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Determination and pharmacokinetic study of meropenem in rat bile using on-line microdialysis and liquid chromatography

Y.L. Chang[a,b], M.H. Chou[b], M.F. Lin[b], C.F. Chen[c], T.H. Tsai[c,d,*]

[a]*Institute of Pharmacology, National Yang-Ming University, Taipei 112, Taiwan*
[b]*Department of Pharmacy, Veterans General Hospital-Taipei, Taipei 112, Taiwan*
[c]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan*
[d]*Institute of Traditional Medicine, National Yang-Ming University, Taipei 112, Taiwan*

Abstract

Meropenem is a carbapenem antibiotic with a wide spectrum of activity against both Gram-positive and Gram-negative bacteria. Because of its clinical efficacy, meropenem is an excellent choice for the treatment of serious infections in both adults and children. The knowledge of tissue concentrations of antibiotic in an infection site is valuable for the prediction of treatment outcome. To investigate the biliary disposition of meropenem, we utilized a minimally invasive sampling technique with a shunt linear microdialysis probe for continuous sampling in the biliary excretion studies. Analysis of meropenem in the dialysates was achieved using a LiChrosorb RP-18 column (Merck, 250×4.6 mm I.D.; particle size 5 μm) maintained at ambient temperature. The mobile phase was 50 m*M* monosodium phosphoric acid–methanol (80:20, v/v, pH 3.0). The UV detector wavelength was set at 298 nm. The area under the concentration–time curve and elimination half-lives of meropenem were about 6144±1494 min μg/ml and 61±17 min, respectively. This study represents a successful application of the microdialysis technique, which is an effective method for pharmacokinetic and biliary drug excretion studies.

Keywords: Meropenem; Antibiotics; Carbapem antibiotics

1. Introduction

Meropenem (Fig. 1) is a broad spectrum carbapenem antibiotic which can be used effectively as monotherapy for the treatment of intra-abdominal infections [1]. Generally, the selection of an antibiotic for the treatment of infection is usually based on information given by the minimum inhibitory concentration (MIC) together with the time versus serum concentration profile of drugs. However, the data obtained from serum may not be appropriate for infections of an extravascular compartment, such as

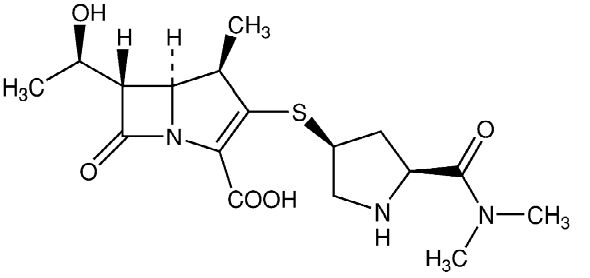

Fig. 1. Chemical structure of meropenem.

*Corresponding author. National Research Institute of Chinese Medicine, 155-1, Li-Nong Street, Section 2, Shih-Pai, Taipei 112, Taiwan. Tel.: +886-2-2820-1999; fax: +886-2-2826-4276.

E-mail address: thtsai@cma23.nricm.edu.tw (T.H. Tsai).

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a biliary tract infection or soft tissue infections [2,3]. In these cases the pharmacokinetic profiles in tissues rather than in serum determine the clinical outcome of antibiotic therapy [4].

Microdialysis is an in vivo sampling technique for the continuous monitoring of analytes in the fluids of interstitial spaces, providing the unique opportunity to obtain near-complete concentration profiles of drugs in anatomically clearly defined tissues and organs. Meropenem levels in serum and different tissues have been determined previously by both microbiological methods [5–7] and high-performance liquid chromatography (HPLC) [8–14]. And some of the HPLC assays with different extraction techniques have been extensively applied to pharmacokinetic studies [15–20]. In this paper, we use microdialysis with a shunt linear probe [21] for continuous sampling of meropenem from rat bile. In addition, to minimize the degradation of meropenem in the physiological environment, an automatic sampling system and a stable analytical condition are required. Therefore, we use an in vivo on-line microdialysis sampling method coupled with the HPLC analytical system for measuring meropenem in rat bile to establish the biliary disposition profile of meropenem.

2. Experimental

2.1. Chemicals and reagents

Meropenem was purchased from Sumitomo (Osaka, Japan). Liquid chromatographic grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Animals

The institutional animal experimentation committee of the National Research Institute of Chinese Medicine reviewed and approved all experimental protocols involving animals. Male specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei, Taiwan. The animals had free access to food (Laboratory rodent diet No. 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being supplied for experiments, at which time only food was removed. These animals were allowed to acclimatize to their environmentally controlled quarters (24±1 °C and 12:12 h light–dark cycle) for at least 5 days before the experiments began. At the start of experiments, the rats were anesthetized with urethane 0.8 g/ml and chloralose 0.08 g/ml (1 ml/kg, i.p.). Throughout the experimental period, anesthesia was maintained by administering one quarter of the initial dose at each hour. The experimental animals were kept warm with a heating pad throughout the experiments.

2.3. Chromatography

The HPLC system consisted of a chromatographic pump (BAS PM-80, Bioanalytical Systems, West Lafayette, IN, USA), an on-line injector (CMA/160, Stockholm, Sweden) equipped with a 20 μl sample loop, and a UV detector (Soma S-3702, Tokyo, Japan). Meropenem dialysate was separated using a LiChrosorb RP-18 column (Merck, 250×4.6 mm I.D.; particle size 5 μm) maintained at ambient temperature. The mobile phase comprised 50 m*M* monosodium phosphoric acid (pH 3.0)–methanol (80:20, v/v), and the flow-rate of the mobile phase was 1 ml/min. The buffer was filtered through a Millipore 0.45 μm filter and degassed prior to use. The detecting UV wavelength was set at 298 nm, and the output signal from the HPLC–UV system was recorded using an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Method validation

All calibration curves of meropenem (external standards) were made prior to the experiments with correlation values of at least 0.995. The intra-day and inter-day variabilities for meropenem were assayed (six replicates) at concentrations of 0.1, 0.5, 1, 5, 10, 50, 100 and 500 μg/ml on the same day and on 6 consecutive 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 (%)=$[(C_{nom}-C_{obs})/(C_{nom})]\cdot 100$. The 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 15% covering the range of actual experimental concentrations were considered acceptable [22].

2.5. Microdialysis experiment

The bile duct microdialysis probes were constructed in the laboratory and were largely based on the design originally described by Scott and Lunte [21]. The detailed construction of the flow-through microdialysis probe has been described in our previous reports [23,24]. In brief, a 7-cm piece of dialysis membrane (spectrum, 150 μm outer diameter with a cut-off at nominal molecular mass of 9000, Laguna Hills, CA, USA) was inserted into a section of the polyethylene tubing (PE-60; 0.76 mm I.D.×1.22 mm O.D.), with the ends of the dialysis membrane connected to a piece of silica tubing (40 μm I.D.× 140 μm O.D., SGE, Australia). A piece of PE-10 tubing (0.28 mm I.D.×0.61 mm O.D.) was then attached to both ends of the PE-60 tubing and all unions were cemented with epoxy. At least 24 h was allowed for the epoxy to dry. After bile duct cannulation, the probe was perfused with normal saline and the flow-rate set at 2 μl/min. Outflows from the bile microdialysis probe were connected to an on-line injector and automatically injected every 10 min. After dialysate levels had stabilized (approximately 2 h), meropenem (50 mg/kg) was intravenously administered via the femoral vein. From each sample, 20 μl of dialysate was assayed using the HPLC system.

2.6. Recovery of microdialysate

For in vivo recovery, normal saline solution containing meropenem (5 or 10 μg/ml) was pumped through the probes at a constant flow-rate (2 μl/min) using the infusion pump (CMA/100). After a stabilization period of 2 h, the inlet (C_{in}) and outlet (C_{out}) concentrations of meropenem were determined by HPLC. The in vivo recovery ratios were then calculated by the following equation [25]: $\text{recovery}_{\text{in vivo}}=1-(C_{out}/C_{in})$

2.7. Pharmacokinetic study

The concentrations of meropenem in rat bile dialysates were determined from the calibration curves. Absolute concentrations in extracellular fluid were calculated from the concentrations in dialysates by the following equation: concentration=dialysate/recovery.

Pharmacokinetic calculations were performed using the observed data. All data were subsequently processed by the computer pharmacokinetic program WinNonlin standard version 1.1 (Science Consulting, Apex, NC, USA) for the calculation of pharmacokinetic parameters according to the non-compartmental model [26]. All data are presented as mean±standard error. The area under the concentration curve (AUC), the area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated by using statistical moments [27]. The mean residence time and clearance (CL) were calculated as follows: MRT=AUMC/AUC, CL=dose/AUC.

3. Results and discussion

The chromatograms obtained using the liquid chromatographic method are shown in Fig. 2. Each analysis was completed within 10 min. Separation of meropenem from endogenous chemicals in bile dialysates was achieved in an optimal mobile phase containing 50 m*M* monosodium phosphate (pH 3.0)–methanol (80:20). Meropenem retention time was 6.2 min (Fig. 2). Peak areas of meropenem were linear (r^2>0.995) over a concentration range of 0.1–500 μg/ml. Fig. 2A shows a typical chromatogram of a standard mixture containing meropenem (5 μg/ml). The blank sample (Fig. 2B) shows that the chromatographic conditions revealed no biological substances that would interfere significantly with the accurate determination of the drug. Fig. 2C depicts a chromatogram of actual meropenem in rat bile. The dialysate sample contains meropenem (3.83 μg/ml) collected from the bile fluid at 10 min following meropenem administration (50 mg/kg, i.v.).

Intra-assay and inter-assay (Table 1) accuracy of meropenem levels fell well within predefined limits of acceptability. All bias and RSD values were

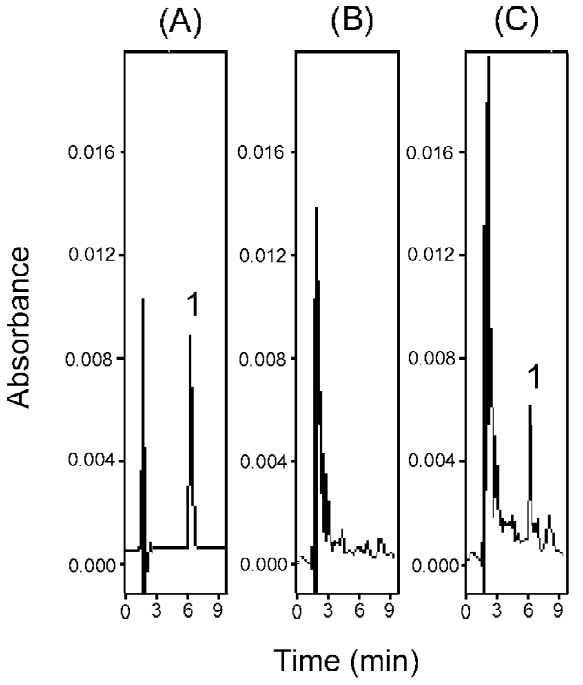

Fig. 2. Typical chromatograms for injection of (A) standard meropenem (5 μg/ml), (B) a blank bile dialysate, and (C) a bile dialysate sample containing meropenem (3.83 μg/ml) collected from bile fluid at 10 min after meropenem administration (50 mg/kg, i.v.). 1: Meropenem.

within 15%. This method has a quantitative limit of 0.1 μg/ml. The in vivo recovery of meropenem is shown in Table 2. It can be seen that this method is sensitive enough to measure meropenem in rat bile for pharmacokinetic study. These data have been corrected for in vivo recoveries. The concentration versus time curve is shown in Fig. 3, and the concentration of meropenem in the bile was increased during the first 10 min following drug administration.

It is generally accepted that appropriate pharmacokinetic/pharmacodynamic information can provide useful data by showing the diffusion of active antibiotic to the infection sites in sufficient quantities to maintain concentrations above the MIC of the relevant pathogens for an adequate time period. In the present study, the unbound drug concentrations were not over 1 μg/ml, which is the MIC relevant to bacteria of intra-abdominal infections, until 180 min after 50 mg/kg meropenem had been administered.

Table 1
Intra-assay and inter-assay accuracy of meropenem

Nominal concentration (μg/ml)	Observed concentration (μg/ml)[a]	RSD (%)	Accuracy (% bias)
Intra-assay (n=6)			
0.10	0.11±0.02	14.3	10.0
0.50	0.50±0.02	4.34	0.8
1.00	1.00±0.02	2.28	0.20
5.00	5.00±0.03	0.59	0.04
10.00	10.02±0.01	0.09	0.04
50.00	49.97±0.06	0.13	0.20
100.00	100.10±0.13	0.13	−0.11
500.00	499.98±0.02	0.004	0.003
Inter-assay (n=6)			
0.10	0.10±0.01	12.35	0.60
0.50	0.49±0.02	3.34	1.60
1.00	0.99±0.02	1.66	0.8
5.00	4.97±0.04	0.90	0.68
10.00	9.99±0.03	0.34	0.02
50.00	49.58±0.33	0.66	0.84
100.00	100.53±0.47	0.47	−0.53
500.00	499.93±0.06	0.01	0.01

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

From these microdialysis sampling data, the pharmacokinetic parameters were calculated using the non-compartmental model (Table 3). The current results were comparable with previous conducted studies, in which bile was obtained either during abdominal surgery or at the time of endoscopic retrograde cholangiography [28,29].

Conventional methods used to measure drug concentration in the bile have been described using bile fluid collection [30]. However, these methods require a relatively complicated clean-up process before samples can be analyzed. Furthermore, the attempts to determine drug concentrations continuously from the bile duct with no bile loss have had limited success. To overcome these drawbacks of traditional methods, we constructed an automatic on-line flow-

Table 2
In vivo microdialysis recoveries (%) of meropenem in rat bile

Concentration (μg/ml)	Recovery (%)
5	83.2±3.1
10	80.2±4.3

Data expressed as mean±SD (n=6).

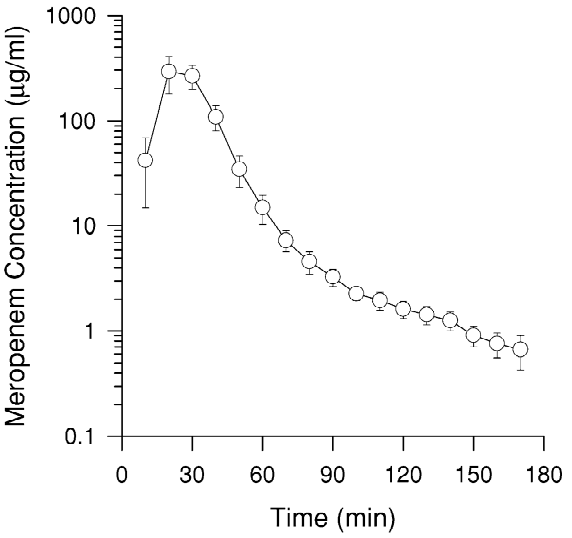

Fig. 3. Bile concentration of meropenem versus time curve after meropenem administration (50 mg/kg). Error bars are means±S.E.M. (n=6).

through microdialysis probe [21,31] for bile duct sampling coupled with an HPLC analytical system. This system can provide near real-time analysis of meropenem in bile dialysate. Although the requirement of high biliary antibiotic concentration for the treatment of biliary tract infection is uncertain [32,33], the application of microdialysis to monitor biliary drug concentrations did provide a dynamic profile, which characterized the disposition of meropenem in the biliary tract. These approaches have been successfully applied in our previous studies [34–37].

In summary, we demonstrated a rapid and sensitive chromatographic method for the determination of meropenem in rat bile fluid using in vivo microdialysis with HPLC–UV. This method results in less tissue damage, fewer animals needed, no biological fluid loss, and exhibits no endogenous interference with sufficient sensitivity. The information derived from our study may be useful for the development of effective preclinical dosage schedules, and it may improve predictions regarding therapeutic outcomes of meropenem in biliary tract infection.

Table 3
Estimated pharmacokinetic parameters of bile following meropenem administration (50 mg/kg, i.v.)

Parameter	Estimated
$t_{1/2}$ (min)	61±17
AUC (min μg/ml)	6144±1494
CL (ml/kg/min)	8.1±1.8
MRT (min)	37±3

Data are expressed as means±standard error of the mean (S.E.M.).

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