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On-line microdialysis coupled with microbore liquid chromatography with ultraviolet detection for continuous monitoring of free cefsulodin in rat blood

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Abstract

A microdialysis method followed by a microbore liquid chromatographic ultraviolet detection procedure has been performed for the assay of unbound cefsulodin in rat blood. A microdialysis probe was inserted into the jugular vein for blood sampling. This method involves an on-line design for submitting dialysate into the liquid chromatographic system. The chromatographic conditions consisted of a mobile phase of methanol–100 m*M* monosodium phosphoric acid (10:90, v/v, pH 5.0) pumped through a microbore reversed-phase column at a flow-rate of 0.05 ml/min. Detection wavelength was set at 265 nm. Microdialysis probes, being laboratory-made, were screened for acceptable in vivo recovery while chromatographic resolution and detection were validated for response linearity as well as intra- and inter-day variabilities. The method was then applied to pharmacokinetics profiling of cefsulodin in the blood following intravenous administration of cefsulodin (20 mg/kg) in rats. Pharmacokinetics were calculated from the corrected data for dialysate concentrations of cefsulodin versus time. Based on pharmacokinetic calculation, cefsulodin best fitted to a two-exponential disposition. This study provided specific pharmacokinetic information for protein-unbound cefsulodin and demonstrated the applicability of this continuous sampling method for pharmacokinetic study.

Keywords: Microdialysis; Kinetic studies; Pharmaceutical analysis; Cefsulodin; Cephalosporins; Antibiotics

1. Introduction

β-Lactam antibiotics are the most widely used class of antimicrobial agents. Cefsulodin is classified as a third-generation cephalosporin having potent activity against *Pseudomonas aeruginosa* [1,2] through the rapid penetration of the outer membrane of this Gram-negative bacteria and binding to a specific penicillin-binding protein [3]. When a drug permeates a cell, it will traverse the cellular plasma membrane only if protein-unbound free drug is in equilibrium across the membranes. The present investigation was undertaken to determine the protein-unbound free cefsulodin concentration in rat blood by an on-line microdialysis technique for additional pharmacokinetic studies. Various methods have been developed for determining cefsulodin in biological fluids, all of which involve various pretreatment procedures prior to liquid chromatography (LC) [4–8]. These pretreatment procedures include deproteination by trichloroacetic acid solution [4] or organic solvent [5,6] from biological samples. These techniques suffer from the need for intensive sam-

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pling of blood, and time consuming clean-up procedures for small volumes of samples. Therefore, in this paper, we use a microdialysis method to exclude the protein-bound compounds out of the microdialysis membrane in order to sample only the protein-unbound free fraction of the drug [9,10]. Such a design was applied to pharmacokinetics profiling of cefsulodin in the blood. Coupling to microbore LC–UV enhanced the resolution and reduced the required sample size, permitting the setting up of an on-line system for convenient and continuous monitoring.

2. Experimental

2.1. Reagents

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

2.2. Liquid chromatography

The microbore liquid chromatographic system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an on-line injector (CMA 160, Stockholm, Sweden) equipped with a 10-μl sample loop and an ultraviolet detector (Dynamax, 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 ambient temperature. The mobile phase consisted of methanol–100 m*M* monosodium phosphoric acid (10:90, 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, and degassed prior to use. The optimal UV detection for cefsulodin was at a wavelength of 265 nm [5]. 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 Center at National Yang-Ming University (Taipei, Taiwan). These animals were specifically 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 exposed for further drug administration. The rat's body temperature was maintained at 37°C with a heating pad.

2.4. Method validation

All calibration curves of cefsulodin (external standards) were made prior to the experiments with correlation values of at least 0.995. The intra- and inter-assay variabilities of cefsulodin were assayed (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 theoretical concentration (C_0) and the mean back value (C_1) and calculated concentration (C_2) as follows: bias (%)=$[(C_1-C_2)/(C_0)]\cdot 100$. The precision relative standard deviation (RSD) was calculated from the observed concentrations as follows: % RSD=[standard deviation (SD)/C_1]·100. Accuracy (% bias) and precision (% RSD) values within ±15% covering the range of actual experimental concentrations were considered acceptable [11].

2.5. Blood and brain microdialysis

Microdialysis probes (active length 10 mm), were made of a silica capillary in a concentric design and covered at the tips by dialysis membranes (150 μm outer diameter with a cut-off at nominal molecular mass of 13000; Spectrum, Laguna Hills, CA, USA). The blood microdialysis probe was positioned within the jugular vein/right atrium (toward the heart) of an anesthetized rat and then perfused with the anticoagulant acid citrate dextrose (ACD) solution (3.5 m*M* citric acid, 7.5 m*M* sodium citrate, 13.6 m*M* dextrose) at a flow-rate of 1 μl/min [12]. Following a 2-h baseline collection of dialysates, cefsulodin (20 mg/kg) was intravenously administered via the femoral vein. Then 10-μl aliquots of the blood

dialysates were automatically injected into the microbore LC–UV chromatographic system for analysis using an on-line injector (CMA/160) at 10-min intervals.

2.6. Recovery of cefsulodin from microdialysis probes

For in vivo recovery determinations, the microdialysis probes were inserted into the rat jugular vein, under anesthesia with sodium pentobarbital. ACD solution containing cefsulodin (1 μg/ml) was perfused through a probe at a constant flow-rate (1 μl/min) using a infusion pump. After a 2-h stabilization period, the inlet (C_{in}) and outlet (C_{out}) concentrations of cefsulodin were determined by LC–UV. The in vivo recovery ($Recovery_{in\ vivo}$) of cefsulodin across the microdialysis probe was calculated by the following equation [13]: $Recovery_{in\ vivo} = [(C_{in} - C_{out})/C_{in}]$.

2.7. Pharmacokinetics

Cefsulodin concentrations were corrected for in vivo recoveries of the respective microdialysis probes. Pharmacokinetic calculations were done by entering the observed data into the computer program WinNonlin (version 1.1; Scientific Consulting, Apex, NC, USA), which subsequently processed all data for the calculation of pharmacokinetic parameters following the two-compartmental model. Protein unbound cefsulodin concentration data were obtained by correcting the microdialytic data for in vivo recovery of the respective microdialysis probes. Pharmacokinetic calculations of unbound cefsulodin in the rat blood was fitted to a biexponential ($C = Ae^{-\alpha t} + Be^{-\beta t}$) decay.

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 from the formation slope determined by the method of the least-square. The areas under the concentration curves (AUCs) were calculated using the trapezoid method. Half-life ($t_{1/2}$) values were calculated using the equation: $t_{1/2,\beta} = 0.693/\beta$ for elimination half-life. The clearance (CL) was calculated as: CL=dose/AUC. The mean residence time (MRT) was calculated as MRT=AUMC/AUC. The volume of distribution (V_{dss}) at steady state was calculated as MRT·CL.

3. Results and discussion

Cefsulodin in blood dialysate was adequately resolved using microbore LC–UV in a relatively short retention time of 4.7 min (Fig. 1). Fig. 1A and B show typical chromatograms of cefsulodin (10 μg/ml) and a blank blood dialysate, respectively. Although a number of peaks appeared in the chromatogram, none of them interfered with the analysis of cefsulodin. Fig. 1C shows the chromatogram of a blood dialysate sample containing cefsulodin (8.7 μg/ml) collected from rat blood 20 min following cefsulodin administration (20 mg/kg, i.v.).

Concentration–response relationship in the present

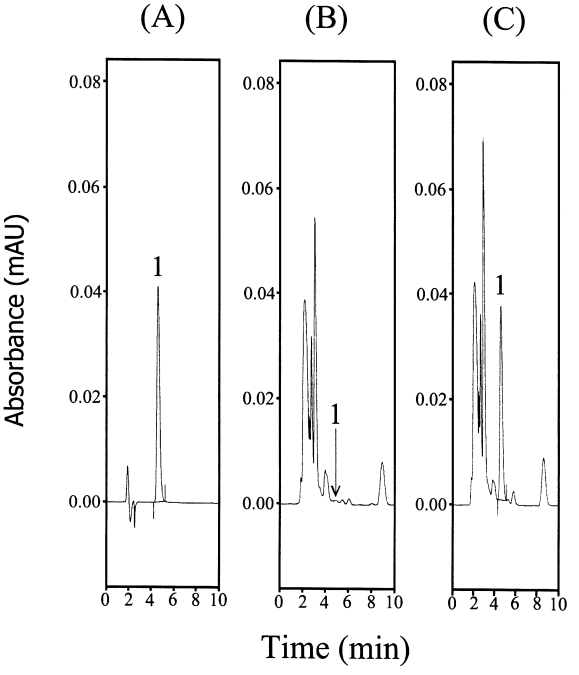

Fig. 1. Typical chromatograms of (A) standard cefsulodin (10 μg/ml), (B) a blank blood dialysate from the microdialysis probe prior to drug administration, and (C) a blood dialysate sample containing cefsulodin (8.7 μg/ml) collected from a rat blood microdialysate 20 min post cefsulodin administration (20 mg/kg, i.v.). Peak 1=cefsulodin.

method indicated linearity (r^2>0.995) over a concentration range of 0.05–10 μg/ml for cefsulodin. The limit of quantitation of the method was 0.05 μg/ml. Intra- and inter-assay precision (% RSD) and accuracy (% bias) of cefsulodin fell well within the predefined limits of acceptability (<15%) (Table 1). In vivo microdialysis recovery of cefsulodin (1 μg/ml) in rat blood was 44.16±5.27% (n=6).

The two pharmacokinetic models (one- and two-compartment) were compared according to Akaike's information criterion (AIC) [14] and the Schwartz criterion (SC) [15], with minimum AIC and SC values were regarded as the best representation of the blood concentration–time course data. A two-compartment model was proposed and validated through the program to explain the apparent biphasic disposition of blood cefsulodin after an intravenous bolus injection. 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 cefsulodin in rats. Based on these microdialysis sampling data, the pharmacokinetics of unbound cefsulodin in blood fitted best to a two-compartment model as follows: $C=47e^{-0.09t}+14e^{-0.02t}$ for cefsulodin intravenous administration (20 mg/kg). Pharmacokinetic parameters of cefsulodin in blood are presented in Table 2. Fig. 2 shows the measured cefsulodin concentrations over time in the blood following cefsulodin administration (20 mg/kg, i.v.), the data having been corrected for in vivo recoveries. These data suggest that the pharmacokinetics of unbound cefsulodin in rat blood fit well with a two-compartment model, which was in agreement with the previous report [16].

Table 2
Pharmacokinetic parameters of cefsulodin in rat blood following 20 mg/kg intravenous administration

Parameter	Estimated[a]
A (μg/ml)	47.5±14.8
B (μg/ml)	14.2±3.6
α (1/min)	0.09±0.02
β (1/min)	0.03±0.004
$t_{1/2,\beta}$ (min)	30.7±6.6
AUC (min μg/ml)	1009.0±77.2
Cl (ml/kg/min)	20.4±1.4
MRT (min)	27.2±4.6
V_{dss}[b] (ml/kg)	547.9±78.8

[a] Data are expressed as mean±S.E.M. (n=6).
[b] V_{dss} is expressed as volume of distribution in steady state.

In summary, on-line microdialysis sampling coupled with microbore LC and ultraviolet detection permitted automated and continuous monitoring which requires no clean-up preparations or consumption of body fluids. This method exhibits no endogenous interference with sufficient sensitivity in blood dialysates. The disposition of cefsulodin in rat blood

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

Theoretical concentration (μg/ml)	Mean concentration[a] (μg/ml)	RSD (%)	Accuracy (% bias)
Intra-assay (n=6)			
0.05	0.053±0.006	11.3	6.0
0.10	0.11±0.01	9.1	10.0
0.50	0.51±0.01	2.0	2.0
1.0	1.01±0.03	3.0	1.0
5.0	4.94±0.05	1.0	−1.2
10.0	10.05±0.02	0.2	0.5
Inter-assay (n=6)			
0.05	0.055±0.007	12.7	10.0
0.10	0.11±0.01	9.1	10.0
0.50	0.49±0.01	2.0	−2.0
1.0	1.01±0.01	1.0	1.0
5.0	4.96±0.05	1.0	−0.8
10.0	10.03±0.02	0.2	0.3

[a] Mean concentration data are expressed as rounded mean±SD.

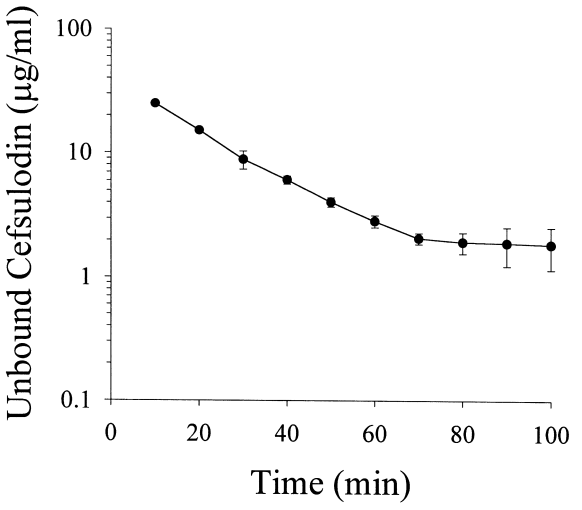

Fig. 2. Unbound cefsulodin concentrations in rat blood following 20 mg/kg intravenous administration. Data are presented as the mean±S.E.M. (n=6).

appears to follow a two-compartment pharmacokinetic model. Finally, this method is applicable to further protein-unbound pharmacokinetic studies of drugs in rats.

Acknowledgements

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