Penetration of Doripenem into Skeletal Muscle and Subcutaneous Adipose Tissue in Healthy Volunteers

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Sufficient antibiotic concentrations at the infection site are a prerequisite for good bacterial killing. This study was performed to determine pharmacokinetics of doripenem in soft tissues and saliva. Six healthy male volunteers received a single intravenous infusion of 500 mg doripenem over 1 h. The concentrations of doripenem were measured over 8 h in saliva, plasma, and extracellular space fluid of skeletal muscle and subcutaneous adipose tissue employing in vivo microdialysis. Unbound drug concentrations were determined using ultra-high-performance liquid chromatography-tandem mass spectrometry. Maximum concentrations of doripenem were 15.3 ± 6.0 mg/liter in plasma, 9.9 ± 2.3 mg/liter in subcutaneous adipose tissue, 6.6 ± 2.9 mg/liter in skeletal muscle, and 0.5 ± 0.2 mg/liter in saliva. Areas under the concentration-time curve (AUC) from 0 to infinity were 26.3 ± 10.1, 20.4 ± 3.8, 12.8 ± 3.0, and 1.0 ± 0.5 mg·h/liter in plasma, adipose tissue, skeletal muscle, and saliva, respectively. Ratios of AUC in adipose tissue, skeletal muscle, and saliva to those in plasma were 0.84 ± 0.28, 0.53 ± 0.19, and 0.04 ± 0.03, respectively. In all six volunteers, a threshold of ≥40% for "time above MIC," an index indicative of good antimicrobial activity, was exceeded in adipose tissue for MICs of ≤2 mg/liter and in skeletal muscle for MICs ≤1.5 mg/liter. Doripenem penetrates well into interstitial space fluid of skeletal muscle and adipose tissue, suggesting good antimicrobial activity in infected soft tissues, whereas it is detectable in relatively low concentrations in saliva.

Doripenem (Doribax; Janssen-Cilag International NV, Beerse, Belgium) is a new parenteral carbapenem antibiotic active against a broad spectrum of Gram-positive and Gram-negative bacteria, with an overall MIC50 value (MIC inhibiting 90% of tested isolates) of ≤1 mg/liter reported for a collection of about 10,000 isolates from different bacterial species (9). Importantly, it is stable against most beta-lactamases, including those derived from isolates producing extended-spectrum beta-lactamase (ESBL) and isolates overexpressing AmpC, and shows good activity against Pseudomonas aeruginosa (7, 13, 19). Doripenem is approved at a dose of 500 mg three times a day (t.i.d.) for the treatment of complicated intra-abdominal and urinary tract infections and also in several countries for ventilator-associated and nosocomial pneumonia (9, 10). It has a low plasma protein binding capacity of about 8% in humans (10, 23). There is no relevant accumulation of doripenem in plasma even after multiple doses of 1,000 mg t.i.d. in healthy volunteers (6, 9).

To obtain optimal antimicrobial efficacy, it is important that the antibiotic achieves sufficient concentrations at the site of infection. Drug concentrations in the relevant tissue, however, may substantially differ from those in plasma (16). Knowledge on pharmacokinetics in plasma alone, therefore, is often not sufficient to estimate if certain bacterial pathogens can be killed at the infected site. Since most bacterial infections occur in the interstitial space, knowing exact antibiotic concentrations in the interstitium of infected organs is important for assessment of bacterial killing.

Therefore, the present study was performed to measure pharmacokinetics of doripenem in plasma and saliva as well as in the interstitial space fluid of subcutaneous adipose tissue and skeletal muscle of healthy volunteers after a single dose of 500 mg. Tissue concentrations were determined using the microdialysis technique, which allows continuous measurement of free, i.e., microbiologically active, drug concentrations (20–22).
end of the study day to determine probe recovery. Samples were kept on ice for a maximum of 1 h, and plasma was obtained from blood samples. All samples were snap-frozen at approximately −20°C and stored at approximately −80°C until analysis.

Quantitative analysis. (i) Sample preparation. To achieve protein precipitation, all collected samples were diluted 1:1 with methanol and centrifuged (13,000 rpm, 10 min, 4°C). An aliquot of the supernatant was diluted with double-distilled water (ddH$_2$O) at the ratio 1:2.5 to lower the methanol concentration with regard to peak symmetry and sensitivity. The mixture was vortexed, and an aliquot of 5 μl was injected immediately for ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis.

(ii) Preparation of calibration standards and quality control sample. A stock solution of doripenem with a concentration of 100 mg/liter was obtained by dissolving in ddH$_2$O. It was serially diluted with drug-free plasma and prepared as described above to achieve the following relevant concentrations: 0.025, 0.05, 0.25, 0.5, 2.5, 5.0, 25.0, and 50.0 mg/liter. The coefficient of variation (CV) varied from 5.01% to 13.93%. The calibration curve correlation coefficient ($r^2$) reached 0.9959. The limit of detection (LOD, signal-to-noise ratio) was 0.005 mg/liter, and the limit of quantification (LOQ, signal-to-noise ratio = 10) was 0.025 mg/liter. The quality control sample was a 1:200 dilution of the stock (500 μg/liter) in ddH$_2$O.

(iii) UHPLC-MS/MS. The UHPLC-MS/MS system consisted of a Dionex UltiMate 3000 Rapid Separation (Germering, Germany) and an Applied Biosystems MDS Sciex API 4000 (MDS Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer fitted with an electrospray ionization source. Chromatographic separation was employed using a Waters Acquity CSH C18, 2.1 by 50 mm, 1.7 μm UHPLC column (Dublin, Ireland). The mobile phase consisted of aqueous formic acid (A) (pH 2.80) and acetonitrile (B). Three percent B was increased to 10% within 1.75 min, followed by 95% B for 2.25 min and reequilibration for 3.70 min. The total run time for each sample was 7.70 min. The flow rate was kept at 0.500 ml/min at a temperature of 23°C. The autosampler was set at 10°C. The retention time of doripenem was 1.49 min ± 0.27 min. The MS detection was performed with multiple reaction monitoring (MRM; positive ion mode) for quantification, and settings were optimized for doripenem analysis: nebulizer gas, heater gas, and curtain gas were set at 30, 30, and 40 liters/min. Turbo IonSpray voltage was 5,500 V at 550°C. The collision activated dissociation (CAD) flow was used at 4 liters/min, and the declustering and entrance potentials were 51 V and 10 V, respectively. The optimized collision energy (CE) for the analyte varied from 21 eV for the quantifier to 23 eV for the qualifier. The dwell time was adjusted to 120 ms per transition. Quantification of doripenem was employed using MRM of the transition m/z 420.9 → 274.3 (quantifier), while the qualifier transition was m/z 420.9 → 342.1. The Analyst 1.5 software (MDS Sciex, Concord, Ontario, Canada) was used to control the UHPLC-MS/MS system and to perform the measurement.

Pharmacokinetic analysis. Noncompartmental pharmacokinetic analysis was performed using Kinetica 2000 (version 3.0; Innaphase, Philadephia, PA). Descriptive statistics were performed, presenting results as means ± standard deviations.

RESULTS
No serious side effects were observed after administration of 500 mg doripenem to six healthy male volunteers (Table 1). The night following the study, one volunteer felt malaise for a few hours, which ceased spontaneously and was possibly related to the study drug. The mean recovery rate of microdialysis probes situated in subcutaneous adipose tissue was 36% ± 11%, and for those in skeletal muscle was 39% ± 17%.

Mean concentration-time curves over 8 h of unbound doripenem in the four tested compartments of the six volunteers are shown in Fig. 1. Maximum concentrations (mean $C_{\text{max}}$) were 15.3 ± 6.0 mg/liter in plasma, 9.9 ± 2.4 mg/liter in subcutaneous adipose tissue, 6.6 ± 2.9 mg/liter in skeletal muscle, and 0.5 ± 0.2 mg/liter in saliva (Table 2). Coincidentally, the highest areas under the free concentration-time curves from 0 to infinity (AUC$_{\text{total}}$) were found in plasma (26.3 ± 10.1 mg · h/liter), followed by subcutaneous adipose tissue (20.4 ± 3.8 mg · h/liter) and skeletal muscle (12.8 ± 3.0 mg · h/liter). The lowest AUC$_{\text{total}}$ values were found in saliva (1.0 ± 0.5 mg · h/liter). Ratios of AUC$_{\text{total}}$s in adipose tissue, skeletal muscle, and saliva to those in plasma were 0.84 ± 0.28, 0.53 ± 0.19, and 0.04 ± 0.03, respectively. The plasma half-life ($t_{1/2}$) of doripenem was comparable between the different compartments (Table 2).

DISCUSSION
Doripenem is approved for clinical treatment of abdominal and urinary tract infections and pneumonia. Most available data regarding tissue penetration rely on animal studies (4, 9, 12, 13) where pharmacokinetics were described for different compartments such as plasma, kidney, lung, liver, bone, cecum, and trachea (4, 9, 12, 13). However, most of these studies measured drug concentrations in tissue homogenates which bear the major problem that extracellular and intracellular drug amounts get mixed together with blood from capillaries. Hence, the concentration resulting from a tissue homogenate cannot be ascribed to a particular compartment and can hardly be interpreted. In humans, pharmacokinetic studies have investigated plasma, urine (5, 6, 7), and saliva (8). Our study is the first to show that the concentration of unbound doripenem in plasma, subcutaneous adipose tissue, skeletal muscle, and saliva of healthy volunteers after administration of 500 mg as a short intravenous infusion over 1 h.

### TABLE 1 Characteristics of healthy male volunteers

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>BMI (kg/m²)</th>
<th>Wt (kg)</th>
<th>Ht (cm)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.2</td>
<td>86.9</td>
<td>182</td>
<td>1.1</td>
<td>121</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>22.9</td>
<td>86.9</td>
<td>195</td>
<td>1.2</td>
<td>117</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>21.0</td>
<td>63.6</td>
<td>174</td>
<td>1.1</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>25.3</td>
<td>78.3</td>
<td>177</td>
<td>1.1</td>
<td>95</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>23.6</td>
<td>83.7</td>
<td>188</td>
<td>1.1</td>
<td>116</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>18.3</td>
<td>62.6</td>
<td>185</td>
<td>1.0</td>
<td>108</td>
<td>21</td>
</tr>
<tr>
<td>Mean</td>
<td>22.9</td>
<td>77.0</td>
<td>183</td>
<td>1.1</td>
<td>108</td>
<td>30</td>
</tr>
<tr>
<td>± SD</td>
<td>2.6</td>
<td>10.2</td>
<td>7</td>
<td>0.1</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>
24), prostatic tissue (26), and peritoneal fluid (14), but there is a lack of data concerning the drug concentrations in soft tissues and the lung. Therefore, we examined the tissue distribution of doripenem in the interstitial space fluid of skeletal muscle and subcutaneous adipose tissue in humans, as well as in saliva.

Some pharmacometric parameters in plasma were similar in magnitude to the data reported by Cirillo et al. (6) for a comparable population while others were different: time to maximum concentration (T\text{max}), 1.0 (0.5 to 1.0) versus 0.92 (0.75 to 1.17) h; t\text{1/2}, 1.2 ± 0.2 versus 1.2 ± 0.1 h; AUC\text{total}, 26.3 ± 10.1 versus 36.3 ± 8.8 mg·h/liter; volume of distribution at steady state (V\text{ss}), 25.6 ± 6.6 versus 16.8 ± 5.5 liters; c\text{max}, 15.3 ± 6.0 versus 23.0 ± 6.6 mg/liter; and clearance (CL), 21.1 ± 7.2 versus 14.6 ± 3.6 liters/h, respectively.

Free maximum concentrations (C\text{max}) of doripenem in subcutaneous adipose tissue were ~0.7-fold those in plasma. C\text{max} of doripenem in muscle were ~0.5-fold those in plasma. The reason for the slight discrepancy in tissue concentrations between muscle and adipose tissue is not clear. The terminal half-life of doripenem of about 1.1 h measured in subcutaneous adipose tissue and muscle tissue was comparable to that in plasma. The relatively short time period until reaching maximum concentrations in tissue (median T\text{max} of 1.2 to 1.3 h after start of infusion over 1 h) (Table 2) indicates relatively rapid drug penetration from plasma to soft tissues.

For doripenem, like for other beta-lactams, the best predictor correlating with the antimicrobial activity of a dosing regimen is the time the free drug concentrations exceed the MIC (f\text{T} > MIC; time above MIC). f\text{T} > MIC is usually presented in percentage of a dosing interval and is more relevant if it is calculated not only for plasma but especially for the site of infection. The required threshold of f\text{T} > MIC differs according to the bacterial species to be eradicated, but there is strong evidence that bactericidal activity of doripenem can be achieved if f\text{T} > MIC is >40% (7, 17, 18). For some, particularly Gram-positive pathogens, f\text{T} > MIC values required for bacterial killing tend to be lower than 40% (1, 3).

Overall, the MIC\text{90} across several relevant bacterial species was reported to be ≤1 mg/liter for doripenem (9). More specifically, Gram-negative bacteria such as ESBL-producing Enterobacteriaceae and Pseudomonas aeruginosa or Haemophilus spp. showed MIC\text{90} of 0.12, 2, and 0.25 mg/liter, respectively (2). Among relevant Gram-positive microbes, Streptococcus pneumoniae was reported to have a MIC\text{90} of 0.5 mg/liter (15) while Staphylococcus aureus (including both oxacillin-susceptible and -resistant isolates) had a MIC\text{90} of 4 mg/liter (11).

Based on our current data, the “conservative” threshold of f\text{T} > MIC of >40% would be reached in subcutaneous adipose tissue of all volunteers for pathogens with a MIC of ≤2 mg/liter and in skeletal muscle for pathogens with a MIC of ≤1.5 mg/liter. Thus, all isolates considered susceptible to doripenem according to the EUCAST breakpoints, i.e., with a MIC of ≤1 mg/liter, would be adequately covered in subcutaneous adipose and muscle tissue (8). In contrast, a therapy with 500 mg doripenem t.i.d. might be insufficient for some strains of oxacillin-resistant Staphylococcus aureus (MIC\text{90} of 2 mg/liter, but MIC\text{90} of >8 mg/liter) (11).

The required threshold of >40% for f\text{T} > MIC to achieve optimal bacterial killing was originally defined for the plasma compartment of different in vivo models. This plasma-based threshold has been used successfully not only to predict the effect of therapy against infections residing in plasma itself, i.e., against bacteremia, but also for infections residing in peripheral compartments (7). However, f\text{T} > MIC values determined in peripheral tissues are often different from those in plasma. For example, in the present study, f\text{T} > MIC was lower in skeletal muscle (≥40%) than in plasma (≥53%) for a MIC of 1.5 mg/liter. Considering that plasma-based f\text{T} > MIC thresholds are also successfully applied to predict the outcome of antibiotic therapy against infections residing in the thigh (7) where local f\text{T} > MIC is lower, it should be discussed if the threshold indicating optimal f\text{T} > MIC must be modified if f\text{T} > MIC is determined directly in the tissue of interest.

Remarkably, doripenem is the only carbapenem with two alternative infusion durations of either 1 h or 4 h. The manufacturer recommends considering the longer infusion duration of 4 h especially for more severe pneumonias and for less susceptible bacteria. Prolonged infusions of doripenem over 4 h instead of 1 h administered every 8 h may help maximizing periods of f\text{T} > MIC, contributing to better coverage of some less susceptible isolates (3, 14, 25). Also, higher overall doses of doripenem, such as 1 g t.i.d., which are already used by some hospitals, can contribute to better antibiotic efficacy, avoiding critical underdosing against less susceptible bacteria. However, it has to be stated that tissue pharmacokinetics may change in severely ill patients due to pathophysiological alterations, limiting unrestrained extrapolation of data obtained from healthy subjects.

The present observation of lower f\text{T} > MICs of doripenem in peripheral compartments than in plasma is different in a previous study, where Ikawa et al. (14) found higher probabilities of target attainment of T > MIC in peritoneal fluid. Apparently, the pathophysiological and pharmacokinetic conditions in peritoneal fluid of these abdominal surgery patients were different than in plasma or soft tissues.

Possible gender differences were not investigated in this study, which was conducted exclusively in male volunteers. The drug approval package available at the FDA website (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022106s000_ClinPharmR_P2.pdf) contains clinical data showing that after infusion of 500 mg doripenem over 1 h to all subjects, females have an

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**TABLE 2 Pharmacokinetic parameters of unbound doripenem in different compartments after a single intravenous infusion of 500 mg over 1 h**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>C\text{max} (mg/liter)</th>
<th>T\text{max} (h)</th>
<th>AUC\text{0-8h} (mg·h/liter)</th>
<th>AUC\text{total} (mg·h/liter)</th>
<th>t\text{1/2} (h)</th>
<th>V\text{ss} (liters)</th>
<th>CL (liters/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>15.3 ± 6.0</td>
<td>1.0 (0.5–1.0)</td>
<td>26.0 ± 9.9</td>
<td>26.3 ± 10.1</td>
<td>1.2 ± 0.2</td>
<td>25.6 ± 6.6</td>
<td>21.1 ± 7.2</td>
</tr>
<tr>
<td>Subcutis</td>
<td>9.9 ± 2.4</td>
<td>1.25 (0.75–1.25)</td>
<td>20.2 ± 3.8</td>
<td>20.4 ± 3.8</td>
<td>1.1 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>6.6 ± 2.9</td>
<td>1.25 (1.25)</td>
<td>12.6 ± 2.9</td>
<td>12.8 ± 3.0</td>
<td>1.1 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>0.5 ± 0.2</td>
<td>1.0 (0.5–1.5)</td>
<td>0.9 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>2.2 ± 2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Means ± standard deviations.

\* Presented as median and range.
AUC which is about 11% higher than males, and elderly (>66 years) male and female subjects have an AUC in serum which is about 49% higher than that in young healthy controls. The higher AUC values previously reported for plasma of female and elderly subjects also suggest that at a standard dose of 500 mg over 1 h, the soft tissue concentrations of doripenem in these populations might be higher than or are at least equal to those currently observed in relatively young male volunteers.

Microdialysis is not feasible in each organ system in healthy subjects. Doripenem is also used for treating pneumonia, but samples of bronchoalveolar fluid are not easily available from human volunteers. Therefore, drug concentrations were additionally measured in saliva/sputum. Although volunteers were asked to expectorate saliva/sputum from the lower airways, in fact, it has to be assumed that primarily saliva from the oral cavity was obtained, a fluid covering the epithelia of the upper respiratory tract.

With $t_{max}$ values ranging from 0.2 to 0.7 mg/liter, doripenem concentrations were significantly lower in saliva than in plasma (~0.04-fold that in plasma) or soft tissues. Considering the resulting $f/T > MIC$ values, doripenem might be reliable against bacteria residing in saliva only if their MIC would be $\leq 0.1$ mg/liter. Indeed, some susceptible respiratory tract pathogens might be adequately covered in saliva, considering the MIC$_{50}$ of $\leq 0.06$ mg/liter reported for Streptococcus pneumoniae, Haemophilus influenzae, oxacillin-susceptible Staphylococcus aureus, and beta-hemolytic streptococci (11). However, the doripenem concentrations measured in saliva may not be applied directly to the conditions in the lung or, more specifically, to bronchoalveolar fluid because the latter has a different chemical composition containing surfactant. Therefore, a pharmacokinetic study focusing on the lower respiratory tract would be interesting, especially in patients with pneumonia or other underlying lung diseases where the pulmonary microcirculation is impaired.

In conclusion, doripenem penetrates well into interstitial space fluid of skeletal muscle and adipose tissue, suggesting good antimicrobial activity in infected soft tissues. In contrast, it is detectable at much lower concentrations in saliva, inciting studies of doripenem pharmacokinetics in bronchoalveolar fluid.

REFERENCES