Microdialysis Study of Imipenem Distribution in the Peritoneal Fluid of Rats with Experimental Acute Pancreatitis

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Imipenem distribution was characterized by microdialysis in the peritoneal fluid of rats with experimental pancreatitis. The ratios of peritoneal fluid to blood area under unbound concentration-versus-time curves were close to unity, suggesting that imipenem was not degraded in peritoneal fluid.

Microdialysis has recently been increasingly used to determine the active unbound concentrations of antibiotics in tissue extracellular fluid (ECF), where most infections occur (1, 7). These concentrations determine treatment efficacy or failure, but if inappropriate, they also may accelerate the development of resistant mutants. Tissue ECF concentrations may be altered by impaired blood flow, changing fluid volumes, and other disease-related factors. However, in most tissues, unbound ECF concentrations of antibiotics should be equal to unbound plasma concentrations at steady state. Therefore, corresponding unbound-concentration-versus-time curves (areas under the curve [AUCs]) after single-dose administrations should also be equal. However, compared to plasma profiles, unbound ECF tissue concentration-versus-time curves should be shifted to the right and peak concentrations should be decreased. Therefore, unbound ECF tissue concentrations should be better predictors of antibiotic efficacy than unbound plasma levels. Furthermore, in several particular situations, tissue AUCs based on unbound concentrations may be lower than corresponding plasma AUCs. This would happen in the presence of active efflux transport systems, such as those encountered at the blood-brain barrier level or in cases of peripheral drug degradation (2). Accordingly, it has recently been shown, using intraperitoneal microdialysis, that the meropenem AUC in the peritoneal fluid (PF) of critical-care patients with peritonitis was lower than the corresponding total plasma AUC (5). Meropenem protein binding is negligible and therefore could not account for this observation. A pharmacokinetic model with peripheral elimination, consistent with the degradation of meropenem observed ex vivo in the PF of other patients, was developed to successfully fit the data. However, in a previous intraperitoneal-microdialysis study in rats, we observed that unbound imipenem concentration-versus-time profiles were almost always superimposed in blood and PF, both in control rats and in rats with an experimental model of peritonitis by cecal ligation and puncture (6). Although it should be confirmed experimentally, the same observation would most likely be made with the more stable meropenem (4). However, the enzymes released, as well as the resulting pH in the PF cavity, should vary with the experimental model of peritonitis, which in turn could have consequences for antibiotic degradation. Therefore, the model of peritonitis by cecal ligation and puncture may not be predictive of what happens in patients, in whom peritonitis is often associated with necrosis or perforation of the upper segments of the gastrointestinal tract. For that reason, it was decided to reproduce the initial study in rats, but using a different model of disease that could be more predictive of the human situation. The selected model was an experimental model of pancreatitis adapted from Xia et al. (10).

Six male Sprague Dawley rats from Janvier Laboratories (Le Genest-St-Isle, France) weighing between 300 and 350 g were used for the experiment, which was conducted in accordance with the NIH principles of laboratory animal care (9a). The day before the experiment, the rats were anesthetized by isoflurane inhalation (Forene; Abbot, Rungis, France) (8, 9), and pancreatitis was induced by an adaptation of a previously described method (10). After a laparotomy, pancreaticobiliary duct retrograde centesis was conducted through the duodenum seromuscular layer. A catheter was advanced into the pancreaticobiliary duct for 5 mm through the papilla of Vater. Special attention was paid to atraumatic surgical technique. Perforation of the duct during cannulation and intrapancreatic hemorrhage during surgical manipulations led to study termination. Five percent sodium taurocholate (0.1 ml · 100 g of body weight−1; Sigma, Saint Quentin Fallavier, France) was injected in the retrograde direction at a flow rate of 0.1 ml · min−1 (CMA 100 microdialysis pump; Phymep, Paris, France). Ten minutes after infusion, the clamp and catheter were removed, and the duodenal wall, with a 4-0 polyamid 6 suture, and the abdomen, with a 3-0 polyester suture, were closed. After surgery, the rats were allowed to recover to a conscious status and had free access to water. On the day of the experiment, the rats were reanesthetized by isoflurane inhalation (8, 9), and polyethylene cannulas were inserted into the left femoral vein for drug administration and into the artery for biochemical-marker determinations. Two CMA/20 probes (polycarbonate; membrane length, 10 mm; 20,000-Da cutoff; CMA microdialysis; Phymep, Paris, France) were inserted into...
the right jugular vein and into the central part of the pancreatic gland within the peritoneal cavity, respectively. The microdialysis study was conducted as previously described (6). After retrodialysis by drug period, in which probes were perfused with Ringer solution (Phymep, Paris, France) containing imipenem at 10 μg · ml⁻¹ (Tienam; Merck Sharp & Dohme-Chibret Laboratories), and a washout period, the rats received an intravenous infusion of imipenem at a dose of 30 mg · kg⁻¹ over a 30-min period. Blood and PF microdialysate samples were collected over 165 min at 10-min intervals during the first 60 min and 15-min intervals during the last 105 min. After collection, the dialysates were diluted to the appropriate volume with a stabilizer (0.5 M HEPES buffer [Sigma Aldrich, Saint Quentin Fallavier, France], pH 6.8, ethylene glycol [Sigma Aldrich, Saint Quentin Fallavier, France], high-performance liquid chromatography grade water [1:0.5:0.5 {vol/vol/vol}]) and directly analyzed as previously described (6). At the end of the microdialysis experimentation, blood samples were also collected in appropriate tubes to determine lipase and amylase concentrations with an automatic analyzer (modular automatic analyzer; Roche, France). Pharmacokinetic parameters were determined in each individual rat by a noncompartmental approach according to standard procedures and with the software WinNonLin 4.0.1 (Pharsight Corporation, Mountain View, CA). Statistical analyses were performed by paired t tests, and significance was set at a P level of <0.05.

In vivo imipenem recovery by loss varied between 21.1% ± 4.7% and 65.4% ± 3.4% in blood and between 21.4% ± 2.3% and 53.7% ± 3.3% in peritoneal fluid but was stable throughout the experiment (165 min) (data not shown). Amylase and lipase concentrations were elevated in rats with pancreatitis (n = 6) (9,777 ± 930 U · liter⁻¹ and 208 ± 75 U · liter⁻¹, respectively) and were significantly higher (P < 0.05; unpaired t test) than corresponding values (2,542 ± 125 U · liter⁻¹ and 7 ± 3 U · liter⁻¹) determined in rats used as controls (n = 7), attesting to pancreatitis development. Unbound imipenem concentration-versus-time profiles in blood and PF were most often almost superimposed (Fig. 1). Pharmacokinetic parameters characteristic of imipenem distribution (volume of distribution) and elimination (clearance) were consistent with previously reported values in rats (Table 1) (3, 7, 9). Peak concentrations (maximum concentration of drug in serum and time to maximum concentration of drug in serum) were not statistically different between the media, attesting to rapid drug distribution. Mean AUCs were not statistically different between media, and the mean blood-to-PF AUC ratio was close to unity (1.08 ± 0.16). These results suggest that no imipenem degradation occurred, at least to a significant extent, in the PF of rats with pancreatitis, consistent with what was previously observed in rats with an experimental model of peritonitis by cecal puncture and ligation (6). Although complementary experiments will be necessary to further investigate this issue, including a study with imipenem in humans, it can be concluded that rats may not be a good predictive animal model for the investigation of antibiotic distribution within infected PF.

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### TABLE 1. Estimated pharmacokinetic parametersa

<table>
<thead>
<tr>
<th>Parameter b</th>
<th>Blood (mean ± SD)</th>
<th>Peritoneal fluid (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg · ml⁻¹)</td>
<td>53.9 ± 25.3</td>
<td>51.8 ± 21.5</td>
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<tr>
<td>Tmax (min)</td>
<td>35.0 ± 0.0</td>
<td>36.7 ± 4.1</td>
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<tr>
<td>V (ml · kg⁻¹)</td>
<td>407 ± 266</td>
<td>307 ± 266</td>
</tr>
<tr>
<td>CL (ml · min⁻¹ · kg⁻¹)</td>
<td>17.0 ± 10.6</td>
<td>17.0 ± 10.6</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>17.3 ± 8.0</td>
<td>17.6 ± 4.4</td>
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a Estimated in blood and PF of rats with pancreatitis (n = 6) after a 30-min intravenous infusion of imipenem at a dose of 30 mg · kg⁻¹. The AUCblood/PF ratio was 1.08 ± 0.16.

b Cmax, maximum concentration of drug in serum; Tmax, time to maximum concentration of blood in serum; V, volume of distribution; CL, clearance; t½, half-life.
REFERENCES


