ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2010, p. 3783–3789 Vol. 54, No. 9
0066-4804/10/$12.00 doi:10.1128/AAC.00903-09

Pharmacokinetic/Pharmacodynamic Investigation of Colistin against *Pseudomonas aeruginosa* Using an *In Vitro* Model[▽]

Phillip J. Bergen,[1] Jurgen B. Bulitta,[2,3] Alan Forrest,[2,3] Brian T. Tsuji,[2] Jian Li,[1]† and Roger L. Nation[1]*†

Facility for Anti-infective Drug Development and Innovation, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia[1]; School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, SUNY, Buffalo, New York[2]; and Ordway Research Institute, Albany, New York[3]

Received 1 July 2009/Returned for modification 13 August 2009/Accepted 19 June 2010

Colistin plays a key role in treatment of serious infections by *Pseudomonas aeruginosa*. The aims of this study were to (i) identify the pharmacokinetic/pharmacodynamic (PK/PD) index (i.e., the area under the unbound concentration-time curve to MIC ratio [*f*AUC/MIC], the unbound maximal concentration to MIC ratio [fC_{max}/MIC], or the cumulative percentage of a 24-h period that unbound concentrations exceed the MIC [$fT_{>MIC}$]) that best predicts colistin efficacy and (ii) determine the values for the predictive PK/PD index required to achieve various magnitudes of killing effect. Studies were conducted in a one-compartment *in vitro* PK/PD model for 24 h using *P. aeruginosa* ATCC 27853, PAO1, and the multidrug-resistant mucoid clinical isolate 19056 muc. Six intermittent dosing intervals, with a range of fC_{max} colistin concentrations, and two continuous infusion regimens were examined. PK/PD indices varied from 0.06 to 18 for targeted fC_{max}/MIC, 0.36 to 312 for *f*AUC/MIC, and 0 to 100% for $fT_{>MIC}$. A Hill-type model was fit to killing effect data, which were expressed as the $\log_{10}$ ratio of the area under the CFU/ml curve for treated regimens versus control. With fC_{max} values equal to or above the MIC, rapid killing was observed following the first dose; substantial regrowth occurred by 24 h with most regimens. The overall killing effect was best correlated with *f*AUC/MIC ($R^2 = 0.931$) compared to fC_{max}/MIC ($R^2 = 0.868$) and $fT_{>MIC}$ ($R^2 = 0.785$). The magnitudes of *f*AUC/MIC required for 1- and 2-$\log_{10}$ reductions in the area under the CFU/ml curve relative to growth control were 22.6 and 30.4, 27.1 and 35.7, and 5.04 and 6.81 for ATCC 27853, PAO1, and 19056 muc, respectively. The PK/PD targets identified will assist in designing optimal dosing strategies for colistin.

Globally there is a growing threat from the emergence of multidrug-resistant (MDR) microorganisms (38), especially among a number of important Gram-negative bacterial pathogens (16, 29, 38). Colistin (polymyxin E) still retains significant activity against many of these MDR Gram-negative pathogens, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, which often leaves it as the only therapeutic option available (19, 26). With very few new chemical entities against Gram-negative infections in the drug development pipeline (29, 30, 38), particularly against *P. aeruginosa* (38), the use of colistin, a once-neglected antibiotic, has increased dramatically over the last 5 years (11, 26).

Colistin is available commercially as colistin sulfate (hereafter referred to as colistin) and sodium colistin methanesulfonate (CMS), which is administered parenterally. CMS is an inactive prodrug of colistin (3) and, after parenteral administration, colistin is formed *in vivo* (21, 27, 33). Despite its newfound importance in therapy, there is a dearth of information on the pharmacokinetic (PK) and pharmacodynamic (PD) properties of colistin, a situation of significant concern given that resistance to colistin is beginning to emerge (1, 15, 18, 26, 28). Thus, the aims of the present study were to utilize an *in vitro* PK/PD model to (i) identify the PK/PD index (i.e., the area under the unbound concentration-time curve to MIC ratio [*f*AUC/MIC], the unbound maximal concentration to MIC ratio [fC_{max}/MIC], or the cumulative percentage of a 24-h period that unbound concentrations exceed the MIC [$fT_{>MIC}$]) that best predicts colistin efficacy and (ii) determine the magnitude of the predictive PK/PD index required to achieve various magnitudes of killing effect.

(Parts of the present study were presented at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Washington, DC, 25 to 28 October 2008, and at the Second American Conference on Pharmacometrics, Mashantucket, CT, 4 to 7 October 2009.)

MATERIALS AND METHODS

Bacterial strains and media. Three strains of *P. aeruginosa* were used in the present study: two reference strains, ATCC 27853 and PAO1 (American Type Culture Collection, Rockville, MD), and an MDR mucoid clinical isolate, 19056 muc. The MICs of colistin, as determined by broth microdilution (6), were 1 μg/ml for ATCC 27853 and PAO1 and 0.5 μg/ml for 19056 muc. All MIC determinations were performed in three replicates on separate days. Storage was in tryptone soy broth (Oxoid, Basingstoke, Hampshire, England) with 20% glycerol (Ajax Finechem, Seven Hills, New South Wales, Australia) at −80°C in cryovials (Simport Plastics, Boloeil, Quebec, Canada).

Chemicals and reagents. Colistin sulfate was purchased from Sigma-Aldrich (lot 095K1048, 20,195 U/mg; St. Louis, MO). Immediately prior to each experiment, colistin stock solutions were prepared by using Milli-Q water (Millipore Australia, North Ryde, New South Wales, Australia), sterilized by filtration with a 0.22-μm-pore-size Millex-GP filter (Millipore, Bedford, MA), and then stored

* Corresponding author. Mailing address: Facility for Anti-infective Drug Development and Innovation, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville Campus, 381 Royal Parade, Parkville, Victoria 3052, Australia. Phone: 61 3 9903 9061. Fax: 61 3 9903 9629. E-mail: roger.nation@pharm.monash.edu.au.

† J.L. and R.L.N. contributed equally to this study.

▽ Published ahead of print on 28 June 2010.

TABLE 1. Colistin dosage regimens and sampling times in the *in vitro* PK/PD model[a]

Parameter	Dosage regimen[b]					
	3 h	4 h	8 h	12 h	24 h	CI
Target fC_{max} (μg/ml)						
ATCC 27853	0.50, 1.5	1.0, 2.0, 3.5, 9.0, 18	3.0*	2.0, 4.5*, 9.0	0.20, 0.25, 0.30, 0.50, 1.0, 1.5, 3.5, 9.0*, 18	1.0, 4.5
PAO1			3.0	3.0, 9.0, 18	0.06, 0.13, 0.25, 0.50, 1.0, 2.0	1.0
19056 muc[c]			3.0*	0.06, 0.13, 0.15, 0.30, 1.0, 1.5, 4.5*	0.03, 0.06, 0.13, 0.15, 0.25, 0.35, 0.50, 1.0, 1.5, 2.0, 9.0*	
Sampling times (h) for microbiological measurements	0, 1, 2, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22, 24	0, 1, 2, 4, 5, 6, 8, 9, 12, 13, 16, 17, 20, 21, 24	0, 1, 2, 4, 6, 8, 9, 16, 17, 24	0, 1, 2, 4, 6, 8, 12, 13, 24	0, 1, 2, 4, 6, 8, 24	0, 1, 2, 4, 6, 8, 12, 24

[a] Dosage regimens involved intermittent administration at the dosage intervals indicated (3 to 24 h) to achieve target fC_{max} or constant concentrations simulating continuous infusion (CI).
[b] *, results taken from the first 24 h of a previous study (2).
[c] For this strain, an additional 6-hourly regimen with an fC_{max} of 1 μg/ml was performed.

at 4°C before use; colistin is stable under these conditions (22). All other chemicals were from suppliers previously described (23).

Binding of colistin in growth medium. The binding of colistin in cation-adjusted Mueller-Hinton broth (CAMHB; Ca^{2+} at 23.0 μg/ml and Mg^{2+} at 12.2 μg/ml; Oxoid, Hampshire, England) was measured by equilibrium dialysis using a Perspex dialysis cell unit containing two chambers (1 ml in each chamber) separated by a semipermeable membrane (Spectra/Por-2, lot 29300; Spectrum Laboratories, Rancho Dominguez, CA). Colistin (sulfate) was spiked into CAMHB (donor chamber) to achieve concentrations of 10 and 30 μg/ml and dialyzed at 37°C against the same volume of isotonic phosphate buffer (0.067 M, pH 7.3) (acceptor chamber); samples were prepared in triplicate. Samples of CAMHB and buffer were removed from each reservoir after 24 h (shown in preliminary studies to be the time required for equilibration) and stored at −80°C until analyzed as described below. The fraction of colistin unbound in CAMHB (f_u) was calculated as follows: (acceptor colistin concentration)/(donor colistin concentration).

***In vitro* PK/PD model and colistin dosing regimens.** Experiments to examine the PK/PD indices driving the microbiological response to colistin were conducted over 24 h using a one-compartment *in vitro* PK/PD model (2). Briefly, the system consisted of four sealed containers (compartments), each containing 100 ml of CAMHB at 37°C and a magnetic stir bar to ensure adequate mixing. One compartment acted as a control to define growth dynamics in the absence of colistin, whereas colistin was delivered into the remaining compartments to achieve the desired intermittent injection or continuous infusion regimens (see below).

Prior to each experiment, strains were subcultured onto horse blood agar (Media Preparation Unit, The University of Melbourne, Parkville, Australia) and incubated at 35°C overnight. One colony was then selected and grown overnight in 10 ml of CAMHB, from which early-log-phase growth was obtained. A 1.0-ml aliquot of this early-log-phase bacterial suspension was inoculated into each compartment at the commencement of each experiment to yield approximately 10^6 CFU/ml.

Both intermittent and continuous infusion dosage regimens of colistin were examined. For dosage regimens involving intermittent administration of colistin, sterile drug-free CAMHB from a central reservoir was pumped through the system at a predetermined rate, displacing CAMHB from each compartment, thus simulating colistin elimination (half-life [$t_{1/2}$] = 4 h) in healthy volunteers (12) and people with cystic fibrosis (21, 34, 35). Flow rates were calibrated prior to experiments and monitored throughout to ensure the system was performing optimally. The appropriate loading dose of colistin (sulfate) was injected into each treatment compartment following bacterial inoculation to achieve the desired steady-state C_{max} ($\equiv fC_{max}$; see Results); intermittent maintenance doses were given at appropriate intervals to achieve the same fC_{max} as after the respective loading dose. This simulated steady-state PK with intermittent dosing. For the continuous-infusion regimens, colistin was spiked into the CAMHB within the central reservoir prior to initiation of the experiment such that all media flowing through the system (with the exception of the growth control compartment) contained a constant concentration of colistin. For both intermittent and continuous regimens, serial samples were collected aseptically, as shown in Table 1, for viable counting and determination of colistin concentrations. Viable counting was performed by using a Whitley automatic spiral plater (WASP; Don Whitley Scientific, West Yorkshire, United Kingdom) and a ProtoCOL colony counter (Synbiosis, Cambridge, United Kingdom); the limits of counting and quantification of the procedure were 20 and 400 CFU/ml, respectively, as specified in the ProtoCOL manual.

Dosing regimens were selected to maximally differentiate among the PK/PD indices under investigation (fAUC/MIC, fC_{max}/MIC, and $fT_{>MIC}$). Overall, six intermittent dosing intervals (every 3, 4, 6, 8, 12, and 24 h) were examined with fC_{max} varied across each schedule; two continuous infusion (CI) regimens were also examined (Table 1). In all, 85 treatments across 37 different combinations of dosage frequency and fC_{max} were examined for the three strains. PK/PD indices varied from 0.06 to 18 for targeted fC_{max}/MIC, from 0.36 to 312 for fAUC/MIC, and from 0 to 100% for $fT_{>MIC}$. The range of fC_{max} used extended to greater than that seen in humans (21) to explore the complete dose-response relationship from essentially no effect to maximum effect.

Quantification of colistin in CAMHB and buffer. Samples (250 μl) collected from the *in vitro* PK/PD model and equilibrium dialysis experiments were analyzed as previously described (2). Concentrations of colistin were measured by using HPLC with derivatization and fluorescence detection (24) with an assay range for colistin sulfate of 0.10 to 6.00 μg/ml; samples were diluted when the expected colistin concentrations were higher than the upper limit of quantification. Analysis of quality control (QC) samples with nominal concentrations of 0.40, 4.00, 9.00, and 18.00 μg/ml (the latter two QC samples required dilution) demonstrated that the accuracy and coefficients of variation were within 15%.

Determination of predictive PK/PD index. The following PK/PD indices were determined for each dosage regimen: fAUC/MIC, fC_{max}/MIC, and $fT_{>MIC}$. The area under the unbound colistin concentration-versus-time curves over 24 h (fAUC; μg · h/ml) was determined by equation 1 (see below), where n is the number of dosing intervals in the 24-h period, and k is the elimination rate constant (0.17 h^{-1}, corresponding to a 4-h half-life). The percentage of time that unbound concentrations exceeded the MIC ($fT_{>MIC}$) was determined by equation 2. Targeted fC_{max}, trough (fC_{min}), and k values were used for all calculations.

$$f\text{AUC} = n \cdot (fC_{max} - fC_{min})/k \quad (1)$$

$$fT_{>MIC} = n \cdot \ln(fC_{max}/\text{MIC})/k/24 \cdot 100\% \quad (2)$$

The area under the curve (AUC_{CFU}) of the time course profile of bacterial numbers (CFU/ml from 0 to 24 h) was calculated by using the linear trapezoidal rule. The killing effect (drug effect) chosen as the measure of efficacy (E) was quantified by the log ratio area method, which compensates for bacterial loss from our model (14):

$$E = \log_{10}\frac{AUC_{CFU(treatment)}}{AUC_{CFU(growth\ control)}} \quad (3)$$

The relationship between killing effect (E) and each of the three PK/PD indices was analyzed by using the Hill equation with a baseline and an inhibitory effect:

$$E = E_0 - \frac{E_{\max} \cdot x^{\gamma}}{EI_{50}^{\gamma} + x^{\gamma}} \quad (4)$$

where E is the observed effect, E_0 is the baseline effect in the absence of colistin, $E_{\max}$ is the maximal effect, x is the PK/PD index under investigation, EI_{50} is the magnitude of the PK/PD index producing 50% of E_{max}, and γ is the sigmoidicity coefficient (Hill's constant).

The parameters of equation 4 were estimated by three different approaches: (i) uniformly weighted least-squares estimation in WinNonlin Professional (version 5.2.1; Pharsight Corp., Mountain View, CA), (ii) a pooled fitting approach based on maximum-likelihood estimation in NONMEM VI (level 1.2), and (iii) nonlinear mixed-effects modeling in NONMEM VI using the first-order conditional estimation method. An additive error model on a log scale was used for approaches ii and iii. The data for all regimens were fit separately for each of the nine combinations of strains and PK/PD indices for approaches i and ii. The data for all regimens and all three strains were comodeled for approach iii for each of the PK/PD indices. Median estimates and 90% nonparametric confidence intervals (5 to 95% percentile) were determined via nonparametric bootstrapping as described previously using 1,000 replicates for each analysis of approaches ii and iii (4). For each bootstrap data set, 44 regimens were randomly chosen for strain ATCC 27853, 26 regimens were randomly chosen for 19056 muc and 15 regimens were randomly chosen for PAO1. The P values for two-sided nonparametric comparisons between PK/PD indices were computed based on the pairwise differences in objective function values between two PK/PD indices for each of the 1,000 bootstrap replicates. Determination of the PK/PD index best characterizing killing effect was assessed by the coefficient of determination (R^2), NONMEM's objective function ($-2 \cdot$ log-likelihood), and visual inspection of the observed versus fitted effect plots.

The drug exposure ($x_{\text{nn log10 effect}}$) required for 1- or 2-$\log_{10}$ reduction in the area under the CFU/ml curve relative to growth control (equation 5) and the drug exposure (EI_{90}) causing 90% of maximal effect (equation 6) were calculated as follows:

$$x_{\text{nn log}_{10}\text{ reduction}} = \frac{EI_{50}}{\left(\frac{E_{\max}}{nn} - 1\right)^{\frac{1}{\gamma}}} \quad (5)$$

$$EI_{90} = \frac{EI_{50}}{\left(\frac{1}{0.9} - 1\right)^{\frac{1}{\gamma}}} \quad (6)$$

The desired extent of the $\log_{10}$ reduction (nn) enters equation 5 as a positive number. Equation 5 yields exposure targets only if $E_{\max}$ is larger than nn.

RESULTS

Binding of colistin in CAMHB. The fractions of colistin unbound in CAMHB (f_u) at equilibrium, with initial concentrations for colistin sulfate of 10 and 30 μg/ml, were 0.96 and 0.95, respectively, indicating practical equivalence of total and unbound concentrations.

PK validation. The mean ± the standard deviation (SD; n = 58) of the absolute percentage relative differences between targeted and achieved colistin $fC_{\max}$ concentrations as determined by high-pressure liquid chromatography (HPLC) was 9.76 ± 14.2, and the mean of the percentage relative differences was −4.64 ± 16.7. The observed mean $t_{1/2}$ for the simulated intermittent dosage regimens was 4.06 ± 0.46 h (n = 47) for the targeted value of 4 h; since the $fC_{\min}$ for some dosage regimens was below the lower limit of quantification of the HPLC assay (0.10 μg/ml), $t_{1/2}$ was not directly measured in all experiments.

Bacterial killing of *P. aeruginosa* in the *in vitro* PK/PD model. Representative killing profiles for each strain are shown in Fig. 1. The initial inocula in control and treatment compartments (mean ± the SD) were 6.21 ± 0.09 (n = 15) and 6.18 ± 0.14 (n = 44) $\log_{10}$ CFU/ml for ATCC 27853, 6.39 (n = 2) and 6.29 ± 0.13 (n = 15) $\log_{10}$ CFU/ml for PAO1, and 5.88 ± 0.41 (n = 4) and 6.08 ± 0.32 (n = 26) $\log_{10}$ CFU/ml for 19056 muc. After 24 h, bacterial numbers in control compartments had increased to 8.06 ± 0.20 (n = 15) $\log_{10}$ CFU/ml for ATCC 27853, 8.21 (n = 2) $\log_{10}$ CFU/ml for PAO1, and 7.74 ± 0.07 (n = 4) $\log_{10}$ CFU/ml for 19056 muc. For all strains there was early dosage-dependent killing, followed by regrowth to various extents (Fig. 1).

Relationships between killing effect and PK/PD indices. Since the differences in the initial inocula were small (see above), we elected not to standardize AUC_{CFU} by dividing by initial inoculum when calculating the killing effect. Parameter estimates for modeling approaches i, ii, and iii were consistent for all three PK/PD indices and robust for fAUC/MIC and $fC_{\max}$/MIC; approach iii yielded the most robust estimates for $fT_{>MIC}$. Modeling by approach iii (Table 2) indicated that between-strain variability was largest for EI_{50} and negligible for the other parameters. The confidence intervals for EI_{50} indicated significantly lower values for strain 19056 muc. The relationships between killing effect and fAUC/MIC, $fC_{\max}$/MIC, or $fT_{>MIC}$ are shown in Fig. 2. Of the three indices, fAUC/MIC best described the killing effect (R^2 = 0.931; Fig. 2A); the relationship between the killing effect and $fC_{\max}$/MIC had a lower R^2 of 0.868 (Fig. 2B). A poorer relationship existed between the killing effect and $fT_{>MIC}$, where a high degree of scatter and systematic deviations from the curve fit was observed (R^2 = 0.785; Fig. 2C). Median (nonparametric 90% confidence interval) objective function values from modeling approach iii were as follows: −79.6 (−111 to −54.4) for fAUC/MIC, −28.3 (−72.1 to 2.9) for $fC_{\max}$/MIC, and 16.4 (−35.9 to 47.5) for $fT_{>MIC}$. The objective function was significantly lower for fAUC/MIC compared to $fC_{\max}$/MIC (P = 0.050, two-sided testing; from 1,000 nonparametric bootstrap replicates) and for fAUC/MIC compared to $fT_{>MIC}$ ($P < 0.01$). Differences between $fC_{\max}$/MIC and $fT_{>MIC}$ were not significant (P = 0.2).

The magnitudes of the fAUC/MIC index required for 1- and 2-$\log_{10}$ reduction in the area under the CFU/ml curve relative to growth control for each strain are shown in Table 3. Near-maximal killing was achieved with fAUC/MIC ratios of approximately 40, 50, and 9 for ATCC 27853, PAO1, and 19056 muc, respectively (Table 3 and Fig. 2A).

DISCUSSION

Colistin, which first became clinically available more than 50 years ago, was never subjected to many of the drug development procedures required of new drugs today. As a consequence, current dosage regimens for CMS/colistin are chosen empirically, and much is still to be learned about the PK, PD, and the PK/PD index that best correlates with antibacterial activity of colistin (26). Such information is important for rational design of optimal dosing strategies.

Previous animal or *in vitro* pharmacodynamic studies have reported regrowth of *P. aeruginosa* with a range of colistin (2, 17) or polymyxin B (39) dosage regimens. Similarly, this was generally observed in the present study despite unbound colistin concentrations far in excess of clinically achievable concentrations in some experiments. Even for the regimens that achieved very extensive bacterial killing after the first dose, substantially less net killing occurred after subsequent doses

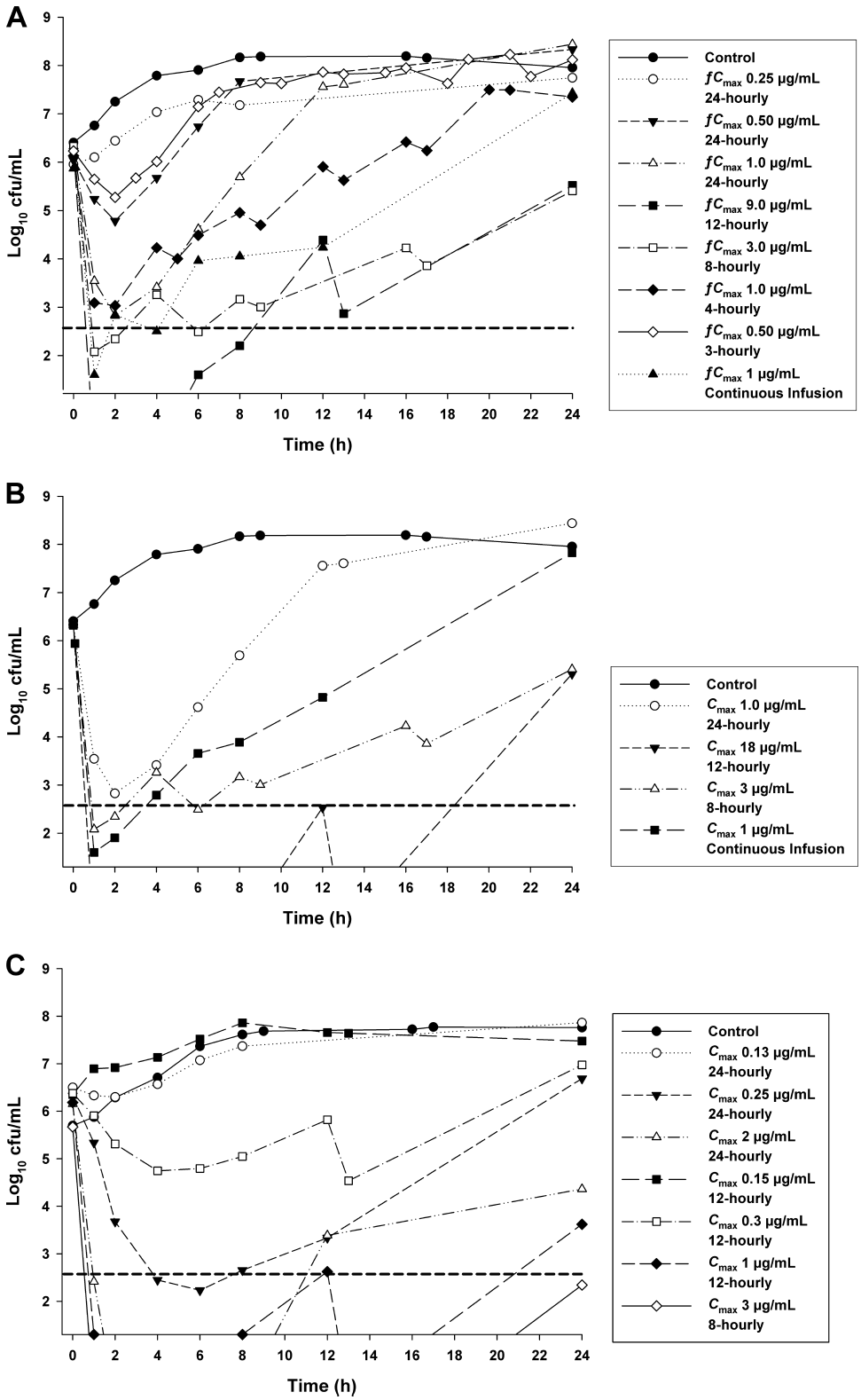

FIG. 1. Typical microbiological responses observed in the *in vitro* PK/PD model simulating the colistin pharmacokinetics of different dosage regimens using ATCC 27853 (MIC = 1 μg/ml) (A), PAO1 (MIC = 1 μg/ml) (B), and the MDR clinical isolate 19056 muc (MIC = 0.5 μg/ml) (C). The *y* axis starts from the limit of counting, and the limit of quantification is indicated by the horizontal broken line.

TABLE 2. Median parameter estimates from 1,000 bootstrap replicates for each of the three PK/PD indices[a]

PK/PD index	Strain	Median parameter estimates (90% nonparametric confidence intervals)			
		E_0	E_{max}	EI_{50}	γ
*f*AUC/MIC	ATCC 27853	–0.232 (–0.349 to −0.139)	3.05 (2.88–3.21)	26.4 (23.8–28.9)	4.77 (3.20–7.27)
	PAO1			31.2 (28.6–35.4)	
	19056 muc			5.91 (4.60–12.5)	
fC_{max}/MIC	ATCC 27853	–0.110 (–0.319 to 0.025)	3.12 (2.81–3.38)	1.82 (1.54–2.25)	3.13 (2.08–12.2)
	PAO1			2.48 (1.74–3.27)	
	19056 muc			0.834 (0.645–1.27)	
$fT_{>MIC}$	ATCC 27853	–0.365 (–0.558 to −0.212)	3.07 (2.68–3.39)	39.6 (31.9–47.1)	2.13 (1.26–4.30)
	PAO1			68.4 (41.0–135)	
	19056 muc			13.6 (9.90–21.7)	

[a] Data for all three strains were comodeled for each PK/PD index (approach iii, see Materials and Methods). Initial models with between-strain variability for all four parameters showed that the variability in E_0, E_{max}, and γ was negligible. Since exclusion of the variability for these three parameters did not affect the objective function significantly, the final model only included between-strain variability for EI_{50}.

(Fig. 1). We have shown previously the presence of resistant subpopulations after a 72-h exposure to colistin in the same *in vitro* PK/PD model (2). We are currently developing a mechanism-based mathematical model that can describe and predict the time course of bacterial growth and killing and which incorporates the emergence of multiple bacterial populations with various colistin susceptibilities.

Three previous studies have addressed issues around the exposure-response relationships for polymyxins. Using a limited dose fractionation design, Tam et al. (39) investigated the PD of polymyxin B against *P. aeruginosa* in an *in vitro* PK/PD hollow-fiber model and suggested that activity was most likely linked to AUC/MIC. The study by Tam et al. (39), however, was not specifically designed to examine the relationship between efficacy and each PK/PD index. In a study of colistin against *P. aeruginosa* in a neutropenic mouse infection model, Ketthireddy et al. (17) concluded that once-daily dosing was most effective and that the data were consistent with C_{max}/MIC being the PK/PD index most predictive of efficacy; PK data, however, were not included in that study. In neutropenic mouse thigh and lung infection models, Dudhani et al. (10) found that *f*AUC/MIC was the index most predictive of efficacy. In the present study, we used a much larger dose fractionation design in an *in vitro* dynamic model to distinguish between PK/PD indices determining colistin efficacy. A Hill-type model was fit to the data using an area-based method whereby all CFU/ml versus time data for each regimen were taken into account. This approach allowed for a measure of the time-averaged drug effect and has been implemented in a previous investigation with vancomycin against *Staphylococcus aureus* (14). The analysis demonstrated that *f*AUC/MIC was most closely correlated with bacterial killing (Fig. 2). Estimates of PD parameters were precise and consistent between all three estimation approaches.

In order to design dosage regimens rationally, it is necessary to know not only which PK/PD index is most predictive of bacterial killing but also the magnitude of that index needed to achieve various extents of kill (7). In the present study, respective values of *f*AUC/MIC of ~25 and 35 for the reference strains were required to achieve 1- and 2-log reductions in the area under the CFU/ml curve relative to growth control (Table 3). These results are in extremely good agreement with those obtained by Dudhani et al. (10) in the neutropenic mouse thigh infection model against the same two strains (*f*AUC/MIC values of ~23 and 34 for 1- and 2-log reductions, respectively). Interestingly, the corresponding *f*AUC/MIC values for the MDR clinical isolate were somewhat lower in our *in vitro* model compared to the neutropenic mouse thigh infection model (~6 and 7 *in vitro* compared to ~16 and 28 *in vivo* for 1- and 2-log reductions, respectively). The explanation for this difference is not known but may relate to differences in growth dynamics of this mucoid strain between *in vitro* and *in vivo* systems. We acknowledge the presence of a washout effect on bacteria with the use of open one-compartment PK/PD systems such as in our study; however, the generally good level of agreement across all strains between the present *in vitro* study and infection models involving neutropenic mice (10) is very reassuring in relation to future clinical applications of the *f*AUC/MIC targets for colistin.

Unfortunately, it is currently not possible to compare the *f*AUC/MIC targets from the present and other (10) preclinical studies with the *f*AUC/MIC values achieved in infected patients receiving currently recommended CMS dosage regimens. The inability to undertake this comparison arises because recent studies have shown that colistin binding in plasma involves the acute-phase reactant α1-acid glycoprotein (AAG), and the unbound fraction of colistin is influenced by the concentrations of both colistin and AAG (9). Since plasma AAG concentrations are influenced by pathophysiological stresses including infection (31, 40), the f_u of colistin in patients is likely to vary depending on the severity and stage of infection and magnitude of plasma colistin concentration. Although the knowledge of total plasma colistin concentrations achieved in patients is increasing (21, 25, 27, 33), there is no information on unbound plasma concentrations. As such information is forthcoming it will be possible to not only assess the ability of current CMS dosage regimens to meet the above-mentioned *f*AUC/MIC targets but also to design optimized dosage regimens.

The use of once-daily doses of CMS has recently been reported (13, 36), presumably based upon the concentration-dependent killing of colistin observed *in vitro* (28). However, we suggest caution with this approach. First, *in vitro* data suggest that the toxicity of colistin is concentration and time de-

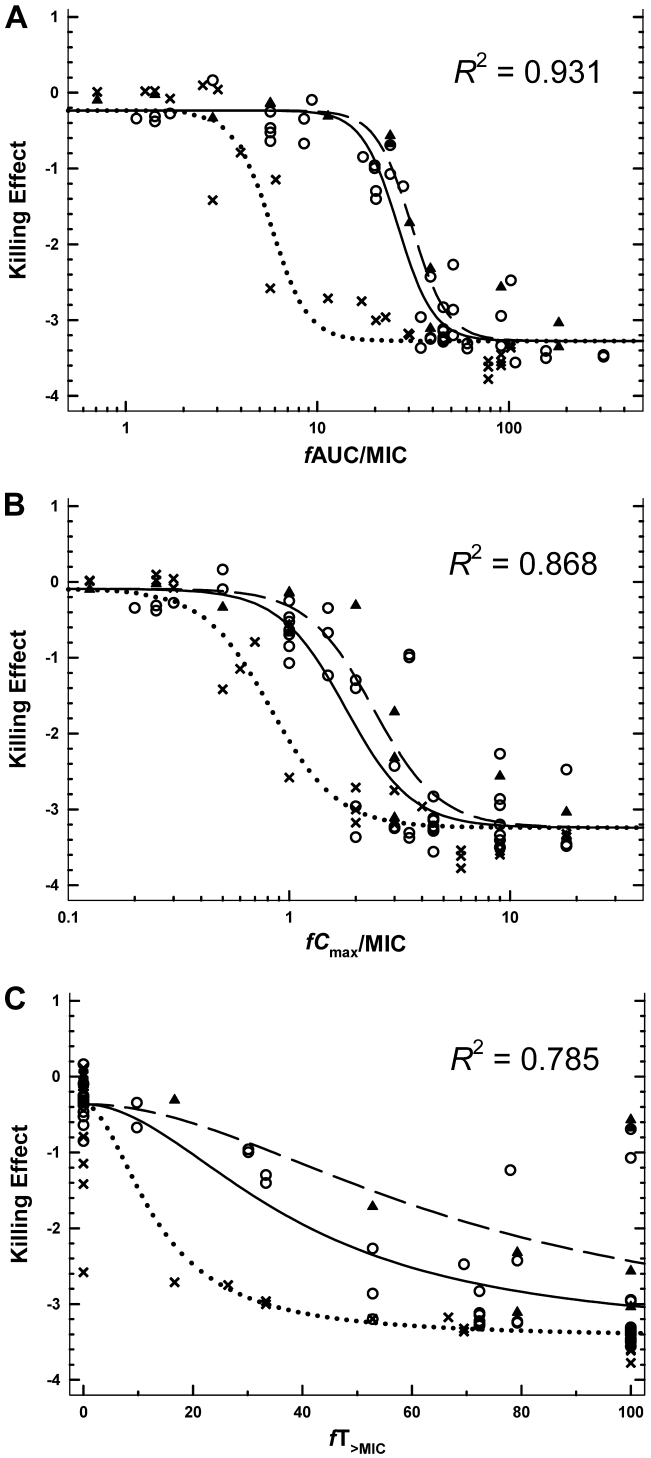

FIG. 2. Relationship between killing effect (log area ratio) against *P. aeruginosa* ATCC 27853 (solid line and open circles), PAO1 (dashed line and solid triangles), and 19056 muc (dotted line and crosses) as a function of three PK/PD indices: *f*AUC/MIC (A), fC_{max}/MIC (B), and $fT_{>MIC}$ (C). Each data point represents the result from a single treatment run. Lines represent model-generated fits using modeling approach iii (see Materials and Methods and Table 2).

TABLE 3. Median target values from 1,000 bootstrap replicates of colistin *f*AUC/MIC for 1- and 2-$\log_{10}$ reductions in the area under the CFU/ml curve relative to growth control and for 90% (EI_{90}) of maximal effect

Killing effect	Median target values (90% nonparametric confidence intervals)		
	ATCC 27853	PAO1	19056 muc
1-$\log_{10}$ reduction	22.6 (19.9–25.7)	27.1 (23.6–29.9)	5.04 (3.93–10.5)
2-$\log_{10}$ reduction	30.4 (27.2–33.0)	35.7 (32.6–41.7)	6.81 (5.21–14.3)
EI_{90}	42.0 (35.3–52.1)	49.3 (40.8–68.5)	9.78 (6.71–20.3)

pendent (20). Moreover, greater nephrotoxicity was observed in rats with a dosage regimen mimicking once-daily dosing of CMS in humans compared to a twice-daily regimen that delivered the same daily dose (41). Second, for both colistin and polymyxin B larger, infrequent doses in *in vitro* models led to greater emergence of resistance in *P. aeruginosa* compared to lower-dose/higher-frequency regimens (2, 39). Third, colistin lacks a significant postantibiotic effect *in vitro* (28, 32), although in a brief report such a phenomenon has been suggested to occur *in vivo* (17); additional studies are needed. Given the recent emergence of resistance to colistin (1, 15, 18, 26, 28), the ability to choose regimens which not only maximize killing but also suppress or minimize the development of resistance may prove crucial in preventing this trend.

In the present study, we simulated a 4-h colistin half-life as observed in healthy volunteers (12) and people with cystic fibrosis (21, 34, 35); a longer half-life has been reported in critically ill patients (27, 33, 37). The PK/PD indices described here are specific for *P. aeruginosa* and may differ for other Gram-negative pathogens; species specific differences in the magnitude of a particular index required to achieve certain levels of killing have been demonstrated for other anti-infectives (8). In addition, *in vitro* PK/PD models lack the defense mechanisms present in patients with intact immune systems; however, they may more adequately reflect drug-related antimicrobial activity in an immunocompromised host. Finally, higher PK/PD target values (e.g., for *f*AUC/MIC) may be required for infections with a high initial inoculum (5).

To our knowledge, this is the first *in vitro* investigation specifically designed to elucidate the relationship between bacterial killing and PK/PD indices for colistin against any organism. We have demonstrated that for colistin *f*AUC/MIC is the PK/PD index most closely correlated with the killing of *P. aeruginosa*. Our findings are in good agreement with those from recent studies in neutropenic mouse infection models. As information on the pharmacokinetics of unbound colistin in patients is obtained, the PK/PD targets reported here will assist in designing optimal dosing strategies for this increasingly important therapeutic option.

ACKNOWLEDGMENTS

The assistance of Chun-Hong Tan and Hui He of the Facility for Anti-infective Drug Development and Innovation, Monash University, Melbourne, Australia, and of Charlemagne Lacza of the School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, is gratefully acknowledged.

The project described was supported by awards R01AI079330 and R01AI070896 from the National Institute of Allergy and Infectious

Diseases. J.L. is an Australian National Health and Medical Research Council R. Douglas Wright Research Fellow.

The content here is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

We have no conflicts of interest to declare.

REFERENCES

1. **Antoniadou, A., F. Kontopidou, G. Poulakou, E. Koratzanis, I. Galani, E. Papadomichelakis, P. Kopterides, M. Souli, A. Armaganidis, and H. Giamarellou.** 2007. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. J. Antimicrob. Chemother. **59:**786–790.
2. **Bergen, P. J., J. Li, R. L. Nation, J. D. Turnidge, K. Coulthard, and R. W. Milne.** 2008. Comparison of once-, twice-, and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with *Pseudomonas aeruginosa* in an in vitro pharmacodynamic model. J. Antimicrob. Chemother. **61:**636–642.
3. **Bergen, P. J., J. Li, C. R. Rayner, and R. L. Nation.** 2006. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **50:**1953–1958.
4. **Bulitta, J. B., S. B. Duffull, M. Kinzig-Schippers, U. Holzgrabe, U. Stephan, G. L. Drusano, and F. Sorgel.** 2007. Systematic comparison of the population pharmacokinetics and pharmacodynamics of piperacillin in cystic fibrosis patients and healthy volunteers. Antimicrob. Agents Chemother. **51:**2497–2507.
5. **Bulitta, J. B., J. C. Yang, L. Yohonn, N. S. Ly, S. V. Brown, R. E. D'Hondt, W. J. Jusko, A. Forrest, and B. T. Tsuji.** 2010. Attenuation of colistin bactericidal activity by high inoculum of *Pseudomonas aeruginosa* characterized by a new mechanism-based population pharmacodynamic model. Antimicrob. Agents Chemother. **54:**2051–2062.
6. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement (M100-S15). Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Craig, W. A.** 2003. Basic pharmacodynamics of antibacterials with clinical applications to the use of beta-lactams, glycopeptides, and linezolid. Infect. Dis. Clin. N. Am. **17:**479–501.
8. **Craig, W. A.** 1995. Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage regimens for broad-spectrum cephalosporins. Diagn. Microbiol. Infect. Dis. **22:**89–96.
9. **Dudhani, R. V., J. Li, and R. Nation, L.** 2009. Plasma binding (PB) of colistin (C) involves multiple proteins and is concentration dependent: potential clinical implications, abstr. A1-576. Abstr. 49th Annu. Intersci. Conf. Antimicrob. Agents Chemother.
10. **Dudhani, R. V., J. D. Turnidge, K. Coulthard, R. W. Milne, C. R. Rayner, J. Li, and R. L. Nation.** 2010. Elucidation of the pharmacokinetic/pharmacodynamic determinant of colistin activity against *Pseudomonas aeruginosa* in murine thigh and lung infection models. Antimicrob. Agents Chemother. **54:**1117–1124.
11. **Falagas, M. E., and S. K. Kasiakou.** 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin. Infect. Dis. **40:**1333–1341.
12. **Gregoire, N., P. Gobin, C. Grignon, D. Frasca, P. Saulnier, W. Couet, and O. Mimoz.** 2008. Pharmacokinetic modeling of colistin methanesulfonate (CMS) and colistin in healthy volunteers after intravenous infusion of CMS, abstr. A-1668, p. 27. Abstr. 48th Intersci. Conf. Antimicrob. Agents Chemother.
13. **Gunderson, B. W., K. H. Ibrahim, L. B. Hovde, T. L. Fromm, M. D. Reed, and J. C. Rotschafer.** 2003. Synergistic activity of colistin and ceftazidime against multiantibiotic-resistant *Pseudomonas aeruginosa* in an in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **47:**905–909.
14. **Harigaya, Y., J. B. Bulitta, A. Forrest, G. Sakoulas, A. J. Lesse, J. M. Mylotte, and B. T. Tsuji.** 2009. Pharmacodynamics of vancomycin at simulated epithelial lining fluid concentrations against methicillin-resistant *Staphylococcus aureus* (MRSA): implications for dosing in MRSA pneumonia. Antimicrob. Agents Chemother. **53:**3894–3901.
15. **Johansen, H. K., S. M. Moskowitz, O. Ciofu, T. Pressler, and N. Hoiby.** 2008. Spread of colistin resistant non-mucoid *Pseudomonas aeruginosa* among chronically infected Danish cystic fibrosis patients. J. Cyst. Fibros. **7:**391–397.
16. **Jones, R. N.** 2001. Resistance patterns among nosocomial pathogens: trends over the past few years. Chest **119:**397S–404S.
17. **Ketthireddy, S., D. G. Lee, Y. Murakami, T. Stamstad, D. R. Andes, and W. A. Craig.** 2007. In vivo pharmacodynamics of colistin against *Pseudomonas aeruginosa* in thighs of neutropenic mice, abstr. A-4, p. 1. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother.
18. **Ko, K. S., J. Y. Suh, K. T. Kwon, S. I. Jung, K. H. Park, C. I. Kang, D. R. Chung, K. R. Peck, and J. H. Song.** 2007. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J. Antimicrob. Chemother. **60:**1163–1167.
19. **Landman, D., C. Georgescu, D. A. Martin, and J. Quale.** 2008. Polymyxins revisited. Clin. Microbiol. Rev. **21:**449–465.
20. **Lewis, J. R., and S. A. Lewis.** 2004. Colistin interactions with the mammalian urothelium. Am. J. Physiol. Cell Physiol. **286:**C913–C922.
21. **Li, J., K. Coulthard, R. Milne, R. L. Nation, S. Conway, D. Peckham, C. Etherington, and J. Turnidge.** 2003. Steady-state pharmacokinetics of intravenous colistin methanesulphonate in patients with cystic fibrosis. J. Antimicrob. Chemother. **52:**987–992.
22. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, and K. Coulthard.** 2003. Stability of colistin and colistin methanesulfonate in aqueous media and plasma as determined by high-performance liquid chromatography. Antimicrob. Agents Chemother. **47:**1364–1370.
23. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, K. Coulthard, and D. W. Johnson.** 2001. A simple method for the assay of colistin in human plasma, using pre-column derivatization with 9-fluorenylmethyl chloroformate in solid-phase extraction cartridges and reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. **761:**167–175.
24. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, T. C. Smeaton, and K. Coulthard.** 2003. Use of high-performance liquid chromatography to study the pharmacokinetics of colistin sulfate in rats following intravenous administration. Antimicrob. Agents Chemother. **47:**1766–1770.
25. **Li, J., and R. L. Nation.** 2006. Comment on: pharmacokinetics of inhaled colistin in patients with cystic fibrosis. J. Antimicrob. Chemother. **58:**222–223.
26. **Li, J., R. L. Nation, J. D. Turnidge, R. W. Milne, K. Coulthard, C. R. Rayner, and D. L. Paterson.** 2006. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect. Dis. **6:**589–601.
27. **Li, J., C. R. Rayner, R. L. Nation, R. Deans, R. Boots, N. Widdecombe, A. Douglas, and J. Lipman.** 2005. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. Antimicrob. Agents Chemother. **49:**4814–4815.
28. **Li, J., J. Turnidge, R. Milne, R. L. Nation, and K. Coulthard.** 2001. In vitro pharmacodynamic properties of colistin and colistin methanesulfonate against *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. Antimicrob. Agents Chemother. **45:**781–785.
29. **Livermore, D. M.** 2004. The need for new antibiotics. Clin. Microbiol. Infect. **10**(Suppl. 4)**:**1–9.
30. **Livermore, D. M.** 2003. The threat from the pink corner. Ann. Med. **35:**226–234.
31. **Morita, K., and A. Yamaji.** 1995. Changes in the serum protein binding of vancomycin in patients with methicillin-resistant *Staphylococcus aureus* infection: the role of serum α1-acid glycoprotein levels. Ther. Drug Monit. **17:**107–112.
32. **Owen, R. J., J. Li, R. L. Nation, and D. Spelman.** 2007. In vitro pharmacodynamics of colistin against *Acinetobacter baumannii* clinical isolates. J. Antimicrob. Chemother. **59:**473–477.
33. **Plachouras, D., M. Karvanen, L. E. Friberg, E. Papadomichelakis, A. Antoniadou, I. Tsangaris, I. Karaiskos, G. Poulakou, F. Kontopidou, A. Armaganidis, O. Cars, and H. Giamarellou.** 2009. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with gram-negative bacterial infections. Antimicrob. Agents Chemother. **53:**3430–3436.
34. **Ratjen, F., E. Rietschel, D. Kasel, R. Schwiertz, K. Starke, H. Beier, S. van Koningsbruggen, and H. Grasemann.** 2006. Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. J. Antimicrob. Chemother. **57:**306–311.
35. **Reed, M. D., R. C. Stern, M. A. O'Riordan, and J. L. Blumer.** 2001. The pharmacokinetics of colistin in patients with cystic fibrosis. J. Clin. Pharmacol. **41:**645–654.
36. **Rosenvinge, A., T. Pressler, and N. Hoiby.** 2005. Colistin intravenously is a safe and effective treatment of multidrug-resistant microorganisms in cystic fibrosis. J. Cyst. Fibros. **4:**S32.
37. **Skiada, A., J. Pavleas, C. Vafiadi, K. Salatas, A. Markoyannakis, P. Tofas, K. Tzanetou, G. Thomopoulos, A. Rigas, K. Rigas, E. Vafiadi, G. L. Petrikkos, and G. L. Daikos.** 2008. Pharmacokinetics of three different dosing regimens of colistin with meaning for optimum use, abstr. A-1670, p. 27. 48th Annu. Intersci. Conf. Antimicrob. Agents Chemother.
38. **Talbot, G. H., J. Bradley, J. E. Edwards, Jr., D. Gilbert, M. Scheld, and J. G. Bartlett.** 2006. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin. Infect. Dis. **42:**657–668.
39. **Tam, V. H., A. N. Schilling, G. Vo, S. Kabbara, A. L. Kwa, N. P. Wiederhold, and R. E. Lewis.** 2005. Pharmacodynamics of polymyxin B against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **49:**3624–3630.
40. **Voulgari, F., P. Cummins, T. I. Gardecki, N. J. Beeching, P. C. Stone, and J. Stuart.** 1982. Serum levels of acute phase and cardiac proteins after myocardial infarction, surgery, and infection. Br. Heart J. **48:**352–356.
41. **Wallace, S. J., J. Li, R. L. Nation, C. R. Rayner, D. Taylor, D. Middleton, R. W. Milne, K. Coulthard, and J. D. Turnidge.** 2008. Subacute toxicity of colistin methanesulfonate in rats: comparison of various intravenous dosage regimens. Antimicrob. Agents Chemother. **52:**1159–1161.