

# Lack of Lung Tissue and Systemic Accumulation after Consecutive Daily Aerosols of Amikacin in Ventilated Piglets with Healthy Lungs

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IN a model of ventilated piglets with healthy lungs, we recently demonstrated a substantial deposition of nebulized amikacin into the distal lung.<sup>1</sup> However, only half of the amount of amikacin available for the lungs was eliminated in the urine; the other half was either fixed in the lungs, eliminated by mucociliary clearance, or distributed in other organs. Consequently, consecutive daily nebulizations of amikacin may result in lung tissue accumulation and systemic accumulation, and the frequency of readministration should be defined in experimental animals before nebulized amikacin can be safely used in humans for treating or preventing ventilator-associated pneumonia. The aim of the present study was to assess lung tissue and plasma pharmacokinetics resulting from the daily nebulization of amikacin during a 4-day period of mechanical ventilation in anesthetized piglets with healthy lungs.

## Materials and Methods

### Animal Preparation

Eighteen healthy Landrace piglets aged 3 to 4 months and weighing  $20 \pm 2$  kg were anesthetized using intravenous propofol (3 mg/kg) and were orotracheally intubated in the supine position with a 7.0 Hi-Lo Jet Mallinckrodt tube (Mallinckrodt Inc., Argyle, NY). Anesthesia was maintained throughout the experiment with a continuous infusion of midazolam,  $0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; pancuronium,  $0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; and fentanyl,  $5 \mu\text{g} \cdot$

$\text{kg}^{-1} \cdot \text{h}^{-1}$ . A 20-gauge catheter was inserted in the ear vein for continuous infusion of 10% dextrose ( $1.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and lactated Ringer's solution ( $3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). A femoral artery catheter (Plastimed, St. Leu la Forêt, France) and a suprapubic urinary catheter (Vesicost; Angiomed, Karlsruhe, Germany) were placed for blood and urine sampling. All animals were treated according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 93-23, revised 1985).

The piglets were then mechanically ventilated for 4 days in the prone position with a César ventilator (Taema, Antony, France) using a fixed tidal volume of 15 ml/kg, a variable respiratory rate adjusted to maintain  $\text{Paco}_2$  between 35 and 45 mmHg, zero end-expiratory pressure, and a fraction of inspired oxygen adjusted to maintain  $\text{Pao}_2$  above 80 mmHg.

### Aerosol Generation and Assessment of Extrapulmonary Deposition

A commercially available ultrasonic nebulizer (Atomisor MegaHertz; Diffusion Technique Française, Saint-Etienne, France), producing small-sized particles required for a distal parenchymal lung deposition, was used as previously described.<sup>1</sup> The daily dose of amikacin ( $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) was set according to previous studies and was based on the determination of the extrapulmonary deposition<sup>1,2</sup> in order to deliver to the respiratory system a dose equivalent to the dose of 15 mg/kg recommended for intravenous administration.

### Study Design

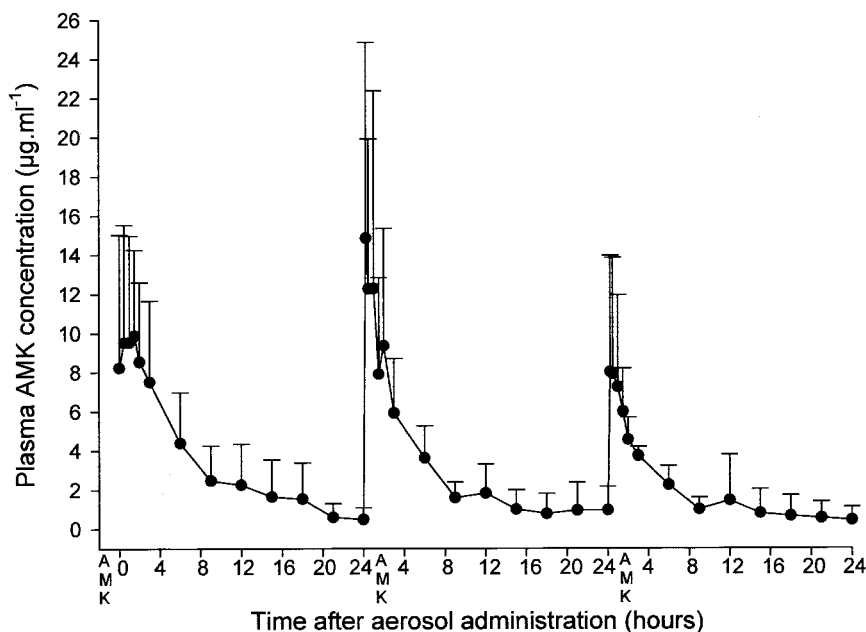
The first amikacin aerosol was performed at steady state, 2 h after the induction of anesthesia. The subsequent aerosols were delivered at 24-h time intervals. In order to detect a possible lung accumulation of amikacin, five piglets were killed 60 min after the first aerosol (group 1), four were killed 60 min after the second aerosol (group 2), and nine were killed 1 h after the fourth aerosol (group 4). In order to identify a possible systemic accumulation, blood samples were collected in group 4 ( $n = 9$ ) at 0, 0.5, 1, 1.5, 2, 3, 6, 9, 12, 15, 18, 21, and 24 h after the end of each daily nebulization. Urine samples were collected every 3 h during the 24 h following each nebulization. Amikacin plasma and urinary

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Fig. 1. Plasma concentrations of amikacin (AMK; mean  $\pm$  SD) following three consecutive aerosols ( $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) in nine piglets ventilated during 4 days. Following each aerosol (black arrows), amikacin plasma concentrations significantly decreased ( $P < 0.0001$ , two-way analysis of variance) in similar proportion (no interaction). Peak plasma and trough amikacin concentrations were not significantly different following the first, second, and third aerosol, suggesting a lack of systemic accumulation.



concentrations were measured by the immunoenzymatic method (TDx; Abbott Laboratories, Abbott Park, IL).

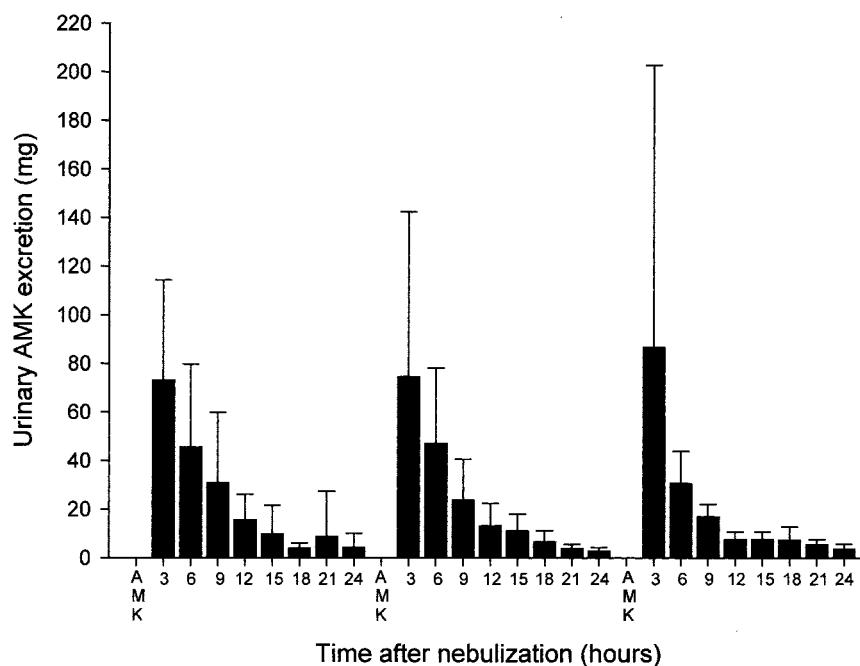
#### Measurement of Lung Tissue Concentrations of Amikacin

At the end of the experiment, the animals were killed as previously described,<sup>2</sup> and five subpleural lung specimens measuring 3 to 4 cm<sup>3</sup> were excised from the upper lobe (S2), the middle lobe (S4), the apical dependent segment of the lower lobe (S6), the anterior non-dependent segment of lower lobe (S8), and the postero-caudal segment of lower lobe (S10). Tissue samples were processed for amikacin lung tissue using the immunoenzymatic method.<sup>1,2</sup>

#### Pharmacokinetic Analysis

The peak plasma concentration ( $C_{\max}$ ) and the trough plasma concentration ( $C_{\min}$ ) were obtained by direct observation of the individual kinetic profiles. The analysis was performed with the Siphar<sup>®</sup> pharmacokinetics software program (Simed, Creteil, France) using a one-compartment pharmacokinetic open model with a first order elimination. The area under the plasma concentration-time curve ( $AUC_{P\ 0 \rightarrow 24\ h}$ ) was calculated using the trapezoidal rule and included all experimental data points. The amount of drug excreted in the urine ( $A_{U\ 0 \rightarrow 24\ h}$ ) was calculated as the sum of amikacin urinary output measured every 3 h.

Fig. 2. Fractional urinary elimination of amikacin (AMK; mean  $\pm$  SD) in nine piglets ventilated during 4 days and receiving a daily amikacin aerosol at a dose of  $40 \text{ mg/kg}$  (black arrows). Following each aerosol, amikacin urinary elimination significantly decreased ( $P < 0.0001$ , two-way analysis of variance) in similar proportion (no interaction). Amikacin elimination time profile was similar after each aerosol, suggesting the absence of systemic and lung accumulation between doses.



**Table 1. Plasma and Urine Pharmacokinetic Parameters after the First, Second, and Third Aerosol (n = 9)**

|  | Day 1           | Day 2           | Day 3           | P    |
|--|-----------------|-----------------|-----------------|------|
| $AUC_{S\ 0 \rightarrow 24\ h}, \mu g \cdot h^{-1} \cdot ml^{-1}$ | $79 \pm 18$     | $68 \pm 12$     | $67 \pm 26$     | 0.32 |
| $C_{max}, \mu g/ml$  | $13 \pm 6$      | $16 \pm 10$     | $11 \pm 6$      | 0.37 |
| $C_{min}, \mu g/ml$  | $0.47 \pm 0.60$ | $0.94 \pm 1.20$ | $0.43 \pm 0.67$ | 0.72 |
| $A_{U\ 0 \rightarrow 24\ h}, mg$                                 | $176 \pm 80$    | $175 \pm 113$   | $165 \pm 125$   | 0.63 |

Values were not significantly different (one-way analysis of variance for  $AUC_{S\ 0 \rightarrow 24\ h}$  and  $C_{max}$ ; Kruskal-Wallis for  $C_{min}$  and  $A_{U\ 0 \rightarrow 24\ h}$ ). All data are expressed as mean  $\pm$  SD.

$A_{U\ 0 \rightarrow 24\ h}$  = amount of drug excreted in the urine in the 24-h period;  $AUC_{S\ 0 \rightarrow 24\ h}$  = area under the plasma concentration-time curve;  $C_{max}$  = peak plasma concentration;  $C_{min}$  = trough plasma concentration.

### Statistical Analysis

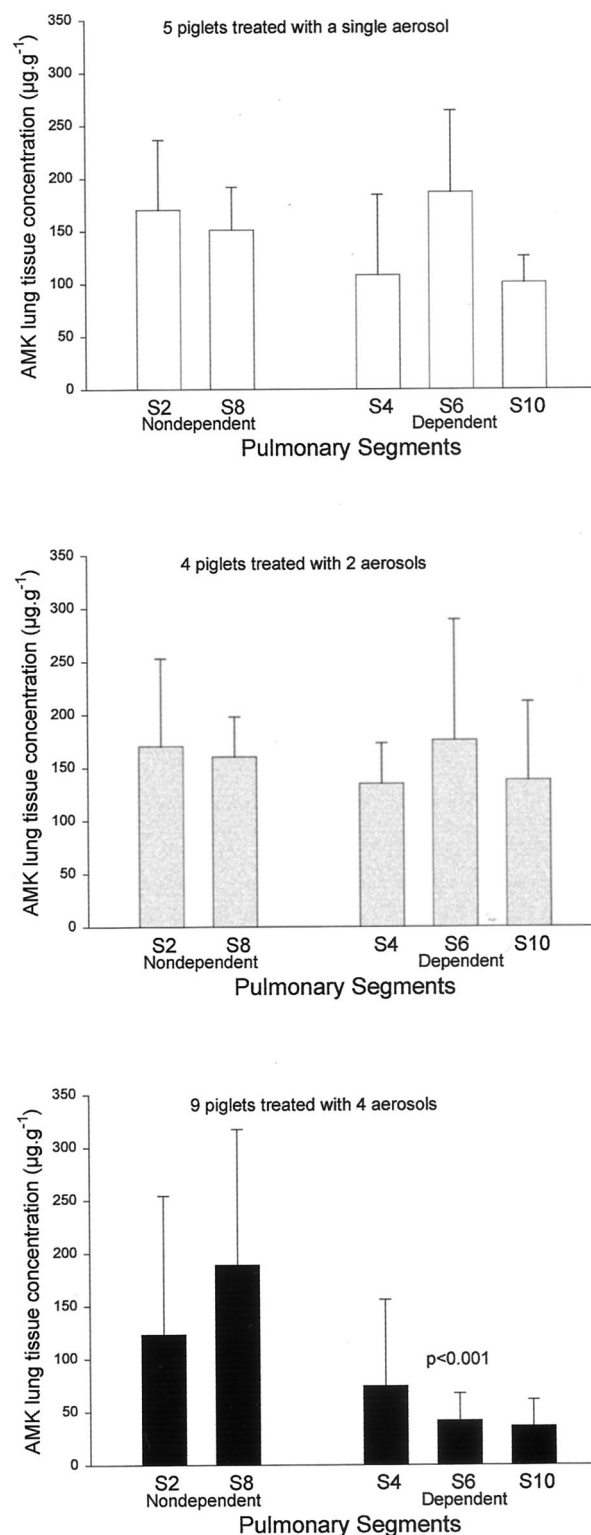
All data are expressed as mean  $\pm$  SD. Plasma amikacin concentrations, amikacin fractional urinary output, and standard pharmacokinetic parameters ( $C_{max}$ ,  $C_{min}$ ,  $AUC_{S\ 0 \rightarrow 24\ h}$ , and  $A_{U\ 0 \rightarrow 24\ h}$ ) were compared using one-way analysis of variance (ANOVA) for repeated measures (day 1, day 2, and day 3) or Kruskal-Wallis test (nonnormal distribution). The regional distribution of lung tissue concentrations according to time was compared using two-way ANOVA for one within factor (lung segment) and one grouping factor (group 1, group 2, group 4). Statistical analysis was performed using Statview<sup>®</sup> 5.2 software (SPSS Inc., Chicago, IL). A *P* value less than 0.05 was considered statistically significant.

## Results

Mean pharmacokinetic parameters ( $C_{max}$ ,  $C_{min}$ ,  $AUC_{S\ 0 \rightarrow 24\ h}$ , and  $A_{U\ 0 \rightarrow 24\ h}$ ) remained unchanged on days 1, 2, and 3 (table 1). The plasma and urine concentrations *versus* the time curves of amikacin are shown in figures 1 and 2. The elimination of amikacin in the last urine fraction was comparable after the first, second, and third aerosol:  $4.4 \pm 5.7$ ,  $2.9 \pm 1.4$ , and  $3.7 \pm 2.1$  mg, respectively. Mean lung tissue amikacin concentrations were not statistically different between groups 1 and 2. As shown in figure 3, in group 4, amikacin lung tissue concentrations remained unchanged in the nondependent pulmonary segments and were significantly decreased in the dependent pulmonary segments (S4, S6, and S10).

## Discussion

In ventilated piglets with healthy lungs, the nebulization of amikacin at a dose of  $40\ mg \cdot kg^{-1} \cdot d^{-1}$  during 4 days resulted in high lung tissue concentrations without producing detectable parenchymal and systemic accumulation.



**Fig. 3.** Regional distribution of amikacin (AMK) lung tissue concentrations measured in piglets with healthy lungs 60 min after the first (white bars), the second (gray bars), and the fourth (black bars) aerosol, showing a significant decrease (two-way analysis of variance) in tissue concentrations in the dependent pulmonary segments after the fourth aerosol. Data are expressed as mean  $\pm$  SD. S2 = upper lobe; S4 = middle lobe; S6 = dependent segment of lower lobe; S8 = nondependent segment of lower lobe; S10 = caudal segment of lower lobe.

### *Lung Tissue Concentrations after Repetitive Amikacin Aerosols*

After 28 h of mechanical ventilation and two aerosols of amikacin, lung tissue concentrations ranged from 50 to 400  $\mu\text{g/g}$  and were homogeneously distributed between dependent and nondependent lung segments. After 75 h of mechanical ventilation and four consecutive aerosols, amikacin lung tissue concentrations remained unchanged in the nondependent lung segments and were significantly decreased in the dependent lung segments, ranging from 20 to 60  $\mu\text{g/g}$ . The observed decrease in lung deposition within the dependent lung segments very likely is explained by a time-dependent reduction in regional lung aeration, as previously described.<sup>2,3</sup> No positive end-expiratory pressure was administered during the period of mechanical ventilation, exposing the dependent lung areas to progressive atelectasis. Further studies are required to assess whether positive end-expiratory pressure, by preventing a time-dependent collapse of dependent lung regions, may maintain a high parenchymal deposition of inhaled amikacin. However, it must be pointed out that despite the decrease in amikacin deposition in dependent pulmonary segments, amikacin lung tissue concentrations remained three to seven times greater than concentrations obtained after intravenous administration.<sup>1</sup>

### *Amikacin Plasma and Urine Pharmacokinetics after Consecutive Daily Aerosols*

Following each aerosol, amikacin peak and trough plasma concentrations and daily urinary elimination remained within similar ranges in each individual piglet. However, an important interindividual variability was observed, confirming previous results obtained in spontaneously breathing patients with cystic fibrosis.<sup>4</sup>

In animals with healthy lungs, the intact alveolar-capillary membrane offers a high resistance to the systemic diffusion of amikacin.<sup>1</sup> Therefore, in the present study, low and delayed amikacin peak plasma concentrations were found, contrasting with high lung tissue concentrations. Every day, only half of the dose administered to the tracheobronchial tree was eliminated in the urine. Consequently, during 3 days of administration, significant amounts of amikacin likely accumulated within the tracheobronchial tree and were removed by successive endotracheal suctioning and/or physiologic mucociliary clearance.

In the present study, amikacin plasma concentrations plateaued from the tenth hour following the second and the third aerosol, and the trough concentrations remained below the threshold for renal toxicity.<sup>5</sup> Of course these findings cannot be automatically extrapolated to bronchopneumonic animals, in which the systemic diffusion of aerosolized amikacin is substantially greater. However, the kinetic profiles of plasma concentration decays following nebulization or intravenous administration of equivalent doses of amikacin are similar in bronchopneumonic animals.<sup>6</sup> Thus, it is reasonable to assume that a unique daily aerosol should be as safe as a unique intravenous administration if renal function is preserved. Additional studies performed in bronchopneumonic animals are required to confirm this hypothesis.

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## References

1. Goldstein I, Wallet F, Robert J, Becquemin MH, Marquette CH, Rouby JJ: Lung tissue concentrations of nebulized amikacin during mechanical ventilation in piglets with healthy lungs. *Am J Respir Crit Care Med* 2002; 165:171-5
2. Elman M, Goldstein I, Marquette CH, Wallet F, Lenaour G, Rouby JJ: Influence of lung aeration on pulmonary concentrations of nebulized and intravenous amikacin in ventilated piglets with severe bronchopneumonia. *ANESTHESIOLOGY* 2002; 97:199-206
3. Marquette CH, Wermert D, Wallet F, Copin MC, Tonnel AB: Characterization of an animal model of ventilator-acquired pneumonia. *Chest* 1999; 115:200-9
4. Illowite JS, Gorvoy JD, Smaldone GC: Quantitative deposition of aerosolized gentamicin in cystic fibrosis. *Am Rev Respir Dis* 1987; 136:1445-9
5. Mingot-Leclercq MP, Tulkens PM: Aminoglycosides: Nephrotoxicity. *Antimicrob Agents Chemother* 1999; 43:1003-12
6. Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Becquemin MH, Marquette CH, Rouby JJ: Lung deposition and efficiency of nebulized amikacin during experimental pneumonia in piglets. *Am J Respir Crit Care Med* 2002; 166:1375-81

## Appendix

The following members of the Experimental ICU Study Group participated in this study: Philippe Lechat, M.D., Ph.D. (Professor, Department of Pharmacology, Pitié-Salpêtrière Hospital, Paris, France); Guy Aymard, Ph.D. (Assistant Professor, Department of Pharmacology, Pitié-Salpêtrière Hospital); Armelle Nicolas Robin, M.D. (Assistant Professor, Réanimation Chirurgicale Pierre Viars, Pitié-Salpêtrière Hospital); Qin Lu, M.D., Ph.D. (Associate Professor, Réanimation Chirurgicale Pierre Viars, Pitié-Salpêtrière Hospital); Pierre Coriat, M.D. (Professor, Department of Anesthesiology, Pitié-Salpêtrière Hospital); Olivier Petitjean, Ph.D. (Professor, Department of Pharmacology, Avicenne Hospital, Bobigny, France); and Kamel Louchahi, Ph.D. (Associate Professor, Department of Pharmacology, Avicenne Hospital).