ANESTHESIOLOGY

Nebulization of Vancomycin Provides Higher Lung Tissue Concentrations than Intravenous Administration in Ventilated Female Piglets with Healthy Lungs

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Intravenously administered vancomycin is the recommended treatment for methicillin-resistant *Staphylococcus aureus* ventilator-associated pneumonia
- High rates of vancomycin treatment failure may be due to poor lung tissue drug penetration
- Administration of nebulized antibiotics can produce high lung tissue concentrations, resulting in more efficient bacterial killing with reduced systemic toxicity

What This Article Tells Us That Is New

- The hypothesis that lung tissue vancomycin concentrations will be higher after administration as an inhaled aerosol than after intravenous administration was tested in healthy, anesthetized, mechanically ventilated female piglets
- One hour after administration of a 37.5 mg/kg aerosol dose, the median lung tissue vancomycin concentration (161 μg/g) was 13 times that after intravenous administration of 15 mg/kg (12 μg/g)
- Twelve hours after aerosol administration, the median lung tissue vancomycin concentration was 63 μg/g, while 12 h after intravenous administration, vancomycin was undetectable in 60% of lung specimens

ABSTRACT

Background: Intravenous vancomycin is used to treat ventilator-associated pneumonia caused by methicillin-resistant *Staphylococcus aureus*, but achieves high rates of failure. Vancomycin nebulization may be efficient to provide high vancomycin lung tissue concentrations. The aim of this study was to compare lung tissue and serum concentrations of vancomycin administered intravenously and by aerosol in mechanically ventilated and anesthetized healthy piglets.

Methods: Twelve female piglets received a single intravenous dose of vancomycin (15 mg/kg) and were killed 1 (n = 6) or 12 h (n = 6) after the end of administration. Twelve piglets received a single nebulized dose of vancomycin (37.5 mg/kg) and were killed 1 (n = 6) or 12 h (n = 6) after the end of the aerosol administration. In each group, vancomycin lung tissue concentrations were assessed on postmortem lung specimens using high-performance liquid chromatography. Blood samples were collected for serum vancomycin concentration measurement 30 min and 1, 2, 4, 6, 8, and 12 h after the end of vancomycin administration. Pharmacokinetics was analyzed by nonlinear mixed effect modeling.

Results: One hour after vancomycin administration, lung tissue concentrations in the aerosol group were 13 times the concentrations in the intravenous group (median and interquartile range: 161 [71, 301] μg/g *versus* 12 [4, 42] μg/g; $P < 0.0001$). Twelve hours after vancomycin administration, lung tissue concentrations in the aerosol group were 63 (23, 119) μg/g and 0 (0, 19) μg/g in the intravenous group ($P < 0.0001$). A two-compartment weight-scaled allometric model with first-order absorption and elimination best fit serum pharmacokinetics after both routes of administration. Area under the time-concentration curve from 0 to 12 h was lower in the aerosol group in comparison to the intravenous group (56 [8, 70] mg · h · l^{-1} *vs.* 121 [103, 149] mg · h · l^{-1}, $P = 0.002$). Using a population model, vancomycin bioavailability was 13% (95% CI, 6 to 69; coefficient of variation = 85%) and absorption rate was slow (absorption half life = 0.3 h).

Conclusions: Administration of vancomycin by nebulization resulted in higher lung tissue concentrations than the intravenous route.

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Methicillin-resistant *Staphylococcus aureus* is one of the main causal agents of ventilator-associated pneumonia.[1,2] This pathogen often presents resistance to other antimicrobials, which is a concern in relation to the treatment options.[3] Vancomycin is the recommended treatment,[4] but this standard therapy results in poor clinical outcomes, not exceeding 57% of clinical success rate.[5–11]

High rates of vancomycin treatment failure may be explained by poor lung tissue penetration of intravenous administration. The large size of the molecule may limit parenchymal diffusion, explaining the observation that vancomycin alveolar lining epithelial fluid concentration is around one sixth that in serum.[5,12,13] Another pharmacokinetic issue is that vancomycin is a time-dependent antibiotic, and sustained concentrations above the minimum inhibitory concentration of the bacteria between dose

intervals are essential for bacteria killing. The limited efficacy of vancomycin may also be associated with diminished bactericidal activity against methicillin-resistant *S. aureus* strains with minimum inhibitory concentration in the superior limit of susceptibility, ranging from 1 to 2 μg/ml. This is a great concern, as mortality of ventilator-associated pneumonia increases as a function of *S. aureus* vancomycin minimum inhibitory concentration.[5]

Nebulization of antibiotics can provide high lung tissue concentration, an efficient bacterial killing with reduced systemic toxicity.[14–17] Clinical studies have demonstrated the effectiveness of nebulized antibiotics to treat ventilator-associated pneumonia, eradicating multidrug-resistant organisms and reducing the pressure for selection of new resistant organisms.[18,19] However, randomized controlled trials establishing the superiority of nebulized *versus* intravenous antibiotics are lacking.[20] Despite the lack of recommendation supporting their use,[21] inhaled colistin and aminoglycosides are widely used in the world for treating ventilator-associated pneumonia and ventilator-associated tracheobronchitis, caused by multidrug-resistant Gram-negative bacteria.[22,23]

There is no experimental study comparing nebulized *versus* intravenous vancomycin for treating ventilator-associated pneumonia and ventilator-associated tracheobronchitis caused by methicillin-resistant *S. aureus*. The aim of the study was to compare the distribution of lung tissue concentrations between the different lung segments, and the serum pharmacokinetics of vancomycin administered either intravenously or by aerosol through vibrating plate nebulizer in four groups of mechanically ventilated piglets with healthy lungs. We hypothesized that vancomycin lung tissue concentration will be greater after nebulization in comparison to intravenous administration. Vancomycin extrapulmonary deposition after nebulization was measured to determine antibiotic lung availability.

Materials and Methods

This study was approved by Research Ethics Committee of the School of Medicine of São Paulo University (São Paulo, Brazil; No. 1001/18). Twenty-eight Landrace and Largewhite crossbred female pigs weighing 26.5 ± 3.7 kg (18 to 31 kg) were used in the study. Animals were fasted for 8 h with free access to water before the experiments.

Animal Preparation

The animals were sedated with intramuscular ketamine 5 mg/kg and midazolam 0.25 mg/kg, then were anesthetized using propofol 3 mg/kg and orotracheally intubated. Anesthesia was maintained with a continuous infusion of propofol 100 μg · kg^{-1} · h^{-1}, midazolam 0.5 mg · kg^{-1} · h^{-1}, pancuronium 0.3 mg · kg^{-1} · h^{-1}, and fentanyl 16 μg · kg^{-1} · h^{-1}. The femoral artery was cannulated with a catheter (Pulsiocath PV2015L20, Pulsion Medical System, Germany) for continuous blood pressure and cardiac output monitoring and intermittent blood sampling. After bladder catheterization, urine output was collected every 3 h. During the experiment, a 3 ml · kg^{-1} · h^{-1} lactated Ringer's solution was administered. The piglets were then placed in the prone position and mechanically ventilated in a volume-controlled mode, tidal volume 8 ml/kg, positive end-expiratory pressure 5 cm H_2O, and fractional inspired oxygen tension 0.50, with a Servo-i ventilator (Maquet Critical Care, Sweden). The prone position, the natural posture of four-legged animals, is associated with a more homogenous ventilation-perfusion distribution resulting from a better ventilation distribution and an increased correlation between regional ventilation and pulmonary blood flow.[24]

Aerosol Generation

A vibrating plate nebulizer (Aeroneb Pro; Aerogen Ltd., Ireland) was positioned in the inspiratory limb, 30 cm proximal to the Y piece. Each nebulization was performed up to 30 min after inserting 37.5 mg/kg of vancomycin diluted in 10 ml of sterile water into the nebulizer chamber. Ventilator settings for the nebulization period were optimized as recommended[21]: volume-controlled mode using constant inspiratory flow; respiratory frequency of 12 breaths/min; inspiratory/expiratory ratio of 50%; tidal volume, 8 to 9 ml/kg; end-inspiratory pause of 20% of the duty cycle; and absence of heat and moisture exchange or conventional humidifier. A filter was added on the distal part of the expiratory limb as recommended.[21] In a preliminary study, one piglet received a single vancomycin nebulization, and ventilator circuits were washed separately with 1 liter of distilled water to assess vancomycin extrapulmonary deposition as previously recommended.[14] The same washing procedure was performed in each animal receiving nebulized vancomycin to assess the vancomycin dose delivered to the respiratory system. The amount of deposited vancomycin in the inspiratory circuits and endotracheal tube was measured by high-performance liquid chromatography.

Study

Twenty-four piglets were distributed into four groups. There was no blinding for analyzing data or randomization in this study. Allocation of animals to each experimental group was

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determined by the day of the week (*e.g.*, Monday, Wednesday, and Friday for 1-h experiments and Tuesday and Thursday for 12-h experiments). Each experiment started at 8:00 AM. Twelve animals received a single dose of vancomycin through intravenous infusion for more than 1 h (15 mg/kg), and animals were killed either 1 (n = 6) or 12 h (n = 6) after the end of administration. Twelve piglets received a single dose of vancomycin through nebulization (37.5 mg/kg in up to 30 min), and animals were killed either 1 (n = 6) or 12 h (n = 6) after the end of administration. In nebulized and intravenous groups killed after 12 h, blood samples were collected for vancomycin concentration measurement 30 min and 1, 2, 4, 6, 8, and 12 h after the end of vancomycin administration. In all groups, the piglets were killed by exsanguination through carotid artery cannulation. Five subpleural specimens of the left lung measuring 1 to 2 cm^3 were excised from the upper lobe (S2), the middle lobe (S4), the apical dependent segment of the lower lobe (S6), the anterior nondependent segment of lower lobe (S8), and the posterior-caudal segment of lower lobe (S10) of the left lung. Postmortem tissue samples were cryomixed in nitrogen. Tissue vancomycin concentrations were measured using high-performance liquid chromatography. Three nontreated animals ventilated for 1 h served as controls.

Serum Vancomycin Concentrations

Serum vancomycin concentrations were measured from noncitrated and centrifuged samples. Tissue and serum vancomycin concentrations were measured using high-performance liquid chromatography with ultraviolet detection.[25] For lung tissue samples, 200 mg of tissue was homogenized in 100 μl of ultrapure water, 40 μl of internal standard caffeine, and 100 μl of trifluoroacetic acid 50% and centrifuged at 5,000 rpm for 3 min, and 20 μL of the supernatant was injected into the high performance liquid chromatography system. The mobile phase consisted of a mixture of 50 mM ammonium phosphate monobasic solution, pH 4.5, and acetonitrile (90:10 v/v), using a flow rate of 1 ml/min. The standard calibration curve for tissue samples was prepared in the 0.6 to 1,000 μg/g concentration range, and tissue vancomycin concentrations were corrected for contaminating blood.[14,26] The peak and trough serum concentrations were obtained by direct observation of the individual kinetic profiles. The area under the serum concentration–time curve from 0 to 12 h was calculated using the trapezoidal rule and included all experimental data points.

Systemic bioavailability of the aerosol, volume of distribution, elimination clearance, and elimination rate constant were calculated from measured vancomycin serum concentrations (Supplemental Digital Content 1, http://links.lww.com/ALN/C243).

Population Pharmacokinetics Analysis

The pharmacokinetic data were analyzed using NONlinear Mixed Effect Modeling software (NONMEM version v7.4; ICON Development Solutions, USA). One- and two-compartmental weight scaled allometric models with first-order absorption and elimination were used to describe the time profile of vancomycin serum concentrations. Structural population parameter estimates [(central volume of distribution (l); peripheral volume of distribution (l); distribution clearance (l/h); elimination clearance (l/h); absorption rate half time (h); bioavailability (%)] were obtained using the first order conditional estimation method. Parameter values were standardized to 70 kg total body weight, as expressed in equation 1,

$$Pi = P_{TVSt} \cdot \left(\frac{W_i}{70}\right)^{PWR} \quad (1)$$

where Pi is the structural parameter in the i^{th} individual, P_{TVSt} is the population parameter estimate standardized for 70 kg, W_i is the weight in the i^{th} individual, and PWR is the exponent for the allometric model and represents a value of 1 for volumes, 0.25 for absorption rate half time, and 0.75 for clearances.

Random effects were included in the model allowing for assessment of between-subject variability and residual variability. Between-subject variability in structural parameters was modeled by exponentiating the random effect variables and is reported as coefficient of variation (%). Residual variability was characterized using a proportional and additive error model.

Model selection was based on visual inspection of data fits, goodness-of-fit plots, standard errors of the estimated parameters, and the minimum value of the objective function (–2 · log-likelihood) provided by NONMEM. A decrease in the objective function of 6.63 for an additional model parameter was considered significant at the $P < 0.01$ level (chi-square distribution). Bootstrap methods, implemented in PLT Tools version 6 (a graphical interface for the NONMEM system, developed by Dennis M. Fisher and Steven L. Shafer, available at www.PLTsoft.com), provided a means to evaluate parameter uncertainty. A total of 1,000 replications was used to estimate parameter CIs.

Description of quality of fit plots of the final two-compartment weight scaled allometric vancomycin pharmacokinetic model is provided in the Supplemental Digital Content 1 (http://links.lww.com/ALN/C243).

Statistical Analysis

The statistical analysis was performed using GraphPad Prism5 (GraphPad Software, USA) statistical software. Data were expressed as median and interquartile range (25 to 75%). The distribution of the data was verified by Shapiro–Wilk normality test.

In this experimental study, we set the number of animals to 24, six per group, based on our previous experimental studies comparing intravenous *versus* nebulized

amikacin in healthy piglets.[27] Measured vancomycin lung concentrations were compared using the Mann–Whitney test. Serum vancomycin concentrations over time in each group were compared using the Friedman test (nonparametric ANOVA) with Dunn's posttest. The comparisons between aerosol and intravenous groups were performed at each time point by the Mann–Whitney test. Two-tailed testing was used, and a P value less than 0.05 was considered significant.

Results

All 24 piglets survived throughout the experimental protocol lasting 1 or 12 h.

Aerosol Delivery

The total duration of each nebulization ranged between 22 and 26 min. In piglet 3, ventricular fibrillation occurred 10 min after beginning nebulization and was immediately defibrillated (a single shock of 100 J). The causative mechanism was not identified: Ventilator settings remained unchanged, and no obstruction of the expiratory filter was documented. After normalization of end-tidal carbon dioxide, nebulization was continued without vancomycin lost during the procedure. As shown in the tables of Supplemental Digital Content 2 (http://links.lww.com/ALN/C244), cardiac index and urine output remained stable throughout the experiment. In piglet 6, moderate diarrhea was present during the experiment without affecting cardiac index, mean arterial pressure, and central venous pressure.

Extrapulmonary Deposition of Vancomycin

The total extrapulmonary deposition was median 38% (interquartile range, 30%, 42%) of the initial dose of vancomycin inserted into the nebulizer (37.5 mg/kg), distributed in different parts of the respiratory circuit: 7% (6%, 10%) was retained in the nebulizer's chamber, 40% (33%, 54%) in the inspiratory limb of the respiratory circuit, 20% (17%, 21%) in the expiratory filter, 14% (8%, 25%) in the expiratory limb of respiratory circuit, and 12% (9%, 14%) in the endotracheal tube. The resulting fraction of vancomycin reaching the respiratory tract was 62% (57%, 70%) of the initial dose, representing a dose equivalent to 24 (22, 26) mg/kg, a value approximately 1.6 times the intravenous dose (15 mg/kg) reaching the pulmonary circulation.

Vancomycin Lung Tissue Deposition

Vancomycin lung tissue concentrations were homogeneously distributed between nondependent and dependent pulmonary segments in aerosol and intravenous groups 1 h after the end of administration of a single dose.

In groups euthanized 1 h after the end of vancomycin administration, lung tissue concentrations were significantly higher in the aerosol group than in the intravenous group (median [interquartile range], 161 [71, 301] μg/g *vs.* 12 [4, 42] μg/g, respectively [$P < 0.0001$]), as shown in figure 1. In the groups euthanized 12 h after the end of vancomycin administration, lung tissue concentrations were also significantly higher in the aerosol group than in the intravenous group (median [interquartile range], 63 [23, 119] μg/g *vs.* 0 [0, 19] μg/g; $P < 0.0001$). In 18 of the 30 subpleural specimens collected in six animals 12 h after the intravenous administration, vancomycin was undetectable.

Figure 2 shows regional distribution of vancomycin lung tissue concentrations 12 h after the end of the aerosol administration. Ninety-seven percent of lung segments had vancomycin lung tissue concentrations above the sensitive methicillin-resistant *S. aureus* minimum inhibitory concentration of 2 μg/ml. Eighty percent of lung segments had vancomycin lung tissue concentrations above the vancomycin-resistant *S. aureus* minimum inhibitory concentration of 16 μg/ml.

Blood contamination represented 5.8 % of lung tissue concentration in the aerosolized groups and 1.2% in the intravenous groups.

Vancomycin Serum Concentrations

Serum pharmacokinetics of vancomycin is represented in figure 3. Thirty minutes and 1, 2, and 4 h after the end of antibiotic administration, vancomycin serum concentrations

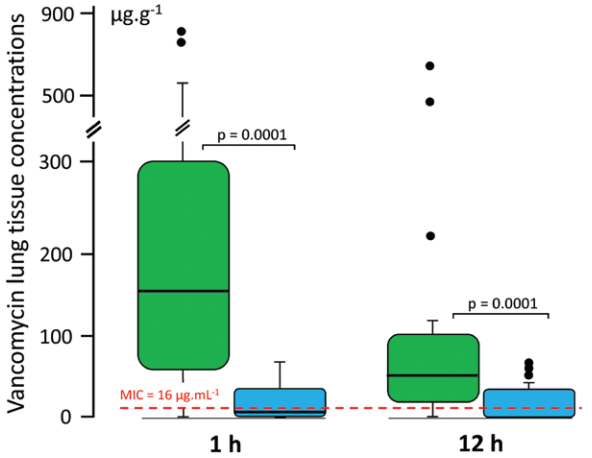

Fig. 1. Lung tissue vancomycin concentrations 1 and 12 h after the end of administration of a single aerosol (*green box plot*) or a single intravenous administration (*blue box plot*). In each group, six piglets were killed 1 h after the end of vancomycin administration and six 12 h after. In each animal, five specimens were collected from the left lung. Vancomycin concentrations were corrected for blood contamination. The *dashed line* indicates the minimal inhibitory concentration (MIC) for strains resistive to vancomycin, 16 μg · ml^{-1}. Data are expressed as median and interquartile range.

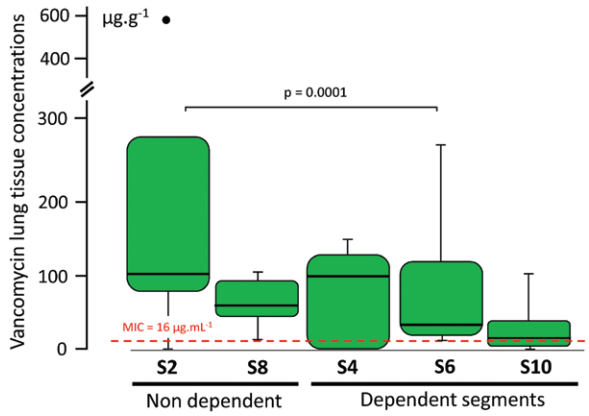

Fig. 2. Regional distribution of vancomycin lung tissue concentrations measured in piglets with healthy lungs (n = 6) 12 h after a single aerosol of vancomycin. Concentrations were measured by high-performance liquid chromatography in dependent and nondependent pulmonary segments. Data are expressed as median and interquartile range. S2, Upper lobe; S4, middle lobe; S6, apical dependent segment of lower lobe; S8, anterior nondependent segment of lower lobe; S10, posterior-caudal segment of lower lobe. MIC, minimal inhibitory concentration.

were significantly lower in the aerosol group, although the administered dose of vancomycin was 1.6 times the intravenous dose. However, there was no statistical difference between aerosol and intravenous serum trough concentrations.

As shown in table 1, area under the serum concentration–time curve was significantly greater in the intravenous group in comparison to the aerosol group. Higher volumes of distribution and clearance were assessed in the aerosol group compared to the intravenous group. Discrepancies between groups are consistent with the 37.4% of systemic bioavailability in the aerosol group compared to 100% in the intravenous group.

Population Pharmacokinetics Analysis: Model Selection

Inhalation and intravenous vancomycin pharmacokinetic data were analyzed simultaneously in a combined data set. A one-compartment weight scaled allometric model with first order absorption and elimination was first used to fit the data. The model had an objective function value of 213.738. Visual inspection of diagnostic plots showed biased predictions with systematic underprediction at low vancomycin concentrations and overprediction at high concentrations (data not shown). A two-compartment weight scaled allometric model with first order absorption and elimination produced a significant improvement in model fit with a decrease in the objective function value of 48.910 points (four additional parameters). Visual inspection of diagnostic plots corroborated the adequacy of model predictions (fig. 4 and Supplemental Digital Content 1, figs. 1–3, http://links.lww.com/ALN/C243).

Population Parameter Estimates

Final population parameter estimates, with their 95% CIs and coefficients of variation, are shown in table 2. In this selected weight scaled allometric model, vancomycin bioavailability (parameter F in table 2) was low (13%; CI 95%, 6 to 69%; coefficient of variation, 85%), with high variability between pigs. The mean estimate of volume of distribution standardized to 70 kg was 118 l.

From figure 4, it is possible to infer that there is a slow release of vancomycin from the lungs to the systemic circulation after nebulization. This slow lung absorption rate is reflected by an absorption halflife of 0.3 h. Current data, however, show wide variations in vancomycin serum concentrations after nebulized vancomycin.

Discussion

In anesthetized and ventilated piglets with healthy lungs, high vancomycin lung tissue concentrations were documented after a single vancomycin aerosol. One hour after nebulization, median vancomycin lung tissue concentration was 13 times the concentrations obtained after intravenous administration, although the nebulized dose delivered to the respiratory system was 1.6 times the intravenous dose delivered to the pulmonary circulation. Twelve hours after intravenous administration, vancomycin was undetectable in 60% of postmortem lung specimens, whereas high tissue concentrations were found after a single aerosol. Vancomycin trough lung tissue concentrations were 8.5 to 50 times the minimum inhibitory concentration of sensitive strains in dependent lung segments and 30 to 50 times in nondependent lung segments.

As a time-dependent antibiotic, trough rather than peak vancomycin lung tissue concentrations are determinant for bacterial killing and clinical efficiency. Twelve hours after a single vancomycin aerosol dose, trough lung tissue concentrations are 10 to 50 times the minimum inhibitory concentration of sensitive strains of methicillin-resistant *S. aureus* and one to six times the minimum inhibitory concentration of resistive strains, depending on pulmonary segments. In comparison, trough lung tissue concentrations 12 h after a single intravenous administration of vancomycin were largely below minimum inhibitory concentration of sensitive strains. Such results suggest that one or two daily administrations of nebulized vancomycin could be enough to provide efficient bacterial killing. These impressive results, however, are to be tempered. It is well-known that pneumonia decreases lung aeration and lung penetration of nebulized antibiotics.[14] Antibiotic concentration in an infected lung can reach one fifth of the nebulized antibiotic concentration in healthy lungs.[27,28] In addition, lung infection increases the alveolar–capillary barrier's

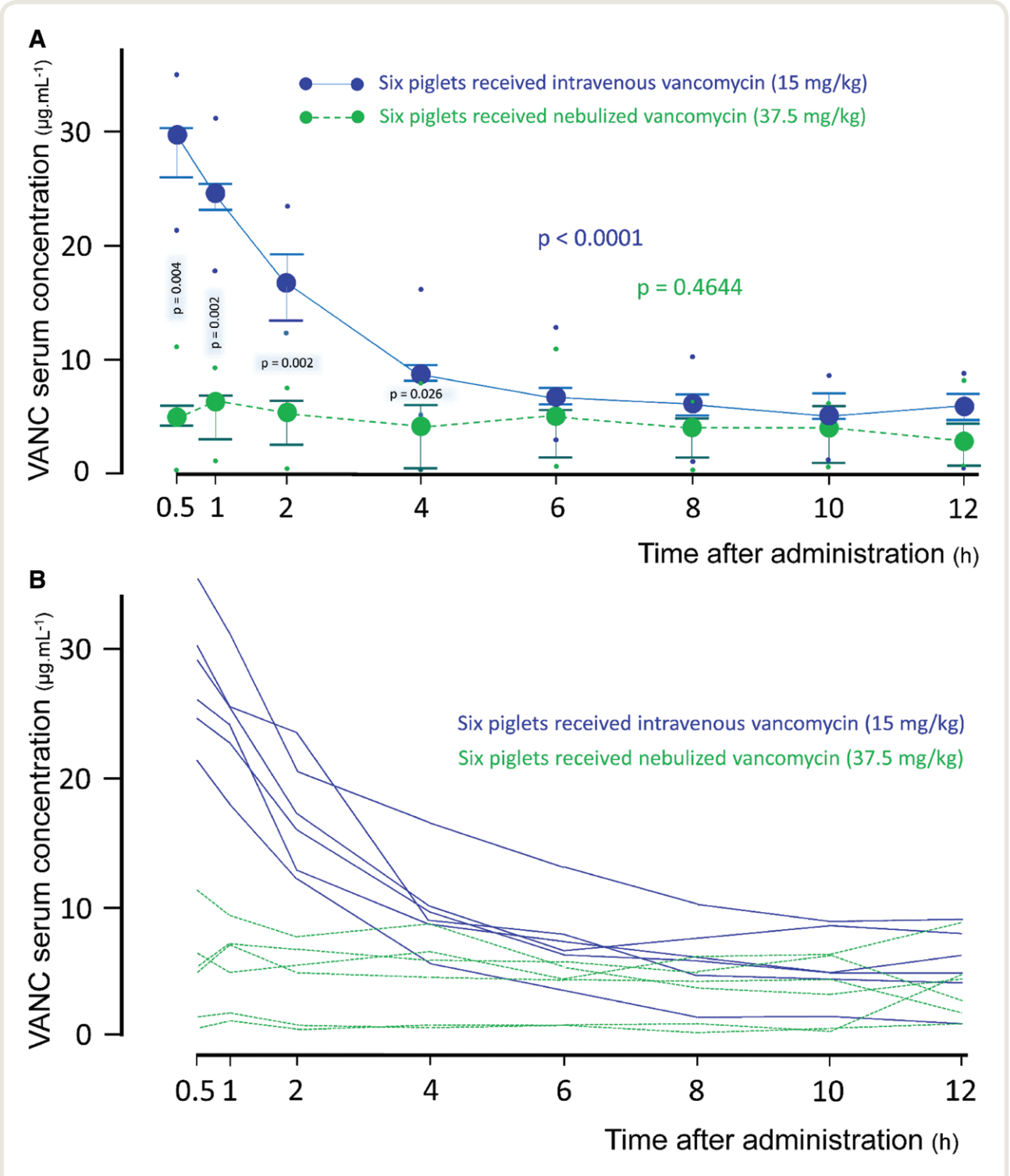

Fig. 3. Vancomycin (VANC) serum concentrations after the end of administration by aerosol (*green circles*) or by intravenous infusion (*blue circles*). (*A*) Median values, interquartile range (*blue and black horizontal lines*) and extreme values (*blue and black dots*). $P < 0.0001$ indicates that there is a highly time-dependent decrease in vancomycin serum concentrations after the intravenous administration (Friedman test). $P = 0.4644$ indicates that there is no time-dependent decrease in vancomycin serum concentrations after the nebulization (Friedman test). *P* values in *blue* indicate significant statistical differences between groups. (*B*) Individual curves after the nebulization or intravenous infusion.

Table 1. Serum Vancomycin Pharmacokinetic Parameters in Intravenous Group (Six Piglets) and Aerosol Group (Six Piglets) Monitored for 12 h after Vancomycin Administration (15 mg · kg^{-1} *vs.* 37.5 mg · kg^{-1}, Respectively)

	IV	Aerosol	*P* Value
Dose (mg · kg^{-1})	15	24 (23, 27)*	0.002
Cmax (mg · l^{-1})	27.8 (22.5, 31.6)	7.8 (2.4, 11)	0.002
Cmin (mg · l^{-1})	4.7 (3.2, 7)	2.2 (0.1, 3.4)	0.041
$T_{1/2}$ (h)	4.4 (3.5, 5.1)	5.1 (4.2, 7.9)	0.259
$AUC_{0\text{-}12h}$ (mg · h · l^{-1})	121 (103, 149)	56 (8, 70)	0.002
AUC_{∞} (mg · h · l^{-1})	143 (116, 202)	75 (14, 91)	0.015
F (%)	—	37.4	—
Vd (l)	18 (15, 20)	142 (62, 637)	0.002
CL (l · h^{-1})	2.5 (2.1, 3.5)	13.6 (2.4, 43)	0.002
Kel	0.16 (0.14, 0.21)	0.14 (0.08, 0.17)	0.259

Median (interquartile range).

*Dose entering the respiratory system after vancomycin extrapulmonary deposition.

$AUC_{0\text{-}12h}$, area under the curve from 0 to 12 h after the dose; AUC_{∞}, area under the curve to infinity; CL, elimination clearance; Cmax, maximum observed vancomycin serum concentration; Cmin, minimum observed vancomycin serum concentration; F, systemic bioavailability of the aerosol; Kel, elimination rate constant; $T_{1/2}$, elimination half life; Vd, volume of distribution.

permeability, which facilitates the diffusion of nebulized antibiotics into the pulmonary blood stream.[14] Therefore, trough tissue concentrations should be measured in animals with inoculation pneumonia to assess whether vancomycin concentrations are high enough at the site of infection and whether the rhythm of nebulization, once or twice a day, is appropriate to maintain trough lung tissue concentrations 5 to 10 times the minimum inhibitory concentration. Studies on nebulized ceftazidime, another time-dependent antibiotic, have demonstrated the need for eight daily nebulizations to maintain adequate lung tissue concentrations in infected pulmonary segments,[15] complicating the clinical administration.[29]

The technique of nebulization was optimized, as recommended[21]: vibrating mesh nebulizers were used, allowing a low vancomycin retention in the chamber; they were positioned 30 cm before the Y piece, allowing a bolus effect; specific ventilator settings were used to limit as much as possible inspiratory flow turbulences; and the humidification system was removed. Sixty-two percent of the dose deposited in the nebulizer chamber reached the respiratory tract, representing an efficient antibiotic delivery. Inhaled vancomycin is a safe, well-tolerated, and efficient treatment for recalcitrant nasal carriage[30] and bronchial colonization by methicillin-resistant *S. aureus.*[31–34] Vancomycin nebulization has also been safely used as a treatment of ventilator-associated tracheobronchitis caused by this pathogen.[35] There are no published studies on the efficiency of vancomycin nebulization for treating ventilator-associated pneumonia caused by methicillin-resistant *S. aureus,* whose eradication from the infected lung parenchyma is more demanding, requiring higher antibiotic lung tissue deposition. Our study is an indispensable step to verify vancomycin deposition in an experimental model before the use of inhaled vancomycin in patients with ventilator-associated pneumonia.

It has been reported that a single intravenous vancomycin dose (1 g) administered to patients undergoing lung carcinoma resection does not produce sustained lung tissue concentrations: vancomycin concentrations peaked by 1 h (9.6 [6, 12] μg/g), fell below the minimum inhibitory concentration of susceptible staphylococci from the fourth hour, and reached 2.8 (0.9, 7.8) μg/g after 12 h, and in three patients, vancomycin was undetectable in lung tissue by 12 h.[12] Our results are similar: vancomycin lung tissue concentration reached 12 (4, 42) μg/g 1 h after the end of the intravenous administration, decreased to 0 (0, 19) μg/g after 12 h, and was undetectable in 63% of lung specimens. Pulmonary antibiotic penetration after intravenous administration is influenced by various factors such as molecular size, lipophilicity, and diffusibility of the drug.[36] While the fenestrated pulmonary capillary bed is expected to permit passive diffusion of antibiotics with molecular weight less than 1.000 Da,[37] vancomycin is a large glycopeptide compound (1.450 Da), with high hydrophilicity.[38] A previous experimental study in rats indicated that vancomycin has a limited ability to cross alveolar-capillary membrane, remaining on the administration side after intravenous injection or nebulization.[39] Polarity of vancomycin at alveolar pH may also hinder the passage of the molecule through physiologic membranes. The alveolar basement membrane is characterized by negative charges,[40] whereas capillary basement membranes are positively charged.[41] As vancomycin is positively charged in blood, it is repelled by the endothelial membrane into the capillary lumen, hindering its passage into interstitial and alveolar spaces.

As expected, 30 min after intravenous administration, serum vancomycin concentrations peaked at median value of 29 μg/ml and then decreased to reach median trough concentrations of 5.4 μg/ml, far below the recommended concentrations of 20 μg/ml required to treat methicillin-resistant *S. aureus* infections.[42] The serum pharmacokinetic profile in animals that received the aerosol showed that 30 min after nebulization, serum vancomycin concentrations reached a median peak value of 5.9 μg/ml and then progressively decreased to median trough concentrations of 2.7 μg/ml. It has to be noted that trough vancomycin concentrations did not differ significantly between both routes of administration, although the nebulized dose reaching the respiratory system was 1.6 higher than the intravenous dose. In accordance with other nebulized antibiotics, the NONMEM analysis revealed that the time-dependent decrease in serum vancomycin concentrations followed biexponential decrease after nebulization. However, the absorption rate constant and bioavailability were much lower, suggesting a low pulmonary absorption.

In this selected weight scaled allometric model, the parameters extrapolated to 70-kg patients are consistent with previous studies.[43] Previously reported vancomycin elimination

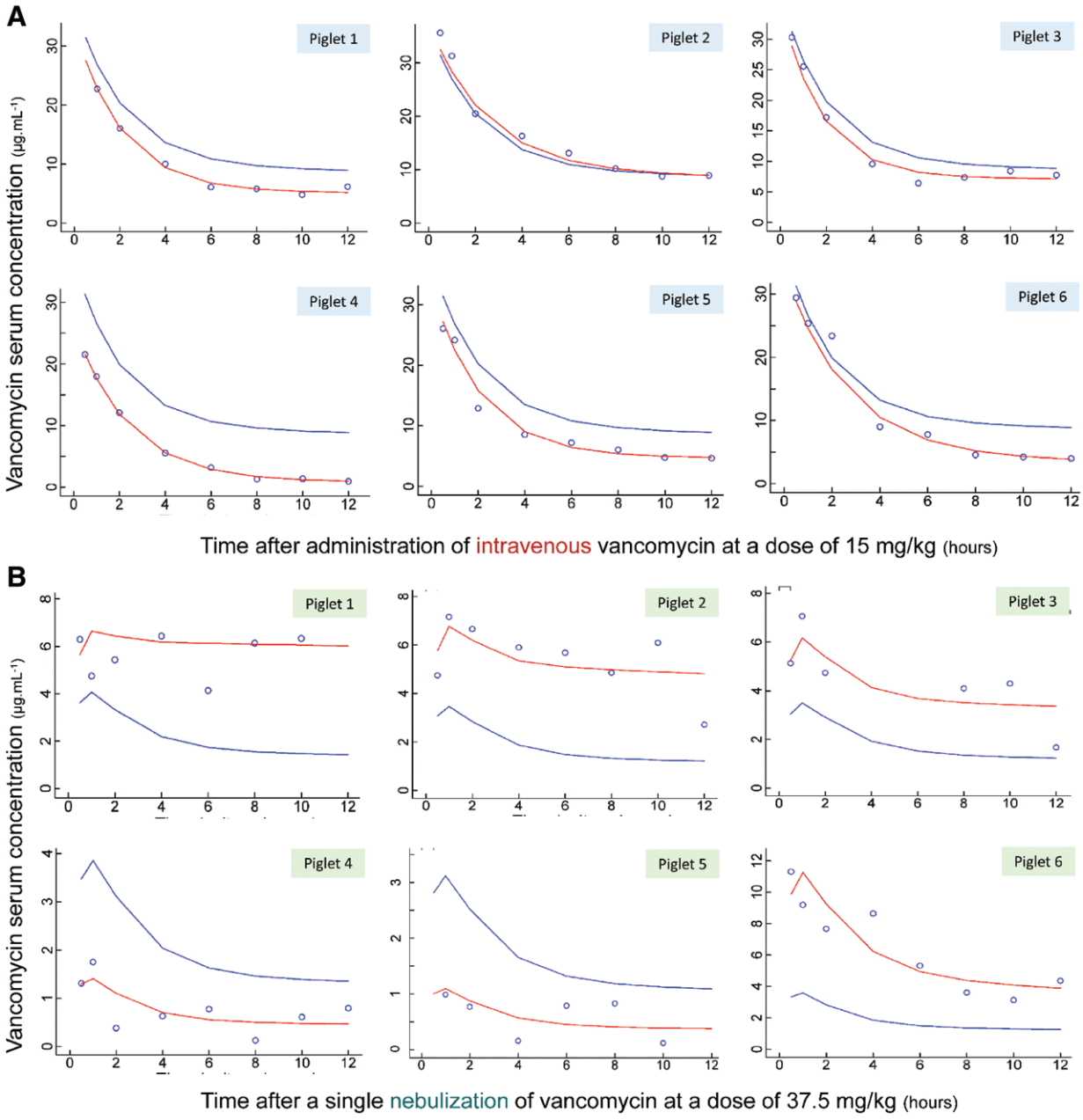

Fig. 4. Vancomycin pharmacokinetic analysis using NONlinear Mixed Effect Modeling (NONMEM) software. *Blue points* are the measured serum concentrations. *Blue lines* are the population-predicted concentrations. *Red lines* are the individual predicted concentrations (*post hoc* predicted concentrations). (*A*) NONMEM analysis for the six piglets that received intravenous vancomycin: The biexponential decrease in serum concentrations fits both individual and population predicted concentrations with a low variability. (*B*) NONMEM analysis for the six piglets that received nebulized vancomycin: The biexponential decrease in serum concentrations fits both individual and population-predicted concentrations with a high variability.

clearance and volume of distribution values in adult patients receiving intravenous vancomycin are highly variable, ranging from 2.17 l/h to 6.02 l/h and 27 l to 142 l.[43] In the current population pharmacokinetic analysis, the mean estimate of volume of distribution standardized to 70 kg is 118 l, which is within previously reported ranges. The mean elimination clearance estimate of 0.85 l/h, however, is low compared with previously reported values. Since vancomycin is eliminated primarily via the renal route, it is possible that a decrease in cardiac output and renal blood flow from the effects of general anesthesia and mechanical ventilation might explain the relatively low clearance estimate.

Table 2. Vancomycin Population Pharmacokinetic Parameter Estimates Using a Weight Scaled Allometric Model Assuming an Exponent of 0.75, 1, or 0.25 and a Standard Weight of 70 kg, for Clearance, Volume of Distribution, and T_{abs}, Respectively (NONlinear Mixed Effect Modeling software)

	Estimate of Structural Parameters	Bootstrap Estimate	95% CI	CV (%)
CL [l/h/(kg/70)$^{0.75}$]	0.85	1.00	0.02–2.99	88.2%
V1 [l/(kg/70)]	35.2	36.4	30.2–53.6	23.1%
CLd (l/h/(kg/70)$^{0.75}$)	8.41	8.88	5.12–13.1	21.1%
V2 [volunteers, l/(kg/70)]	72.8	81.2	27.7–183	116%
T_{abs} [h/(kg/70)$^{0.25}$]	0.32	0.27	0.01–0.48	—
F (%)	13.0	19.0	6–69	85%
Additive residual error (mg/l)	0.68	0.58	0.24–1.19	—
Proportional residual error (%)	9.9	13.7	3.16–20.0	—

Bootstrap estimate equals median of 1,000 bootstrap replications; 95% CI obtained by bootstrap. Volume of distribution is the sum of V1 and V2.

CL, elimination clearance; CLd, distribution clearance; CV, between-subject variability, expressed as an apparent coefficient of variation; F, bioavailability; T_{abs}, absorption rate half time; V1, central volume of distribution; V2, peripheral volume of distribution.

The NONMEM population pharmacokinetic model shows a 13% vancomycin bioavailability with a high coefficient of variation (85%), which differs from the 37.4% bioavailability calculated in the descriptive pharmacokinetic analysis. It has to be noted that a 37% bioavailability of nebulized colistin was previously reported. The variability of bioavailability in our population model was related to two piglets, which had very low serum vancomycin concentrations after nebulization (fig. 4). Interindividual variability can be influenced by multiple factors, such as the aerodynamic characteristics of the aerosol, the pulmonary blood flow, and the chemical characteristics of the antibiotic molecule.[44]

This study has a number of limitations. It is an experimental study in piglets, and the results cannot be automatically extrapolated to humans. Only female piglets were included in this research. Literature lacks studies that adequately describe sex differences in anatomy and physiology of the respiratory system in Landrace and Largewhite crossbreed piglets. More studies should be conducted to describe possible particularities. Nevertheless, we do not expect any influence of sex steroids in vancomycin lung tissue deposition, once the female piglets were 3 to 4 months of age, which is before sexual maturity in swine.[45] Second, the study was performed with a single dose, and it was not possible to assess pulmonary and systemic accumulation of vancomycin over a longer period of administration, both issues impacting toxicity. Third, the nebulized dose that reached the trachea was 1.6 times the intravenous dose that entered the pulmonary circulation. Equivalent doses could have resulted in lower pulmonary and serum concentrations. Fourth, the current data obtained in healthy lungs cannot be extrapolated to infected lungs. This data may apply to the prevention of pulmonary infections and to the early stages of ventilator-associated pneumonia where the lungs remain well aerated. Therefore, more studies are required to investigate pulmonary deposition of vancomycin in a lung parenchyma severely infected with methicillin-resistant *S. aureus* strains, especially with a minimum inhibitory concentration greater than 1.5 μg/ml.[11]

Conclusions

In conclusion, our study provides evidence that a single dose of 24 mg/kg of nebulized vancomycin administered to the respiratory system produces high lung tissue concentrations with low trough serum concentrations in piglets with normal lungs, which are possibly explained by low bioavailability and slow absorption rates. The results of the current study are encouraging, suggesting that one or two daily administrations of nebulized vancomycin could be enough to maintain tissue concentrations largely above the minimum inhibitory concentration of methicillin-resistant *S. aureus* and provide efficient bacterial killing. Further studies are required to confirm these benefits in infected lungs.

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Competing Interests

The authors declare no competing interests.

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Alypin: An Antithetical Anesthetic

The name of alypin, a local anesthetic synthesized in 1905 as a substitute for cocaine, derived from the Greek word *alypeo*, meaning "free from pain." Touted as cheaper, safer, and easier to sterilize than cocaine, alypin gained popularity in ophthalmology, otolaryngology, and dentistry. In spite of its etymology, however, alypin was not quite pain free. Infiltration could be caustic, triggering tissue injury. Oral administration left a bitter taste; surplus nasolacrimal drainage from alypin eye drops was thus unpalatable. While alypin, unlike cocaine, did not cause mydriasis, it could still irritate the cornea, inducing hyperemia and turbidity. Its failure to live up to its name was pronounced in urology, where urethral administration led to several fatal or near-fatal cases. Unmitigated local anesthetic toxicity—with hallucinations, seizures, apnea, and even cardiac arrest—could suddenly ensue. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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