In Vitro Pharmacodynamics of Ceftazidime against Pseudomonas aeruginosa Isolates from Cystic Fibrosis Patients

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The concentration/MIC (C/MIC) ratio maximizing the bactericidal activity of ceftazidime against 10 Pseudomonas aeruginosa isolates from cystic fibrosis patients was identified. Bactericidal activity was assessed by determining the percent difference in the area under the killing curve at each C/MIC ratio for all of the isolates from that of their growth control. The percent effect at each C/MIC ratio was fitted to a sigmoidal E\textsubscript{max} model with maximum bactericidal activity defined as the C/MIC ratio that produced an effect that was 90% of the E\textsubscript{max}. Our results suggest that at least some isolates may require higher C/MIC ratios than previously reported for maximal activity.

Beta-lactam antibiotics have been shown to have time-dependent antibacterial activity such that an optimal effect may be achieved by maintaining concentrations in serum above the MIC for the offending pathogen (6). Elucidation of this relationship in vitro has led to trials comparing continuous infusion and intermittent dosing of beta-lactams; several in vitro and in vivo studies have produced data suggesting that continuous infusion of ceftazidime may have efficacy equivalent or superior to that of intermittent dosing (8–10, 17, 21, 22). Despite these promising results, some issues require clarification. It is not known whether time-dependent bactericidal action is achieved at any concentration above the MIC or whether some dose dependency is operative; it has been reported that the bactericidal activity of beta-lactams was somewhat concentration-dependent and was maximized at a concentration/MIC (C/MIC) ratio of 4 to 5 (6, 18, 24). The objective of this study was to determine whether a particular ceftazidime C/MIC ratio could be identified that maximizes bactericidal activity against clinical isolates of Pseudomonas aeruginosa isolated from cystic fibrosis (CF) patients.

The microorganisms tested included 10 isolates of P. aeruginosa (5 mucoid and 5 nonmucoid) from 10 different CF patients and P. aeruginosa ATCC 27853, which served as a control. The final inoculum (approximately 5 x 10^5 CFU/ml) used in all tests was prepared in accordance with National Committee for Clinical Laboratory Standards guidelines and verified by using a spiral plater (Spiral Systems Inc., Cincinnati, Ohio) (19). Mueller-Hinton broth (lot 71464JB; Difco Laboratories, Detroit, Mich.), adjusted to 25 mg of Ca\textsuperscript{2+} per liter and 12.5 mg of Mg\textsuperscript{2+} per liter, was used for susceptibility and time-kill tests. Standard solutions of ceftazidime with 10% (wt/wt) sodium carbonate were prepared in sterile water for injection. Working stock solutions were diluted to the appropriate concentration in cation-adjusted Mueller-Hinton broth and consisted of ≤5% (vol/vol) additional aqueous solution. Ceftazidime analytical-grade powder (lot 426019) and sodium carbonate (lot UMH511) were supplied by Glaxo Wellcome (Research Triangle Park, N.C.). Colony count and Etest (AB Biodisk, Solna, Sweden) MIC determinations were performed on antibiotic-free 15-cm-diameter Mueller-Hinton agar plates (BBL, Cockeysville, Md.). MICs were identified in triplicate by using the Etest method with an inoculum spectrophotometrically matched to a 0.5 McFarland standard (approximately 1.5 x 10^8 CFU/ml). The plates were incubated at 35°C for 24 h, and the MIC was defined in accordance with the manufacturer’s guidelines. Modal values were used as the basis for the time-kill study.

Time-kill studies were conducted by using the broth macrodilution technique in accordance with National Committee for Clinical Laboratory Standards guidelines (20). The C/MIC ratios studied were 1, 2, 4, 8, and 16, which corresponded to a concentration range of 2 to 96 µg/ml. At 0, 1, 2, 4, 8, 12, and 24 h, samples were withdrawn from each tube for colony count determination. Samples that were anticipated to have colony counts greater than 4 x 10^3 CFU/ml, based on preliminary time-kill experiments, were transferred into a sterile container, and serial 10-fold dilutions were prepared. A 50-µl aliquot of a diluted or undiluted sample was placed onto a Mueller-Hinton agar plate by using a spiral plater. The plates were incubated for 18 to 24 h at 35°C, and surviving colonies were counted. For data analysis, colony counts were not plotted versus time to assess differences in bactericidal activity. All plots were evaluated for extent of killing. The time at which ≥99.9% killing of the initial inoculum was first observed at each C/MIC ratio was noted. The C/MIC ratio that minimized the time to 99.9% killing (sampling point when noted) and prevented regrowth was determined for all of the isolates. The extent of killing was evaluated by calculating the area under the kill curve (AUKC) from the \textit{log}_{10} colony count versus time curves by using the linear trapezoidal rule, and the C/MIC ratio that resulted in the smallest
AUC from time zero to 24 h (AUC_{0–24h}) was noted. The AUC_{0–24h} for each C/MIC ratio tested was then transformed to a percent effect based on the individual growth curves for each isolate by using the equation % Effect = [(A - B)/A] \times 100, where A is the area under the growth curve and B is the AUC_{0–24h} at each C/MIC ratio tested. Calculated effects and the C/MIC ratios were fitted to a sigmoidal E_{max} model (14) by using BOOMER, a modeling and simulation software program (4). This relationship is expressed as Effect = E_{max} \times C^3/EC_{50}^2 + C^3, where C is the C/MIC ratio required for the observed effect, E_{max} is the maximum achievable effect that could be achieved at any C/MIC ratio, EC_{50} is the C/MIC ratio at which 50% of the E_{max} was observed, and n is the sigmoidicity factor. E_{max} was constrained between 0 and 75, as the maximum measurable effect (MME), based upon our initial inoculum and sampling scheme, ranged from 68 to 71% (calculated as instantaneous killing to the limit of detection by the first sample point with no subsequent regrowth). EC_{50} was constrained between 0.1 and 10 to represent the outer limits at which the EC_{50} can be observed. The sigmoidicity factor (n) was fixed at 2 to best characterize the nature of the data. Concentration-effect relationships were evaluated for goodness of fit by assessing the coefficient of determination, Akaike’s information criterion, and the weighted sum square of the residuals. Relationships that produced a coefficient of determination closest to 1.000 and the lowest Akaike’s information criterion and weighted sum square of the residuals were considered to represent the best fit. The C/MIC ratios that resulted in the E_{max} (EC_{max}), 90% of the E_{max} (EC_{90}), and 50% of the E_{max} (EC_{50}) were calculated for each of the individual isolates tested. A Wilcoxon signed-rank test was used to assess the differences in the outcome measurements (EC_{max}, EC_{90}, and EC_{50} and the corresponding effects) between mucoid and nonmucoid strains. The Kruskal-Wallis test was used to assess the differences across the C/MIC ratios tested among all of the isolates. Significance was defined, a priori, as P < 0.05.

MICs ranged from 2 to 6 μg/ml. A lag time prior to killing was apparent with all of the test organisms. The lag time for four of the five mucoid strains was 4 h, whereas that for ATCC 27853 and all of the nonmucoid strains was 2 h. In 24 of 25 instances (five organisms, five C/MIC ratios tested), 99.9% killing of the initial inoculum was generally achieved with nonmucoid strains within 8 h. For the mucoid strains, 99.9% killing was achieved in 19 of 25 instances, but often not until 12 or 24 h. The geometric mean C/MIC ratios that resulted in no regrowth were 6.1 and 5.3 for the mucoid and nonmucoid strains, respectively. A C/MIC ratio of 8 resulted in no regrowth for strain ATCC 27853. The geometric mean C/MIC ratios that minimized the AUC were 3.5 and 3.0 for the mucoid and nonmucoid strains, respectively. A C/MIC ratio of 16 minimized the AUC for strain ATCC 27853. The C/MIC ratio-effect relationships for the nonmucoid and mucoid strains are illustrated in Fig. 1 and 2. Overall, the mucoid strains exhibited more variability in the C/MIC ratio-effect relationships than did the nonmucoid strains. E_{max} was significantly higher (95% confidence interval, 1.05 to 8.50; P = 0.04) for the nonmucoid strains (Table 1). When E_{max} was expressed as a percentage of the MME, the mean effects were 89 and 84 for the nonmucoid and mucoid strains, respectively. The EC_{max}, EC_{90}, and EC_{50} were not statistically significantly different between the nonmucoid and mucoid strains (P = 0.69); however, the C/MIC ratios were higher for the mucoid strains. The highest EC_{max}, EC_{90}, and EC_{50} observed were 66, 6.6, and 2.2, respectively.

The length of time that β-lactam concentrations remain above the MIC has been shown to correlate with efficacy in vivo (2, 11, 12, 16, 23). It appears that simply exceeding the MIC does not maximize antibacterial activity; it has been suggested that a concentration equivalent to four times the MIC would maximize antibacterial activity for penicillins and cephalosporins (7, 18, 24). We did not find that this observation applied to the activity of ceftazidime against all of the clinical isolates of P. aeruginosa from CF patients examined in our study.

Because a maximum bactericidal effect may be described in a number of ways, the C/MIC ratio that results in maximum activity can vary. For example, we demonstrated that the average (geometric mean) C/MIC ratios that minimized the time to 99.9% killing, prevented regrowth, and minimized the AUC_{0–24h} for all of the isolates were 2.0, 5.8, and 3.8, respectively. The percent effect calculation used in our analysis takes into account relevant parameters by using the area under the time-kill curve and relating to the individual growth controls. Thus, when a range of C/MIC ratios are tested, the C/MIC ratio-effect relationship can be characterized by fitting the data to a mathematical model, in this case, the sigmoidal E_{max}.
model. Maximum activity can then be defined as the C/MIC ratio that results in the $E_{\text{max}}$. However, the calculated C/MIC ratio at $E_{\text{max}}$ can result in large values, as exhibited in this study, and may not be achievable in vivo. Mathematically, these high C/MIC ratios are calculated when values have not reached an absolute plateau at the highest C/MIC ratio tested, therefore producing a positive slope, and the difference between the C/MIC ratios at $E_{\text{max}}$ and 90% $E_{\text{max}}$ can be quite large. Consequently, we chose to identify the C/MIC ratio that resulted in 50 and 90% of the $E_{\text{max}}$ and we defined maximum activity can then be defined as the C/MIC ratio that maximizes the effect by using a dose ranging study (1, 17, 24, 26). However, there was a fair degree of variability among the isolates tested, indicating that some strains would require a higher C/MIC ratio for this degree of effect. Unlike these in vitro studies, various C/MIC ratios have not been purposefully studied in humans. The majority of in vivo studies reported to date identified the C/MIC ratio associated with efficacy, but none identified the optimal C/MIC ratio that maximized the effect by using a dose ranging study (1, 8–10, 15, 21, 25). For example, results from studies with CF patients suggested that continuous infusion of ceftazidime was efficacious; however, ceftazidime was not administered to achieve a specific multiple of the MIC for infecting pathogens and the resultant C/MIC ratios ranged from 5 to 13 (10, 15). In a comparison of nonmucoid and mucoid strains, the latter required a higher C/MIC ratio for the $E_{\text{max}}$, as well as for 50 and 90% of the $E_{\text{max}}$. These findings are in keeping with other studies which have suggested that mucoid strains of $P. aeruginosa$ are more resistant to antibiotic action than are their nonmucoid counterparts (3, 13).

In summary, we found 6.6 to be the optimal C/MIC ratio that would maximize activity, as it exhibited an effect that was greater than or equal to 90% of the $E_{\text{max}}$ for all of the strains evaluated. Although the study of larger numbers of CF-derived isolates is necessary before firmer conclusions can be drawn, it appears that at least some CF-derived isolates require higher C/MIC ratios (than previously reported for $\beta$-lactam antibiotics) for maximal killing. Ultimately, host defense status and severity and site of infection will likely need to be taken into consideration in this relationship (18, 26). Our observations may provide a framework for further work to clarify the optimal C/MIC ratio which maximizes the antibiotic effect in vivo and the ultimate implications for dosing, including that with constant infusion.

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REFERENCES


