.01±0.1	1.0	0.1

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

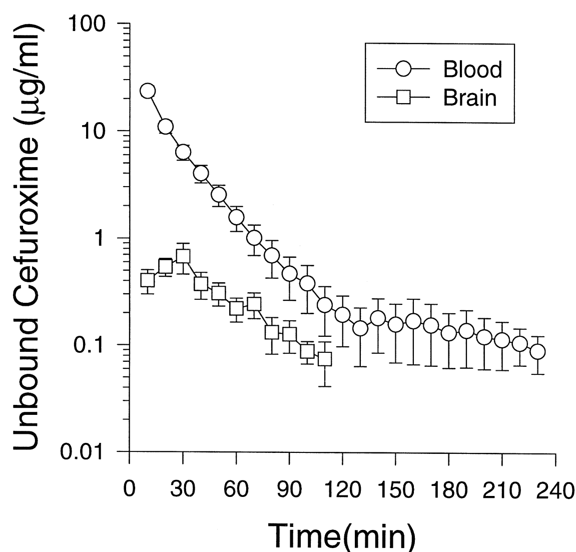

Fig. 4. Unbound cefuroxime concentrations in rat blood and brain following a 20 mg/kg intravenous administration of cefuroxime. Data are presented as the mean±S.E.M. ($n=6$).

in rat blood vessel and brain for peripheral and central pharmacokinetic studies.

Fig. 4 shows the corrected cefuroxime concentrations over time in rat blood and brain after cefuroxime administration (20 mg/kg, i.v.). These data were corrected by in vivo recovery of each probe. The mean concentration of cefuroxime in the brain increased during the first 10 min and reached the peak concentration at 30 min following drug administration. The pharmacokinetic parameters of cefuroxime in blood and brain are given in Table 3. Based on the pharmacokinetic parameters, the AUC ratio of protein-unbound cefuroxime in rat brain and blood was about 4.2%. The penetration of cefuroxime across the blood–brain barrier was previously reported [2,3]. The mean CSF/serum ratios of cefuroxime were between 3.7 to 31.8% in patient with meningitis [3]. Our data demonstrated that unbound cefuroxime brain/blood AUC ratios after an intravenous administration of cefuroxime (20 mg/mg) in the rat was 4.2%. These results provided further evidence with agreement that cefuroxime could penetrate blood–brain barrier in others reported [2,3,20,21]. It also rationalized that cefurox-

Table 2
In vivo microdialysis recoveries (%) of cefuroxime in rat blood and brain

Concentration	Recovery (%)[a]
In rat blood	
1 μg/ml	26.5±3.9
5 μg/ml	34.0±3.4
In rat brain	
50 ng/ml	9.2±1.0
100 ng/ml	9.3±0.5

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

Table 3
Pharmacokinetic parameters of cefuroxime in rat blood and brain following cefuroxime administration (20 mg/kg, i.v., $n=6$)

Parameter	Estimated[a]
Blood	
AUC (min mg/ml)	0.78±0.10
AUMC (min mg/ml)	14.83±3.91
CL (l/min/kg)	0.03±0.00
MRT (min)	17.70±2.25
Brain	
AUC (min mg/ml)	0.033±0.0065
AUMC (min mg/ml)	1.44±0.38
MRT (min)	41.81±5.23

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

ime may prevent the neurosurgical wound infections [20].

Cefuroxime concentrations in brain dialysates were detectable to at least approximately 10 ng/ml at 110 min following cefuroxime administered for all animals. Further investigation will be needed to measure trace cefuroxime (<1 ng/ml) in brain dialysates. In early studies, problems with sensitivity, accuracy and precision of pre-treatment procedures were responsible for the lack of brain pharmacokinetic studies in cefuroxime. Henceforth, we may focus our aims on cefuroxime concentrations in brain and their therapeutic effects in experimental animals.

In conclusion, our results indicate that cefuroxime rapidly (within 10 min) enters the extracellular fluid of brain striatum following an i.v. administration of cefuroxime. The results suggest that the quantitative analysis of unbound cefuroxime in brain and blood can be simultaneously carried out using microbore LC with ultraviolet detection. It is possible to apply this validated method for studying the drug that is able to across the blood–brain barrier using microdialysis.

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