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Short communication

High-performance liquid chromatography with ultraviolet detection for real-time therapeutic drug monitoring of meropenem in plasma

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Abstract

A simple, rapid, and precise high-performance liquid chromatography (HPLC) method using ultrafiltration to remove plasma protein was developed to determine meropenem concentrations in human plasma in a clinical setting. Plasma was separated by centrifugation at 4 °C from blood collected in heparinized vacuum tubes, and meropenem was stabilized by immediately mixing the plasma with 1 M 3-morpholinopropanesulfonic acid buffer (pH 7.0) (1:1). Ultrafiltration was used for plasma deproteinization. Meropenem was detected by ultraviolet absorbance at 300 nm with no interfering plasma peak. The calibration curve of meropenem in human plasma was linear from 0.05 to 100 μg/mL. Intraday and interday precision was less than 7.17% (CV), and accuracy was between 97.7% and 106.3% over 0.05 μg/mL. The limit of detection was 0.01 μg/mL. The assay has been clinically applied to a real-time therapeutic drug monitoring in pediatric patients and pharmacokinetic studies.
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1. Introduction

Meropenem (Fig. 1) is a widely used carbapenem that has antibacterial activity against a broad range of Gram-positive and -negative bacteria [1]. Pharmacokinetic studies have been done from various viewpoints [2–5]. For these studies, several high-performance liquid chromatography (HPLC) assays with ultraviolet (UV) detection have been done with solvent extraction [6], solid phase extraction [7,8], or column switching method [9,10] for sample preparation. Recently, HPLC assays using mass spectrometry have been reported for determination of plasma levels of meropenem [11–13]. The liquid chromatography (LC)–mass spectrometry methods are superior to LC–UV method but are expensive and not generally available.

In a clinical setting, therapeutic drug monitoring (TDM) of meropenem is necessary but has been hardly done. One of the reasons is the complex process involved in determining meropenem. Some of them require special mechanical devices, whereas others require time and/or highly skilled personnel. But other carbapenems such as biapenem and imipenem have been measured in plasma using HPLC with ultrafiltration [14–16]. Ultrafiltration is a simple and rapid method. Thus, for real-time TDM, we describe here a sensitive HPLC method of measuring meropenem that uses ultrafiltration for plasma deproteinization.

2. Experimental

2.1. Reagents and materials

Standard meropenem was provided by Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan) and 1 M 3-morpholinopropanesulfonic acid (MOPS) buffer (pH 7.0) was

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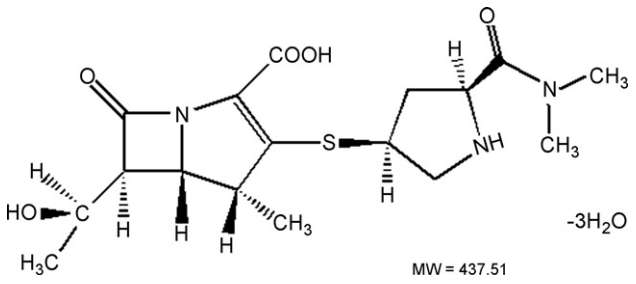

Fig. 1. Structure of meropenem.

purchased from Dojindo Laboratories (Kumamoto, Japan). $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}2H_2O$ were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and acetonitrile was purchased from Sigma–Aldrich, Japan (Tokyo, Japan). All chemicals were of analytical grade. The Nanosep 10 K centrifugal filter device was purchased from PALL Corporation (New York, USA).

2.2. Equipment and chromatographic conditions

The HPLC system comprised 600E system controller, 700 Satellite WISP auto-sampler (Waters Corporation, Milford, MA, USA), UV spectrophotometric detector SPD-6A (Shimadzu Corporation, Kyoto, Japan), Chromatocorder 21 (System Instruments Co., Ltd., Tokyo, Japan), and a column heater U-620 Type30 (Sugai Chemical Industry Co., Ltd., Wakayama, Japan).

The samples were separated on a μBondasphere C_{18} 5 μm (3.9 mm × 150 mm) column (Waters Corporation, Milford, MA, USA). The mobile phase was a mixture of 10 mM phosphate buffer (pH 7.4) and acetonitrile (90:10, v/v), and the pump flow rate was 1.0 mL/min. The auto sampler was set to 4 °C, and the injection volume was 20 μL. The column temperature was 40 °C. The meropenem peak was detected by UV absorbance at 300 nm.

2.3. Sample preparation

Blood samples were collected into heparinized vacuum tubes (Nipro Corporation, Osaka, Japan) and separated by centrifugation at 1000 × *g* for 10 min at 4 °C. Plasma samples were mixed with the same volume of 1 M MOPS buffer as a stabilizer and stored at −40 °C until analysis. Control human plasma was a mixture of equal volume of plasma from six healthy volunteers and stored at −40 °C.

A working stock solution of meropenem was prepared daily at a concentration of 1 mg/mL in 50 mM MOPS buffer. Control plasma was spiked with meropenem with the final concentrations corresponded to 0.05, 0.1, 0.5, 1.0, 5.0, 25.0, 50.0, and 100.0 μg/mL. Samples (200 μL) were then mixed with 200 μL of 1 M MOPS buffer (pH 7.0) and transferred to a Nanosep 10 K centrifugal filter device. Clinical plasma samples that were mixed with the same volume of 1 M MOPS buffer and stored at −40 °C were thawed to room temperature. A 400-μL aliquot was transferred to a Nanosep 10 K centrifugal filter device. The devices were centrifuged at 12,000 × *g* for 10 min at room temperature. Filtrate (20 μL) was injected into the HPLC system for analysis.

2.4. Method validation

This method was evaluated for linearity, specificity, recovery, stability, accuracy, and precision. Plasma standard samples (0.05, 0.1, 0.5, 1.0, 5.0, 25.0, 50.0, and 100.0 μg/mL) were prepared using control plasma, and intraday and interday assay precision and accuracy were determined. The limit of detection (LOD) of meropenem was defined as the sample concentration of meropenem that resulted in peak heights of threefold the standard deviation of the noise level, S_N. The limit of quantitation (LOQ) of meropenem was determined from validation data.

The recovery of meropenem by ultrafiltration was determined by comparing the peak heights from plasma standards with those from meropenem standards that were similarly prepared, except that meropenem aqueous solution replaced spiked control plasma and they were not filtered.

In addition, in order to examine the influence of different individual plasma on accuracy, precision, and recovery of meropenem, meropenem was spiked into each plasma from six different individuals at 1.0, 25.0, and 100.0 μg/mL.

2.5. Application to pharmacokinetic studies in patients

Bacteria constitute an important cause of infection in neutropenic patients with cancer and are also an important cause of morbidity [17,18]. Particularly in pediatric patients, real-time TDM is very important because the individual difference is great in body weight and kidney function, etc. Pediatric leukemia or other cancer patients aged 12 and 14 years were infused with 500 mg of meropenem over 1-h infusion every 8 h. Plasma concentrations of meropenem were measured at 0, 1, 2, 4, and 6 h after starting the infusion. Changes in the plasma meropenem concentration were fitted to a two-compartment model and analyzed with the nonlinear least-squares computer program (MULTI-Win) [19]. The duration of time that the drug concentration remains the minimum inhibitory concentration (MIC) for microorganisms ($T > MIC$) (% of 24 h) for these patients were determined according to the method for calculation of $T > MIC$ [20]. This study was approved by the Ethics Committee at Hiroshima University Hospital.

3. Results

3.1. Typical chromatograms

Fig. 2 illustrates the biological matrix with blank control plasma (Fig. 2a) and control plasma spiked with 0.5 μg/mL of meropenem (Fig. 2b), as well as a plasma sample from a patient with a concentration of 1.1 μg/mL (Fig. 2c). Interfering peaks were not evident and the retention time for meropenem was 3.8 min. At high sensitivity, most plasma has a peak of an endogenous component around 5.8 min (Fig. 2). This peak might come in succession in the following chromatogram at short analysis time. We adapted a 7-min analysis time to avoid this in Fig. 2.

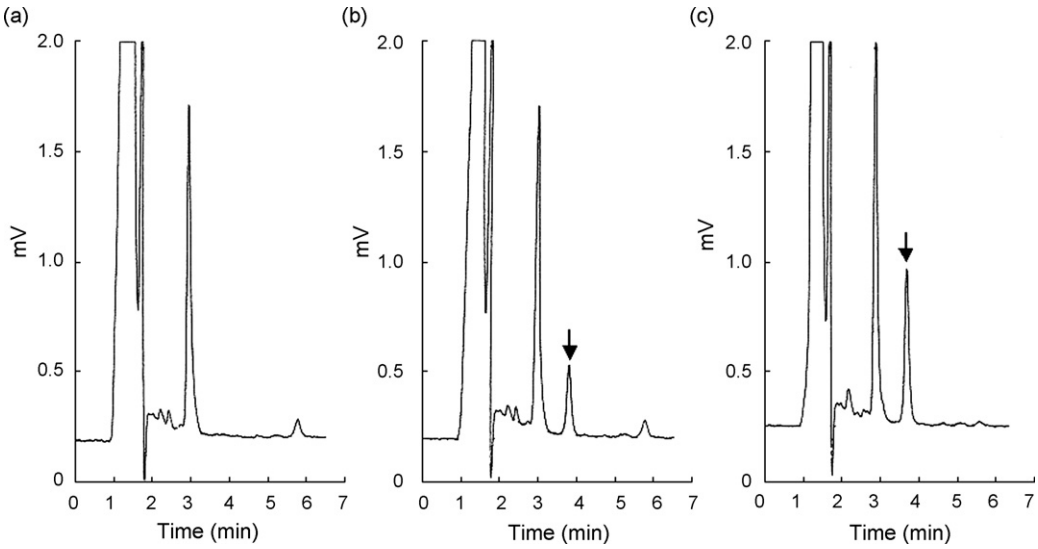

Fig. 2. Representative chromatograms of (a) blank control plasma, (b) control plasma spiked with 0.5 μg/mL meropenem, (c) patient plasma sample (1.1 μg/mL). Arrows indicate meropenem peaks.

But we can analyze around 4.5-min analysis time only to avoid overlapping this peak with the meropenem peak.

3.2. Method validation

The linearity of the ultrafiltration method was good between 0.05 and 100 μg/mL ($r^2 = 0.9999$ (mean) $\pm 1.0 \times 10^{-4}$ (S.D.) ($n = 5$)). Table 1 summarizes the reproducibility and accuracy at each calibration standard. The LOQ was determined as the lowest concentration of standard (0.05 μg/mL (C.V.: 6.65%, accuracy: 106.3%, intraday assay, $n = 6$)) and the LOD, defined in Section 2.4, was 0.01 μg/mL, using a 20-μL injection volume.

The recovery of meropenem was $96.1 \pm 2.9\%$ (S.D.) ($n = 6$), $93.9 \pm 1.0\%$ (S.D.) ($n = 6$), $94.7 \pm 0.7\%$ (S.D.) ($n = 6$), $95.1 \pm 0.5\%$ (S.D.) ($n = 6$), $93.7 \pm 0.8\%$ (S.D.) ($n = 6$), $93.4 \pm 0.4\%$ (S.D.) ($n = 6$), and $93.3 \pm 1.2\%$ (S.D.) ($n = 6$) at 0.1, 0.5, 1.0, 5.0, 25.0, 50.0, and 100.0 μg/mL, respectively.

When we calculated C.V. using the data from six individuals; it was 1.65%, 1.30%, and 0.52% at 1.0, 25.0, and 100.0 μg/mL of meropenem, respectively. And we calculated accuracy using the same data, it was $100.8 \pm 1.7\%$ (S.D.), $101.0 \pm 1.3\%$ (S.D.), and $99.9 \pm 0.5\%$ (S.D.) at 1.0, 25.0, and 100.0 μg/mL of meropenem, respectively. In spite of using each plasma from six different individuals, precision and accuracy were equivalent to that using control plasma. Absolute recovery using the data from six individuals was $94.9 \pm 1.6\%$ (S.D.), $93.6 \pm 1.2\%$ (S.D.), and $92.6 \pm 0.5\%$ (S.D.) at 1.0, 25.0, and 100.0 μg/mL of meropenem, respectively. These data were also equivalent to those using control plasma.

Table 1
Accuracy and intra- and interday precision data for measurement of meropenem in human plasma

Concentration added (μg/mL)	Concentration found (mean ± S.D.) (μg/mL)	C.V. (%)	Accuracy (%)
Intraday assay ($n = 6$)			
0.05	0.053 ± 0.004	6.65	106.3
0.1	0.105 ± 0.003	2.98	105.0
0.5	0.510 ± 0.005	1.06	102.1
1.0	1.017 ± 0.008	0.77	101.7
5.0	5.147 ± 0.029	0.57	102.9
25.0	25.61 ± 0.217	0.85	102.4
50.0	50.90 ± 0.219	0.43	101.8
100.0	99.39 ± 1.317	1.33	99.4
Interday assay ($n = 5$)			
0.05	0.049 ± 0.004	7.17	97.7
0.1	0.103 ± 0.005	4.40	102.9
0.5	0.513 ± 0.009	1.72	102.5
1.0	1.013 ± 0.020	2.02	101.3
5.0	5.085 ± 0.061	1.21	101.7
25.0	25.52 ± 0.443	1.74	102.1
50.0	50.18 ± 0.605	1.21	100.4
100.0	99.68 ± 0.494	0.50	99.7

S.D.: standard deviation; C.V. (%): coefficient of variation.

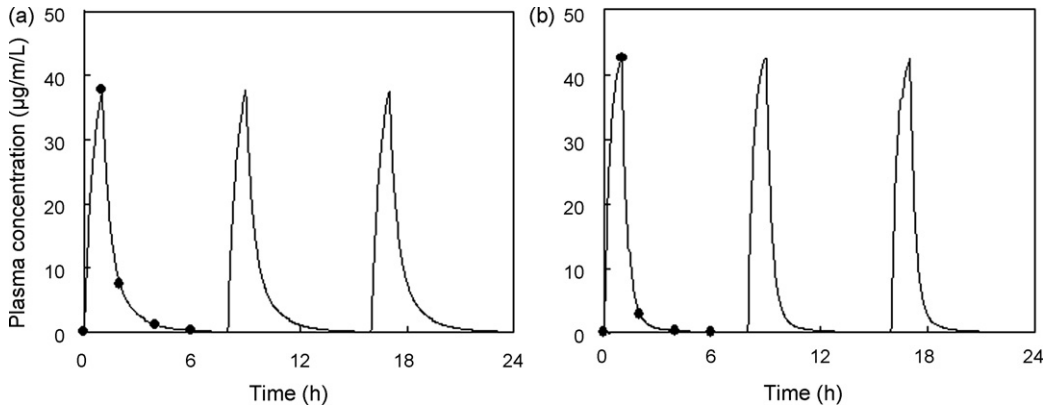

Fig. 3. Time course of plasma meropenem concentrations in two pediatric patients infused with 500 mg meropenem for 1 h every 8 h. (a) Patient A. (b) Patient B. Symbols, measured data; lines, change in plasma meropenem concentration fitted to two-compartment model.

To evaluate for specificity, six blank plasma samples from different individuals were investigated for interference of endogenous matrix components, and no interference peak was observed. Specificity was also assessed in the presence of other β-lactams: biapenem, imipenem, cefepime, cefozopran, or cefotiam at a concentration of 20 μg/mL. None of the chromatograms revealed any limitations for the assay.

The stability of the plasma samples at 1.0, 5.0, 50.0, and 100.0 μg/mL of meropenem in control plasma was examined using an equal volume of 1 M MOPS buffer (pH 7.0) as a stabilizer after storage at −40 °C. The concentrations at 60 d were 101.5 ± 1.2% (S.D.) (n = 4), 100.9 ± 0.3% (S.D.) (n = 4), 98.3 ± 1.1% (S.D.) (n = 4), and 104.5 ± 0.5% (S.D.) (n = 4) of the initial concentration at 1.0, 5.0, 50.0, and 100.0 μg/mL, respectively.

3.3. Real-time therapeutic drug monitoring

Fig. 3 shows the results of real-time TDM and MULTI-Win [19] analyses of two pediatric patients. Table 2 shows the pharmacokinetic parameters determined in this study. The efficiency of meropenem has been thought to closely correlate with T > MIC. The T > MIC targets required for bacteriostatic and bacteriocidal effects were considered to be 20% and 40%, respectively [21,22]. Table 2 also shows the T > MIC (% of 24 h) for these patients at the MIC of 4 μg/mL.

Peak plasma concentrations of meropenem were 37.6 and 42.7 μg/mL for pediatric patients A and B, respectively. Since patients A and B weighed 51.1 and 39.1 kg, respectively, the peak plasma meropenem concentration was higher in patient B than in A due to body weight. However, the T > MIC (% of 24 h) at the MIC of 4 μg/mL was somewhat larger in patient A than in B (Table 2). Kidney function appeared to be involved in this finding. Creatinine clearance (Clcr) was calculated from serum creatinine values [23], and Clcr of patient A was 6.1 L/h and Clcr of B was 10.9 L/h. Patient B had better kidney function than A, thus the elimination rate constant (k_{10}) of patient B was larger than that of A (Table 2).

4. Discussion

The LOD and LOQ values of this method were 0.01 and 0.05 μg/mL, respectively. LC–mass spectrometry showed that the LOQ for human plasma was 0.019 μg/mL [12], but this procedure is not practical for bedside TDM. On LC–UV method, the LOD was around 0.1 μg/mL [7,9] and the LOQ was 0.25 μg/mL [6] to 2.5 μg/mL [8]. The sensitivity of our method was better than that of these LC–UV methods. The comparatively improved sensitivity was apparently due to the high recovery and the absence of dilution, except for the equal volume of 1 M MOPS buffer (pH 7.0) included as a stabilizer.

In this method, meropenem-bound plasma protein cannot be separated by ultrafiltration and cannot be recovered. An internal standard might be added to correct this recovery. However, as shown in Section 3.2, accuracy and precision were not influenced by individual plasma. The recovery of meropenem in spiked plasma samples was around 95% and it was hardly influenced by the plasma protein content. This might be due to low protein binding of meropenem: only 2% (Merrem package insert; AstraZeneca Pharmaceuticals, Wilmington, DE, USA) to 5% [24]. Therefore, we consider that internal standard was not needed in this method. We think

Table 2
Pharmacokinetic parameters of meropenem (500 mg) over 1 h infusion

Patient	Vd (L)	k_{21} (1/h)	k_{12} (1/h)	k_{10} (1/h)	$AUC_{0-24\,h}$ (μg h/mL)	T > MIC (%)[a] (MIC = 4)
A	5.70	1.10	0.43	1.75	150.0	32
B	3.58	0.99	0.20	3.01	139.4	23

k_{21} and k_{12}: first-order transfer rate constants between central and peripheral compartments. k_{10}: first-order elimination rate constant from the central compartment. AUC: area under the concentration–time curve.

[a] Predicted T > MIC (% of 24 h) when meropenem (500 mg) was administrated over 1 h infusion every 8 h

that the advantage of this method is its simplicity. Moreover, to confirm that meropenem binding to plasma proteins might minimally influence the ultrafiltration method, we examined the influence of a variation in plasma protein content upon recovery using control plasma and control plasma diluted 1:2 at 25 μg/mL of meropenem. Under clinical conditions, the protein content of plasma might fall to about 50% at most. There was no significant difference in terms of the recovery in control plasma and in control plasma diluted 1:2 (not shown in detail).

A low percentage of organic solvent in the mobile phase was used for the separation of meropenem, a consequence of its hydrophilic property. We consider that plasma deproteinization by ultrafiltration without dilution with organic solvent is suitable to meropenem because of its high hydrophilic property and low protein-binding ratio. We consider that this deproteinization method will be suitable for many hydrophilic drugs with low protein-binding ratio. Especially for unstable drugs, it is likely to be able to process it without degradation because operation time is short.

We obtained these results within 1 h from two pediatric patients using real-time TDM after final blood sampling with time-management analysis and individualized real-time medical treatment. As described in the Section 1, meropenem is a popular carbapenem drug, the pharmacokinetics of which has been studied in detail. However, the TDM of meropenem has not been thoroughly investigated in the clinical setting. One reason is that meropenem is not easy to measure, although many investigators have described various methods of determination. Some of them require special mechanical devices, whereas others require time and/or highly skilled personnel. We developed a simple method of removing plasma protein using ultrafiltration so that meropenem levels can be determined in human plasma more easily and rapidly than by conventional means. After a 10-min centrifugation, filtrates can be subjected to HPLC, which requires only a few minutes and no special technique. We could predict $T > \mathrm{MIC}$ within 1 h after final blood sampling, so subsequent medication could be changed according to the TDM results. We believe that this method will be very useful in the clinical setting.

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