

## Greprofloxacin against penicillin-resistant pneumococci in the rabbit meningitis model

Cynthia M. Gerber<sup>a</sup>, Luis Tovar<sup>b</sup>, Marianne Cottagnoud<sup>a</sup>, Klaus A. Neftel<sup>b</sup>, Martin G. Täuber<sup>c</sup> and Philippe Cottagnoud<sup>b\*</sup>

<sup>a</sup>Department of Internal Medicine, Zieglerspital, Berne, <sup>b</sup>Department of Internal Medicine, Inselspital, Freiburgstrasse, 3010 Berne and <sup>c</sup>Institute for Medical Microbiology, University of Berne, Berne, Switzerland

**Greprofloxacin, a new fluoroquinolone, produced bactericidal activity comparable to that of vancomycin and ceftriaxone in the treatment in rabbits of meningitis caused by a pneumococcal strain highly resistant to penicillin (MIC 4 mg/L) ( $\Delta\log_{10}$  cfu/mL-h for greprofloxacin,  $-0.32 \pm 0.15$ ; dose, 15 mg/kg iv;  $\Delta\log_{10}$  cfu/mL-h for vancomycin,  $-0.39 \pm 0.18$ ; dose,  $2 \times 20$  mg/kg iv;  $\Delta\log_{10}$  cfu/mL-h for ceftriaxone,  $-0.32 \pm 0.12$ ; dose, 125 mg/kg iv). Higher doses of greprofloxacin (30 mg/kg and  $2 \times 50$  mg/kg) did not improve the killing rates. The combination of greprofloxacin with vancomycin was not significantly superior to monotherapies ( $P > 0.05$ ). *In vitro*, greprofloxacin was bactericidal at concentrations above the MIC. Using concentrations around the MIC, addition of vancomycin to greprofloxacin showed synergic activity.**

### Introduction

During the last four decades,  $\beta$ -lactam antibiotics were usually the treatment of choice for pneumococcal infections. Since 1970, the continuous worldwide spread of penicillin-resistant pneumococci has considerably reduced the efficacy of this class of antibiotics. In 1997 the highest rates of penicillin-resistant strains (MIC  $> 2$  mg/L) in western Europe were registered in Spain and in France, with resistance rates in excess of 40% in Toulouse and Barcelona (Alexander Project).<sup>1</sup>

However, these antibiotics remain the first-line drugs for most pneumococcal infections, provided that their penetration into infected tissues is sufficient (e.g. in the lung).<sup>2</sup> As a result of the limited penetration of penicillin into the subarachnoid space, the treatment of meningitis caused by resistant strains (MIC  $> 2$  mg/L) is more difficult. A combination of high-dose cephalosporins (i.e. cefotaxime or ceftriaxone) and vancomycin is recommended.<sup>3</sup>

The problem of penicillin-resistant isolates is commonly complicated by the associated resistance to cephalosporins. Cases of treatment failure with cefotaxime and ceftriaxone have already been reported.<sup>4,5</sup> Confronted with the limitations of the actual treatment recommendations, new therapeutic options are needed. Promising candidates among recently developed agents are the new quinolones. These

substances are active against many Gram-positive microorganisms, including penicillin-resistant pneumococci.<sup>6–8</sup>

Greprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid hydrochloride), a new broad-spectrum quinolone, has excellent activity against penicillin-sensitive and penicillin-resistant pneumococci *in vitro*<sup>9</sup> and has been successfully evaluated in respiratory tract infections.<sup>10–12</sup> Little is known about the efficacy of greprofloxacin in meningitis caused by penicillin-resistant strains.

In the present study, we tested greprofloxacin alone and in combination with vancomycin against a pneumococcal strain resistant to penicillin (MIC  $> 2$  mg/L) in the rabbit meningitis model and *in vitro*.

### Materials and methods

#### Rabbit meningitis model

The meningitis model originally described by Dacey & Sande<sup>13</sup> was used, with slight modifications. In brief, young New Zealand white rabbits weighing 2–2.5 kg were anaesthetized by intramuscular injections of ketamine 30 mg/kg and xylazine 15 mg/kg and were immobilized in stereotactic frames for induction of meningitis and cerebrospinal fluid (CSF) samplings. An inoculum containing  $1 \times 10^5$  cfu of a

\*Corresponding author. Tel: +41-316322111; Fax: +41-316323847; E-mail: pcottagn@insel.ch

penicillin-resistant pneumococcus serotype 6 originally isolated from a patient with pneumonia at the University Hospital of Berne, Switzerland, was injected directly into the cisterna magna. The MICs for this strain were as follows: penicillin G, 4 mg/L; vancomycin, 0.12–0.25 mg/L; ceftriaxone, 0.5 mg/L; and grepafloxacin, 0.06 mg/L. A long-acting anaesthetic (ethylcarbamate, 3.5 g/rabbit) was injected subcutaneously. The animals were then returned to their cages. Fourteen hours later, the cisterna magna was punctured again for periodic CSF sampling at 0, 0.75, 2.5, 4, 6 and 8 h. Antibiotics were administered through a peripheral ear vein as bolus injections at the following concentrations: grepafloxacin, 15, 30 and 50 mg/kg; vancomycin, 20 mg/kg; ceftriaxone, 125 mg/kg. Grepafloxacin (15 and 30 mg/kg) and ceftriaxone were injected at 0 h. Vancomycin and grepafloxacin (50 mg/kg) were administered at 0 and 4 h. Untreated controls received the same volume of saline.

Bacterial concentrations were measured by 10-fold serial dilutions of CSF samples, plated on blood agar plates containing 5% sheep blood and incubated overnight at 37°C. The antimicrobial activity of the regimens during the 8 h treatment was calculated by linear regression analysis and expressed as the decrease in log<sub>10</sub> cfu per millilitre per hour ( $\Delta\log_{10}$  cfu/mL·h). In parallel, 20  $\mu$ L of undiluted CSF were plated (limit of detectability: 50 cfu/mL). The different dilutions were compared in order to exclude significant carryover effects during therapy. We arbitrarily assigned a value of 1.7 (log<sub>10</sub> of the limit of detectability) to the first sterile CSF sample and a value of 0 to any subsequent sterile sample. The results are expressed as mean  $\pm$  standard deviation. Statistical significance was determined by the Newman–Keuls test.

#### Measurement of antibiotic concentrations in the CSF

Grepafloxacin concentrations in the CSF were determined by an agar diffusion method using antibiotic medium 11 (Difco Laboratories, Detroit, MI, USA). Standard curve experiments were performed in saline with 5% rabbit serum in order to mimic CSF protein concentrations during meningitis. *Bacillus subtilis* (ATCC 6633) was used as the test strain.<sup>14</sup> The variability between days was <10%. The limit of detection was 0.2 mg/L.

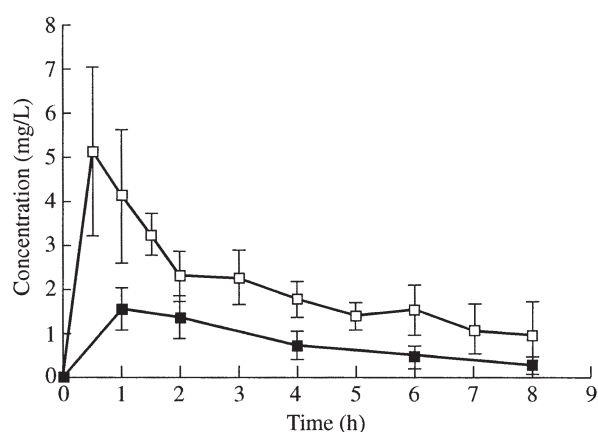
#### In vitro assays

The pneumococcal strain was grown in C + Y medium<sup>15</sup> to an optical density of 0.3 at 590 nm and then diluted 40-fold to 10<sup>6</sup> cfu/mL, corresponding to the CSF bacterial titre in rabbits before initiation of therapy. Grepafloxacin (MIC 0.06 mg/L) was added to a final concentration of 0.06–0.3 mg/L. The combination of grepafloxacin 0.06 mg/L and vancomycin 0.12 mg/L was also tested. Bacterial titres were determined at 0, 2, 4 and 6 h by serial dilution of

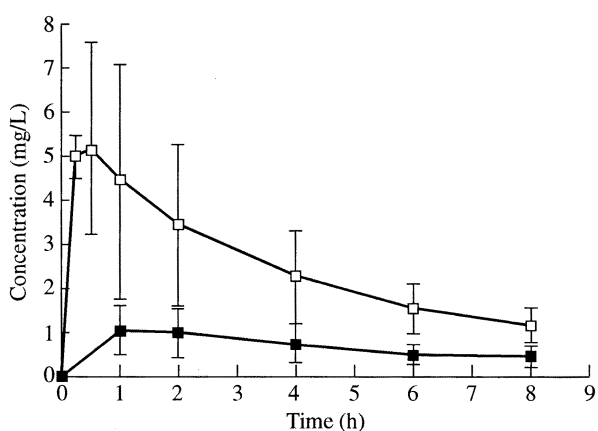
samples, plated on agar plates containing 5% sheep blood and incubated at 37°C for 24 h. Experiments were performed in triplicate and results expressed in mean  $\pm$  standard deviation. Synergy was defined as bactericidal effect of a drug combination significantly exceeding the sum of the bactericidal effects of each agent alone.

## Results

The kinetic data for grepafloxacin are presented in Figures 1–3. Grepafloxacin 15 mg/kg produced a peak serum concentration of *c.* 5 mg/L decreasing slowly to 1 mg/L 8 h later. The peak CSF concentration was 1.6 mg/L with a trough of *c.* 0.3 mg/L. Following a single injection of 30 mg/kg, the serum and CSF concentrations peaked at 5.2 and 1.1 mg/L, respectively. The serum and CSF trough concentrations were 1.4 and 0.45 mg/L, respectively. The highest dose



**Figure 1.** Grepafloxacin concentration in serum (□) and CSF (■) for 8 h after iv injection of grepafloxacin 15 mg/kg. The concentration of grepafloxacin remained above the MIC (0.06 mg/L) for the entire treatment period.

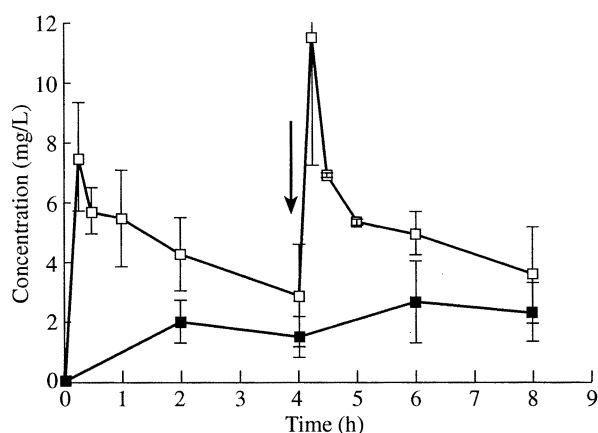


**Figure 2.** Grepafloxacin concentration in serum (□) and CSF (■) for 8 h after a single iv injection of grepafloxacin 30 mg/kg.

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tested in this model was  $2 \times 50$  mg/kg. After the first injection, the serum peak concentration reached 7.6 mg/L, decreasing to 2.8 mg/L 4 h later. The second injection produced a peak concentration of *c.* 12 mg/L and a trough of 3.6 mg/L. During the therapy period, CSF concentrations ranged between 2.8 and 1.6 mg/L. CSF penetration of grepafloxacin was calculated for each animal by comparison of serum and CSF areas under the curve. In our model the penetration of grepafloxacin into the CSF was  $16 \pm 5.7\%$ . The CSF concentrations remained above the MIC (0.06 mg/L) for the entire experimental period for all three doses used. The CSF:MIC ratio ranged between 26 and 5 for the 15 mg/kg group, between 18 and 7 for the 30 mg/kg group and between 46 and 26 for the highest dose ( $2 \times 50$  mg/kg).

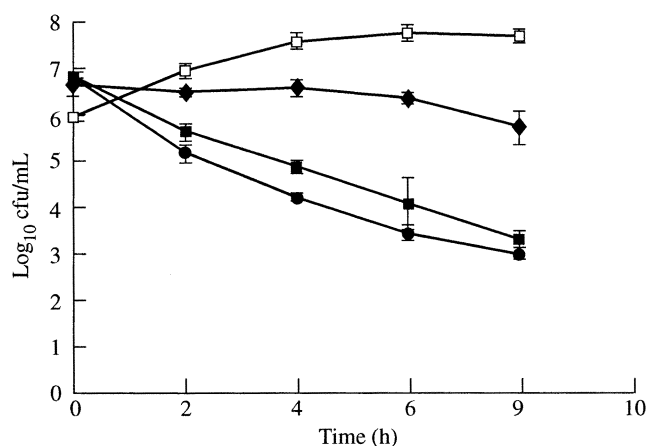
The killing rates of the different doses are summarized in the Table. All three grepafloxacin doses produced similar



**Figure 3.** Grepafloxacin concentration in serum (□) and CSF (■) for 8 h after two iv injections of grepafloxacin 50 mg/kg. The arrow indicates the second injection after 4 h.

bactericidal activity. Although the higher doses (30 mg and  $2 \times 50$  mg/kg) showed a slightly higher efficacy, the differences were not significant ( $P > 0.05$ ). The addition of vancomycin increased the killing rates of grepafloxacin only marginally ( $P > 0.05$ ).

*In vitro*, grepafloxacin showed excellent bactericidal activity at concentrations above the MIC (2.5 and  $5 \times$  MIC) (see Figure 4). Combination therapy with vancomycin was also tested. For this purpose, antibiotic concentrations equal to the MIC were chosen deliberately, producing only a negligible bactericidal effect as monotherapies (grepafloxacin,  $-0.8 \Delta \log_{10}$  cfu/mL·h; vancomycin,  $-1.7 \Delta \log_{10}$  cfu/mL·h). The grepafloxacin–vancomycin combination was synergic ( $-3.8 \Delta \log_{10}$  cfu/mL·h) (see Figure 5).

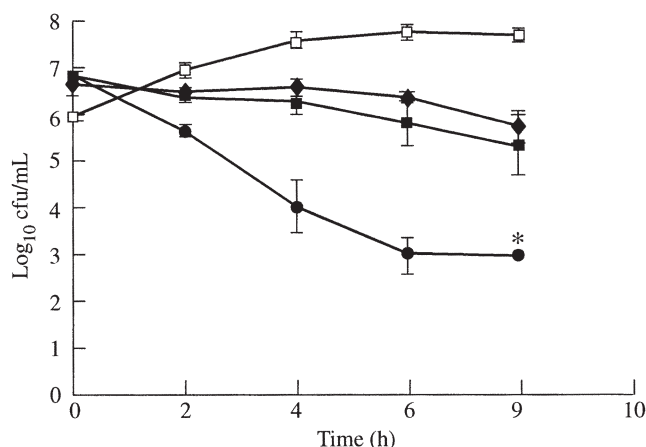


**Figure 4.** Killing rates of grepafloxacin *in vitro* at  $1 \times$  MIC (◆),  $2.5 \times$  MIC (■) and  $5 \times$  MIC (●) compared with untreated controls (□). Experiments were performed in triplicate and killing rates were expressed as mean  $\pm$  s.d.

**Table.** Grepafloxacin compared with ceftriaxone and vancomycin alone and in combination in experimental meningitis caused by *Streptococcus pneumoniae* resistant to penicillin

Antibiotic	Rabbits	Initial titre $\log_{10}$ cfu/mL mean $\pm$ s.d.	Killing rate $\Delta \log_{10}$ cfu/mL·h mean $\pm$ s.d.
Controls	10	$6.30 \pm 0.64$	$+0.04 \pm 0.10^a$
Vancomycin	12	$6.30 \pm 1.12$	$-0.36 \pm 0.19$
Ceftriaxone	9	$5.64 \pm 0.73$	$-0.32 \pm 0.12$
Grepafloxacin			
15 mg/kg	11	$5.74 \pm 1.09$	$-0.32 \pm 0.15$
30 mg/kg	9	$6.10 \pm 0.39$	$-0.41 \pm 0.21$
$2 \times 50$ mg/kg	9	$6.46 \pm 0.46$	$-0.41 \pm 0.23$
Grepafloxacin 30 mg/kg + vancomycin	12	$6.87 \pm 1.71$	$-0.45 \pm 0.10$

<sup>a</sup> $P < 0.05$  versus all other groups; by Newman–Keuls multiple comparison test.



**Figure 5.** Killing rates of grepafloxacin 0.06 mg/L ( $1 \times \text{MIC}$ ) (◆) and vancomycin 0.12 mg/L ( $1 \times \text{MIC}$ ) (■) compared with the combination of the two substances (●) *in vitro*. □, Untreated controls. The experiments were performed in triplicate and results were expressed as means  $\pm$  s.d. \* $P < 0.05$  versus monotherapy.

## Discussion

*Streptococcus pneumoniae* is one of the most common microorganisms causing respiratory infections, otitis media and meningitis in adults. Before the emergence of penicillin-resistant strains, the therapy of these infections was straightforward and  $\beta$ -lactam antibiotics were usually the antibiotics of choice. In the case of meningitis, the emergence of cephalosporin- and penicillin-resistant strains has complicated the therapy, because of the limited penetration of these antibiotics into the subarachnoid space. Treatment failures with cephalosporin monotherapy have already been reported,<sup>4,5</sup> underlining the need for alternative therapies. The recommendation for the treatment of these infections is a combination of a cephalosporin (ceftriaxone or cefotaxime) with vancomycin.<sup>16</sup> Among the potential candidate drugs, newer quinolones seem promising. High doses of trovafloxacin are very bactericidal against penicillin-resistant pneumococci,<sup>17</sup> but doses corresponding to CSF concentrations achievable in humans were less efficacious ( $-0.31 \Delta \log_{10} \text{cfu/mL}\cdot\text{h}$ ). Recently, we have shown that addition of vancomycin significantly improved the killing rates of trovafloxacin.<sup>18</sup> Grepafloxacin has excellent activity against penicillin-sensitive and -resistant pneumococci *in vitro*.<sup>6-9</sup> Numerous studies document the efficacy of grepafloxacin in community-acquired respiratory tract infections, including community-acquired pneumonia, acute bacterial exacerbations of chronic bronchitis<sup>10-12,19</sup> and sinusitis (Olafsson, E., St-Pierre, C., Niekerk, G., Elie, W., unpublished results). In the rabbit meningitis model we studied the kinetic of grepafloxacin into the CSF and its efficacy against penicillin-resistant pneumococci. Since the grepafloxacin dosage has not been defined for meningitis in humans, we chose several doses leading to sufficient grepafloxacin concentrations in the

CSF. Doses ranging from 15 to  $2 \times 50$  mg/kg produced CSF concentrations far above the MIC for the entire treatment period. CSF peak concentrations varied between 2.8 and 1.6 mg/L and trough concentrations between 1.6 and 0.3 mg/L. The lowest dose (15 mg/kg) produced serum concentrations similar to that produced by a single dose of 600 mg grepafloxacin per os in healthy male volunteers.<sup>20,21</sup> In our model, 30 mg/kg slightly increased the serum concentrations over 8 h without significantly increasing the CSF concentration. The average penetration of grepafloxacin into the CSF was  $16 \pm 5.7\%$ , which is slightly inferior to trovafloxacin (19–27%).

The doses of ceftriaxone ( $1 \times 125$  mg/kg) and vancomycin ( $2 \times 20$  mg/kg) were standard doses that have been used in previous studies in the same model;<sup>22</sup> they correspond to high-dose regimens in humans.<sup>3,23,24</sup>

Grepafloxacin 15 mg/kg had good bactericidal activity *in vivo*, comparable to that of vancomycin and ceftriaxone monotherapies.<sup>22,25</sup> Higher doses (30 mg or  $2 \times 50$  mg/kg) improved the killing rates only marginally. The reasons for the lack of efficacy of the higher doses are not clear but might be explained by the dose-dependent killing effects of quinolones in the CSF.<sup>17</sup> The highest concentration used in this study ( $2 \times 50$  mg/kg) produced CSF:MIC ratios far above the dose-dependent killing range.

Addition of vancomycin tended to enhance the killing rate of grepafloxacin, although the differences were not statistically significant ( $P < 0.05$ ).

To confirm the results obtained *in vivo*, combinations were also tested *in vitro*. For the time-kill experiments over 8 h, we selected concentrations around the MIC that led to marginal killing rates with monotherapies. In this *in vitro* setting, addition of vancomycin produced a synergic effect (Figure 5).

The efficacy of this new quinolone in our animal model and its low side effect profile<sup>26,27</sup> qualify grepafloxacin as a potential candidate for the treatment of meningitis with resistant strains. On the other hand, the addition of vancomycin did not produce a substantial benefit *in vivo*.

## Acknowledgement

This work was supported by grants from Glaxo Wellcome Corporation and the Wander Foundation, Berne, Switzerland.

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Received 12 July 1999; returned 2 November 1999; revised 22 December 1999; accepted 23 February 2000

