ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2005, p. 1633–1635 Vol. 49, No. 4
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.4.1633–1635.2005

Mutant Prevention Concentrations of ABT-492, Levofloxacin, Moxifloxacin, and Gatifloxacin against Three Common Respiratory Pathogens

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Received 20 May 2004/Returned for modification 15 September 2004/Accepted 23 November 2004

The purpose of this study was to compare the mutant prevention concentration (MPC) of ABT-492 to those of levofloxacin, moxifloxacin, and gatifloxacin against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The fluoroquinolones had comparable mutation selection windows, which is the ratio of MPC/MIC, for all isolates.

Community-acquired respiratory tract infections account for over two-thirds of antibiotic prescriptions and comprise approximately 80% of all infectious presentations (12). Antimicrobial resistance has impeded the treatment of common bacterial respiratory tract infections. The fluoroquinolones are widely used in the treatment of such infections, and the potential for developing resistance to the fluoroquinolones is becoming a concern.

The mutant prevention concentration (MPC) has been suggested as a useful parameter for selecting appropriate dosing of antimicrobials to prevent the selection of resistant bacteria during therapy (5, 6, 13, 14). The purpose of this investigation was to determine the MPC of ABT-492, an investigational fluoroquinolone, versus those of levofloxacin, moxifloxacin, and gatifloxacin against common community-acquired respiratory pathogens: *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.

Twenty-four MPC determination experiments with ABT-492, levofloxacin, moxifloxacin, and gatifloxacin against *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were performed. Three clinical *S. pneumoniae* strains were selected based on their various penicillin (PCN) susceptibilities (MICs of 0.012, 1, and 8 mg/liter). Two *H. influenzae* clinical strains were chosen based on the presence and absence of beta-lactamase production. Lastly, the *M. catarrhalis* clinical isolate was beta-lactamase positive. All isolates were obtained from the Clinical Microbiology Laboratory at Regions Hospital (St. Paul, Minn.) except the PCN-nonsusceptible *S. pneumoniae* isolate, which was kindly provided by M. J. Rybak (Wayne State University and Detroit Receiving Hospital, Detroit, Mich.).

Stock solutions of ABT-492 (Abbott Laboratories, Abbott Park, Ill.), levofloxacin (Ortho-McNeil Pharmaceutical, Inc., Raritan, N.J.), moxifloxacin (Bayer Corporation, West Haven, Conn.), and gatifloxacin (Bristol-Myers Squibb, Princeton, N.J.) were prepared according to the manufacturer's instructions and frozen at −80°C until needed.

Susceptibility to ABT-492, levofloxacin, moxifloxacin, and gatifloxacin was tested in duplicate for each isolate at inocula of 10^5 CFU/ml. Susceptibility testing was performed via broth microdilution according to NCCLS guidelines.

The MPC methodology was an adaptation of methods outlined by Blondeau et al. (2). Agar dilution plates were prepared by adding aliquots of stock antibiotic solution to molten agar and by dispensing the molten agar into 15- by 150-mm sterile petri dishes. The agar used included the following: Trypticase soy agar (Becton Dickinson Microbiology Systems, Sparks, Md.) plus 5% defibrinated sheep blood for *S. pneumoniae* and *M. catarrhalis*, and GC agar base (Becton Dickinson Microbiology Systems) plus 2% hemoglobin solution and supplement B for *H. influenzae*. Five doubling dilution concentrations were prepared for each antibiotic-bacteria combination. The isolate to be tested was subcultured to three blood agar plates (chocolate agar used for *H. influenzae*) and incubated for 20 to 24 h at 37°C in 5 to 10% CO_2, producing a dense lawn of growth. All growth from each plate was inoculated into three separate flasks, each containing 200 ml of broth (*Haemophilus* test medium for *H. influenzae*, Todd-Hewitt broth for *S. pneumoniae*, cation-adjusted Mueller-Hinton broth for *M. catarrhalis*), and incubated for 18 to 20 h at 37°C in 5 to 10% CO_2. The combined volume, 600 ml, was divided into 50-ml portions and centrifuged. The supernatant was decanted, and all pellets were combined to make the final suspension. Inocula were determined using serial saline dilution and plating techniques.

Two hundred microliters of the concentrated bacterial suspension was used to inoculate a control plate plus five agar dilution plates per concentration, resulting in a total of 1 ml exposed to each concentration. The plates were incubated at 37°C in 5 to 10% CO_2 and examined for growth after 24 h, 48 h, and 5 days. The MPC was reported as the lowest concentration preventing all growth after 5 days.

Susceptibility testing results are presented in Table 1. The average number of bacteria in the concentrate was as follows: the PCN-susceptible *S. pneumoniae* isolate had a mean of 1.2

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TABLE 1. MPC/MIC ratios

Isolate[a]	MPC/MIC [mg/liter (ratio)]			
	ABT-492	Levofloxacin	Moxifloxacin	Gatifloxacin
Sp-S	0.015/0.002 (8)	4/1 (4)	2/0.25 (8)	2/0.5 (4)
Sp-NS	0.015/0.002 (8)	8/1 (8)	2/0.25 (8)	2/0.5 (4)
Sp-R	0.03/0.004 (8)	4/1 (4)	2/0.25 (8)	2/0.5 (4)
Hi-N	0.008/0.0005 (16)	0.125/0.015 (8)	0.125/0.03 (4)	0.25/0.015 (16)
Hi-P	0.008/0.0005 (16)	0.06/0.015 (4)	0.25/0.03 (8)	0.25/0.03 (8)
Mc-P	0.008/0.002 (4)	2/0.125 (16)	2/0.125 (16)	0.5/0.06 (8)

[a] Sp, *S. pneumoniae;* Hi, *H. influenzae;* Mc, *M. catarrhalis;* S, NS, and R reflect penicillin-susceptible, -nonsusceptible, and -resistant isolates, respectively; N, beta-lactamase negative; P, beta-lactamase positive.

$\times 10^9$ CFU/ml; the PCN-nonsusceptible *S. pneumoniae* isolate had a mean of 2.4×10^9 CFU/ml; the PCN-resistant *S. pneumoniae* isolate had a mean of 1.8×10^9 CFU/ml; the beta-lactamase-positive *H. influenzae* isolate had a mean of 3.2×10^{10} CFU/ml; the beta-lactamase-negative *H. influenzae* isolate had a mean of 1.5×10^{10} CFU/ml; and the beta-lactamase-positive *M. catarrhalis* isolate had a mean of 6.3×10^9 CFU/ml. MPC results are shown in Table 1. All four of the fluoroquinolones have comparable mutation selection windows (MSWs), which is the ratio of MPC/MIC, for all isolates tested.

Antibiotic pharmacodynamics have been used in attempts to identify outcome parameters to predict antimicrobial efficacy in vivo. Thus far, three major parameters, all of which link a pharmacokinetic parameter to the bacterial MIC, have been suggested in the literature. These three parameters include the following: the area under the antibiotic concentration-time curve (AUC)-to-bacterial MIC ratio, or AUC/MIC; the antibiotic peak concentration-to-bacterial MIC ratio, or peak/MIC; and the time antibiotic concentration remains above the bacterial MIC (T>MIC).

More recently, the MPC concept has been suggested as a useful tool for guiding appropriate dosing of antimicrobials to minimize the selection of resistant bacteria (5, 6, 13, 14). MPC is defined as the minimum concentration necessary to prevent the proliferation and enrichment of single-step mutants (2). MIC testing utilizes an inoculum size of approximately 10^5 to 10^6 CFU/ml, which has certain disadvantages. MIC testing does not emulate the bacterial load associated with many infections and may not detect resistant mutant subpopulations that may be present. Because MPC testing uses a higher inoculum size (typically 10^{10} to 10^{11} CFU/ml or sizes approximating the inverse of the frequency of mutation for fluoroquinolones), this technique may prove useful in determining the efficacy of antibiotics in preventing the selection of resistant mutants. The inocula achieved in this study were lower than those reported by other investigators. However, the mutational frequency for fluoroquinolones is 1 in 10^6 to 1 in 10^9 cells (8), meaning the inocula we achieved were adequate to detect mutant subpopulations. A discussion regarding the difficulty in achieving an inoculum of 10^{11} CFU/ml can be found elsewhere (9).

While the MPC concept should not be applied to all antimicrobials and all bacteria (9, 11), the concept represents a consideration for fluoroquinolone activity versus various pathogens (1–4, 7, 10, 15). One of the main mechanisms of resistance to fluoroquinolones involves chromosomally mediated alterations in the target sites, topoisomerase II and topoisomerase IV. Previous studies suggest that these mutations occur in a stepwise manner (8). The MPC concept applies to the fluoroquinolones because the theory suggests that achieving the MPC will prevent further growth of first-step mutants. Thus, maintaining a concentration above the MPC, outside of the MSW, is desirable.

The free peak concentrations achieved with clinical dosing of levofloxacin (8.5 mg/liter at a dose of 750 mg every 24 h), moxifloxacin (2 mg/liter at a dose of 400 mg every 24 h), and gatifloxacin (4 mg/liter at a dose of 400 mg every 24 h) are at or above the MPCs for all isolates in this study. Clinical dosing for ABT-492 remains to be determined. Firsov et al. suggest that enrichment of resistant subpopulations of *Staphylococcus aureus* will occur if fluoroquinolone concentrations remain within the MSW for >20% of the dosing interval (7). While ABT-492 offers no advantage regarding the size of the MSW, because the MPC is considerably lower than those of levofloxacin, moxifloxacin, or gatifloxacin, clinical dosing of ABT-492 may be able to achieve a concentration above the MPC, outside the MSW, for a longer portion of the dosing interval. In conclusion, we suggest that although the size of the MSW is important (smaller is better), the primary clinical consideration should be whether drug concentrations at the site of infection are above the MPC. Therefore, the peak/MPC ratio or the time the concentration remains above the MPC (T>MPC), outside of the MSW, may be more clinically relevant than the MSW.

This work was supported by Abbott Laboratories (Abbott Park, Ill.).

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