Cerebrospinal fluid impairs antimicrobial activity of fosfomycin in vitro

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Received 4 March 2009; returned 20 May 2009; revised 29 May 2009; accepted 29 June 2009

Objectives: Fosfomycin penetrates well into cerebrospinal fluid (CSF) and is considered for treatment of infections of the central nervous system (CNS). This study evaluated the influence of human CSF on the antimicrobial activity of fosfomycin.

Methods: Time–kill curves were performed in Mueller–Hinton broth (MHB) and in pooled human CSF using fosfomycin concentrations ranging from 0.25× to 8× MIC for a clinical Staphylococcus aureus isolate. To estimate the activity of fosfomycin at the target site, the concentration–time curve measured in CSF of a patient at steady state was simulated in vitro in human CSF using two S. aureus isolates.

Results: In CSF a higher fosfomycin concentration (8× MIC) was required to achieve sustained bacterial killing than in MHB (1× MIC). In vitro simulation of the pharmacokinetic profile measured in CSF of the selected patient showed initial killing, but terminal re-growth of both test strains.

Conclusions: The antibacterial activity of fosfomycin is lower in CSF than in MHB, and drug concentrations slightly exceeding the MIC may not be sufficient to achieve bactericidal effects in the CNS.

Keywords: CSF, time–kill curves, PK/PD simulation, pH, CO2

Introduction

While numerous studies have identified antibiotics that penetrate the blood–brain barrier little attention has been directed to the influence of cerebrospinal fluid (CSF) on antimicrobial activity. However, relating antibiotic CSF concentrations to the MICs determined in broth may be misleading because CSF can affect bacterial growth and killing.1,2

Fosfomycin has a relatively low molecular mass (138.1) and acts by inhibiting the UDP-N-acetylgulcosamine-enolpyruvyl-transferase required for the synthesis of murein for the bacterial cell wall. The fosfomycin concentrations measured in CSF of patients with extraventricular drainage-associated infections at steady state (mean Cmax 62 ± 38 mg/L) were considered sufficient for therapy of common CSF infections.3 To test this assumption we evaluated the influence of human CSF on the activity of fosfomycin in vitro.

Methods

Media and strains

Remnants of ~300 CSF samples from patients who did not receive antibiotics were collected, pooled and sterile-filtered. Mueller–Hinton broth (MHB) was supplemented with glucose-6-phosphate (G-6-P). A clinical isolate of Staphylococcus aureus (SA 16) obtained from a blood culture at an internal medicine ward was chosen because it had the highest MIC still classified as susceptible by the CLSI (16 mg/L, ‘worst case’). Besides SA 16, the S. aureus ATTC 29213 isolate with an MIC of 2 mg/L (SA 2) was used for...
the pharmacokinetic/pharmacodynamic (PK/PD) simulation, representing a previously reported MIC50 value.4

Static time–kill curves

Bacterial time–kill curves were performed in MHB, in CSF in ambient air and in CSF in air with 5% CO2 using fosfomycin concentrations below and above the MIC. CO2 served to maintain physiological pH in CSF.2,5

PK/PD simulation

To estimate bacterial killing at the target site in the worst case, the PK profile of a ventriculitis patient with the lowest drug concentrations in CSF at steady state in a previous study (broken line in Figure 1c) was simulated.3 In the CSF of this patient the AUC0–8 was 112 mg·h/L and the half-life was 8.7 h after multiple doses of 8 g of fosfomycin every 8 h. Test strains were exposed to the selected PK profile in vitro in CSF in air with 5% CO2. Rising drug concentrations were obtained by addition of fosfomycin, and falling concentrations by addition of CSF. Bacterial counts were determined over time and back-extrapolated to the original volume to account for the respective dilution.

Results and discussion

The media used had a major influence on bacterial growth and killing of fosfomycin. In MHB sustained bactericidal effects were achieved at 1× MIC (Figure 1a). In CSF with 5% CO2 killing occurred between baseline and 8 h at fosfomycin concentrations of 1× MIC or higher, but bacterial re-growth was detected at 1× to 4× MIC after 24 h (Figure 1b). To achieve sustained killing 8× MIC was required in CSF with 5% CO2. In CSF without CO2 even at 8× MIC fosfomycin had no relevant antimicrobial effect because its activity is impaired at higher pH (not shown).

When the concentration–time curve of the selected patient was simulated in vitro in CSF fosfomycin was bactericidal to

Figure 1. Bacterial killing of S. aureus (SA 16) by fosfomycin at various concentrations (n=3) in MHB (a), and in human CSF incubated in air with 5% CO2 (b). (c) PK/PD simulation with growth inhibition curves (n=5) of S. aureus isolates SA 16 (MIC 16 mg/L) and SA 2 (MIC 2 mg/L) in CSF with 5% CO2. Isolates were exposed in vitro to the fosfomycin concentration–time curve (broken line) measured at steady state in CSF of a selected patient with extraventricular drainage-associated infection. Means ± SD.
SA 2 during the first hours of the experiment (Figure 1c). After 24 h marked re-growth was detected although the fosfomycin concentrations fluctuated between 5 and 16 MIC. Both bacterial killing and growth of SA 16 were marginal compared with SA 2. This is comprehensible because the simulated fosfomycin concentrations corresponded to 0.7 to 2 MIC, a range that was also insufficient in the static time–kill curves in CSF.

Thus, time–kill curves and PK/PD simulations suggest that the fosfomycin concentrations in CSF must exceed at least 8 MIC during the entire dosing interval to obtain a lasting reduction of S. aureus. This is in agreement with previous studies reporting that fosfomycin monotherapy is effective in rabbit meningitis caused by Streptococcus pneumoniae, only if a minimum concentration of 8 to 10 MIC is constantly achieved at the target site.6,7 The analogous results of the rabbit studies with our in vitro experiments suggest that bacterial time–kill curves may predict the magnitude of antibiotic concentrations needed in vivo in the CNS if they are performed in pH-adjusted CSF.

Lower growth rates may be responsible for reduced antimicrobial activity in CSF because fosfomycin inhibits synthesis of the bacterial cell wall, and its activity was assumed to be growth and time dependent, rather than concentration dependent.5,8 Also the lack of G-6-P may explain reduced activity, but as G-6-P is not detectable in human CSF it was not added in the experiment.9

Considering the fosfomycin concentrations reported for human CSF at steady state,3 CNS infections caused by strains for which the MIC is ≥4 mg/L may hardly be treated with fosfomycin. Anyway, fosfomycin is combined with other antimicrobial agents in clinical practice to avoid emergence of resistant isolates. In experimental meningitis fosfomycin enhanced the efficacy of vancomycin or ceftriaxone.3

Among other limitations, the present study investigated neither changing nutrient and G-6-P concentrations in CSF, nor various bacterial species and combinations of fosfomycin with other antibiotics.

Summarizing, the activity of fosfomycin against S. aureus is notably reduced in human CSF, and drug concentrations constantly exceeding a multiple of the MIC are required to achieve sustained antimicrobial effects.

Acknowledgements

We thank Christian Joukhadar for providing pharmacokinetic data, and Johann Hausdorfer for his practical support during the study.

Fosfomycin in CSF

Funding

This project was funded by the Department of Clinical Pharmacology of the Medical University of Vienna. Consumable supplies were in part provided by the Innsbruck Medical University.

Transparency declarations

None to declare.

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