Microdialysis Study of Imipenem Distribution in the Intraperitoneal Fluid of Rats with or without Experimental Peritonitis

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Received 23 August 2005/Returned for modification 27 September 2005/Accepted 11 October 2005

The purpose of this study was to extend the use of microdialysis to the investigation of antibiotic distribution into the intraperitoneal fluid of rats with or without peritonitis. Microdialysis probes were inserted into the jugular vein and peritoneal cavity of control rats or rats with intra-abdominal sepsis (n = 8 in each group) induced by cecal ligation and punctures. Imipenem (IPM) probe recoveries were determined in each rat by retrodialysis by drug. IPM was infused intravenously at a dose of 30 mg · kg⁻¹ over 30 min, microdialysis samples were collected for 120 min, and IPM concentrations were determined by high-performance liquid chromatography. Intra-peritoneal infection had no statistically significant effect on IPM clearance (11.9 ± 2.3 ml · min⁻¹ · kg⁻¹ in control rats versus 10.9 ± 2.1 ml · min⁻¹ · kg⁻¹ in rats with peritonitis) or the volume of distribution (296 ± 47 ml · kg⁻¹ in control rats versus 310 ± 49 ml · kg⁻¹ in rats with peritonitis). IPM concentration profiles in intraperitoneal fluid and blood were virtually superimposed in control rats, whereas in infected animals, the mean intraperitoneal IPM concentrations were apparently slightly lower than corresponding blood levels. However, the areas under the concentration-versus-time curve estimated in intraperitoneal fluid and blood were not significantly different in both groups, with the corresponding ratios close to unity (1.01 ± 0.19 and 0.89 ± 0.28 in control rats and rats with peritonitis, respectively). In conclusion, IPM distribution in intraperitoneal fluid is rapid and complete both in control rats and in rats with peritonitis.

Pharmacokinetic studies of antibiotics most often rely on the determination of total concentrations in plasma. However, since most infections occur in tissue extracellular fluid, free antibiotic concentrations in the interstitial fluid at the target site are more useful for predicting the time course of antimicrobial activity and optimal drug dosing regimens (14). Microdialysis is a reliable technique for investigating the kinetics of antibiotics in different tissues, and it has been used on many occasions in various tissues, such muscle, lung, brain, and skin, in both animals and humans (18). However, no microdialysis antibiotic-distribution study has ever been conducted into the intraperitoneal fluid of rats with or without peritonitis, to our knowledge, although the technique has been used to monitor intraperitoneal concentrations of markers of ischemia, such as lactate, mainly in pigs (13, 22, 24), but also in humans (8, 19) and rats (2, 10, 11, 12).

Intra-abdominal infections represent a common clinical problem, characterized by the presence of purulent secretions and tissue inflammation in the intra-abdominal cavity. According to The Surgical Infection Society guidelines, imipenem (IPM)/cilastatin is indicated in the presence of high-severity intra-abdominal infections (3, 17, 21). Imipenem is the leading compound of the carbapenem antibiotic family. It possesses a broad spectrum of antibacterial activity against most gram-positive and gram-negative aerobic and anaerobic bacteria, including Escherichia coli, Pseudomonas aeruginosa, and Bacteroides fragilis (6), which have been commonly reported to cause peritonitis (4, 7, 9). It has demonstrated its effectiveness in the treatment of nosocomial and community-acquired bacterial infections and has a favorable cost-effectiveness relationship compared to other antibiotic therapies for the treatment of intra-abdominal infections, in particular, nosocomial peritonitis (1). Interestingly, IPM is also a good drug candidate for microdialysis studies (16).

The objective of the present study was therefore to investigate the intraperitoneal-fluid distribution of IPM by microdialysis in healthy rats and in rats with experimental peritonitis caused by cecal ligation and puncture.

**MATERIALS AND METHODS**

**Chemicals.** IPM monohydrate-sodium cilastatin salt (Tienam; Merck Sharp & Dohme-Chibret Laboratories, Paris, France) was used to prepare IPM solutions in 0.9% NaCl or Ringer’s solution for intravenous administration or probe perfusion, respectively. All chemicals used were of analytical grade, and solvents were high-performance liquid chromatography (HPLC) grade.

**Animals.** Sixteen male Sprague-Dawley rats from Janvier Laboratories (Le Genest-St-Isle, France), weighing between 300 and 350 g, were used for pharmacokinetic experiments and were divided into two groups, a peritonitis group (n = 8) and a control group (n = 8). Six extra rats, three infected and three uninfected, of the same weight were used to evaluate the stability of IPM recovery in the peritoneum as a function of time. All animals were acclimatized in wire cages in a 12-h light-dark cycle for a minimum of 5 days before the beginning of the experiment to allow them to adjust to the new environment. During this period, they had free access to food (A03; Safe, Villemonais-sur-Orge, France) and water. This work was done in accordance with the Principles of Laboratory Animal Care (18a).

**Induction of peritonitis.** The day before the experiment, rats from the infected group were anesthetized by isoflurane (Forene; Abbot, Rungis, France) inhalation (16), and peritonitis was induced as previously described (5, 25) with a 100% success rate. Through a midline laparotomy, the cecum was ligated just below the ileocecal valve, and the surface was punctured twice with a 25-gauge needle...
below the ligature; the bowel was then placed back into the peritoneal cavity, and the abdomen was closed. If the cecum was empty, it was filled with feces before the ligature by milking the stools back from the colon. At the end of surgery, the rats were allowed to recover consciousness. Food was withdrawn approximately 24 h before the experiment, but the animals had free access to water until the beginning of the second part of the surgery.

Catheter and probe insertion. On the day of the experiment, the rats were anesthetized by isoflurane (Forene; Abbott, Rungis, France) inhalation. Polyethylenyl cannulas were inserted into the left femoral vein and artery for drug administration and lactate determinations. At the end of the catheter insertion, a blood sample was collected in a tube containing fluoride and then centrifuged at 1,000 × g for 10 min at 4°C. The supernatants were used for lactate determinations (Lactate PAP; bioMérieux, Lyon, France). A CMA/20 probe (polycarbonate; membrane length, 10 mm; 20,000-Da cutoff; CMA microdialysis; Phymep, Paris, France) was inserted into the right jugular vein as previously described (15, 16). Another, similar CMA/20 probe was perfused with Ringer’s solution (perfusion fluid T1; CMA microdialysis; Phymep, Paris, France) at a flow rate of 3.5 µl·min⁻¹ (CMA 100 microdialysis pump; Phymep, Paris, France) and inserted into the rat peritoneal cavity between intestinal loops through laparotomy (11). After insertion, the intraperitoneal microdialysis probe was flushed at 10 µl·min⁻¹ for approximately 15 min to remove bubbles. The flow rate was then decreased to 3.5 µl·min⁻¹ until the end of surgery. The probe was then sutured to the abdominal muscle, and the abdominal cavity was closed.

Stability of IPM probe recoveries with time. The experiment started with a retrodialysis by drug period, during which the probes were perfused (CMA 100 microdialysis pump; Phymep, Paris, France) for 45 min at 2 µl·min⁻¹ and for 15 min at 1 µl·min⁻¹ with Ringer’s solution containing 10 µg·ml⁻¹ IPM (10 µg·ml⁻¹) to equilibrate the system. The flow rate was maintained at 1 µl·min⁻¹ for the duration of the experiment. After this equilibration period, microdialysate samples were collected automatically by a CMA/140 microfraction collector (CMA microdialysis; Phymep) for 150 min by fractions corresponding to 10-min intervals. Directly after collection, the microdialysates in the peritoneum and blood were collected over 120 min (i.v. injections of 20% ethylcarbamate). Directly after collection, the microdialysates (Lactate PAP; bioMérieux, Lyon, France) were diluted (1:3 [vol/vol] for dialysates collected at 10-min intervals and 1:2 [vol/vol]) for the rest of the intervals) with a stabilizer (0.5 M HEPES buffer, pH 6.8, ethylene glycol, HPLC grade water [1:0.5:0.5 {vol/vol/vol}]). Standard curves were prepared with IPM in a previous HPLC assay (16). The mean recovery in each rat was then obtained from these 15 consecutive determinations (Lactate PAP; bioMérieux, Lyon, France). A CMA/20 probe (polycarbonate; membrane length, 10 mm; 20,000-Da cutoff; CMA microdialysis; Phymep, Paris, France) was inserted into the right jugular vein as previously described (15, 16). Another, similar CMA/20 probe was perfused with Ringer’s solution (perfusion fluid T1; CMA microdialysis; Phymep, Paris, France) at a flow rate of 3.5 µl·min⁻¹ (CMA 100 microdialysis pump; Phymep, Paris, France) and inserted into the rat peritoneal cavity between intestinal loops through laparotomy (11). After insertion, the intraperitoneal microdialysis probe was flushed at 10 µl·min⁻¹ for approximately 15 min to remove bubbles. The flow rate was then decreased to 3.5 µl·min⁻¹ until the end of surgery. The probe was then sutured to the abdominal muscle, and the abdominal cavity was closed.

Noncompartmental pharmacokinetic analysis. Pharmacokinetic parameters were determined for each individual rat by a noncompartmental approach according to standard procedures and with WinNonLin 4.0.1 software (Pharsight Corporation, Mountain View, California). Total unbound body clearance (CL_u) was calculated as follows: CL_u = dose/AUC_free, blood. Where AUC_free, blood is the total area under the free-blood concentration-versus-time curve calculated using the linear trapezoidal rule. The area remaining under the curve after the last measured concentration, C(last), was determined from C(last) = Clood, blood. The elimination rate constant, k1,2, blood, and its corresponding half-life (1/2, blood) were estimated by least-squares fit of datum points (log concentration time) in the terminal phase of the decline. The volume of distribution (V_c) was also estimated from Clooood, blood. The AUC and CL_U2 were determined for the same time period and were determined by the same procedure and are referred as AUC_free, blood and CL_u, respectively.

RESULTS

Stability of imipenem probe recoveries with time. The average relative RL of IPM over a period of 150 min determined for

![](https://example.com/image.png)
control rats (P mated for rats with peritonitis were significantly higher than for recovery was stable for the duration of any particular experi-
trend between control rats and rats with peritonitis, and this of IPM differed between rats for the same medium and be-
64.6% respectively, 56.0
posed (Fig. 2a). Concentrations at the end of infusion were,
exponential, and the concentration profiles were almost superim-
three dedicated control rats varied between 28.3% ± 2.1% and
66.4% ± 3.0% in blood and between 17.1% ± 3.0% and
31.2% ± 3.0% in the peritoneum. Corresponding values for three dedicated rats with peritonitis were, respectively, 39.7% ±
3.2% and 68.7% ± 2.4% in blood and 21.9% ± 2.5% and
64.6% ± 2.1% in the peritoneum. Therefore, recovery by loss of IPM differed between rats for the same medium and be-
tween blood and peritoneal fluid for the same rat, but there was no trend between control rats and rats with peritonitis, and this recovery was stable for the duration of any particular experiment (150 min) (Fig. 1a and b).

Lactate concentrations in plasma. Lactate concentrations estimated for rats with peritonitis were significantly higher than for control rats (P < 0.05), equaling 3.85 ± 0.61 mmol · liter⁻¹ and 2.22 ± 0.32 mmol · liter⁻¹, respectively.

Pharmacokinetic study. Whatever the group, the IPM RL varied between 20.5% ± 1.6% and 71.7% ± 6.4% in blood and between 13.5% ± 6.9% and 41.7% ± 2.1% in the peritoneum.

(i) Control group. The decay of free-IPM concentrations in blood and in intraperitoneal fluid with time were monoe-
xponential, and the concentration profiles were almost superimposed (Fig. 2a). Concentrations at the end of infusion were, respectively, 56.0 ± 9.4 and 52.4 ± 20.8 μg · ml⁻¹ in blood and intraperitoneal fluid (not significant [NS]). Pharmacokinetic parameter values obtained for the control group are presented in Table 1. Half-lives and AUCs were not statistically different between blood and intraperitoneal fluid, and AUC ratios were close to 1 (1.01 ± 0.19) (Table 1).

(ii) Peritonitis group. The decay of free IPM concentrations in blood and intraperitoneal fluid with time were still mono-
exponential, but mean IPM concentrations in intraperitoneal fluid were apparently slightly lower than the corresponding blood levels (Fig. 2b). However, AUCs estimated in intraperitoneal fluid and blood were NS, as were maximum concentrations (54.0 ± 8.3 and 44.6 ± 1.79 μg · ml⁻¹ in blood and intraperitoneal fluid, respectively). Furthermore, intraperitoneal fluid-to-blood AUC ratios were still close to unity (0.89 ± 0.28) and were NS compared to the corresponding value ob-
tained in the control group (1.01 ± 0.19). IPM clearance and volume of distribution were also not significantly different be-
tween groups.

**DISCUSSION**

The well-known experimental model of peritonitis by cecal ligation and puncture (5, 25) used in the present study was easily reproduced, with increased lactate concentrations, as well as characteristic clinical symptoms (tissue necrosis and a nauseating smell), attesting to peritonitis. Several intraperi-
neal microdialysis studies of rats have been reported, but all were conducted by the same group (2, 10, 11, 12). However, the technique, and in particular the introduction and position-
ing of microdialysis probes in the intraperitoneal fluid of anes-
thetized rats, could also be reproduced without any particular difficulty. Under these conditions, probe recoveries varied widely between rats and between media (blood and intraperi-
toneal fluid), but this variability was comparable to that previ-
ously observed in muscle using similar CMA/20 probes (16) and was adequately corrected by preliminary determination of the recovery by loss in each individual rat and medium. This procedure is time-consuming and may be difficult to handle with infected hospitalized patients. Therefore, average recovery values have sometimes been used to correct for individual probe recoveries, as in the case of a study with imipenem (23). Our present and past experiences (16) with this compound clearly indicate that this procedure is not appropriate.

Pharmacokinetic parameters derived from blood during this study also compare favorably with values previously obtained by our group in comparable situations, in particular, clearance and volume of distribution, which was as usual close to total extracellular body water (16). Both were not affected by infec-
tion (Table 1).

However, the major finding of the present study was that unbound IPM AUCs estimated in intraperitoneal fluid and blood were not statistically different for both control and in-

- **Table 1.** Values of pharmacokinetic parameters obtained in blood and intraperitoneal fluid of control rats and rats with peritonitis after a 30-min intravenous infusion of imipenem at a dose of 30 mg · kg⁻¹

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control rats (n = 8)</th>
<th>Rats with peritonitis (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, free (μg · ml⁻¹)</td>
<td>56.0 ± 9.4</td>
<td>52.4 ± 20.8</td>
</tr>
<tr>
<td>CLr (ml · min⁻¹ · kg⁻¹)</td>
<td>11.9 ± 2.3</td>
<td>10.9 ± 2.1</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>17.3 ± 2.5</td>
<td>18.3 ± 3.1</td>
</tr>
<tr>
<td>AUC0–t (ml · mg⁻¹ · min⁻¹)</td>
<td>20.7 ± 3.8</td>
<td>21.9 ± 4.6</td>
</tr>
</tbody>
</table>

*Values for AUCblood/AUCfree, blood were 1.01 ± 0.19 for control rats and 0.89 ± 0.28 for rats with peritonitis.*
ected rats, demonstrating that infection has no effect on the distribution of IPM in this physiological liquid. This observation was favored by the use of blood microdialysis, which allows direct comparisons between multiple unbound blood and tissue concentrations without blood sampling (16). It corroborates previous results observed in other tissues without physiological barriers, such as muscle or lung tissue in healthy rats (16), and is in agreement with basic pharmacokinetic concepts, considering that at equilibrium unbound drug concentrations should be identical on both sides of these semipermeable membranes (20). Although these observations should be confirmed in patients, taken together, they suggest that unbound-IPM concentrations in blood—and even total concentrations, since protein binding is limited for this compound—should reflect the time course of the drug at the infection site and should therefore be appropriate to predict the time course of effect.

In conclusion, this study has first confirmed the feasibility of microdialysis for investigating intraperitoneal-fluid distribution over time course of effect. Intra-abdominal isolates at operation: a predictor of postoperative infection. Antimicrob. Agents Chemother. 46:2132–2136.
