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Use of a New Mouse Model of *Acinetobacter baumannii* Pneumonia To Evaluate the Postantibiotic Effect of Imipenem

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***Acinetobacter baumannii* is responsible for severe nosocomial pneumonia. To evaluate new therapeutic regimens for infections due to multiresistant strains and to study the pharmacodynamic properties of various antibiotics, we developed an experimental mouse model of acute *A. baumannii* pneumonia. C3H/HeN mice rendered transiently neutropenic were infected intratracheally with 5×10^6 CFU of *A. baumannii*. The mean $\log_{10}$ CFU/g of lung homogenate (± the standard deviation) were 9 ± 0.9, 9.4 ± 0.8, 8.6 ± 1.2, and 7.7 ± 1.4 on days 1, 2, 3, and 4 postinoculation. The lung pathology was characterized by pneumonitis with edema and a patchy distribution of hemorrhages in the peribronchovascular spaces of both lungs. Abscesses formed on days 3 and 4. Four days after inoculation, subacute pneumonitis characterized by alveolar macrophage proliferation and areas of fibrosis was observed. The cumulative mortality on day 4 was 85%. This new model was used to study the effects of 1, 2, or 3 50-mg/kg doses of imipenem. Imipenem concentrations in lungs were above the MIC for 2 h after the last dose. The in vivo postantibiotic effect (PAE) was determined during the 9-h period following the last dose; it decreased in duration with the number of doses: 9.6, 6.4, and 4 h after 1, 2, and 3 50-mg/kg doses, respectively. In contrast, no in vitro PAE was observed. This model offers a reproducible acute course of *A. baumannii* pneumonia. The presence of a prolonged in vivo PAE supports the currently recommended dosing intervals of imipenem for the treatment of human infections due to *A. baumannii*, i.e., 15 mg/kg three times a day.**

Acinetobacter baumannii is the *Acinetobacter* species responsible for the majority of nosocomial pulmonary infections (1). Based on specific diagnostic techniques, two recent investigations demonstrated that 15 and 24% of all episodes of pneumonia in mechanically ventilated patients included at least one *Acinetobacter* species (12, 26).

Difficult-to-treat *A. baumannii* infections are the consequence of the widespread diffusion of strains in hospitals and their increasing resistance to antibiotics. Animal models represent an essential step between in vitro sensitivity testing and clinical studies. Because of the increasing resistance of *A. baumannii* to many antibiotics, an experimental model was needed to evaluate the efficacy of new antibiotic regimens. However, no *Acinetobacter* respiratory infection model was available. Therefore, by using an approach that has recently been developed to establish acute respiratory infections due to *Streptococcus pneumoniae* (28) or *Haemophilus influenzae* (11, 27), we developed a mouse model of *A. baumannii* pneumonia with similarities to the human disease (2, 4). Imipenem is active against nearly 100% of *Acinetobacter* strains and is the "gold standard" therapy for these severe nosocomial infections (29). Despite a half-life of only 1 h, this antibiotic is usually administered at 6- or 8-h intervals, which might suggest a postantibiotic effect (PAE). Therefore, we used this new model to assess the pharmacodynamic properties of imipenem.

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MATERIALS AND METHODS

Challenge organisms and antibiotic. A cephalosporinase-hyperproducing strain of *A. baumannii* (SAN-94040), resistant to aminoglycosides and fluoroquinolones and isolated from the blood cultures of a patient with nosocomial pneumonia, was stored at −80°C in Trypticase soy (TS) broth containing 5% glycerol. Imipenem was provided by Merck Sharp & Dohme (West Point, Pa.). *A. baumannii* ATCC 1506 was used as the reference standard for in vitro studies.

Determination of virulence in mice. *A. baumannii* was cultured in TS broth and inoculated intraperitoneally (i.p.) into mice (five per group) as 0.5 ml of the pure inoculum and diluted 1/2, 1/10, 10^{-2}, 10^{-3}, 10^{-5}, and 10^{-7}. The 50% lethal dose (LD_{50}) was estimated by the probit method from the number of mice surviving on day 5 of observation (6).

In vitro tests. (i) MICs and minimum bactericidal concentrations (MBCs). MICs and MBCs were determined by the agar dilution and broth dilution methods by using geometric twofold serial dilutions in Mueller-Hinton broth (MHB). The final inoculum was 10^6 CFU/ml. The MIC was determined after incubation at 30°C for 18 h. MBC end points were determined by subculturing 100 μl from the first cloudy tube onto MHB agar. The MBC was defined as the antibiotic concentration inducing a 99.9% reduction in CFU per milliliter (≤10 CFU per plate) (25).

(ii) In vitro bactericidal effect and PAE. The rate of killing and the presence of a PAE were determined by using a log-phase inoculum of 10^7 CFU/ml to reproduce the in vivo inoculum at the start of therapy. Shaking cultures were incubated at 37°C for 18 h. The bactericidal effect on strain SAN-94040 was determined by using imipenem concentrations of one-half, one, two, four, and eight times the MIC. Aliquots were sampled and quantified at 2, 6, 9, and 24 h.

The in vitro PAE of imipenem was investigated for both strains (SAN-94040 and ATCC 1506) by exposing the bacteria to the drug at a concentration equal to four times the MIC for 2 h. The antibiotic was removed from culture by repeated centrifugations and resuspension in fresh MHB (9, 20). Absence of the antibiotic was verified by disk diffusion. The antibiotic-free bacterial suspension was then incubated at 37°C. Aliquots were withdrawn for microorganism quantification at time zero, immediately after removal of the antibiotic, and hourly for the next 8 h. The experiment was repeated twice on different days. The in vitro PAE was quantitated by calculating the difference between the times required for the numbers of drug-exposed and untreated organisms to increase 10-fold above the numbers present immediately after removal of the antibiotic (9, 18).

Experimental animals. To select the most suitable mouse strain for experimental infection (with susceptibility to infection and a high and reproducible rate of mortality), three strains of 6-week-old, specific-pathogen-free, female mice

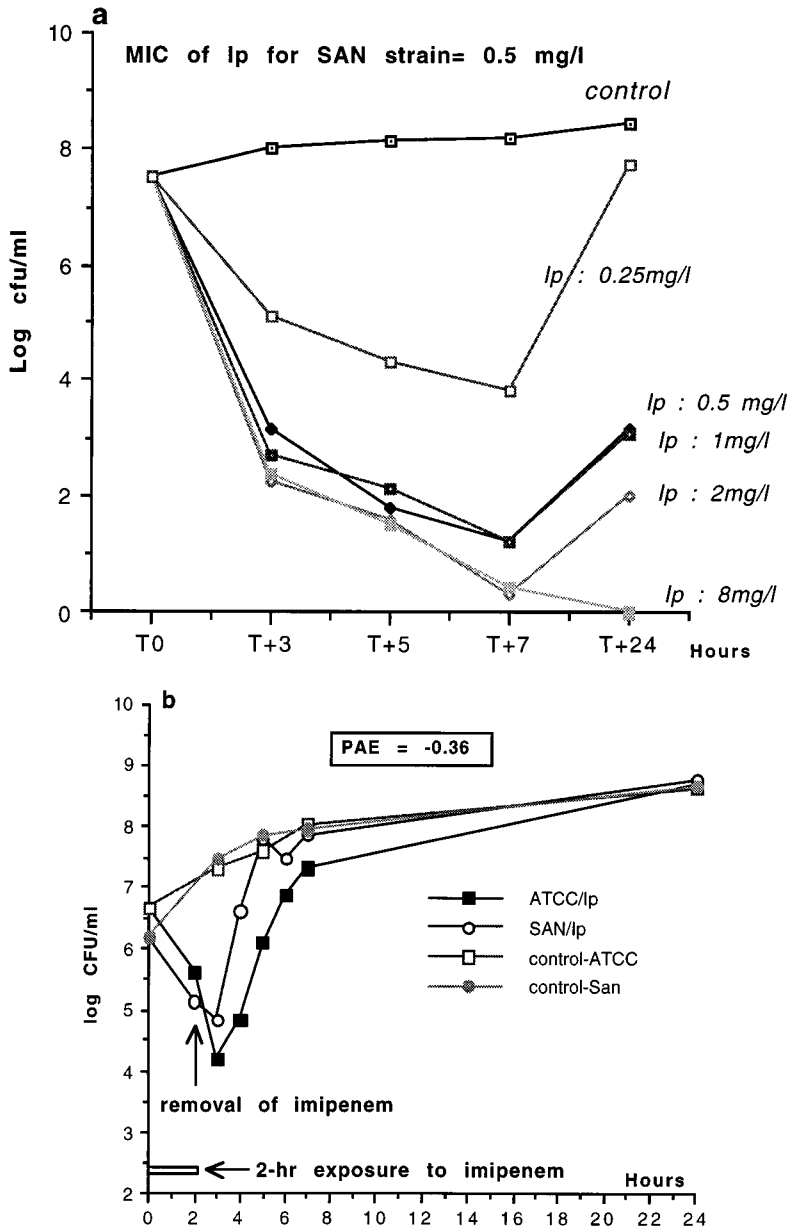


FIG. 1. (a) In vitro bactericidal activity of imipenem (Ip), at one-half, one, two, four, and sixteen times the MIC, against *A. baumannii* SAN-94040 (SAN). (b) PAE of imipenem against *A. baumannii* SAN-94040 and ATCC 15606 (ATCC) after 2 h of exposure to the drug at four times the MIC. Imipenem produced no in vitro PAE against the tested strains (mean value ± SD, −0.36 ± 0.19 h).

were used: Swiss Webster (24 to 25 g), C57BL/6N (16 to 17 g), and C3H/HeN (18 to 20 g) (Iffa-Credo Laboratories, L'Arbresle, France).

The mice were rendered transiently neutropenic by injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, Ind.) i.p. (150 mg/kg of body weight) in a volume of 0.2 ml at 4 and 3 days before *A. baumannii* inoculation (day 0). The mice were anesthetized by i.p. injection of 0.2 ml of 0.65% sodium pentobarbital before bacterial inoculation. Animals were infected by intratracheal instillation via the mouth as previously described (11). Briefly, the trachea was cannulated with a blunt needle and 50 μl of a bacterial suspension containing 10^8 CFU/ml (spectrophotometrically controlled) was instilled. Inoculum size was confirmed by quantitative cultures. Inoculation efficacy was systematically tested by quantitation of viable organisms in the lungs removed from two control infected animals immediately after bacterial inoculation and 3 h later.

For each experiment, the animals were separated into two groups. For the first ($n = 74$), mice were weighed daily and weight changes were noted; mortality was studied until day 7; for the second ($n = 36$), mice were killed by exposure to CO_2 on days 1, 2, 3, 4, and 7 postinoculation and exsanguinated by cardiac puncture. The total number of circulating neutrophils was determined by counting in a hemacytometer with the Unopette system (Beckton Dickinson, Paramus, N.J.). The lungs were then removed for quantitative bacteriological and histopathological studies.

Histopathological examination. After removal, the lungs were immersed in Bouin's fixative until examination. Fixed lung slices approximately 5 mm thick were dehydrated in graded alcohol solutions, embedded in paraffin, and cut into 6-μm-thick sections. Mounted sections were stained for light microscopy with hematoxylin and eosin (HE).

Bacteriological analysis. For quantitative bacteriological studies, blood and lung samples were collected daily from day 1 to day 7 from groups of six mice. Lungs were removed, weighed, and homogenized in 10 ml of saline. Serial 10-fold dilutions of the homogenates were plated onto TS agar (0.1 ml on 9-cm-diameter plates). Results are expressed as mean $\log_{10}$ CFU per gram of lung ± the standard deviation (SD). The lower limit of detection was 5×10^2 CFU/g of lung. Quantitative blood cultures were carried out by plating 0.1 ml of whole blood onto 9-cm-diameter TS agar plates.

Pharmacodynamic properties of imipenem. Pharmacokinetic parameters were evaluated in infected C3H/HeN mice. Concentrations of imipenem in lungs and

TABLE 1. Comparison of susceptibilities of three strains of nonneutropenic mice to *A. baumannii* 48 h after intratracheal inoculation of 5×10^6 CFU

Mouse strain (mean wt [g] ± SD)	No. of deaths/no. of mice tested	Mean maximum wt loss (g) ± SD (%)	Mean $\log_{10}$ CFU/g of lung ± SD
Swiss Webster (26.28 ± 1.5)	3/15[a]	2.74 ± 2 (10.42)[a]	8.73 ± 0.68
C3H/HeN (19.5 ± 0.96)	9/15	4.19 ± 1 (21.4)	9.1 ± 0.8
C57BL/6N (16.44 ± 0.5)	10/14	3.23 ± 1.1 (19.6)	9.26 ± 0.3

[a] $P = 0.02$ versus the other strains of mice.

sera were determined after administration of single doses of 50 and 100 mg/kg at 3 h after infection. Sera and lungs were collected from groups of three mice at 5, 10, 15, 30, 60, and 120 min postadministration of imipenem. Animals were killed by exposure to CO_2 and exsanguinated by cardiac puncture. Serum was separated and immediately stored at −80°C. Lungs were removed from exsanguinated mice, briefly washed in sterile water, weighed, and cryohomogenized. Antibiotic concentrations were determined by an agar well microbiological assay with *Bacillus subtilis* spores (Difco, Detroit, Mich.) as the indicator organism and antibiotic medium 2 (Diagnostic Pasteur, Marnes-la-Coquette, France). Standard samples were prepared in sulfonate buffer, pH 6 (17). The calibration curve was linear from 0.06 to 64 mg/liter, and the limit of sensitivity was 0.06 mg/liter. Variation within replicates was ≤5%. Results are expressed as milligrams per liter for sera and milligrams per kilogram for lungs. Pharmacokinetic analyses were performed by using standard methods (5). The peak drug concentration in serum, the mean time to the peak drug concentration in serum, the elimination half-life, and the area under the concentration-time curve (AUC) were determined.

The in vivo PAE of imipenem was evaluated after administration of 1, 2, or 3 cumulative doses of 50 mg/kg three times a day, starting at 3 h postinoculation of the SAN-94040 strain. During the 9-h period following the last injection, lungs were removed and divided into two blocks for bacterial counting and determination of the imipenem concentration (three animals per data point). The in vivo PAE was calculated as described by Gudmundsson et al. (16), by using the following equation: $PAE = T - C - M$, where M is the time that the antibiotic level in the lungs exceeded the MIC, T is the time required for the mean bacterial count in the lungs to increase 1 $\log_{10}$ above the count at time M, and C is the time needed for the bacterial count in the lungs of control animals to increase by 1 $\log_{10}$ from time zero.

RESULTS

In vitro studies. (i) MICs and MBCs. Strain SAN-94040 overproduced cephalosporinase and was resistant to cephalosporins, aminopenicillins, and ureidopenicillins; the MIC and MBC of imipenem were 0.5 and 0.5 mg/liter, respectively, for the reference strain and 0.5 and 1 mg/liter for SAN-94040.

(ii) Killing curves and in vitro determination of PAE. Imipenem produced a rapid bactericidal effect against SAN-94040 (Fig. 1a) at one to eight times the MIC, and no dose effect was observed in the range of these concentrations. After exposure for 2 h, imipenem had no PAE against the clinical isolate and the ATCC strain. Compared with control cultures without the antibiotic, both *Acinetobacter* strains grew much more rapidly during the first hour after drug removal (negative PAE), catching up with the former by 7 h (Fig. 1b).

TABLE 2. Characteristics and duration of *A. baumannii* lung infection in C3H/HeN mice

Postinfection day	No. of neutrophils/mm^3	Mean body wt (g) ± SD	Cumulative mortality (%)[a]	Mean CFU/g of lung ± SD
0	300	19.3 ± 1.06	0	7.3 ± 0.2
1	220	17.7 ± 1.2	8	9 ± 0.9
2	4,120	16.7 ± 1.4	45	9.4 ± 0.8
3	12,000	17.3 ± 1.6	81	8.6 ± 1.2
4	4,000	17.8 ± 1.5	85	7.7 ± 1.4
7	5,200	19.2 ± 1.1	85	$<5 \times 10^2$

[a] Seventy-four mice were tested.

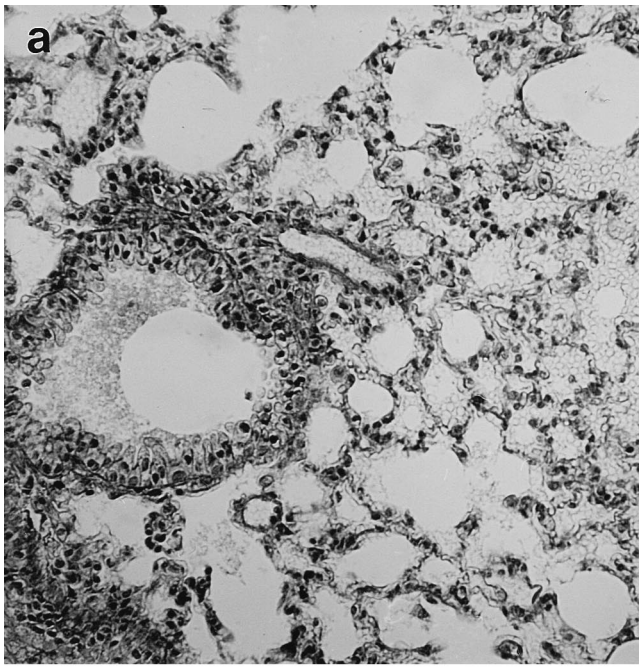
Virulence of *A. baumannii* for mice. The virulence of SAN-94040 was evaluated in two mouse strains. The LD_{50}s ranged from 0.54×10^7 to 3.15×10^7 bacteria per mouse for normal C3H/HeN and Swiss mice, without significant differences between groups. The LD_{50} for neutropenic mice treated with cyclophosphamide (10^7 bacteria per mouse) was not significantly different from that of normal mice (2.4×10^7 and 3.15×10^7 bacteria per mouse, respectively, for Swiss Webster and C3H/HeN mice).

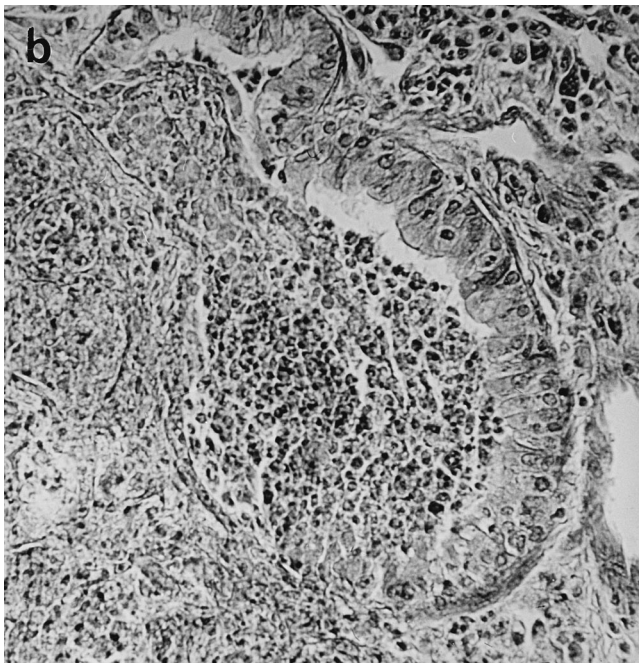
Susceptibilities of different strains of mice to *A. baumannii*. We compared the susceptibilities of three strains of nonneutropenic mice to *Acinetobacter* pneumonia. On the basis of preliminary studies, each mouse was inoculated with 5×10^6 CFU. As shown in Table 1 a significant difference in susceptibility to *Acinetobacter* was found between the Swiss Webster strain and C3H/HeN and C57BL/6N mice (mortality, $P = 0.02$; weight loss, $P = 0.02$). In contrast, among the three strains of mice, no significant differences in the mean titer of bacteria recovered from the lungs were found ($P > 0.05$). Because C57BL/6N mice were more sensitive to anesthesia, the mouse infection model was developed with C3H/HeN mice.

Mouse infection model. (i) Clinical findings, neutrophil counts, and outcome. Inoculated mice became weak and less active as early as day 1 after inoculation. Body weight loss was maximum on day 2 (mean ± SD, 13% ± 8%). Most animals developed bilateral conjunctivitis. Physical improvement was noted on day 4, and 7 days after bacterial challenge, all survivors had normal activity and had recovered their initial weight. Mice were neutropenic only during the first 2 days of infection (Table 2). Transient leukocytosis (12,000/mm^3) was observed on day 3. The overall mortality was 63 (85%) of 74; nearly all deaths occurred between days 2 and 3 postinoculation.

(ii) Quantitative bacteriological findings. Two mice were killed approximately 15 min after inoculation to quantitate the initial bacterial counts in the lungs. *A. baumannii* was able to establish a lung infection, since the mean bacterial count increased from 3×10^7 CFU/g immediately after intratracheal inoculation (the standard error relative to the theoretical inoculum was <3%; $n = 10$) to 6×10^9 CFU/g on day 1 and remained at the same level until day 2. By day 4, counts had decreased to 7×10^7 CFU/g, and no organisms were recovered from the lungs on day 7 after inoculation (Table 2). Blood cultures of 10 (71%) of the 14 mice tested on day 2 were positive. The mean concentration of bacteria isolated from blood was 5.3×10^2 CFU/ml.

(iii) Histopathological findings. Macroscopical observation showed large areas of consolidation associated with a patchy distribution of hemorrhagic lesions in both lungs as early as day 1 after inoculation. The histopathology of *A. baumannii* lung infection was characterized by severe alveolar interstitial pneumonia. On days 1 and 2, the lesions consisted of pneumonitis with edema and a patchy distribution of hemorrhages in the peribronchovascular spaces in both lungs (Fig. 2a). Abscess formation, with extensive infiltration of polymorphonuclear leukocytes and exudate in alveolar spaces and bronchial lu-





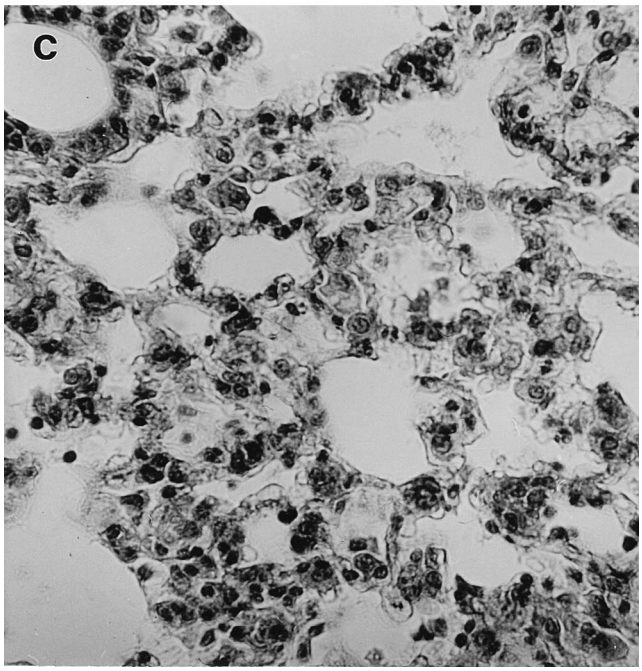


FIG. 2. Histological evolution of experimental *A. baumannii* pneumonia in mice. (a) Lung section showing hemorrhagic and edematous alveolitis, typical of days 1 and 2 postinfection (HE stain; magnification, ×100). (b) Microscopic lung architecture on day 3 postinoculation (HE stain; magnification, ×400). (c) Alveolar macrophage proliferation on day 4 postinfection (HE stain; magnification, ×100).

mens, was noted on days 3 and 4 (Fig. 2b). On day 4, subacute pneumonitis characterized by alveolar macrophage proliferation and areas of fibrosis was observed (Fig. 2c).

Pharmacokinetic parameters of single-dose imipenem. After one dose of imipenem-cilastatin (50 or 100 mg/kg), peak levels in plasma were around 80 and 168 mg/liter, respectively, and the serum half-life was 9 min with both dosages. Since the 50-mg/kg dose provided a peak concentration in serum close to that observed in humans, this dose was chosen for determination of pharmacokinetic parameters. The duration of a level of imipenem in serum above the MIC for the test microorganism was 1 h. Peak imipenem levels in lung tissue were 20 times the MIC and 10 times the MBC (10 mg/kg of lung 7 min postinjection) and remained higher than the MIC for 2 h. Three hours after injection, concentrations in lungs were <0.06 mg/kg. The AUC, the AUC/MIC ratio, and the peak/dose ratio were 19.2 mg/h/liter, 0.36, and 1.72, respectively, after a 50-mg/kg i.p. injection.

In vivo PAE. The in vivo PAE of imipenem was evaluated after administration of 1, 2, or 3 doses of 50 mg/kg three times daily, starting at 3 h postinoculation. Bacterial counts were determined during the 9-h period following the last dose. After a single dose of 50 mg/kg, the mean $\log_{10}$ CFU per gram of lung fell from 7.7 ± 0.3 before treatment to 6.3 ± 0.38 ($P < 0.05$) 3 h after the dose. Over the next 5 h, bacterial counts in the lungs remained stable. Imipenem levels in the lungs exceeded the MIC for 2 h and fell below it at a calculated time of 2.4 h. Bacterial counts rose from 5.8 ± 1.05 CFU/g at 9 h to 8.3 ± 0.3 CFU/g at 24 h after injection. Thus, an in vivo PAE of 9.6 h was demonstrated (Fig. 3a). After two 50-mg/kg doses, the mean $\log_{10}$ CFU/g of lung fell from 6.8 ± 0.45 before treatment to 4.14 ± 0.65 ($P < 0.05$) 6 h after the second dose. Over the next 3 h, bacterial counts in lungs remained stable. Bacterial counts rose from 4.4 ± 2.1 CFU/g at 12 h to 6.9 ± 0.8 CFU/g 24 h after the second injection. The PAE following a second dose was 6.4 h (Fig. 3b). After three doses of imipenem, the mean $\log_{10}$ CFU per gram of lung fell from 6.6 ± 0.9 before treatment to 2.9 ± 0.2 ($P < 0.05$) 5 h after the last dose and then rose to 5.5 ± 1.3 CFU/g at 24 h after the last injection. The in vivo PAE following a third dose was 3.9 h (Fig. 3c). No change in the MIC of imipenem was observed for strains isolated at 24 h posttreatment.

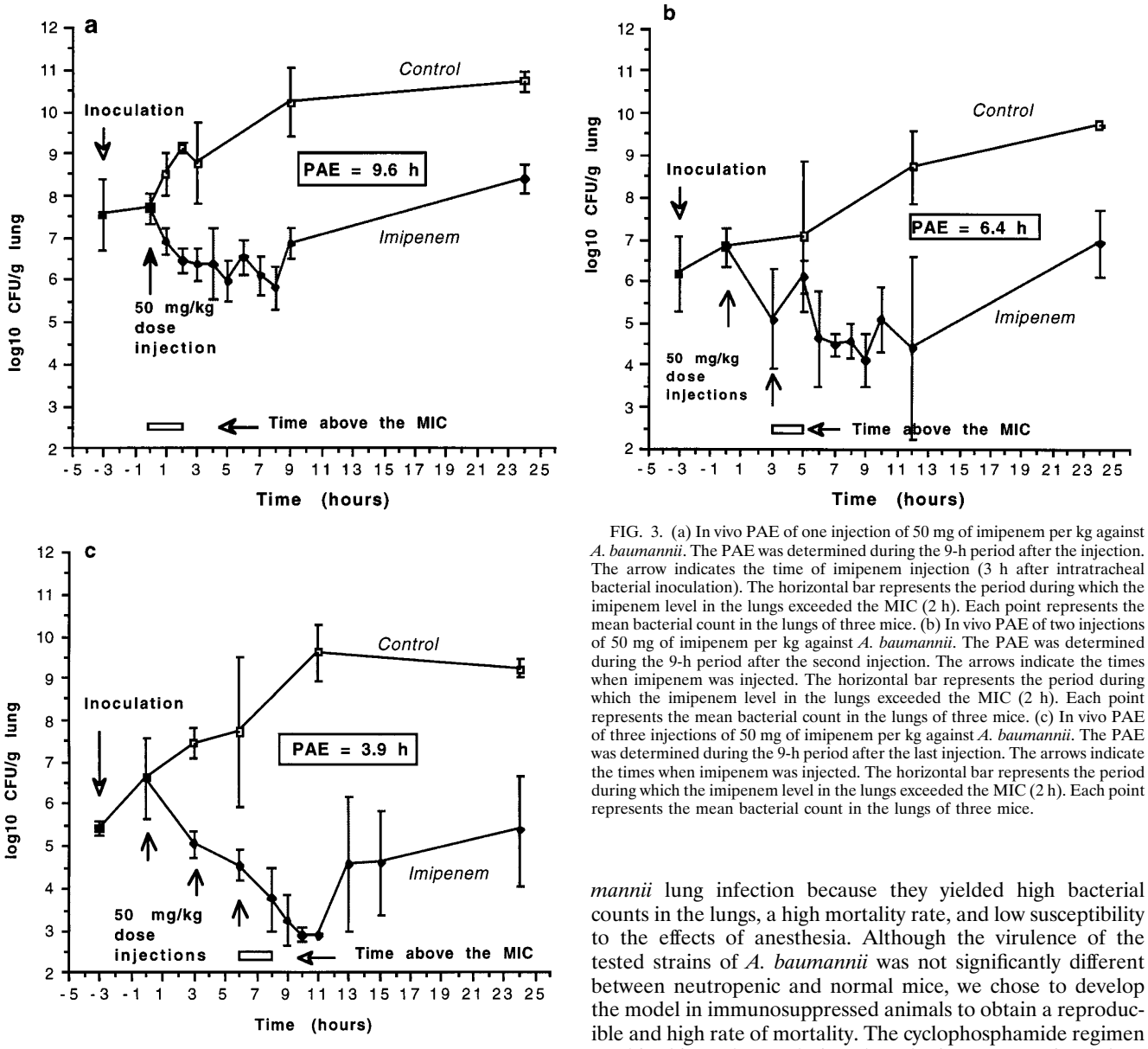


FIG. 3. (a) In vivo PAE of one injection of 50 mg of imipenem per kg against *A. baumannii*. The PAE was determined during the 9-h period after the injection. The arrow indicates the time of imipenem injection (3 h after intratracheal bacterial inoculation). The horizontal bar represents the period during which the imipenem level in the lungs exceeded the MIC (2 h). Each point represents the mean bacterial count in the lungs of three mice. (b) In vivo PAE of two injections of 50 mg of imipenem per kg against *A. baumannii*. The PAE was determined during the 9-h period after the second injection. The arrows indicate the times when imipenem was injected. The horizontal bar represents the period during which the imipenem level in the lungs exceeded the MIC (2 h). Each point represents the mean bacterial count in the lungs of three mice. (c) In vivo PAE of three injections of 50 mg of imipenem per kg against *A. baumannii*. The PAE was determined during the 9-h period after the last injection. The arrows indicate the times when imipenem was injected. The horizontal bar represents the period during which the imipenem level in the lungs exceeded the MIC (2 h). Each point represents the mean bacterial count in the lungs of three mice.

DISCUSSION

A. baumannii is an emerging pathogen among hospital-acquired infections, especially in patients under mechanical ventilation. Because of its increasing resistance to many available antibiotics (19, 22), *A. baumannii* is now one of the most difficult-to-treat pathogens. A major limitation to the study of the pharmacodynamic properties of antibiotics active against *A. baumannii* is the lack of relevant animal models. Although the virulence of clinical isolates of *Acinetobacter* spp. has been studied in a mouse model of acute systemic infection induced by i.p. injection (22, 23), a model of *A. baumannii* pneumonia has not been reported. The present study describes a new experimental model of acute *A. baumannii* pneumonia leading to death in 85% of the animals within 3 days. Since the three tested strains of normal mice were only moderately susceptible to infection, animals were rendered transiently neutropenic. C3H/HeN mice were chosen as suitable for studies of *A. baumannii* lung infection because they yielded high bacterial counts in the lungs, a high mortality rate, and low susceptibility to the effects of anesthesia. Although the virulence of the tested strains of *A. baumannii* was not significantly different between neutropenic and normal mice, we chose to develop the model in immunosuppressed animals to obtain a reproducible and high rate of mortality. The cyclophosphamide regimen used in this study was designed to obtain neutropenia of short duration but able to facilitate the onset of the infectious process. Although the majority of animals were bacteremic, quantitative blood cultures yielded relatively low bacterial counts compared with those in the lungs. This finding suggests that pneumonia was the primary focus of infection. The histological patterns observed in this model were dependent on the neutrophil levels in blood. During the early phase of infection, when animals were neutropenic, the main lesions were edema and hemorrhages. Abscesses formed later, when animals had recovered from neutropenia and developed leukocytosis. Most animals died during this stage of infection with histological patterns close to that observed in *A. baumannii* pneumonia in nonneutropenic humans. Surviving animals cleared bacteria after day 4. Although *A. baumannii* is well documented as a nosocomial pathogen (8), community-acquired *Acinetobacter* pneumonia has also been reported. The mouse model described here shares some similarities with the community-acquired *A. baumannii* pneumonia previously described (2, 4). Patients were bacteremic, and in all cases pneumonia was

fulminant, with 64% mortality. The mean duration of symptoms was 3 days for survivors and 2 days for those who died. Although our model does not cause 100% mortality, it offers a reproducible acute course of pneumonia and is therefore suitable for the study of antibiotic pharmacokinetics and pharmacodynamics within the first 48 h of infection.

Imipenem is the gold standard therapy for *Acinetobacter* nosocomial infections, but no information on the pharmacodynamics and in vivo bactericidal activity of imipenem is available. In contrast with the absence of an in vitro PAE, we were able to demonstrate a prolonged in vivo PAE. The mechanisms responsible for a PAE after β-lactam administration are not clear. β-Lactam antibiotics consistently induce a PAE against gram-positive cocci, but there is no effect or a minimal effect against gram-negative bacilli. Gottfredsson et al. (15) measured the rate of DNA synthesis in bacteria after exposure to various antimicrobial agents and suggested the existence of multiple mechanisms for a PAE. Imipenem is unique among the β-lactams because it can induce significant in vitro and in vivo PAEs against *Pseudomonas aeruginosa* (7, 16). However, some variations have been noted both in vitro and in vivo (16, 18). It is commonly accepted that the in vitro PAE is the consequence of a direct interaction between the bacteria and the antibiotic, for example, prolongation of the lag time and/or an increase of the generation time. It has been suggested that the PAE of penicillin on *Streptococcus pyogenes* is caused by irreversible binding of penicillin to penicillin-binding proteins (PBPs) 1 to 3 and represents the time necessary for the synthesis of new PBPs required for normal growth (31). The preferential binding of imipenem to PBP-2 leads to spheroplast formation, and the observed PAE on *P. aeruginosa* could represent the time required for the spheroplasts to synthesize a new cell wall (20, 21). Other mechanisms could be involved to explain the lack of concordance between in vivo and in vitro findings. These differences could be explained in part by the interaction of the bacteria with host defense mechanisms and other events, such as different bacterial growth rates which are probably slower in vivo than in vitro. In nonneutropenic animals, it has been suggested that the antibiotic may modify the cell surface, thereby enhancing bacterial susceptibility to phagocytosis, intracellular killing, or both (postantibiotic leukocyte enhancement) (9, 14). However, in our model, animals had less than 400 neutrophils/mm^3 during the PAE study (i.e., 12 h) and there were few neutrophils in lungs (Fig. 2a). An in vivo PAE, although shorter, has also been observed in neutropenic mice (13). Some strains of *A. baumannii*, generally isolated from systemic infections, have the ability to produce slime which enhances their virulence and is cytotoxic to neutrophils and macrophages (23). As with other species, exposure to imipenem can affect bacterial interactions with leukocytes and macrophages and the microorganisms could be more susceptible during the postantibiotic phase.

It has to be emphasized that we observed a shortening of the duration of in vivo PAE with the repetition of doses. Why the duration of the in vivo PAE induced by imipenem was dependent on the number of doses remains unclear. One possible reason for the loss of the PAE in our experiment may have been changes in susceptibility following exposure. However, we did not observe any change in the MIC of imipenem in strains isolated at 24 h posttreatment. Adaptive resistance, which causes reduced bactericidal activity following a second antibiotic exposure during the postantibiotic phase and has been described in vivo with aminoglycosides (10), could be one explanation for our findings. A diminished in vitro killing rate has also been noted with β-lactams when they were added at concentrations below the MIC early during the postantibiotic phase (24). The clinical significance of the in vivo PAE of imipenem described in this study has not been clearly established. However, it should prove beneficial since imipenem is routinely given at intervals of 6 to 8 h despite its known very low concentration at the infection site 2 h after the last injection (3).

In conclusion, we have described a mouse model of *A. baumannii* pneumonia which offers a reproducible acute course of pneumonia with similarities to the human disease. Because of the difficulties in treating nosocomial infections caused by *A. baumannii*, this model should be useful to evaluate new antibiotic regimens. The efficacy of the currently recommended imipenem dosing intervals for the treatment of human *A. baumannii* pneumonia (6 to 8 h) may be due to the time during which drug concentrations in the lungs remain above the MIC, which is associated with a prolonged in vivo PAE. However, keeping in mind that the duration of the PAE decreased with the number of doses, a longer interval between doses cannot be recommended.

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