Degradation Kinetics of Imipenem in Normal Saline and in Human Serum

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Received 26 September 1985/Accepted 27 February 1986

Imipenem (N-formimidoyl thienamycin) is a carbapenem antibiotic with an unusually broad spectrum of antimicrobial activity (5, 10). In the kidney, imipenem is metabolized by breakage of the beta-lactam bond by dehydropeptidase I present in the brush border of the renal proximal tubule (4). To inhibit the renal metabolism of imipenem, it is combined in a 1:1 ratio with an inhibitor of the dipeptidase, cilastatin. Coadministration of imipenem and cilastatin increases the urinary recovery of imipenem to more than 70% of the dose, which is associated with a significant increase in the plasma area under the curve (2, 7).

The renal handling of imipenem is attributed to a combination of glomerular filtration and tubular secretion (2, 3). However, pharmacokinetic studies have shown that 20 to 25% of an imipenem dose is unaccounted for by renal elimination. These data suggest some degree of nonrenal systemic metabolism. Whether extrarenal biotransformation of imipenem occurs, not caused by the dehydropeptidase I enzyme, remains unanswered. Metabolites may be excreted renally, as greater than 99% of radioactivity is found in urine after a dose of radiolabeled imipenem (9). Another possible fate of the nonrenally excreted imipenem is spontaneous degradation in serum. As the compound is known to be stable for only short periods after reconstitution, the purpose of this study was to characterize the relative stability of unbuffered imipenem in 0.9% sodium chloride and in human serum over the range of temperatures likely to be encountered both in solution and in vivo.

The degradation kinetics of imipenem were determined at an initial concentration of 2.5 mg/ml in 0.9% sodium chloride at controlled temperatures of 2, 24, 37, and 50°C. Analyses were performed by using high-pressure liquid chromatography. A Waters 600A solvent delivery system, Waters Associates model 710B WISP, and a Schoeffel Spectroflow 770 UV detector were used for the assay. Chromatography was performed on a Waters µ-Bondapak C-18 column. The mobile phase was 0.1 M sodium phosphate buffer adjusted to pH 6.21 at a flow rate of 1.0 ml/min. The column effluent was monitored at 300 nm, and an attenuation of 0.02 absorbance units, full scale, was used in all procedures. Integration of peak areas and height was performed by a Hewlett-Packard 3388 integrator. Tyramine was used as the internal standard. All samples were assayed in duplicate. Saline media were injected onto the column directly, and an acetoniure-methylene chloride cleanup procedure was used to extract serum before chromatography.

**Stability in saline.** To determine the effect of temperature on the stability of imipenem, aliquots of spiked (2.5 mg/ml) saline were monitored at controlled temperatures. Samples were periodically assayed, and semilogarithmic plots of concentration versus time were constructed to determine the rate and order of imipenem degradation.

The degradation rate constant was temperature dependent in that the half-life was 2.0 h at 37°C. The kinetic analysis revealed an apparent first-order process, with the most rapid degradation observed at the elevated temperatures. These results are shown in Table 1.

**Stability in serum.** The degradation rate kinetics of imipenem were also determined in human serum (CDA) under identical assay conditions at controlled temperatures of 20 and 37°C. Serum was spiked with imipenem to an initial concentration of 100 µg/ml. The results are shown in Table 2. The half-life of imipenem in serum at 37°C was approximately 10 h.

An Arrhenius plot was constructed with the apparent first-order rate constant as a function of the reciprocal of temperature. The Arrhenius relationships were linear over the temperature range 2 to 50°C ($r^2$ in saline = 0.9985). Slopes yield activation energy of 17 kcal (ca. 71,000 J/mol) in normal saline and 18 kcal (ca. 75,000 J/mol) in serum. These values are consistent with hydrolysis reactions, and the loss of activation energy is an indication of the involvement of a single proton.

**TABLE 1. Degradation rate kinetics of imipenem (2.5 mg/ml) in 0.9% sodium chloride at controlled temperatures**

<table>
<thead>
<tr>
<th>Temp °C</th>
<th>Degradation rate constant (h⁻¹)</th>
<th>Half-life (h)</th>
<th>No. of assay determinations</th>
<th>Correlation coefficient ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>275 0.016</td>
<td>44.42</td>
<td>6</td>
<td>0.987</td>
</tr>
<tr>
<td>25</td>
<td>298 0.112</td>
<td>6.21</td>
<td>7</td>
<td>0.998</td>
</tr>
<tr>
<td>37</td>
<td>310 0.339</td>
<td>2.05</td>
<td>9</td>
<td>0.967</td>
</tr>
<tr>
<td>50</td>
<td>323 0.802</td>
<td>0.86</td>
<td>11</td>
<td>0.962</td>
</tr>
</tbody>
</table>

* Stability studies were conducted over 12 to 24 h. Activation energy: 17 kcal (ca. 71,000 J/mol).
**TABLE 2. Degradation rate kinetics of imipenem (100 µg/ml) in human serum at controlled temperatures**

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Degradation rate constant (h⁻¹)</th>
<th>Half-life (h)</th>
<th>No. of assay determinations</th>
<th>Correlation coefficient (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.012</td>
<td>58.72</td>
<td>6</td>
<td>0.969</td>
</tr>
<tr>
<td>37</td>
<td>0.065</td>
<td>10.73</td>
<td>7</td>
<td>0.943</td>
</tr>
</tbody>
</table>

* Stability studies were conducted over 12 to 24 h. Activation energy: 18 kcal (ca. 75,000 J/mol).

of microbiologic activity (1) suggests cleavage of the beta-lactam ring of imipenem.

The stability of deproteinated, extracted imipenem standards was determined in the presence of morpholinepropanesulfonic acid (MOPS; pH 6.8). This compound is added to imipenem as a stabilizing buffer (8). The results of the analysis indicated first-order degradation of the stabilized deproteinated extract at 24°C with a 17.6% loss over 4 h. For nonstabilized deproteinated extract, there was a 19.8% loss over 4 h. These results indicated that the addition of 0.22 M MOPS immediately before deproteinization and extraction of imipenem serum standards has no effect on the stability profile. In addition, the observed loss in nonstabilized extract had no significant effect on the degradation kinetics of imipenem determined in serum. The time lapse between extraction and injection of imipenem serum standards was held constant from 20 to 30 min, thus minimizing any potential effect.

These results indicated the degradation rate kinetics of imipenem in 0.9% sodium chloride and unbuffered human serum are first order and significant at higher temperatures. The pharmacokinetic data of imipenem degradation in the present study are consistent with the stability data of previous studies (1, 3, 6). The Arrhenius concept is valid over the temperature range 2 to 50°C with activation energy indicating hydrolysis, a likely mechanism of imipenem degradation.

Our finding of a 10.7-h half-life for imipenem in human serum at 37°C indicated biotransformation in serum not due to the renal dipeptidase-dehydropeptidase I. Previous pharmacokinetic data demonstrated imipenem renal clearance accounts for approximately 70% of total clearance, suggesting extrarenal biotransformation (2, 7). Our data suggest that a portion of this nonrenal clearance is due to in vivo degradation of imipenem in serum. The design of pharmacokinetic studies must consider imipenem degradation as an elimination process influencing the overall elimination rate constant.

**LITERATURE CITED**


