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In Vitro Pharmacodynamic Properties of Colistin and Colistin Methanesulfonate against *Pseudomonas aeruginosa* Isolates from Patients with Cystic Fibrosis

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The in vitro pharmacodynamic properties of colistin and colistin methanesulfonate were investigated by studying the MICs, time-kill kinetics, and postantibiotic effect (PAE) against mucoid and nonmucoid strains of *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis. Twenty-three clinical strains, including multiresistant strains, and one type strain were selected for MIC determination. Eleven strains were resistant; MICs for these strains were >128 mg/liter. For the susceptible strains, MICs of colistin ranged from 1 to 4 mg/liter, while the MICs of colistin methanesulfonate were significantly higher and ranged from 4 to 16 mg/liter. The time-kill kinetics were investigated with three strains at drug concentrations ranging from 0.5 to 64 times the MIC. Colistin showed extremely rapid killing, resulting in complete elimination at the highest concentrations within 5 min, while colistin methanesulfonate killed more slowly, requiring a concentration of 16 times the MIC to achieve complete killing within 24 h. Colistin exhibited a significant PAE of 2 to 3 h at 16 times the MIC against the three strains after 15 min of exposure. For colistin methanesulfonate, PAEs were shorter at the concentrations tested. Colistin methanesulfonate had lower overall bactericidal and postantibiotic activities than colistin, even when adjusted for differences in MICs. Our data suggest that doses of colistin methanesulfonate higher than the recommended 2 to 3 mg/kg of body weight every 12 h may be required for the effective treatment of *P. aeruginosa* infections in cystic fibrosis patients.

Cystic fibrosis (CF) is one of the most common inherited diseases in western countries and is characterized by recurrent lower respiratory tract infections. *Pseudomonas aeruginosa* is the predominant respiratory pathogen and is isolated from about 80% of patients over their life times (9). *P. aeruginosa* plays an important role in the progressive lung destruction and subsequent respiratory failure that occurs in CF patients (9).

Colistin (or polymyxin E), a polypeptide antibiotic, was first isolated in Japan from *Bacillus polymyxa* subsp. *colistinus* in 1947 and became available for clinical use in 1959. Colistin is a multicomponent antibiotic consisting of several closely related decapeptides with a general structure composed of a cyclic heptapeptide moiety and a side chain acetylated at the N terminus by a fatty acid. The two main components, which have been identified by the composition of their amino acids and fatty acids (1), are colistin A (polymyxin E1) and colistin B (polymyxin E2). They have the same amino acids but a different fatty acid (6-methyloctanoic acid and 6-methylheptanoic acid, respectively). Colistins are bactericidal to gram-negative bacteria by a detergent-like mechanism, interfering with the structure and function of the outer and cytoplasmic membranes of bacteria. This mechanism involves interaction with lipopolysaccharides and phospholipids of the outer membrane and electrostatic interference with the outer membrane by competitively displacing divalent cations (calcium and magnesium) from the negatively charged phosphate groups of membrane lipids (13). The resultant damage to the osmotic barrier leads to leakage of intracellular contents.

Colistin has many characteristics which favor its use in the treatment of multiresistant *P. aeruginosa* isolates from patients with CF, including rapid bactericidal activity, purportedly rare development of resistance, and a narrow spectrum of activity. It is currently enjoying a resurgence, with use via both the inhalational and intravenous routes, in the treatment of chronic infection and acute exacerbations of CF due to multiresistance to other agents. It is used therapeutically as colistin methanesulfonate, which has sulfomethyl moieties attached to the five amine functional groups.

Early clinical reports showed a high incidence of toxicity with colistin, but further studies suggested that this conclusion resulted from inappropriate patient selection, higher-than-recommended doses, and inappropriate monitoring (4). The most appropriate dosing schedule of an antibiotic depends on its pharmacodynamic parameters, including the MICs for the target pathogens, time-kill kinetics, and postantibiotic effect (PAE). Because there are potency differences between colistin and colistin methanesulfonate (11), our studies were carried out with both forms. The aims of our study were to examine the in vitro pharmacodynamic properties, namely, bacterial killing and PAE, of colistin and colistin methanesulfonate and to compare the magnitudes of pharmacodynamic properties with levels achieved in vivo.

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TABLE 1. MICs of colistin and colistin methanesulfonate against *P. aeruginosa* isolates from CF patients

Mucoid status	Susceptibility pattern	No.of strains	Colistin form	MIC (mg/liter)	
				Range	Geometric mean
All strains[a]	Susceptible	13	Sulfate	1–4	3.1
			Na methanesulfonate	4–16	7.1
	Resistant	11	Sulfate	>128	>128
			Na methanesulfonate	>128	>128
Mucoid	Susceptible	5	Sulfate	1–4	2.8
			Na methanesulfonate	4–8	5.6
Nonmucoid[a]	Susceptible	8	Sulfate	1–4	3.3
			Na methanesulfonate	4–16	8.0

[a] Includes type strain ATCC 27853.

MATERIALS AND METHODS

Bacterial strains and antibiotics. Twenty-three clinical isolates of *P. aeruginosa*, both mucoid and nonmucoid strains, were selected from routine clinical isolates from patients with acute exacerbations of CF. Isolates were selected sequentially from different patients as they presented with acute exacerbations; both mucoid and nonmucoid strains may have come from the same patient. Strains were identified by colonial morphology, characteristic pigment production, and resistance to C-390 (7). Sixty-one percent of these strains were resistant to at least three of the following agents: aztreonam, ceftazidime, meropenem, piperacillin, ticarcillin, gentamicin, tobramycin, and ciprofloxacin. In addition, 74% were resistant to tobramycin and 52% were resistant to ticarcillin, the principal agents used in our hospital for acute exacerbations of CF. A type culture of *P. aeruginosa* (ATCC 27853) was also studied. Subcultures were maintained on horse blood agar. Colistin (sulfate) was obtained from Sigma (St. Louis, Mo.). Colistin methanesulfonate (sodium) was obtained from Dumex (Copenhagen, Denmark).

MIC determination. MICs were determined by both broth macrodilution and microdilution in cation-adjusted Mueller-Hinton broth (Oxoid, Hampshire, England) according to NCCLS standards (16). Strains were considered resistant to colistin and colistin methanesulfonate if the MICs were ≥32 mg/liter.

Time-kill kinetics. The time-kill kinetics of four strains, ATCC 27853 and three clinical isolates, two of which were mucoid, were examined. The clinical isolates were selected in order to have a range of MICs within the susceptible category. The MICs of colistin and colistin methanesulfonate, respectively, for the four strains were as follows: ATCC 27853, 4 and 16 mg/liter; 18982, 4 and 8 mg/liter; 19056, 1 and 8 mg/liter; and 20223, 4 and 16 mg/liter. Colistin and colistin methanesulfonate were added to a logarithmic-phase broth culture of approximately 10^6 CFU/ml to yield concentrations of 0, 0.5, 1, 2, 4, 8, 16, 32, and 64 times the MIC for the strain under study. Subcultures for viable counts were performed on nutrient agar (Oxoid) at 0, 5, 10, 15, 20, 25, 30, 45, and 60 min and 2, 3, 4, and 24 h after antibiotic addition. Viable counts were determined after 24 h of incubation of subcultures at 37°C.

PAE. The in vitro PAE was determined by the standard in vitro method (5) for two of the three clinical strains noted above and the ATCC strain with both agents. For each experiment, *P. aeruginosa* (≈10^6 CFU/ml) in logarithmic phase growth was exposed for 15 min (for colistin) or 1 h (for colistin methanesulfonate) in Mueller-Hinton broth (Oxoid) to the antibiotics at concentrations of 0.5, 1, 2, 4, 8, and 16 times the MIC. Fifteen minutes of exposure was used for colistin due to its very rapid bactericidal effect, to ensure that there were adequate numbers of bacteria for sampling at the end of the exposure interval. Antibiotic was removed by twice centrifuging at 3,000 × *g* for 10 min, decanting the supernatant, and resuspending in prewarmed broth. Viable counts were performed at 0, 1, 2, 3, 4, 5, 6, and 24 h on nutrient agar (Oxoid). A growth control was performed in the same fashion but without exposure to antibiotic. The colonies were counted after 24 h of incubation at 37°C. PAE was determined by comparing regrowth of treated and growth control cultures, using the standard formula of the time for the control culture to increase 10-fold subtracted from the time for the treated culture to do the same (5).

Statistical and mathematical analysis. Comparisons of MICs were done using the unpaired *t* test with pooled variance on log-transformed values in Systat version 9.0 (SPSS Inc., Chicago, Ill.). The killing effects of colistin and colistin methanesulfonate at different concentrations were quantified by calculation of the mean survival time over 4 h ($MST_{240\ min}$) using the equation $MST_{240\ min} = AUMC_{0–240\ min}/AUC_{0–240\ min}$, where $MST_{240\ min}$ is in minutes, $AUMC_{0–240\ min}$ is the area under the curve of CFU per milliliter multiplied by time of sampling in minutes from 0 to 240 min, and $AUC_{0–240\ min}$ is the area under the curve of CFU per milliliter from 0 to 240 min. Areas were determined using the trapezoidal rule.

RESULTS

MICs. Table 1 illustrates the MICs obtained with colistin and colistin methanesulfonate. There was essentially no difference in results obtained by macro- versus microdilution testing. For all susceptible strains, colistin was threefold more active (geometric mean MIC, 3.1 mg/liter) than colistin methanesulfonate (geometric mean MIC, 7.1 mg/liter) ($P = 0.004$). The colistin and colistin methanesulfonate MICs, respectively, for the four strains used in time-kill and PAE experiments were as follows: 18982 (mucoid), 4 and 8 mg/liter; 19056 (mucoid), 1 and 8 mg/liter; 20223 (nonmucoid), 4 and 16 mg/liter; and ATCC 27853 (standard nonmucoid strain), 4 and 16 mg/liter.

Time-kill kinetics. In time-kill studies, both colistin and colistin methanesulfonate were bactericidal in a concentration-dependent manner (Fig. 1). With colistin, bacteria became undetectable after 4 h at all concentrations except 0.5 times the MIC. At the highest multiples of the MIC, the bactericidal rate of colistin was so high that at 64 times the MIC no bacteria could be detected within 5 to 10 min for the three strains where sampling was done at these early times. At the same multiples of the MIC, colistin methanesulfonate was less rapidly bactericidal than colistin. It was still rapidly bactericidal at the highest concentrations, with counts falling to undetectable numbers in 1 to 4 h at 16 to 64 times the MIC. Below these concentrations, colistin methanesulfonate failed to eliminate bacteria at 24 h. The control laboratory strain, ATCC 27853, was an exception to this, being rapidly eliminated by concentrations of colistin methanesulfonate of 1 to 64 times the MIC.

$MST_{240\ min}$ values were calculated at different multiples of the MIC for both agents. $MST_{240\ min}$ values of the four strains with colistin ranged from 3.7 to 84 min at 0.5 times the MIC. At 64 times the MIC, $MST_{240\ min}$ values ranged from <0.001 to 0.003 min. With colistin methanesulfonate the $MST_{240\ min}$ ranges were 140 to 185 minutes at 0.5 times the MIC, and 0.002 to 7.4 min at 64 times the MIC. Results for all concentrations tested are plotted in Fig. 2. Both the abscissa and the ordinate of Fig. 2 are plotted on a logarithmic scale to clarify relationships between strains and drugs. Again it is clear that colistin

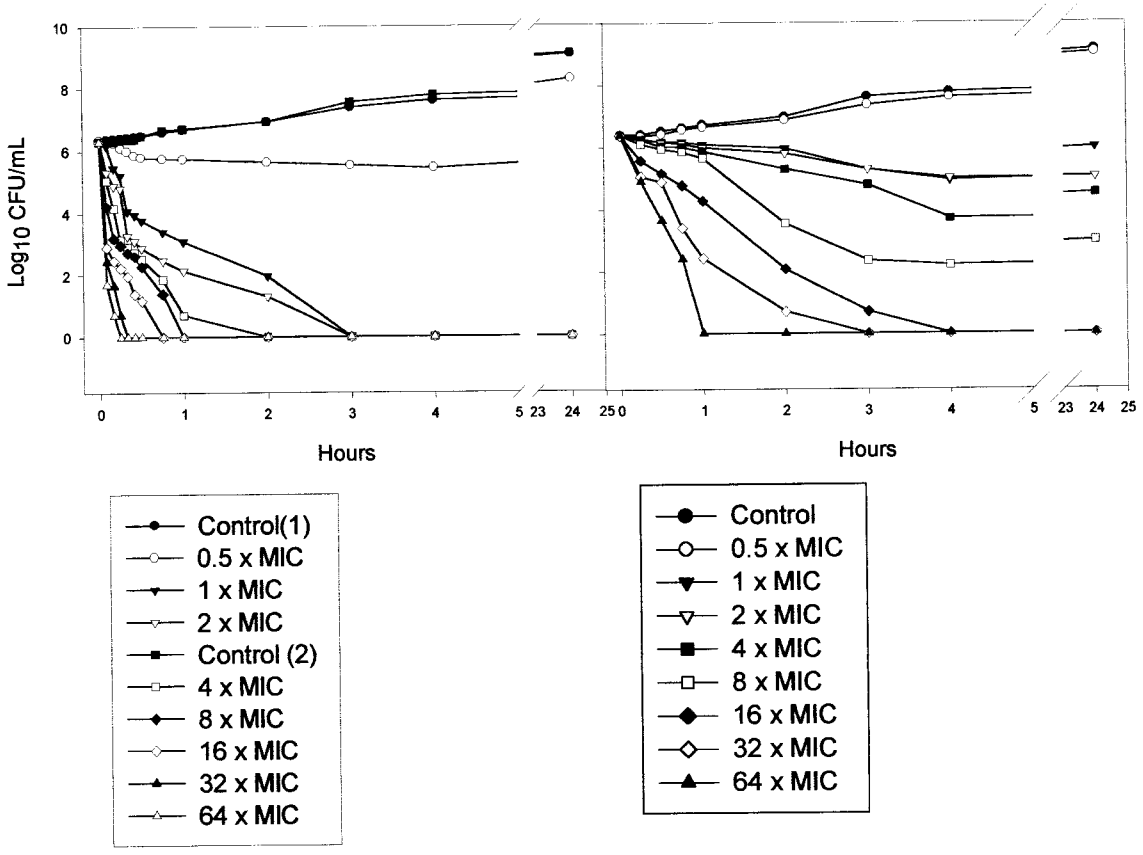

FIG. 1 Killing curves for a mucoid strain of *P. aeruginosa* (18982) by colistin (left) and colistin methanesulfonate (right).

methanesulfonate is less bactericidal than colistin at the same multiples of the MIC. For instance, geometric $MST_{240\ min}$ values of the four strains at 4 times the MIC were 0.07 min for colistin versus 34.7 min for colistin methanesulfonate. Apart from a single nonmucoid strain which appeared to be highly susceptible to the bactericidal action of colistin, the three clinical strains had MSTs similar to those of each other but longer than those of the standard laboratory strain at most concentrations of both agents. A minimum MST was not observed for either agent, suggesting that higher concentrations would have resulted in even more rapid killing. There was also an apparent biphasic bactericidal action for both agents, with lower reductions in MSTs over the 1- to 4-fold MIC range and higher reductions at the 8- to 64-fold MIC range.

PAE. Figure 3 illustrates the PAE of colistin and colistin methanesulfonate on three strains. The rapid bactericidal ac-

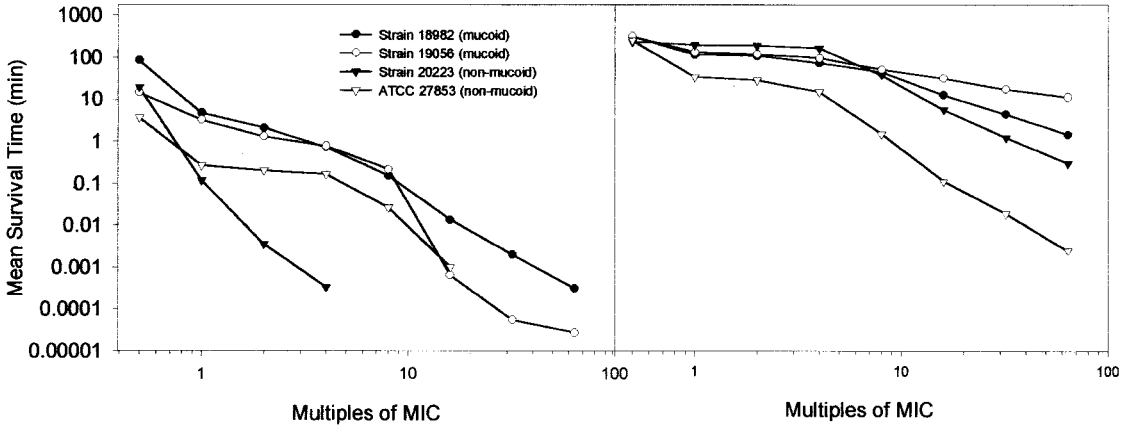

FIG. 2. Bactericidal activities of colistin (left) and colistin methanesulfonate (right) against four strains of *P. aeruginosa* as measured by MST.

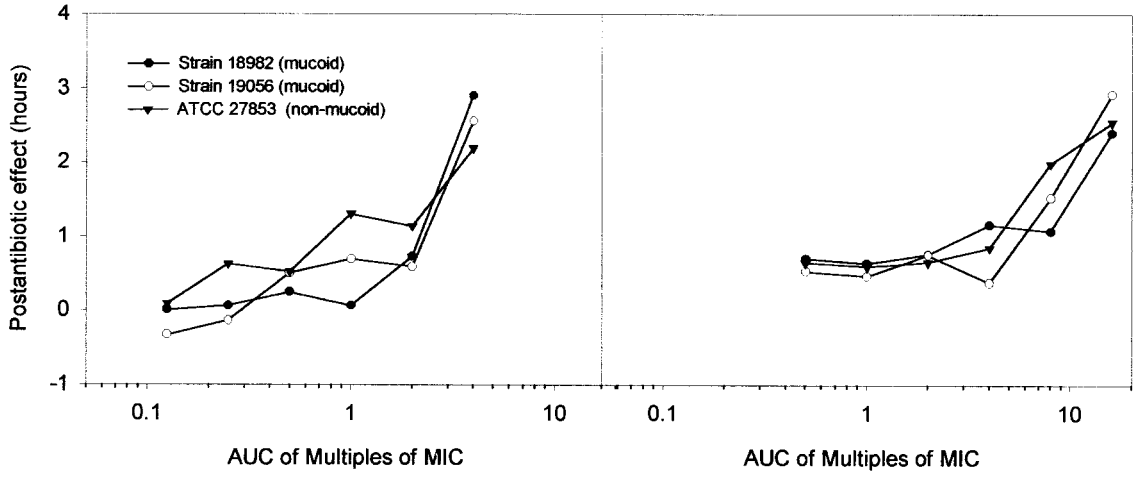

FIG. 3. PAE of colistin (left) and colistin methanesulfonate (right) against three strains of *P. aeruginosa*. AUC of multiple of MIC, product of multiple of MIC and duration of exposure in hours.

tivity of colistin required that exposure be limited to 15 min to ensure sufficient bacteria at the end of exposure for accurate determination of the PAE. For colistin methanesulfonate a more conventional 1-h exposure prior to drug removal was used. Similar profiles were obtained with the three strains examined when compared for drug exposure by multiples of the MIC. In order to make a meaningful comparison between drugs, the product of concentration and time of exposure (area under the curve of drug exposure) was used as the exposure variable. Colistin and colistin methanesulfonate produced a significant PAE (greater than 1 h) against the three strains only at the highest concentrations studied. However, it is clear that the maximum PAE was not achieved for either drug against any strain. Based on drug exposure, colistin was about four times more active than colistin methanesulfonate.

DISCUSSION

Due to the small number of strains included in our study, the prevalence of resistance to colistin in these strains is high compared to the overall rate observed in our patient population. Colistin resistance is prevalent in *P. aeruginosa* strains from our patients (~19%). This level of resistance may result in part from the frequent use of inhaled colistin methanesulfonate in our clinic. Previous suggestions that there is a low risk of resistance emergence with *P. aeruginosa* appear to precede the current widespread use of long-term inhalational therapy with colistin in some CF clinics.

As noted in the past, there is a significant difference between the in vitro activities of colistin and colistin methanesulfonate, with colistin methanesulfonate having lower activity than colistin (8). This difference is important, as colistin may often be used for susceptibility testing in vitro, whereas it is the sodium salt of colistin methanesulfonate that is used clinically. Furthermore, colistin and colistin methanesulfonate are both unequal mixtures of the salts of two components, colistins A and B (1), which may well have different in vitro activities. Even more problematically, the colistin methanesulfonate derivatives are undefined mixtures of the mono-, di-, tri-, tetra-, and pentasubstituted compounds, which may well differ between manufacturers and may completely or partially hydrolyze to colistin in solution (11). That there may be a difference between manufacturers' preparations is supported by a recent study which examined the activity of a colistin methanesulfonate preparation manufactured in the United Kingdom against *P. aeruginosa*, and found lower MICs overall than our study (3) and lower than would be expected by normal variation between strains or laboratories.

In an attempt to quantify the bactericidal activity of colistin in a way that would allow us to directly compare the effects of different antibiotic concentrations, we developed a model-independent parameter of killing, the $MST_{240\ min}$. This parameter is based on statistical moments and is analogous although not identical to the mean residence time parameter used in pharmacokinetics (12). It has the advantage over simple area-based methods for measuring bactericidal response (14) that it is not affected by variations in starting inoculum between experiments. $MST_{240\ min}$ is calculated from the bacterial concentrations (CFU per milliliter) observed in the time-kill curves. It uses the area under the first moment of the curve (i.e. plot of CFU per milliliter times time versus time) divided by the area under the time-kill curve (plot of CFU per milliliter versus time), with both areas calculated by the trapezoidal rule for the 4 h of the experiment. Use of the $MST_{240\ min}$ also allowed us to quantitate the difference in bactericidal activities between the colistin and colistin methanesulfonate.

In our study, colistin demonstrated very rapid concentration-dependent killing. Indeed, to get detailed information on the rapidity of onset of killing, sampling was required every 5 min in the first half-hour. For colistin methanesulfonate, killing was a little slower but there was still had significant concentration-dependent killing. The rapid bactericidal activities of both colistin and colistin methanesulfonate are presumably related to their permeabilizing action on the cell membrane following self-promoted uptake generated by the action of the drug on the outer membrane (13). Others have recently examined the bactericidal activities of colistin methanesulfonate at two con-

centrations (0.5 and 5 mg/liter) and have demonstrated more rapid killing with the higher concentration (14).

Intravenous administration of 2 to 2.5 mg of colistin methanesulfonate per kg of body weight results in peak concentrations in plasma of 6 to 15.5 mg/liter (mean, 9.6) following a 20- to 30-min infusion (2). Higher values (10 to 36 mg/liter [mean, 18.0]) have been seen with doses of 3 mg/kg infused over 5 min in patients with renal failure (7). A recent study in patients with CF using higher doses of 1.8 to 4.3 mg/kg (160 mg) every 8 h (mean dose, 8.8 mg/kg/day) showed a mean peak level of 12.3 mg/liter (4). Peak levels were lower than those anticipated from previous studies and were attributed to larger volumes of distribution frequently noted in patients with CF. Overall, studies have found peak concentrations that are not much higher than the MICs that we noted for isolates of *P. aeruginosa* from CF patients (4 to 16 mg/liter) and substantially less than the 16 times the MIC (64 to 256 mg/liter) required for complete in vitro killing within 24 h by colistin methanesulfonate. The precise concentrations achieved in infected sputum with inhaled colistin are unknown, but they are known to vary somewhat between lung regions (10).

Both colistin and colistin methanesulfonate produced a moderate PAE at higher concentrations. Unfortunately, due to the rapid killing at higher concentrations, a maximum PAE was not observed, and thus maximum values may well be substantially higher. The area under the curve of drug exposure, rather than time of exposure or concentration alone, has been shown to correlate best with the duration of the PAE with beta-lactams (15), and this is likely to be true also for colistin methanesulfonate.

It is clear from our studies that colistin methanesulfonate shows lower potency than colistin, even when corrected for MICs, as demonstrated in our time-kill and PAE experiments. Overall, our findings suggest that doses higher than the usually recommended 2 to 3 mg/kg every 12 h may be necessary, at least for the preparation we were examining, to maximize efficacy of colistin methanesulfonate when given intravenously for the treatment of acute exacerbations of *P. aeruginosa* infection in CF patients. Clearly, this must be weighed against the potential for increased toxicity.

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