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Elucidation of the Pharmacokinetic/Pharmacodynamic Determinant of Colistin Activity against *Pseudomonas aeruginosa* in Murine Thigh and Lung Infection Models▽

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Colistin is increasingly used as last-line therapy against Gram-negative pathogens. The pharmacokinetic (PK)/pharmacodynamic (PD) index that best correlates with the efficacy of colistin remains undefined. The activity of colistin against three strains of *Pseudomonas aeruginosa* was studied in neutropenic mouse thigh and lung infection models. The PKs of unbound colistin were determined from single-dose PK studies together with extensive plasma protein binding analyses. Dose-fractionation studies were conducted over 24 h with a dose range of 5 to 160 mg/kg of body weight/day. The bacterial burden in the thigh or lung was measured at 24 h after the initiation of treatment. Relationships between antibacterial effect and measures of exposure to unbound (*f*) colistin (area under the concentration-time curve [*f*AUC/MIC], maximum concentration of drug in plasma [fC_{max}]/MIC, and the time that the concentration in plasma is greater than the MIC [$fT > MIC$]) were examined by using an inhibitory sigmoid maximum-effect model. Nonlinearity in the PKs of colistin, including its plasma protein binding, was observed. The PK/PD index that correlated best with its efficacy was *f*AUC/MIC in both the thigh infection model ($R^2 = 87\%$) and the lung infection model ($R^2 = 89\%$). The *f*AUC/MIC targets required to achieve 1-log and 2-log kill against the three strains were 15.6 to 22.8 and 27.6 to 36.1, respectively, in the thigh infection model, while the corresponding values were 12.2 to 16.7 and 36.9 to 45.9 in the lung infection model. The findings of this *in vivo* study indicate the importance of achieving adequate time-averaged exposure to colistin. The results will facilitate efforts to define the more rational design of dosage regimens for humans.

Infections caused by multidrug-resistant Gram-negative bacteria, in particular, *Pseudomonas aeruginosa*, are presenting a critical challenge, and they are associated with a high mortality rate if they are not treated promptly and effectively (3). The Antimicrobial Availability Task Force of the Infectious Diseases Society of America, the FDA, and other organizations have highlighted the urgent need to develop new antibiotics with activity against Gram-negative organisms, including *P. aeruginosa* (4, 27). Unfortunately, novel agents with activity against *P. aeruginosa* may not be available in the next 9 to 11 years (24). In the meantime, colistin (polymyxin E) is often the only available active antibiotic and is increasingly used as the last line of therapy against Gram-negative "superbugs" (8, 13, 18).

Colistin is a cationic lipopeptide antibiotic with concentration-dependent bactericidal activity against *P. aeruginosa* and other Gram-negative bacteria (18). It is administered parenterally in the form of its inactive prodrug, colistin methanesulfonate (CMS) (2). CMS/colistin entered clinical use in the late 1950s, but it fell out of favor in the 1970s due to concerns about the potential for nephrotoxicity and neurotoxicity (8, 18). Since CMS/colistin was never subjected to contemporary drug development procedures, there has been a substantial lack of preclinical and clinical pharmacokinetic (PK) and pharmacodynamic (PD) data that may be used to guide the selection of appropriate dosage regimens (18). As a result, the dosage regimens in use today were selected empirically and are not based upon solid PK/PD data. The urgency to establish optimized dosage regimens is highlighted by recent reports of colistin resistance (11, 27).

To assist with the optimization of dosage regimens, the aims of the present study were to elucidate the PK/PD index of colistin, the antibacterially active form of CMS (2), that correlates best with efficacy against *P. aeruginosa* by using mouse thigh and lung infection models and to determine the target values of the index for various magnitudes of antibacterial effects.

(This study was presented, in part, at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, September 2007, abstr. A-5.)

MATERIALS AND METHODS

Bacterial strains and media. Three strains of *P. aeruginosa* were employed: two reference strains, ATCC 27853 and PAO1 (American Type Culture Collec-

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tion, Manassas, VA), and a multidrug-resistant clinical mucoid strain, strain 19056, from a patient with cystic fibrosis. The MICs of colistin (sulfate), as determined by broth microdilution (5), were 1 mg/liter for ATCC 27853 and PAO1 and 0.5 mg/liter for 19056. All strains were stored in tryptone soy broth (Oxoid Australia, West Heildelberg, Victoria, Australia) with 20% glycerol (Ajax Finechem, Seven Hills, New South Wales, Australia) at −80°C in cryovial storage tubes (Simport Plastics, Boloeil, Quebec, Canada). Prior to each experiment, the strains were subcultured onto horse blood agar (Media Preparation Unit, University of Melbourne, Parkville, Victoria, Australia) and incubated overnight at 37°C. A colony was then selected and grown overnight in 10 ml of cation-adjusted Mueller-Hinton broth (CAMHB; Oxoid, Hampshire, England), from which early-logarithmic-phase growth was obtained.

Chemicals and reagents. Colistin sulfate (lot 123K1382; 20,227 U/mg) was purchased from Sigma-Aldrich (St. Louis, MO). Colistin solutions were freshly prepared in water before each experiment, sterilized by passage through a 0.2-μm-pore-size syringe filter (Minisart; Sartorius Stedim Biotech GmbH, Goettingen, Germany), and stored at 4°C prior to use. Under these conditions, colistin is stable for up to 60 days (15). All other chemicals were from the suppliers described previously (16).

Animals. Six-week-old, specific-pathogen-free, female Swiss mice (weight, 22 to 26 g) were obtained from Monash Animal Services (Clayton, Victoria, Australia). The mice were fed normal mouse chow and water *ad libitum* and were housed in Microenvironmental comfort, Isolation, Containment, and Enrichment (MICE) cages (Australian Animal Care Systems Pty Ltd., Keilor, Victoria, Australia) at room temperature (20 to 23°C). The animals were maintained in accordance with the criteria of the Australian code of practice for the care and use of animals for scientific purposes, and the study methodology for use of the animals was approved by the Monash Institute of Pharmaceutical Sciences animal ethics committee.

Neutropenic mouse thigh and lung infection models. Mice were rendered neutropenic by injecting two doses of cyclophosphamide (Endoxan; Baxter Healthcare Pty Ltd., New South Wales, Australia) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) prior to experimental infection (9). Before the inoculum was introduced into the thighs or lungs, the mice were anesthetized briefly with isoflurane by inhalation. Thigh infection was produced by injecting 50 μl of an early-logarithmic-phase bacterial suspension (~10^7 CFU/ml) intramuscularly into each posterior thigh muscle. Lung infection was produced by introducing intranasally, via a 29-gauge needle, 50 μl of a suspension of bacterial cells (~10^8 CFU/ml) in early logarithmic phase. The bacterial suspension was delivered in ~10-μl volumes, which, when they were placed directly in the nares of the anesthetized mouse, were inhaled spontaneously; this procedure was repeated until the animals received the entire 50 μl of the bacterial suspension. Thereafter, the animals were held in a vertical position with their head up for 1 min. In both models, colistin treatment commenced 2 h after inoculation, by which time an infection was reproducibly established (mean bacterial burden for the three strains, ~6.2 to 6.9 $\log_{10}$ CFU per thigh or lung).

Pharmacokinetics of colistin in neutropenic infected mice. Single-dose PK studies were performed with neutropenic thigh-infected mice after subcutaneous administration (dose volume, 0.2 ml) of colistin (sulfate; 5, 10, 20, or 40 mg/kg). The animals were humanely killed, and blood samples were collected from a central vein and placed into heparinized (sodium) tubes for a total of six to eight time points per dose level ($n = 4$ animals per time point) over 10 h. The samples were centrifuged at 10,000 × *g* for 10 min, and the plasma was stored at −80°C until analysis. The concentration of colistin in each plasma sample was determined by a validated reversed-phase high-performance liquid chromatography (HPLC) method (16), with minor modifications. The calibration range was 0.10 to 4.00 mg/liter. If concentrations were above the range of the calibration concentrations, the samples were diluted prior to analysis. Intraday analysis of quality control samples of 0.30 and 3.50 mg/liter ($n = 6$ of each) returned results of 0.29 ± 0.01 mg/liter and 3.44 ± 0.18 mg/liter, respectively. The corresponding interday accuracy and reproducibility were 0.29 ± 0.03 mg/liter and 3.46 ± 0.39 mg/liter, respectively ($n = 6$). The limit of quantification was 0.10 mg/liter.

Plasma binding. The unbound fraction (f_u) of colistin in the plasma of neutropenic mice with thigh infections was measured by using equilibrium dialysis; plasma (1 ml) was dialyzed across a semipermeable membrane (Spectra/Por-2 dialysis membrane, lot 29300; molecular weight cutoff, 12,000 to 14,000) against an equal volume of pH 7.4 isotonic phosphate buffer (0.067 M) for 21 h at 37°C. At the time of dialysis equilibrium, the colistin concentrations in plasma and buffer were measured by HPLC. The f_u of colistin was determined for nine equilibrium plasma colistin concentrations across the range of 0.97 to 30.0 mg/liter. The extensive nonspecific binding to commonly used membranes (17) excluded the use of ultrafiltration in this study.

Pharmacodynamics of colistin in neutropenic mouse thigh and lung infection models. In both the thigh and the lung infection studies, colistin treatment was initiated 2 h following bacterial inoculation. For the thigh-infected animals, the colistin (sulfate) regimens involved subcutaneous doses over a range of 5 to 40 mg/kg; and these doses were administered at intervals of 3, 6, 8, 12, or 24 h. Because of acute toxicity, the largest dose able to be administered at a given time was 40 mg/kg; the range of daily doses was 5 to 160 mg/kg/day. Each dosage regimen involved two mice (i.e., four datum points). The bacterial burden in untreated control animals was determined 2 h after inoculation and 24 h later to define the bacterial load at the start time of colistin therapy (in the treated mice) and the overall level of bacterial growth in the absence of colistin, respectively. The mice were humanely killed 24 h after the initiation of colistin treatment. Both entire posterior thigh muscles, including the bone, from each mouse were aseptically collected and individually homogenized (Polytron tissue homogenizer; Kinematica, Switzerland) with 2 ml sterile normal saline in a polystyrene round-bottom tube (Becton Dickinson). The homogenate was mixed with a further 2 ml of sterile saline and filtered through a sterile filter bag (pore size, 280 μm; Bagpage; Interscience, France). The filtrate was serially diluted with saline, and 50-μl aliquots were plated on nutrient agar plates with a WASP2 spiral plater (Don Whitley Scientific Ltd., England). Following incubation at 37°C for 24 h, the colonies were counted by using a Synbiosis protoCOL colony counter (Don Whitley Scientific Ltd.). The numbers of CFU were counted for each thigh and expressed as the number of $\log_{10}$ CFU per thigh. The lower limit of counting was 100 CFU per thigh (equivalent to one colony per plate). For lung-infected mice, the colistin regimens were as described above, with the exception of a 3-h dosing interval. At 2 h after inoculation (untreated controls) and 24 h later (untreated controls plus colistin-treated mice), the animals were humanely killed. The lungs were collected aseptically and homogenized in 2 ml of normal saline in a polystyrene round-bottom tube. The counts of viable bacteria in the right and left lungs were determined as described above. The lower limit of counting was 220 CFU per lung (equivalent to one colony per plate).

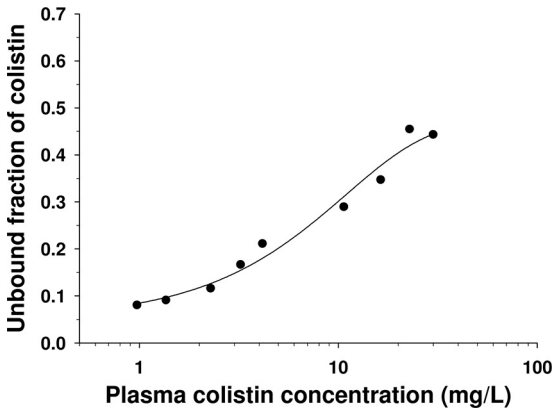

FIG. 1. f_u of colistin against the end-dialysis plasma concentration of colistin in the equilibrium dialysis study. The solid line is a four-parameter model fit obtained by nonlinear least-squares regression ($R^2 = 98\%$) of the experimental data: $f_u = -3.45 + 3.91/(1 + \exp\{-[x - (-21.41)]\}/10.09)$, where x is the plasma colistin concentration.

Data analysis. The time course of total (i.e., protein-bound plus unbound) plasma colistin concentration arising from each single dose (5, 10, 20, or 40 mg/kg) was used to generate the corresponding time course for unbound colistin in plasma. The equilibrium dialysis study showed that the f_u of colistin increased from ~0.05 at <1 mg/liter of colistin in plasma to ~0.45 at a plasma colistin concentration of 30 mg/liter (Fig. 1); this range of total plasma concentrations encompassed those observed in the PK study. The relationship between the f_u of colistin and the end dialysis concentration in the plasma binding study was very well described by a four-parameter sigmoidal equation (coefficient of determination [R^2] = 98%) by the use of the SigmaPlot program (version 8.02; Systat Software Inc., San Jose, CA) (Fig. 1). Thus, the time course for the unbound concentration of colistin in plasma for each single dose was obtained by multiplying the total plasma concentration at each time point by the f_u of colistin at the corresponding total plasma (end dialysis) concentration from the equilibrium dialysis study. The values of the PK parameters were then obtained by noncompartmental analysis (WinNon-

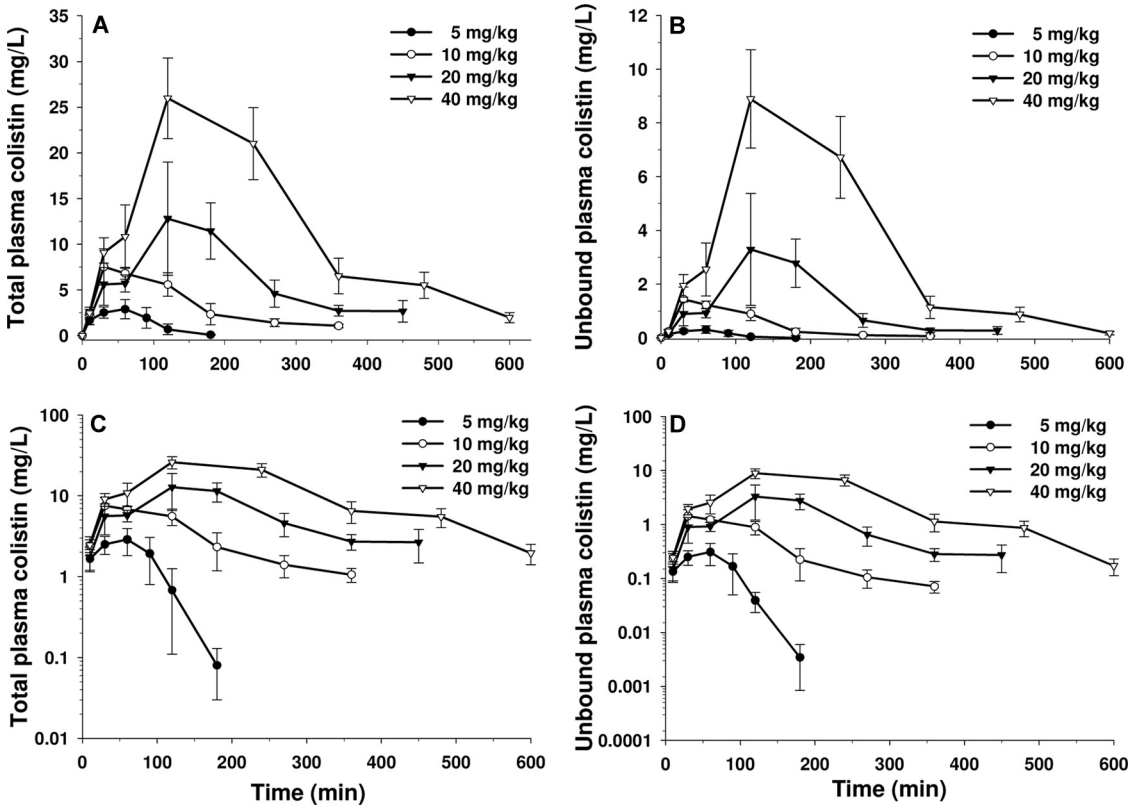

FIG. 2. Total (A) and unbound (B) plasma colistin concentrations versus time after administration of single subcutaneous doses of 5, 10, 20, or 40 mg/kg colistin (sulfate) in neutropenic infected mice. (C and D) Corresponding data on semilogarithmic coordinates. Each symbol represents the mean ± standard deviation for four mice.

lin software, version 5.2.1; Pharsight Corporation). Estimates, referenced to the unbound fraction of colistin in plasma, included the maximum concentration in plasma (C_{max}) and the time of occurrence of C_{max} (T_{max}), the terminal half-life ($t_{1/2}$), the apparent volume of distribution at steady state (V/F, where F is the bioavailability), and apparent body clearance (CL/F).

The superposition principle (10) was applied to the single-dose unbound plasma concentration-time data (described above) to generate the corresponding time course over 24 h, for multiple administrations of the respective dose at the various dosage intervals used in the PK/PD studies. From each resulting multiple-dose profile for unbound plasma colistin over the 24-h treatment period, it was possible to determine fC_{max} (the mean value of the unbound peaks across the 24-h treatment was used), the area under the concentration-time curve (fAUC; determined by use of the trapezoidal rule), and the time that the drug concentration remained above the MIC ($fT >$ MIC) (expressed as a percentage) for each colistin regimen. Subsequently, the PK/PD analysis was conducted by using the inhibitory sigmoid dose-effect model, $E = E_0 - E_{max} \cdot X^{\gamma}/(X^{\gamma} + EC_{50}^{\gamma})$, where E is the measure of effect (i.e., the $\log_{10}$ CFU per thigh or lung at 24 h), X is the value of the relevant PK/PD index (fC_{max}/MIC, fAUC/MIC, or $fT >$ MIC), E_0 is the effect in the absence of drug, E_{max} is the maximal drug effect, EC_{50} is the value of the target PK/PD index required to achieve 50% of E_{max}, and γ is the Hill coefficient of the PK/PD index-effect curve. The relationship between efficacy and each of the three PK/PD indices was determined for each strain by unweighted nonlinear least-squares regression (WinNonlin software, version 5.2.1). R^2 was used to estimate the variance due to regression for each of the PK/PD indices; the goodness of fit was also assessed by visual examination. The magnitude of the most predictive PK/PD index corresponding to various magnitudes of effect (i.e., stasis [suppression of bacterial growth to a level at which the number of viable bacterial cells in the thigh or lung after 24 h of treatment was equivalent to that at the time of initiation of colistin treatment] and 1-, 2-, and 3-$\log_{10}$ kill) was estimated from the use of the inhibitory-effect sigmoid E_{max} model equation and the parameters (E_0, E_{max}, EC_{50}, and γ) obtained from the nonlinear least-squares regression.

RESULTS

Pharmacokinetics of colistin in neutropenic infected mice. The f_u of colistin in the plasma of neutropenic infected mice was highly concentration dependent. The values of f_u varied over an ~10-fold range across the total plasma colistin concentrations observed in the single-dose PK study. A sigmoidal equation described well the relationship between f_u and the total plasma colistin concentration (Fig. 1) and afforded a means of converting the total plasma concentrations to the corresponding unbound concentrations in the single-dose PK study. The total and unbound plasma colistin concentration-time courses in neutropenic infected mice following single subcutaneous doses of 5, 10, 20, or 40 mg/kg are shown in Fig. 2, and the derived values of PK parameters for unbound colistin are presented in Table 1.

Relationships between PK/PD indices and antibacterial activity. (i) Thigh infection model. At the start of treatment (i.e., 2 h after inoculation), the mean ± standard deviation bacterial loads in the mice were 6.14 ± 0.25, 6.35 ± 0.31, and 6.26 ± 0.10 $\log_{10}$ CFU of ATCC 27853, PAO1, and 19056 per thigh, respectively. Over the next 24 h in the untreated control mice, the bacterial numbers increased by 2.33 ± 0.35, 2.35 ± 0.10,

TABLE 1. Values of pharmacokinetic parameters for unbound colistin following subcutaneous administration of single doses (5 to 40 mg/kg) in neutropenic infected mice

Parameter	Result for a single subcutaneous dose of:			
	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
C_{max} (mg/liter)	0.31	1.44	3.29	8.89
T_{max} (min)	60	30	120	120
V/F (L/kg)	10.1	4.63	4.88	3.27
CL/F (ml/min/kg)	169	46.8	29.2	16.4
$t_{1/2}$ (min)	17.9	68.7	80.2	83.6

and 2.01 ± 0.33 $\log_{10}$ CFU/thigh, respectively. The most effective colistin dosage regimens (i.e., at the upper end of the daily doses studied) reduced the bacterial burden, relative to the values obtained 2 h after inoculation, by 3.17 ± 0.14, 4.01 ± 0.11, and 3.69 ± 0.07 $\log_{10}$ CFU/thigh for ATCC 27853, PAO1, and 19056, respectively. No samples had bacterial burdens below the counting limit. The relationships between the effect against ATCC 27853 and each of the PK/PD indices *f*AUC/MIC, *f*C_{max}/MIC, and *fT* > MIC are shown in Fig. 3; similar relationships were observed for PAO1 and 19056 (data not shown). The strongest relationship was observed between the bacterial burden and *f*AUC/MIC, with R^2 being 87% (Fig. 3). The PK/PD model parameter estimates for each strain in the thigh infection model are shown in Table 2.

(ii) Lung infection model. Two hours after inoculation (i.e., at the time of commencement of colistin treatment), the bacterial burdens were 6.64 ± 0.18, 6.92 ± 0.12, and 6.43 ± 0.07 $\log_{10}$ CFU/lung for ATCC 27853, PAO1, and 19056, respectively. In untreated animals, over the next 24 h the bacterial numbers increased by 2.63 ± 0.15, 2.16 ± 0.19, and 2.30 ± 0.18 $\log_{10}$ CFU/lung, respectively. The most effective colistin dosage regimens (i.e., at the upper end of the daily doses studied) resulted in reductions, relative to the bacterial numbers at the start of colistin treatment (i.e., 2 h after inoculation), of 3.58 ± 0.26, 3.09 ± 0.13, and 3.11 ± 0.21 $\log_{10}$ CFU/lung, respectively. No samples had bacterial burdens below the counting limit. The relationships between the effect of colistin against ATCC 27853 and each of the PK/PD indices in the lung infection model are shown in Fig. 4; similar relationships were observed for strains PAO1 and 19056 (data not shown). The strongest relationship was observed between the antibacterial effect and *f*AUC/MIC, with R^2 being 89% (Fig. 4). The PK/PD model parameter estimates for each strain in the lung infection model are shown in Table 2.

Magnitude of *f*AUC/MIC associated with various magnitudes of antibacterial effect. Table 3 shows the values of *f*AUC/MIC required for bacteriostasis and 1-, 2-, and 3-$\log_{10}$ reductions in the bacterial burden. There was little difference across the strains in the *f*AUC/MICs required to achieve a given magnitude of effect in the respective infection models. For the three strains, the *f*AUC/MIC ratios necessary to achieve a 1-$\log_{10}$ reduction in bacterial counts were 1.3- to 1.9-fold (in the thigh infection model) and 2.5- to 3.1-fold (in the lung infection model) larger than the ratios necessary to achieve a static effect. The magnitude of the index required to produce a 2-$\log_{10}$ reduction in bacterial numbers varied among the strains from being 1.8- to 3.3-fold (in the thigh infection model) and 5.9- to 9.1-fold (in the lung infection model) greater than the *f*AUC/MIC required to achieve a bacteriostatic effect.

DISCUSSION

The increased use of CMS/colistin in recent years has been driven by the rise in multidrug resistance among Gram-negative bacteria, including *P. aeruginosa* (8, 13, 18), and the shortage of new antibiotics with activities against these organisms

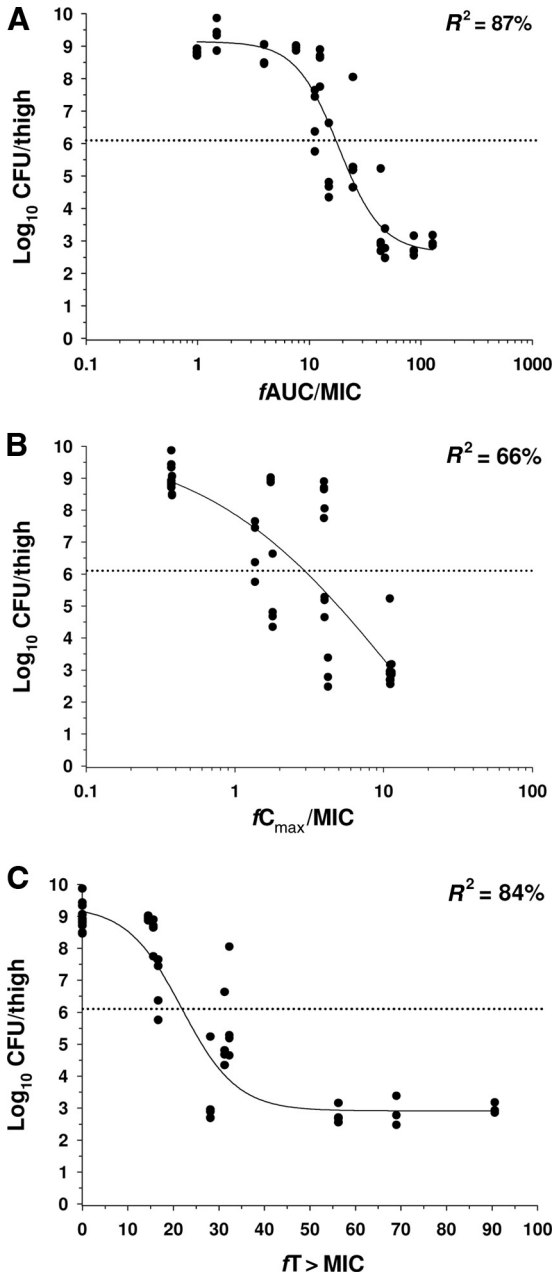

FIG. 3. Relationships for *P. aeruginosa* ATCC 27853 between the $\log_{10}$ CFU per thigh at 24 h and the PK/PD indices *f*AUC/MIC (A), *f*C_{max}/MIC (B), and *fT* > MIC (C). Each symbol represents the datum from a single thigh. The dotted lines represent the mean bacterial burden in the thighs at the start of treatment.

TABLE 2. PK/PD model parameter estimates predicting viable counts at 24 h for the *f*AUC/MIC index for colistin against all three strains of *P. aeruginosa* in the thigh and lung infection models

Model and strain	E_{max} (log_{10} CFU/organ)	E_0 (log_{10} CFU/organ)	EC_{50}	γ
Thigh infection				
ATCC 27853	6.29 (8.2)[a]	8.97 (2.9)	18.8 (11.8)	2.36 (23.1)
PAO1	5.97 (6.1)	8.34 (1.9)	22.7 (12.6)	1.51 (16.2)
19056[b]	6.23 (10.1)	7.98 (3.0)	19.5 (20.4)	1.13 (24.0)
Lung infection				
ATCC 27853	7.58 (16.1)	9.34 (3.4)	16.8 (48.8)	0.61 (20.2)
PAO1	7.36 (26.1)	8.97 (3.4)	31.7 (87.9)	0.54 (30.0)
19056[b]	6.86 (12.7)	8.85 (2.9)	12.4 (40.0)	0.54 (18.4)

[a] Data in parentheses are the percent relative standard error.
[b] Multidrug-resistant mucoid strain.

reaching the clinic (4, 27). Unfortunately, the preclinical and clinical pharmacological information needed to design effective CMS dosage regimens remains inadequate. In the present study, we used two murine infection models to determine, for the first time *in vivo*, the PK/PD index most predictive of activity against *P. aeruginosa* and the magnitude of the predictive index required for various magnitudes of killing effect. Colistin (sulfate) was employed, as this is the active antibacterial entity formed *in vivo* after the administration of CMS (2); and the research was facilitated by use of a specific, accurate, and reproducible HPLC method for determination of the PK properties (16).

Nonlinearity was a feature of the unbound PK of colistin in neutropenic infected mice. Both the apparent volume of distribution and the apparent clearance (both of which were referenced to the unbound plasma concentrations) decreased as the dose increased in the single-dose PK study (Table 1). Colistin has been reported to bind extensively to a range of tissues (6), and the trend observed for the unbound apparent volume of distribution (Table 1) is consistent with an increase in the unbound fraction in tissues possibly resulting from the saturation of tissue binding sites at the higher doses (21). The clearance of colistin is almost exclusively by as yet uncharacterized nonrenal elimination pathways (18); the decrease in unbound clearance with increasing dose (Table 1) is consistent with the saturation of intrinsic clearance pathways (32). It is also possible that increased bioavailability at higher subcutaneous doses may have contributed to the trends observed in the unbound apparent volume of distribution and unbound apparent clearance. The PK nonlinearity in this study was observed over the very wide range of colistin doses (and plasma colistin concentrations) needed to fully characterize the PK/PD relationships; we are not aware of any evidence of the occurrence of such nonlinearity across the more restricted range of colistin concentrations used in human patients. The nonlinear PKs observed in mice were inconsequential in regard to the PK/PD analysis, since they relied on measures of exposure (e.g., fC_{max} and *f*AUC). The superposition principle was applied to the single-dose unbound plasma colistin concentration-time curves to generate the unbound plasma concentrations for the various dosage regimens across the 24-h treatment period. While this approach is strictly applicable to drugs with linear pharmacokinetics, its use in the present study was acceptable since there was little accumulation.

Elucidation of the binding of colistin in plasma from neutropenic infected mice was pivotal to the conduct of the PK/PD study. The f_u of colistin in plasma increased markedly with the total plasma concentration (Fig. 1) across the range of concentrations observed in the PK/PD study. It has recently been demonstrated that colistin binds not only to albumin but also to the acute-phase plasma protein alpha-1-acid glycoprotein (AAG); the increase in f_u with the total plasma colistin con-

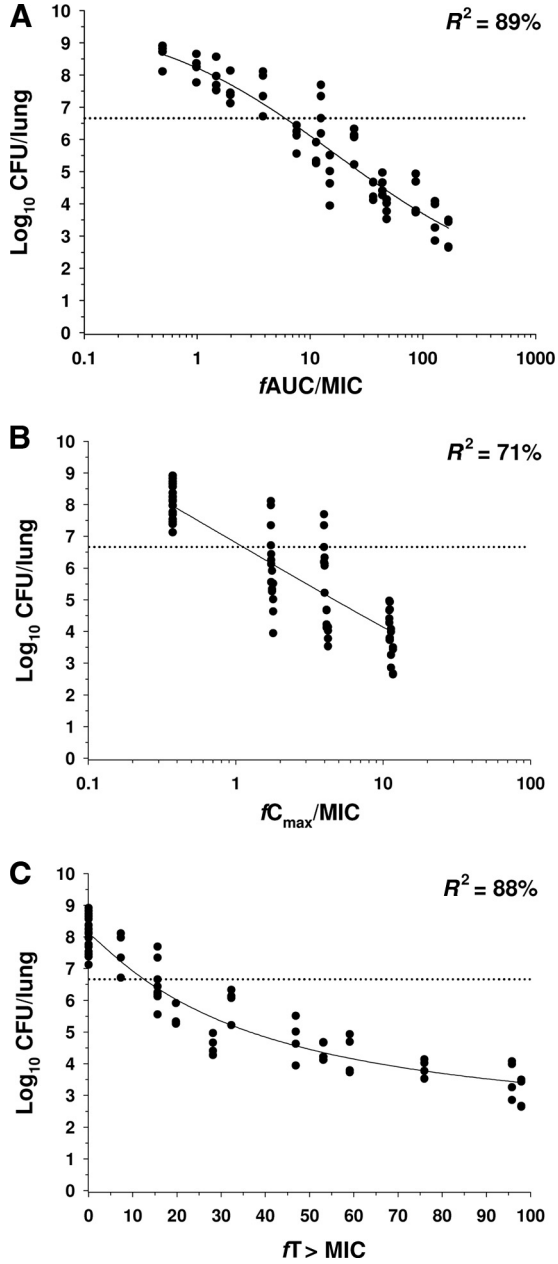

FIG. 4. Relationships for *P. aeruginosa* strain ATCC 27853 between the log_{10} CFU per lung at 24 h and the PK/PD indices *f*AUC/MIC (A), fC_{max}/MIC (B), and $fT >$ MIC (C). Each symbol represents the datum from a single lung. The dotted lines represent the mean bacterial burden in the lungs at the start of treatment.

TABLE 3. Target values of colistin *f*AUC/MIC for stasis and 1-, 2-, and 3-$\log_{10}$ kill against all three *P. aeruginosa* strains in the thigh and lung infection models

Model and kill effect	Target value of colistin *f*AUC/MIC for strain:		
	ATCC 27853	PAO1	19056
Thigh infection			
Static effect	17.3	14.4	8.34
1-$\log_{10}$ kill	22.7	22.8	15.6
2-$\log_{10}$ kill	31.2	36.1	27.6
3-$\log_{10}$ kill	55.1	66.7	53.3
Lung infection			
Static effect	6.43	5.42	4.07
1-$\log_{10}$ kill	15.6	16.7	12.2
2-$\log_{10}$ kill	37.9	45.9	36.9
3-$\log_{10}$ kill	105	135	141

centration (Fig. 1) arises because of the saturation of binding sites as the molar concentration of colistin approaches the molar concentration of AAG (7). The mathematical relationship between f_u and the total plasma concentration (Fig. 1) was the key to converting plasma concentration-time courses for total (i.e., bound plus unbound) plasma colistin to the corresponding time courses for unbound colistin. This, in turn, allowed the PK/PD analysis to be based upon unbound indices (i.e., fC_{max}/MIC, *f*AUC/MIC, and $fT >$ MIC).

The PK/PD analyses for both the thigh and the lung infection models revealed that *f*AUC/MIC was the PK/PD index that was the most predictive of the antibacterial effect of colistin against *P. aeruginosa* (Fig. 3 and 4). In our models, the *f*AUC/MIC ratio appeared to be only slightly more predictive than $fT >$ MIC of *in vivo* bacterial killing, on the basis of the R^2 values, although visual examination of the relationships revealed a relatively large scatter in the relationship for $fT >$ MIC in the thigh model at $fT >$ MIC values of 20 to 30% (Fig. 3). The observation of potent concentration-dependent killing against *P. aeruginosa in vitro* (20) would suggest that the *f*AUC/MIC ratio would be more likely to be predictive of activity *in vivo*. Similar observations in regard to the discrimination between PK/PD indices have been made by use of the mouse thigh infection model with erythromycin and *Streptococcus pneumoniae* and of a long dosing interval (for a mouse) of gentamicin and *Escherichia coli* (29). The lack of production of a significant postantibiotic effect by colistin against *P. aeruginosa* (20) may have been a factor contributing to the correlation with $fT >$ MIC in the present study. That the *f*AUC/MIC ratio was the most predictive index is consistent with the results of a colistin dose-fractionation study conducted against *P. aeruginosa* in an *in vitro* PK/PD model (Bergen et al., submitted). Tam et al. (28) used a limited dose-fractionation design to investigate the PDs of polymyxin B against *P. aeruginosa* in an *in vitro* PK/PD hollow-fiber model; while the study was not designed to identify the PK/PD index predictive of an antibacterial effect, the authors concluded that activity was most likely linked to AUC/MIC (28). In a study reported in abstract form only, Kethireddy et al. (12) investigated the PDs of colistin against *P. aeruginosa* in a neutropenic mouse thigh infection model. It was concluded that once-daily dosing was most effective and that the data were consistent with C_{max}/MIC being the PK/PD index that is the most predictive of efficacy. It should be noted, however, that PK data were not included in that study (12) and the nonlinear PK behavior observed in the present study would be expected to have confounded the interpretation of the data. In the present study with murine thigh and lung infection models (Fig. 3 and 4) and also in an *in vitro* PK/PD model (Bergen et al., submitted), *f*AUC/MIC was superior to fC_{max}/MIC in regard to the ability to predict activity against *P. aeruginosa*. This indicates that time-averaged exposure to colistin is more important than the achievement of high peak concentrations from the administration of large doses less frequently. This is consistent with the concentration-dependent killing observed in an *in vitro* static study (20). In dosage regimen design, consideration must also be given to the potential for the emergence of resistance and also nephrotoxicity and neurotoxicity. It has been shown in an *in vitro* PK/PD model that simulated human dosing regimens incorporating higher doses of colistin administered once daily produced the emergence of resistance at levels higher than those achieved with a thrice-daily regimen involving the administration of essentially the same total daily dose (1). In rats, the intravenous administration of CMS in regimens mimicking twice- and once-daily administration of a clinically relevant daily dose for humans revealed a greater range and severity of renal lesions in the regimen corresponding to once-daily dosing, indicating that the potential for renal toxicity may be greater with extended-interval dosing and large individual doses (31).

There are some important points in regard to the quantitative aspects of the relationships between the antibacterial effect and *f*AUC/MIC for the various strains in the two infection models. First, examination of the data in Table 3 for all three strains in a given infection model reveals very small interstrain variability in the *f*AUC/MIC values associated with bacterial stasis and 1-, 2-, and 3-$\log_{10}$ kill. For example, in the thigh infection model, an *f*AUC/MIC of 27.6 to 36.1 was required for 2-$\log_{10}$ kill across the three strains, while in the lung infection model, the corresponding range of *f*AUC/MIC values was 36.9 to 45.9. Interestingly, these values are remarkably similar to the *f*AUC/MIC of 32.8 required for 2-$\log_{10}$ kill of *P. aeruginosa* ATCC 27853 and PAO1 in an *in vitro* PK/PD model (Bergen et al., submitted). Second, the *f*AUC/MIC required for stasis in the lung infection model was generally lower than that required in the thigh infection model, while the *f*AUC/MIC required for 3-$\log_{10}$ kill in the lung infection model was generally higher than that required in the thigh infection model. Differences between the models in the dynamics of bacterial growth would have contributed to the finding relating to stasis, as evidenced by the larger increase in bacterial numbers that occurred in untreated animals across the 24-h treatment period in the thigh compared with that which occurred in the lung (Fig. 3 and 4). The higher *f*AUC/MIC required for 3-$\log_{10}$ kill in the lung is consistent with the lower Hill coefficients for that model (Table 2). This may reflect differences in bacterial behavior (e.g., different phenotypic states and the relative extent of biofilm formation) between the two sites and/or the somewhat restricted access of colistin to the infection site in the lung relative to the level of access to the infection site in the thigh. Another possible contributor to the differences in the PK/PD targets between the two infection models might be the slightly

higher bacterial burden in the lung compared to that in the thigh at the start of treatment.

In theory, it should be possible to compare the *f*AUC/MIC required for various magnitudes of antibacterial effect observed in the murine models in this study with the *f*AUC/MIC achieved with currently used CMS dosage regimens in patients. Unfortunately, this comparison is not possible at the moment because, although there is increasing information on the total plasma colistin concentrations occurring in CMS-treated patients (14, 19, 22, 25, 26), there is a total lack of knowledge on the unbound plasma concentrations of colistin. As reported here, the f_u of colistin in mouse plasma is highly concentration dependent. Colistin binds to the acute-phase plasma protein AAG (7). Thus, the f_u of colistin in the plasma of infected patients may be expected to be influenced by both the concentration of colistin and that of AAG, the latter of which may be affected by various pathophysiological stresses, including infection (23, 30).

In conclusion, this study has first demonstrated in two mouse infection models that *f*AUC/MIC is the PK/PD index that is the most strongly linked to the antibacterial effect, indicating the importance of achieving adequate time-averaged exposure to colistin across the day. While the PDs of colistin were generally similar between the thigh and the lung infections, there were also some differences possibly linked to differences in bacterial dynamics at the two infection sites and/or the relative access of colistin to these sites. The study has also defined *f*AUC/MIC targets for achieving various magnitudes of bacterial kill. Studies are ongoing to determine the unbound plasma concentrations of colistin in human patients receiving CMS. As that information becomes available, the *f*AUC/MIC targets reported in this study will facilitate the design of dosage regimens to optimize the antibacterial effect.

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