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EXPERIMENTAL

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Nebulized ceftazidime in experimental pneumonia caused by partially resistant *Pseudomonas aeruginosa*

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Abstract *Purpose:* Ventilator-associated pneumonia caused by *Pseudomonas aeruginosa* with impaired sensitivity to ceftazidime is frequent in critically ill patients. The aim of the study was to compare lung tissue deposition and antibacterial efficiency between nebulized and intravenous administrations of ceftazidime in ventilated piglets with pneumonia caused by *Pseudomonas aeruginosa* with impaired sensitivity to ceftazidime. *Methods:* Ceftazidime was administered 24 h following the intra-bronchial inoculation of *Pseudomonas aeruginosa* (minimum inhibitory concentration = 16 μg ml^{-1}), either by nebulization (25 mg kg^{-1} every 3 h, $n = 6$) or by continuous intravenous infusion (90 mg kg^{-1} over 24 h after an initial rapid infusion of 30 mg kg^{-1}, $n = 6$). Four non-treated inoculated animals served as controls. All piglets were killed 48 h (intravenous and control groups) or 51 h (aerosol group) after inoculation. Lung tissue concentrations and lung bacterial burden were assessed on multiple post-mortem subpleural lung specimens [(lower limit of quantitation = 10^2 colony forming unit (cfu g^{-1})]. *Results:* Ceftazidime trough lung tissue concentrations following nebulization were greater than steady-state lung tissue concentrations following continuous intravenous infusion [median and interquartile range, 24.8 (12.6–59.6) μg g^{-1} vs. 6.1 (4.6–10.8) μg g^{-1}] ($p < 0.001$). After 24 h of ceftazidime administration, 83% of pulmonary segments had bacterial counts $<10^2$ cfu g^{-1} following nebulization and only 30% following intravenous administration ($p < 0.001$). In control animals, 10% of lung segments had bacterial counts $<10^2$ cfu g^{-1} 48 h following bronchial inoculation.
Conclusion: Nebulized ceftazidime provides more efficient bacterial killing in ventilated piglets with pneumonia caused by *Pseudomonas aeruginosa* with impaired sensitivity to ceftazidime.

Keywords Nebulization · Ceftazidime · Pneumonia · *Pseudomonas aeruginosa* · Mechanical ventilation · Treatment

Introduction

Ventilator-associated pneumonia is one of the most frequent nosocomial infections in the intensive care setting [1] and represents the main reason for the prescription of antibiotics in critically ill patients [2]. *Pseudomonas aeruginosa*, often multiresistant to antibiotics, is one of the major causative microorganisms [3]. Even though ceftazidime is a cephalosporin whose activity is specifically directed against *Pseudomonas aeruginosa*, impaired sensitivity of *Pseudomonas aeruginosa* to ceftazidime is constantly rising [4, 5].

In order to rapidly eradicate partially resistant strains of *Pseudomonas aeruginosa* from the infected lung, ceftazidime concentration at the site of infection should remain permanently far above the elevated minimum inhibitory concentration (MIC) [6]. Although increasing the dose up to 90 mg kg^{-1} day^{-1} and administering ceftazidime through a continuous intravenous infusion have been shown to provide steady-state lung tissue concentrations 16 times greater than the trough concentrations following an intermittent infusion of 30 mg/kg three times a day [7], such concentrations remain <16 μg ml^{-1}, a value characterizing the MIC of most partially resistant *Pseudomonas aeruginosa* infecting the lungs of ventilated patients.

Nebulization of antibiotics offers the possibility of delivering high lung tissue concentrations of antibiotics in normal and infected lungs [8, 9]. The nebulization of amikacin, a concentration-dependent antibiotic, provides a rapid and efficient bacterial killing in piglets with *Escherichia coli* inoculation pneumonia [10]. In a previous study, we reported high peak lung tissue concentrations after a single nebulization of 1-g ceftazidime in mechanically ventilated piglets with *Pseudomonas aeruginosa* pneumonia [11]. A time-dependent antibiotic like ceftazidime, however, is efficient for killing bacteria only if plasma concentrations in the infected parenchyma are permanently maintained far over MIC. In this preliminary study [11], the ability of nebulization to maintain high trough lung tissue concentrations was not investigated.

The aim of the present study was to assess trough lung tissue concentrations and the bactericidal activity following eight ceftazidime nebulizations per day in a porcine model of inoculation pneumonia caused by *Pseudomonas aeruginosa* with impaired sensitivity to ceftazidime. The data were compared with those obtained in a second group of animals treated by a continuous intravenous administration of ceftazidime.

Methods

Animal preparation and bronchial inoculation

Sixteen bred domestic Largewhite-Landrace piglets, aged 3–4 months and weighing 19 ± 1 kg, were anesthetized using propofol 3 mg kg^{-1} and orotracheally intubated in the supine position. Anesthesia was maintained with a continuous infusion of midazolam 0.3 mg kg^{-1} h^{-1}, pancuronium 0.3 mg kg^{-1} h^{-1} and fentanyl 5 μg kg^{-1} h^{-1}. The femoral artery was cannulated with a a 3-F polyethylene catheter (Plastimed, St Leu la Forêt, France) for blood sampling. After technical preparation, the piglets were placed in the prone position and mechanically ventilated in a volume-controlled mode (tidal volume 10 ml kg^{-1}, positive end-expiratory pressure 5 cmH_2O) with a Cesar ventilator (Taema, Antony, France). All animals were treated according to the guidelines of the Department of Experimental Research of the Lille University and the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 93-23, revised 1985).

Forty milliliters of a suspension containing 10^6 colony-forming units ml^{-1} (cfu ml^{-1}) of *Pseudomonas aeruginosa* with impaired sensitivity to ceftazidime (MIC = 16 μg ml^{-1}) were bronchoscopically inoculated in both lungs (10 ml in the upper lobes, 10 ml in the middle lobes and 20 ml in the lower lobes).

Aerosol generation

Each nebulization was performed over 30 min after inserting 25 mg kg^{-1} of ceftazidime powder diluted in 10 ml of sterile water into the chamber of a vibrating plate nebulizer (Aeroneb Pro®; Aerogen Ltd., Galway, Ireland) positioned on the inspiratory limb 15 cm proximal to the Y-piece [12]. During the nebulization, the following ventilator settings were used: absence of heat and moisture exchanger, volume-controlled mode, administration of a constant inspiratory flow, respiratory rate of 15 breaths/min, inspiratory/expiratory ratio of 50%, an end-inspiratory pause representing 20% of the duty cycle, and positive end-expiratory pressure of 5 cmH_2O. In six piglets, after completion of a single nebulization, ventilatory circuits were washed separately in a fixed volume of distilled water to assess ceftazidime extrapulmonary deposition as recently described [12].

Study design

Twenty-four hours after the inoculation, six piglets received an intravenous rapid infusion of ceftazidime (30 mg kg^{-1} in 30 min) followed by a continuous infusion of 90 mg kg^{-1} day^{-1}. In six piglets, eight aerosols of ceftazidime (25 mg kg^{-1} every 3 h over 30 min) were administered. In the aerosol group, blood samples were collected: 30 min, 1, 1.5 and 2 h after the first and second aerosols, and before (trough plasma concentration) and 30 min (peak plasma concentration) after the third and eighth aerosols. In the intravenous group, blood samples were collected 30 min, 2, 4, 8, 16 and 24 h after initiation

of therapy. The piglets were killed by exsanguination through direct cardiac puncture after sternotomy [11]. In the intravenous group, animals were killed at the 24th h after initiation of therapy, whereas in the aerosol group, animals were killed 3 h after the eighth aerosol in order to measure ceftazidime trough lung tissue concentration. Four non-treated inoculated animals ventilated for 48 h served as controls.

Immediately after killing, five "subpleural" specimens were excised from the upper, middle and lower lobes. Each specimen was divided into three contiguous blocks. The first tissue block was cryomixed in liquid nitrogen, weighed and homogenized; ceftazidime tissue concentration was measured by high-performance liquid chromatography [13] with correction for contaminating blood [14]. The lower limit of quantitation for ceftazidime concentration was 1 mg l^{-1}. The coefficient variations for ceftazidime of 1, 5, 25 and 50 mg l^{-1} were respectively 3.7, 6, 4.6 and 5.5%, and remained stable over time. On the second block, the quantitative lung tissue bacterial burden was measured according to the reference method [15]. The lower limit of quantitation for bacterial counts in the lung was 10^2 cfu g^{-1}. The third block was used for histological examination. Bronchopneumonic lesions characterizing each secondary pulmonary lobule were classified into two categories: mild pneumonia, defined as the presence of bronchiolitis and/or focal and interstitial pneumonia, or severe pneumonia, defined as the presence of confluent and/or necrotizing purulent pneumonia [16]. The percentage of each category was calculated as the number of secondary lobules of the category divided by the total of lobules analyzed.

Statistical analysis

The statistical analysis was performed using SigmaStat 2.03 (SPSS Inc., San Rafael, CA) statistical software. Data were expressed as mean ± SD or median and interquartile range (IQR) (25–75%) when the distribution was abnormal. The distribution of the data was verified by Kolmogorov–Smirnov normality test. The percentage of infected secondary pulmonary lobules in the aerosol and intravenous groups was compared by chi-square test. Ceftazidime lung tissue concentrations measured in aerosol and intravenous groups in different lung segments were analyzed using two-way analysis of variance (ANOVA) for one grouping factor (intravenous or aerosol) and one within factor (lung segments). Comparisons of ceftazidime lung concentrations between the intravenous and aerosol groups and between severe and mild pneumonias were performed by Mann–Whitney rank sum test. Lung tissue bacterial burden in the aerosol, intravenous and control groups was compared using the Kruskall–Wallis test followed by Dunn's test. Plasma concentrations following each aerosol were compared by one-way ANOVA for repeated measures. A p value ≤ 0.05 was considered as significant.

Results

Pathologic findings

Forty-eight hours after the inoculation, 75% of secondary pulmonary lobules were infected in control piglets (45% with mild pneumonia and 30% with severe pneumonia), attesting to the presence of lung infection in the inoculated lungs. After 24 h treatment by ceftazidime, the percentages of secondary pulmonary lobules of mild and severe pneumonias were 70 and 23% in the aerosol group, and 63 and 17% in the intravenous groups. The severity of pneumonia was not significantly different between the aerosol and intravenous groups. Figure 1 shows macroscopic and histological aspects of the lungs in a control piglet 48 h after the intrabronchial inoculation.

Extrapulmonary deposition of ceftazidime

Of the initial amount of ceftazidime inserted into the nebulizer (25 mg kg^{-1}), 10.1 ± 6.7% was retained in the nebulizer's chamber, 29.0 ± 7.3% in the inspiratory limb of the respiratory circuit, 2.8 ± 0.6% in the endotracheal tube and 17.4 ± 9.1% in the expiratory filter. The total extrapulmonary deposition was 59.3 ± 7.1%. The resulting fraction of ceftazidime reaching the respiratory tract was 40.7 ± 7.1% of the initial 25 mg kg^{-1} placed in the nebulizer chamber, representing a dose equivalent to 86 mg kg^{-1} day^{-1} delivered to the respiratory tract, a value similar to the intravenous dose (90 mg kg^{-1} day^{-1}).

Lung tissue deposition of ceftazidime

Trough lung tissue concentrations in the aerosol group were significantly higher than steady-state lung tissue concentrations in the intravenous group [median (IQR) 24.8 (12.6–59.6) vs. 6.1 (4.6–10.8) μg g^{-1})] ($p < 0.001$), respectively. Distribution of lung tissue concentrations between lung segments was similar in each group of piglets (Fig. 2).

In the aerosol group, trough lung tissue concentrations at the end of the experiment were significantly greater in lung areas with mild pneumonia than in lung areas with severe pneumonia (Fig. 3). In lung segments with severe pneumonia, however, mean trough lung tissue concentrations remained >16 μg ml^{-1}, the MIC of inoculated *Pseudomonas aeruginosa*. Steady-state lung tissue concentrations after intravenous ceftazidime were not influenced by the histological grade of pneumonia.

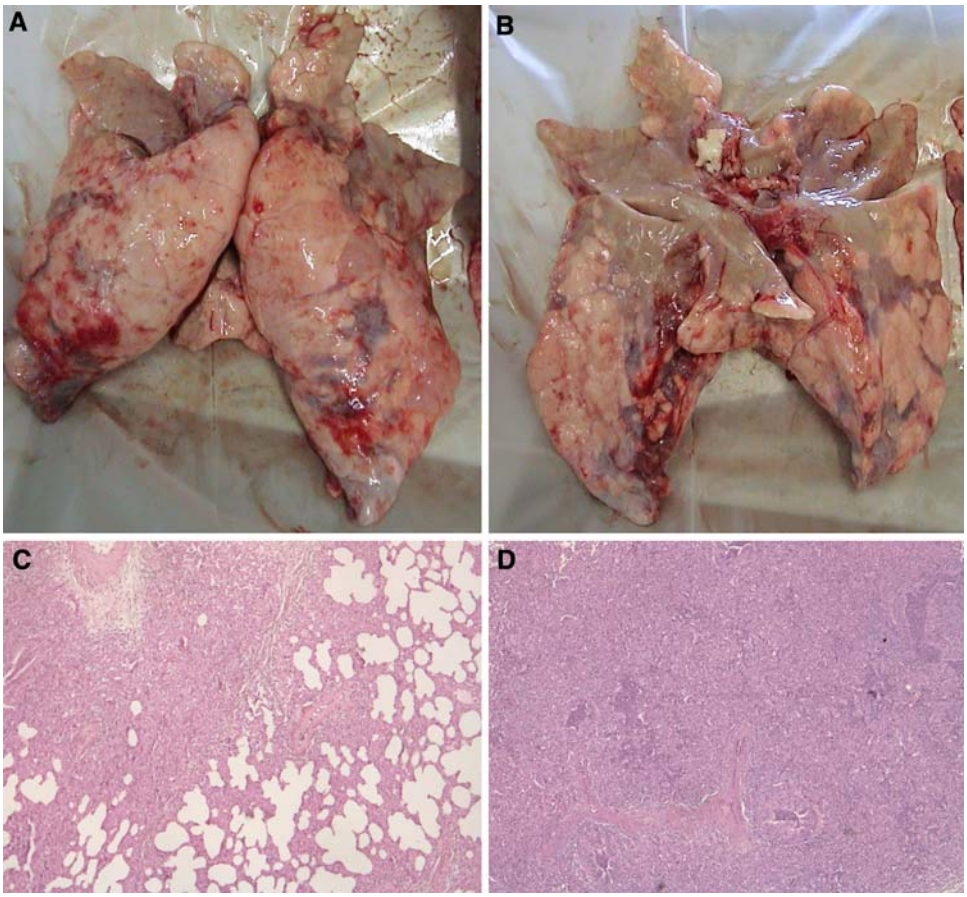

Fig. 1 Macroscopic (*upper panels*) and histological (*lower panels*) aspects of the lungs in a control piglet 48 h after the intrabronchial inoculation of 40 ml of a solution containing 10^6 colony-forming units ml^{-1} of *Pseudomonas aeruginosa*. **a** Nondependent regions of right and left lungs. **b** Dependent regions of right and left lungs. **c**, **d** Confluent pneumonia in dependent lung regions: segment 8 (*left picture*) and segment 10 (*right picture*)

Bactericidal activity of ceftazidime

As shown in Fig. 4, after 24 h of antibiotic administration, the *Pseudomonas aeruginosa* lung bacterial burden was significantly lower in the aerosol group than in intravenous and control groups: median (IQR): 0 (0–0) versus 8.5×10^2 ($0–5 \times 10^4$) cfu g^{-1} and 2×10^4 (1.3×10^3–7.5×10^6) cfu g^{-1}, respectively. In the aerosol group, 83% of segments were characterized by bacterial counts ranging between 0 (sterile) and 10^2 cfu g^{-1} versus 30% in intravenous and 10% in control groups.

Serum pharmacokinetics

Pharmacokinetics of ceftazidime plasma concentrations are represented in Fig. 5. Peak and trough plasma concentrations were similar after each successive aerosol, suggesting the lack of ceftazidime accumulation. In the aerosol group, mean peak plasma concentrations were significantly lower than steady-state plasma concentrations in the intravenous group.

Discussion

This study, performed in ventilated piglets with inoculation pneumonia caused by partially resistant *Pseudomonas aeruginosa* shows that tissue concentrations of nebulized ceftazidime achieved in sub-pleural lung regions were fourfold greater than those obtained after continuous intravenous administration. Such a high lung deposition was associated with rapid and powerful bactericidal activity. Confirming previous data reported in piglets with *Escherichia coli* pneumonia treated by nebulized amikacin [10], ceftazidime distal lung deposition decreased with the severity of pneumonia. However, in lung areas with extensive infection and massive loss of aeration, tissue concentrations remained $\geq$16 μg g^{-1}, the MIC of the inoculated *Pseudomonas aeruginosa*.

Ceftazidime lung tissue concentrations and bactericidal activity

Pseudomonas aeruginosa is one of the most common gram-negative pathogens causing ventilator-associated

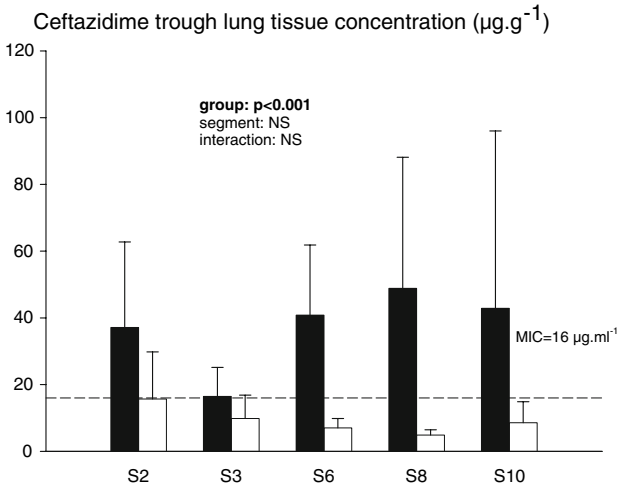

Fig. 2 Trough lung tissue concentrations of ceftazidime measured 3 h after the eighth nebulization in the aerosol group (*black bars*, $n = 6$ anesthetized and ventilated piglets) and steady-state lung tissue concentrations of ceftazidime measured 24 h after the start of continuous intravenous infusion in the intravenous group (*white bars*, $n = 6$ anesthetized and ventilated piglets). Ceftazidime lung tissue concentrations were measured on lung specimens obtained in different lung segments (S) representative of each lobe: upper lobe (*S2*), middle lobe (*S3*), nondependent (*S6*), dependent (*S8*) and posterocaudal (*S10*) segments of lower lobe. Significantly higher lung tissue concentrations were found in the aerosol group. *Dashed line* represents minimum inhibitory concentration (MIC) of inoculated *Pseudomonas aeruginosa*

pneumonia [17]. Unfortunately, its resistance to available anti-pseudomonal agents reaches 30% in critically ill patients [18]. In pneumonia caused by sensitive *Pseudomonas aeruginosa*, a continuous intravenous infusion of 90 mg kg^{-1} day^{-1} is recommended to optimize the lung pharmacodynamic profile [7, 19]. In the present study where pneumonia was caused by partially resistant strains, lung bacterial burden did not decrease following such a continuous intravenous infusion (Fig. 4), whereas ceftazidime nebulization repeated eight times a day achieved a rapid and efficient bacterial killing.

It is well known that bactericidal activity of concentration-dependent aminoglycosides depends on peak antibiotic interstitial lung tissue concentrations transitorily reached in the infected parenchyma. On the other hand, time-dependent antibiotics like ceftazidime are efficient for killing bacteria only if interstitial lung tissue concentrations in the infected parenchyma are permanently maintained far over MIC. As a consequence, trough concentrations are much more important than peak concentrations, and continuous administration is preferred to intermittent administration. In a previous study, we reported high peak lung tissue concentrations after a single nebulization of 1-g ceftazidime in mechanically ventilated piglets with *Pseudomonas aeruginosa* pneumonia [11]. However, the ability of nebulized ceftazidime to maintain high trough lung tissue concentrations was not investigated in this study.

As far as bactericidal activity is concerned, it is recommended to sustain antibiotic interstitial lung tissue concentrations fivefold above the MIC during the whole treatment period [20], and, ideally, continuous nebulization should be performed during the whole period of treatment. For technical and safety reasons, however, continuous nebulization cannot be performed during prolonged mechanical ventilation. Therefore, intermittent nebulizations whose periodicity should be optimized appear to be the only applicable technique in clinical practice.

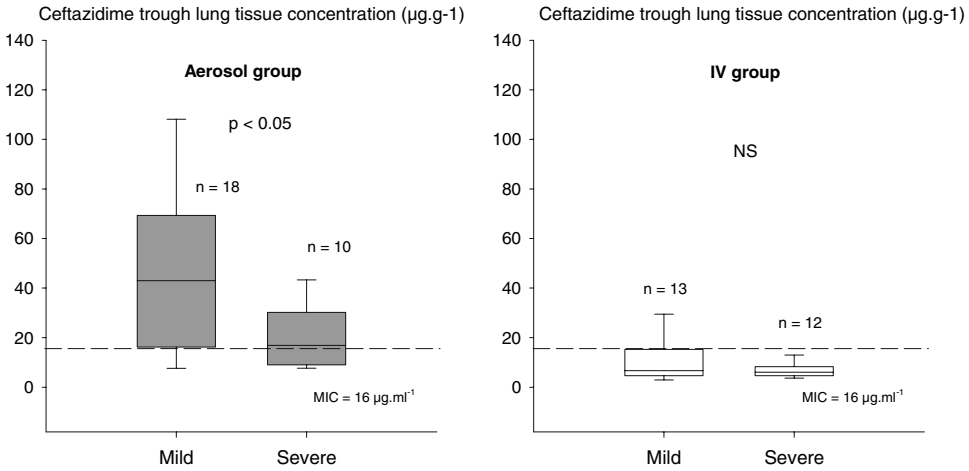

Fig. 3 Ceftazidime trough lung tissue concentrations according to the histological severity of pneumonia in the aerosol (*dark gray boxes, left panel*) and continuous intravenous infusion (*white boxes, right panel*) groups. *Dashed lines* represent minimum inhibitory concentration (MIC) of inoculated *Pseudomonas aeruginosa. Mild* mild pneumonia, *Severe* severe pneumonia, *n* number of lung segments in each histological category, *IV* continuous intravenous infusion. In the aerosol group, ceftazidime trough lung tissue concentrations were significantly greater in lung segments with mild pneumonia ($p < 0.05$)

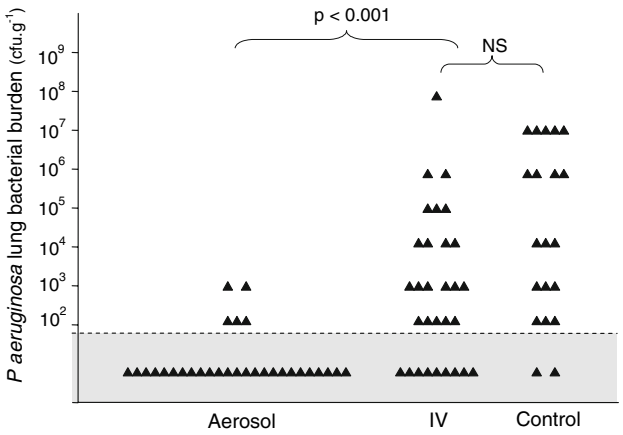

Fig. 4 Lung bacterial burden of *Pseudomonas aeruginosa (P aeruginosa)* after 24 h of ceftazidime administration. Lung segments (*triangles*) were sampled 3 h after the eighth aerosol in the aerosol group, 24 h after the start of continuous infusion in the intravenous group (IV) and 48 h after the bacterial inoculation in the untreated control group. Lung bacterial burden was significantly lower in the aerosol group compared to the intravenous and control groups. The difference was not statistically significant between intravenous and control groups. The *grey area* indicates the lower limit of quantitation for bacterial counts. Lung segments in this area are characterized by bacterial counts ranging between 0 (sterile) and 10^2 cfu g^{-1}

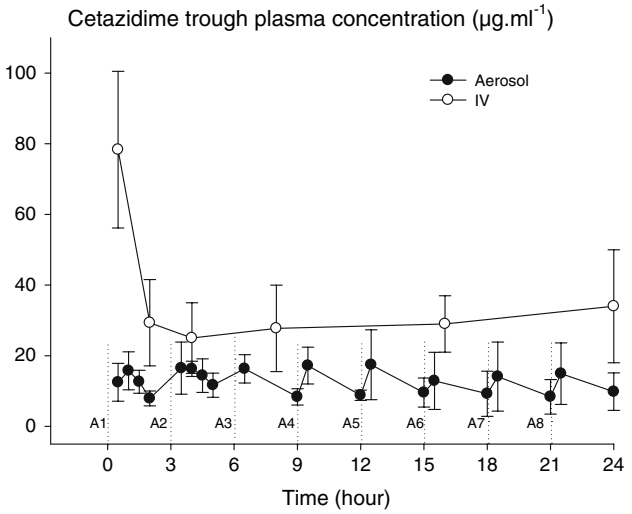

Fig. 5 Comparison of time profiles of mean ceftazidime plasma concentrations in the intravenous group (continuous infusion of 90 mg/kg per day preceded by an initial rapid infusion of 30 mg/kg, *open circles*, six anesthetized and ventilated piglets with inoculation pneumonia) and the aerosol group (intermittent administration by nebulization of 25 mg/kg every 3 h, *closed circles*, six anesthetized and ventilated piglets with inoculation pneumonia). *A1* to *A8* = First to eighth aerosol. *IV* intravenous continuous infusion

In a preliminary study using the same experimental model, we found that ceftazidime nebulization at a dose of 50 mg kg^{-1} every 6 h (4 g day^{-1}) was not able to achieve adequate trough lung tissue concentrations and provide efficient bacterial killing. Therefore, in the present study, the same dose (4 g day^{-1}) was nebulized every 3 h (25 mg kg^{-1}), with the hope of reaching sufficient trough lung tissue concentrations. Due to an extrapulmonary deposition of 59, 41% of the initial ceftazidime dose placed into the nebulizer chamber entered the respiratory system at the distal end of the endotracheal tube. This pulmonary dose of 86 mg kg^{-1} day^{-1} was equivalent to the dose administered intravenously. It is therefore of note that trough lung tissue concentrations following ceftazidime nebulizations were fourfold greater than those obtained after continuous intravenous administration allowing a major bactericidal effect in 83% of subpleural lung specimens cultured after eight aerosol sequences.

It should be pointed out that lung tissue concentrations obtained from homogenized lung biopsies, as reported in the present study, likely underestimate lung interstitial tissue concentrations, the true concentrations that determine ceftazidime's bactericidal effect. Lung tissue concentrations measured in homogenates represent the total amount of ceftazidime coming from both interstitial and cell compartments. Because β-lactams have been shown to have virtually no ability to fill the intracellular space, the cell component of the homogenate acts as diluting space [21], and ceftazidime concentrations measured in homogenates underestimate interstitial concentrations [21–23]. The value of this diluting factor can be estimated from one of our previous work performed in the same experimental model [7]. Following a 24-h continuous intravenous infusion of 90 mg kg^{-1} ceftazidime, plasma levels were 3.5-fold greater than lung tissue concentrations measured in homogenized lung biopsies [7]. According to the pharmacokinetic theory, following continuous intravenous infusion, ceftazidime plasma-free and lung interstitial-free fractions are equal at steady state [21, 22]. Since ceftazidime plasma protein binding is low (15–20%), it can be reasonably estimated that lung interstitial space represented 30% of the volume of the homogenate. Such a theoretical approach was confirmed in dogs receiving continuous infusion of cefepime [24]. This estimated value of 30% is also in accordance with values of 25 and 40% reported for the extracellular compartment of lung parenchyma measured in dogs [25] and rabbits [26] and with the value of 40% reported for the extracellular compartment of the human bronchial mucosa [27].

Therefore, lung tissue concentrations measured in homogenates should be multiplied by 3.5 to estimate lung interstitial concentrations. As a consequence, interstitial trough tissue concentrations were likely 3- to 13-fold greater than MIC following nebulization and 1 to 2.5-fold greater than MIC following continuous intravenous infusion. This reasoning allows understanding of the major and rapid (within 24 h) bactericidal effect observed following ceftazidime nebulization, contrasting

with the lack of bactericidal effect following continuous intravenous administration. It also outlines the need to reach tissue interstitial concentrations at least fivefold the MIC.

Confirming previous findings [10, 16, 28, 29], lung deposition of nebulized ceftazidime was significantly lower in lung segments with severe pneumonia compared to segments with mild pneumonia. The presence of confluent and necrotizing purulent bronchopneumonia with multiple purulent plugs obstructing distal bronchioles and intra-alveolar inflammatory cells markedly reduces lung aeration, thereby explaining decreased ceftazidime lung deposition [30, 31]. In three lung segments with lung aeration <10%, ceftazidime trough lung tissue concentrations in homogenates were less than 10 $\mu g\ g^{-1}$, corresponding to interstitial lung tissue concentrations $\leq$2-fold the MIC; in four segments with mild pneumonia sampled in dependent lung regions, trough lung tissue concentrations ranged between 7 and 13 $\mu g\ g^{-1}$, corresponding to interstitial lung tissue concentrations ranging between 1.5 and 2.8-fold of the MIC. These findings could explain the lack of bactericidal effect in 5 of 30 lung segments in the aerosol group.

Clinical implications

In patients with *Pseudomonas aeruginosa* pneumonia treated by intravenous antibiotics, recurrence caused by a resistant strain is frequently observed [32–34]. Persistent colonization [35], bacterial adherence to the endotracheal tube and respiratory tract [36], increased apoptosis in neutrophils by the type III secretory system [37] and lung tissue concentrations insufficient for preventing bacterial growth [20] are considered as the main mechanisms of recurrent pneumonia. The present study demonstrates that repetitive nebulizations of ceftazidime provide the possibility of ensuring high trough lung tissue concentrations, a condition that may limit the risk of selecting resistant strains of *Pseudomonas aeruginosa*. In addition, the route of administration reduces upper airway colonization and acts efficiently against the bacterial inoculum coming from the oropharynx [38, 39].

In anesthetized and paralyzed piglets, aerosols repeated every 3 h were well tolerated, and ceftazidime-induced bronchospasm was not observed. In the first pre-study experiments, several episodes of complete obstruction of the expiratory filter were observed and associated with a sudden increase in peak inspiratory pressure and severe arterial hypotension. One animal died from bilateral compressive pneumothorax due to expiratory limb obstruction following completion of the third aerosol. Following this lethal complication, we decided to systematically change the expiratory filter following each aerosol. During the experiments, no complications were observed.

If the nebulization of time-dependent antibiotics is to have a clinical future, it should be outlined that the technique is demanding: nebulizations have to be repeated every 3 h with a standardized technique in order to guarantee distal lung deposition of aerosolized particles. Optimizing nebulization requires removal of moisture exchanger, reduced tidal volume, low respiratory frequency and prolonged inspiratory time, all factors aimed at providing a laminar inspiratory flow and maximal lung deposition [9]. To avoid turbulent inspiratory flows that promote impaction of aerosolized particles in the ventilator circuits and trachea, the patient should always remain synchronized with the ventilator and, if necessary, sedated during the nebulization period. Last but not least, the expiratory filter should be changed systematically after each nebulization. In order to ensure the clinical feasibility of nebulization of time-dependent antibiotics in the intensive care unit, physicians and nurses should follow a specific training aimed at providing indispensable skills.

In conclusion, in anesthetized piglets mechanically ventilated for an inoculation pneumonia caused by *Pseudomonas aeruginosa* with impaired sensitivity to ceftazidime, eight aerosols of ceftazidime per day are much more efficient in terms of lung deposition and bacterial killing than a continuous intravenous infusion of an equivalent dose. Further clinical studies are required to assess the efficiency of nebulized ceftazidime combined with nebulized amikacin in the treatment of pneumonia caused by *Pseudomonas aeruginosa*.

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Members of the Experimental ICU Study

The following members of the Experimental ICU Study Group participated in this study: Marc Tonnellier and Ivan Goldstein (Réanimation Polyvalente, Hôpital Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Université Pierre et Marie Curie-Paris6), Kamel Louchahi (Département de Pharmacologie, Hôpital Avicenne, Bobigny, France); Marie-Hélène Becquemin (Explorations Fonctionelles Respiratoires UPRES 2397, Hôpital Pitié-Salpêtrière et Université Paris VII, Paris, France); Gilles Lenaour, (Département d'anatomopathologie, Hôpital Pitié-Salpêtrière, Paris, France).

References

1. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M (1995) The prevalence of nosocomial infection in intensive care units in Europe Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA 274:639–644
2. Bergmans DC, Bonten MJ, Gaillard CA, van Tiel FH, van der Geest S, de Leeuw PW, Stobberingh EE (1997) Indications for antibiotic use in ICU patients: a 1-year prospective surveillance. J Antimicrob Chemother 39:527–535
3. Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F (2007) *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect 13:560–578
4. Obritsch MD, Fish DN, MacLaren R, Jung R (2005) Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. Pharmacotherapy 25:1353–1364
5. Cavallo JD, Leblanc F, Fabre R (2000) Surveillance of *Pseudomonas aeruginosa* sensitivity to antibiotics in France and distribution of beta-lactam resistance mechanisms: 1998 GERPB study. Pathol Biol (Paris) 48:472–477
6. Alou L, Aguilar L, Sevillano D, Gimenez MJ, Echeverria O, Gomez-Lus ML, Prieto J (2005) Is there a pharmacodynamic need for the use of continuous versus intermittent infusion with ceftazidime against *Pseudomonas aeruginosa*? An in vitro pharmacodynamic model. J Antimicrob Chemother 55:209–213
7. Girardi C, Tonnellier M, Goldstein I, Sartorius A, Wallet F, Rouby JJ (2006) Lung deposition of continuous and intermittent intravenous ceftazidime in experimental *Pseudomonas aeruginosa* bronchopneumonia. Intensive Care Med 32:2042–2048
8. Goldstein I, Wallet F, Robert J, Becquemin MH, Marquette CH, Rouby JJ (2002) Lung tissue concentrations of nebulized amikacin during mechanical ventilation in piglets with healthy lungs. Am J Respir Crit Care Med 165:171–175
9. Rouby JJ, Goldstein I, Lu Q (2006) Inhaled antibiotic therapy. In: Tobin MJ (ed) Principles and practice of mechanical ventilation, chap 64, 2nd edn. McGraw-Hill Medical Publishing Division, New York, pp 1311–1332
10. Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Marquette CH, Rouby JJ (2002) Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. Am J Respir Crit Care Med 166:1375–1381
11. Tonnellier M, Ferrari F, Goldstein I, Sartorius A, Marquette CH, Rouby JJ (2005) Intravenous versus nebulized ceftazidime in ventilated piglets with and without experimental bronchopneumonia: comparative effects of helium and nitrogen. Anesthesiology 102:995–1000
12. Ferrari F, Liu ZH, Lu Q, Becquemin MH, Louchahi K, Aymard G, Marquette CH, Rouby JJ (2008) Comparison of lung tissue concentrations of nebulized ceftazidime in ventilated piglets: ultrasonic versus vibrating plate nebulizers. Intensive Care Med 34:1718–1723
13. Myers CM, Blumer JL (1983) Determination of ceftazidime in biological fluids by using high-pressure liquid chromatography. Antimicrob Agents Chemother 24:343–346
14. Dahlberg E (1983) Estimation of the blood contamination of tissue extracts. Anal Biochem 130:108–113
15. Baselski VS, Wunderink RG (1994) Bronchoscopic diagnosis of pneumonia. Clin Microbiol Rev 7:533–558
16. Elman M, Goldstein I, Marquette CH, Wallet F, Lenaour G, Rouby JJ (2002) Influence of lung aeration on pulmonary concentrations of nebulized and intravenous amikacin in ventilated piglets with severe bronchopneumonia. Anesthesiology 97:199–206
17. Agodi A, Barchitta M, Cipresso R, Giaquinta L, Romeo MA, Denaro C (2007) *Pseudomonas aeruginosa* carriage, colonization, and infection in ICU patients. Intensive Care Med 33:1155–1161
18. Flamm RK, Weaver MK, Thornsberry C, Jones ME, Karlowsky JA, Sahm DF (2004) Factors associated with relative rates of antibiotic resistance in *Pseudomonas aeruginosa* isolates tested in clinical laboratories in the United States from 1999 to 2002. Antimicrob Agents Chemother 48:2431–2436
19. Boselli E, Breilh D, Rimmele T, Poupelin JC, Saux MC, Chassard D, Allaouchiche B (2004) Plasma and lung concentrations of ceftazidime administered in continuous infusion to critically ill patients with severe nosocomial pneumonia. Intensive Care Med 30:989–991
20. Mouton JW, den Hollander JG (1994) Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an in vitro pharmacokinetic model. Antimicrob Agents Chemother 38:931–936
21. Nix DE, Goodwin SD, Peloquin CA, Rotella DL, Schentag JJ (1991) Antibiotic tissue penetration and its relevance: impact of tissue penetration on infection response. Antimicrob Agents Chemother 35:1953–1959
22. Ryan DM, Cars O (1983) A problem in the interpretation of beta-lactam antibiotic levels in tissues. J Antimicrob Chemother 12:281–284
23. Nix DE, Goodwin SD, Peloquin CA, Rotella DL, Schentag JJ (1991) Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. Antimicrob Agents Chemother 35:1947–1952
24. Bayat S, Louchahi K, Verdiere B, Anglade D, Rahoui A, Sorin PM, Tod M, Petitjean O, Fraisse F, Grimbert FA (2004) Comparison of 99mTc-DTPA and urea for measuring cefepime concentrations in epithelial lining fluid. Eur Respir J 24:150–156
25. Talor AE, Guyton AC, Bishop VS (1965) Permeability of the alveolar membrane to solutes. Circ Res 16:353–362
26. Gengo FM, Schentag JJ, Jusko WJ (1984) Pharmacokinetics of capacity-limited tissue distribution of methicillin in rabbits. J Pharm Sci 73:867–873
27. Baldwin DR, Wise R, Andrews JM, Honeybourne D (1992) Concentrations of cefpodoxime in serum and bronchial mucosal biopsies. J Antimicrob Chemother 30:67–71
28. Goldstein I, Bughalo MT, Marquette CH, Lenaour G, Lu Q, Rouby JJ (2001) Mechanical ventilation-induced air-space enlargement during experimental pneumonia in piglets. Am J Respir Crit Care Med 163:958–964
29. Wermert D, Marquette CH, Copin MC, Wallet F, Fraticelli A, Ramon P, Tonnel AB (1998) Influence of pulmonary bacteriology and histology on the yield of diagnostic procedures in ventilator-acquired pneumonia. Am J Respir Crit Care Med 158:139–147

30. Fabregas N, Torres A, El-Ebiary M, Ramirez J, Hernandez C, Gonzalez J, de la Bellacasa J, de Anta J, Rodriquez-Roisin R (1996) Histopathologic and microbiologic aspects of ventilator-associated pneumonia. Anesthesiology 84:757–759
31. Rouby JJ, Martin De Lassale E, Poete P, Nicolas MH, Bodin L, Jarlier V, Le Charpentier Y, Grosset J, Viars P (1992) Nosocomial bronchopneumonia in the critically ill histologic and bacteriologic aspects. Am Rev Respir Dis 146:1059–1066
32. Hauser AR (2008) *Pseudomonas aeruginosa*: an uninvited guest refuses to leave. Am J Respir Crit Care Med 178:438–439
33. Rello J, Mariscal D, March F, Jubert P, Sanchez F, Valles J, Coll P (1998) Recurrent *Pseudomonas aeruginosa* pneumonia in ventilated patients: relapse or reinfection? Am J Respir Crit Care Med 157:912–916
34. Visscher S, Schurink CA, Melsen WG, Lucas PJ, Bonten MJ (2008) Effects of systemic antibiotic therapy on bacterial persistence in the respiratory tract of mechanically ventilated patients. Intensive Care Med 34:692–699
35. Valles J, Mariscal D, Cortes P, Coll P, Villagra A, Diaz E, Artigas A, Rello J (2004) Patterns of colonization by *Pseudomonas aeruginosa* in intubated patients: a 3-year prospective study of 1,607 isolates using pulsed-field gel electrophoresis with implications for prevention of ventilator-associated pneumonia. Intensive Care Med 30:1768–1775
36. Kobayashi H (2005) Airway biofilms: implications for pathogenesis and therapy of respiratory tract infections. Treat Respir Med 4:241–253
37. El Solh AA, Akinnusi ME, Wiener-Kronish JP, Lynch SV, Pineda LA, Szarpa K (2008) Persistent infection with *Pseudomonas aeruginosa* in ventilator-associated pneumonia. Am J Respir Crit Care Med 178:513–519
38. Palmer LB, Smaldone GC, Chen JJ, Baram D, Duan T, Monteforte M, Varela M, Tempone AK, O'Riordan T, Daroowalla F, Richman P (2008) Aerosolized antibiotics and ventilator-associated tracheobronchitis in the intensive care unit. Crit Care Med 36:2008–2013
39. Falagas ME, Siempos II, Bliziotis IA, Michalopoulos A (2006) Administration of antibiotics via the respiratory tract for the prevention of ICU-acquired pneumonia: a meta-analysis of comparative trials. Crit Care 10:R123