HPLC Method for Measuring Meropenem and Biapenem Concentrations in Human Peritoneal Fluid and Bile: Application to Comparative Pharmacokinetic Investigations

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Abstract

A high-performance liquid chromatography (HPLC) method using ultrafiltration to pretreat peritoneal fluid and bile samples is developed to measure meropenem and biapenem concentrations in human peritoneal fluid and bile. Meropenem or biapenem in peritoneal fluid or bile samples is stabilized by mixing with 1 mol/L 3-morpholinopropanesulfonic acid buffer (pH 7.0) (1:1). The mixture is transferred to a Nanosep 10K centrifugal filter device; after centrifugation, the filtrate is subjected to reversed-phase HPLC, and the eluate is monitored at 300 nm. No interference from endogenous substances is observed. The lower limits of quantification are 0.05 µg/mL for peritoneal fluid and 0.1 µg/mL for bile. The new method has been applied to comparative site-specific-pharmacokinetic investigations in surgery patients.

Introduction

Meropenem and biapenem are parenteral carbapenems with a broad activity spectrum against Gram-positive and Gram-negative bacteria. These drugs are often used for the treatment and prophylaxis of intra-abdominal and biliary tract infections (1). Because meropenem and biapenem act at the site of infection, it is important to monitor these drugs' concentrations in peritoneal fluid and bile after intravenous administration (2,3). It is also clinically important to clarify differences in the peritoneal and biliary pharmacokinetics between meropenem and biapenem in order to select the appropriate carbapenem for treatment. However, pharmacokinetic investigations and therapeutic drug monitoring of these drugs have predominantly focused on plasma (4–6). As this is in part due to the lack of a reliable assay, there is a clear need for a meropenem and biapenem assay that is compatible not only with plasma but also with peritoneal fluid and bile samples.

To date, several methods have been described for measuring meropenem concentrations in these two types of biological samples: a microbiological assay (7) and liquid chromatography–tandem mass spectroscopy (LC–MS–MS) method (8) for peritoneal fluid; and a different microbiological assay (9) and a high-performance liquid chromatography (HPLC) method using solid-phase extraction for sample pretreatment (10) for bile. No method has been described for measuring biapenem concentrations in human peritoneal fluid or bile. Microbiological assays lack a sufficiently low limit of quantification and cannot differentiate meropenem from other antibiotics when coadministered. LC–MS–MS has the advantage of very sensitive detection, but it requires expensive instrumentation, time, and highly skilled personnel, and thus is not as commonly used as HPLC in clinical settings. As for sample pretreatment, solid-phase extraction procedures for meropenem (10,11) could be time consuming. Instead, simple ultrafiltration has been used as a sample pretreatment step for measuring carbapenems in human plasma (12–14).

The present study aimed to develop a HPLC method using ultrafiltration for sample pretreatment to measure meropenem and biapenem concentrations in human peritoneal fluid and bile.

Experimental

Reagents and materials

Standard meropenem and biapenem were provided by Dainippon Sumitomo Pharma Corporation (Osaka, Japan) and Meinji Seika Kaisha (Tokyo, Japan), respectively. 3-Morpholinopropanesulfonic acid (MOPS) buffer (1 mol/L, pH 7.0) was purchased from Dojindo Laboratories (Kumamoto,

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Japan). $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot {}^2H_2O$, acetic acid, and sodium acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan), and acetonitrile was purchased from Sigma Aldrich Japan (Tokyo, Japan). All other chemicals were analytical-grade. The Nanosep 10K centrifugal filter device was purchased from Pall Corporation (East Hills, New York).

Equipment

The HPLC system was comprised of an LC-9A pump, an SIL-10ADVP auto-sampler, a SPD-20A UV spectrophotometric detector (Shimadzu, Kyoto, Japan), a U-620 Type30 column heater (Sugai Chemical Industry, Wakayama, Japan), and a Chromatocorder 21 (System Instruments, Tokyo, Japan).

Chromatographic conditions

Preliminary studies confirmed that optimized chromatographic conditions for the separation of meropenem (14) and biapenem (13) from plasma components can apply to peritoneal fluid and bile samples. Thus, meropenem was separated on a Waters Symmetry C_{18} (4.6 × 150 mm, 5 µm) column (Milford, MA). The isocratic mobile phase was a mixture of 10 mM sodium phosphate buffer (pH 7.4) and acetonitrile (100:10, v/v) pumped at a rate of 1.0 mL/min. The auto sampler was set to 4°C, the injection volume was 20 µL, and the column temperature was 40°C. The meropenem peak was detected by UV absorbance at 300 nm. For biapenem, the same chromatographic conditions were used except that the isocratic mobile phase was 100 mM sodium acetate buffer (pH 4.6) and acetonitrile (197:3, v/v).

Peritoneal fluid and bile samples

Peritoneal fluid samples were collected from patients through an intra-abdominal drain. The sample was mixed with the same volume of 1 mol/L MOPS buffer to ensure stabilization of meropenem (14,15) and biapenem (13,16) and stored at –40°C until analysis. A mixture of equal volumes of peritoneal fluid from three patients not treated with any antibiotics was used as the control human peritoneal fluid.

Bile samples and control human bile were collected from patients through a biliary drain and prepared in the same manner as the peritoneal fluid.

Analytical procedure

A working stock solution of meropenem or biapenem was prepared daily at a concentration of 1 mg/mL in 50 mM MOPS buffer. Control peritoneal fluid or bile was spiked with each drug at a final concentration of 0.05, 0.1, 0.5, 1.0, 5.0, 25.0, 50.0, and 100.0 µg/mL. The control sample (225 µL) was mixed with 225 µL of 1 mol/L MOPS buffer (pH 7.0). A 400-µL aliquot of the mixed control sample was pipetted into the sample reservoir of a Nanosep 10K centrifugal filter device and then centrifuged at 12,000 × *g* for 10 min at room temperature (Centrifuge LN9527-27; Abbott Laboratories, Abbott Park, IL). A 400-µL aliquot of thawed clinical peritoneal fluid or bile sample was also transferred to a Nanosep 10K device and centrifuged similarly. After the centrifugation, ~100 µL of filtrate was collected in the filtrate reservoir of the device. Finally, an 80-µL aliquot of each sample or drug-spiked filtrate was placed into an autosampler tube for the HPLC (injection volume, 20 µL).

Method validation

The method was evaluated for linearity, accuracy, and precision [expressed as the percent coefficient of variation (CV (%))]. Standard samples (0.05, 0.1, 0.5, 1.0, 5.0, 25.0, 50.0,

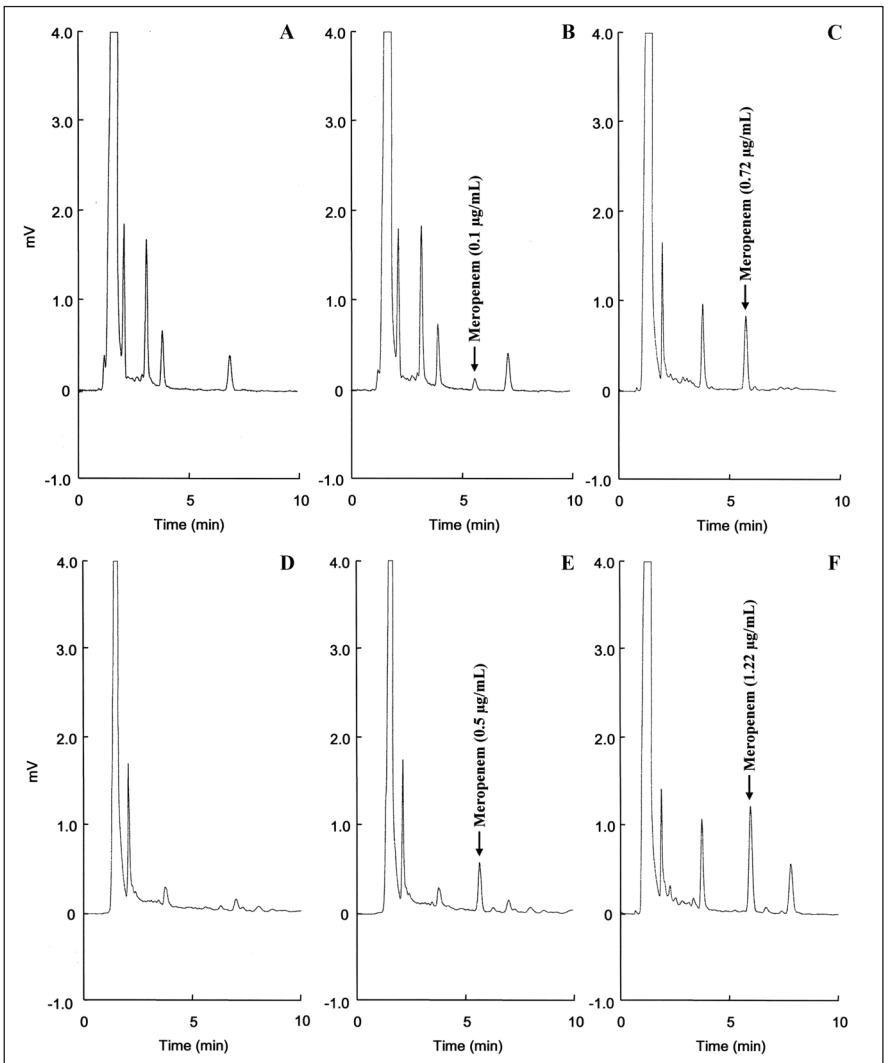

Figure 1. Typical chromatograms for meropenem: (A) blank control peritoneal fluid; (B) control peritoneal fluid spiked with 0.1 µg/mL meropenem; (C) a single patient's peritoneal fluid sample (0.72 µg/mL); (D) blank control bile; (E) control bile spiked with 0.5 µg/mL meropenem; and (F) a single patient's bile sample (1.22 µg/mL).

and 100.0 µg/mL of meropenem or biapenem) were prepared using control peritoneal fluid or bile, and intra- and inter-day assay precision and accuracy were determined. Linear regression analysis of the calibration data was performed using the equation $y = mx + b$ with a weighting of 1/y where y is the peak area ratio, x is the concentration of each drug, and m and b are the slope and intercept, respectively. The limit of detection (LOD) was defined as the sample concentration of each drug that resulted in peak heights three times the standard deviation of the noise level. The lower limit of quantification (LLOQ) was determined from the validation data, according to the Guidance for Industry Bioanalytical Method Validation (May 2001; the U.S. Food and Drug Administration, Rockville, MD).

Recovery

The recovery of meropenem or biapenem by ultrafiltration was determined by comparing the peak heights from peritoneal fluid or bile standards with those from drug standards that were similarly prepared, except that unfiltered aqueous solutions of the appropriate drug were used rather than spiked control peritoneal fluid or bile samples.

Stability

The stability of the peritoneal fluid or bile samples (5.0, 25.0, and 50.0 µg/mL of meropenem or biapenem, $n = 4$ for each) was examined using an equal volume of 1 mol/L MOPS buffer as a stabilizer after storage at –40°C. Control peritoneal fluid or bile samples were spiked to contain 5.0, 25.0, or 50.0 µg/mL of either meropenem or biapenem. An equal volume of 1 mol/L MOPS buffer was added, and the samples were mixed, divided into 0.5-mL aliquots, and stored at –40°C. The concentration of each drug was determined after 0, 7, 15, and 30 days of storage.

Application to site-specific-pharmacokinetic investigations in patients

The previously mentioned method was used to determine the comparative site-specific pharmacokinetics of meropenem and biapenem in four surgery patient groups ($n = 3$ for each). Patients with an intra-abdominal drain (group A) and patients with a biliary drain (group B) each received a 0.5-h infusion of 500 mg meropenem while a separate set of patients with an intra-abdominal drain (group C) or a biliary drain (group D) each received a 0.5-h infusion of 300 mg biapenem. Peritoneal fluid, bile, and plasma samples were collected at 0.5–5.5 h after the start of the infusion. Plasma concentrations of meropenem (14) and biapenem (13) were determined using validated HPLC methods. Using the MULTI program (17), data collected for each drug concentration were analyzed with a three-compartment pharmacokinetic model to estimate the maximum drug concentration (C_{max}) and the area under the drug concentration-time curve from 0 to 8 h ($AUC_{0–8\,h}$).

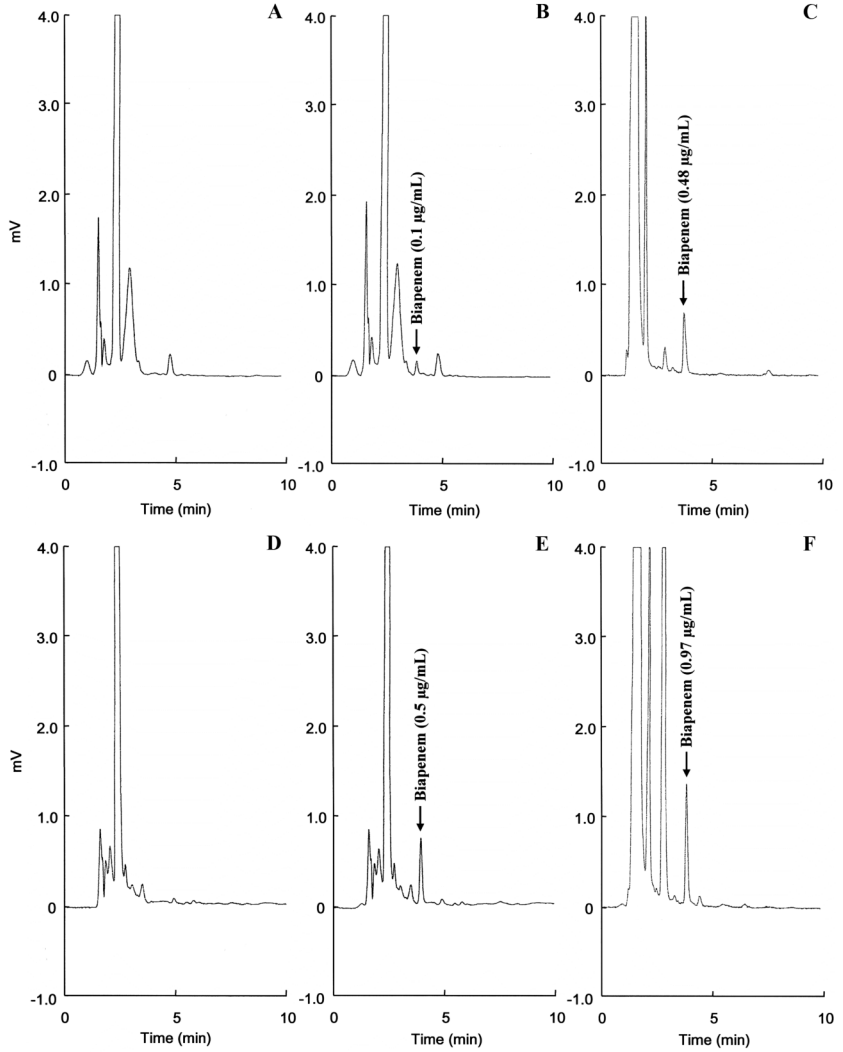

Figure 2. Typical chromatograms for biapenem: (A) blank control peritoneal fluid; (B) control peritoneal fluid spiked with 0.1 µg/mL biapenem; (C) a single patient's peritoneal fluid sample (0.48 µg/mL); (D) blank control bile; (E) control bile spiked with 0.5 µg/mL biapenem; and (F) a single patient's bile sample (0.97 µg/mL).

Results

Typical chromatograms

Figures 1 and 2 show typical HPLC traces. For meropenem, no interfering peaks were evident, and the retention time of meropenem was 5.5 min for both peritoneal fluid (Figures 1B–1C) and bile (Figures 1E–1F) samples. Interfering peaks were also not evident for biapenem, which had a retention time of 3.8 min for both peritoneal fluid (Figures 2B–2C) and bile (Figures 2E–2F) samples.

Method validation

Data from a calibration plot for the peak-area ratio for varying amounts of meropenem and biapenem are given in

Table I. The method provided satisfactory linearity at 0.05–100 µg/mL for peritoneal fluid and 0.1–100 µg/mL for bile with a mean r^2 of 0.9999. The LOD (defined as the concentration of each drug giving a signal-to-noise ratio of > 3:1) were 0.01 µg/mL for peritoneal fluid and 0.02 µg/mL for bile using a 20-µL injection volume. Based on the validation data for meropenem (Table II) and biapenem (Table III), the LLOQ (the drug concentration at which both a CV of < 20% and an accuracy of 80–120% are fulfilled in both the intra- and inter-day assays) were determined to be 0.05 µg/mL for peritoneal fluid and 0.1 µg/mL for bile.

Table I. Analytical and Statistical Parameters for the Measurement of Meropenem and Biapenem in Peritoneal Fluid and Bile (n = 5)

	Calibration curve ($y = mx + b$)*					
	Slope m (Mean ± SD)	Intercept b (Mean ± SD)	r^2 (Mean ± SD)	Range (µg/mL)	LOD (µg/mL)	LLOQ (µg/mL)
Meropenem						
Peritoneal fluid	53776 ± 483	1492 ± 735	$0.9999 \pm 0.4 \times 10^{-5}$	0.05–100	0.01	0.05
Bile	48931 ± 1233	2338 ± 1534	$0.9999 \pm 0.5 \times 10^{-4}$	0.1–100	0.02	0.1
Biapenem						
Peritoneal fluid	54659 ± 749	1512 ± 493	$0.9999 \pm 0.4 \times 10^{-4}$	0.05–100	0.01	0.05
Bile	49693 ± 1636	2573 ± 1518	$0.9999 \pm 1.4 \times 10^{-4}$	0.1–100	0.02	0.1

* $y = mx + b$ with a weighting of $1/y$; y, the peak area ratio; x, the concentration of meropenem or biapenem; m, the slope; b, the intercept.

Recovery

As shown in Table IV, the mean recoveries of meropenem and biapenem in peritoneal fluid and bile by ultrafiltration were 95.2–101.7% at 0.5–50.0 µg/mL.

Stability

For meropenem, the concentrations after 30 days storage at –40°C were 103.8 ± 0.5%, 100.8 ± 1.0%, and 100.7 ± 0.1% [mean ± standard deviation (SD), n = 4] of the initial concentration (5.0, 25.0, and 50.0 µg/mL, respectively) in peritoneal fluid, and 94.9 ± 4.8%, 94.5 ± 3.5%, and 94.6 ± 2.4% of the corresponding initial concentration in bile.

For biapenem, the concentrations after 30 days at –40°C were 99.5 ± 2.3%, 99.3 ± 0.8%, and 100.2 ± 0.8% (n = 4) of the initial concentration (5.0, 25.0, and 50.0 µg/mL, respectively) in peritoneal fluid, and 101.1 ± 3.3%, 97.0 ± 4.0%, and 101.6 ± 3.5% of the corresponding initial concentration in bile.

Site-specific pharmacokinetic investigations in patients

Figure 3 shows time courses of peritoneal fluid and bile concentrations of meropenem and biapenem in surgery patients. For each patient, all drug con-

Table II. Accuracy and Intra- and Inter-day Precision Data for the Measurement of Meropenem in Peritoneal Fluid and Bile

Meropenem conc. added (µg/mL)	Meropenem conc. found (µg/mL) (Mean ± SD, n = 6)	CV (%)	Accuracy (%)
Peritoneal fluid: Intra-day assay			
0.05	0.054 ± 0.005	8.78	108.8
0.1	0.108 ± 0.005	4.30	107.8
0.5	0.520 ± 0.012	2.36	103.9
5.0	5.044 ± 0.072	1.43	100.9
50.0	50.26 ± 0.286	2.57	100.5
100.0	99.91 ± 0.743	0.74	99.9
Peritoneal fluid: Inter-day assay			
0.05	0.054 ± 0.004	7.32	107.2
0.1	0.103 ± 0.007	6.48	103.0
0.5	0.505 ± 0.014	2.80	101.1
5.0	4.929 ± 0.082	1.67	98.6
50.0	50.12 ± 0.536	1.07	100.2
100.0	99.91 ± 0.313	0.31	99.9
Bile: Intra-day assay			
0.05	0.052 ± 0.003	5.93	103.8
0.1	0.104 ± 0.009	8.61	103.6
0.5	0.500 ± 0.030	6.06	99.9
5.0	4.999 ± 0.162	3.25	100.0
50.0	49.87 ± 0.554	1.11	99.7
100.0	100.06 ± 0.985	0.98	100.1
Bile: Inter-day assay			
0.05	0.062 ± 0.020	32.29	124.8
0.1	0.107 ± 0.012	10.77	104.8
0.5	0.514 ± 0.019	3.64	102.8
5.0	5.070 ± 0.143	3.00	101.4
50.0	50.14 ± 1.327	2.65	100.3
100.0	99.71 ± 0.683	0.69	99.7

Table III. Accuracy and Intra- and Inter-day Precision Data for the Measurement of Biapenem in Peritoneal Fluid and Bile

Biapenem conc. added (µg/mL)	Biapenem conc. found (µg/mL) (Mean ± SD, n = 6)	CV (%)	Accuracy (%)
Peritoneal fluid: Intra-day assay			
0.05	0.053 ± 0.007	13.21	105.3
0.1	0.103 ± 0.009	8.39	107.8
0.5	0.512 ± 0.014	2.36	103.9
5.0	5.100 ± 0.128	2.50	100.9
50.0	50.60 ± 1.372	2.71	101.2
100.0	99.62 ± 2.976	2.99	99.6
Peritoneal fluid: Inter-day assay			
0.05	0.047 ± 0.004	8.08	93.8
0.1	0.095 ± 0.006	6.02	95.2
0.5	0.501 ± 0.024	4.81	100.2
5.0	5.015 ± 0.085	1.70	100.3
50.0	49.90 ± 0.761	1.52	99.8
100.0	100.04 ± 0.470	0.47	100.0
Bile: Intra-day assay			
0.05	0.058 ± 0.009	15.96	116.6
0.1	0.109 ± 0.010	9.19	108.8
0.5	0.514 ± 0.013	2.44	102.8
5.0	5.078 ± 0.077	1.51	101.6
50.0	49.76 ± 0.772	1.55	99.5
100.0	100.08 ± 1.702	1.70	100.1
Bile: Inter-day assay			
0.05	0.074 ± 0.014	19.49	138.5
0.1	0.105 ± 0.008	7.74	104.8
0.5	0.516 ± 0.020	3.80	103.2
5.0	5.091 ± 0.139	2.72	101.8
50.0	50.34 ± 1.277	2.54	100.7
100.0	99.85 ± 2.637	2.64	99.8

centrations were determined within 1 h after the final sampling (i.e., 6.5 h) and were in the range of the calibration curves (0.05–100 µg/mL for peritoneal fluid and 0.1–100 µg/mL for bile). The pharmacokinetic analyses demonstrated that the mean C_{max} of meropenem was 30.8 µg/mL at 0.68 h in peritoneal fluid (Figure 3A) and 8.9 µg/mL at 1.01 h in bile (Figure 3B) after 500 mg dosing. For biapenem, the mean C_{max} was 16.9 µg/mL at 0.74 h in peritoneal fluid (Figure 3C) and 4.0 µg/mL at 1.13 h in bile (Figure 3D) after 300 mg dosing. As summarized in Table V, the C_{max} ratios to plasma were similar for meropenem and biapenem but different between peritoneal fluid and bile. The same tendency was shown for the $AUC_{0–8\ h}$ ratios to plasma.

Table IV. Recovery Experiments

Concentration added (µg/mL)	Recovery (%) (Mean ± SD, *n* = 6)
Meropenem: Peritoneal fluid	
0.5	101.1 ± 2.4
5.0	101.7 ± 1.5
50.0	96.6 ± 0.5
Meropenem: Bile	
0.5	99.2 ± 3.3
5.0	97.4 ± 3.4
50.0	96.2 ± 2.5
Biapenem: Peritoneal fluid	
0.5	99.8 ± 2.8
5.0	98.7 ± 2.5
50.0	98.3 ± 2.7
Biapenem: Bile	
0.5	100.6 ± 2.5
5.0	95.5 ± 1.4
50.0	95.2 ± 1.5

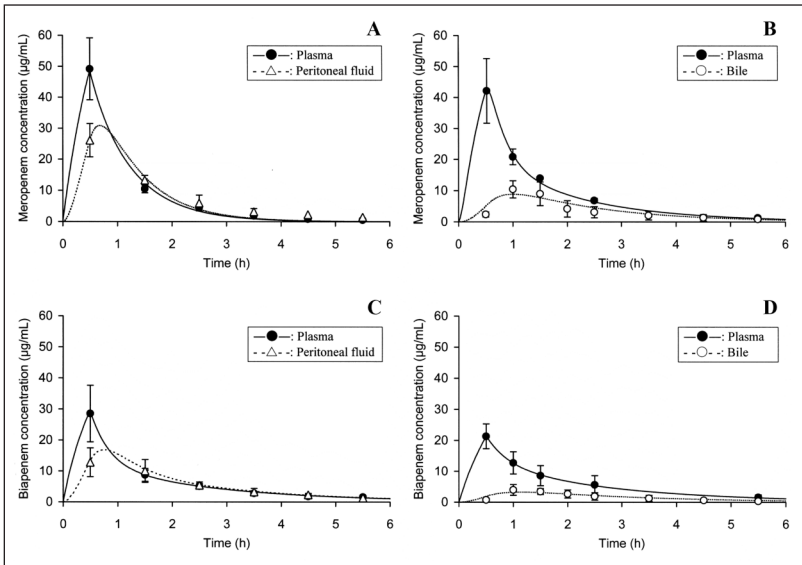

Figure 3. Observed concentrations of meropenem and biapenem in peritoneal fluid, bile, and plasma of four surgery patient groups (mean ± SD, *n* = 3 for each): (A) patient group A (0.5-h infusion of 500 mg meropenem); (B) patient group B (0.5-h infusion of 500 mg meropenem); (C) patient group C (0.5-h infusion of 300 mg biapenem); and (D) patient group D (0.5-h infusion of 300 mg biapenem).

Discussion

In the present study, a HPLC method using ultrafiltration for sample pretreatment was developed to monitor meropenem and biapenem in human peritoneal fluid and bile. The validated method was applied to comparative site-specific pharmacokinetic investigations in surgery patients.

Peritoneal fluid is a clear and colorless fluid from the peritoneal cavity. In contrast, bile is a yellow-green secretion fluid, which contains cholic acid, fats, bile salts, and bilirubin. These biliary components, more than the peritoneal fluid components, could affect the HPLC baseline and compromise the CV (> 20%) and accuracy (> 120%) for intra- and inter-assays at the lowest drug concentration tested (0.05 µg/mL) (Tables II–III). Consequently, both LOD and LLOQ were higher in bile than in peritoneal fluid (Table I).

For the measurement of meropenem in human peritoneal fluid, the LOD of our method (0.01 µg/mL) was better than that of a microbiological assay (0.1 µg/mL) (7), and the LLOQ of our method (0.05 µg/mL) was comparable to that of a LC–MS–MS method (0.03 µg/mL) (8). For the measurement of meropenem in human bile, the LOD of our method (0.02 µg/mL) was better than those of a microbiological assay (0.25 µg/mL) (9) and a HPLC method using solid-phase extraction for sample pretreatment (0.28 µg/mL) (10). For biapenem, no validated quantification method has been reported for human peritoneal fluid and bile.

The mean recoveries of meropenem and biapenem in peritoneal fluid and bile by ultrafiltration were 95.2–101.7% in the range 0.5–50.0 µg/mL (Table IV). The near 100% recovery could be due to low protein binding to meropenem (2% binding according to the MERREM package insert, AstraZeneca Pharmaceuticals, Wilmington, DE; 5% binding according to Moczygemba (18)] and biapenem [2.3% binding (19); 3.7% binding (20)]. Therefore, we concluded that the current method does not require an internal standard to correct for the influence of peritoneal fluid and bile proteins on meropenem and biapenem recovery as was also the case with our previous method for measuring meropenem (14) and biapenem (13) in plasma.

The validated method was successfully applied to surgery patients, indicating that it has clinically acceptable sensitivity for the determination of meropenem and biapenem concentrations in human peritoneal fluid and bile and that the methodology is applicable for pharmacokinetic investigations and real-time therapeutic drug monitoring in patients receiving these drugs. Moreover, comparison of the site-specific pharmacokinetics (Figure 3 and Table V) suggests that the rate and extent of tissue penetration may vary by the infection site but not by the carbapenem, although firm conclusions cannot be drawn as this was a preliminary study using a small number of patients.

Table V. Patient Information and Pharmacokinetic Data from the Four Patient Groups (*n* = 3 for each)

			Peritoneal fluid		Bile	
Patient group	Administered drug	Weight (kg) (Mean ± SD)	C_{max} ratio to plasma (Mean ± SD)	AUC_{0-8} h ratio to plasma (Mean ± SD)	C_{max} ratio to plasma (Mean ± SD)	AUC_{0-8} h ratio to plasma (Mean ± SD)
A	Meropenem	53.7 ± 11.2	0.56 ± 0.06	0.91 ± 0.01	—	—
B	Meropenem	45.6 ± 6.1	—	—	0.23 ± 0.12	0.42 ± 0.17
C	Biapenem	50.9 ± 8.0	0.54 ± 0.08	0.84 ± 0.05	—	—
D	Biapenem	52.8 ± 16.3	—	—	0.17 ± 0.07	0.30 ± 0.04

Conclusion

Peritoneal fluid and bile samples were pretreated using ultrafiltration. After 10 min centrifugation, the filtrate could be subjected to HPLC and the analyte of interest separated within a few minutes from the other components of the samples. Meropenem and biapenem concentrations in samples from patients were determined within 1 h after final sampling, providing LLOQ of 0.05 µg/mL for peritoneal fluid and 0.1 µg/mL for bile. This new method is useful especially for real-time therapeutic drug monitoring as well as for pharmacokinetic investigations.

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