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Induction of *in vitro* resistance to BMS-284756 by *Streptococcus pneumoniae*

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Sir,

BMS-284756 (T-3811) is a novel quinolone that lacks a fluorine at the C-6 position, and is active *in vitro* against *Streptococcus pneumoniae*, including strains with elevated ciprofloxacin MICs.[1,2]

Because the various quinolones differ in both target affinity and their activation of efflux pumps, one can speculate that the phenotypic expression of quinolone resistance will also differ. Studies have shown that *in vitro* fluoroquinolone resistance can be selected for in pneumococci, a property that may have clinical significance.[3]

The purpose of the present study was to clarify whether a quinolone that lacks a fluorine at the C-6 position would have faster or slower development of resistance compared with other fluoroquinolones. Previous authors have demonstrated that quinolones with a methoxy moiety at the C-8 position (e.g. gatifloxacin and moxifloxacin) have an increased antibacterial activity, especially against first-step gyrase- and topoisomerase IV-resistant mutants. Recently, Drlica and colleagues[4,5] have reported that compounds with a C-8 methoxy moiety differ in their ability to restrict the selection of resistant mutants. Quinolones with a C-8 methoxy group were shown to have an enhanced ability to block mutant growth and to kill mutant cells in *Escherichia coli*, *Staphylococcus aureus* and *Mycobacterium* spp., and fewer resistant mutants are selected when bacteria are challenged with C-8 methoxy quinolones than with C-8 hydrogen derivatives.

In this study, 50 distinct clinical isolates of *S. pneumoniae* were repeatedly exposed to BMS-284756, ciprofloxacin, gatifloxacin, levofloxacin and gemifloxacin during a 6 day period. Mean MICs[6] for the original isolates were as follows (mg/L): gemifloxacin, 0.014; BMS-284756, 0.030; gatifloxacin, 0.122; levofloxacin, 0.566; and ciprofloxacin 0.590.

Approximately 5×10^8 cfu of each of the 50 strains were added to tubes containing 9.9 mL brain–heart infusion (BHI) broth supplemented with 5% bovine serum and two-fold dilutions of the quinolones (range 0.001–64 mg/L) (in analogy to MIC determination). The tubes were then incubated for 24 h at 37°C. Aliquots from those tubes containing the highest drug concentration that still permitted visible growth (i.e. 0.5 × MIC) were used, following a 1:10 dilution, to inoculate a second set of serial drug dilutions. After overnight incubation, the bacteria were transferred again. Finally, after six serial transfers, the bacteria with the highest MICs were collected, stored and subcultured on quinolone-free agar for 10 days to assess the stability of resistance.

S. pneumoniae MICs increased from 0.030 to 0.142 mg/L in BMS-284756-containing medium, from 0.014 to 0.250 mg/L in gemifloxacin-containing medium, from 0.122 to 0.574 mg/L in gatifloxacin-containing medium, from 0.566 to 4.724 mg/L in levofloxacin-containing medium and from 0.590 to 15.562 mg/L in ciprofloxacin-containing medium.

Based on a breakpoint of ⩽1 mg/L (⩽2 mg/L for levofloxacin), 100% of the 300 selected mutants (50 strains incubated over 6 days) were inhibited by BMS-284756, 100% were inhibited by gemifloxacin, 98% by gatifloxacin, 70% by levofloxacin and 22% by ciprofloxacin.

In order to analyse the rate of resistance development, we converted the MIC values of all clinical strains and selected mutant isolates to a log scale (base 2). Initial values at time zero (original MIC values) were subtracted from subsequent MICs on days 1–6. The slope of the MIC increase over the 6 day period was computed by linear regression and compared using ANOVA with the Student–Newman–Keuls *post hoc* test; comparisons were regarded as statistically significant if $P < 0.05$. Judging from the squared correlation coefficients R^2 (mean ± S.E.M.: BMS-284756, 0.766 ± 0.019; ciprofloxacin, 0.922 ± 0.008; gatifloxacin, 0.764 ± 0.019; gemifloxacin, 0.931 ± 0.009;

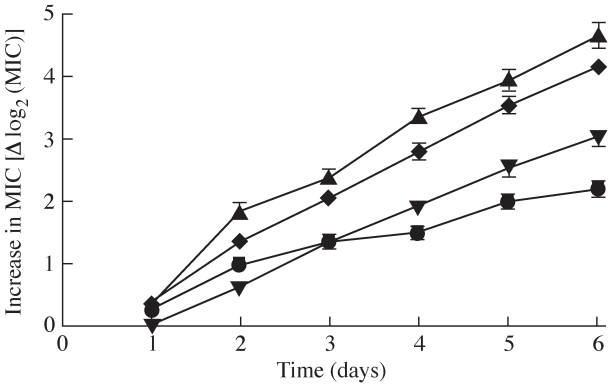

Figure 1. Increases in MICs relative to individual initial MICs were evaluated for the five drugs during 6 days after exposure. Data presented are mean ± S.E.M. (n = 50). The lines for gatifloxacin and BMS-284756 are almost identical. Symbols: ▲, ciprofloxacin; ◆, gemifloxacin; ▼, levofloxacin; ■, gatifloxacin; ●, BMS-284756.

levofloxacin, 0.908 ± 0.009), the linearity of the individual lines was 'good', meaning that the regression coefficients were a reasonable measure of the resistance development rate. The relatively low R^2 values of gatifloxacin and BMS-284756 reflect their low slope values rather than a lack of linearity.

Nearly identical lines were found for ciprofloxacin and gemifloxacin, both reaching rather high $\log_2$ MIC increases after 6 days (ciprofloxacin, 0.841 ± 0.032; gemifloxacin, 0.756 ± 0.030), while lower increases were measured for levofloxacin (0.620 ± 0.027), gatifloxacin (0.373 ± 0.020) and BMS-284756 (0.384 ± 0.018). The rates of resistance development are significantly different between the drugs tested (Figure 1). These differences, measured on the log scale, correspond to x-fold increases in the initial MIC values per day: BMS-284756 1.294-fold; gatifloxacin 1.295-fold; levofloxacin 1.537-fold; gemifloxacin 1.689-fold; and ciprofloxacin 1.791-fold (Figure 2). Thus, BMS-284756 and gatifloxacin displayed the lowest propensity for causing resistance development.

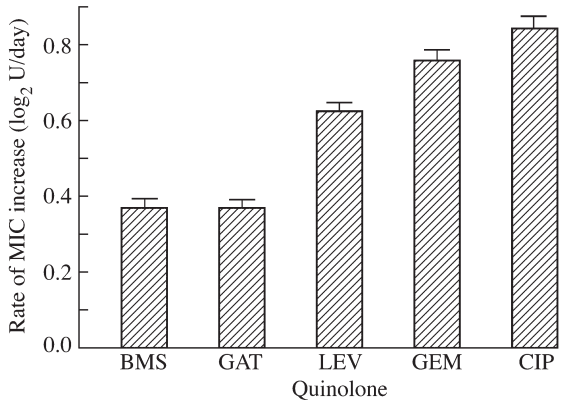

Figure 2. The rate of resistance development as measured by the slopes of individual time-dependent increases in MICs for five quinolones. Data presented are mean ± S.E.M. BMS-284756 and gatifloxacin exhibit the lowest MIC increase with a mean rate of <0.4 $\log_2$ U/day (corresponding to a 1.3-fold increase per day), while ciprofloxacin shows the highest mean slope with >0.8 $\log_2$ U/day (corresponding to a 1.8-fold increase per day). Abbreviations: BMS, BMS-284756; GAT, gatifloxacin; LEV, levofloxacin; GEM, gemifloxacin; CIP, ciprofloxacin.

Our data concerning the low propensity of gatifloxacin to select quinolone-resistant mutants are supported by investigations by Drlica and colleagues,[4,5] who showed that gatifloxacin was less affected by changes in either DNA gyrase or topoisomerase IV. Similar results with respect to the propensity of a quinolone to select resistant mutants within a period of 6 days are now also shown for BMS-284756 which lacks a methoxy group at C-8 and also a fluorine at position C-6. Thus, the chemical structure at a special position of a quinolone has a direct impact on the propensity for resistance development, but other factors, such as the spontaneous mutation rate or the antibacterial activity in general, seem to be at least as important as a special chemical structure at C-8 or at another position.

In summary, the novel des-fluoro(6) quinolone BMS-284756 exhibits *in vitro* activity against *S. pneumoniae* isolates and selected mutants and also has a low propensity for resistance development.

References

1. Fung-Tomc, J. C., Minassian, B., Kolek, B., Huczko, E., Aleksunes, L., Stickle, T. *et al.* (2000). Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS-284756. *Antimicrobial Agents and Chemotherapy* **44**, 3351–6.

2. Takahata, M., Mitsuyama, J., Yamashiro, Y., Yonezawa, M., Araki, H., Todo, Y. *et al.* (1999). In vitro and in vivo antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone. *Antimicrobial Agents and Chemotherapy* **43**, 1077–83.

3. Boos, M., Mayer, S., Fischer, A., Köhrer, K., Scheuring, S., Heisig, P. *et al.* (2001). In-vitro development of resistance to six quinolones in *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy* **45**, 938–42.

4. Dong, Y., Xu, C., Zhao, X., Domagala, J. & Drlica, K. (1998). Fluoroquinolone action against mycobacteria: effects of C-8 substituents on growth, survival, and resistance. *Antimicrobial Agents and Chemotherapy* **42**, 2978–84.

5. Lu, T., Zhao, X. & Drlica, K. (1999). Gatifloxacin activity against quinolone-resistant gyrase: allele-specific enhancement of bacteriostatic and bactericidal activities by the C-8-methoxy group. *Antimicrobial Agents and Chemotherapy* **43**, 2969–74.

6. National Committee for Clinical Laboratory Standards. (1998). *Performance Standards for Antimicrobial Susceptibility Testing: Eighth Informational Supplement M100-S8*. NCCLS, Villanova, PA.