Aerosolization of imipenem/cilastatin prevents pseudomonas-induced acute lung injury


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Aerosolization of imipenem/cilastatin was compared with continuous intravenous infusions of the antibiotic for pharmacokinetic/pharmacodynamic analysis. The concentrations of imipenem/cilastatin in bronchoalveolar lavage fluids (BAL) obtained from rats exposed to the aerosolized antibiotic were significantly greater than the concentrations in BAL in the rats that had received intravenous infusions of imipenem/cilastatin. The two methods of antibiotic delivery were compared for their effects on bacterial-induced lung injury in rats that had Pseudomonas aeruginosa instilled into their airspaces. The aerosolization of antibiotic was associated with significantly decreased bacterial-induced lung injury. The high concentrations of antibiotic in the airspaces secondary to aerosolization appears to kill bacteria more quickly and preserve lung epithelial and endothelial integrity better than systemic delivery of the same antibiotic.

Introduction

Colonisation occurs in nearly all patients who are critically ill; a percentage of these colonised patients then develop nosocomial pneumonia (de Latorre, et al., 1995). A pervasive hypothesis is that if colonisation could be prevented, then nosocomial pneumonia would be prevented. Strategies that have been proposed to decrease colonisation include selective decontamination of the gut and oropharynx. In one prospective, controlled study, decontamination therapy significantly decreased the incidence of Gram-negative pneumonia in patients who were mechanically ventilated. (Gastinne, et al. 1992).

Pseudomonas aeruginosa lung infections are a major cause of nosocomial infection, and associated with the highest mortality in nosocomial pneumonia (Kollef et al., 1995). A technique that might decrease P. aeruginosa colonisation of the trachea and lung is the aerosolization of antibiotics to those patients at risk for nosocomial pneumonia. The increased concentrations of antibiotic achieved via aerosolization might lead to...
increased bacterial killing in the airspaces of the lung and prevention of bacterial-induced lung injury.

This study compared antibiotic levels achieved in lung airspaces after aerosolization of impenem/cilastatin versus continuous intravenous administration of the drug. In addition, the correlation between the mode of administration and antibiotic levels with subsequent bacterial-induced lung injury was investigated.

Methods

Rats

Specific pathogen free Sprague Dawley male rats (300–375 g, Simonsen, Gilroy, CA, USA) were used for the entire study. All animal experiments were done in compliance with the Committee on Animal Research at the University of California at San Francisco.

Bacteria

*P. aeruginosa*, PAK (generously provided by D. Frank), was used (Kudoh et al., 1994). The strain was stored as a bacterial stock at −70°C in a 10% sterile skimmed milk solution. After the inoculation of the bacteria on VBMM (Vogel Bonner minimal medium) plate for 36 h at 37°C, the bacteria were cultured in tryptic soy broth for 13 h at 32°C in a shaking incubator (Nicas & Iglewski, 1984). We have found that bacteria in early stationary phase are more virulent (Kudoh et al., 1994). The concentration of bacteria was determined spectrophotometrically and cultures were diluted with Ringer’s lactate solution to make a 1 × 10^6 cfu/mL solution. Quantitative cultures were performed on all instillates to assure accurate inoculations. The MIC (mg/L) of this *Pseudomonas* to imipenem/cilastatin was <0.0005 mg/mL.

Administration of imipenem/cilastatin and determination of imipenem concentration

Imipenem/cilastatin (Primaxin; Merck & Co., Inc., NJ, USA) was administered to rats either via an aerosol (200 mg) for 1 h or via a continuous intravenous infusion (25 mg/kg/h throughout the study over 5 h). The intravenous dose was chosen to achieve similar plasma and tissue levels as found in humans; this was done by increasing the human dose approximately two-fold, as the plasma half-life of β-lactams in rodents is approximately 15 min compared with 1 h in humans (Hajdu, et al., 1985). The aerosol dose was chosen based on studies utilizing aerosolized tobramycin (Cooney et al., 1994). As rodent species have dehydropeptidase activity in their lungs and metabolize imipenem/cilastatin faster than primate species (whose dehydropeptidase activity is mainly in the kidney) (Hajdu et al., 1985), intravenous imipenem/cilastatin was administered continuously throughout the study. For aerosolization, rats were placed in a nose-only aerosol chamber (Intox Products, Albuquerque, NM, USA) and remained in the chamber for 60 min exposed to imipenem/cilastatin solution in buffered saline (20 mg/mL, pH 7.0). Aerosols were generated by an Aerotech II nebulizer (CIS-US, Bedford, MA, USA), driven by compressed air at a flow rate of 10 L/min. The particle size of the aerosol was measured by a cascade impactor (Intox Products). Under these conditions, the mass median aerodynamic diameter was 1.5 μm.
Approximately 0.1% of what is aerosolized reaches the airspaces of the lungs of the rodents (Hashimoto et al., 1996).

To determine the plasma and bronchoalveolar fluid concentrations of imipenem/cilastatin after the 1 h aerosolization or after the intravenous administration of the antibiotic, rats were exsanguinated immediately after the delivery of the antibiotic; bronchoalveolar lavage was then performed using a total of 30 mL of buffered saline. The volume of BAL obtained was similar in all animals, approximately 28 mL, and all animals were exsanguinated prior to lavage being performed. The BAL fluid was measured (mL) and frozen immediately in liquid nitrogen. Plasma samples were also frozen and stored at −70°C until assayed. The BAL fluid and plasma concentrations of imipenem/cilastatin achieved in the experiments with intravenous administration were measured 5 h after continuous administration of the drug. Only three animals were utilized for these investigations as the concentrations in the plasma and in the BAL were found to be very similar between animals. The concentrations of imipenem/cilastatin in both plasma and in the BAL fluid were determined by an assay using reverse-phase high-pressure liquid chromatography (Gravallese et al., 1984; Myers & Blumer, 1984). The recovery of imipenem/cilastatin in the BAL fluid was determined by multiplying the amount of fluid recovered by the concentration of the drug in that fluid. Almost 80% of the drug was recovered in the first lavage. The third sample of lavage fluid contained less than 5% of the total quantity of antibiotic measured in the sum of all the recovered fluid.

Surgical procedure for lung injury studies

All surgical procedures were carried out while the rats were anaesthetized. Anaesthesia was achieved using 30 mg of pentobarbital given intraperitoneally (ip). Anaesthesia was maintained with 12 mg of ip pentobarbital given every 2 h. A polyethylene tube (PE240, Clay Adams, Parsippany, NJ, USA) was inserted into the trachea via tracheostomy. The rats were ventilated mechanically with a constant-volume animal respirator (Harvard Apparatus, South Natick, MA, USA) with 100% oxygen and 3 cm H₂O positive end-expiratory pressure. The respiratory rate was adjusted to maintain an arterial PCO₂ between 35 and 45 mmHg. The right carotid artery was cannulated with a polyethylene tube (PE50 Clay Adams), and used for arterial blood pressure measurements and blood sampling. The right jugular vein was cannulated with a polyethylene tube (PE50), and used for the administration of the antibiotic or of buffered saline. Pancuronium bromide (0.3 mg/kg) was given intravenously every 2 h for neuromuscular blockade. The rats were placed in the right lateral decubitus position after the surgery in preparation for the airspace instillation of bacteria.

Experimental protocol for lung injury studies

For the studies assessing the effect of the antibiotic pretreatments on bacterial-induced lung injury, the following protocol was used. After a 30 min interval of stabilization, 3 μCi of ¹³¹I labeled human albumin (Merck-Frosst, Quebec, Canada) was injected via the venous line. Blood samples for radioactivity were obtained 15 and 30 min after the ¹³¹I injection. The preparation and instillation of protein solution was performed as we have reported previously (Wiener-Kronish et al., 1993; Kudoh et al., 1994). The
airspace instillate consists of Ringer's lactate, 5% of rat albumin, 0.5 mg of anhydrous Evan's blue and 3 μCi of 125I labeled human albumin (Merck-Frosst).

For studies using bacteria *P. aeruginosa* PAK was added to the airspace instillate and adjusted to 5 × 10⁸ cfu/mL. Instillation of this solution (3 ml/kg) was done via a PE-50 tube over 30 min. The unilateral distribution (right lung) of the instillation was confirmed after each experiment by observing the distribution of the Evan's blue in lung. Also, the recovery of 125I-albumin from the left lungs was always less than 0.2% of the total instillate radioactivity.

Blood samples for radioactivity and blood gas measurement were obtained every hour after the instillation. Four hours after the administration of the instillate, the animals were anaesthetized deeply. Their abdomens were opened and the rats were exsanguinated. Urine, right pleural fluid, and a piece of the liver were obtained from each animal for radioactivity measurements and bacterial culture. The lungs were removed and all remaining airspace fluid was collected using a PE50 tube. After weighing and adding a known amount of sterile distilled water, both right and left lungs were homogenized separately for total protein measurement, haemoglobin measurement, wet-to-dry weight ratio measurement, radioactivity counts and quantitative bacteriological culture.

**Specific Protocols for lung injury studies**

**Group 1a. No pre-treatment; instillate without bacteria (n = 5).**

Control rats were anaesthetized and received an airspace instillate without bacteria. Rats were ventilated for 4 h and then exsanguinated and processed as described above.

**Group 1b. Pretreatment with aerosol imipenem/cilastatin; instillate without bacteria (n = 5).**

To determine the effect of aerosolized imipenem on the lung, these rats were exposed to a 1 h aerosol of imipenem/cilastatin. The rats were then anaesthetized and received an airspace instillate without bacteria. The rats were ventilated for 4 h and processed as group 1a rats.

**Group 2. No pre-treatment; instillate contains bacteria (n = 5).**

Rats were anaesthetized and received an airspace instillate containing 5 × 10⁸ cfu of PAK in addition to the radioactive protein tracers. Four hours later the rats were processed as in the above experiments.

**Group 3. Pretreatment using aerosolized imipenem/cilastatin; instillate contains bacteria (n = 5).**

The rats in this group were exposed to a 1 h aerosol of imipenem/cilastatin and then were anaesthetized. The airspace instillate contained 5 × 10⁸ cfu of PAK. Four hours later the rats were processed as in the above experiments.
Group 4. *Pretreatment using iv imipenem/cilastatin; instillate contains bacteria (n = 5).* These rats received a continuous intravenous administration of imipenem/cilastatin which was started 1 h prior to the airspace instillation. The rats were processed 4 h later as they had been in the above experiments.

**Measurements for the lung injury studies**

Systemic arterial pressures, airway pressures and arterial blood gases were recorded hourly throughout the experiment. Total protein concentrations of plasma, instillate, and final alveolar fluid were measured by biuret method. Protein permeability of alveolar epithelial and endothelial barriers were assessed by measuring the bi-directional flux of albumin across the barriers using two different albumin tracers, as we have done previously (Wiener-Kronish, *et al.*, 1993; Kudoh *et al.*, 1994). We used $^{125}$I-albumin as an alveolar protein tracer and $^{131}$I-albumin as a vascular protein tracer. Briefly, the total quantity of $^{125}$I-albumin (the alveolar protein tracer) instilled in the lung was determined by measuring duplicate samples of the instilled solution for total counts (cpm/g) and multiplying this by the total weight of the instillate. The total quantity of $^{125}$I-albumin remaining in the right lung, in the left lung, in the remaining alveolar fluid, in the pleural fluid, and in the urine were also calculated in the same manner. The total quantity of $^{125}$I-albumin that had left the airspaces and entered the circulating plasma was determined by multiplying the total counts (cpm/g) in the plasma after 4 h times the estimated plasma volume (body weight × 0.07 [1-haematocrit]).

The quantity of $^{125}$I-albumin in the right lung homogenate and in the aspirated fluid were added to calculate the recovery of the instilled $^{125}$I-albumin remaining in the lung after 4 h. Calculations for the total recovery of $^{125}$I-albumin for each experiment averaged 97.9 ± 2.2%. The quantity of unbound $^{125}$I-albumin was measured by adding 20% trichloroacetic acid (TCA) to each sample. The percentage of unbound $^{125}$I from all experiments was never greater than 0.2% in the instillate and 0.6% in the plasma samples, even 4 h after the instillation.

The counts of $^{131}$I-albumin (the vascular protein tracer) in the plasma over the course of the experiment were measured and averaged. The ratio of the $^{131}$I-albumin counts in the final alveolar sample to the averaged plasma counts provides a good index of equilibration of the vascular protein tracer into the alveolar compartment (Wiener-Kronish *et al.*, 1993). Also, we estimated the accumulation of the vascular protein tracer in the extravascular space as the extravascular plasma equivalents. This was used as an index of the endothelial permeability (Wiener-Kronish *et al.*, 1993).

**Table 1. Plasma concentration and total recovery from BAL fluid of imipenem after 1 h intravenous administration or 1 h aerosolization**

<table>
<thead>
<tr>
<th>Condition</th>
<th>no.</th>
<th>Plasma concentration (mg/L)</th>
<th>Recovery from BAL (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iv imipenem/cilastatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg/kg/h intravenous infusion</td>
<td>3</td>
<td>56.0 ± 9.0¹</td>
<td>25.8 ± 0.8⁵</td>
</tr>
<tr>
<td>Aerosol imipenem/cilastatin</td>
<td>5</td>
<td>0.3 ± 0.01</td>
<td>107.9 ± 20.7</td>
</tr>
</tbody>
</table>

Mean ± S.E.

¹P < 0.01; ⁵P < 0.05.
Table II. The number of bacteria in the instillate and in the experimental lung 4 h after the bacterial instillation

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Bacteria in instillate (cfu × 10^6)</th>
<th>Bacteria in experimental lung (cfu × 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pre-treatment</td>
<td>5 2.6 ± 0.1</td>
<td>6.9 ± 2.2</td>
</tr>
<tr>
<td>Aerosol imipenem/cilastatin</td>
<td>5 3.1 ± 0.3</td>
<td>&lt;0.1*</td>
</tr>
<tr>
<td>iv imipenem/cilastatin</td>
<td>5 3.0 ± 0.3</td>
<td>&lt;0.1*</td>
</tr>
</tbody>
</table>

Mean ± S.E.

*Significant at 95% vs no pre-treatment.

Extravascular plasma equivalents are expressed as milliliters and are calculated by dividing the total counts of extravascular 131I-albumin in experimental lung (right lung) by the average 131I-albumin cpm/g in plasma.

Statistics

The data were expressed as mean and standard error. One-way analysis of variance for factorial or repeated measures experiments were used. Fisher's PLSD (protected least significant difference) procedure was used for post-hoc multicomparison analysis among the groups. We regarded the data as statistically significant with a P value of <0.05.

Results

Concentration of imipenem/cilastatin after aerosol delivery of continuous iv infusion

The concentrations of imipenem/cilastatin after aerosol delivery or after a continuous iv administration are presented in Table I. The plasma concentration of imipenem/cilastatin after 5 h of continuous intravenous infusion was 200-fold higher than that achieved after a 1 h aerosol of the drug. However, continuous intravenous infusion for 5 h resulted in a concentration of imipenem/cilastatin in the airspaces of the lung that was only one-fourth of that achieved by the 1 h aerosol. Aerosolization to rodents was determined to be inefficient; less than 0.1% of the amount of aerosolized imipenem/cilastatin was measured in the airspaces of the rats.

Table III. Movement of alveolar tracer, 125I-albumin, from the lung into the blood circulation over 4 h

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>no.</th>
<th>% 125I in lung</th>
<th>% 125I in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pre-treatment without bacteria</td>
<td>5</td>
<td>97.2 ± 1.4</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Aerosol imipenem/cilastatin without bacteria</td>
<td>3</td>
<td>96.8 ± 1.4</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>No pre-treatment with bacteria</td>
<td>5</td>
<td>91.0 ± 1.2*</td>
<td>7.0 ± 0.9*</td>
</tr>
<tr>
<td>Aerosol imipenem/cilastatin with bacteria</td>
<td>5</td>
<td>97.6 ± 0.9*</td>
<td>0.6 ± 0.2*</td>
</tr>
<tr>
<td>iv imipenem/cilastatin with bacteria</td>
<td>5</td>
<td>93.1 ± 0.9*</td>
<td>3.2 ± 0.8*</td>
</tr>
</tbody>
</table>

Mean ± S.E.

*Significant at 95% vs no pre-treatment without bacteria.

*Significant at 95% vs no pre-treatment with bacteria.
Table IV. Movement of vascular tracer, $^{131}$I-albumin, from the circulation into the lung over 4 h

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>No.</th>
<th>Ratio of $^{131}$I alveolar/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pre-treatment without bacteria</td>
<td>5</td>
<td>$0.07 \pm 0.01$</td>
</tr>
<tr>
<td>Aerosol imipenem/cilastatin without bacteria</td>
<td>3</td>
<td>$0.04 \pm 0.01$</td>
</tr>
<tr>
<td>No pre-treatment with bacteria</td>
<td>5</td>
<td>$0.34 \pm 0.05^*$</td>
</tr>
<tr>
<td>Aerosol imipenem/cilastatin with bacteria</td>
<td>5</td>
<td>$0.08 \pm 0.02^*$</td>
</tr>
<tr>
<td>iv imipenem/cilastatin with bacteria</td>
<td>5</td>
<td>$0.13 \pm 0.03^*$</td>
</tr>
</tbody>
</table>

Mean ± S.E.
$^*$Significant at 95% vs no pre-treatment without bacteria.
$^*$Significant at 95% vs no pre-treatment with bacteria.

Bacterial-induced lung injury

Pretreatment utilising either aerosolized imipenem/cilastatin or intravenous imipenem/cilastatin was successful in killing all bacteria instilled in the airspaces (Table II). In contrast, untreated rats had a three-fold increase in the bacterial inoculum during the 4 h experiment (Table II).

Aerosolization of imipenem/cilastatin did not increase the permeability of the alveolar epithelium to protein; there was no increased escape of $^{125}$I-albumin from the airspaces of the rats that were exposed to aerosolized antibiotic (Table III). Furthermore, there was no increase in the influx of $^{131}$I-albumin from the circulation into the lungs of these rats (Table IV). These data suggest that the rats exposed to the aerosolized antibiotic suffered no lung injury from the drug itself.

Pretreatment with either the aerosolized imipenem/cilastatin or the intravenous imipenem/cilastatin led to a decrease in the escape of $^{125}$I-albumin from the bacterial-instilled lung (Table III). In contrast, untreated rats challenged with airspace bacteria had 7% of their instilled radioactive protein tracer leave the airspaces and enter the circulation over the 4 h interval (Table III). Aerosolization of imipenem/cilastatin led to the smallest egress of $^{125}$I-albumin into the circulation, suggesting that the least amount of alveolar epithelial damage occurred in this group of rats (Table III).

The influx of the vascular tracer, $^{131}$I-albumin, from the circulation into the lung is used as a measure of the lung endothelial and epithelial permeability to protein. In untreated rats exposed to airspace bacteria, there was approximately a three-fold increase in the influx of the vascular tracer (Table IV). In contrast, either aerosolization of imipenem/cilastatin or intravenous administration of this antibiotic decreased this influx of the $^{131}$I-albumin into the lung. There was no statistically significant difference between the influx of this radioactive protein in the pretreated rats; however, the rats pretreated with the aerosolized antibiotic had the same percentage of vascular tracer influx as rats that were not exposed to airspace bacteria (Table IV). Therefore, aerosolization of imipenem/cilastatin completely protected the alveolar epithelium and endothelium from any increases in permeability secondary to airspace bacteria.

Discussion

Imipenem ($N$-formimidoyl thienamycin), a potent $\beta$-lactam antibiotic, has a broad spectrum of activity versus Gram-negative pathogens, including $P$. aeruginosa (Barza,
In addition, the antibiotic interacts with bacterial cell walls in a manner that results in less endotoxin release compared with other β-lactam antibiotics (Hodson, Penketh & Batten, 1981).

Aerosolization of imipenem/cilastatin did not result in an increase in the permeability of either the alveolar epithelium or the lung endothelium (Tables III and IV). Gas exchange in the rats exposed to the aerosol was not perturbed (data not shown). Therefore, we could not detect any physiological problems caused by this method of drug delivery. Furthermore, aerosolization of the antibiotic resulted in selectively high concentrations of the drug in the airspaces of the lung (Table I), suggesting that for infections limited to the airspaces of the lung this may be a superior method of drug delivery.

Both aerosolization of imipenem/cilastatin and the intravenous administration of this antibiotic led to similar numbers of bacteria in the lung after the 4 h experiment (Table II). However, the results from the physiological parameters of lung injury suggest that the killing of the bacteria may have occurred earlier in the rats exposed to the aerosolized antibiotic, as less \(^{125}\text{I}\)-albumin and \(^{131}\text{I}\)-albumin left or entered the lungs in the rats pretreated with aerosolized antibiotic. This conclusion seems likely given the antibiotic levels achieved in the airspaces after aerosolization; the antibiotic levels were four-fold higher at the initiation of the experiment in the rats pretreated with the aerosolized antibiotic (Table I). In contrast, the animals that received a continuous intravenous infusion for 5 h had an antibiotic level in their airspaces that was one-half of their plasma concentration (Table I). Their airspace concentration was still only one-fourth of that achieved by the 1 h aerosol delivery (Table I). Therefore, aerosolization of imipenem/cilastatin was much more efficacious in achieving high levels of antibiotic in the airspaces of the lung than intravenous therapy and the higher level of antibiotic was associated with significantly less lung injury, measured by several independent measures of protein permeability.

The administration of antibiotics via aerosol has become popular for the treatment of chronic colonisation of bacteria in cystic fibrosis patients and in AIDS patients (Hodson et al., 1981; Debs et al., 1987; Montgomery et al., 1987; Strandvik et al., 1988; Flamholc et al., 1992; Gonda, 1992; Littlewood, Smye & Cunliffe, 1993; Cooney et al., 1994). However, this study evaluated the utility of aerosolized imipenem/cilastatin in protecting against a subsequent airspace bacterial-injury. We have shown previously that the instillation of airspace bacteria leads to a dose-related increase in the permeability of the alveolar epithelium and lung endothelium, secondary to the exoproducts produced by the \(P.\ aeruginosa\) (Wiener-Kronish et al., 1993; Kudoh et al., 1994). This investigation shows that the injurious effects of the bacterial instillate could be prevented completely by the pretreatment of the rats with aerosolized imipenem/cilastatin. These results suggest that aerosolization with imipenem/cilastatin or a similar drug might be a useful prophylactic treatment of patients at high risk for colonisation and/or pneumonia in intensive care units. The advantage of the aerosolization of this antibiotic is that plasma levels of the antibiotic are low, minimising systemic exposure.

The present study did not investigate the utility of aerosolized imipenem/cilastatin after the establishment of a bacterial airspace infection. Once the alveolar epithelium is damaged and permeability is increased, it is unknown whether the aerosolization of antibiotics results in different pharmacokinetics/pharmacodynamics compared with intravenous antibiotics.
In conclusion, the aerosol administration of imipenem/cilastatin prior to the instillation of airspace bacteria completely protected the lung from bacterial-induced epithelial or endothelial injury. The pretreatment did not perturb lung physiological parameters; aerosolization of the antibiotic was associated with higher concentrations of the antibiotic in the airspaces and better preservation of the lung epithelial and endothelial permeability than the intravenous administration of the antibiotic. The results cannot be extrapolated to humans without further investigation; however, these experiments suggest that imipenem/cilastatin, could be considered for aerosolization to patients just as tobramycin and gentamicin are now being utilized.

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References


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