Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-associated ventriculitis

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Objective: The present study was performed to test the ability of fosfomycin to penetrate into the CSF of neurointensive care patients with ventriculostomy-associated ventriculitis.

Patients and methods: Six patients requiring neurointensive care monitoring, including extraventricular drainage due to secondary obstructive hydrocephalus, were enrolled into the study. All patients received 8 g of fosfomycin intravenously three times a day over a period of at least 5 days. Concentrations of fosfomycin in the CSF and plasma were measured after single-dose administration and at steady state.

Results: Mean values of the fosfomycin area under the time–concentration curves for the dosing interval of 8 h (AUC8) were 929 ± 280 and 225 ± 131 mg·h/L for plasma and CSF after single-dose administration, respectively (P < 0.03). The ratios of the AUC8 for CSF to the AUC8 for plasma were 0.23 ± 0.07 after a single dose and 0.27 ± 0.08 following multiple doses (P > 0.05, not significant). Additional in vitro experiments have shown that fosfomycin exerts non-concentration-dependent microbial growth inhibition. At steady state, the time above MIC (t > MIC) values were 98%, 92% and 61% for pathogens with MIC values of 8, 16 and 32 mg/L, respectively.

Conclusion: The present pharmacokinetic study indicates that 8 g of fosfomycin three times per day should provide sufficient antimicrobial concentrations in the CSF for the overall treatment period. Thus, the co-administration of fosfomycin could be useful for the treatment of ventriculitis caused by susceptible pathogens.

Keywords: CSF, intensive care, pharmacokinetics, humans

Introduction

Severe brain infections require immediate administration of antibiotics that effectively combat prevalent pathogens. However, in some cases empirical antibiotic therapy fails to kill the causative pathogen. This can be attributed to an inadequate choice of antimicrobial agent, impaired penetration of the drug into tissues, or to the development of bacterial resistance during antimicrobial therapy. In particular, impaired penetration of antibiotics into brain tissue and CSF has been observed in patients with extraventricular drainage (EVD) and presenting with ventriculitis.1

Fosfomycin is a bactericidal antimicrobial agent with high in vitro activity against a range of Gram-positive and distinct Gram-negative bacteria, such as Pseudomonas aeruginosa,2 Escherichia coli3 and other Enterobacteriaceae.4 Tissue concentrations of fosfomycin were identical to plasma levels in healthy volunteers,5 and differed only moderately in critically ill patients and diabetics.5,6 Thus, although there was almost complete plasma-to-tissue equilibration for tissues in these patients, we had no knowledge of concentrations of fosfomycin in the CSF of patients with ventriculitis. Previous pharmacokinetic (PK)/pharmacodynamic (PD) simulations have raised the speculation that optimal microbial killing by fosfomycin in tissues is
not necessarily linked to high \( C_{\text{min}} \) values, as fosfomycin exerts non-concentration-dependent microbial growth inhibition.\(^5,6\) These PK/PD characteristics of fosfomycin would, theoretically, compensate for impaired penetration of fosfomycin into brain tissues as long as concentrations in brain tissue and CSF exceed MIC values for causative pathogens for a distinct period.

Thus, the present study addressed the ability of fosfomycin to penetrate into the CSF of patients with EVD-associated ventriculitis after single-dose administration and at steady state. In addition, we aimed to show in vitro that fosfomycin exerts concentration-independent growth inhibition of a select model strain of methicillin-susceptible \textit{Staphylococcus aureus}.

Materials and methods

The study protocol was approved by the Ethics Committee of the University of Innsbruck Medical School and was performed in accordance with the Declaration of Helsinki (1964) in the revised version of 1996 (Somerset-West), the Guidelines of the International Conference of Harmonization, the Good Clinical Practice Guidelines and the Austrian drug law. The study met all criteria set by the local ethics committee for patients who were unable to give written consent because of incapacity or being comatose.

Patients

Six Caucasian patients (two women and four men) with severe haemorrhage infarction (\( n = 1 \)) or acute subarachnoid haemorrhage (\( n = 5 \)) requiring EVD due to obstructive hydrocephalus were treated with broad-spectrum intravenous antibiotic therapy. Patients’ demographic and laboratory data are shown in Tables 1 and 2. Bacterial ventriculitis was diagnosed based on the isolation of pathogens in the CSF, on inflammatory parameters in plasma, and elevated counts of leucocytes and protein levels in the CSF. Methicillin-susceptible \textit{Staphylococcus aureus} (MSSA) was identified in two patients, and methicillin-susceptible \textit{Staphylococcus epidermidis} (MSSE) was found in two other patients. No pathogen was isolated in the CSF of two patients (Table 2). In these two patients, ventriculitis was diagnosed based on a significant increase in granulocytes in the liquor, a decrease in the ratio of glucose in liquor to serum, an increase in protein concentration in liquor and positive MSSE blood cultures. Fosfomycin was administered at a dosage of 8 g three times a day. No interaction of fosfomycin with concomitantly administered drugs occurred. Each patient underwent common diagnostic and therapeutic intensive care measures.

Measurement of fosfomycin concentrations

Total fosfomycin levels in plasma and CSF were determined by a previously published but modified gas chromatography method.\(^7\) The coefficients of variation were 7.0% at 6 mg/L, 6.6% at 240 mg/L and 2.2% at 480 mg/L (\( n = 4 \)). The limit of detection was 1 mg/L. Intra-day and inter-day coefficients of variations were <7%.

Study protocol

Patients received 8.0 g of fosfomycin three times a day over a period of at least 5 days. Eight grams of fosfomycin dry powder (Fosfomycin ‘Biochemie’, 8.0 g, Trockenstechampulle, Biochemie, Vienna, Austria) was reconstituted with 200 mL of sterile water just before dosing and administered to patients. Study drug was administered over a period of \( \sim 30 \) min. The sampling interval was 1–2 h over a period of 8 h after the first drug administration of fosfomycin. Thereafter, the blood and CSF samples were collected at 2 h intervals. After 24 h of treatment, steady-state conditions of fosfomycin were reached and the sampling interval was extended to 4 h. Sampling of CSF and plasma was continued for up to 180 h after first drug administration. All samples were stored at \(-80^\circ\text{C} \) until analysis.

PK calculations

PK analysis was performed using the commercially available computer software Kinetica 3.0 (Innaphase Sarl, Paris, France). All parameters were calculated using non-compartmental analysis. Area under the concentration-time–curve (AUC) values for plasma and CSF were calculated from non-fitted data by employing the linear trapezoidal rule. The volume of drug distribution (\( V \)) and clearance (\( CL \)) were calculated for plasma by use of standard formulae as follows: \( V = \text{dose}/\text{AUC}_{\text{total}} \times \kappa_{\text{cl}} \); \( CL = \kappa_{\text{el}} \times V \); where \( \kappa_{\text{el}} \) represents the elimination rate constant. The half-life calculated for the terminal slope \( (t_{1/2}) \) was calculated by the equation \( t_{1/2} = \ln(2)/\beta \).

The following PK parameters were determined: AUC, maximum concentration \( (C_{\text{max}}) \) and time to maximum concentration \( (T_{\text{max}}) \). Main PK data are summarized in Table 3.

In vitro experiments

\textbf{Bacterial strain and antibiotic.} \textit{S. aureus} was obtained from the American Type Culture Collection (ATCC 29213). Between experiments, \textit{S. aureus} was stored in liquid nitrogen until use. Fosfomycin was purchased from Biochemie (Austria) and was stored, handled and prepared according to the manufacturer’s guidelines. In all experiments, glucose-6-phosphate (Boehringer Mannheim, Germany) was added at a concentration of 25 mg/mL according to the NCCLS guidelines.\(^10\)

\textbf{Susceptibility tests.} The MIC was determined by a two-fold serial Mueller–Hinton microdilution method, according to NCCLS criteria.\(^10\) \textit{S. aureus} was pre-cultured overnight on a Columbia agar plate (Columbia + 5% sheep blood; bioMérieux, France) and then introduced into Mueller–Hinton broth (MHB; Mikrobiologische Mueller–Hinton, Bouillon, Merck, Germany) at an initial inoculum of \( 5 \times 10^5 \text{cfu/mL} \). The lowest concentration of fosfomycin, which inhibited visible bacterial growth after 20 h of incubation at 37°C, was defined as the MIC value.

\textbf{Time–kill curves.} Bacterial growth inhibition curves were obtained by inoculating MHB with \( 5 \times 10^5 \text{cfu/mL} \) of \textit{S. aureus} ATCC 29213 at defined fosfomycin concentrations. Samples were drawn and bacteria counted at defined time-points over a period of 8 h. The bacterial growth inhibition period of 8 h was chosen because of the three-times daily dosing regimen of fosfomycin with a dosing interval of 8 h. After vortexing the culture tubes, two 50 \( \mu \)L samples were drawn and serially diluted with 0.9% sodium chloride. Twenty microlitres of each fraction was plated onto Columbia agar plates, which were incubated for 24 h at 37°C. Afterwards the colonies were counted and back-extrapolated to the original volume to determine the number of cfu/mL. Controls present bacterial growth in MHB when no antibiotic was added.

Statistical calculations

For statistical comparison of PK parameters, paired Wilcoxon tests were employed. All PK data are presented as means ± S.D. A two-sided \( P \) value <0.05 was considered significant.

Results

Patients

Six patients were enrolled in the study. Patient diagnosis on admission identified pathogens for EVD ventriculitis; the demographic and
mycin in the CSF (Figure 1) resulted from the flushing of the CSF somewhat from the time schedule. The individuals, sampling of specimens was incomplete or deviated. Specimens were collected at defined time-points. However, in some in vivo experiment completed the study and were included in safety analysis.

Laboratory data are presented in Tables 1 and 2. All patients completed the study and were included in safety analysis.

### In vivo experiment

Specimens were collected at defined time-points. However, in some individuals, sampling of specimens was incomplete or deviated somewhat from the time schedule. The ‘sawtooth’ levels of fosfomycin in the CSF (Figure 1) resulted from the flushing of the CSF drain every 2 h with ~3 mL of physiological saline solution. This was carried out to avoid blocking of the drainage system by blood cells. For this reason, 3 mL of CSF was drawn every 2 h and the drain was subsequently rinsed with saline solution. This procedure, however, is not routine in all intensive care units and concentrations of fosfomycin are expected to be ~15% higher in liquor if the EVD is not periodically flushed with saline solution.

The time-concentration courses of fosfomycin for plasma as well as for CSF were not significantly different after single and multiple doses (Figure 1; \( P > 0.12 \); not significant). Concentrations of fosfomycin in CSF were consistently lower than corresponding plasma concentrations following single- and multiple-dose administration (Table 3 and Figure 1; \( P < 0.03 \)). Steady-state conditions of fosfomycin were reached in plasma and CSF after administration of the third intravenous dose. The mean ratios of the AUC8 for CSF to the AUC8 for plasma were 0.23 \( \pm 0.07 \) and 0.27 \( \pm 0.08 \) after single and multiple doses, respectively (Table 3; \( P = 0.17 \), not significant). Mean \( C_{\text{max}} \) values were 260 \( \pm 85 \) mg/L and 43 \( \pm 20 \) mg/L for plasma and CSF, respectively, following single dose administration of 8 g of fosfomycin (\( P < 0.03 \)). After multiple doses of fosfomycin, mean \( C_{\text{max}} \) values were 307 \( \pm 101 \) mg/L for plasma and 62 \( \pm 38 \) mg/L for CSF (\( P < 0.03 \)). Main PK parameters of fosfomycin for plasma and CSF are presented in Table 3.

### Safety and tolerability

Study drug administration was well tolerated by all patients. No study drug-related severe adverse events were observed during the patients’ stay at the neurointensive care unit.

### Bacterial growth inhibition experiment

Bacterial growth inhibition of \( S. \) aureus ATCC 29213 by fosfomycin at different concentrations is presented in Figure 2. Initial inhibition of growth of \( S. \) aureus was observed for all fosfomycin concentrations used. Similar and effective bacterial growth inhibition was detected for all fosfomycin concentrations that were equal to or higher than the strain’s MIC value (2 mg/L), which indicates that fosfomycin exerts non-concentration-dependent antimicrobial effects.

At steady state, the calculated time above MIC (\( \tau > \text{MIC} \)) values for CSF were 98%, 92% and 61% for pathogens with MIC values of 8, 16 and 32 mg/L, respectively.

### Discussion

One of the most important mainstays in the therapy of severe brain infections is the administration of antimicrobial agents that are known to reach effective concentrations in the CSF and brain tissue. This issue becomes particularly important when looking at recent reports that have linked the development of bacterial resistance to

### Table 1. Demographic data are presented as means ± S.D.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female/male)</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 ± 8</td>
<td>53 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 ± 9</td>
<td>ND</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 1.6</td>
<td>ND</td>
</tr>
<tr>
<td>Leucocyte count (10⁹/L)</td>
<td>12.5 ± 2.4</td>
<td>9.1 ± 1.5</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>616 ± 72</td>
<td>470 ± 46</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.82 ± 0.24</td>
<td>0.82 ± 0.23</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>13.2 ± 4.5</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>Cell count in liquor/mm³</td>
<td>757 ± 421</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>Protein in liquor (g/L)</td>
<td>146 ± 35</td>
<td>64 ± 27</td>
</tr>
<tr>
<td>Glucose liquor/serum ratio</td>
<td>0.39 ± 0.08</td>
<td>0.77 ± 0.07</td>
</tr>
</tbody>
</table>

ND, not determined.

### Table 2. Patients’ characteristics and concomitant therapy

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Underlying disease</th>
<th>Pathogen</th>
<th>Antibiotic co-treatment</th>
<th>Haemodynamically active drugs</th>
<th>Duration of fosfomycin therapy (days)</th>
<th>Duration of ventriculitis (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>47</td>
<td>subarachnoid</td>
<td>MSSA</td>
<td>amikacin ceftazidime</td>
<td>dopamine, dobutamine, phenylephrine</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>61</td>
<td>subarachnoid</td>
<td>MSSSE</td>
<td>penicillin G clindamycin amikacin ceftazidime</td>
<td>dopamine, dobutamine, norepinephrine</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>50</td>
<td>subarachnoid</td>
<td>MSSSE</td>
<td>amikacin ceftazidime</td>
<td>dopamine, dobutamine, norepinephrine</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>63</td>
<td>subarachnoid</td>
<td>none</td>
<td>netilmicin ceftazidime</td>
<td>dopamine, phenylephrine</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>43</td>
<td>cerebral infarcture</td>
<td>none</td>
<td>penicillin G clindamycin ceftazidime</td>
<td>none</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>56</td>
<td>subarachnoid</td>
<td>MSSA</td>
<td>amikacin ceftazidime</td>
<td>none</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

*aSix patients were studied.
MSSA, methicillin-susceptible *Staphylococcus aureus*.
MSSE, methicillin-susceptible *Staphylococcus epidermidis*.
subinhibitory antibiotic concentrations at the target site. Against this background, we set out the present study and addressed the penetration properties of fosfomycin into the CSF in neurointensive care patients with EVD-associated ventriculitis.

In the present study, we found that fosfomycin exerts non-concentration-dependent bacterial growth inhibition of *S. aureus* (Figure 2). The antimicrobial effect of fosfomycin on Gram-negative bacteria has not been explored in the present study, but previous experiments from our group have shown that fosfomycin kills strains of *Enterobacter cloacae* and *Serratia marcescens* comparable with *S. aureus*. Thus, we expect that the antimicrobial effect of fosfomycin on susceptible Gram-negative bacteria is comparable with Gram-positive pathogens. Steady-state conditions were observed after the third intravenous dose of fosfomycin, but the plasma-to-CSF equilibration was not complete (Figure 1). Concentrations of fosfomycin in the CSF were ∼four-fold lower in comparison with plasma (P < 0.03). This is indicated by the ratio of the AUC8 for CSF to the AUC8 for plasma of 0.23 ± 0.07 and 0.27 ± 0.08 after single and multiple dose administration, respectively. Peak concentrations of fosfomycin increased by ∼18%–40% in plasma and CSF at steady state compared with single-dose administration, although the limited number of patients and the high inter-individual variability did not allow for statistical confirmation of this trend (P not significant).

This important finding confirms previous data derived from patients with brain infections. Thus, there is evidence that penetration of fosfomycin into the CSF is restricted. This, most probably, is due to the hydrophilic character of fosfomycin, the partially intact blood–brain barrier and the presence of transport pumps, such as P-glycoprotein that actively act against plasma-to-tissue equilibration.

Our observation of incomplete fosfomycin plasma-to-CSF equilibration might be of clinical relevance because β-lactams and fosfomycin exert optimal bacterial killing when the time above the MIC (> MIC) of the respective target is optimized (Figure 2). Exceeding the MIC over a dosing interval of 60%–70% (> MIC) is considered the lower threshold value for favourable clinical and bacteriological outcome. Several recent clinical studies suggested that > MIC should almost cover 100% of the dosing interval for ‘difficult-to-treat’ pathogens, although studies in animal infection models have found that > MIC does not necessarily need to reach this value to exert a significant antimicrobial effect, providing that unbound drug levels are used for these calculations. Thus, at steady state, fosfomycin appears to be highly effective when considering calculated > MIC values of unbound fosfomycin in the CSF as values of 98%, 92% and 61% will be reached for bacteria with MIC values of 8, 16 and 32 mg/L, respectively. These MIC values do not include *S. aureus* (MRSA), *MSSA*, *Streptococcus* species and *Enterobacteriaceae*. Nevertheless, it is recommended to administer fosfomycin in combination with other classes of antimicrobial agents to improve antimicrobial activity and to avoid the development of resistance. Cephalosporins as a class have been suggested for combined use with fosfomycin because of their similar tissue and plasma PK profiles in healthy volunteers and critically ill patients. The antimicrobial efficacy of the simultaneous administration of cephalosporins with fosfomycin versus monotherapy with fosfomycin to intensive care patients has currently been demonstrated in vitro.

One might expect the ability of hydrophilic compounds such as fosfomycin—and the class of cephalosporins—to penetrate the CSF

![Figure 1. Time-concentration profiles of fosfomycin for plasma (open circles) and CSF (filled squares) after single and multiple intravenous doses of 8 g over 30 min in neurointensive care patients (*n* = 6). Each arrow indicates intravenous administration of fosfomycin. The solid horizontal lines represent MIC values for pathogens. Data are shown as means ± s.d.](image_url)

**Table 3. Main PK parameters calculated for the study population (*n* = 6)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Single dose</th>
<th></th>
<th>Multiple doses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plasma</td>
<td>CSF</td>
<td>plasma</td>
<td>CSF</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;CSF&lt;/sub&gt;/AUC&lt;sub&gt;plasma&lt;/sub&gt;</td>
<td>0.23 ± 0.07</td>
<td>0.27 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;8&lt;/sub&gt; (mg·h/L)</td>
<td>929 ± 280&lt;sup&gt;a&lt;/sup&gt;</td>
<td>225 ± 131</td>
<td>1035 ± 383&lt;sup&gt;b&lt;/sup&gt;</td>
<td>295 ± 179</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>260 ± 85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43 ± 20</td>
<td>307 ± 101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 ± 38</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 1.8</td>
<td>1.5 ± 1.0</td>
<td>4.5 ± 2.7</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2p&lt;/sub&gt; (h)</td>
<td>3.0 ± 1.0</td>
<td>ND</td>
<td>4.0 ± 0.5</td>
<td>ND</td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>7.4 ± 2.3</td>
<td>ND</td>
<td>5.0 ± 2.0</td>
<td>ND</td>
</tr>
<tr>
<td>V (L)</td>
<td>30.8 ± 10.2</td>
<td>ND</td>
<td>26.3 ± 9.7</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are presented as means ± s.d.

<sup>a</sup>P < 0.05 compared with CSF single dose.

<sup>b</sup>P < 0.05 compared with CSF multiple dose.

C<sub>max</sub>, the maximum concentration of fosfomycin; T<sub>max</sub>, the time to reach C<sub>max</sub>; t<sub>1/2p</sub>, the terminal elimination half-life; AUC, area under the concentration–time curve; CL, total body clearance; V, apparent volume of distribution; ND, not determined.
infections in neurointensive care patients with ventriculostomies. Thus, CSF pharmacokinetics of fosfomycin indicate that effective concentrations of fosfomycin can be demonstrated in the CSF, covering a range of clinically relevant bacteria, associated with MSSE, effective concentrations of fosfomycin can be demonstrated in the CSF, covering a range of clinically relevant bacteria, including MRSA, MSSA, Streptococcus species and Enterobacteriaceae. Nevertheless, fosfomycin should be administered in combination with other classes of antimicrobial agents to avoid the development of bacterial resistance.

In conclusion, the present study has shown that, with the exception of MSSE, effective concentrations of fosfomycin can be demonstrated in the CSF, covering a range of clinically relevant bacteria, including MRSA, MSSA, Streptococcus species and Enterobacteriaceae. Thus, CSF pharmacokinetics of fosfomycin indicate that fosfomycin might qualify for the management of severe brain infections in neurointensive care patients with ventriculostomy-associated ventriculitis. Nevertheless, fosfomycin should be administered in combination with other classes of antimicrobial agents to avoid the development of bacterial resistance.

References


Figure 2. Growth inhibition versus time curves of a select S. aureus ATCC 29213 strain, which was exposed to different fosfomycin concentrations over a period of 8 h.