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Concurrent quantification and pharmacokinetic analysis of cefotaxime in rat blood and brain by microdialysis and microbore liquid chromatography

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Abstract

A simple but effective coupling of microdialysis and microbore liquid chromatograph with UV detection technique was applied to the simultaneous studying of the pharmacokinetics of cefotaxime in both the peripheral compartment and central nervous system. The mobile phase consisted of methanol–100 m*M* monosodium phosphoric acid (25:75, v/v, pH 5.5) pumped through a C_{18} microbore column at a flow-rate of 0.05 ml/min. Detection of cefotaxime was set at a UV wavelength of 254 nm. Microdialysis probes were inserted into the jugular vein and striatum of the rat. Following stabilization of microdialysate levels, rats received cefotaxime (20 mg/kg, i.v., $n=6$) via the femoral vein, and complete concentration versus time profiles for blood and striatum were constructed. The results indicated that cefotaxime rapidly (within 10 min) entered the extracellular fluid of brain striatum following intravenous administration. Noncompartmental pharmacokinetics analysis indicated that the area under the concentration versus time ratio of cefotaxime in rat brain and blood was 6.9%, suggesting appreciable blood–brain barrier penetration. The method was relatively simple, imposed minimal physiological perturbance as it involved no body fluid consumption and sampled in particular protein-unbound drugs, generally believed to be the active fraction.

Keywords: Cefotaxime

1. Introduction

Pharmacokinetic studies provide valuable information regarding drug disposition helpful in the determination of dosage regimens. In general, most drugs exert their therapeutic effects not in the protein bound form but in the protein-unbound form. Therefore, the total concentration of a drug (protein bound and unbound) in the bloodstream does not directly reflect its effective concentration at the cellular level. In the case of central nervous system acting drugs, which must penetrate the blood–brain barrier, their presence in the tissue fluid should be indicated [1].

Cefotaxime, a third generation cephalosporin anti-

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biotic, is characterized by broad spectrum of activity and increased resistance to β-lactamases [2]. To evaluate its disposition in peripheral circulation and the central nervous system, there is a need to detect protein-unbound cefotaxime in the blood and brain. Various methods have been developed to determine cefotaxime in biological fluids, all of which involve various pretreatment procedures prior to reversed-phase liquid chromatography (LC) [3–6] or capillary electrophoresis [7]. These pretreatment procedures include liquid-phase [6] or solid-phase [6,8,9] extraction, and deproteination by organic solvent [10,11], or anion-exchange extraction [12]. These techniques generally suffer from the need for intensive sampling of body fluids, and time-consuming clean-up procedures for small samples. To simplify the sample clean-up steps, microdialysis, in which macromolecules would be excluded, was coupled to microbore high-performance liquid chromatography (HPLC) and ultraviolet detection. Such a technique was applied for the simultaneous determinations of unbound cefotaxime in rat blood and brain.

2. Experimental

2.1. Reagents

Cefotaxime (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.

2.2. Liquid chromatography

The liquid chromatographic system consisted of a chromatographic pump [BAS PM-80, Bioanalytical System (BAS), West Lafayette, IN, USA], an on-line injector (CMA 160, Stockholm, Sweden) equipped with a 10-μl sample loop and an ultraviolet detector (Linear Model LC305, San Jose, CA, USA). Dialysates were chromatographed using a reversed-phase C_{18} microbore column (150×1 mm I.D., particle size 5 μm, BAS) maintained at ambient temperature. The mobile phase, consisting of methanol–100 m*M* monosodium phosphoric acid (25:75, v/v, pH 5.5), was filtered through a 0.22-μm Millipore membrane, and then degassed prior to being pumped through the system at a flow-rate of 0.05 ml/min. The detection UV wavelength was set at 254 nm in accordance with a previous report [13]. Output data were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

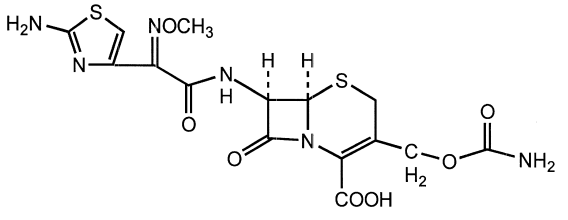

Fig. 1. Chemical structure of cefotaxime.

2.3. Animals

Adult male Sprague–Dawley rats (280–320 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). These animals were specific pathogen-free and were allowed to acclimate in their environmentally controlled quarters (24±1°C and 12:12 h light–dark cycle) for at least 5 days before experimentation. The rats were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and remained anesthetized throughout the experimental period. The femoral vein was canulated for drug administration. The rat's body temperature was maintained at 37°C with a heating pad.

2.4. Method validation

Stock cefotaxime solution was made up by dissolving cefotaxime in methanol and diluted in Ringer's solution to desired concentrations when used. Calibration curves (external standards) using standard cefotaxime solutions and spiked plasma in concentration ranges covering the expected experimental concentrations with correlation values of at least 0.995 were established. The intra- and inter-day variabilities of cefotaxime were assessed by assaying cefotaxime (six replicates) at concentrations of 0.1, 0.5, 1, 5 and 10 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})]×100. The precision relative standard deviation (RSD) was calculated from the observed concentrations as follows: % RSD=[standard deviation (SD)/C_{obs}]×100. Accuracy (% Bias) and precision (RSD) values of within ±20% covering the range of actual experimental concentrations were considered acceptable [14]. The limit of detection (LOD) is the smallest concentration that can be distinguished from the noise level, at a signal-to-noise ratio of 3:1. The limit of quantification (LOQ) is defined as the lowest concentration on the calibration curve that can be measured with acceptable precision, with an RSD not exceeding 20% [14].

2.5. Blood and brain microdialysis

Blood and brain microdialysis systems consisted of a CMA/100 microinjection pump (CMA/Microdialysis, Stockholm, Sweden) and microdialysis probes. Dialysis probes for blood (10 mm in length) and brain (3 mm in length) were made of silica capillary in a concentric design and covered at the tips by dialysis membranes (Spectrum, 150 μm O.D. with a cut-off at nominal molecular mass of 13 000, Laguna Hills, CA, USA) [15]. The blood microdialysis probe was positioned within the jugular vein/right atrium (toward the heart) while the striatal probe was implanted using coordinates (AP 0.2 mm; ML 3.2 mm; DV 7.0 mm) provided by the Paxinos and Watson atlas [16]. The position of each brain microdialysis probe was verified at the end of the experiments [17,18]. The anti-coagulant ACD solution (3.5 m*M* citric acid; 7.5 m*M* sodium citrate; 13.6 m*M* dextrose) and Ringer's solution (147 m*M* Na^+; 2.2 m*M* Ca^{2+}; 4 m*M* K^+; pH 7.0) were used to perfuse the blood and striatal probes, respectively, both at a flow-rate of 1 μl/min [15]. Following a 2-h baseline collection of dialysates, cefotaxime (20 mg/kg) was intravenously administered via the femoral vein. Blood dialysates were injected into the microbore chromatographic system by an on-line injector (CMA/160) at 10-min intervals while the striatal dialysates were collected by a fraction collector (CMA/140) at 10-min intervals. Aliquots of 10 μl were then assayed by microbore LC.

2.6. Recovery of microdialysis probe

For in vivo recovery determinations, the blood and brain microdialysis probes were inserted into the rat jugular vein and striatum, respectively, under anesthesia with sodium pentobarbital. ACD solution (for blood microdialysis) containing cefotaxime (50 or 100 ng/ml) or Ringer's solution (for brain microdialysis) containing cefotaxime (1 or 2 μg/ml) was respectively perfused through the probes 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 cefotaxime were determined by HPLC. The in vivo recovery ($Recovery_{in\ vivo}$) of cefotaxime across a microdialysis probe was calculated by the following equation [19]: $Recovery_{in\ vivo}=[(C_{in}-C_{out})/C_{in}]$.

2.7. Pharmacokinetics

Cefotaxime concentrations were corrected by in vivo recoveries of the respective microdialysis probes. Pharmacokinetic calculations were obtained by treatment of observed data. All data were subsequently processed by the computer program WinNonlin standard version 1.1 (Science Consulting, Apex, NC, USA) for the calculation of pharmacokinetic parameters according to the non-compartmental model [20]. All data are presented as means±standard errors. The area under the concentration (AUC) and the area under the moment versus time (AUMC) curves were calculated using the trapezoid method. The mean residence time (MRT) was calculated as follows: MRT=AUMC/AUC (see Table 3).

3. Results and discussion

Cefotaxime in blood and brain dialysates was adequately resolved using microbore LC. The retention time of cefotaxime was 7.1 min (Fig. 2), enabling on-line injection in the case of blood analysis. Fig. 2A and B show, respectively, typical chromatograms of standard cefotaxime (5 μg/ml) and that of a blank blood dialysate. None of the observed peaks interfered with the analysis of cefotaxime. Fig. 2C shows a chromatogram of a

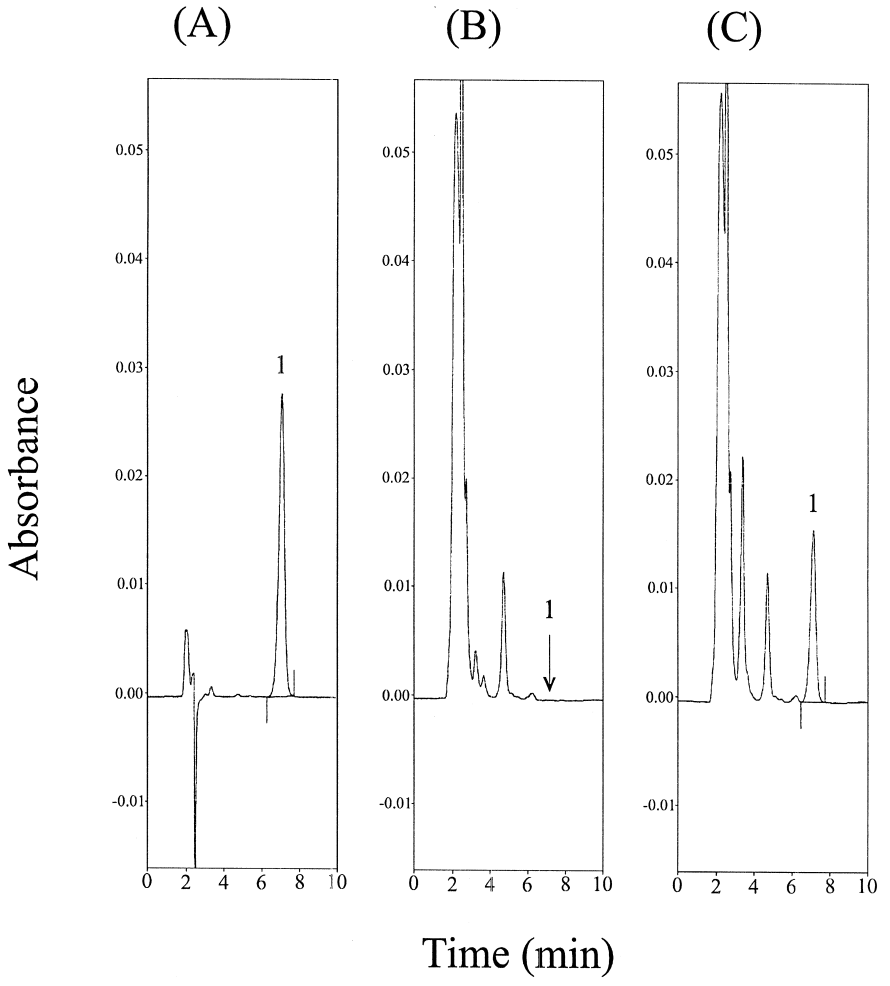

Fig. 2. Typical chromatograms of (A) standard cefotaxime (5 μg/ml), (B) a blank blood dialysate from the microdialysis probe prior to drug administration, and (C) a blood dialysate sample containing cefotaxime (2.73 μg/ml) collected from rat blood microdialysate 20 min post cefotaxime administration (20 mg/kg, i.v.). 1: Cefotaxime.

blood dialysate sample containing cefotaxime (equivalent to 2.73 μg/ml) 20 min following cefotaxime administration (20 mg/kg, i.v.).

Fig. 3A and B show, respectively, typical chromatograms of standard cefotaxime (0.05 μg/ml) and that of a blank brain dialysate. Again none of the observed peaks interfered with the analysis of cefotaxime. Fig. 3C shows a chromatogram of a brain dialysate sample containing cefotaxime (equivalent to 0.06 μg/ml) collected from a rat brain 20 min following cefotaxime administration (20 mg/kg, i.v.). Detector response was linear (r^2>0.995) over a concentration range of 0.01–10 μg/ml for cefotaxime. The intra- and inter-day precision and accuracy of cefotaxime fell well within the predefined limits of acceptability (<10%). All % bias and RSD values were within ±10% (Table 1). The LOD and LOQ for cefotaxime were 0.005 μg/ml and 0.01 μg/ml, respectively, within the pre-defined level [14]. Such a detection technique was sufficiently sensitive to allow coupling, either on- or off-line, depending on the availability of analytical instruments, with microdialysis for the detection of unbound cefotaxime in rat blood vessel and brain in the course of a pharmacokinetic study.

Regarding the merits, demerits and precautions in

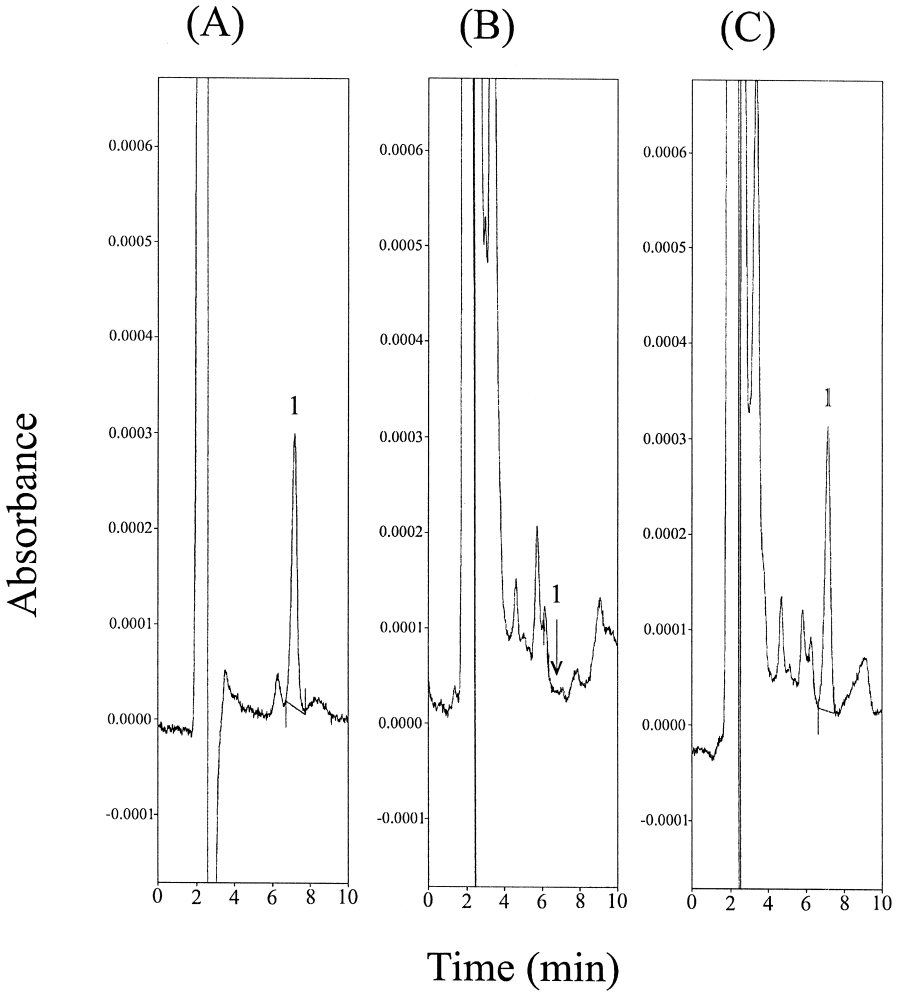

Fig. 3. Typical chromatograms of (A) standard cefotaxime (0.05 μg/ml), (B) a blank brain dialysate from the microdialysis probe prior to drug administration, and (C) a brain dialysate sample containing cefotaxime (0.06 μg/ml) collected from rat brain microdialysate 20 min post cefotaxime administration (20 mg/kg, i.v.). 1: Cefotaxime.

the application of microdialysis, while it offers the distinct advantage of not consuming body fluid and is therefore ideally suitable for applications where multiple sampling are required, recovery efficiency and variability are major concerns. In general, in vitro recoveries are affected by physical factors such as materials, dimensions of the probe and perfusion rate. Thus, each probe has to be calibrated prior to experiments and all physical constants have to be kept as constant as possible. Results from the present study, indicated that in vivo recovery in blood was higher than that in the brain (Table 2). In vivo microdialysis recovery involves additional factors such as body temperature, nature of the tissue matrix (virtuosity) and fraction volume. Due to the difficulty and certain uncertainties in the in vivo calibration, many workers simply report their findings as percentage changes relative to a reference level. However, in pharmacokinetics studies such as the present one, there is a need for conversion to real values. Thus each and every probe used in our study was calibrated for in vivo recovery prior to data collection and the results corrected for recovery according to its own efficiency.

Table 1
Intra- and inter-day accuracy and precision of the HPLC method for determination of cefotaxime

Nominal concentration (μg/ml)	Observed concentration[a] (μg/ml)	RSD (%)	Accuracy (% Bias)
Intra-assay			
0.01	0.011±0.001	9.1	10.0
0.05	0.051±0.001	2.0	2.0
0.1	0.097±0.002	2.0	−3.0
0.5	0.51±0.01	2.0	2.0
1	0.98±0.07	7.1	−2.0
5	5.06±0.28	5.5	1.2
10	10.01±0.16	1.6	0.1
Inter-assay			
0.01	0.011±0.001	9.1	10.0
0.05	0.051±0.002	3.9	2.0
0.1	0.099±0.004	4.0	−1.0
0.5	0.49±0.004	0.8	−2.0
1	1.01±0.01	1.0	1.0
5	4.93±0.14	2.8	−1.4
10	10.07±0.26	2.6	0.7

[a] Observed concentration data are expressed as rounded means±SD (n=6).

Fig. 4 shows the measured cefotaxime concentrations over time in rat blood and the brain after cefotaxime administration (20 mg/kg, i.v.). These data had been corrected for in vivo recoveries. Consistent with other reports indicating quick penetration of the blood–brain barrier by cefotaxime [21,22] the mean concentration of cefotaxime in the brain increased during the first 10 min, reaching a peak concentration at 20 min, which is compatible with the notion of peripheral administration for the prevention of bacterial meningitis as reported by Modai [23]. Nevertheless, the AUC ratio (see Table 3) for cefotaxime in rat brain and blood was 6.9%, indicating that unbound concentrations of cefotaxime in rat blood were much higher than the unbound cefotaxime concentrations in the extracellular space of brain, accounting for the fact that brain level of cefotaxime fell to below detectable limits much sooner than in the blood. It is reasonable to assume that such a system can be applied to the simultaneous

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

Concentration	Recovery (%)
In rat blood	
1 μg/ml	27.1±2.6
5 μg/ml	24.4±1.3
In rat brain	
50 ng/ml	12.0±1.5
100 ng/ml	12.9±3.6

[a] Data are expressed as mean±SD (n=6).

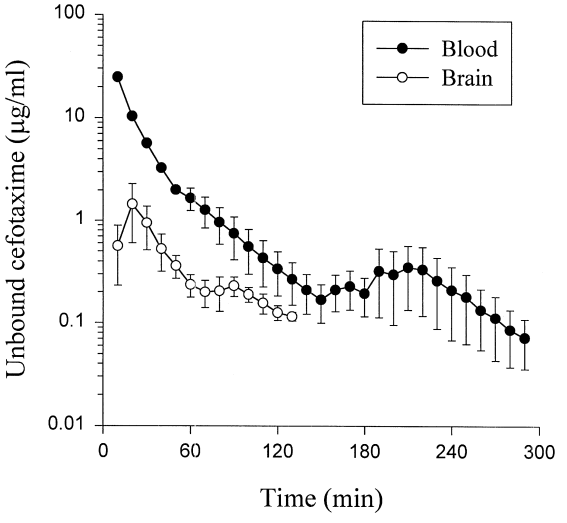

Fig. 4. Unbound cefotaxime concentrations in rat blood and brain following 20 mg/kg i.v. administration. Data are presented as the mean±S.E.M. (n=6).

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

Parameters	Estimated
Blood	
AUC (min μg/ml)	841±71
AUMC (min μg/ml)	17 510±4631
MRT (min)	20±3
Brain	
AUC (min μg/ml)	58±19[b]
AUMC (min μg/ml)	4094±1018[b]
MRT (min)	84±21[b]

[a] Data are expressed as mean±S.E.M. ($n=6$).
[b] The mean was significantly different from the blood sample ($P<0.05$).

blood and brain monitoring of drugs that have putative central nervous system action [1]. The drug concentrations in the blood and brain that are not declining in parallel may by reason of the different metabolic environment. The major metabolic organ in the peripheral circulation is the liver and it processes involved complicated phase 1 and phase 2 metabolism of drug. These processes may not happen in the central brain system that may account for the concentrations difference between blood and brain.

In conclusion, our results indicate that cefotaxime rapidly (within 10 min) entered the extracellular fluid of brain striatum following i.v. administration. The results also suggest that cefotaxime may penetrate blood–brain barrier. Quantitative analysis of unbound cefotaxime in brain and blood can be simultaneously determined using microbore LC with ultraviolet detection.

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