# Antibacterial activity of grepafloxacin

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Grepafloxin has an extremely broad spectrum of activity. Its activity against Gram-positive bacteria exceeds that of currently available quinolones. Grepafloxacin-resistant mutants seem to occur less frequently than ciprofloxacin- or ofloxacin-resistant mutants, and the increase in minimum inhibitory concentration (MIC) against the former mutants is less than that of the latter. This applies only to the relative differences (in dilution steps); the absolute values are similar. Grepafloxacin kills Gram-positive bacteria at concentrations little above the MIC. Its pharmacodynamic profile against pneumococci is promising, favouring use of this drug for respiratory tract infections.

**Key Words:** *Gram-positive bacteria, Grepafloxacin, Quinolones*

## L'activité antibactérienne de la grépafloxacine.

**RÉSUMÉ :** Le spectre d'activité de la grépafloxacine est très large. Son activité contre les bactéries gram-positives excède celle des quinolones actuellement disponibles. Les mutants résistants à la grépafloxacine semblent moins fréquents que les mutants résistants à la ciprofloxacine ou à l'ofloxacine, et l'augmentation de la concentration minimale inhibitrice (CMI) pour les premiers est plus faible que pour les seconds. Ceci s'applique seulement aux différences relatives (dans les phases de dilution) ; les valeurs absolues sont similaires. La grépafloxacine tue les bactéries gram-positives à des concentrations excédant légèrement la CMI. Son profil pharmacodynamique contre les pneumocoques est prometteur, privilégiant ainsi l'utilisation de ce médicament pour le traitement des infections des voies respiratoires.

4-Quinolones are potent inhibitors of DNA synthesis (1). Their main targets are two structurally homologous enzymes, DNA gyrase (2,3) and DNA topoisomerase IV (4,5). In *Escherichia coli* and probably other Gram-negative bacteria, DNA gyrase is the primary target for quinolones (6-8), but in Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, topoisomerase IV seems to be the primary target (9,10). However, the specificity for a target can vary with the drug (11). Thus, structural alterations in the quinolone molecule can dramatically affect the in vitro and in vivo activity, eg, the antibacterial pharmacodynamics, the pharmacokinetics and the toxicity. Fluorinated quinolones like ciprofloxacin and ofloxacin have gained a wide acceptance for use in the treatment of urinary tract infections, sexually transmitted diseases, skin and soft tissue infections, but are not used so widely for the treatment of respiratory tract infections. This is because of their limited potency against clinically important organisms, including *S pneumonia* and staphylococci, as well as enterococci (12). Grepafloxacin is a recently developed fluorinated quinolone with enhanced activity against these pathogens and with altered pharmacokinetics. Compared with ciprofloxacin, the most potent fluoroquinolone clinically available, grepafloxacin carries two additional methyl groups, one at position C-5′ of the quinolone ring and the other at position C-3′ of the piperazinyl moiety. These modifications enhance activity against Gram-positive bacteria. In addition, the latter methyl group controls pharmacokinetic properties (13,14). In this paper the pattern of activity, the pharmacodynamics and the kill kinetics of grepafloxacin will be reviewed, and the development of resistance will be discussed.

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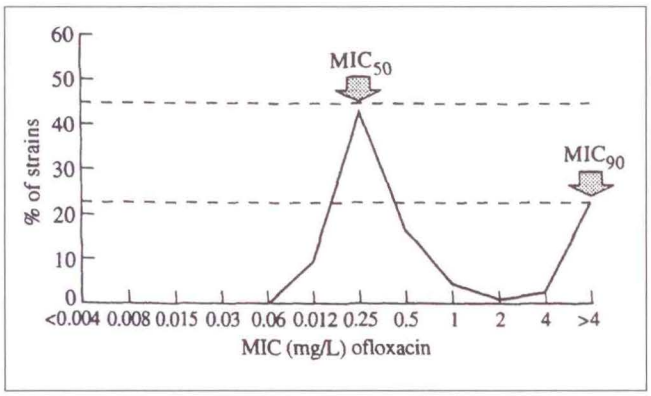


**Figure 1)** *Distribution of ofloxacin minimum inhibitory concentration against 768* Staphylococcus aureus *strains (19)*

## SPECTRUM OF ACTIVITY OF GREPAFLOXACIN

Antibacterial activity is usually assessed by determining minimum inhibitory concentrations (MICs), which are presented as $MIC_{50}$, $MIC_{90}$ and MIC range. However, the distribution of MICs is much more informative. For example, the $MIC_{90}$ is often used to compare related and unrelated drugs, but this value is strongly influenced by the total number of resistant strains. If 10% of tested strains are resistant, especially with quinolones, the $MIC_{90}$ may be 64-fold higher or more than that for 9% resistant strains. As demonstrated in Figure 1, where ofloxacin activity against *S aureus* is given as an example, the $MIC_{50}$ represents the peak of the naturally sensitive population without acquired resistance, and therefore represents the real intrinsic activity of the drug much better. More than 20% of the strains are resistant to ofloxacin. Thus, the $MIC_{90}$ represents the resistant population. The intrinsic activity, however, is represented by the $MIC_{50}$, as the $MIC_{50}$ is at the exact centre of the Gaussian distribution of the naturally occurring population of sensitive strains without mutation in the *grlA* or *gyrA* genes. The activity of grepafloxacin will therefore be discussed here in terms of $MIC_{50}$s, as these are not influenced by the number of resistant strains. The development of resistance has to be considered separately, as these two phenomena are not related.

Grepafloxacin is active against both Gram-positive and Gram-negative bacteria (15-18). Table 1 lists bacteria according to their $MIC_{50}$s. Gram-negative bacteria such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *E coli*, *Shigella* species and *Moraxella catarrhalis* are most sensitive. However, the majority of important pathogens, such as staphylococci, especially *S aureus* (methicillin-resistant or -sensitive), but also most Gram-negative bacteria, including Enterobacteriaceae and *Acinetobacter* species, are inhibited by 0.03 mg/L grepafloxacin. Streptococci are inhibited by 0.125 mg/L and enterococci by 0.25 mg/mL, comparable to the less sensitive Gram-negative bacteria, *Pseudomonas aeruginosa*, *Serratia marcescens* and others. Some *Enterococcus faecium* strains have MICs of 4 mg/L, while *Enterococcus faecalis* and *Enterococcus avium* are inhibited by 0.5mg/L. The activity against anaerobes is also low (eg, 2 mg/L for *Bacteroides fragilis*). Thus, most species are included in grepafloxacin's spectrum of activity if a more sensitive or more resistant breakpoint (1 mg/L or less and 4 mg/L or more, respectively) is assumed from the values obtained with other quinolones.

**MIC distributions (19):** Figure 2 shows the distribution of MICs againsg *E coli*; it can be seen that ciprofloxacin and gre-

**TABLE 1**
**Intrinsic activity of grepafloxacin given as minimum inhibitory concentration ($MIC_{50}$) values (15-18)**

| $MIC_{50}$(mg/L) | Gram-positive bacteria | Gram-negative bacteria |
|---|---|---|
| 0.004 | – | *Haemophilus influenzae* (15) |
| 0.008 | – | *Escherichia coli* (15), *Shigella* species (15), *Neisseria gonorrhoeae* (15), *Moraxella catarrhalis* (15) |
| 0.016 | *Bacillus cereus* (15) | *Yersinia enterocolitica* (15), *Acinetobacter* species (15), *Salmonella enteritidis* (15) *Citrobacter diversus* (15), *Enterobacter agglomerans* (15) |
| 0.031 | *Staphylococcus aureus* (15), *Streptococcus equisimilis* (15), *Staphylococcus haemolyticus* (15), *Staphylococcus hominis* (16) | *Citrobacter freundii* (15), *Enterobacter aerogenes* (15,16), *Enterobacter cloacae* (15,16), *Enterobacter salazakii* (16), *Salmonella* species (16), *Klebsiella oxytoca* (15,16), *Klebsiella pneumoniae (15,16), Citrobacter diversus* (16), *Acinetobacter baumanii (16), Acinetobacter lwoffii* (16) |
| 0.062 | *Staphylococcus epidermidis* (16), *S haemolyticus* (16) | *Morganella morganii* (15), *Proteus vulgaris* (15,16), *C freundii* (16), *Aeromonas hydrophila* (16) |
| 0.125 | *C jekeiium* (15), group B streptococci (15), *Streptococcus* species (16) *Streptococcus pneumoniae* (15), *Streptococcus saprophyticus* (16) | *Proteus mirabilis* (15), *Providencia stuarti* (15), *Proteus rettgeri* (15) *Serratia marcescens* (15), *Serratia liquefaciens* (15), *M morganii (16)* |
| 0.25 | *Enterococcus faecalis* (15,18), group A,C,F,G streptococci (15) | *P aeruginosa* (15), *P mirabilis* (16), *S marcescens* (16), *Chlamydia pneumoniae* (17) |
| 0.5 | *E faecalis* (16), *Enterococcus avium* (16), *Enterococcus faecium* (18) | *Stenotrophomonas maltophilia* (15,16), *P aeruginosa* (16), *Flavobacterium* species (16) |
| 1 | – | – |
| 2 | *E faecium* (16) | *Bacillus fragilis* (15), *P stuartii* (16), *Bacteriodes bivius diseins* (15) |
| 4 | *E faecium* (15) | *P rettgeri* (16) |

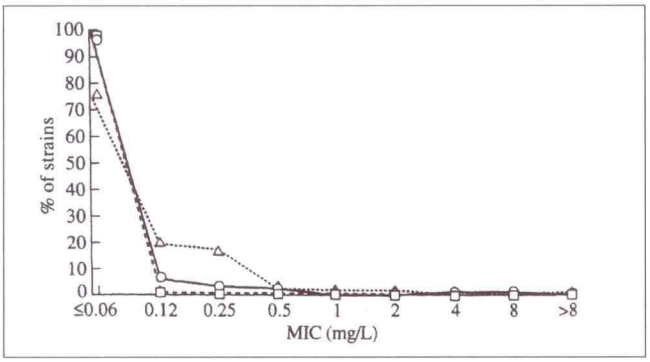


**Figure 2)** *Distribution of ciprofloxacin (O), grepafloxacin (□) and ofloxacin (Δ) minimum inhibitory concentration against 1008* Escherichia coli *strains (19)*

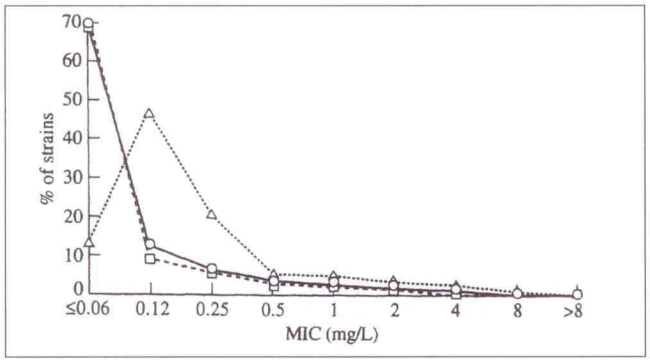


**Figure 3)** *Distribution of ciprofloxzcin (O), grepafloxacin (□) and ofloxacin (Δ) minimum inhibitory concentrations against 387* Klebsiella pneumoniae *strains (19)*

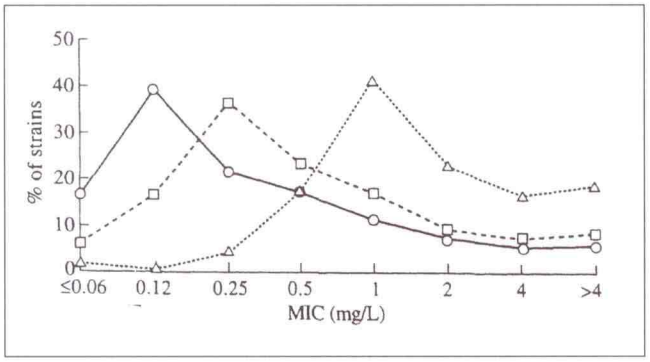


**Figure 4)** *Distribution of ciprofloxzcin (O), grepafloxacin (□), and ofloxacin (Δ) minimum inhibitory concentrations against 651* Pseudomonas aeruginosa *strains (19)*

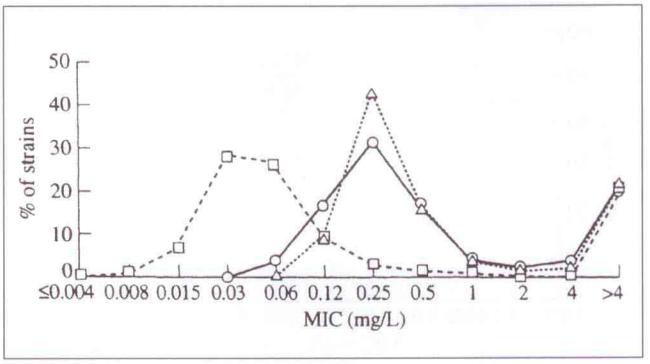


**Figure 5)** *Distribution of ciprofloxzcin (O), grepafloxacin (□) and ofloxacin (Δ) minimum inhibitory concentrations against 768* Staphylococcus aureus *strains (19)*

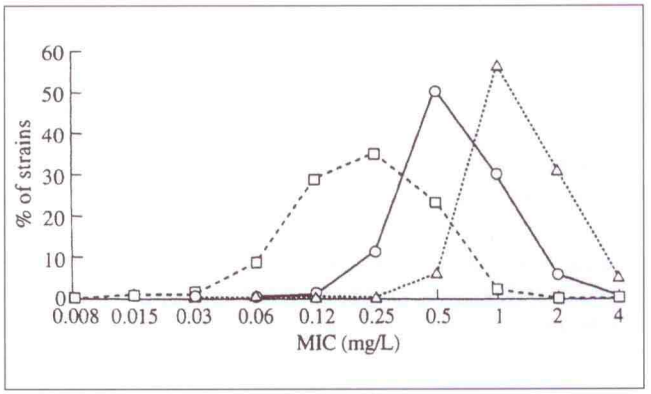


**Figure 6)** *Distribution of ciprofloxzcin (O), grepafloxacin (□) and ofloxacin (Δ) minimum inhibitory concentrations against 119 strains of group A and B streptococci (19)*

pafloxacin have nearly identical activity, while the activity of ofloxacin is slightly lower. The profile for *K pneumoniae* (Figure 3) is similar. Against *P aeruginosa*, grepafloxacin is only half as active as ciprofloxacin, but four times more active that ofloxacin (Figure 4). Against Gram-positive bacteria like *S aureus* grepafloxacin is four to eight times more active than other drugs (Figure 5), even if methicillin-resistant strains are included. Most methicillin-resistant strains are resistant to quinolones as well, the MICs for all drugs tested being 4 mg/L or greater (15). Against group A and B streptococci, grepafloxacin is twice as active as ciprofloxacin and four times as active as ofloxacin (Figure 6). the improvement towards Gram-positive bacteria is best shown with *S pneumoniae* (Figure 7; AL Barry, personal communication), where grepafloxacin is four times more active than ciprofloxacin and 16 times more active than fleroxacin. There were no differences in grepafloxacin MICs against penicillin-sensitive, -intermediate and -resistant strains (Table 2). Grepafloxacin is one of the most effective fluoroquinolones against these species (Table 3), only trovaflxacin and the new derivative DU-6859a being more active (21).

## DEVELOPMENT OF RESISTANCE

In the early days of fluorinated quinolones it seemed as if some bacteria would never become resistant towards these new drugs. However, after several years of widespread use of fluoroquinolones even *E coli* has developed significant resistance towards fluoroquinolones (22-24). By sophisticated methods it has been shown that, in *E coli*, several mutations are necessary to render an *E coli* strain clinically resistant to ciprofloxacin: a double mutation in the *gyrA* gene encoding subunit A of DNA gyrase (25), additional mutations in the *parC* gene encoding topoisomerase IV (7), and alterations in the outer membrane are needed (26). Following serial exposure to grepafloxacin or to ciprofloxacin, Gram-negative bacilli develop resistance to the selected agents. Those resistant mutants showed cross-resistance to all other fluoroqui-

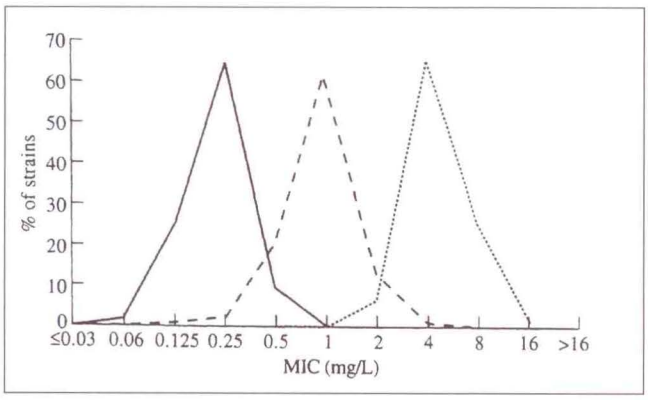


**Figure 7)** *Distribution of ciprofloxzcin (---), grepafloxacin (—), and fleroxacin (····) minimum inhibitory concentrations against 698* Streptococcus pneumoniae *strains (20 and AL Barry, personal communications)*

**TABLE 2**
**In-vitro activity of grepafloxacin against penicillin-susceptible, -intermediate and -resistant *Streptococcus pneumoniae* isolates (N=698) (18)**

| Organism | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Number | 514 | 117 | 66 |
| MIC range (mg/L) | <0.03-1 | 0.125-0.5 | 0.125-0.5 |
| $MIC_{50}$ (mg/L) | 0.25 | 0.25 | 0.25 |
| $MIC_{90}$ (mg/L) | 0.25 | 0.25 | 0.25 |
| % <0.25 mg/L | 91 | 93 | 94 |
| % susceptible | 100 | 100 | 100 |

*MIC Minimum inhibitory concentration*

**TABLE 3**
**In-vitro susceptibility (mg/L) of pneumococci (21)**

| Drug | MIC range | $MIC_{50}$ | $MIC_{90}$ |
|---|---|---|---|
| Ciprofloxacin | 0.5-4.0 | 2 | 4 |
| Ofloxacin | 0.5-4.0 | 2 | 4 |
| Levofloxacin | 0.5-2.0 | 1 | 2 |
| Lomefloxacin | 2-16 | 4 | 8 |
| Tosulfloxacin | 0.064-0.5 | 0.25 | 0.25 |
| Sparfloxacin | 0.064-1.0 | 0.25 | 0.5 |
| DU-6859a | <0.008-0.125 | 0.064 | 0.125 |
| Grepafloxacin | 0.125-1.0 | 0.25 | 0.5 |
| Trovafloxacin | 0.064-0.5 | 0.125 | 0.25 |

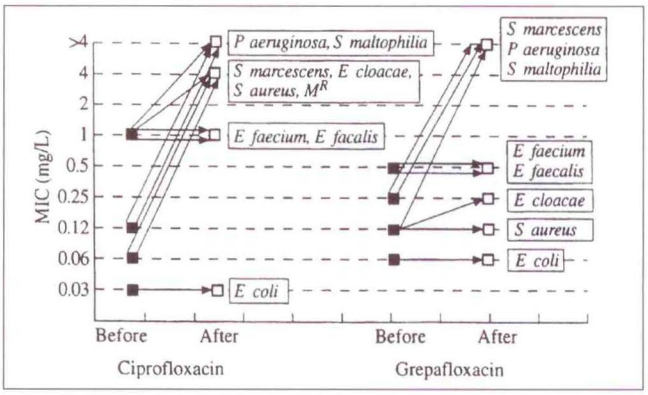


**Figure 8)** *Development of resistant mutants of bacterial strains following 10 serial transfers in subinhibitory concentrations of grepafloxacin or ciprofloxacin (18)*

nolones (Figure 8) and to nalidixic acid (18). Spontaneously occurring resistant mutants will be observed at a frequency of $10^{-7}$ to less than $10^{-11}$ with Gram-negative bacilli. Such mutants are rare among multiresistant *S aureus* and enterococci. The results of these experiments vary with the experimental conditions, but in general it seems that ciprofloxacin and grepafloxacin do not select resistant *E coli*, *E faecium* or *E faecalis* in the presence of subinhibitory conditions. With grepafloxacin *S aureus* did not show an increase of the MIC while *E faecalis* did, but only by one dilution step. With ciprofloxacin, however, the increases in the MIC using the same procedure were five to seven serial dilution steps. Similar results were obtained by Sader et al (19) with the lowest frequency of mutation seen in *E faecalis* (less than $9\times10^{-10}$) and highest in *P aeruginosa* ($1.8\times10^{-7}$). Whether the selection of resistant mutants in vivo will follow the in vitro results is questionable and difficult to evaluate because of the simultaneous use of several quinolones in most hospitals.

Defined mutants of *E coli* did not show significantly different responses to ciprofloxacin and grepafloxacin (Table 4 [27]). Two different mutations in the *gyrA* gene increased the ciprofloxacin and grepafloxacin MICs by five and four dilutions, respectively. The same held true for the acquisition of a second *gyrA* mutation in a *gyrA–parC* double mutant and a *parC* mutation in a *gyrA* double mutant. The same difference in the MIC increase occurred after acquisition of a *marR* deletion.

## KILL KINETICS

According to Barry (18), ciprofloxacin and grepafloxacin-kill bacteria most effectively at the MIC or a 2× MIC (Figure 9). Only one-fifth of experiments revealed a minimum bactericidal concentration (MBC) at 4× MIC. Differences between grepafloxacin and ciprofloxacin were not significant. Ciprofloxacin, however, initially killed more rapidly. With both drugs regrowth frequently occurred after 24 to 48 h incubation at these low concentrations (18). Wakebe and Mitsuhashi (28) compared the bactericidal activity of grepafloxacin with those of ciprofloxacin and ofloxacin against *S aureus*, *E coli* and *P aueruginosa*. The pattern of killing was very similar for all drugs, if concentrations between 0.25× MIC and 2× MIC were used in the experiments. For *S aureus*, however, the MIC was much lower, indicating that grepafloxacin is more potent than the other drugs tested.

To determine the pharmacodynamic properties of a drug it is necessary to simulate its pharmacokinetics. We used *S pneumoniae*, *M catarrhalis* and *H influenzae* as the clinically most important respiratory tract pathogens in an in-vitro model (29) simulating the pharmacokinetics of a 400 mg dose of grepafloxacin. Figure 10 demonstrates the high activity of the drug. Even after a single dose of the drug the viable cell counts of all three bacterial species were steadily reduced by nearly five to six orders of magnitude within 24 h, *M catarrhalis* being the most sensitive. The long half-life of the drug en-

**TABLE 4**
**Comparative activity of ciprofloxacin and grepafloxacin against mutants of *Escherichia coli* (27)**

| | | | | Change in MIC (serial twofold dilution steps) | |
|---|---|---|---|---|---|
| *gyrA* | *gyrB* | *parC* | *marR* | Ciprofloxacin | Grepafloxacin |
| A | – | – | – | 5 | 4 |
| A, E | – | E | E | 5 | 4 |
| E, E | – | A | – | 5 | 4 |
| E | – | – | A | 3 | 2 |
| E | E | E | A | 1 | 1 |

*A Additional mutation; E Existing mutation; MIC Minimum inhibitory concentration*

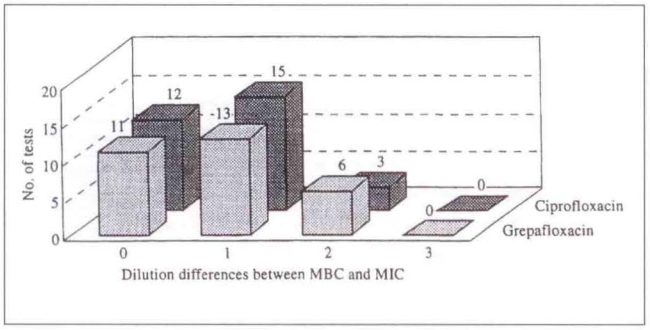


**Figure 9)** *Bactericidal concentrations of grepafloxacin and ciprofloxacin. The number of twofold dilutions below the minimum inhibitory concentration (MIC) (increasing concentration) was tested for 30 strains including three strains of each of the following:* Staphylococcus aureus *(methicillin resistant),* Enterococcus faecalis, Enterococcus faecium, Streptococcus agalactiae, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa *and* S maltophilia *(18)*

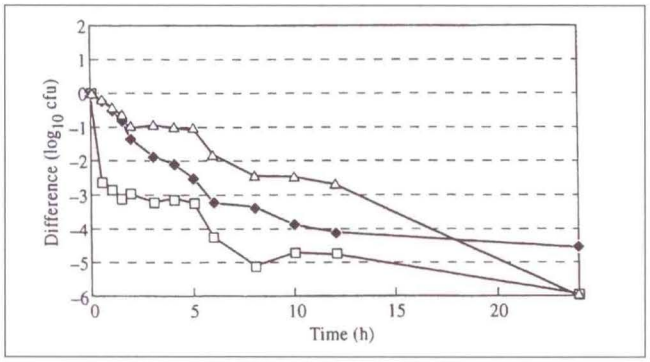


**Figure 10)** *The kill-kinetics of* Haemophilus influenzae *(Δ),* Streptococcus pneumoniae *(♦) and* Moraxella catarrhalis *(□) after simulation of a single 400 mg dose of grepafloxacin with 24 h (29). The pharmacokinetic data were from Glaxo Wellcome (1996)* Grepafloxacin Consultants' Manual, *with* $C_{max}$*=1.4 mg/L and* $t_{1/2}$*=13.7 h*

sured that regrowth did not occur at any time during the experiment (24 h). Comparing these data with a 500 mg dose of ciprofloxacin against *S pneumoniae* (Figure 11), it is obvious that the better pharmacodynamic properties of grepafloxacin result in superior activity. Ciprofloxacin reduced the inoculum by only one order of magnitude before the

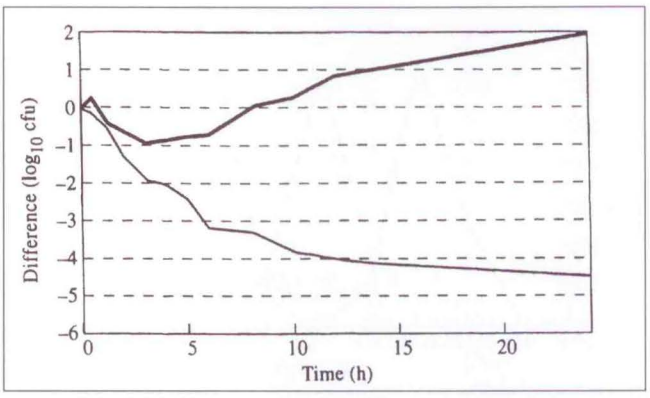


**Figure 11)** *The kill kinetics within 24 h against* Streptococcus pneumoniae *strains after application of a single 500 mg dose of ciprofloxacin (upper curve) in comparision with that of a single dose of 400 mg grepafloxacin (lower curve)*

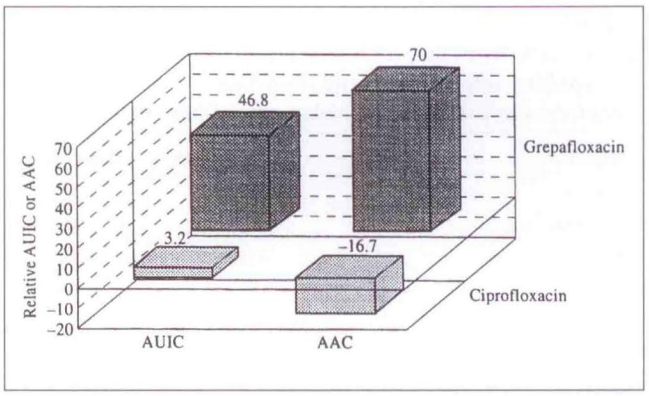


**Figure 12)** *Comparison of relative inhibitory area under the curve (AUIC) and area above the curve (AAC) values for grepafloxacin and ciprofloxacin demonstrating the superior antibacterial activity of grepofloxacin towards* Streptococcus pneumoniae

number of viable bacteria increased. We calculated the area under the inhibitory curve (AUIC) and, as a measure of the number of killed bacteria, the area above the curve (AAC), making the area below the inoculum positive, and that above the inoculum negative (as this means an increase in the number of bacteria). Both parameters demonstrate the favourable pharmacodynamic properties of grepafloxacin as compared with ciprofloxacin (Figure 12). Schentag (30) proposed an AUIC value of more than 125 as predictive for therapeutic success. As our data in contrast to Shentag's patient data are obtained from in vitro experiments AUIC values cannot be evaluated accordingly.

## CONCLUDING REMARKS

Grepafloxacin contains two more methyl groups than ciprofloxacin, and this broadens its antimicrobial spectrum to include a wider range of Gram-positive bacteria, including pneumococci. The favourable pharmacokinetic parameters may be possible and MICs indicate once daily dosing may be possible. The pharmacodynamic activity of grepafloxacin against *S pneumoniae* indicates that this drug may be promising for the treatment of respiratory tract infections.

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