Evaluation of fusidic acid in therapy of experimental *Staphylococcus aureus* meningitis

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Objectives: Combination therapy that includes fusidic acid, an antimicrobial agent highly active against staphylococci, has been recommended in the treatment of patients with *Staphylococcus aureus* meningitis. The aim of this study was to evaluate the pharmacokinetic, CSF bactericidal and anti-inflammatory properties of fusidic acid.

Methods: The pharmacokinetics, treatment efficacy and parameters of the meningeal inflammatory response were studied in rabbits, using an experimental meningitis model against *S. aureus* (MICs of fusidic acid and methicillin were 0.125 and 1 mg/L, respectively).

Results: Fusidic acid entered the CSF, with peak values within 0.5–1 h of the intravenous bolus injection/infusion and with a percentage penetration (AUC<sub>CSF/AUC<sub>serum</sub></sub>) into uninfected and purulent CSF of 1.9% ± 0.7 and 4.5% ± 0.7, respectively. Rabbits treated with antibiotics [fusidic acid 80 mg/kg/6 h (n = 6), methicillin 80 mg/kg/3 h (n = 7) and the two combined (n = 6)] had significantly higher bacterial kill rates than untreated controls (n = 6, P < 0.05). Combination therapy was less effective, with significantly less killing after 6 h of treatment than methicillin alone (P < 0.05). CSF white blood cells and CSF levels of interleukin-8 (IL-8), glucose, lactate and protein were altered during staphylococcal meningitis, but with no significant difference between antibiotic-treated and untreated rabbits.

Conclusions: Antagonism between methicillin and fusidic acid was observed in staphylococcal meningitis.

Keywords: fusidic acid, *Staphylococcus aureus*, meningitis, methicillin, antagonism

Introduction

Although *Staphylococcus aureus* meningitis is a severe disease with high mortality, it is rare and few clinical treatment studies are available. One such study, which included 104 consecutive cases with *S. aureus* meningitis, showed that patients who were treated with fusidic acid in combination with another antistaphylococcal agent (in most cases methicillin) had a better outcome than patients receiving any other recommended treatment regimen, including treatment with methicillin alone.1

Fusidic acid is an antimicrobial agent with high *in vitro* activity against staphylococci, including methicillin-resistant *S. aureus*.2 That fusidic acid, in combination with methicillin in *S. aureus* meningitis, is possibly superior, may be the consequence of the synergic CSF bactericidal effect of fusidic acid. Another benefit of fusidic acid could be its high penetration and accumulation within the central nervous system, as previously documented in other body fluids/tissues.3 In addition, the anti-inflammatory and immunosuppressive effects of fusidic acid may reduce meningeal inflammation.

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and thereby improve outcome. However, experimental documentation for these hypotheses is not available.

In the present study, we investigated the pharmacokinetic and treatment efficacy of fusidic acid alone and in combination with methicillin, using an experimental staphylococcal meningitis model in rabbits. In addition, we report data on meningeal inflammation during antibiotic treatment of experimental staphylococcal meningitis.

Materials and methods

Bacteria

*S. aureus* E2371 (fusidic acid MIC 0.125 mg/L; methicillin MIC 1 mg/L), isolated from a blood culture, was used in all experiments.

Antimicrobial agents

Sodium fucidate (fusidic acid, Fucidin) and methicillin (Lucopenin) were obtained from Leo Pharmaceutical Products, Ballerup, Denmark, and DuraScan Medical Products, Odense, Denmark, respectively.

Time–kill experiments

*S. aureus* [∼10^5 cfu/mL in MHB (Statens Serum Institut, Copenhagen, Denmark)] was challenged with fusidic acid or methicillin at two-fold dilutions from 64.0 to 0.5 × MIC, or with methicillin at concentrations of 0–32 × MIC, together with fusidic acid at a concentration of 16 × MIC. Bacterial counts were determined after –1, 0, 1, 3, 6 and 24 h. Time–kill experiments were performed in triplicate. Lower detection levels were 10 cfu/mL.

Determination of fusidic acid concentrations in CSF and serum

The concentration of fusidic acid in CSF and serum samples was determined by bioassays. Lower detection limits were 0.2 and 3.7 mg/L for fusidic acid in CSF and serum, respectively. The inter- and intra-assay coefficients of variation for the standard concentrations tested were <7% and <1.5%, respectively.

Determination of bacterial concentration

Bacterial concentrations were determined in CSF and blood by plating 50 µL of undiluted and 10-fold serial dilutions on 5% blood agar plates (Statens Serum Institut). The lowest detectable bacterial counts were 20 cfu/mL. Comparison of the bacterial counts in different dilutions of CSF was performed to exclude significant carryover phenomena.

The rabbit meningitis model

A rabbit meningitis model, previously described for pneumococci, was modified for the use of staphylococci. Rabbits were inoculated intracisternally with 1 × 10^7 cfu of *S. aureus*, and antibiotic therapy was started 6 h after the bacterial challenge. After testing for bacterial concentration, CSF and blood samples were centrifuged at 3000 g for 10 min and the supernatants stored at –80°C for subsequent analysis.

CSF bactericidal effect of antibiotics in staphylococcal meningitis. Rabbits were treated with fusidic acid 80 mg/kg (n = 6), methicillin 80 mg/kg (n = 7) and the two combined (n = 6). Six untreated rabbits served as a control group. Fusidic acid was given at 6 and 12 h after the bacterial challenge, as an intravenous infusion over 2 h. Methicillin was administered intravenously over 10–15 min at 6, 9 and 12 h after the bacterial inoculation. Bacterial concentrations in CSF and blood samples were determined at 0, 3, 6, 9, 12 and 15 h after the bacterial inoculation.

Indices of meningeal inflammation

CSF samples, taken at 0, 3, 6, 9, 12 and 15 h after the bacterial inoculation, were analysed for white blood cell (WBC), interleukin-8 (IL-8) protein, lactate and glucose concentrations, according to methods described previously.

Statistical analysis

All data are presented as medians and 25/75 percentiles, except pharmacokinetic parameters, which are provided as means ± S.D. Pharmacokinetic parameters were calculated using the GraphPad Prism program. The Mann–Whitney test was used to compare two groups. For comparisons between more than two groups, the Kruskal–Wallis test was used. When tests were significantly different, Dunn’s multiple comparison test was performed to compensate for multiple comparisons. The synergism of two drugs was defined as a statistically significant increase in efficacy compared with the most effective drug alone. Antagonism was defined when the efficacy of two drugs had decreased statistically significantly, compared with the most effective drug alone. Comparison of paired data was performed by the Wilcoxon signed rank test. P < 0.05 was considered significant.
Fusidic acid against *Staphylococcus aureus* meningitis

**Results**

*Antibiotic efficacy in vitro and in vivo*

In time–kill studies, a bacteriostatic effect was observed at fusidic acid concentrations $>8 \times$ MIC, whereas no inhibition of bacterial growth was seen at concentrations below this (data not shown). Methicillin alone showed time-dependent killing with no additional efficacy at concentrations $>4 \times$ MIC, whereas compared with methicillin alone, the combination with fusidic acid induced significantly less killing of *S. aureus* (Wilcoxon test, $P < 0.05$, Figure 1a). In the meningitis model, rabbits treated with antibiotics had significantly higher reductions in CSF bacterial concentration at all time points (3, 6 and 9 h) after start of therapy than untreated rabbits (Mann–Whitney test, $P < 0.05$, see Figure 1b). Combination therapy with fusidic acid and methicillin was less effective in reducing CSF bacterial concentrations than therapy with methicillin alone at all time points, reaching statistical significance 6 h after start of therapy $[-3.0 \text{Δlog}_{10} \text{cfu/mL} (-1.2 \text{ to } -3.6)$ versus $-1.0 \text{Δlog}_{10} \text{cfu/mL} (-0.3 \text{ to } -1.9)$, respectively, Dunn’s multiple comparison test, $P < 0.05$, see Figure 1b].

*Indices of meningeal inflammation*

Within 6 h of bacterial inoculation, CSF levels of WBCs, IL-8, protein (data not shown) and lactate (data not shown) had increased, whereas there was no significant alteration in CSF glucose levels (see Figure 2). During 9 h of antibiotic treatment, CSF levels of WBCs, protein and lactate remained elevated, whereas IL-8 levels were normal at the end of the study. A decrease in CSF glucose levels was observed in rabbits treated with the fusidic acid and methicillin combination. No significant difference was observed in any of the CSF biochemical parameters above, between untreated and treated rabbits, or among the three groups treated with antibiotics. However, there was a significantly lower CSF glucose concentration in rabbits treated with the combination than in rabbits treated with methicillin alone at 12 and 15 h (Dunn’s multiple comparison test, $P < 0.05$, see Figure 2).

*Pharmacokinetics of fusidic acid*

$C_{\text{max}}$ of fusidic acid into inflamed and uninflamed CSF was observed within 0.5–1 h after dosing. After a 15 min bolus injection, $C_{\text{max}}$ was higher (6.0 mg/L ± 1.5 versus 2.7 mg/L ± 1.2), $t_{1/2}$ was longer (83.0 min ± 20.3 versus 70.0 min ± 7.6), and the CSF penetration (AUC<sub>CSF</sub>/AUC<sub>serum</sub>) higher (4.5% ± 0.7 versus 1.9% ± 0.7) with inflamed than with uninflamed meninges, respectively. The CSF concentration of fusidic acid remained above the MIC during the 6 h study period.

**Discussion**

We found that fusidic acid and methicillin combined had an antagonistic CSF bactericidal effect, compared with therapy with methicillin alone, in experimental *S. aureus* meningitis. Therapy with bacteriostatic and bactericidal drugs combined...
has resulted previously in a high mortality rate in patients with pneumococcal meningitis. It is well-known that β-lactams such as methicillin express their bactericidal activity only on growing bacteria, whereas fusidic acid expresses bacteriostatic activity by inhibiting bacterial growth. Therefore, it is not surprising that the antagonistic CSF bactericidal effect of therapy with fusidic acid and methicillin combined was confirmed in time–kill experiments. But whether the statistically significant antagonism documented in vivo and in vitro is of clinical importance remains to be defined, since our meningitis model is not designed for the evaluation of long-term adverse outcome (e.g. mortality, neurological sequelae).

To our knowledge, only one other study has previously evaluated combination therapy with fusidic acid in vivo. In contrast to our findings, Fantin et al., using a rabbit S. aureus endocarditis model, found indifferent in vivo activity after 4 days of therapy with fusidic acid when combined with vancomycin, despite the observation of an antagonistic effect in time–kill studies. We have no explanation for this, except that it could be the result of differences in animal models, bacterial strains or antibiotics studied.

In vitro studies have shown previously that fusidic acid has immunomodulating effects similar to cyclosporin A (e.g. attenuates the release of IL-1 from activated mononuclear cells). Moreover, fusidic acid could have a beneficial role in infectious diseases, since treatment with fusidic acid improves survival in animal models, such as lipopoly saccharide-induced septic shock and septic shock due to acute necrotizing pancreatitis. In these studies, fusidic acid attenuated systemic levels of the proinflammatory cytokines, tumour necrosis factor-α and IL-8. In staphylococcal meningitis, we found no significant alteration in the meningeal inflammatory response during therapy with fusidic acid, except for a higher decrease in CSF glucose levels during combination therapy. Thus, treatment with fusidic acid may not influence local levels of inflammatory parameters.

Previous studies have shown that fusidic acid penetrates well into other body fluids/tissues (with corresponding fluid/tissue levels relative to serum of 23–83%). The penetration of fusidic acid into the CSF (AUC CSF/serum ratios) was poor, with values of ~4.5% into inflamed meninges and ~2% into uninflamed meninges. There are no reports in meningitis patients of CSF penetration by fusidic acid; CSF penetration was lower than brain tissue penetration in a limited number of non-meningitis patients undergoing surgery for brain tumour (~0–0.5% versus 1–10%). We found that the brain tissue concentration of fusidic acid was higher than the corresponding CSF levels (data not shown). However, this was probably the result of blood contamination, since measurement of haemoglobin content showed that brain tissue consisted of ~33% blood (data not shown). Thus, we believe that treatment of meningitis patients with the recommended dose of fusidic acid (~8 mg/kg), which is 10 times lower than the dose used here, will not result in CSF concentrations significantly above the MIC for most pathogens.

Figure 2. Indices of meningeal inflammation during antibiotic treatment of experimental staphylococcal meningitis. Rabbits were inoculated with 10⁷ cfu of S. aureus intracisternally at 0 h, and antibiotic treatment was initiated 6 h after the bacterial inoculation. No significant difference was observed between the groups, except for significantly lower CSF glucose levels in rabbits treated with the combination at 12 and 15 h (Dunn’s multiple comparisons test, P < 0.05).
In conclusion, we found significant antagonism of combination therapy with fusidic acid in experimental staphylococcal meningitis. Further studies are required to investigate the influence of time-span studied on the bacterial killing during such therapy.

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References