Interface-Area-to-Volume Ratio of Interstitial Fluid in Humans Determined by Pharmacokinetic Analysis of Netilmicin in Small and Large Skin Blisters

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Human pharmacokinetics of netilmicin during multiple dosing were studied in serum and in the fluid of skin blisters with two different ratios of interface area to fluid volume. The kinetics in the blisters followed the serum concentration-time curve with a delay but with a similar elimination half-life of 2.4 h. The kinetics in the 40-μl blisters followed closely the theoretically calculated concentrations of the peripheral compartment of a two-compartment model. In contrast, the concentrations in the 120-μl blisters increased less rapidly, lower peaks were achieved, and concentrations decreased with a significantly longer delay. A very similar area-specific flow or clearance rate of 1.6 μl · h⁻¹ · mm⁻² was calculated for the interface area between the serum compartment and either the small or large blisters. The observed rapid mass transfer between serum and blister fluid suggests similar oscillations of concentrations in serum and in small interstitial fluid compartments.

Antibiotic penetration into interstitial fluid has been studied in various compartments providing different ratios of surface area for drug exchange to compartment volume. Comparative studies have been performed both in vitro and in animal models of infection (6, 8). In the study presented here, pharmacokinetics were determined in humans in the fluid of skin blisters with two different ratios of interface area to fluid volume during multiple dosing of netilmicin.

Serum kinetics of aminoglycosides can be simulated by a two-compartment model over a time period of more than 12 h following administration (1, 9). The parameters of the model are determined such that the concentration-time curve of the central compartment mimics the measured concentrations in serum. However, no anatomical compartments in which the concentration-time curve follows the calculated concentrations of the peripheral compartment of the model have been identified. The kinetics of netilmicin in the fluid of small and large skin blisters were compared with the kinetics calculated for the peripheral compartment of a two-compartment model.

MATERIALS AND METHODS

Study design. The serum kinetics and penetration of netilmicin into interstitial fluid in six healthy male volunteers (body weight, 56 to 66 kg; age, 22 to 26 years) were studied. Doses of 2 mg of netilmicin per kg of lean body weight (doses of 116 to 141 mg) were administered intramuscularly twice and thrice daily over a 1-day period (1). Injections were administered alternately on the left and right ventrolateral sides. A crossover design was used, with two dosage schedules separated by 3 weeks. The concentration-time course in serum was documented with 27 serum samples. Tissue penetration in 10 skin blisters of two different sizes was studied. The participants of the study were hospitalized twice for 48 h each time.

Blisters. Skin suction blisters were produced 3 h before administration of the first dose of each dosage schedule of netilmicin in all participants. Two plastic blocks containing five holes each were strapped on the forearm after the skin was cleansed with alcohol. Half-spherical blisters were formed after controlled suction of 180 mm Hg (1 mm Hg = 133.3 Pa) over a period of 2 h (4). Five blisters had a diameter of 6 mm (mean volume sampled, 40 μl; interface area between blister fluid and tissue, 28 mm²) and five blisters had a diameter of 8 mm (volume, 120 μl; interface area between blister fluid and tissue, 50 mm²). The interface-area-to-volume ratios were 2.4 and 1.4 μl/mm² for the small and large blisters, respectively. The fluid was aspirated from each blister only once by a syringe. During the twice-daily dosing regimen, blister fluid was sampled at 0.75, 1.7, 3, 6, 12, 19, and 26 h; during the thrice-daily dosing regimen, it was sampled at 0.5, 1, 2, 4, 8, 16, and 26 h.

Assay. All samples were stored frozen until assayed for netilmicin by a radioenzymatic assay (9). Pooled human serum was used for the calibration of serum sample measurements, and a mixture of 1 part serum and 3 parts physiological saline was used for the calibration of blister fluid assays. The coefficient of variation of the assay was <4% for concentrations of netilmicin of >0.5 mg/liter, and the sensitivity limit was 0.04 mg/liter (1).

Modeling. A three-compartment model with first-order absorption was used to fit both serum and blister fluid data simultaneously over a 12-h period (Fig. 1):

\[
V_C \cdot \frac{dC_C}{dt} = D \cdot k \cdot e^{-kt} - (Q + CI + F) \cdot C_C + Q \cdot C_p + F \cdot C_b \\
V_p \cdot \frac{dC_p}{dt} = Q \cdot (C_C - C_p) \\
V_b \cdot \frac{dC_b}{dt} = F \cdot (C_C - C_b)
\]

\(V\) represents the volume of distribution, \(D\) is the dose, and \(k\) is the time constant for exponential absorption from the intramuscular depot. \(Q, CI,\) and \(F\) are flow rates, and the indices \(C, p,\) and \(b\) refer to the central, peripheral, and blister compartments, respectively. \(V_b\) was set to 40 and 120 μl for the small and large blisters, respectively.

For the simulation of the concentrations in serum alone, without fitting the blister fluid levels, the third equation was
omitted and the flow rate $F$ was set to zero. This reduced model represents a two-compartment model with first-order absorption.

The pharmacokinetic parameters of this model were adapted to the experimental data obtained within the first 12 h by a nonlinear fitting program which minimizes the sum of weighted squared deviations (5). The weighting function of the squared differences between the observed and predicted values was based on the precision of the assay. Over the concentration range of interest, the relative error of the assay was almost constant. Accordingly, the weighting function was chosen to be inversely proportional to the squares of the absolute concentration values.

RESULTS

Figure 2 shows the drug concentrations in serum and blister fluid following administration of 2 mg of netilmicin per kg. The kinetics of the blisters followed the serum concentration-time curve with a delay, but with a similar half-life of 2.4 h. The figure also shows the calculated concentration-time curves of the central and peripheral compartments of a two-compartment model used to fit the serum data. The concentration-time curve of the central compartment of this model closely fitted the serum data. The following parameters (± standard deviations of the estimates) were calculated: $V_1 = 10.3 ± 1.3$ liters; $V_2 = 4.7 ± 0.9$ liters; $Cl = 82 ± 1$ ml/min; $k = 3.6 ± 0.7$ h$^{-1}$; and $Q = 5.4 ± 0.9$ liters/h. The concentration-time curve calculated with this model for the peripheral compartment closely mimicked the kinetics of the 40-μl blisters, despite the fact that these blister fluid data were not fitted. In contrast, the concentrations in the 120-μl blisters increased less rapidly, lower peaks were achieved, and concentrations decreased with a significantly longer delay ($P < 0.05$ [sign test]) compared with the values calculated for the peripheral compartment.

A three-compartment model used to simultaneously fit concentrations both in serum and in the fluid of either small or large blisters showed close agreement between measured and fitted concentrations (Table 1). For the small blisters, a flow or clearance rate ($F$) of $43 ± 2.6$ μl/h (mean ± standard deviation) was determined, and a rate of $81 ± 5.8$ μl/h was determined for the large blisters. The larger flow rate determined for the larger blisters relates to the greater interface area, which separated the larger blister fluid compartments from the tissue. The interface area between blister fluid and tissue was 28 mm$^2$ for the small blisters and 50 mm$^2$ for the larger blisters. Dividing the flow rates by the respective interface area, a very similar area-specific flow rate of 1.54 or 1.62 μl · h$^{-1}$ · mm$^{-2}$ was calculated for the interface area between serum and small or large blisters, respectively.

![FIG. 1. Three-compartment model for analysis of netilmicin concentrations in serum and blister fluid (for abbreviations, see text).](image)

![FIG. 2. Concentration of netilmicin in serum (mean from six persons) and blister fluid (mean from three to six persons) after intramuscular injection of netilmicin (2 mg/kg of lean body mass). A two-compartment model was used to fit the serum data. The solid line represents the concentration time curve of the central compartment; the broken line represents concentrations calculated for the peripheral compartment. The concentrations in the blisters were not considered for this fitting procedure. Nevertheless, the kinetics calculated for the peripheral compartment of the model fit closely the kinetics of the small blisters, whereas the increase and decrease of concentrations in the larger blisters were more delayed.](image)

**TABLE 1. Measured and calculated concentrations of netilmicin in 40- and 120-μl blisters following an intramuscular dose of 2 mg/kg of lean body mass**

<table>
<thead>
<tr>
<th>Blister vol</th>
<th>Time (h)</th>
<th>Measured concn [mg/liter, mean ± SD (no. of persons)]</th>
<th>Residual* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 μl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>2.0 ± 1.0 (3)</td>
<td></td>
<td>-10.5</td>
</tr>
<tr>
<td>0.75</td>
<td>3.5 ± 2.0 (3)</td>
<td></td>
<td>7.4</td>
</tr>
<tr>
<td>2</td>
<td>5.1 ± 1.1 (5)</td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>3.6 ± 0.5 (5)</td>
<td></td>
<td>-1.5</td>
</tr>
<tr>
<td>8</td>
<td>0.8 ± 0.6 (5)</td>
<td></td>
<td>-9.3</td>
</tr>
<tr>
<td>12</td>
<td>0.3 ± 0.1 (5)</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>120 μl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.3 ± 1.5 (5)</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>1.7</td>
<td>4.1 ± 2.0 (6)</td>
<td></td>
<td>-4.2</td>
</tr>
<tr>
<td>4</td>
<td>3.9 ± 0.3 (6)</td>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>2.2 ± 0.5 (4)</td>
<td></td>
<td>-5.2</td>
</tr>
</tbody>
</table>

* Residual between the mean measured concentration and the concentration calculated by the three-compartment model as a percentage of the measured value.
time constant, defined as flow rate to volume, was determined to be $1.1 \text{ h}^{-1}$ for the small blisters and $0.67 \text{ h}^{-1}$ for the large blisters.

The total protein content of the blister fluid was $17 \pm 3$ g/liter (mean ± standard deviation). The protein consisted of $61.6\% \pm 3.0\%$ albumin, $3.9\% \pm 0.8\%$ $\alpha_1$-globulin, $7.0\% \pm 1.7\%$ $\alpha_2$-globulin, $12.7\% \pm 2.0\%$ $\beta$-globulin, and $14.8\% \pm 1.8\%$ gamma globulin. These percentage values were within the 95% confidence intervals established at our institution for serum, except that the $\alpha_1$-globulin average percentage of 3.9% measured in blister fluid was above the normal range of 1.4 to 3.3% for serum.

Drug accumulation did occur to only a limited extent during the multiple-dosing regimens. For example, the concentration in the 40-µl blisters averaged $2.2 \pm 0.5$ mg/liter 6 h after the first dose and $2.2 \pm 0.3$ mg/liter 7 h after the second dose during the twice-daily dosing regimen. Samples drawn from the 40-µl blisters 26 h after the onset of dosing had somewhat higher drug concentrations than did samples obtained within the first dosing interval (Table 1), reflecting drug accumulation and/or a slight reduction of drug clearance from blisters that were more than 1 day old. During the thrice-daily dosing regimen, 10 h after the third dose, the concentration averaged $0.91 \pm 0.28$ mg/liter, and during the twice-daily dosing regimen, 14 h after the second dose, the concentration averaged $0.39 \pm 0.13$ mg/liter.

**DISCUSSION**

The pharmacokinetics of netilmicin in serum and blister fluid could be fitted closely with a model which simplifies the complex anatomical and physiological situation in vivo by a linear three-compartment model. Because of the good renal clearance of the young participants, the relatively short dosing intervals, and the limited observation period, renal drug accumulation in a deep compartment could be omitted from the model (2, 9).

Similar area-specific flow rates were observed for small and large blisters interfacing with the central compartment. Thus, the ratio of interface area to volume of the compartment determined the delay by which the concentrations in the blisters followed the concentrations in serum. Compartmental analysis of the measured data suggested that the time constant of mass transfer of netilmicin between the central and peripheral compartments corresponded to the situation with 40-µl blisters.

The volume of distribution of the central compartment was estimated as 10 liters, which is approximately four times the volume of plasma in adults (3). Aminoglycosides are hydrophilic and do not penetrate intracellularly except by adsorptive pinocytosis by proximal tubular cells (7). Anatomically, the central compartment of the model includes both the intravascular space (excluding blood cells) and interstitial fluid compartments with excellent permeability. The peripheral compartment also represents part of the interstitium. However, drug penetration to the latter interstitial compartments follows a kinetic with slower time constants. Mass transfer between serum and the various anatomical spaces with interstitial fluid might be represented more realistically by a model which includes multiple interstitial compartments with various time constants. However, data analysis by the proposed model allows for adequate simulation of the observed concentrations by splitting the interstitial distribution volumes into only two compartments. The calculated kinetics of the peripheral compartment represents the superposition of the kinetics in multiple interstitial spaces with relatively slow drug exchange. However, even in these spaces the concentrations followed the serum kinetics with only a minor delay. Peak and trough levels observed in serum can therefore be considered as representative for well-vascularized interstitial fluid compartments. These considerations suggest that once-daily aminoglycoside treatment of soft tissue infections might result in both high levels in tissue and prolonged periods with subinhibitory concentrations at the site of infection. In contrast, the length of the dosing interval might be less critical at infection sites with low ratios of interface area to volume or in the presence of interface areas with poor vascularization or permeability, resulting in less-pronounced oscillations of drug concentrations within a dosing interval.

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**REFERENCES**


