Antimicrobial activity of cefepime and rifampicin in cerebrospinal fluid in vitro

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Objectives: Though used for infections of the central nervous system, the pharmacodynamics of antimicrobial agents is commonly evaluated only in commercially available bacterial growth media. In the present study, the effects of cerebrospinal fluid (CSF) on bacterial killing by cefepime and rifampicin were investigated.

Methods: CSF was collected from patients who did not receive antibiotics. Time–kill curves were performed over 24 h using drug concentrations of 0.25-, 0.5-, 1-, 2-, 4- and 8-fold the respective MIC for the Staphylococcus aureus test strain. Killing curves were performed in Mueller–Hinton broth (MHB), in CSF incubated in ambient air (CSFAIR) and in CSF in air with 5% CO2 (CSFCO2). CO2 served to adjust the pH of CSF to physiological values.

Results: Sustained bacterial killing was achieved by cefepime at lower drug concentrations in CSFCO2 than in MHB. In contrast, rifampicin concentrations above the MIC were required to exert sustained killing in CSFCO2. Both drugs were least effective in CSFAIR.

Conclusions: Standard susceptibility tests may lead to over- or underestimation of the activity of distinct antibiotics in CSF. Evaluation of the antimicrobial activity in pH-adjusted CSF can provide useful information on drugs considered for the treatment of bacterial infections residing in CSF.

Keywords: bacterial killing, CSF, pH, CO2

Introduction

In the treatment of infections of the central nervous system (CNS), it is important to reach adequate antibiotic concentrations in cerebrospinal fluid (CSF). The levels of various antibiotics in CSF have been measured to determine their eligibility to treat CNS infections such as shunt-associated ventriculitis or meningitis. In order to estimate the efficacy of these drugs in vivo, the pharmacokinetic data obtained in CSF have been related to the MICs for relevant pathogens determined in vitro in Mueller–Hinton broth (MHB). However, the use of susceptibility data determined in MHB could be inappropriate for bacterial infections residing in CSF because it has been reported that CSF may affect bacterial growth, bacterial killing and the post-antibiotic effect of antibiotics.

The present study set out to determine the influence of CSF on bacterial killing of Staphylococcus aureus by cefepime and rifampicin. These drugs were chosen for this in vitro study because they have documented good penetration into CSF and they represent different antibiotic classes.

Methods

Media

MHB (Merck, Darmstadt, Germany) was used as the reference medium in ambient air. During routine investigations, over 200 CSF samples were collected from patients who did not receive antibiotics. The CSF remnants were stored at −80°C. Samples with visible contamination and samples showing antimicrobial activity in a bioassay with Bacillus subtilis ATCC 6633 were excluded. The CSF samples were pooled and sterile-filtered before use. Experiments were performed in ambient air (CSFAIR) and in an atmosphere containing 5% CO2 (CSFCO2) to adjust pH to...
physiological values. Indicator strips (Merck) were used to monitor the pH of CSF. Concentrations of protein, glucose, sodium and potassium were determined in CSF before and after experimental use.

Bacterial test strain

*S. aureus* (ATCC 29213) was used because this species frequently causes shunt-associated ventriculitis. MICs of rifampicin (Aventis, Vienna, Austria) and cefepime (Bristol–Myers Squibb, Vienna, Austria) were determined by the microdilution method.

Time–kill curves

Bacterial killing curves were performed by inoculating the test strain in 1 mL of MHB, CSF<sub>AIR</sub> or CSF<sub>CO2</sub> to achieve \( \sim 8 \times 10^5 \) cfu/mL. After 40–60 min of incubation, the antibiotic was added at concentrations representing 0.25-, 0.5-, 1-, 2-, 4- and 8-fold the respective MIC. At baseline, 4, 8 and 24 h after the addition of the antibiotic, bacterial counts were determined. Each experiment was performed in triplicate and included a growth control.

Results

**pH-monitoring of CSF**

Throughout the experiments, pH continuously showed values of 9–10 in CSF<sub>AIR</sub> and values of 7–8 in CSF<sub>CO2</sub>.

**Nutrients in CSF**

Pooled CSF from one growth control tube was subjected to laboratory analysis before use and 24 h after the start of the experiment. While there was a notable reduction of glucose in CSF from 45 mg/dL before use to 24 mg/dL after 24 h, electrolytes and protein levels remained relatively constant over time.

**Time–kill curves**

Killing of *S. aureus* by cefepime and rifampicin was tested in MHB, CSF<sub>AIR</sub> and CSF<sub>CO2</sub> at concentrations ranging from 0.25- to 8-fold the MIC (Figures 1 and 2). The media used had a major influence on bacterial killing. Cefepime was bactericidal at a lower concentration in CSF<sub>CO2</sub> (lowest concentration, 0.5 × MIC) than in MHB and CSF<sub>AIR</sub> (lowest concentrations, 2 × MIC and 4 × MIC, respectively). In contrast, rifampicin was bactericidal at a lower concentration in MHB (lowest concentration, 1 × MIC) than in CSF<sub>CO2</sub> (lowest concentration, 2 × MIC) and CSF<sub>AIR</sub> (no bactericidal activity at any of the concentrations tested).

However, if killing by cefepime occurred, it was more rapid in MHB than in CSF<sub>CO2</sub> (\( \sim 10\)-fold lower bacterial counts in MHB than in CSF<sub>CO2</sub> after 8 h), whereas rifampicin tended to kill more rapidly in CSF<sub>CO2</sub> than in MHB.

Discussion

Although several studies have estimated antibiotic CSF penetration, only a few studies have attempted to assess how human CSF influences the antimicrobial activity of antibiotics compared with conventional growth media.\(^2,7,8\)

The present experiments confirmed that the medium used has a profound influence on both bacterial growth and the killing of
Effect of CSF on antibiotic activity

*S. aureus*. Bacterial growth in CSF was slower and less pronounced than in MHB when no antibiotics were added, resulting in bacterial counts that were ~10-fold lower in CSF than in MHB after overnight incubation. This might be attributed to the lower content of nutrients such as glucose or select electrolytes in CSF.

The antimicrobial activity of both antibiotics was strongly reduced in CSF if incubated in ambient air, but not in an atmosphere containing 5% CO2. It is known that the antibiotic activity of cefepime and rifampicin is reduced at higher pH values. Presumably, the reduced antimicrobial activity in CSF is related to the fact that CSF is barely buffered and that the pH of CSF decreases rapidly in ambient air to values of 9–10 by loss of CO2. The fact that the almost physiological pH of 7–8 can be maintained in CSF by incubation in an atmosphere containing CO2 probably explains why bacterial killing was higher in CSF than in MHB.

Owing to these findings, we conclude that only the results obtained in CSF with a pH of 7–8 achieved by 5% CO2 incubation are most relevant because this setting reflects the conditions in vivo, whereas microbiological results obtained in CSF alone are not representative. This implies that previous findings postulating distinct effects of CSF on antibiotic activity are doubtful if the results were from studies undertaken in ambient air.

In addition, Cunniffe et al. recommended the adjustment of pH to physiological values by incubation in air with 5% CO2 when working with CSF. This measure raises detection rates of bacterial pathogens in routine cultures by avoiding oxidative stress on bacterial cells. For similar reasons, Wilcox et al. recommended an atmosphere with 5% CO2 for microbiological studies with peritoneal dialysis fluid.

Since the activity of cefepime is ‘growth-dependent’, better activity might be expected in MHB, where bacterial growth is higher than in CSFCO2 (Figure 1). Surprisingly, in spite of slower bacterial growth in CSFCO2, the cefepime concentration required to achieve bactericidal effects was ~4-fold (two log2) lower in CSFCO2 than in MHB. However, if killing occurred, the reduction of bacterial counts by cefepime was more rapid in MHB than in CSFCO2. We speculate that killing by cefepime is faster in MHB than in CSF due to the higher bacterial growth rates, as can be expected for a ‘growth-dependent’ antibiotic. Yet, due to the lower nutrient concentrations, bacterial cells may be more vulnerable in CSFCO2, allowing cefepime concentrations below the MIC to be bactericidal in CSFCO2.

Interestingly, the killing characteristics of ‘growth-independent’ rifampicin were the converse of those of cefepime. Killing by rifampicin tended to be faster in CSFCO2 than in MHB, whereas a slightly (one log2) higher drug concentration was required in CSFCO2 than in MHB to achieve sustained killing (Figure 2). Since growth is not a prerequisite for the activity of rifampicin, the weakness of the bacteria in nutrient-poor CSFCO2 may have enhanced the velocity of killing.

Most importantly, this study suggests that the clinical efficacy of antibiotics in CSF might be over- or underestimated, if based only on MIC values determined in MHB. Although the present tests were limited to two drugs, we propose that the activity of antibiotics that are considered for CNS infections should also be evaluated in vitro in pH-adjusted human CSF. Although the results of this in vitro study cannot be directly applied to in vivo conditions, the results of such tests may be helpful to confirm or reject certain treatments. For example, in the present case, the indication of cefepime for the treatment of CSF infections caused by *S. aureus* may be confirmed by its good antibacterial activity in CSFCO2 at low concentrations. In addition, the quality and the power of in vitro pharmacokinetic/pharmacodynamic simulations can be improved by using the appropriate target medium, i.e. human CSF.

The limitations of this study have to be considered. Owing to the limited availability of human CSF, it was not possible to test further bacterial species or antimicrobial agents. Loss of nutrients in CSF with sinking glucose levels over time in vitro may be less pronounced in vivo in individual patients due to turnover and regeneration of CSF. On the other hand, reduced glucose levels are also a typical finding of bacterial infections located in CSF in vivo, and therefore, the consumption of glucose in vitro might be interpreted as a minor limitation. However, severe bacterial CNS infections are often associated with a range of further metabolic changes, such as an increase in protein and white blood cell counts in CSF. Such conditions may additionally modify the activity of antimicrobial agents, especially of compounds with high protein binding.

In summary, bacterial killing by antimicrobial drugs can notably diverge in pH-adjusted human CSF compared with standard microbiological media. Hence, the use of MIC values determined in MHB alone might lead to over- or underestimation of the antibiotic activity at the target site. We conclude that testing the antimicrobial activity in pH-adjusted human CSF in vitro might deliver useful information about agents considered for the treatment of CNS infections.

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Transparency declarations

None to declare.

References


