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Efficacy of β-Lactams for Treating Experimentally Induced Pneumonia Due to a Carbapenem-Hydrolyzing Metallo-β-Lactamase-Producing Strain of *Pseudomonas aeruginosa*

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A rat pneumonia model was established with a *Pseudomonas aeruginosa* strain that produced the plasmid-encoded metallocarbapenemase VIM-2. A significant decrease in lung bacterial titers was observed when imipenem, cefepime, ceftazidime, and piperacillin-tazobactam were given at the highest doses recommended for humans, despite their high MICs. Aztreonam at high doses produced a similar decrease in bacterial titers.

The carbapenems imipenem and meropenem possess the broadest spectrum of activity among β-lactams. They are active against most gram-negative rods that produce clavulanate-inhibited extended-spectrum serine β-lactamases (11). However, *Pseudomonas aeruginosa* isolates that produced metallo-β-lactamases (MβLs) are increasingly being reported (9). These clavulanate-resistant MβLs have a very broad hydrolysis profile, including carbapenems and extended-spectrum cephalosporins but not the monobactam aztreonam (17). The MβLs of the IMP series are from gram-negative aerobes, mostly in southeastern Asia (3, 6–8, 19, 22), whereas those of the VIM series have been reported more recently from *P. aeruginosa* isolates in southern Europe and in Taiwan (4, 10, 14–16, 20, 21). The suitable antibiotic therapy for treating infections due to MβL-producing *P. aeruginosa* strains remains unknown.

Since *P. aeruginosa* is a predominant cause of nosocomial pneumonia (18), we compared the in vivo antibacterial activities of five β-lactams for treating experimental pneumonia due to *P. aeruginosa* strain COL-1, which produces the plasmid-mediated MβL VIM-2 (16).

MICs of β-lactams for *P. aeruginosa* COL-1 were determined as described elsewhere (16). MICs of imipenem, cefepime, ceftazidime, piperacillin-tazobactam, and aztreonam were 128, 64, 256, 16, and 0.25 μg/ml, respectively, indicating that this isolate was resistant to imipenem, ceftazidime, and cefepime, moderately susceptible to piperacillin-tazobactam, and susceptible to aztreonam.

Pharmacokinetics of β-lactams were determined in Wistar rats, as previously described (12, 13). These rats were rendered neutropenic by the intraperitoneal administration of cyclophosphamide and renally insufficient by subcutaneous uranyl nitrate administration (13). The pharmacokinetic parameters of the antibiotics were similar to those observed when a 1-g imipenem dose, a 2-g cefepime or ceftazidime dose, a 4.5-g piperacillin-tazobactam dose, or 1- to 2-g aztreonam doses are given intravenously to healthy humans (Table 1).

The pneumonia model was as previously developed in our laboratory (12, 13). A 0.5-ml portion of a bacterial suspension containing 7.8 $\log_{10}$ CFU of *P. aeruginosa* COL-1 was injected intratracheally into anaesthetized animals. Among the 120 animals of this study, 110 were alive 3 h after bacterial inoculation. At that time, rats were randomly assigned to a control group (no antibiotic) and six treatment groups. Treatment groups received intraperitoneal injections of either imipenem-cilastatin (30 mg/kg of body weight/8 h), cefepime (60 mg/kg/8 h), ceftazidime (60 mg/kg/8 h), piperacillin-tazobactam (120 and 15 mg/kg/6 h, respectively), or aztreonam (30 or 60 mg/kg/6 h) (1, 2, 12, 13). These dosages were retained to mimic plasma concentrations observed with highest recommended doses of these β-lactams in humans. Therapy began 3 h after bacterial inoculation and continued for 24 h.

Viable bacteria in lungs of sacrificed rats were counted after 18 h of growth on Mueller-Hinton agar (bioMérieux). Bacterial counts in the lungs, leukocyte counts, and creatinine levels in the control and treatment groups were compared by one-way nonparametric analysis of variance (Kruskal-Wallis test). When the value of this test was statistically significant, each treatment group was compared to the control group and to each of the other treatment groups by using the Mann-Whitney U test. For all tests, a P value of <0.05 was considered significant.

At sacrifice, creatinine levels and leukocyte counts in plasma were not statistically different between groups (Table 2). At that time, bacterial counts in untreated animals were 5 $\log_{10}$ (range, 3.0 to 9.7) CFU/g of lungs. Rats treated with low aztreonam doses had bacterial counts in their lungs similar to those of untreated animals (Table 3). Imipenem, cefepime, ceftazidime, piperacillin-tazobactam, and high aztreonam doses resulted in a significant decrease of lung bacterial counts

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TABLE 1. Pharmacokinetics for antibiotics given intraperitoneally to noninfected rats with uranyl nitrate-induced renal impairment

β-Lactam	No. of animals	Dose (mg/kg)	Median (range) antibiotic level in plasma (μg/ml)		Median (range) half-life (h)	Median (range) area under curve (μg · h/ml)
			Peak (30 min after dosing)	Trough (8 h after dosing)		
Imipenem	5	30	83 (66–104)	<0.5 (<0.5–1)	1.0 (0.7–1.3)	112 (95–145)
Cefepime	6	60	160 (125–220)	12 (6–28)	2.0 (1.4–2.8)	422 (188–615)
Ceftazidime	4	60	197 (139–214)	15 (5–24)	2.2 (1.9–2.6)	346 (266–382)
Piperacillin-tazobactam[a]	6	120	310 (198–382)	1 (<1–3)	1.1 (0.7–1.4)	436 (312–726)
Aztreonam	6	60	149 (95–181)	<5 (<5–<5)	1.3 (0.8–1.7)	359 (161–548)

[a] The dose and levels in plasma are those of piperacillin.

compared to the untreated group. Treatments with imipenem, cefepime, and high aztreonam doses also resulted in a significant decrease of the lung bacterial titers compared to low-dose-aztreonam-treated animals.

A second set of similar experiments was done in order to confirm these surprising results. Of 120 rats, 100 were still alive 3 h after bacterial inoculation. Compared to controls (5.3 [3 to 8.9] $\log_{10}$ CFU/g), a significant decrease in lung bacterial titers was observed when imipenem (3.2 [2 to 5.8], $P = 0.04$), cefepime (2.9 [2 to 5.8], $P = 0.02$), ceftazidime (3.1 [2.1 to 5.9], $P = 0.03$), and piperacillin-tazobactam (3.5 [2 to 9], $P = 0.04$) were given at the highest doses recommended for humans. Aztreonam at high doses produced a similar decrease in bacterial titers (3.1 [2.2 to 6.3], $P = 0.02$) but not at low doses (4.3 [2.8 to 8.7], $P = 0.3$).

The failure of the antibacterial activity of low aztreonam doses could not be explained by inadequate aztreonam concentrations in plasma, since they remained above the MIC virtually throughout the dosing interval. In contrast, treatment with imipenem at its highest doses recommended for humans significantly reduced bacterial titers in rat lungs, although the strain was imipenem resistant. In order to confirm this result, additional experiments with infected rats treated with the same imipenem dose were performed, with similar results (data not shown). The stability of the plasmid-mediated β-lactamase gene in *P. aeruginosa* COL-1 was assessed in vivo by plating lung bacteria of control and imipenem-treated groups onto imipenem-free and imipenem (128 μg/ml)-containing Mueller-Hinton agar and then determining the MICs of β-lactams for strains arising from these culture media.

Similar paradoxical results were observed by Ernst et al. with an experimental model of pneumonia due to an imipenem-resistant *P. aeruginosa* strain (5). In that study, the mechanism of imipenem resistance was likely impermeability related. As stated, a lower aztreonam distribution into rat lungs is unlikely to explain these results, since the penetration of aztreonam and imipenem into the lung tissue is similar (5). However, an inoculum effect has been reported for aztreonam for 10^7 to 10^8 CFU/ml, whereas imipenem efficacy is not affected by the inoculum size up to 10^8 CFU/ml (1, 2). In our cases, the aztreonam MIC remained at 32 μg/ml with an inoculum of 10^7 CFU/ml, a value below the peak level of this antibiotic (Table 1). Moreover, differential protein binding of aztreonam (50 to 60%) and imipenem (10 to 20%) may explain, at least in part, the paradoxical effects observed (1, 2).

Results similar to those obtained for imipenem were observed in rats receiving cefepime, ceftazidime, and piperacillin-tazobactam. Whereas β-lactamase VIM-2 significantly hydrolyzes these β-lactams in vitro (16), these antibiotics retained some activity in vivo. In vivo, a low level of expression of the β-lactamase VIM-2 or a low catalytic efficacy (possibly due to inhibitors) may explain the paradoxical efficacy of these β-lactams.

In conclusion, only aztreonam at high doses seemed to be an active regimen for treating pneumonia due to a VIM-2-producing *P. aeruginosa* isolate. In our model, aztreonam is the only β-lactam that had an in vitro activity that mirrored its activity in vivo. This result may be extended to infections due to *P. aeruginosa* strains that produce other MβLs.

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TABLE 2. Creatinine levels and leukocyte counts in rat plasma observed at sacrifice, i.e., 24 h after therapy initiation

β-Lactam[a]	No. of animals	Median (range) creatinine level in plasma (μmol/liter)	Median (range) leukocyte count in plasma (/mm^3)
None	15	253 (188–465)	310 (210–750)
Imipenem	15	263 (175–440)	280 (160–480)
Cefepime	16	229 (171–412)	335 (150–880)
Ceftazidime	16	336 (225–496)	350 (170–740)
Piperacillin-tazobactam	16	263 (181–538)	260 (120–350)
Aztreonam			
LD	16	233 (157–333)	280 (80–540)
HD	16	265 (179–421)	260 (70–750)

[a] LD, low dose; HD, high dose.

TABLE 3. Titers of VIM-2 producing *P. aeruginosa* in rat lungs according to treatment groups and statistical differences between groups

Treatment[b]	No. of animals	Median (range) lung bacterial titer ($\log_{10}$ CFU/g)	Statistical difference[a] (*P* value) compared to:	
			Control group	Low-dose aztreonam group
Control	15	5.0 (3.0–9.0)		0.25
Imipenem	16	3.3 (2.3–4.1)	**0.004**	**0.001**
Cefepime	16	3.3 (2.6–5.7)	**0.01**	**0.009**
Ceftazidime	16	3.3 (2.3–7.2)	**0.04**	0.13
Piperacillin-tazobactam	16	3.3 (2.3–6.0)	**0.03**	0.26
Aztreonam				
LD	16	4.0 (3.3–6.3)	0.25	
HD	15	2.8 (2.3–6.4)	**0.002**	**0.003**

[a] Values showing a significant difference are in bold.
[b] LD, low dose; HD, high dose.

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