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Efficacy of Cefepime and Imipenem in Experimental Murine Pneumonia Caused by Porin-Deficient *Klebsiella pneumoniae* Producing CMY-2 β-Lactamase

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Previous studies have shown decreased in vitro activity of zwitterionic cephalosporins and carbapenems against porin-deficient *Klebsiella pneumoniae* expressing a plasmid-mediated AmpC-type β-lactamase (PACBL). The in vitro and in vivo activities of cefepime and imipenem were evaluated against the porin-deficient strain *K. pneumoniae* C2 and its CMY-2-producing derivative [*K. pneumoniae* C2(pMG248)]. The MICs (in micrograms/milliliter) of cefepime and imipenem against *K. pneumoniae* C2 were 0.125 and 0.25, respectively, while the corresponding values against *K. pneumoniae* C2(pMG248) were 8 and 16. Cefepime showed a greater inoculum effect than imipenem against both strains. Imipenem showed a significant post-antibiotic effect (>2 h) against *K. pneumoniae* C2(pMG248) at 1×, 2×, 4×, 6×, and 8× MIC. The maximum concentrations of drug in serum of cefepime and imipenem in a pneumonia model using mice were 124.1 and 16.9 μg/ml, respectively. ΔT/MIC for *K. pneumoniae* C2 and C2(pMG248) were 1.29 h and 0.34 h for imipenem and 2.96 h and 1.27 h for cefepime. Both imipenem (30 mg/kg of body weight every 3 h) and cefepime (60 mg/kg every 4 h), administered for 72 h, increased the survival rate (86.6% and 100%) compared with untreated control animals (26.6%, $P < 0.003$) infected with *K. pneumoniae* C2. For the CMY-2-producing strain, imipenem, but not cefepime, increased the survival rate compared to the controls (86.6% and 40% versus 40%, $P < 0.01$). Bacterial concentration of the lungs was significantly decreased by both antimicrobials. In conclusion, imipenem was more active in terms of survival than cefepime for the treatment of murine pneumonia caused by a porin-deficient *K. pneumoniae* expressing PACBL CMY-2.

Beta-lactamase production is the most important resistance mechanism to β-lactam antibiotics in gram-negative bacteria. Initial resistance to expanded-spectrum cephalosporins was mediated by hyperproduction of chromosomal class C β-lactamases in a limited number of species, such as *Escherichia coli*, *Enterobacter* spp., *Serratia marcescens*, *Citrobacter freundii*, and *Pseudomonas aeruginosa* (18, 30). In those bacteria unable to produce AmpC (*K. pneumoniae*, *Proteus mirabilis*, *Salmonella* spp.), resistance to expanded-spectrum cephalosporins was mediated by extended-spectrum β-lactamases (ESBL) belonging to the TEM, SHV, or CTX-M types (13, 14, 15, 31). These ESBLs were active against oxyimino-cephalosporins but not against 7-α-metoxy-cephalosporins, β-lactamase inhibitors, or carbapenems (13, 14, 18).

The continued use of cephamycins and combinations of β-lactam–β-lactamase inhibitors are potential contributors to the appearance of plasmids which encode class C β-lactamases (PACBL) in *K. pneumoniae*, *Escherichia coli*, and, to a lesser extent, other enterobacteria (22). These enzymes are not inactivated by clinically used β-lactamase inhibitors and confer a spectrum of activity greater than that observed in ESBLs.

Since the first description of MIR-1 and CMY-1, more than 20 PACBLs have been described in different countries of Europe, America, and Asia, most of them being discovered in *K. pneumoniae* strains (4, 26). CMY-2 is one of the most prevalent and most widely distributed PACBLs and has been found in several countries (27). Taking into consideration the difficulty of detecting PACBLs, the real prevalence of these enzymes is probably underestimated. In one United States study which included 25 states, PACBLs were found in 8.5%, 6.9%, and 4% of *K. pneumoniae*, *K. oxytoca*, and *E. coli*, respectively (1).

Strains expressing PACBL are consistently resistant to penicillins, oxyimino-cephalosporins, and cephamycins. Most of them are also resistant to aztreonam and β-lactamase inhibitor combinations, with the occasional exception of piperacillin-tazobactam (27). Nevertheless, susceptibility to cefepime, cefpirome, and carbapenems was not affected by these enzymes (27).

Loss of outer membrane porins in PACBL expressing *K. pneumoniae* can increase the MICs of carbapenems to the resistant category (20). Moreover, in such strains, the MICs of cefepime and cefpirome show inoculum dependence exceeding values of 256 μg/ml at an inoculum of 10^7 CFU/ml (12). Clinical strains of both *K. pneumoniae* and *E. coli*, whose imipenem MICs are as high as 64 μg/ml owing to porin loss and PACBLs

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(CMY-4, ACT-1), have been described already (5, 6, 33). The potential use of zwitterionic cephalosporins (i.e., cefepime and cefpirome) and carbapenems in infections caused by strains expressing both resistance mechanisms is currently unknown.

The purpose of this study is to compare the use of cefepime and imipenem in a murine pneumonia model caused by PACBL (CMY-2)-expressing *K. pneumoniae* and lacking major porins.

MATERIALS AND METHODS

Bacterial strains. *K. pneumoniae* C2 is a previously described (20, 21) ceftazidime-susceptible strain, derived in vitro from the clinical isolate *K. pneumoniae* NEDH-1 (deficient in porins OmpK35 and OmpK36 and producing SHV-2). *K. pneumoniae* C2(pMG248) is a transconjugant derived from K. *pneumoniae* C2, containing the plasmid pMG248, which codes for PACBL CMY-2 (1). Plasmid pMG248 was introduced into *K. pneumoniae* C2 by conjugation, as previously described (20). *K. pneumoniae* C2(pMG248) does not lose pMG248 upon repeated subculturing in an antimicrobial-free medium (data not shown).

Antimicrobial agents. Imipenem and imipenem plus cilastatin were obtained from Merck and Sharp and Dohme (Madrid, Spain) for the in vitro and the in vivo experiments, respectively, and cefepime was from Bristol-Myers Squibb (Madrid, Spain).

Susceptibility testing and time-kill curve experiments. MICs of cefepime and imipenem against *K. pneumoniae* strains C2 and C2(pMG248) were determined by microdilution according to NCCLS guidelines (24). The activities of the three β-lactams were tested using three different inocula: 10^5, 10^6, and 10^7 CFU/ml. Minimal bacterial concentrations (MBCs) were determined by subculturing onto antimicrobial-free Mueller-Hinton agar (MHA) 100-μl aliquots of wells containing antimicrobial concentrations greater than or equal to the MIC of the corresponding agent. Plates were incubated at 35°C for 48 h, and viable colonies were counted. MBCs were determined as the concentration that killed ≥99.9% of the initial inoculum.

Time-kill kinetic assays were conducted on the Mueller-Hinton broth (MHB) at drug concentrations of 1× and 4× MIC. A control without using antibiotics was evaluated in parallel. The starting inoculum was 10^6 CFU/ml. Cultures were incubated at 37°C, without shaking. Viable counts were determined by serial dilution at 0 h, 2 h, 4 h, 8 h, and 24 h after adding the drug. Viable counts were determined by plating 100 μl of control or test cultures at the indicated times onto MHA plates. Plates were incubated for 48 h, and the colony count, the $\log_{10}$ of viable cells (CFU/ml), was determined.

Determination of postantibiotic effect (PAE) of imipenem and cefepime. The in vitro PAE of imipenem and cefepime was investigated for both strains [*K. pneumoniae* C2 and C2(pMG248)] by exposing the bacteria (10^7 CFU/ml) to the drug at a concentration of equal to 1×, 2×, 4×, 6×, and 8× MIC for 1.5 h (19). The antibiotic was removed from the culture by three centrifugations and resuspended in fresh MHB. The antibiotic-free bacterial resuspension was then incubated at 37°C and shaken. After removal of the drug, viable counts were performed hourly for the next 8 h. A growth control, prepared and treated in a similar way to the test solution but without being exposed to antibiotics, was included in each experiment. The PAE was calculated as previously described (7).

Animals. Immunocompetent C57BL/6 female mice weighing 14 to 16 g were used. They were obtained from the University of Seville's facility, had a sanitary status of MPF (murine pathogen free), and were assessed for genetic authenticity. Animals were housed in regulation cages and given free access to food and water.

Drug pharmacokinetics. The plasma levels of imipenem and cefepime were determined after administration of a single intramuscular (i.m.) dose of each antimicrobial. The dosage administered was 30 mg/kg of body weight for imipenem and 60 mg/kg for cefepime. After 10, 15, 30, 60, 90, 120, and 150 min, blood was extracted from the periorbital plexus of three anesthetized mice per time point. The total plasmatic drug concentrations were measured by the bioassay method, using *Micrococcus luteus* ATCC 9341 as the indicator strain. The intraday and interday variations of the assays were 3.43% ± 0.75% and 4.48% ± 2.42% for cefepime and 2.62% ± 2.44% and 3.22% ± 1.91% for imipenem; the linearity (r^2) of the assay was 0.96 ± 0.69 and 0.91 ± 0.02, respectively; the lower limits of detection were 0.9 and 0.01 μg/ml. The maximum plasma concentration (C_{max}, in milligrams per liter) and terminal half-life ($t_{1/2}$, in hours) were calculated using the PKCALC program (32). The time during which the plasma concentration remained above the MIC (ΔT/MIC, in hours) was estimated by extrapolation from the regression line of plasma elimination (9) using the MIC obtained with an inoculum of 10^5 CFU/ml.

Experimental pneumonia model in mice. A modification of the Esposito and Pennington model (8) performed by our group (29) was used to produce pneumonia. The mice were anesthetized with an intraperitoneal (i.p.) injection of 5% sodium thiopental. They were suspended vertically, and the tracheae were then cannulated with a blunt-tipped metal needle. The feel of the needle tip against the tracheal cartilage confirmed the intratracheal location. A microliter syringe (Hamilton Co., Reno, NV) was used for the inoculation. After inoculation, the mice remained in a vertical position for 3 min and then in a 30° position until they awoke.

Klebsiella pneumoniae C2 and C2(pMG248) were used in the experimental pneumonia. The animals were inoculated with 50 μl of the *K. pneumoniae* bacterial suspension, obtained through an 18-h culture in trypticase soy broth (Becton-Dickinson Microbiology Systems) at 37°C and mixed 1:1 with porcine mucin diluted to 10% in saline solution (M-2378; Sigma Chemical Co., St. Louis, MO), with a final inoculum size of approximately 10^8 CFU/ml.

Antimicrobial treatment. To evaluate the effectiveness of the different treatment regimens, 50 mice were inoculated with the C2 strain and 50 mice were inoculated with the C2(pMG248) strain. They were then divided into three groups, which received the following treatments for 72 h: control (not treated, $n = 20$); imipenem, 30 mg/kg every 3 h, i.m. ($n = 15$); and cefepime, 60 mg/kg every 4 h, i.m. ($n = 15$); the doses of imipenem and cefepime were chosen to obtain a ΔT/MIC referring to *K. pneumoniae* C2 which was higher than 40% of the dosing interval. The first dose of every antibiotic was administered 4 h after inoculation with the following dose schedule: 30 mg/kg every 3 h for imipenem and 60 mg/kg every 4 h for cefepime. The animals were observed for 72 h for mortality, and surviving mice were killed 4 h after the last dose by i.p. administration of 5% sodium thiopental (Braun Medical, Barcelona, Spain). All the mice were analyzed immediately after death. Once the animals were dead, thoracotomy was carried out. The heart and lungs were extracted together, and the lungs were later separated onto a sterile petri plate and weighed. The lungs were processed for quantitative culture after being homogenized in 2 ml of sterile saline solution (Stomacher 80 Tekmar Co., Cincinnati, Ohio). After 10-fold dilution, aliquots of 100 μl were plated onto Columbia sheep-blood agar plates for 24 h at 37°C. Sterile cultures were considered to have ≤1 log CFU/g of lung. The results were expressed as the means ± standard deviations of the log CFU/gram of the lung.

Lung samples were processed for histological study in five mice from each control group. The lungs were fixed with 10% formaldehyde for pathological study. The lung blocks were embedded in paraffin and cut into 4-μm-thick sections. The slices included all the pulmonary lobes to be studied by optical microscopy. They were processed according to standard methods for hematoxylin-eosin, periodic acid-Schiff, Gram, Masson's Trichromic, and silver reticulin stains.

In order to confirm that imipenem and cefepime were not toxic to the animals, groups of 10 noninfected mice were each given the antibiotics for 72 h.

The use of the experimental pneumonia model was approved by the Ethics Committee of the Hospitales Universitarios Virgen del Rocío, Seville, Spain.

Selection and characterization of antimicrobial-resistant mutants. Selection of mutants derived from either *K. pneumoniae* C2 or C2(pMG248) was performed by inoculating the lung homogenates in either medium containing 4× MIC of cefepime or imipenem against the tested strain. Lungs from 15 animals treated with cefepime or imipenem were used in these assays. Plates were incubated at 35°C for 48 h, and the colonies were counted. In a parallel experiment, mutants were also selected under the same conditions, using a suspension of bacteria grown in trypticase soy broth (35°C, 20 h) as an inoculum. Frequency of mutation was expressed as the ratio between the number of colonies on plates containing 4× MIC of the corresponding agent and the original inoculum. Up to eight different colonies on every plate were selected for further study. Bacteria were subcultured twice on antimicrobial agent-free Mueller-Hinton agar. The activity of cefepime or imipenem by disk diffusion (25) was determined for every colony. True mutants were defined as those whose inhibition diameter around cefepime (30 μg; Oxoid) or imipenem (10 μg) decreased to ≥10 mm.

Statistical analysis. Numbers of surviving animals were evaluated with the Fisher's exact test. The CFU/gram of lung tissue was analyzed using the homogeneity of variance and posthoc tests (Tukey-Kramer and Dunnet test). The SPSS 11.5 statistical package (SPSS Inc., Chicago, IL) was used. A P value of <0.05 was considered significant.

RESULTS

In vitro studies. MICs and MBCs of cefepime and imipenem against *K. pneumoniae* C2 and *K. pneumoniae* C2(pMG248)

TABLE 1. MICs and MBCs of cefepime and imipenem against two strains of *K. pneumoniae* at three different inocula.

Strain	Antimicrobial	Inoculum (CFU/ml)	MIC (μg/ml)	MBC (μg/ml)
C2	Imipenem	10^5	0.25	0.25
		10^6	0.5	0.5
		10^7	1	1
	Cefepime	10^5	0.125	0.25
		10^6	0.125	0.125
		10^7	4	8
C2(pMG248)	Imipenem	10^5	16	16
		10^6	32	32
		10^7	32	32
	Cefepime	10^5	8	16
		10^6	16	16
		10^7	32	32

containing the bla_{CMY-2} gene are shown in Table 1. For both strains, imipenem showed a lower inoculum effect than cefepime, but these differences were not significant for the CMY-2-producing strain. The results of time-kill curve experiments with cefepime and imipenem were the following. First, against *K. pneumoniae* C2, imipenem showed a higher bactericidal effect than did cefepime. Bacterial regrowth was observed for 1× MIC of cefepime and imipenem at 4 and 8 h, respectively. Second, imipenem produced a rapid bactericidal effect against *K. pneumoniae* C2(pMG248) at both 1× and 4× MIC. After 24 h, regrowth was not noted. Cefepime produced a lower bactericidal effect than imipenem against this strain, and bacterial regrowth was observed after 4 to 8 h of incubation. Against this strain, the time required to obtain a 3 log reduction of the initial inoculum was 8 h for cefepime and 2 h for imipenem.

In vitro determination of PAE. After exposure for 1.5 h, imipenem at 1×, 2×, 4×, 6×, and 8× MIC showed a PAE against *K. pneumoniae* C2(pMG248) of 2 h when compared to control cultures without antimicrobial agents (Fig. 1). PAE was not observed with imipenem against *K. pneumoniae* C2. Cefepime did not show any PAE against either *K. pneumoniae* C2 or C2(pMG248).

Pharmacokinetic studies. The pharmacokinetic and pharmacodynamic parameters of each antimicrobial drug used in the mice (C_{max}, $t_{1/2}$, and ΔT/MIC) are shown in Table 2. The ΔT/MICs of imipenem and cefepime for the C2 strain were more than 40% of the interval between doses.

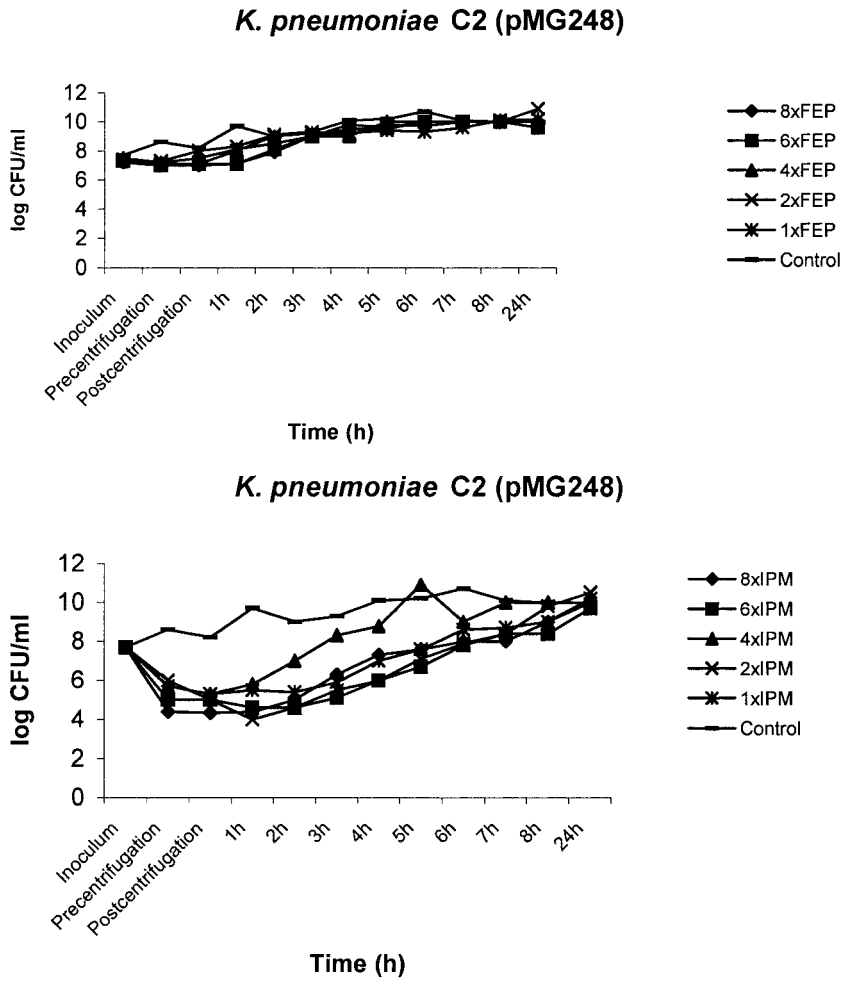

FIG. 1. PAE of imipenem and cefepime against *K. pneumoniae* C2(pMG248) after 1.5 h of exposure to 1×, 2×, 4×, 6×, and 8× MIC of cefepime (FEP) and imipenem (IPM).

TABLE 2. C_{max} and $t_{1/2}$ after a single dose of cefepime and imipenem and ΔT/MIC of both agents for two strains of *K. pneumoniae*

Drug (dose)	C_{max} (mg/liter)	$t_{1/2}$ (h)	ΔT/MIC (h) for strain:	
			C2	C2(pMG248)
Imipenem (30 mg/kg)	16.9	0.15	1.29	0.34
Cefepime (60 mg/kg)	124.06	0.28	2.96	1.27

Pathological studies. The mice groups inoculated with strains C2 and C2(pMG248) showed alterations compatible with acute pneumonia. They presented acute inflammation, characterized by diffuse and/or focal affectation of all lobes, with a mild to severe inflammatory infiltration of polymorphonuclear cells, sometimes forming segmentary abscesses, and with mild to moderate infiltration of alveolar macrophages. Gram-negative bacterial colonies and alveolar hemorrhagic areas were also observed.

Therapeutic efficacy in experimental pneumonia. The survival rate of each group of mice at 72 h is shown in Table 3. Survival rates in the control groups were 26.7% (95% inhibitory concentration [IC_{95}], 4.3 to 49.1) and 40% (IC_{95}, 15.2 to 64.8) for strains C2 and C2(pMG248), respectively. In the treated groups, the survival rates of animals infected with strain C2 were 86.6% (IC_{95}, 69.4 to 100) for imipenem and 100% for cefepime. The two treatment groups showed an improved survival rate when compared with the control group ($P < 0.003$), with no difference between either antimicrobial. For strain C2(pMG248), the survival rates were 86.6% (IC_{95}, 69.4 to 100) for imipenem and 40% (IC_{95}, 15.2 to 64.8) for cefepime. Imipenem was the only therapy that significantly decreased the mortality rate when compared with both the control group and the group treated with cefepime ($P < 0.01$).

Bacterial counts found in the lungs of the different groups are shown in Table 3 and Fig. 2. For *K. pneumoniae* C2, cefepime and imipenem significantly cleared the bacteria from the lungs when compared with the control group (1.74 ± 0.75 and 3.38 ± 1.06 versus 9.16 ± 2.16 CFU/g, $P < 0.01$), with cefepime being the most active agent ($P < 0.001$). For CMY-2 producing *K. pneumoniae* C2(pMG248), both agents showed significant clearance of bacteria, reducing lung bacterial counts when compared with the controls (4.33 ± 1.65 for cefepime and 4.06 ± 1.20 for imipenem versus 9.07 ± 2.75 log CFU/g for controls, $P < 0.001$).

There was no difference between either group treated with imipenem when survival rates and bacterial clearance from the lungs were compared. Survival with cefepime was higher in the group infected with *K. pneumoniae* C2 than in that infected with *K. pneumoniae* C2(pMG248) ($P < 0.0001$). In the same way, bacterial clearance was better with cefepime in the *K. pneumoniae* C2 group when compared with *K. pneumoniae* C2(pMG248) ($P < 0.01$).

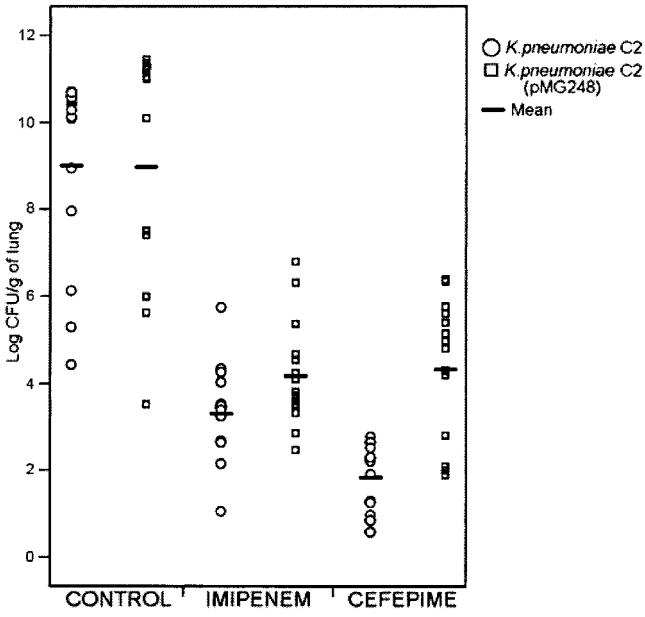

FIG. 2. Effect of antibiotic therapy on the clearance of *K. pneumoniae* from mice lungs.

Selection of resistant mutants. When lung homogenates from 15 mice infected with *K. pneumoniae* C2 and treated with cefepime or imipenem were used for selection of resistant mutants, there were no colonies recovered on plates containing 4× MIC. No bacterial growth was obtained from any lung homogenate of the 15 animals treated with imipenem and infected with *K. pneumoniae* C2(pMG248). For the group treated with cefepime, mutants appeared at a frequency of 10^{-2} to 10^{-3} in 5 of the 15 animals. When disk diffusion assay was used to evaluate the activity of cefepime against these mutants, zone diameter decreased from 16 mm (in the parental strain) to ≤6 mm for all strains tested.

When MHB-grown *K. pneumoniae* C2 was used for selection of resistant mutants, frequency of mutation was 5×10^{-7} and 3.5×10^{-7} for imipenem and cefepime, respectively. When *K. pneumoniae* C2(pMG248) was used, no mutants were detected for imipenem, although a frequency of mutation of 2×10^{-5} was observed for cefepime. Twenty independent colonies were

TABLE 3. Effect of antibiotic therapy on the survival and clearance of *K. pneumoniae* from mouse lungs

Treatment	Dosage	*K. pneumoniae* C2			*K. pneumoniae* C2(pMG248)		
		n	Survival (%)	Log CFU/g of lung	*n*	Survival (%)	Log CFU/g of lung
Control		15	4 (26.7)	9.16 ± 2.16	15	6 (40)	9.07 ± 2.75
Imipenem	30 mg/kg every 3 h	15	13 (86.6)[a]	3.38 ± 1.06[a]	15	13 (86.6)[a]	4.06 ± 1.20[a]
Cefepime	60 mg/kg every 4 h	15	15 (100)[a]	1.74 ± 0.75[a,b]	15	6 (40)[a,b]	4.33 ± 1.65[a]

[a] $P < 0.01$ compared with the control group.
[b] $P < 0.01$ compared to imipenem.

selected from individual plates with bacterial growth, as previously indicated, but in all cases zone diameters were equal to parental strains. This indicated that none of the chosen colonies contained true (as herein defined) antibiotic-resistant mutants.

DISCUSSION

PACBLs are particularly frequent in *K. pneumoniae* and *K. oxytoca* strains isolated in the hospital setting, although they have also been detected in other enterobacteria (1, 5, 6, 10, 11, 12, 26, 27). *K. pneumoniae* strains expressing PACBL are usually susceptible in vitro to zwitterionic cephalosporins and carbapenems, unless other mechanisms of resistance coincide (5, 6, 20, 33, 17, 23). This may be related to the increased stability of such compounds with respect to AmpC-type beta-lactamases and their excellent penetration through the outer membrane (18, 20). Recently, carbapenem-resistant *K. pneumoniae* isolates have been described whose resistance was attributed to the lack of an outer membrane protein in combination with PACBL ACT-1 and CMY-4 (5, 6, 33). In some PACBL-producing *K. pneumoniae* strains which lack major porins, cefepime was active, although a marked inoculum effect was observed (20). CMY-2 is one of the most prevalent PACBLs (27). Strains expressing this enzyme are usually susceptible to both cefepime and imipenem.

In the present study, when CMY-2 was expressed in *K. pneumoniae* deficient in the two major porins (OmpK35 and OmpK36), the strain became resistant to imipenem and an important increase in the cefepime MIC was observed. At a high inoculum, *K. pneumoniae* C2(pMG248) became resistant to both agents, although the effect was slightly more pronounced for cefepime. The in vivo efficacy of cefepime and imipenem against CMY-2 producing *K. pneumoniae* deficient in major porins is currently unknown. To answer this question, a pneumonia model using mice was chosen in the present study because of its resemblance to the pathogenesis of pneumonia in human beings (8). The pathological findings in the model confirmed that animals developed pneumonia independently of CMY-2 expression. The bacterial counts of control animals were significantly higher than those obtained for treated animals, permitting evaluation of the therapeutic value of the two β-lactams tested in this study. For the animal model, doses of 30 mg/kg every 3 h and 60 mg/kg every 4 h were chosen for imipenem and cefepime, respectively, in order to obtain a ΔT/MIC against *K. pneumoniae* C2 higher than 40% of the dosing interval with both antibiotics. Both β-lactams tested showed in vivo activity against *K. pneumoniae* C2, as inferred from the significantly higher survival rates and the lower lung bacterial counts in treated mice when compared to controls. The two treatment groups showed a similar improved survival rate when compared to the control group. On the other hand, in terms of lung bacterial count, while both antibiotics decreased the bacterial lung concentration with respect to the untreated mice, cefepime clearance was significantly greater than that of imipenem. Although time above MIC was >40% of the dosing interval for both antibiotics, it was 74% for cefepime and 43% for imipenem, which may explain this difference.

When animals infected with the CMY-2-producing strain were used, the survival rate was significantly greater for imipenem than for cefepime, and survival with the latter agent did not increase in comparison to that obtained in the controls. The cefepime results for animals infected with the CMY-2-producing strain were worse than those obtained for animals infected with the parental strain lacking this beta-lactamase, although lung bacterial counts in animals subjected to both treatments were similar (and lower than those of the controls), while time above MIC was 11.3% and 31.7% for imipenem and cefepime, respectively. We don't think there is any discrepancy between the results of cefepime in terms of survival or bacterial clearance because, in both variables, cefepime showed less therapeutic effect against C2(pMG248) than against the C2 strain. It seems, therefore, that, in this model, survival is a more sensitive end point than bacterial clearance from the lungs. We think that the poor results in terms of survival with cefepime, in the group infected by the C2(pMG248) strain, is due to the lower bactericidal activity of this agent and for its capability to induce the appearance of resistant mutants; moreover, the low survival in this group may not be a consequence of endotoxin release, because of the high survival in the group infected with the C2 strain in which the bactericidal in vivo activity was higher. The in vivo activity of imipenem, however, was similar against both strains in terms of survival rate and bacterial clearance of the lung, which may be explained by several factors discussed below.

The in vitro data of the activity of cefepime and imipenem support these in vivo results and expand our previous (20) observations. The MICs indicate good activity of both cefepime and imipenem against *K. pneumoniae* C2. Both agents, however, showed reduced activity (particularly using high inocula) against the CMY-2 producing transconjugant, with the inoculum effect being slightly more pronounced for cefepime. Time-kill studies also supported these findings. Cefepime was less bactericidal than imipenem. Bacterial regrowth was observed at 8 h incubation with 1× MIC of cefepime for the CMY-2-producing strain. This regrowth was not observed for imipenem even after 24 h of incubation. The clinical significance of bacterial regrowth is currently unclear but reinforces the importance of an adequate cefepime administration schedule for maintaining concentrations which are above the MIC between dosage intervals (2). Finally, imipenem, but not cefepime, showed a PAE effect against CMY-2-producing *K. pneumoniae*. It was surprising that imipenem did not show any PAE effect against the parental *K. pneumoniae* C2 strain. Although we do not have a precise explanation for this finding, it could related, at least in part, to the fact that PAE was calculated as a function of the MIC of imipenem for either *K. pneumoniae* C2 or its CMY-2-producing derivative, and for this reason the absolute amounts of imipenem were much higher for the CMY-2-producing strain.

An additional explanation for therapy failure with antimicrobial agents might be the emergence of resistant mutants. We used two different approaches for mutant selection and, in general, similar results were obtained with both methods. Although bacterial growth was noted on plates containing 4× MIC of cefepime (both strains) and imipenem (CMY-2-producing strain), the only true mutants were isolated by cefepime from the in vivo grown *K. pneumoniae* C2(pMG248). In the other cases, the organisms were not actual mutants, and it is

possible that they represent organisms able to survive the bactericidal activity of cefepime by mechanisms yet to be determined (16). The genetic basis of the truly cefepime-resistant mutants is not known at this moment, but preliminary results indicate that they are overproducers of β-lactamase. Further studies on this matter and on the possible clinical relevance of these mutants are in progress.

In summary, both in vitro and in vivo results obtained from this study indicate that, in terms of animal survival, imipenem is superior to cefepime in the treatment of murine pneumonia caused by a porin-deficient *K. pneumoniae* expressing CMY-2. Furthermore, it has been shown that CMY-2 should be more able than other β-lactamases to develop the ability to confer higher levels of cefepime resistance (3), which might endanger the future use of this agent in infections caused by CMY-2-producing bacteria.

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