Penetration of Netilmicin in the Lower Respiratory Tract after Once-Daily Dosing*

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A major criticism of the use of aminoglycosides for the treatment of pneumonia is the poor penetration in infected airways. Once-daily dosing of aminoglycosides results in higher peak plasma concentrations without increasing toxic reactions and with optimization of pharmacodynamic properties. To predict intrapulmonary antimicrobial activity after once-daily dosing of aminoglycosides, it is necessary to determine the respective bronchial and alveolar disposition. We prospectively conducted a pharmacokinetic study of netilmicin following the first intravenous administration of a once-daily dosing schedule in 20 ventilated patients with pneumonia. A bronchoscopic sampling of bronchial secretions and a subsegmental bronchoalveolar lavage (BAL) were performed 60, 90, 120, and 180 min (five patients at each time point) on the first treatment day after intravenous administration over 30 min of 450 mg of netilmicin. The netilmicin concentrations in the alveolar lining fluid (ALF) were calculated using urea as an endogenous marker of dilution. In bronchial secretions, a peak concentration of 3.00 (SEM: 0.26) mg/L or 6 percent of the 30-min plasma concentration was reached at 120 min. In ALF, much higher levels were found. At 120 min, a peak ALF concentration of 14.7 (SEM: 2.22) mg/L or 41 percent of the 30-min plasma concentration was reached. Spearman's rank correlation testing failed to show a correlation between bronchial and ALF concentrations. Higher plasma concentrations of netilmicin after once-daily dosing give rise to ALF concentrations exceeding the minimum inhibitory concentration of susceptible respiratory pathogens involved in nosocomial pneumonia, while bronchial concentrations remain low. Aminoglycoside concentrations in bronchial secretions cannot be used to predict alveolar concentrations. Low diffusibility cannot no longer be considered as a disadvantage of aminoglycosides for treating pneumonias.

\[ \text{Chest}\ 1992;\ 101:1023-32 \]

**MIC = minimum inhibitory concentration**

Despite the introduction of several groups of antimicrobial drugs during the last decade, aminoglycosides continue to play a valuable therapeutic role in the treatment of Gram-negative bacillary infections. Nosocomial pneumonia caused by Gram-negative microorganisms is one of the leading causes of hospital-acquired infections and is associated with a persistently high mortality. These therapeutic failures frequently occur despite in vitro susceptibility of the infecting pathogens to the aminoglycosides administered. A reason frequently mentioned could be the poor penetration of aminoglycoside into infected airways. The importance of maximizing local peak concentrations of aminoglycosides was emphasized by several recent reviews. The rapid bactericidal mode of action, the high killing rate, and the postantibiotic effect were indeed shown to be concentration-dependent pharmacodynamic benefits of aminoglycosides. On the other hand, the clinical utility of aminoglycosides is hampered by toxic reactions, requiring judicious therapeutic monitoring. Less frequent daily dosage schedules were subsequently shown to optimize the pharmacodynamic characteristics without increasing organ toxic reactions, to ameliorate therapeutic efficacy, and to minimize the risk of selecting resistant bacterial subpopulations. Thus, in vitro findings and clinical data support the concept of once-daily administration rather than a continuous infusion or three times daily administration of the same dose of aminoglycosides.

The first aim of the present study was to evaluate if the currently used once-daily administration of aminoglycosides yields adequate concentrations in the bronchial and alveolar compartment. Secondly, the concentrations in both compartments were mutually compared and related to plasma concentrations.

**Methods**

**Patients**

Twenty consecutive patients hospitalized in the intensive care unit who were intubated and ventilated for a variety of reasons and who had to receive antibiotics for the development of pneumonia were included in the study. The diagnosis of pneumonia was confirmed by the following criteria: radiologic consolidation, positive Gram-stain or culture of a relevant sputum or endotracheal sample, and fever (temperature ≥ 38°C). Patients' ages ranged from 26 to 78 years. All patients had normal renal function at the start of the study. The fiberbronchoscopy was performed for diagnostic purposes and the investigational procedures were performed following the necessary diagnostic investigations. The study was approved by the Ethical Committee of the University Hospital and informed consent was obtained from patients' relatives prior to inclusion.

**Drug Administration**

All patients received an initial empirical treatment with once-
daily dosed netilmicin combined with a β-lactam compound. Netilmicin (Netromycin, Schering Corporation) 450 mg was infused intravenously over 30 min on the first day of treatment. There was no prior administration of aminoglycosides. Further once-daily doses were adjusted to plasma through levels (≤1.5 mg/L). The pharmacokinetic analysis was performed within the first treatment day, following the first administration of a once-daily dosing schedule of netilmicin.

**Blood Samples**

Blood samples were obtained immediately before and at 30, 60, 90, 120, 180, 240, 300, and 360 min after the start of the initial netilmicin administration. Within 30 min of blood collection, the samples were centrifuged (10 min, 3,000 rpm, 0°C). The plasma was frozen at −70°C until netilmicin and urea assay.

**Bronchoscopic Procedure and Sample Collection**

The 20 patients were assigned at random to one of four groups. The five patients of groups 1, 2, 3, and 4 underwent fiberbronchoscopy at 60, 90, 120, and 180 min, respectively, after the start of the netilmicin infusion. Bronchoscopy (Olympus BF 1T10) was performed through the endotracheal tube. First, endobronchial secretions were aspirated and collected separately. Subsequently, the tip of the bronchoscope was wedged into a subdivision of a segmental bronchus of the right middle lobe or the lingula. Bronchoalveolar lavage (BAL) was then performed by instillation of 50 ml of sterile isotonic saline solution through the aspiration channel of the bronchoscope. The instilled fluid was then reaspirated by gentle manual suction. The dwell time of the instilled fluid in the alveolar acini averaged 15 s. The collected endobronchial secretions were homogenized using papain. In preliminary studies we have shown that papain had no influence on netilmicin concentrations. Both BAL samples and homogenized bronchial secretions were centrifuged in a refrigerated centrifuge (10 min, 3,000 rpm, 0°C). The supernatants were stored at −70°C until netilmicin and urea assay.

**Netilmicin and Urea Assay**

The netilmicin concentrations in bronchial secretions, BAL fluid samples, and plasma were determined using a fluorescence polariza-

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21642/)

**Figure 1.** Netilmicin plasma concentration-time curve (squares) and concentrations in the alveolar lining fluid (ALF) (circles) at 60, 90, 120, and 180 min (patient group 1, 2, 3, and 4, respectively.)

zation immunoassay as described previously. The automated analyzer (TDx Analyzer, Abbott, Diagnostic Division) was used for this purpose, with a sensitivity of 0.20 mg/L for both plasma and lavage fluid.

An enzymatic kinetic UV-method was used to determine urea concentrations in plasma and BAL fluid as described previously. This method was adapted to an automatic analyzer (Hitachi 717, Hitachi LTD, Tokyo, Japan) with a sensitivity of 0.10 mg/dl. The concentration of netilmicin in the alveolar lining fluid (ALF) was calculated using urea as an endogenous marker of dilution, as previously validated by several studies.

**Pharmacokinetic Analysis**

Half-lives of netilmicin in plasma were calculated for each patient with linear regression analysis, as the semilogarithmic plot of the concentration-vs-time curve showed a linear course.

**Statistical Analysis**

The netilmicin concentrations in plasma, bronchial secretions, and ALF between the several time points were compared by the Friedman two-way analysis of variance. The netilmicin concentrations in plasma and ALF at the individual time points were compared by the Mann-Whitney U test, and p<0.05 was regarded as significant. The correlations between concentrations in bronchial secretions and ALF, between concentrations in plasma and ALF, and between concentrations in plasma and bronchial secretions were analyzed by Spearman's rank correlation test.

**Results**

The mean plasma netilmicin concentrations (± SEM) of the 20 patients at each time point and the calculated concentrations in ALF for the four groups of patients (groups 1, 2, 3, and 4 with bronchoscopic sampling at 60, 90, 120, and 180 min after the start of infusion, respectively) are shown in Figure 1.

Urea concentrations in plasma and BAL fluid samples, used to calculate netilmicin concentrations in
ALF, showed no significant differences among the four patient groups. The concentrations of urea averaged 34 (SEM: 2.6) and 1.22 (SEM: 0.20) mg/dl in plasma and in BAL samples, respectively.

At the end of the 30-min infusion of 450 mg of netilmicin, a mean plasma concentration of 36.0 (SEM: 1.32) mg/L was reached. Thereafter the plasma concentrations declined progressively, as 21.4 (SEM: 1.19), 15.3 (SEM: 0.85), 12.0 (SEM: 0.71), 8.3 (SEM: 0.64), 6.4 (SEM: 0.45), 4.8 (SEM: 0.42), and 3.7 (SEM: 0.39) mg/L at 60, 90, 120, 180, 240, 300, and 360 min after start of infusion, respectively. Analysis of variance showed a significant (p<0.01) difference of plasma concentrations among the different time points. No significant difference in plasma concentrations could be demonstrated among the four groups of patients when compared at each time point. The mean calculated plasma half-life of netilmicin was 160 (SEM: 11) min. According to therapeutic netilmicin trough levels during subsequent once-daily administration, no dose adjustments were required in any patient.

The mean netilmicin concentrations in ALF calculated from the reaspirated lavage fluid were 7.5 (SEM: 1.04), 9.6 (SEM: 0.30), 14.7 (SEM: 2.22), and 9.3 (SEM: 0.59) mg/L for group 1, 2, 3, and 4, respectively (Table 1). Analysis of variance showed a significant difference among the ALF concentrations at the several time points (p<0.05). When individual time points were compared with the Mann-Whitney U test, the ALF concentrations in group 3 (at 120 min) were significantly higher than the concentrations at the other time points (p<0.05).

The mean concentrations of netilmicin in the bronchoscopically aspirated bronchial secretions of group 1, 2, 3, and 4 were 1.07 (SEM: 0.37), 1.66 (SEM: 0.31), 2.00 (SEM: 0.26), and 1.93 (SEM: 0.33) mg/L, respectively (Table 1). Analysis of variance failed to show a difference of these concentrations at the several time points.

Spearman’s rank correlation test showed no significant correlation between the netilmicin concentrations in bronchial secretions and those in ALF.

The degree of lower respiratory tract inflammation as evaluated by the percentage of neutrophils in the cellular material of the aspirated BAL fluid showed no significant difference among the four groups. The neutrophil percentage in BAL averaged 70 percent (SEM: 1.9) for the 20 patients evaluated (Table 1).

The peak concentrations of netilmicin in bronchial secretions and ALF reached 6 percent and 41 percent of the 30-min plasma concentrations, respectively.

At each time point, there was a significant correlation between the 30-min plasma concentrations and the ALF concentrations (p<0.01), as shown by Spearman’s rank correlation testing. In contrast, no correlation was observed between concentrations in plasma and those in bronchial secretions.

**Discussion**

It is generally accepted that the concentration of free, bioactive antimicrobial drug at the site of bacterial multiplication is a major determinant for therapeutic efficacy. There is evidence from several human clinical studies that this supposition holds for respiratory tract infections. Most studies evaluating respiratory tract penetration of antibiotics are based on the comparison of concentrations in plasma with those in sputum or bronchial secretions. However, as conducting airways have clearly different anatomic and functional properties when compared with the alveolar spaces, the prediction of alveolar concentrations from those in sputum and bronchial secretions is doubtful. The BAL procedure samples the cells and molecular components in the fluid lining the alveolar surface. The advantages of BAL for determination of drug concentrations in ALF are (1) the

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bronchial Secretions, mg/L</th>
<th>ALF, mg/L</th>
<th>Neutrophils, %</th>
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<tr>
<td>Group 1</td>
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<tr>
<td>1</td>
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<td>7.4</td>
<td>72</td>
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<tr>
<td>4</td>
<td>1.94</td>
<td>7.1</td>
<td>68</td>
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<tr>
<td>5</td>
<td>0.92</td>
<td>4.1</td>
<td>51</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>1.07 (0.37)</td>
<td>7.5 (1.0)</td>
<td>69 (4.5)</td>
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<td>10.0</td>
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<tr>
<td>Mean (SEM)</td>
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<td>9.6 (0.3)</td>
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<tr>
<td>Mean (SEM)</td>
<td>1.93 (0.33)</td>
<td>9.3 (0.6)</td>
<td>68 (1.6)</td>
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**Table 1** — Concentration of Netilmicin in Bronchial Secretions and Alveolar Lining Fluid (ALF), and the Percentage of Neutrophils in Bronchoalveolar Lavage Fluid for Patient Groups 1, 2, 3, and 4 at 60, 90, 120, and 180 min, respectively, after Once-Daily Dosage of 450 mg of Intravenous Netilmicin
To attribute to a particular anatomic site (the lung acinus) allowing assessment of drug transfer across the alveolar-capillary membrane, and (2) the possibility to distinguish cellular and extracellular concentrations. To overcome the dilutional effect of BAL, a marker of dilution is used to calculate the actual drug concentration in the ALF, in situ. Urea, which is easily measured, has a low molecular weight and diffuses freely throughout the body, including the alveolar wall, and has been validated as a reliable marker for this purpose.\textsuperscript{10-17} Hence, this procedure was applied in the present investigation of aminoglycoside disposition in the respiratory tract.

Although it was shown that achieving high peak plasma concentrations of aminoglycosides was linked to improved clinical outcome in patients with Gram-negative sepsis and nosocomial pneumonia, these studies mentioned that it is not definitely known whether high plasma concentrations of aminoglycosides will also lead to high concentrations in the alveoli.\textsuperscript{20,27} On theoretical grounds, it is assumed that higher plasma concentrations will automatically result in better tissue penetration. However, as the disposition of antibiotics in the respiratory tract is influenced by several host- and drug-related factors, effectiveness of antimicrobial drug therapy may not be predicted by plasma concentrations alone.\textsuperscript{2,3,24} Moreover, antimicrobial drugs can be biologically altered by local pH changes, anaerobic conditions, or enzymatic inactivation.\textsuperscript{24,28} Therefore, it is far more relevant to evaluate the local concentrations directly at the site of the respiratory infectious process.

It is generally accepted that aminoglycoside concentrations in sputum and bronchial secretions are only 5 to 20 percent of peak plasma concentrations.\textsuperscript{2,22} The present human study was intended to evaluate the penetration of netilmicin from the vascular space into the ALF and bronchial secretions after the first once-daily administration. The low penetration of netilmicin in bronchial secretions was confirmed, reaching only 6 percent of the 30-min plasma concentration or 2.00 mg/L (SEM: 0.26). These concentrations are very similar to those found in other studies, in which much lower plasma concentrations were reported.\textsuperscript{22} These findings suggest that the transfer of aminoglycosides across the blood-bronchus barrier could be a saturable process. Furthermore, regarding the netilmicin breakpoint for susceptibility of 12 mg/L,\textsuperscript{20} respiratory pathogens multiplying in the bronchi and reported to be susceptible by in vitro testing may not be eradicated by the low bronchial concentrations. Consequently, once-daily dosing of aminoglycosides does not seem to be appropriate for the treatment of severe bronchial infections, such as infectious exacerbations of cystic fibrosis caused by \textit{Pseudomonas aeruginosa}.

On the contrary, the alveolar disposition of aminoglycosides was significantly higher and showed a good correlation with higher plasma concentrations after the first administration of a once-daily dosing schedule. The peak concentration in ALF was calculated to be 14.7 (SEM: 2.22) mg/L or 41 percent of the 30-min plasma concentration. This peak concentration was reached 120 min after the start of netilmicin administration, when plasma concentrations were already declining. High local concentrations persisted until the end of the BAL study (180 min) despite further elimination of netilmicin out of the vascular space. The minimum inhibitory concentration (MIC) range of netilmicin for the common pathogens in nosocomial pneumonia are 0.2 to 25.0 mg/L, 0.2 to 6.3 mg/L, 0.2 to 6.3 mg/L, 0.4 to 50.0 mg/L, and 0.05 to 0.8 mg/L for \textit{P aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{Escherichia coli}, Serratia, and \textit{Staphylococcus aureus}, respectively.\textsuperscript{30}

The high alveolar disposition found in our study is in accordance with the high ALF penetration of tobramycin.\textsuperscript{31} The concentrations in the ALF could not be predicted by those in bronchial secretions, as the correlation of netilmicin concentrations between these two anatomic sites was not significant. These findings illustrate the different pharmacokinetic characteristics of antimicrobial drugs in crossing the alveolar-capillary membrane and the blood-bronchus barrier. The higher disposition in the ALF can be explained by the more extended and thinner surface of the alveolar-capillary membrane and the higher blood supply when compared with the blood-bronchus barrier, facilitating the netilmicin transport across. Furthermore, the high degree of alveolar wall inflammation in the setting of pneumonia, as illustrated by the high percentage of neutrophils in the BAL fluid, may enhance netilmicin disposition in the ALF. Our findings also suggest that the bronchial-alveolar tree can be regarded as a deep tissue compartment with different (ie, slower) elimination characteristics when compared with the vascular space.

It can be concluded from this study that the higher peak plasma concentrations of netilmicin obtained with once-daily administration of the total daily dose reliably give rise to high concentrations in the ALF, exceeding the MIC of susceptible respiratory pathogens. There is an excellent correlation between peak plasma netilmicin concentrations and ALF concentrations. Moreover, high ALF concentrations persist despite declining plasma concentrations. On the contrary, netilmicin concentrations in bronchial secretions remained low, were not predictive for concentrations in ALF, and were not correlated with plasma concentrations.

The concept of low diffusibility as a disadvantage of aminoglycosides only holds for the treatment of infections in the conducting airways and does not apply to...
the treatment of pneumonic processes. Monitoring of peak plasma concentrations seems sufficient to predict alveolar drug penetration as an index of local therapeutic efficacy.

REFERENCES
1 Craven D, Barber T, Steger K, Monteclavo M. Nosocomial pneumonia in the 1990s: update of epidemiology and risk factors. Semin Respir Infect 1990; 5:157-72