Penetration of ertapenem into skeletal muscle and subcutaneous adipose tissue in healthy volunteers measured by in vivo microdialysis

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Objectives: Ertapenem is FDA approved for the treatment of skin and skin-structure infections (SSSI), but its in vivo penetration into the interstitial space of soft tissues is unknown. The present microdialysis study was conducted to measure free, protein-unbound ertapenem concentrations in muscle and subcutaneous tissue.

Volunteers and methods: In a single-centre, prospective, open-label study six healthy volunteers (three females, 22–37 years) were treated with 1 g ertapenem given as a single intravenous dose. Microdialysis and plasma samples were collected before and at different time points up to 12 h after medication. Drug concentrations were determined by a validated LC–MS-MS method.

Results: No serious or microdialysis-associated adverse events were observed. Ertapenem concentrations in plasma reached a maximum (Cmax) of 103.3 – 26.3 mg/L, a terminal elimination half-life (t1/2) of 3.8 – 0.6 h and an AUC0–1 of 359.7 – 66.5 mg · h/L. Mean peak concentrations of free, protein-unbound ertapenem in interstitial space fluid of skeletal muscle and subcutaneous adipose tissue were much lower (Cmax = 6.7 – 4.1 and 4.0 – 1.6 mg/L, respectively). This degree of tissue distribution is consistent with high concentration-dependent plasma protein binding of ertapenem (84–96%). AUC0–1 values for both muscle and adipose tissue were lower as well (39.7 – 24.8 and 18.6 – 4.6 mg · h/L). However, unbound interstitial fluid concentrations exceeded MIC90 values for the important SSSI pathogens for 7 (subcutis) and 10 h (muscle) after dosing.

Conclusions: These results support the previously observed clinical efficacy of ertapenem in the treatment of SSSI.

Keywords: subcutis, distribution, target site, pharmacokinetics

Introduction

In clinical practice, empirical antibiotic therapy is initiated as the first measure dealing with bacterial infection. In case of therapeutic failure, however, it may become necessary to measure the susceptibility of the causative agent and to adjust antibiotic dosage schedules with the aim to attain serum drug concentrations above the MIC for the respective pathogen throughout the dosing interval.1 Although this approach is suitable for conditions where the central compartment is the main site of infection, e.g. septicemia, for localized organ or tissue infections drug concentrations in the interstitial space rather than in serum determine the clinical outcome of antimicrobial therapy.2,3 This is particularly relevant for skin and skin-structure infections (SSSI), which may occur due to surgical wound contamination, after trauma or in diabetic patients and may result in severe necrotizing limb- and even life-threatening infections.4–6 Hence, to be clinically effective an antibiotic should reach pharmacologically active, i.e. unbound, soft tissue concentrations high enough to eradicate the causative pathogen.7,8 A class of antibiotics that has been shown to qualify for the treatment of SSSI is the carbapenems. Ertapenem is a long-acting parenteral...
1-β-methyl-carbapenem, which was selected for clinical development partially based on its pharmacokinetics. Owing to its long plasma half-life, which reflects a high plasma protein binding, ertapenem can be administered once daily. Although clinical studies have demonstrated the effectiveness of ertapenem for SSSI treatment, the in vivo penetration and the resulting free protein-unbound concentrations in interstitial space of soft tissues, such as skeletal muscle and subcutaneous adipose tissue, have not been reported, mainly due to a lack of appropriate methodology. One technique that has been proven suitable for the measurement of target tissue concentrations of a variety of substances in vivo in humans is clinical microdialysis. This method is a minimally invasive technique for the measurement of unbound drug concentrations in virtually every tissue and organ. The present microdialysis study was conducted to measure and compare the free protein-unbound ertapenem concentrations in the interstitial space fluid of two peripheral target sites, skeletal muscle and subcutaneous adipose tissue, following the administration of 1 g infusion, and to compare them with the respective plasma concentrations.

Volunteers and methods

Volunteers

Six healthy volunteers (three men, three women; four Asians, two Caucasians), between 22 and 37 years of age, average height 160.0 ± 8.8 cm, average body weight 64.7 ± 6.8 kg, average body mass index 25.3 ± 2.4 kg/m² and average body surface area 1.68 ± 0.16 m², participated in the study. All had normal renal and hepatic function; the mean creatinine clearance was 100.5 ± 21.1 mL/min · 1.73 m². All volunteers included in the study had normal findings from physical examination, electrocardiogram and laboratory tests (including haematological and biochemical parameters, urinalysis and negative pregnancy test). The mean albumin serum concentration in the volunteers included in the study was 3.7 ± 0.4 g/L. Further exclusion criteria were regular use of medications, abuse of alcoholic beverages, symptoms of significant illness within 3 months before the study period, history of liver or kidney disease potentially interfering with metabolism or excretion of the drug, history of CNS disorders, allergy or hypersensitivity to the study drug, blood donation of more than 500 mL during the previous 3 months, participation in a clinical trial within 3 months before the study period and pregnancy. The study was conducted in the General Clinical Research Center at Shands Hospital, University of Florida, approved by Shands’ Hospital Institutional Review Board (IRB-01) and was performed in accordance with the Declaration of Helsinki. All volunteers were given a detailed description of the study, and their written consent was obtained.

Study design and protocol

The study was conducted as a single-centre, prospective, open-label trial. Volunteers were hospitalized from the evening before start of the study until 12 h post-dosing. On the study day, the volunteers were kept under fasting conditions for 10 h prior to the start of the experiments until 2 h after drug administration. Each volunteer received one dose of 1 g ertapenem as an intravenous infusion over 30 min. Tolerability and safety assessments, clinical chemistry, haematological tests and urinalysis, and the measurement of vital signs (blood pressure, heart rate) and ECG were included in the study. Vital signs were taken before administration of the drug and 15 min, 1, 2, 12 h thereafter. All data relating to drug safety were recorded throughout the study.

Sample collection

To measure the unbound fraction of ertapenem in the interstitial space fluids, microdialysis probes (CMA 60; CMA Microdialysis AB, Solna, Sweden) with a molecular cut-off of 20 kDa were used. Microdialysis probes were inserted after cleaning and thorough disinfection of the skin. One dialysis probe was inserted into the medial vastus muscle and one was inserted into the subcutaneous layer of the thigh. The microdialysis system was flushed with lactated Ringer’s solution and then connected to a microinfusion pump. The principles of microdialysis have been described in detail previously. Briefly, microdialysis is based on sampling of the non-protein-bound fraction and, therefore, the pharmacologically active fraction of analytes from the interstitial space with a semipermeable membrane at the tip of a microdialysis probe. The probe is constantly perfused with a physiological solution (perfusionate) at a flow rate of 2 μL/min. Once the probe is implanted into the tissue, substances present in the extracellular fluid at a particular concentration (C_tissue) are filtered out of the interstitial space fluid by perfusion into the probe, resulting in a concentration (C_dialysate) in the perfusate. Samples are collected and analysed. For most analytes, equilibrium of the concentration between extracellular tissue fluid and the dialysate is incomplete; therefore, C_tissue > C_dialysate. The factor by which the concentrations are interrelated is termed recovery. To obtain absolute concentrations in the interstitial space fluid from the concentrations in unbond dialysate, microdialysis probes were calibrated for in vivo recovery rates by the retrodialysis method. The retrodialysis procedure was performed in each subject before dosing of the drug. The principle of this method relies on the assumption that the diffusion process is quantitatively equal in both directions through the semipermeable membrane. Therefore, ertapenem was added to the perfusate at a concentration of 5.0 mg/L, and the disappearance rate (delivery) through the membrane was taken as the in vivo recovery. The in vivo percentage recovery was calculated as

\[
\text{Recovery (\%)} = 100 - \left( \frac{\text{Concentration}_{\text{dialysate}}}{\text{Concentration}_{\text{perfusate}}} \times 100 \right)
\]

After a 30 min baseline perfusion period, in vivo calibration was performed as described previously for a period of 60 min. A sample taken over the last 30 min of this period was used to calculate the in vivo recovery. After the calibration period was completed, a 60 min washout period was observed. Microdialysis samples were analysed at 60 min intervals up to 12 h post-dose.

Blood samples (5 mL) were collected in lithium heparinate-coated tubes, via a venous plastic cannula (JELCO; Johnson-Johnson, Arlington, TX, USA), before ertapenem infusion, and 0.5, 1, 2, 3, 4, 6, 8 and 12 h after the start of infusion. Samples were centrifuged at 1300 g for 10 min at 4°C. Plasma was separated and stored at −80°C until analysis.

Drug assay

Quantitative determination of ertapenem in plasma and in interstitial space fluid of skeletal muscle and subcutaneous adipose tissue was performed by validated SPE-liquid chromatography–tandem mass spectrometry methods (LC–MS–MS). 0.1 M MES buffer, pH = 6.5 (MES free acid, 19.52 g, dissolved in 900 mL of double distilled water, adjusted pH to 6.5 by adding 5 M ammonium hydroxide, then water added to 1000 mL), was added to all samples (v:v = 1:1)
Tissue penetration of ertapenem

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis with the pharmacokinetic software program (WinNonlin, Pharsight). The maximum observed plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) after drug administration were determined directly from the plasma concentration–time curves. The area under the plasma concentration–time curve from time 0 (the start of infusion) until the last quantifiable plasma concentration (AUC_{0–last}) was calculated using the log-linear trapezoidal rule. AUC_{0–∞} was derived by adding C_{last}/λ_{z} to AUC_{0–last}. The terminal elimination rate constant (λ_{z}) was estimated from the slope of the terminal exponential phase of the logarithmic plasma concentration–time profile using no fewer than three data points. The apparent terminal elimination half-life (t_{1/2z}) was calculated as 0.693/λ_{z}. The mean residence time (MRT) was calculated as AUMC_{0–∞}/AUC_{0–∞}, where AUMC_{0–∞} is the area under the first moment of the concentration–time curve, determined by integrating the product of time and concentration from zero to infinity. Apparent total clearance (CL_{tot}) was calculated as dose/AUC_{0–∞}. The apparent volume of distribution (V_{app}) was calculated as (CL_{tot}/λ_{z}). Protein-unbound ertapenem concentrations in the extracellular skeletal muscle and subcutaneous adipose tissue fluid were calculated from measured microdialysate concentrations and individual probe recovery, determined in our in vivo experiments. The parameters, such as C_{max}, T_{max}, AUC_{0–last}, AUC_{0–∞} and t_{1/2z}, were calculated using the same formulae as for plasma samples. The tissue penetration was calculated as the ratio of the unbound AUC_{0–∞} in skeletal muscle or subcutaneous adipose tissue fluid to the total AUC_{0–∞} in plasma (AUC_{tissue,free}/AUC_{plasma,total}). All data are presented as geometric means ± SD, with the exception of T_{max}, for which only median and minimum–maximum ranges are given.

Results

Safety

All six enrolled volunteers completed the study in accordance with the protocol. The microdialysis procedure and treatments were well tolerated. No serious or severe adverse events or microdialysis-associated side effects were observed. Two volunteers reported headache, not related to the study drug. There were no clinically significant changes in electrocardiograms, blood pressure or pulse. Similarly, there were no clinically important findings in haematology, clinical chemistry or urinalysis.

Pharmacokinetics

The average recoveries from muscle and subcutaneous tissue were found to be 55 ± 14% and 65 ± 7%, respectively (means ± SD). Each subject’s dialysate concentrations were corrected by the respective recovery to determine actual tissue concentrations. The time versus concentration profiles of ertapenem in plasma (total concentration) and in the interstitial space fluid of skeletal muscle and subcutaneous adipose tissue after administration of a single intravenous dose of 1 g over 30 min to healthy volunteers (n = 6) are shown in Figure 1. Pharmacokinetic parameters are listed in Table 1.

Discussion

Bacterial SSSI are among the most frequently seen infectious diseases in the community and occasionally in the hospital setting. Larger and profound lesions are usually secondarily mixed-infected with aerobic Gram-positive and Gram-negative bacteria including Staphylococcus aureus, Streptococcus species, Enterobacteriaceae and anaerobic bacterial pathogens. Apart from surgical and general care measures (wound cleaning) a therapy with highly effective antimicrobial agents is indicated.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma</th>
<th>Muscle</th>
<th>Subcutis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (mg/L)</td>
<td>103.3 ± 26.3</td>
<td>6.71 ± 4.14</td>
<td>3.96 ± 1.63</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>0.5 (NA)</td>
<td>1.5 (0.5–2.5)</td>
<td>0.5 (0.5–1.5)</td>
</tr>
<tr>
<td>C_{12} (mg/L)</td>
<td>7.93 ± 4.15</td>
<td>1.13 ± 0.68</td>
<td>0.31 ± 0.16</td>
</tr>
<tr>
<td>AUC_{0–last} (mg · h/L)</td>
<td>316.1 ± 49.1</td>
<td>35.3 ± 22.3</td>
<td>16.5 ± 3.6</td>
</tr>
<tr>
<td>AUC_{0–∞} (mg · h/L)</td>
<td>359.7 ± 66.5</td>
<td>39.7 ± 24.8</td>
<td>18.6 ± 4.6</td>
</tr>
<tr>
<td>Terminal half-life (h)</td>
<td>3.77 ± 0.60</td>
<td>3.38 ± 0.68</td>
<td>3.63 ± 0.85</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>4.58 ± 0.88</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>V_{z} (L)</td>
<td>15.5 ± 3.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CL_{tot} (L/h)</td>
<td>2.88 ± 0.51</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AUC_{interstitium}/AUC_{total plasma}</td>
<td>–</td>
<td>0.13 ± 0.09</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

Data are presented as geometric means ± SD, with the exception of T_{max}, for which median and minimum–maximum ranges are given. C_{12}, ertapenem concentrations obtained 12 h after start of infusion.
because infected SSSI are often the starting point of phlegmonous inflammations and in the worst case also of a septic syndrome. For the selection of a suitable antibiotic drug the antibacterial spectrum and the concentrations reached at the site of infection are very important. Many studies have shown that plasma concentrations may not be an ideal parameter for the prediction of the clinical efficacy of antibiotics because most infections occur at the tissue sites. In addition, it has been found that only free protein-unbound antibiotic concentrations at the infection sites are responsible for the antibacterial activity. Therefore, inadequate interstitial tissue concentrations can lead to therapeutic failure and bacterial resistance.

Owing to its wide range of antibacterial activity against most Gram-positive, Gram-negative and anaerobic bacteria, ertapenem is a candidate for the treatment of SSSI. However, there is only limited information on the ability of ertapenem to penetrate into different organ tissues, such as lung or pancreatic tissue. With reference to the treatment of SSSI, one study investigated ertapenem penetration into suction-induced skin blister fluids in 12 healthy young volunteers. Drug concentrations in skin blister fluids exceeded 4 mg/L (the MIC at which 90% of isolates tested are eliminated) throughout the entire dosing interval of 24 h. However, extrapolation of these data to the concentrations in infected tissues should be done with extreme caution. One problem is that skin blisters are formed before the administration of the antibiotic. The blister thus serves as a large third compartment with a surface-to-volume ratio which is hardly representative of that for tissue. Besides, other variables must be taken into account, such as the barrier between the blister and the skin, which may change over time, and the presence of proteins in blister fluid.

The main purpose of our study was to measure the concentrations of the non-protein-bound ertapenem in interstitial fluids of two different SSSI target sites (skeletal muscle and subcutaneous adipose tissue) by microdialysis after administration of a single intravenous standard dose (1 g/day). The results show that ertapenem reaches measurable concentrations in both target tissues. As expected, time versus concentration profiles indicate that free ertapenem concentrations in interstitial space fluids were lower than the corresponding total concentrations in plasma (Figure 1). The tissue levels measured in our study corresponded approximately to the free protein-unbound fraction of ertapenem in plasma (4–16%). Free interstitial/plasma concentrations ratios for skeletal muscle and subcutaneous adipose tissue were not congruent, a finding that has been observed previously and that might be explained by differences in local blood flow between the two tissues.

Finally, the question arises—are the free, protein-unbound ertapenem concentrations in the interstitial space fluids of muscle and subcutaneous adipose tissue high enough to kill the bacteria effectively? Like other β-lactam antibiotics, carbapenems exert their killing effect in a time-dependent manner. In this category of antibacterial drugs, increasing the concentrations above 4–5× MIC for the bacteria no longer adds a proportional increase in the killing effect. Therefore, maximum killing is obtained by optimizing the time of exposure of the drug to the bacteria so that the concentrations remain above the MIC as long as possible. The main pharmacokinetic/pharmacodynamic (PK/PD) parameter for β-lactams is the proportion of time of the dose interval during which the drug concentration exceeds the MIC (T > MIC). For carbapenems a T > MIC of 30–40% of the dose interval has been previously suggested to be effective due to their rapid bactericidal activity. In vitro studies demonstrated that ertapenem inhibited 90% (MIC90) of methicillin-susceptible S. aureus strains at 0.25 mg/L. Against Streptococcus spp., ertapenem had a minimal inhibitory concentration of 0.5 mg/L and against extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae MIC90 values ranged from 0.03 to 0.06 mg/L. Ertapenem MIC90 values for Bacteroides fragilis and other anaerobic bacteria were ≤1.0 mg/L. In the present study, protein-unbound ertapenem concentrations 12 h after a single intravenous administration of 1 g were 1.13 ± 0.68 mg/L for muscle tissue and 0.31 ± 0.16 mg/L for subcutaneous adipose tissue. Therefore, a 1 g dose once daily results in muscle tissue concentrations higher than MICs for most of the above-mentioned SSSI pathogens for at least 50% of the entire dosing interval. Also the mean concentrations in subcutaneous adipose tissue exceeded the MIC90s for SSSI pathogens for at least 30% of the dosing interval (Figure 1).

From this finding we can conclude that the data obtained in the study suggest adequate free, protein-unbound ertapenem concentrations in the non-infected interstitial fluid of muscle and subcutaneous adipose tissue. Our results support the previously observed clinical efficacy of ertapenem in the treatment of SSSI.

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Transparency declarations

None to declare.

References