High fosfomycin concentrations in bone and peripheral soft tissue in diabetic patients presenting with bacterial foot infection

Michael V. Schintler1†, Friederike Traunmüller1,2†, Julia Metzler1, Gerhard Kreuzwirt1, Stephan Speland1, Oliver Mauric2, Martin Popovic2,3, Erwin Scharnagl1 and Christian Joukhadar1,2,4,5*

1Department of Surgery, Division of Plastic Surgery, Medical University of Graz, Auenbruggerplatz 29, A-8036 Graz, Austria; 2J&P MEDICAL RESEARCH LTD, Auhostrasse 15/8-9, A-1130 Vienna, Austria; 3Department of Radiology, Division of Cardiovascular and Interventional Radiology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria; 4Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA; 5Harvard Medical School, Boston, MA 02115, USA

Received 6 May 2009; returned 14 May 2009; revised 8 June 2009; accepted 9 June 2009

Objectives: Appropriate antimicrobial therapy and surgical intervention may be required in diabetic patients presenting with severe bacterial foot infection. Methicillin-resistant Staphylococcus aureus (MRSA) agents such as fosfomycin are increasingly in demand because of recent concern regarding vancomycin and daptomycin efficacy and constant use. Intravenous fosfomycin is approved for the therapy of severe soft tissue infections and is highly active against methicillin-susceptible S. aureus and MRSA. In the present study we investigated fosfomycin’s ability to penetrate bone tissue in diabetic patients suffering from severe bacterial foot infection.

Patients and methods: The well established microdialysis technique was utilized to determine fosfomycin concentrations in metatarsal bone in nine patients scheduled for partial bone resection due to bacterial foot infection and osteomyelitis. Plasma and unaffected subcutaneous adipose tissue served as reference compartments.

Results: After a single intravenous dose of approximately 100 mg of fosfomycin per kg of body weight, the mean Cmax, Tmax and AUC0–6 for bone were 96.4 mg/L, 3.9 h and 330.0 mg · h/L, respectively. The degree of tissue penetration as determined by the ratios of the AUC0–6 for bone to plasma and for subcutaneous adipose tissue to plasma were 0.43 ± 0.04 and 0.76 ± 0.05, respectively.

Conclusions: On the basis of relevant pharmacokinetic–pharmacodynamic indices, it seems that fosfomycin is an effective antibiotic for the treatment of deep-seated diabetic foot infections with osseous matrix involvement.

Keywords: single-dose pharmacokinetics, diabetic foot infection, MRSA, osteomyelitis

Introduction

Severe acute bacterial infection of the foot is a frequently observed late diabetic complication. It is the underlying diagnosis in 60%–70% of lower extremity amputations in industrial nations.1 To prevent the loss of an affected limb, radical surgical debridement with excision of necrotic tendons, resection of sequestrating bone and the early intravenous administration of effective antimicrobial agents are mandatory.2 As diabetic foot infection (DFI) is usually of polymicrobial nature, at times including methicillin-resistant Staphylococcus aureus (MRSA) or other multidrug-resistant organisms, clinicians sometimes face problems selecting the appropriate antimicrobial agent.3–5 In this regard, concerns about the ability of vancomycin to penetrate tissues were recently raised.6 This finding prompted others to investigate daptomycin, which is of comparable...
molecular size, exerts high bactericidal activity and shows plasma protein binding of >90%. In contrast to peptide antibiotics, fosfomycin has a unique low molecular weight structure and is virtually unbound to plasma proteins. Therefore, fosfomycin penetrates well into the interstitial space fluid of unaffected and inflamed soft tissues of healthy subjects, diabetics and critically ill patients. At therapeutically relevant concentrations, fosfomycin exerts excellent in vitro bactericidal activity against a wide spectrum of Gram-positive and Gram-negative bacteria. Among the most important pathogens are *S. aureus* including MRSA, *Staphylococcus epidermidis*, *Enterococcus faecalis*, members of the Enterobacteriaceae and the majority of *Pseudomonas aeruginosa* strains. Additionally, fosfomycin is thought to bind to osseous tissue due to its structural similarity to hydroxyapatite.

Reaching adequate concentrations of antibiotics at the target site may be considered a prerequisite for clinical success in antimicrobial therapy. For fosfomycin, concentrations attained in the interstitial space fluid of soft tissues were demonstrated to mimic corresponding unbound plasma concentrations. However, in the medical literature, data on fosfomycin concentrations in bone are exclusively available from single-point measurements from biopsies collected after administration of therapeutically non-relevant doses. The present study aimed at providing a full description of concentration versus time profiles of fosfomycin in plasma and metatarsal bone in DFI after the administration of an approved single intravenous dose of 100 mg/kg of body weight (BW). The microdialysis technique, which is a well established, highly reliable and reproducible method for determining antibiotic concentrations in the interstitial space fluid of tissues (including bone), was utilized in the present investigation. Unaffected, healthy subcutaneous adipose tissue of the lower limb served as the reference tissue in order to eliminate bias from a reduction of blood flow due to peripheral vascular disease, a frequent complication in diabetes patients.

**Patients and methods**

The study was approved by the Ethics Committee of the Medical University of Graz, Austria, and was performed at the Division of Plastic Surgery, Department of Surgery, Landeskrankehaus Universitätsklinikum Graz (State Hospital University Clinic of Graz), Graz, Austria. Analytical work was performed at the laboratory of J&P MEDICAL RESEARCH Ltd, Vienna, Austria.

**Study subjects**

Nine patients with diabetes [three females and six males, aged 48–83 years, body mass index (BMI) 22.4–37.1 kg/m²] presenting with deep-seated bacterial foot infection (stage 3B and 4B according to the University of Texas classification system) were included in the study after written informed consent was obtained. The patients required surgical debridement with partial metatarsal bone resection and adjuvant systemic antimicrobial therapy. Exclusion criteria were known allergy to fosfomycin, pre-treatment with fosfomycin within 1 week before the screening visit, pregnancy, serum creatinine >141 μmol/L (>1.6 mg/dL), severely impaired liver function and severe cardiac insufficiency. All patients had received non-invasive, conservative treatment for DFI prior to inclusion into the study. Six of the nine patients had a history of percutaneous transluminal angioplasty. During the conduct of the present study, the co-administration of antimicrobial agents or medications other than the study drug was permitted, if medically indicated. All subjects suffered from type 2 diabetes. Treatment for diabetes in our patients ranged between 11 and 35 years. The level of angiopathy was not determined consistently in our subjects prior to the start of the study.

**Microdialysis and sampling procedures**

The principles of microdialysis are described in detail elsewhere. In brief, during surgery, a microdialysis probe (shaft length 50 mm, membrane length 10 mm, molecular cut-off 20000; CMA Microdialysis AB, Stockholm, Sweden) was implanted into metatarsal bone close to the resection margin. For this purpose a channel of ~2 mm in diameter and 2 cm in length was drilled into the bone. Under continuous visual control, the probe was inserted into cortical and/or cancellous healthy bone using an adapted plastic cannula (Venflon®, Becton Dickinson, Heidelberg, Germany). The plastic cannula served as a guidance tool to ensure that the membrane at the tip of the probe was properly and safely placed into the target tissue. Suturing and other procedures related to the management of the surgical wound were not hampered or delayed by probe implantation. A second probe was inserted into an unaffected area of subcutaneous adipose tissue of the lower limb. The probes were connected via tubing to a precision pump (SP101i syringe pump, WPI Inc., Sarasota, FL, USA) and constantly perfused with 0.9% sterile saline solution at a flow rate of 1.5 μL/min. After a 60 min equilibration period, a single dose of ~100 mg/kg of fosfomycin per kg of BW (Fosfomycin Sandoz™, Sandoz, Kundl, Austria) was administered to the patient intravenously over ~30 min. Subsequently, microdialysates were collected at 20 min intervals from 0 to 3 h, and at 30 min intervals from 3 to 6 h. Samples of venous blood were drawn from an indwelling intravenous cannula at defined timepoints. In vivo calibration of the individual probes was performed by using the ‘reverse dialysis method’ as previously described. Microdialysates and plasma aliquots collected from venous blood after centrifugation at 1600 g for 10 min were stored at −70°C until analysis.

**Chemical analysis**

Fosfomycin concentrations in plasma and microdialysates were measured by HPLC utilizing indirect ultraviolet detection according to the method of Hu et al. The samples were diluted as necessary with blank human plasma and 0.9% saline, respectively. Plasma samples were prepared by ultrafiltration using centrifugal filter devices (Ultrafree-MC, Millipore, Bedford, MA, USA). The lower limit of quantification was 5 μg/L. The coefficients of inaccuracy (relative error) and imprecision (relative standard deviation) ranged between 0.8% and 12.0%. Individual values of plasma protein binding were not determined as the scientific literature documents fosfomycin’s negligible protein binding in humans and animals. Pharmacokinetic (PK) analysis

PK calculations were carried out by use of commercially available computer software (Kineta, version 3.0; Innaphase, PA, USA). The concentrations at 12 h were calculated by mathematical extrapolation using the formula: $C_{12} = C_0 \times e^{-k \times 12}$, where $C_0$ is the concentration at 6 h after the start of infusion and $k$ is the elimination rate constant at the β-phase of elimination. The AUC0–6 and AUC0–12...
Schintler et al.

in plasma and interstitial fluid were calculated by use of the linear trapezoidal rule. The time that fosfomycin concentrations remained above the MIC for selected bacteria \(T_{\text{MIC}}\) was calculated for plasma and subcutis by the formula: \(T_{\text{MIC}} = \ln \left(C_t / \text{MIC}\right) / k + 1\). The calculation of \(T_{\text{MIC}}\) for bone was corrected accordingly.

Results

The present study set out to provide better understanding of the clinical value of the anti-MRSA drug fosfomycin by determining its concentration in metatarsal bone. The microdialysis technique was utilized in nine patients with severe DFI scheduled to undergo major surgical intervention. No adverse events related to the study drug or to microdialysis probe insertion were observed.

In vivo recovery of fosfomycin in microdialysates was 81.2% ± 5.6% and 67.7% ± 8.6% for subcutaneous tissue and bone, respectively. The concentration versus time profiles of fosfomycin in plasma, subcutaneous adipose tissue and bone are depicted in Figure 1. The main PK parameters are summarized in Table 1. Fosfomycin penetrated well into osseous tissue and equilibrated fully with plasma after 3–4 h following the start of infusion (Figure 1). Compared with a healthy study population, a moderate prolongation of half-life was detected in our patients, which is most probably due to reduced glomerular filtration rates frequently seen in diabetics and in the critically ill. The ratios of the AUC\(_{0–6}\) for bone to plasma and for subcutaneous adipose tissue to plasma were 0.37–0.48 and 0.70–0.86, respectively, indicating that interindividual differences in fosfomycin’s ability to penetrate tissues are low.

It is noteworthy to mention that the microdialysis probe was implanted into vital, macroscopically unaffected bone tissue located at the margin of surgically resected, inflamed and sequestrating bone structures. Theoretically, the PK profile of fosfomycin in inflamed and actually investigated bone may be slightly different. However, from previous clinical investigations in patients presenting with septic shock, ventriculitis, deep-seated abscesses, cellulitis and diabetic foot syndrome, we have seen that the intravenous dose of ~100 mg/kg of BW was administered appropriately. Importantly, the concentrations of fosfomycin peaked in bone between 79 and 124 mg/L and equilibrated fully with plasma after 3–4 h following the start of infusion (Figure 1). Compared with a healthy study population, a moderate prolongation of half-life was detected in our patients, which is most probably due to reduced glomerular filtration rates frequently seen in diabetics and in the critically ill. The ratios of the AUC\(_{0–6}\) for bone to plasma and for subcutaneous adipose tissue to plasma were 0.37–0.48 and 0.70–0.86, respectively, indicating that interindividual differences in fosfomycin’s ability to penetrate tissues are low.

Discussion

Fosfomycin’s peak concentrations in plasma and subcutaneous adipose tissue were in line with those found in previous investigations in healthy subjects and critically ill patients, confirming that the intravenous dose of ~100 mg/kg of BW was administered appropriately. Importantly, the concentrations of fosfomycin peaked in bone between 79 and 124 mg/L and equilibrated fully with plasma after 3–4 h following the start of infusion (Figure 1). Compared with a healthy study population, a moderate prolongation of half-life was detected in our patients, which is most probably due to reduced glomerular filtration rates frequently seen in diabetics and in the critically ill. The ratios of the AUC\(_{0–6}\) for bone to plasma and for subcutaneous adipose tissue to plasma were 0.37–0.48 and 0.70–0.86, respectively, indicating that interindividual differences in fosfomycin’s ability to penetrate tissues are low.

It is noteworthy to mention that the microdialysis probe was implanted into vital, macroscopically unaffected bone tissue located at the margin of surgically resected, inflamed and sequestrating bone structures. Theoretically, the PK profile of fosfomycin in inflamed and actually investigated bone may be slightly different. However, from previous clinical investigations in patients presenting with septic shock, ventriculitis, deep-seated abscesses, cellulitis and diabetic foot syndrome, we have seen that the intravenous dose of ~100 mg/kg of BW was administered appropriately. Importantly, the concentrations of fosfomycin peaked in bone between 79 and 124 mg/L and equilibrated fully with plasma after 3–4 h following the start of infusion (Figure 1). Compared with a healthy study population, a moderate prolongation of half-life was detected in our patients, which is most probably due to reduced glomerular filtration rates frequently seen in diabetics and in the critically ill. The ratios of the AUC\(_{0–6}\) for bone to plasma and for subcutaneous adipose tissue to plasma were 0.37–0.48 and 0.70–0.86, respectively, indicating that interindividual differences in fosfomycin’s ability to penetrate tissues are low.

It is noteworthy to mention that the microdialysis probe was implanted into vital, macroscopically unaffected bone tissue located at the margin of surgically resected, inflamed and sequestrating bone structures. Theoretically, the PK profile of fosfomycin in inflamed and actually investigated bone may be slightly different. However, from previous clinical investigations in patients presenting with septic shock, ventriculitis, deep-seated abscesses, cellulitis and diabetic foot syndrome, we have seen that the intravenous dose of ~100 mg/kg of BW was administered appropriately. Importantly, the concentrations of fosfomycin peaked in bone between 79 and 124 mg/L and equilibrated fully with plasma after 3–4 h following the start of infusion (Figure 1). Compared with a healthy study population, a moderate prolongation of half-life was detected in our patients, which is most probably due to reduced glomerular filtration rates frequently seen in diabetics and in the critically ill. The ratios of the AUC\(_{0–6}\) for bone to plasma and for subcutaneous adipose tissue to plasma were 0.37–0.48 and 0.70–0.86, respectively, indicating that interindividual differences in fosfomycin’s ability to penetrate tissues are low.

Table 1. Key PK parameters of fosfomycin following intravenous administration of 100 mg/kg (n = 9) in plasma and tissues

<table>
<thead>
<tr>
<th>Compartment</th>
<th>(C_{\text{max}}) (mg/L)</th>
<th>(T_{\text{max}}) (h)</th>
<th>(t_{1/2k}) (h)</th>
<th>AUC(_{0–6}) (mg · h/L)</th>
<th>AUC(_{0–12}) (mg · h/L)</th>
<th>AUC(<em>{0–6}) tissue/AUC(</em>{0–6}) plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>377.3 ± 73.2</td>
<td>0.5 ± 0.0</td>
<td>3.6 ± 1.2</td>
<td>785.1 ± 107.2</td>
<td>1013.8 ± 108.4</td>
<td>–</td>
</tr>
<tr>
<td>Subcutis</td>
<td>185.1 ± 34.2</td>
<td>1.0 ± 0.2</td>
<td>3.7 ± 1.4</td>
<td>592.7 ± 77.5</td>
<td>821.3 ± 91.3</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>Bone</td>
<td>96.4 ± 14.5</td>
<td>3.9 ± 0.4</td>
<td>2.2 ± 0.6</td>
<td>330.0 ± 55.3</td>
<td>511.0 ± 100.7</td>
<td>0.43 ± 0.04</td>
</tr>
</tbody>
</table>

Values represent means ± SD.

*Obtained by mathematical extrapolation.
Fosfomycin in diabetic foot infection

PK data presentation among studies, their results cannot be directly compared. Reported outcome can be confusing as it comprises data obtained from single-point measurements versus concentration–time profiles, multiple dosing versus single dosing, total drug concentrations versus free drug concentrations, peak concentration versus AUC, in vitro versus in vivo data, or biopsy results versus microdialysis outcome. Moreover, in distinct investigations sample sizes were too low to allow for statistical comparison of data.

Consequently, 100-fold differences in reported fosfomycin concentrations in bone are not surprising, particularly when biopsy and microdialysis data are directly compared. This discrepancy is probably due to the differing methods applied in quantifying fosfomycin concentrations in bone. The elution of weighed and homogenized bone tissue biopsies might overestimate free fosfomycin concentrations as bone is made of microscopic crystals of hydroxylapatite attached to a matrix of collagen fibrils. There is circumstantial evidence that fosfomycin binds preferentially to these osseous matrices, which subsequently act as a drug depot from where fosfomycin is slowly released to infected sites. However, the nature and strength of this binding is not yet fully described. Furthermore, our data from the present experiment do not support this assumption.

For fosfomycin, the ‘time above the MIC’ [expressed as a percentage of the dosing interval (%T–MIC)] is the predominant PK–pharmacodynamic (PD) parameter showing a high predictive value of antimicrobial killing. According to experiments performed by Craig, it may be inferred that effective bacterial killing for ‘time-dependent’ antibiotics such as fosfomycin may be expected when the MIC for the pathogen is exceeded for at least 40% of the dosing interval. Considering the currently recommended dosing interval of 12 h, in all of our patients this criterion is met for bone for MICs of up to 32 mg/L. Thus, many clinically relevant susceptible strains including S. aureus are covered in all compartments investigated. Confirming this concept, a recent survey from the USA reported fosfomycin’s MIC50 and MIC90 for S. aureus to be 8 and 16 mg/L, respectively, regardless of the level of susceptibility to methicillin. In DFI, however, the possible spectrum of causative pathogens also comprises less susceptible staphylococci, enteric bacteria and P. aeruginosa, for which fosfomycin MICs of >32 mg/L were shown. Such cases may require optimization of fosfomycin’s dosing regimen, where the reduction of the dosing interval to 8 h may be an option. Alternatively, because of documented synergistic or additive effects the use of fosfomycin in combination with other antimicrobial agents, such as third-generation cephalosporins, penicillins, carbapenems, fluoroquinolones and others, may be considered. In addition, the combination of fosfomycin with another antimicrobial agent is considered to substantially reduce the likelihood of development of resistance to fosfomycin. However, this observation is based on experimental data only. Well-designed, controlled trials in humans demonstrating that such combinations are superior to fosfomycin monotherapy are currently not available in the scientific literature. Fosfomycin is naturally inactive against anaerobic bacteria such as Bacteroides spp., which do play a role in DFIs. This fact further supports combination therapy with fosfomycin.

In summary, considering PK–PD aspects, the currently approved intravenous dose of 100 mg/kg of fosfomycin per kg of BW 2–3 times a day appears to result in tissue concentrations that are sufficiently high to cover MRSA and other relevant aerobic bacterial pathogens in diabetic patients presenting with severe foot infection and osteomyelitis.

Funding

The present work was supported by an unrestricted grant from Sandoz GmbH, Vienna, Austria.

Transparency declarations

F. T., O. M. and M. P. are employees/consultants of J&P MEDICAL RESEARCH LTD, which is an independent research institute basically operating according to the Public-Private-Partnership concept. C. J. is managing director of J&P MEDICAL RESEARCH LTD, and owns 100% options. C. J. is also a consultant for pharmaceutical companies. All other authors declare having no relationship with companies that make products relevant to the manuscript and have no conflicts of interest with the present work.

References


577
of a single bolus intravenous, intramuscular and subcutaneous dose of  

Schintler et al.


