Determination of Imipenem and Cilastatin in Serum by High-Pressure Liquid Chromatography

CAROLYN M. MYERS AND JEFFREY L. BLUMER*
Division of Pediatric Pharmacology and Critical Care, Rainbow Babies and Childrens Hospital, and Departments of Pediatrics and Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106

Received 16 January 1984/Accepted 1 May 1984

Imipenem and cilastatin concentrations in serum were determined by using reverse-phase high-pressure liquid chromatography. Serum samples were stabilized with 0.5 M morpholineethanesulfonic acid buffer (pH 6.0) and subjected to ultrafiltration before chromatography. The elution solvent consisted of water or potassium phosphate buffered to pH 2.5 and methanol. The imipenem and cilastatin peaks were detected at 300 and 220 nm, respectively. Recovery from serum was 99% for both imipenem and cilastatin, and the limits of detectability for the two compounds were 0.3 and 0.5 μg/ml, respectively. The assay may be readily applied to pharmacokinetic analysis of imipenem and cilastatin biodisposition in patients.

Imipenem, a stable derivative of thienamycin, is a new carbapenem antibiotic derived from Streptomyces cattleya (3). It is an antibiotic with an unusually broad spectrum of antimicrobial activity that includes isolates resistant to most other agents (1, 7, 8). This breadth of antimicrobial activity is probably due to resistance to hydrolysis by bacterial β-lactamases (6).

The pharmacokinetics of imipenem in normal volunteers have been evaluated by using microbiological assay techniques (5). These studies have revealed a highly variable urinary recovery, despite rapid renal clearance of the drug. This probably results from the metabolism of imipenem by the dipeptidase dehydropeptidase I, located on the brush border of the proximal tubule (2). For clinical purposes, imipenem has been combined with an inhibitor of this enzyme to enhance urinary recovery of the drug (4).

We report here a rapid and sensitive high-pressure liquid chromatographic (HPLC) procedure for the determination of both imipenem and the dehydropeptidase inhibitor, cilastatin, in serum. The methodology may be easily applied to pharmacokinetic analyses of imipenem and cilastatin biodisposition in patients.

MATERIALS AND METHODS

Reagents. Imipenem and the dehydropeptidase inhibitor cilastatin (Fig. 1) were provided by Merck Sharp & Dohme, West Point, Pa. Sodium hydroxide, sodium carbonate, ethylene glycol, and HPLC-grade phosphoric acid (95%) were purchased from Fisher Scientific Co., Pittsburg, Pa.; glass-distilled methanol was from Burdick & Jackson Laboratories, Inc., Muskegon, Mich.; and 2-[N-morpholine]ethanesulfonic acid (MES) and morpholinepropanesulfonic acid were from Sigma Chemical Co., St. Louis, Mo.

HPLC equipment and conditions. Analyses were performed on a Varian Instruments model 5040 ternary liquid chromatograph (Varian Associates, Palo Alto, Calif.) equipped with an automated Rheodyne model 7030 column switching valve and a 50-μl injection loop. The conditions for chromatography are summarized in Table 1. A guard column (4 mm by 4 cm) filled with Vydec 40-μm pellicular reverse-phase packing was placed between the injector and the column. Chromatography was performed on a Micro Pak MCH 10 reverse-phase column (4 mm by 30 cm) which was electronically temperature controlled with a column heater.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Imipenem</th>
<th>Cilastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column type</td>
<td>MCH 10</td>
<td>MCH 10</td>
</tr>
<tr>
<td>Flow (ml/min)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mobile phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol (%)</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>0.10 M potassium phosphate, pH 2.5 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (%)</td>
<td>93</td>
<td>220</td>
</tr>
<tr>
<td>Detection (nm)</td>
<td>300</td>
<td>16</td>
</tr>
<tr>
<td>Attenuation</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Chart speed (cm/min)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Injection (μl)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Calculation</td>
<td>Peak area</td>
<td>Peak area</td>
</tr>
</tbody>
</table>

* Corresponding author.
an angle head rotor. The YMT membrane in the ultrafiltration system retains >99.9% of serum proteins but <5% of l-thyroxine. Since the protein binding of both imipenem and cilastatin is ≤25% (data on file at Merck Research Laboratories, West Point, Pa.) and recovery of both agents from spiked serum samples exceeds 90% (see below), the drug concentrations in the filtrate are considered to represent total drug. Centrifugation was performed for 30 min at 1,000 to 2,000 × g. If the sample obtained was plasma or contained ethylene glycol, a longer period of centrifugation was required and less filtrate was obtained. Filtrates were brought to room temperature and vortexed immediately before injection. Imipenem in the filtrate was stable on ice for at least 6 h. The filtrate could be stored at −20°C for later determination of cilastatin.

**Standards.** A 1-mg/ml stock solution of imipenem was prepared in 0.25 morpholinepropanesulfonic acid buffer (pH 6.8). The 1-mg/ml stock solution of cilastatin was prepared in 0.2% sodium carbonate. Standards for HPLC determination were prepared in 1:1 mixtures of serum and 0.5 M MES (pH 6.0) and were frozen at −70°C.

**Calculations.** No internal standard was needed because of the direct method of sample preparation. Good coefficients of variation supported the use of an external standard. Peak areas were measured in both methods. All concentrations are expressed per milliliter of original undiluted serum sample.

**RESULTS AND DISCUSSION**

Chromatography is shown in Fig. 3 and 4. The analysis for both assays was linear to 100 μg/ml. Duplicate stabilized serum samples at 0.5, 2.5, 20, 40, 75, and 100 μg/ml yielded correlation coefficients of 0.99992 and 0.99958 between peak area and serum concentration for imipenem and cilastatin, respectively. The mean absolute deviation from a straight line was 0.31 and 1.3 μg/ml, and the limit of sensitivity was 0.3 and 0.5 μg/ml for imipenem and cilastatin, respectively.

The recovery for both drugs from serum was 99%, and the recovery from plasma was 94%. It is recommended that serum be collected instead of plasma because the serum ultrafiltration time is shorter, more filtrate is obtained, and the recovery of both drugs is greater.

**FIG. 1.** Structures of (A) imipenem and (B) the dehydropeptidase inhibitor cilastatin.

**FIG. 2.** Absorption spectra: dashed lines indicate 20 μg of imipenem per ml in 7% methanol–93% water; solid line indicates 20 μg of cilastatin per ml in 24% methanol–76% potassium phosphate (pH 2.5).

**FIG. 3.** HPLC of imipenem in patient sera. (A) 0 μg/ml; (B) 30 μg/ml; (C) 0.6 μg/ml.

**FIG. 4.** Absorption spectra of imipenem standards prepared in 0.25 morpholinepropanesulfonic acid buffer (pH 6.8). The line indicates instrument noise.
The same-day coefficient of variation for imipenem in stabilized serum at 20 µg/ml (n = 16) was 1.8 and at 2 µg/ml (n = 18) was 6.2. The between-day coefficient of variation at 20 µg/ml (n = 15) was 3.2.

For cilastatin, the same-day coefficient of variation at 20 µg/ml (n = 20) was 3.4 and at 2 µg/ml (n = 15) was 5.4. The between-day coefficient of variation at 20 µg/ml (n = 10) was 3.9.

Imipenem in buffered serum was completely stable at −70°C over the 90-day study period. However, during the same period at −20 and 4°C, peak areas decreased more than 90% (Fig. 5). Imipenem in serum at a final concentration of 0.25 M MES and 25% ethylene glycol showed a 15% decrease in peak height over 90 days at −20°C. Cilastatin in buffered serum did not deteriorate when stored at 4, −20, or −70°C for 90 days. Stability of imipenem and cilastatin in buffered plasma was essentially identical to the stability in serum.

Studies were conducted to determine whether other compounds interfered with the methods described above. Neither the compounds tested (Table 2) nor unknown peaks in pooled sera eluted at the same time as either imipenem or cilastatin. In our experience with the chromatography of patient sera for imipenem, no peaks later than 24 min were large enough to interfere with later chromatograms. However, in the method for cilastatin, late-eluting peaks, including caffeine, occasionally appeared in subsequent chromatograms. Therefore, we included a wash at 50% methanol and 50% pH 2.5 potassium phosphate at the end of each sample injection.

The assays described above have been useful for the determination of pharmacokinetic parameters. A 20-year-old patient with cystic fibrosis received 7.5 mg of both compounds per kg intravenously, and blood samples were drawn at 0, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 h after the start of the 30-min infusion. The serum concentrations after the first dose of both drugs are shown in Fig. 6. Pharmacokinetic parameters calculated from these data showed an elimination rate constant of 0.80 h⁻¹, an apparent volume of
distribution of 0.36 liter/kg, and a plasma clearance of 216.4 ml/min per 1.73 m².

We have presented a rapid and sensitive HPLC assay for imipenem and the renal dehydropeptidase inhibitor cilastatin. It requires less than 0.5 ml of serum to perform both analyses. The pharmacokinetics of imipenem have been reported previously by Norrby et al. (5). Their calculated pharmacokinetic parameters, determined by a microbiological assay on samples from normal volunteers, were indistinguishable from those obtained by studying imipenem biodisposition in a group of patients receiving the drug for acute pulmonary exacerbations of cystic fibrosis (manuscript in preparation). Nevertheless, methodology for the determination of cilastatin in biological fluids has not been reported. Our procedure should permit the further pharmacokinetic evaluation of this fixed-combination agent.

### LITERATURE CITED