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Imipenem, doxycycline and amikacin in monotherapy and in combination in *Acinetobacter baumannii* experimental pneumonia

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***Acinetobacter baumannii* is a common cause of nosocomial pneumonia and other nosocomial infections. Multiresistant *A. baumannii* has also a high prevalence, which can make effective treatment difficult. We designed a new model of *A. baumannii* experimental pneumonia using C57BL/6 immunocompetent mice. This model was used to compare the efficacy of imipenem, doxycycline and amikacin in monotherapy, and the combination of imipenem plus amikacin and doxycycline plus amikacin. Doxycycline plus amikacin were synergic *in vitro* after 24 h incubation, whereas imipenem plus amikacin showed no *in vitro* synergy. The number of sterile lungs and the lung clearance of *A. baumannii* were greater in the group treated with imipenem than in those treated with amikacin or doxycycline in monotherapy ($P < 0.05$). The combination of imipenem plus amikacin and doxycycline plus amikacin was no more effective than imipenem alone in the clearance of organisms from lungs (2.42 ± 1.46 cfu/g versus 2.7 ± 1.5 cfu/g versus 1.23 ± 1.02 cfu/g). These results suggest that the addition of amikacin does not improve the results obtained by imipenem monotherapy. Doxycycline plus amikacin is an alternative to imipenem in the therapy of *A. baumannii* pneumonia.**

Introduction

Acinetobacter baumannii is a ubiquitous microorganism that colonizes the skin (25%) and pharynx (7%) of healthy, non-hospitalized adults.[1,2] At present, it is an endemic microorganism in many intensive care units (ICU),[3,4] producing intestinal colonization in 71% of patients with a length of stay in the ICU of one week.[5] Currently, it is a significant pathogen among nosocomial infections. The respiratory tract is the most frequent site of infection by *A. baumannii*, mainly as nosocomial pneumonia. In ventilator-associated pneumonia, *Acinetobacter* aetiology is a risk factor for hospital mortality.[6,7]

One of the main problems related to infection by *A. baumannii* is the recent appearance of multiresistant strains,[8–10] including resistance to imipenem.[8,10–14] This makes any type of effective treatment difficult at times. Infections produced by multiresistant *A. baumannii* strains have an attributable mortality of 25–34%;[3,11] the use of inappropriate treatment being a factor associated with poor prognosis.[11,15]

Imipenem and tetracycline are among the most active drugs against multiresistant *A. baumannii*. Vila *et al.*[9] studied the susceptibility of 54 *A. baumannii* strains to antimicrobial drugs. These were sensitive to imipenem and doxycycline in 100% and 98% of instances, respectively. On the other hand, only 55% of the strains were sensitive to ceftazidime, and 52% to ampicillin/sulbactam. In a study carried out in 1993,[11] 54% of 79 bacteraemia-producing *A. baumannii* strains were sensitive to imipenem and doxycycline, 11% to ceftazidime and 74% to ampicillin/sulbactam.

The purpose of this study was to compare the *in vitro* and *in vivo* activity of imipenem, a drug clinically effective against *A. baumannii*, with doxycycline, usually not used for infections produced by Gram-negative bacilli (GNB),

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but to which *A. baumannii* is susceptible *in vitro*. The efficacy of monotherapy was compared with combined treatment with amikacin. For these experiments we have developed a new *Acinetobacter* experimental pneumonia model in immunocompetent mice.

Material and methods

In vitro studies

Microorganisms. A. baumannii strains isolated from blood cultures were used and identified by the MicroScan system (Baxter Health Care, West Sacramento, CA, USA), the API2ONE system (bioMérieux, Marcy 1'Étoile, France) and growth tests.[16] After preliminary tests, strain 731015 of *A. baumannii* was selected for the study. This strain was selected because of its capacity to produce invasive infection in humans, its sensitivity to the antibiotics evaluated in the study, and its capacity to produce pneumonia in the experimental model. In order to increase the virulence of this strain before it was used in the experimental model, two successive intraperitoneal injections were made in mice, administering 0.5 mL of the bacterial suspension with McFarland 0.5 and recovering *A. baumannii* 24 h later from the splenic homogenate.

Antibiotics. Imipenem was obtained from Merck, Sharp & Dohme (Madrid, Spain), doxycycline from Pfizer (Madrid, Spain) and amikacin from Normon, SA (Madrid, Spain). These agents were used as standard laboratory powders and prepared for the *in vitro* studies according to the guidelines from the National Committee for Clinical Laboratory Standards.[17]

Determination of MICs and MBCs. MICs were measured in Mueller–Hinton II Broth Cation-Adjusted (MHBCA, Becton-Dickinson, Cockeysville, MD, USA) by the tube dilution method.[17] Each tube contained antibiotic concentrations ranging from 0.06 to 128 mg/L for imipenem and amikacin and from 0.06 to 1024 mg/L for doxycycline, with a final bacterial concentration of 5×10^5 cfu/mL. Quantification of the initial inoculum and bacterial growth was carried out by serial dilutions in saline solution and subcultures on agar–blood plates 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 was defined as the lowest concentration of antibiotic at which no growth was visible to the naked eye. The MBC was defined as the lowest concentration of antibiotic resulting in the killing of 99.9% of the original inoculum.[18]

Time–kill curves. The *in vitro* bactericidal activity of the different antimicrobials and the possible *in vitro* synergy of the combination of imipenem plus amikacin and doxycycline plus amikacin were evaluated by time–kill curves.[18] We used 20 mL of MHBCA, with concentrations of each drug equivalent to 1 × MIC, 2 × MIC and 4 × MIC, and an inoculum size of 5×10^5 cfu/mL of the *A. baumannii* strain. Tubes with 20 mL of MHBCA, the bacterial inoculum and no antimicrobial drug were used as growth controls. A tube with 20 mL of MHBCA broth and containing neither bacterial inoculum nor drug was used as sterility control. The bacterial growth in both test and control tubes was counted at 0, 2, 4, 8 and 24 h after incubation at 37°C. Ten-fold dilutions were made and 100 μL was subcultured on to sheep blood agar plates (Agar-Sangre Columbia, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) incubated for 24 h at 37°C. An antibiotic was considered bactericidal if a $\geqslant 3 \log_{10}$ decrease in cfu/mL was reached relative to the initial inoculum.[18] The lowest concentrations of the antimicrobial agents that showed bactericidal activity in the time–kill studies were chosen for the synergy studies, being 1 × MIC for imipenem and amikacin and 4 × MIC for doxycycline. Synergy was defined as a 2 $\log_{10}$ decrease in cfu/mL when using the drug combination, relative to the most active component alone.[18]

In vivo studies

Mice. The experiments were performed in immunocompetent C57BL/6 female mice weighing 14–16 g. The mice were obtained from BK Universal Ltd (Barcelona, Spain); they had a sanitary status of MPF (murine pathogen free) and were assessed for genetic authenticity.

Drug pharmacokinetics. The levels of each drug in plasma were determined after the administration of single doses. Imipenem and amikacin were administered intramuscularly (im), while doxycycline was administered intraperitoneally (ip). The dosage administered was 30 mg/kg for imipenem, 7.5 mg/kg for amikacin and 25 mg/kg for doxycycline. After 10, 15, 30, 60, 90, 120 and 150 min, blood was extracted from an incision in the periorbital plexus in anaesthetized mice, in groups of three mice for every time-point. The drug concentrations in plasma were measured by a bioassay method,[19] using *Micrococcus luteus* ATCC 9341 for imipenem, *E. coli* ATCC 10536 for doxycycline and *Staphylococcus epidermidis* ATCC 27626 for amikacin. The maximum concentration (C_{max}; mg/L), the area under the concentration–time curve (AUC; μg·h/L), the terminal half-life ($t_{1/2}$; h) and the time during which the plasma concentration remained >MIC (ΔT/MIC, h) were determined.

Experimental pneumonia from A. baumannii. A modification of Esposito and Pennington's model[20] was used for the production of pneumonia. The mice were anesthetized by an ip injection of 5% sodium thiopental. They were suspended vertically and the trachea was then cannulated with a blunt-tipped metal needle. The feel of the needle tip against the tracheal cartilage confirmed the intratracheal

location. A microlitre syringe (Hamilton Co., Reno, NV, USA) was used for the inoculation. The mice remained in a vertical position for 3 min and then in a 30° position until awake. To obtain experience with this technique, we performed preliminary experiments using instillation of methylene blue due, confirming by autopsy the distribution of the dye in both lungs and its absence in the upper gastrointestinal tract.

The inoculum size was of approximately 10^8 cfu/mL, obtained through a 4 h culture of *A. baumannii* in trypticase soy broth (TSB, Becton-Dickinson Microbiology Systems) at 37°C. The size of the inoculum was determined for each experiment. In preliminary experiments the inoculum that was 100% lethal was determined by using two types of inoculum: (A) 50 μL bacterial suspension (10^8 cfu/mL), (B) 25 μL bacterial suspension (10^8 cfu/mL) plus 25 μL porcine mucin diluted to 10% in saline solution (M-2378; Sigma Chemical Co., St Louis, MO, USA). Two groups of 40 and 41 mice, were inoculated with each type of inoculum, respectively. The animals were observed for 72 h and the cumulative survival rates were recorded every 12 h. The surviving mice were killed after 72 h. All the mice were analysed immediately after death. Lung and blood samples were taken, as detailed below. Of the lung samples, 50% were processed for microbiological studies and the other 50% for histological studies. All the blood samples were processed for microbiological studies.

Six mice were inoculated with 50 μL porcine mucin diluted to 10% in saline solution to confirm that the mucin was not directly toxic to these animals (mucin control group).

Antimicrobial treatment. To evaluate the effectiveness of the different treatment regimens, 95 mice were inoculated with the bacterial suspension plus porcine mucin, diluted to 10%, and then divided into six groups. The first group ($n = 20$) did not receive antimicrobial treatment for 72 h (control group). The other five groups received over 72 h the following treatments: imipenem im, 120 mg/kg/day, tid ($n = 10$); amikacin im, 15 mg/kg/day, bid ($n = 14$); doxycycline ip, 50 mg/kg/day, bid ($n = 14$); imipenem plus amikacin ($n = 14$) and doxycycline plus amikacin ($n = 15$). The first dose of every antibiotic was administered 4 h after inoculation of the organism. In order to confirm that these drugs were not directly toxic to the animals, groups of ten uninfected mice were given each antibiotic for 72 h (uninfected treated groups).

The animals were observed for 72 h of treatment and the cumulative survival rates were recorded every 12 h. The surviving mice were killed 4 h after the last dose by ip administration of 5% sodium thiopental. Lung and blood samples were taken, as detailed below.

Microbiological and histopathological studies. After the animals' death, thoracotomy was carried out. Blood samples were collected by cardiac puncture. Then the heart and both lungs were extracted together and the lungs were later separated on a sterile Petri dish and weighed.

In the microbiological study the lungs were processed for quantitative culture, after being homogenized for 2 min in 2 mL of sterile saline solution (Stomacher 8, Tekmar Co., Cincinnati, OH, USA). After ten-fold dilutions, aliquots of 100 μL were plated on Columbia sheep blood agar plates for 24 h at 37°C. The results are expressed as mean ± S.D. of the $\log_{10}$ cfu/g of tissue. When the cultures were sterile they were assigned the value of $\log_{10}$ corresponding to the sensitivity level of the method (1 cfu). For the blood cultures the samples were inoculated in sterile tubes with 3 mL of TSB and incubated for 24 h at 37°C, then 100 μL of each sample was plated on sheep blood agar plates and incubated for another 24 h at 37°C. The results of the blood cultures are expressed as positive or negative.

Histopathological study. The lungs were fixed with 10% formaldehyde for histopathological study. The lung blocks were embedded in paraffin wax and cut into 4 μm sections. The slices included all the pulmonary lobes to be studied by optical microscopy. They were processed according to standard methods for haematoxylin and eosin, PAS, Gram, Masson's trichromic stain and silver reticulin stains.

Statistical analysis. The following variables were analysed: survival, cfu/g of lung tissue, lung and blood sterility. The two-tailed Fisher's test, test of homogeneity of variances, and the post-hoc tests (Dunnet and Newman-Keuls tests) were used. A P value <0.05 was considered significant.

Results

Characterization of experimental pneumonia by A. baumannii

In the group of mice receiving inoculum (A) (50 μL of the bacterial suspension), 30 of 40 mice (75%) died 12–60 h after the inoculation (25% survival). In the group of mice receiving inoculum (B) (50 μL of a mixture of the bacterial solution plus porcine mucin), 100% of the mice died in the first 48 h after inoculation. Therefore, this second lethal inoculum was used in all subsequent experiments. There was no mortality in the group inoculated with 50 μL of porcine mucin without bacterial suspension.

In group A the microbiological study was carried out on the lungs of 21 mice (16 dead and 5 killed). *A. baumannii* was isolated in all the lungs, with a count of 7.96 ± 3.34 cfu/g of lung. Differences were found in the $\log_{10}$ cfu/g of lung among the dead animals and those killed (9.47 ± 1.27 versus 3.12 ± 3.37 $\log_{10}$ cfu/g, $P = 0.002$). In group B the microbiological study was carried out for the lungs of 20 mice. *A. baumannii* was isolated in all the lungs, with a count of 10.35 ± 0.4 $\log_{10}$ cfu/g of lung. There was no difference in the bacterial count between the dead mice in group A and

group B. All the mice of groups A and B were bacteraemic, and *A. baumannii* was recovered from intracardiac blood.

Histopathological studies were made of the lungs of 19 mice from group A (14 dead and 5 killed) and 21 mice from group B. All the mice in groups A and B showed alterations compatible with pneumonia (Figure). Two histopathological patterns were present: acute and chronic inflammation. Acute inflammation was characterized by diffuse and/or

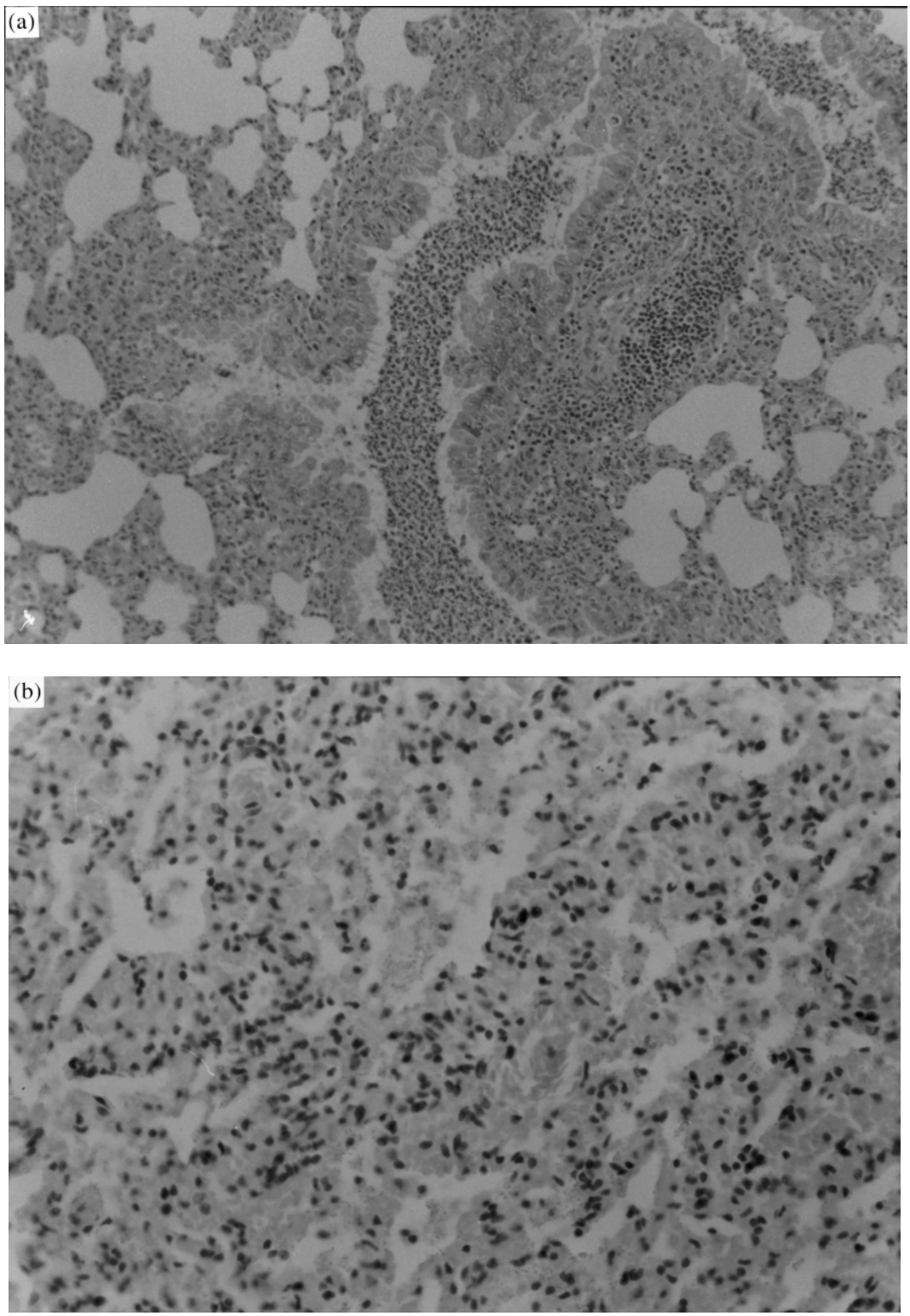

Figure. Histopathological findings for lung tissues of mice inoculated with 50 μL of 10^8 cfu/mL of *A. baumannii* solution. H&E × 100. (a) Acute bronchial and alveolar inflammation. (b) Severe congestion in pulmonary vessels.

focal affectation of all lobes, with mild to severe inflammatory infiltration of polymorphonuclear cells, sometimes forming segmentary abscesses, and mild to moderate infiltration of alveolar macrophages. Accumulation of Gram-negative bacterial colonies in alveoli and, to a lesser extent, in bronchioles, mild to severe congestion in vessels and alveolar septa, and alveolar haemorrhagic areas were also observed. In the chronic inflammation there was focal involvement, with mild peribronchial and perivascular lymphocytic infiltration, as well as a moderate presence of alveolar macrophages. Alveolar collapse, desquamation and mild fibrosis were present. There was also mild acute inflammation in localized areas and an absence of bacterial colonies. The pattern of acute inflammation was evident in all the dead mice of groups A and B, with a discrete decrease in the polymorphonuclear cell infiltration in the mice of group B. The pattern of chronic inflammation was found in those mice of group A that survived the pneumonia.

In vitro tests

Determination of MIC and MBC. The *A. baumannii* strain used was sensitive to the three antimicrobial drugs tested. Imipenem was the most active drug evaluated: MIC 0.12 mg/L and MBC 0.25 mg/L (Table I).

Time–kill curves. Both imipenem and amikacin showed bactericidal activity in the time–kill curves. Imipenem was bactericidal after 8 h at any concentration $\geq 1 \times$ MIC. Amikacin, however, showed a clear dose–effect relationship, being bactericidal after 4 h for concentrations of $1 \times$ MIC and $2 \times$ MIC, and after 2, 4, 8 and 24 h for a concentration of $4 \times$ MIC. Doxycycline plus amikacin was synergic after 24 h of incubation, whereas imipenem plus amikacin showed no *in vitro* synergy.

Table I. MICs and MBCs of antibiotics against *A. baumannii* 731015 strain

Antibiotics	MIC (mg/L)	MBC (mg/L)
Imipenem	0.12	0.25
Amikacin	2	16
Doxycycline	0.25	256

Pharmacokinetics. The pharmacokinetic parameters of each antimicrobial drug used in the mice are shown in Table II. The C_{max} was reached after 10 min for doxycycline and amikacin and after 15 min for imipenem.

Therapeutic efficacy

Survival. Survival in each group of mice is shown in Table III. The survival was 0% in the control group, all mice dying in the first 48 h. In the treated groups the survival rates after 76 h were 90% for imipenem, 79% for amikacin, 50% for doxycycline, 86% for imipenem plus amikacin and 73% for doxycycline plus amikacin. All the treatment groups showed an improved survival rate compared with the control group ($P < 0.01$), with no difference between the different groups of antimicrobial drugs. Survival in the uninfected treated groups was 100%.

Bacterial clearance from lung. There were no sterile lungs in the control group nor in those treated with doxycycline alone. In the remaining treated groups the lungs were sterile in 55.5% of those treated with imipenem, 9% of those who had received amikacin, 33% of those who had received imipenem plus amikacin and 27% of those treated with doxycycline plus amikacin (Table IV). The number of sterile lungs was greater in the groups treated with imipenem alone, imipenem plus amikacin or doxycycline plus amikacin ($P < 0.05$) than in the control group or the groups treated with doxycycline or amikacin.

The bacterial counts found in the lungs of the two different groups are shown in Table IV. All the groups receiving treatment showed higher lung clearance of *A. baumannii* than did the control group ($P < 0.05$). Imipenem monotherapy was better than amikacin or doxycycline ($P < 0.05$). There were no differences in the lung clearance of *A. baumannii* among the imipenem, imipenem plus amikacin and doxycycline plus amikacin groups.

Bacterial clearance from blood. All the mice in the control group were bacteraemic. In the group treated with doxycycline 71% of the surviving mice were bacteraemic, with

Table II. Antibiotic pharmacokinetics in mice

Antibiotic	Mice plasma					
	doses (mg/kg)	C_{max} (mg/L)	$t_{1/2}$ (h)	AUC (mg·h/L)	Δt/MIC (h)	C_{max}/MIC
Imipenem	30	16.9	0.15	8.25	2.01	140
Amikacin	7.5	18.88	0.61	18.25	1.38	9.44
Doxycycline	25	15.99	0.2	5.48	0.86	63.9

Table III. Effect of antibiotic therapy on survival

Time after infection (h)	No. of survivors (%)					
	control ($n = 20$)	imipenem ($n = 10$)	amikacin ($n = 14$)	doxycyclin ($n = 14$)	imipenem + amikacin ($n = 14$)	doxycyclin + amikacin ($n = 15$)
12	20 (100)	10 (100)	13 (93)	13 (93)	13 (93)	15 (100)
24	10 (50)	10 (100)	13 (93)	13 (93)	13 (93)	14 (93)
36	1 (5)	10 (100)	13 (93)	11 (78.5)	12 (86)	12 (80)
48	0	10 (100)	12 (86)	7 (50)	12 (86)	11 (73)
60		10 (100)	11 (79)	7 (50)	12 (86)	11 (73)
72		9 (90)	11 (79)	1 (50)	12 (86)	11 (73)
76		9 (90)[a]	11 (79)[a]	7 (50)[a]	12 (86)[a]	11 (73)[a]

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

Table IV. Effect of antibiotic therapy on the clearance of *A. baumannii* from the lungs and blood

Antibiotic	Lung $\log_{10}$ cfu/g means ± S.D.	Lung no. sterile / no. analysed (%)	Blood no. negative / no. blood cultures (%5)
Control (20)	10.35 ± 0.4	0/20 (0)	0/41 (0)
Imipenem (10)	1.23 ± 1.02[a]	5/9 (55.5)*a*	9/9 (100)[a]
Amikacin (14)	3.64 ± 1.52[a,b]	1/11 (9)	11/11 (100)[a]
Doxycyclin (14)	4.43 ± 1.52[a,b]	0/7 (0)	2/7 (28.5)
Imipenem + amikacin (14)	2.42 ± 1.46[a]	4/12 (33)[a]	12/12 (100)[a]
Doxycyclin + amikacin (15)	2.7 ± 1.5[a]	3/11 (27)[a]	11/11 (100)[a]

[a] $P < 0.05$ compared with the control group.
[b] $P < 0.05$ compared with the imipenem group.

no differences from the control group (Table IV). No surviving mice had bacteraemia in the remaining treated groups; this was a statistically significant difference compared with the control mice and the group treated with doxycycline ($P < 0.01$).

Discussion

In the treatment of *A. baumannii* pneumonia in human beings there are no data relating to whether or not a combination of imipenem with an aminoglyoside is better than imipenem alone. Therefore, the main goal of this study was to compare the *in vitro* and *in vivo* efficacy of imipenem alone or in combination with amikacin in the treatment of *A. baumannii* pneumonia, using a new experimental model in immunocompetent mice. Owing to the frequency of imipenem-resistant strains and the need to find new therapeutic approaches, we have compared these antibiotics, used frequently in infections caused by this organism, with doxycycline alone and in combination with amikacin. Doxycycline is an antibiotic not commonly used for *A. baumannii* infections, but to which a high percentage of *A. baumannii* strains are susceptible.[9,11]

The experimental pneumonia model in immunocompetent mice used in this study is, to our knowledge, the first model of *A. baumannii* pneumonia with these characteristics. Recently, Joly-Guillou[21] described a model for experimental *A. baumannii* murine pneumonia, using neutropenic mice. However, the use of immunocompetent mice permits a more exact reproduction of the lung infection produced by this organism, since *A. baumannii* pneumonia occurs more frequently in immunocompetent patients undergoing invasive treatment, such as mechanical ventilation.[11,22] On the contrary, pneumonia caused by *A. baumannii* is much less frequent in neutropenic patients. The inoculation method used in this model is a variation of the classic pneumonia model by aspiration described by Esposito and Pennington.[20] This form of inoculation is reproducible, as we have proven with the preliminary experiments with methylene blue dye.

Preliminary studies showed that an inoculum size of

10^7 cfu/mL of *A. baumannii* did not produce pneumonia in all the mice (data not shown). In the final experiments the transtracheal instillation of 10^8 cfu/mL of *A. baumannii* caused a high mortality rate (75%). This mortality was related to the microbiological findings (9.47 $\log_{10}$ cfu/g in the dead mice versus 3.12 $\log_{10}$ cfu/g in the survivors) and to the histopathological pattern of acute pulmonary inflammation. The mortality was similar to that obtained in studies made on immunocompetent mice using other GNB[20,23] or on neutropenic mice using *A. baumannii*.[21]

In order to achieve a fatal pneumonia in 100% of the challenged immunocompetent mice and to permit more effective evaluation of the efficacy of the antimicrobial drugs, we inoculated the mice with a mixture of the bacterial suspension and mucin. The mortality was 100% in the first 48 h, with an *A. baumannii* concentration of 10.35 $\log_{10}$ cfu/g of lung. Mucin has been used previously in animal models, such as the murine model of systemic infection through intraperitoneal inoculation of pneumococci,[24] *A. baumannii*, and other GNB.[25] In these models the use of mucin also achieved an increase in the pathogenicity of the inoculum, producing greater mortality or reducing the LD100. The mucin was not a nutritional factor facilitating bacterial growth. Thus, there were no differences in the number of cfu/g of lung between the dead animals inoculated with the bacterial suspension alone and those inoculated with the mixture of mucin in our experiments. The mucin control group proves that mucin alone has no influence on mortality.

In the *in vitro* studies the bactericidal activities determined by the MBC and the time–kill curves did not agree for imipenem and amikacin. Both antimicrobials showed bactericidal activity in the time–kill curves at a concentration equal to the MIC. However, the MBC of imipenem was twice the MIC and the MBC of amikacin was eight times the MIC. This difference has been reported in different studies; the bactericidal activity evaluated by time–kill curves is the parameter that correlates best with *in vivo* activity.[18,26]

Imipenem demonstrated bactericidal activity after 8 h in the time–kill curves at any concentration $\geq 1 \times$ MIC, in agreement with the pharmacodynamics of the β-lactams, which show concentration-independent or time-dependent bactericidal activity.[26] However, the results with amikacin also suggest concentration-dependent bactericidal activity.[26] As expected, doxycycline showed tolerance (MBC/MIC > 32) and no bactericidal activity in the time–kill curve.

All the treatment regimens used decreased mortality significantly in comparison with the control group, without differences among them. However, imipenem was better than amikacin or doxycycline in pulmonary bacterial clearance and the number of mice with sterile lungs. In addition, the results indicate that monotherapy with imipenem is just as effective as therapy with both imipenem and amikacin (in accordance with the lack of synergy found in the time–kill curve for the imipenem–amikacin combination). So, although studies in humans are necessary to confirm these results, the treatment of pneumonia caused by *A. baumannii* strains susceptible to imipenem and not showing *in vitro* synergy with amikacin should be carried out with imipenem alone.

The worst *in vivo* results appeared in the group treated with doxycycline alone. Bacterial clearance from lungs and blood was lower than with the other treatments used. However, the combination of amikacin and doxycycline improved the results obtained with either doxycycline or amikacin alone, in parallel with the synergy demonstrated in the time–kill curves. These *in vivo* results were similar to those found with imipenem.

Imipenem, like other β-lactams, has a time-dependent bactericidal activity, the most important parameter being the time during which the activity was >MIC (ΔT/MIC).[27] However, in contrast to other β-lactams, imipenem has both an *in vivo* and *in vitro* post-antibiotic effect (PAE) against *Pseudomonas aeruginosa*,[28,29] and an *in-vivo* effect in neutropenic mice against *A. baumannii*.[21] It may be speculated that the PAE may be even greater in immunocompetent mice owing to the post-antibiotic leucocyte enhancement.[30] Therefore, even though the ΔT/MIC reached was less than that obtained in humans,[31] imipenem was the most active antimicrobial drug in monotherapy for this model of pneumonia.

Doxycycline is a bacteriostatic antibiotic and showed neither time- nor concentration-dependent bactericidal activity. However, as with other antibiotics with an action mechanism based on the inhibition of protein synthesis, it has a prolonged PAE against GNB.[27,30,32] Amikacin has a concentration-dependent bactericidal activity,[26] showing the highest activity when the C_{max} exceeds 8–10 $\times$ MIC,[27] as we achieved in the present study. As with other aminoglycosides, amikacin also expresses a prolonged PAE against GNB.[30,33]

In conclusion, this experimental model permits the study of the *in vivo* activity of antimicrobial drugs in *A. baumannii* pneumonia in immunocompetent mice. The studies made *in vivo* agree with the results obtained *in vitro*. Imipenem monotherapy was the most effective treatment, and combining it with amikacin did not improve the results. The combination of doxycycline plus amikacin was as effective as imipenem. Although the data obtained in experimental models must be considered carefully, they show that the combination of doxycycline plus amikacin could be an alternative therapy in cases of pneumonia caused by *A. baumannii* strains resistant to imipenem.

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