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Pharmacokinetic/pharmacodynamic assessment of the in-vivo efficacy of imipenem alone or in combination with amikacin for the treatment of experimental multiresistant *Acinetobacter baumannii* pneumonia

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ABSTRACT

A guinea-pig pneumonia model involving imipenem-susceptible and imipenem-resistant strains of *Acinetobacter baumannii* was developed to assess the in-vitro and in-vivo activities of imipenem, alone or in combination with amikacin, and the pharmacokinetic and pharmacodynamic parameters. Serum levels were measured by bioassay (imipenem) or immunoassay (amikacin), followed by calculation of pharmacokinetic and pharmacodynamic parameters (C_{max}, AUC, $t_{1/2}$, C_{max}/MIC, AUC/MIC, and Δt/MIC). In-vivo efficacy was evaluated by comparing bacterial counts in the lungs of treatment groups with end-of-therapy controls by ANOVA and post-hoc tests. Decreases in the C_{max} (13.4%), AUC (13%), $t_{1/2}$ (25%) and Δt/MIC (11.8–32.2%) of imipenem were observed when it was administered with amikacin, compared with administration of imipenem alone. Similarly, decreases in the C_{max} (34.5%), AUC (11.6%), C_{max}/MIC (34.5%) and AUC/MIC (11.7%) of amikacin were observed when it was administered with imipenem. Bacterial counts in lungs were reduced by imipenem (p 0.004) with the imipenem-susceptible strain, and by amikacin (p 0.001) with the imipenem-resistant strain. The combination of imipenem plus amikacin was inferior to imipenem alone with the imipenem-susceptible strain (p 0.01), despite their in-vitro synergy, and was inferior to amikacin alone with the imipenem-resistant strain ($p < 0.0001$). In summary, combined use of imipenem with amikacin was less efficacious than monotherapy, probably because of a drug–drug interaction that resulted in decreased pharmacokinetic and pharmacodynamic parameters for both antimicrobial agents.

Keywords *Acinetobacter baumannii*, amikacin, experimental pneumonia, imipenem, pharmacodynamics, pharmacokinetics

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INTRODUCTION

Treatment of nosocomial infections caused by *Acinetobacter baumannii* is of major concern for clinicians. The natural resistance of *A. baumannii* to adverse environmental conditions, combined with its ability to develop resistance to multiple antimicrobial agents, gives this organism all the properties necessary to be a successful nosocomial pathogen [1,2]. Nosocomial pneumonia is the most frequent infection caused by *A. baumannii*, accounting for 15–24% of all episodes of ventilator-associated pneumonia at some institutions [3,4]. Pneumonia is the major source of *A. baumannii* bacteraemia, and is associated with increased mortality [5,6]. Combination therapy with a β-lactam plus an aminoglycoside is considered the best choice for the treatment of severe infections caused by Gram-negative bacilli [7,8]. However, no controlled clinical studies have been performed to examine this approach with respect to infections caused by *A. baumannii*. Imipenem has been considered the treatment of choice for *A. baumannii* pneumonia [9,10], but is gradually

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losing activity against an increasing number of *A. baumannii* isolates at many institutions [11,12].

The aims of the present study were to compare the in-vitro and in-vivo activities of imipenem in the treatment of experimental pneumonia induced by multiresistant strains of *A. baumannii* with different susceptibilities to carbapenems, and to assess the possible in-vivo therapeutic benefit of combining imipenem with amikacin. For this purpose, a model of *A. baumannii* acute pneumonia was developed in immunocompetent guinea-pigs.

MATERIALS AND METHODS

Bacteria

Two multiresistant strains of *A. baumannii*, isolated originally from the respiratory tract specimens of two patients with nosocomial pneumonia, were used. Both strains were identified by the MicroScan system (Baxter Diagnostics, Deerfield, MA, USA), the API 20NE system (bioMérieux, Marcy L'Etoile, France) and growth tests [13]. Both strains were used to induce acute pneumonia in the experimental model (see below). The first strain (SS) was susceptible to imipenem and amikacin (MICs/MBCs of 1/2 and 4/8 mg/L, respectively), while the second strain (RS) was resistant to imipenem and susceptible to amikacin (MICs/MBCs of 16/64 and 4/4 mg/L, respectively). Both strains were resistant to piperacillin, ampicillin, ticarcillin, amoxycillin–clavulanate, piperacillin–tazobactam, ceftriaxone, cefotaxime, ceftazidime, aztreonam, gentamicin, tobramycin, netilmicin, ofloxacin and ciprofloxacin.

Animals

Dunkin–Hartley female guinea-pigs (250–300 g) were used for in-vivo studies. These were certified pathogen-free and were assessed for genetic authenticity.

Drug pharmacokinetics and pharmacodynamics

Antimicrobial agents were laboratory standard powders and were used immediately after being dissolved and diluted. Serum levels of imipenem (Merck Sharp & Dohme, Madrid, Spain) and amikacin (Normon SA, Madrid, Spain) were measured after a single intramuscular injection in the thigh of the animals, following administration either alone (imipenem 60 mg/kg or amikacin 15 mg/kg) or in combination (at the same dosages, with each drug in opposite thighs). Blood was taken from anaesthetised guinea-pigs by intracardiac needle-aspiration after 10, 15, 30, 60, 90, 120 and 150 min, with groups of three animals for each time-point.

Drug concentrations in sera were measured by bioassay for imipenem [14] and by immunoassay for amikacin (Emit Amikacin Test; Syva, Cupertino, CA, USA) [15]. The bioassay used *Micrococcus luteus* strain ATCC 9341 as the reference standard; the standard curve of imipenem was a straight line for the range 0.25–2 mg/L, and was used to calculate the imipenem concentrations in guinea-pig serum samples by the linear regression model. For determining imipenem levels after combined administration, and in order to inactivate amikacin, polyanetholesulphonic acid 1% w/v (Sigma Chemical Co., St Louis, MO, USA) was added before the bioassay was performed [16].

The detection limit, intra- and inter-assay variability, and lineality for imipenem were 0.09, 0.93, 7.36 and 0.96 mg/L, respectively. The maximum concentration (C_{max}; mg/L), area under the concentration–time curve (AUC; mg.h/mL) and terminal half-life ($t_{1/2}$; h) were then calculated using the program PKCALC [17]. The pharmacodynamic (PD) parameters known to correlate with the in-vivo efficacy of the antimicrobial agents were calculated for both strains [18]. Thus, the time above MIC (Δt/MIC, h) was calculated for imipenem by the construction of a regression line on the concentration–time curve, using the method of Frimodt-Möller *et al.* [19], and the C_{max}/MIC and the AUC/MIC (h) ratios were calculated for amikacin.

In-vitro studies

MICs and MBCs were measured by the tube dilution method with geometric two-fold serial dilutions of the antimicrobial agents (ranging from 0.06 to 128 mg/L) in Mueller-Hinton II Broth Cation-Adjusted (MHBCA; Becton-Dickinson, Cockeysville, MD, USA) and a final inoculum of 5×10^5 CFU/mL. Quantification of the initial inoculum and bacterial growth was by subculture on blood agar plates (Agar-Sangre Columbia; Becton Dickinson) incubated for 18–24 h at 37°C in air. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as control strains. The MIC and MBC were defined as the lowest concentration of antibiotic at which no growth was visible, and the concentration that resulted in the killing of 99.9% of the original inoculum, respectively [20].

The bactericidal activity of antibiotics was assessed by time-kill curves [21]. Tubes containing 20 mL of MHBCA without antibiotics (controls), and with concentrations of each antibiotic equivalent to the C_{max} reached in guinea-pig plasma, were inoculated separately with an aliquot from a 6-h culture of the SS and RS strains to give a final density of 5×10^5 CFU/mL. Bacterial growth in tubes was assessed after incubation for 0, 2, 4, 8 and 24 h at 37°C by subculturing 100-μL aliquots on blood agar plates and incubating for 24 h at 37°C.

The synergy of antimicrobial combinations was evaluated by the chequerboard technique, with concentrations of both antimicrobial agents ranging from 0.0625 to 1 × MIC [22]. Fractional inhibitory concentrations (FICs) of the antimicrobial agents were calculated as follows: the FIC of drug A (FIC_A) = (MIC of drug A in combination/MIC of drug A alone); similarly, the FIC of drug B (FIC_B) = (MIC of drug B in combination/MIC of drug B alone). The sum of both FICs ($\Sigma FIC = FIC_A + FIC_B$) was used to classify the combination as synergistic ($\Sigma FIC \leq 0.5$), partially synergistic ($\Sigma FIC > 0.5$ and < 1), additive ($\Sigma FIC = 1$), indifferent ($\Sigma FIC > 1$ and ≤ 4) or antagonistic ($\Sigma FIC > 4$) [22].

The pneumonia model

Pneumonia was induced by the Esposito and Pennington technique [23]. Guinea-pigs were anaesthetised by an intraperitoneal injection of ketamine (Ketolar; Parke-Davis, Barcelona, Spain) 100 mg/kg and a subcutaneous injection of lidocaine (Lincaína 5%; Braun Medical, Barcelona, Spain)

0.2 mL in the anterior portion of the neck. After aseptic surgical exposure of the cervical trachea, 0.25 mL of bacterial suspension was introduced slowly by trans-tracheal inoculation with a 25G needle. The neck incision was then surgically sutured and, finally, the animals were held in a vertical position for 1 min to ensure efficient distribution of the inoculum into both lungs.

The inoculum was measured in every experiment for both strains. First, a bacterial suspension was prepared by culture for 4 h in trypticase soy broth (TSB; Becton-Dickinson). The final inoculum was obtained by centrifuging 50 mL of the TSB culture and resuspending the bacterial pellet in 10 mL of TSB. Finally, the concentrated bacterial suspension was mixed with 10 mL of porcine mucin solution (Sigma Chemical Co.), diluted to 10% v/v in saline solution, in order to increase pathogenicity [24].

Two groups of six guinea-pigs were inoculated with the SS and RS strains, respectively, and then killed humanely after 4 h in order to confirm the development of pneumonia. For each group, the lungs of four animals were processed for microbiological studies, and those of the remaining two animals for histological studies.

Treatment

Animals were treated for 24 h, starting 4 h after bacterial challenge. Imipenem was administered intramuscularly at 60 mg/kg, three-times-daily, while amikacin was administered intramuscularly at 15 mg/kg four-times-daily. These dosages were chosen according to the results of pharmacokinetic (PK) studies and PD parameters (Δt/MIC for imipenem and C_{max}/MIC for amikacin). Eight groups of animals were evaluated, four inoculated with the SS strain and four with the RS stain. Twelve animals were assigned to each treatment group (control without treatment; treatment with imipenem or amikacin alone; and treatment with imipenem + amikacin). Animals in combination groups received imipenem and amikacin in opposite thighs in order to avoid drug interactions at the site of injection.

The guinea-pigs were killed humanely 4 h after the last dose (including the control groups, which were designated as end-of-therapy controls) and the thoracic wall was opened aseptically to expose the lungs and mediastinum. The lungs and heart were extracted *en bloc*, after which both lungs were separated and weighed. Lung tissue was processed for quantitative culture as described previously [24].

To assess any potential toxicity of the antimicrobial agents, three groups of 12 healthy guinea-pigs were given imipenem, amikacin, or imipenem + amikacin at the same dosages and intervals as the treatment groups for 72 h. Toxicity was assessed by evaluating survival rates 5 days after the first dose of antibiotics.

Statistical analysis

Quantitative lung cultures (CFU/g ± SD) of the control and treatment groups were analysed. The ANOVA test and post-hoc tests (Tukey and Dunnet) were used for group comparisons. The sizes of the groups were designed to detect a difference of 1.5 $\log_{10}$ CFU/g of lung tissue culture with α and β errors of 0.05 and 0.2, respectively. The correlation between PD (C_{max}/MIC, AUC/MIC and Δt/MIC) and in-vivo efficacy parameters was assessed using Pearson's *r* coefficient. For this purpose, treatment groups were divided into those in which imipenem was used and those in which amikacin was used. A p value of < 0.05 was considered significant.

RESULTS

In-vitro studies

Both antimicrobial agents showed bactericidal activity against the SS strain. Against the RS strain, imipenem showed bactericidal activity at 24 h, despite the relatively high imipenem MIC, but amikacin was not bactericidal. The observed discrepancy with imipenem probably reflects the different techniques used to determine MIC (broth microdilution) and bactericidal activity (time-kill curves). Results obtained with these two techniques are not directly comparable. The combination of imipenem + amikacin demonstrated synergy against the SS strain, with ΣFIC = 0.156 (with imipenem at $0.125 \times$ MIC, and amikacin at $0.25 \times$ MIC), and ΣFIC = 0.375 (with imipenem at $0.25 \times$ MIC, and amikacin at $0.125 \times$ MIC). The combination was indifferent (ΣFIC = 2) against the RS strain.

Drug PK and PD parameters

PK and PD parameters for each drug (administered alone or in combination) are shown in Tables 1 and 2. A reduction in all PK and PD parameters of imipenem was observed when imipenem was administered in combination with amikacin. Reductions in C_{max} (13.4%), AUC (13%) and $t_{1/2}$ (25%) led to decreases in Δt/MIC (11.8–32.2%). Similarly, reductions in C_{max} (34.5%) and AUC (11.6%) for amikacin were observed when amikacin was administered in combination with imipenem; these reductions resulted in subsequent reductions in C_{max}/MIC (34.5%) and AUC/MIC (11.7%).

Table 1. Pharmacokinetic parameters of imipenem and amikacin administered alone or in combination, showing the differences (%) with respect to the values reached after their independent administration

Antimicrobial agent	Doses (mg/kg)	C_{max} (mg/L)	AUC (mg.h/L)	$t_{1/2}$ (h)
Imipenem	60	21.6	23	0.5
Imipenem (+ amikacin)[a]	60	18.7 (– 13.4)	20 (– 13)	0.375 (– 25)
Amikacin	15	33	30.8	0.54
Amikacin (+ imipenem)[a]	15	21.6 (– 34.5)	27.2 (– 11.6)	0.9 (+ 66)

[a]Combined pharmacokinetics.

Antimicrobial agent	Doses (mg/kg)	C_{max}/MIC		AUC/MIC (h)		Δt/MIC (h)	
		SS	RS	SS	RS	SS	RS
Imipenem	60	NA	NA	NA	NA	2.28	0.93
Imipenem (+ amikacin)	60[a]	NA	NA	NA	NA	2.01 (– 11.8)	0.63 (– 32.2)
Amikacin	15	8.2	8.2	7.7	7.7	NA	NA
Amikacin (+ imipenem)	15[a]	5.4 (– 34.5)	5.4 (– 34.5)	6.8 (– 11.7)	6.8 (– 11.7)	NA	NA

SS, carbapenem-susceptible strain; RS, carbapenem-resistant strain; NA not applicable.
[a]Combined pharmacokinetics.

Table 2. Pharmacodynamic parameters of imipenem and amikacin administered alone or in combination, showing the differences (%) with respect to the values reached after their independent administration

Characteristics of the pneumonia model

Both strains of *A. baumannii* were isolated from the lungs of every group, with counts of 8.21 ± 0.60 and 8.63 ± 0.48 CFU/g of lung for the SS and RS strains, respectively. Histopathological studies of lungs from animals inoculated with the SS strain showed bilateral and multifocal areas of severe acute inflammatory infiltration of polymorphonuclear cells, mainly in peribronchial and perivascular spaces, with extensive areas of haemorrhagic necrosis. Similarly, the lungs of animals inoculated with the RS strain showed acute inflammatory alterations, although no necrosis was evident, moderate-to-severe congestion in vessels and alveolar septa, scarce areas of alveolar haemorrhage, and acute bronchitis with focal necrosis of the bronchial wall.

In-vivo efficacy of antimicrobial treatment

The in-vivo efficacy parameters of the different treatments are detailed in Tables 3 and 4. Most animals survived the surgical procedure for > 4 h, and 83.3–100% survived during the 24-h treatment period. In groups inoculated with the SS strain, imipenem was the only treatment that reduced bacterial counts in lungs with respect to controls (6.11 ± 0.61 vs. 6.92 ± 0.55 CFU/g; p 0.004); the combinations of imipenem + amikacin were inferior to monotherapy with imipenem (6.89 ± 0.29 vs. 6.11 ± 0.61 CFU/g; p 0.01). In groups inoculated with the RS strain, amikacin was the only treatment that reduced the bacterial counts in lungs (5.35 ± 0.45 vs. 7.31 ± 1.14 CFU/g; p 0.01). Amikacin achieved a significantly higher bacterial count reduction in lungs than did imipenem + amikacin (5.35 ± 0.45 vs. 6.59 ± 0.22 CFU/g; p 0.0001). All animals in the toxicity control groups survived.

No correlation was found between the Δt/MIC of imipenem and the in-vivo efficacy parameters. A negative correlation was found between the C_{max}/MIC (Pearson's $r = -0.22$; p 0.03) and the AUC/MIC (Pearson's $r = -0.2$; p 0.03) of amikacin and the bacterial counts in lungs from treatment groups receiving amikacin.

DISCUSSION

This study developed a new discriminative model with low mortality rates for acute pneumonia

Treatment	Animals (no.)	Inoculum (log CFU/mL)	Survival from surgery	Survival (%)	Lung cultures (log CFU/g, mean ± SD)
Controls	12	9.52	12	11 (91.7)	6.92 ± 0.55
Imipenem	12	9.59	10	10 (100)	6.11 ± 0.61[a]
Amikacin	12	9.07	12	10 (83.3)	6.58 ± 0.56
Imipenem + amikacin	11	9.56	10	10 (100)	6.89 ± 0.29[b]

[a]p 0.004 vs. control group; [b]p < 0.05 vs. imipenem group.

Table 3. Comparative efficacies of imipenem, amikacin and the combination in groups of guinea-pigs inoculated with a carbapenem-susceptible strain

Treatment	Animals (no.)	Inoculum (log CFU/mL)	Survival from surgery	Survival (%)	Lung cultures (log CFU/g, mean ± SD)
Control	12	9.47	12	10 (83.3)	7.31 ± 1.14
Imipenem	12	9.47	8	7 (87.5)	6.77 ± 0.32[a]
Amikacin	12	9.74	10	10 (100)	5.35 ± 0.45[b]
Imipenem + amikacin	12	9.34	10	9 (90)	6.59 ± 0.22[a]

[a]p < 0.05 vs. amikacin group; [b]p < 0.05 vs. control group.

Table 4. Comparative efficacies of imipenem, amikacin and the combination in groups of guinea-pigs inoculated with a carbapenem-resistant strain

caused by multiresistant *A. baumannii* in immunocompetent guinea-pigs. Trans-tracheal inoculation of bacteria was followed by the development of bilateral pneumonia in all animals, thereby resembling the pathogenesis of *A. baumannii* pneumonia in humans, and resulted in high bacterial concentrations in lungs at 28 h following inoculation. The model demonstrated that imipenem was the only treatment which reduced bacterial concentrations in the lungs of guinea-pigs inoculated with the SS strain of *A. baumannii*, while only amikacin reduced bacterial concentrations in the lungs of guinea-pigs inoculated with the RS strain. In both cases, the combination of imipenem + amikacin was inferior to monotherapy, despite demonstrated in-vitro synergy. A drug–drug interaction was apparent between imipenem and amikacin when they were used in combination in the in-vivo experiments, with a decrease in the Δt/MIC of imipenem and the C_{max}/MIC of amikacin. The decline in these key PD parameters could explain the absence of any in-vivo benefit of the combination in comparison to monotherapy.

A correlation was found between the C_{max}/MIC of amikacin and its in-vivo efficacy parameters. As already known, C_{max}/MIC is a key predictor of the antibacterial activity of aminoglycosides, and there is consensus that it should reach a value of ≥ 8 in order to ensure clinical efficacy [18]. In the present study, the C_{max}/MIC ratios were 8.4 in groups treated with amikacin monotherapy, and 5.4 in groups treated with imipenem + amikacin. In addition, the AUC/MIC of amikacin correlated with a reduction in bacterial counts in the lungs of animals treated with amikacin. This latter parameter is also known to be a reliable predictor of the clinical efficacy of aminoglycosides [18]. However, no significant correlation was found between the Δt/MIC of imipenem and its in-vivo efficacy parameters. This was surprising, considering that a Δt/MIC of at least 30–40% of dosing intervals correlates strongly with the antibacterial activity of β-lactams [18]. With respect to carbapenems, even a lower Δt/MIC (25–30%) could be enough to ensure in-vivo efficacy [18,25]. The low Δt/MIC of imipenem in the groups inoculated with the RS strain (11.6%), and the drug–drug interaction, which conditioned reductions in this PD parameter for groups treated with the combination, could account for the lack of correlation between Δt/MIC and the in-vivo activity of imipenem.

The results obtained in the present study were similar to those observed in the treatment of other experimental infections caused by *A. baumannii* [24,26]. Several possibilities could explain the observed drug–drug interaction. It is already known that aminoglycosides interact chemically with β-lactams [27,28]. This reaction results in the opening of the β-lactam ring and acylation of the amino group of the aminoglycoside, and the subsequent loss of activity of both. For this reason, these different types of antimicrobial agents should not be mixed in the same solution before infusion. However, in the present study, the antimicrobial agents were prepared independently and were injected into opposite thighs in order to avoid any interaction at the injection site. In addition, an interaction in the PK process is unlikely, knowing the PK of imipenem and amikacin [25,29], given that the reduction of virtually all PK parameters of both drugs would imply interactions in various steps (absorption, distribution, metabolism and excretion) of the PK profile. Therefore, it seems that the most likely explanation would be an in-vivo chemical interaction between the two antimicrobial agents, which would occur both in intravascular and extravascular compartments, thereby determining a decrease in PD parameters and a subsequent loss of activity of both antimicrobial agents. Such an in-vivo interaction has also been observed in anephric animals following administration of carbenicillin for 24 h in combination with different aminoglycosides [30].

There have been few studies on the clinical importance of such drug–drug interactions. Several studies have demonstrated different rates of aminoglycoside inactivation by different β-lactams (carbenicillin, ticarcillin, moxalactam) in patients treated topically or systemically [31,32], in healthy volunteers [33], or in volunteers with chronic renal failure [34,35]. Other studies failed to demonstrate any clinical benefit when combinations of β-lactams plus aminoglycosides were compared with β-lactam monotherapy for the treatment of severe infections caused by Gram-negative bacilli [36–38]. Further clinical studies analysing combined treatment with β-lactams and aminoglycosides, focusing specifically on clinical PK/PD parameters, are needed to elucidate this issue fully.

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