

Efficacy of gatifloxacin alone and in combination with cefepime against penicillin-resistant *Streptococcus pneumoniae* in a rabbit meningitis model and *in vitro*

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Gatifloxacin penetrated well into cerebrospinal fluid (CSF) ($49 \pm 11\%$), measured by comparison of AUC_{CSF}/AUC_{serum} , and showed good bactericidal activity (leading to a decrease of $0.75 \pm 0.17 \log_{10}$ cfu/mL/h) in the treatment of experimental meningitis in rabbits caused by a penicillin-resistant pneumococcal strain (MIC 4 mg/L). It was significantly more effective than the standard regimen, ceftriaxone with vancomycin, which led to a decrease of $0.53 \pm 0.17 \log_{10}$ cfu/mL/h. The addition of cefepime to gatifloxacin slightly improved the killing rates (giving a decrease of $0.84 \pm 0.14 \log_{10}$ cfu/mL/h). *In vitro*, synergy was demonstrated between cefepime and gatifloxacin by the chequerboard method (fractional inhibitory concentration index = 0.5) and by viable counts over 8 h.

Introduction

The global increase in penicillin-resistant pneumococci has complicated the treatment of pneumococcal infections. In the USA, a recent survey showed rates of penicillin resistance of around 29.5%, with 17.4% of isolates having intermediate resistance.¹ The combination of vancomycin with a cephalosporin is recommended for meningitis caused by resistant strains.² Reliable monotherapy would represent a major advantage. Gatifloxacin, a new quinolone, is a good candidate for monotherapy: it has extended activity against many Gram-positive microorganisms, including penicillin-resistant pneumococci.³ The aim of this study was to test its bactericidal activity against a penicillin-resistant pneumococcal strain in a rabbit model of meningitis. Recently, we have shown that quinolones, e.g. trovafloxacin, act synergically with cephalosporins, e.g. ceftriaxone, in experimental meningitis.⁴ In the present study gatifloxacin was combined with cefepime, the most efficacious cephalosporin in our model.⁵ The standard regimen consisted of ceftriaxone combined with vancomycin.

Materials and methods

Rabbit meningitis model

The experimental protocol was approved by the Veterinärämamt des Kantons Bern. Young New Zealand White rabbits weighing 2–2.5 kg were anaesthetized by im injections of ketamine 30 mg/kg and xylazine 15 mg/kg and were immobilized in stereotactic frames for induction of meningitis and collection of samples of cerebrospinal fluid (CSF). An inoculum containing 1×10^5 cfu of penicillin-resistant pneumococci, serotype 6, was injected directly into the cisterna magna. The pneumococcal strain had originally been isolated from a patient with pneumonia at the University Hospital of Berne, Switzerland. The MICs/MBCs (mg/L) were as follows: penicillin, 4/4; ceftriaxone, 0.5/0.5; cefepime, 0.5/0.5; vancomycin, 0.12–0.25/0.25; and gatifloxacin, 0.12/0.25. A long-acting anaesthetic, ethylcarbamate (also called urethane; 3.5 g/rabbit), was injected subcutaneously and animals were returned to their cages. Fourteen hours later, the cisterna magna was punctured again for collection of CSF samples before and 1, 2, 4, 6 and 8 h after initiation of therapy. A catheter was introduced into a

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femoral artery for collection of serum samples. Antibiotics were administered through a peripheral ear vein as bolus injections at the following doses: gatifloxacin, 15 mg/kg; ceftriaxone, 125 mg/kg; vancomycin, 20 mg/kg; cefepime, 100 mg/kg. Ceftriaxone and gatifloxacin were injected once at 0 h and vancomycin and cefepime at 0 and 4 h according to Gerber *et al.*⁵ Untreated controls received saline. Cefepime, ceftriaxone and vancomycin were purchased commercially and gatifloxacin was kindly provided by Grünenthal GmbH, Germany.

Bacterial titres were measured by 10-fold serial dilutions of CSF samples, plated on tryptic soy agar plates (Difco; Becton Dickinson, Sparks, MD, USA) containing 5% sheep blood and incubated overnight at 37°C. In parallel, 20 µL of undiluted CSF samples was plated (limit of detection: 50 cfu/mL). Comparison between different dilutions of CSF was used to exclude any significant effects of carry-over during therapy. The antimicrobial activity of the regimens during the 8 h treatment was determined by measuring the decrease in viable cell count. This was calculated by linear regression analysis and expressed as the change (Δ) in \log_{10} cfu/mL/h. A value of 1.7 (\log_{10} of the limit of detectability) was assigned to the first sterile CSF sample and a value of 0 to any subsequent sterile sample. The results are expressed as mean \pm s.d. Statistical significance was determined by the Newman–Keuls test.

Measurement of antibiotic concentrations in CSF and serum

Antibiotic concentrations in the CSF were determined by an agar diffusion method. Standard curve experiments were performed in saline with 5% rabbit serum in order to mimic CSF protein concentration.⁶ *Escherichia coli* ATCC 25922 in Antibiotic Medium 1 (Difco) was used as test strain for cefepime. *Bacillus subtilis* ATCC 6633 was used in Antibiotic Medium 1 (Difco) for vancomycin and in Antibiotic Medium 11 (Difco) for gatifloxacin. The intra- and interday variability of this method was <10%. The limit of detection was 0.5 mg/L for vancomycin, 1 mg/L for ceftriaxone and 0.25 mg/L for gatifloxacin.

In vitro assays

The pneumococcal strain was grown in conditioned medium with yeast extract (Difco; 'C+Y medium')⁷ to an optical density of 0.3 at 590 nm and then diluted 40-fold to 10^6 cfu/mL, corresponding to the CSF bacterial titre in rabbits before initiation of therapy. Gatifloxacin was added in concentrations corresponding to $1 \times$, $5 \times$ and $10 \times$ MIC (MIC = 0.12 mg/L). Combinations of cefepime (at 0.5 mg/L, which is equivalent to the MIC) with gatifloxacin (0.12 mg/L) were also tested. Bacterial titres were determined at 0, 2, 4, 6 and 8 h by serial dilution of samples, plated on agar plates containing 5% sheep blood and incubated at 37°C

for 24 h. Experiments were performed in triplicate; results are expressed as mean \pm s.d. A drug combination was said to show synergy when the bactericidal effect of the drug combination was significantly greater than the sum of the bactericidal effects of each agent alone.

Determination of fractional inhibitory concentration (FIC) index

The same isolate used in the time–kill experiments and in the animal model was grown in C+Y medium until logarithmic growth phase and then back-diluted. Suspensions of bacteria (containing $0.5\text{--}1 \times 10^6$ cfu) were pipetted into microtitre trays containing the same medium and concentrations of each antibiotic that ranged from $1/32 \times$ MIC to $2 \times$ MIC. Microtitre trays were incubated at 37°C for 24 h. After 6, 12 and 24 h, inhibition of bacterial growth on the plates was assessed. Experiments were performed in triplicate. FIC indices were calculated according to the method of Eliopoulos & Moellering.⁸ Synergy was defined as an FIC index of ≤ 0.5 , indifference as an FIC of >0.5 to ≤ 4 and antagonism as an FIC of >4 .

Results

Figure 1 shows the pharmacokinetics of gatifloxacin after a single dose of 15 mg/kg. The concentration of gatifloxacin in the serum peaked at a mean of 4.4 mg/L, and then declined slowly to 0.5 mg/L 8 h later. In the CSF the highest concentration achieved was 1.4 mg/L and the concentration decreased progressively to 0.4 mg/L by the end of the treatment period. Throughout the therapy period, concentrations in the CSF remained above the MIC (0.12 mg/L).

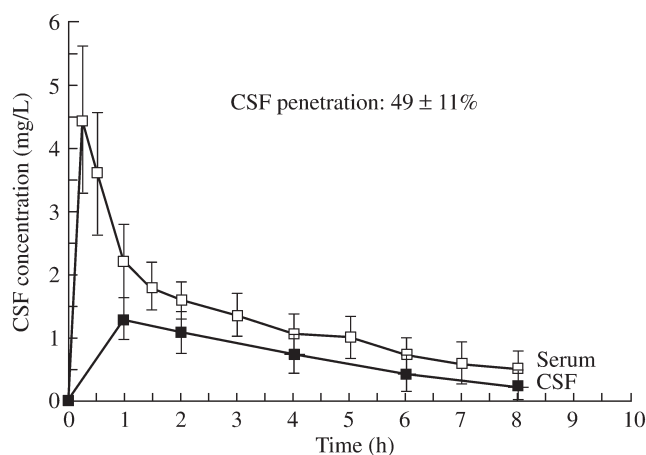


Figure 1. Gatifloxacin concentrations in serum (\square) and CSF (\blacksquare) over an 8 h period after iv injection of gatifloxacin 15 mg/kg. The CSF concentration of gatifloxacin remained above the MIC (0.12 mg/L) for most of the treatment period. The penetration of gatifloxacin into inflamed meninges was $49 \pm 11\%$, determined by comparison of serum and CSF areas under the curve.

Gatifloxacin versus *S. pneumoniae* rabbit meningitis

Table. Single drug and combination therapy against penicillin-resistant *S. pneumoniae* in experimental meningitis

Antibiotic	<i>n</i>	Initial titre (log ₁₀ cfu/mL) (mean ± s.d.)	Killing rate (Δlog ₁₀ cfu/mL/h) (mean ± s.d.)	Killing rate/8 h (log ₁₀ cfu/mL) (mean ± s.d.)
Controls	10	6.10 ± 0.77	+0.02 ± 0.19 ^a	+0.32 ± 0.22 ^a
Gatifloxacin	9	5.69 ± 0.85	-0.75 ± 0.17 ^b	-5.30 ± 0.91 ^b
Cefepime	12	5.77 ± 1.00	-0.62 ± 0.17	-4.63 ± 1.00
Vancomycin + ceftriaxone	10	6.00 ± 0.80	-0.53 ± 0.17 ^{b,c}	-4.18 ± 0.78 ^{b,c}
Gatifloxacin + cefepime	10	5.65 ± 0.77	-0.84 ± 0.14 ^c	-5.36 ± 0.91 ^c

^aSignificantly different from all groups ($P < 0.05$).

^bGatifloxacin significantly different from vancomycin + ceftriaxone ($P < 0.05$).

^cGatifloxacin + cefepime significantly different from vancomycin + ceftriaxone ($P < 0.05$).

Penetration of gatifloxacin into the CSF was calculated by comparison of the serum and CSF areas under the curve (AUC) for each animal. In our model, gatifloxacin penetration into the subarachnoid space was $49 \pm 11\%$. Two injections of cefepime (100 mg/kg) achieved CSF concentrations ranging between 12 and 6.5 mg/L (data not shown). The vancomycin concentration in the CSF ranged between 3.9 and 1.5 mg/L and the ceftriaxone concentration in the CSF between 5 and 3 mg/L. The CSF peak level/MIC ratios ranged between 2.8 and 0.8 for gatifloxacin and between 24 and 13 for cefepime.

The killing rates of the different treatment groups are summarized in the Table. In untreated controls, bacterial titres remained almost constant for 8 h (increasing by $0.02 \pm 0.19 \log_{10}$ cfu/mL/h). Gatifloxacin showed great antibacterial activity and sterilized the CSF in eight out of nine rabbits after 8 h. Two of 10 CSF samples were sterile in the ceftriaxone + vancomycin group, five of 10 in the cefepime group and nine of 10 in the gatifloxacin + cefepime group.

In vitro, gatifloxacin showed good antibacterial activity with concentrations above the MIC (0.6 and 1.2 mg/L) in time–kill assays over 8 h (see Figure 2). Concentrations of 5 and 10 × MIC led to a dose-dependent decrease in viable cell counts over 8 h (4.2 and $7.1 \log_{10}$ cfu/mL, respectively).

Combination therapy with cefepime was also tested. For this purpose, antibiotic concentrations equal to the MIC were chosen, producing only a negligible bactericidal effect as monotherapies. The combination showed a time-dependent synergic activity ($4.5 \log_{10}$ cfu/mL over 8 h). The synergic activity of the combination was also confirmed by the FIC index (0.5), determined using the checkerboard technique.

Discussion

Gatifloxacin is a recently developed fluoroquinolone with an extended activity against penicillin-sensitive and -resist-

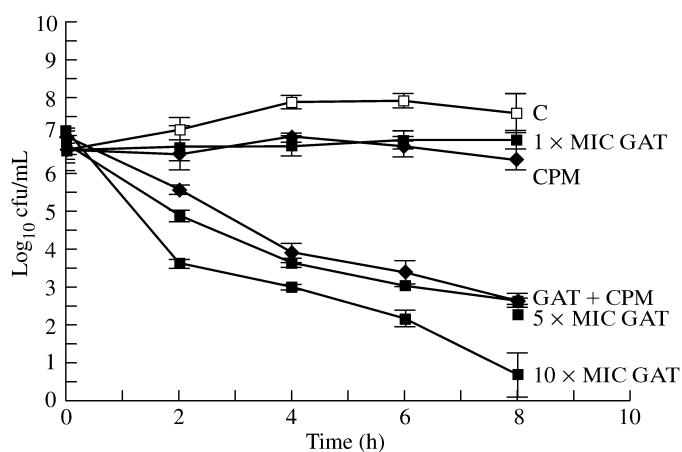


Figure 2. Killing rates of gatifloxacin (■) *in vitro* at concentrations ranging from 0.12 (1 × MIC) to 1.2 mg/L (10 × MIC). Decrease in viable cell count after addition of gatifloxacin (GAT), cefepime (CPM) and cefepime combined with gatifloxacin (CPM+GAT), using concentrations equal to the MIC. Open squares represent untreated controls (C). Experiments were performed in triplicate; killing rates are expressed as mean + s.d.; $P < 0.05$).

ant pneumococci.³ The usually recommended dose (400 mg iv) in humans gives peak concentrations of around 5.7 mg/L, decreasing slowly to 1.8 mg/L 8 h later.⁹ The dose used in our rabbit meningitis model (15 mg/kg iv) gave serum concentrations comparable to those obtained in humans (peak concentration 4.5 mg/L in rabbits; after 8 h, trough concentrations were 1.8 mg/L in humans and 0.6 mg/L in rabbits). The doses of ceftriaxone, vancomycin and cefepime were standard doses that have been used in previous studies in the same model.⁵ In the present study, gatifloxacin penetrated into the CSF very well, confirming recently published data by Lutsar *et al.*,¹⁰ and had pronounced antibacterial activity *in vivo*, sterilizing the CSF in eight of nine animals after 8 h. All animals in the

gatifloxacin group survived. Gatifloxacin was significantly superior to the standard regimen. Gatifloxacin combined with cefepime was the most effective combination therapy, being significantly better ($P < 0.05$) than ceftriaxone combined with vancomycin. Addition of cefepime improved the bactericidal activity of gatifloxacin *in vivo*, but not enough for the combination to be classified as synergic, although synergy between cefepime and gatifloxacin was demonstrated *in vitro* in time-kill experiments and by FIC index.

Gatifloxacin 15 mg/kg showed greater bactericidal activity than trovafloxacin 15 mg/kg and grepafloxacin 30 mg/kg, two quinolones previously tested in the same model.^{11,12}

Based on the good penetration of gatifloxacin into the CSF (49%) and its efficacy in our animal model and *in vitro*, alone or in combination with cefepime, gatifloxacin deserves further investigation.

Acknowledgements

This study was partially supported by a grant of the Wander Foundation, Berne, Switzerland.

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Received 19 July 2000; returned 30 September 2000; revised 26 October 2000; accepted 20 November 2000