

New Dosing Strategies for an Old Antibiotic: Pharmacodynamics of Front-Loaded Regimens of Colistin at Simulated Pharmacokinetics in Patients with Kidney or Liver Disease

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Increasing evidence suggests that colistin monotherapy is suboptimal at currently recommended doses. We hypothesized that front-loading provides an improved dosing strategy for polymyxin antibiotics to maximize killing and minimize total exposure. Here, we utilized an *in vitro* pharmacodynamic model to examine the impact of front-loaded colistin regimens against a high bacterial density (10^8 CFU/ml) of *Pseudomonas aeruginosa*. The pharmacokinetics were simulated for patients with hepatic (half-life [$t_{1/2}$] of 3.2 h) or renal ($t_{1/2}$ of 14.8 h) disease. Front-loaded regimens ($n = 5$) demonstrated improvement in bacterial killing, with reduced overall free drug areas under the concentration-time curve (fAUC) compared to those with traditional dosing regimens ($n = 14$) with various dosing frequencies (every 12 h [q12h] and q24h). In the renal failure simulations, front-loaded regimens at lower exposures (fAUC of 143 mg · h/liter) obtained killing activity similar to that of traditional regimens (fAUC of 268 mg · h/liter), with an ~97% reduction in the area under the viable count curve over 48 h. In hepatic failure simulations, front-loaded regimens yielded rapid initial killing by up to 7 log₁₀ within 2 h, but considerable regrowth occurred for both front-loaded and traditional regimens. No regimen eradicated the high bacterial inoculum of *P. aeruginosa*. The current study, which utilizes an *in vitro* pharmacodynamic infection model, demonstrates the potential benefits of front-loading strategies for polymyxins simulating differential pharmacokinetics in patients with hepatic and renal failure at a range of doses. Our findings may have important clinical implications, as front-loading polymyxins as a part of a combination regimen may be a viable strategy for aggressive treatment of high-bacterial-burden infections.

Early efficacious antimicrobial treatment is the cornerstone in combating infections in critically ill patients (1). However, there is a dearth of new and effective antimicrobial agents available to combat extensively drug-resistant (XDR) Gram-negative strains, particularly for *Pseudomonas aeruginosa* (2–5). This has renewed interest in the once-abandoned polymyxin antibiotic colistin, also known as polymyxin E, which is often the only therapeutic option against XDR *P. aeruginosa* strains (6–9). However, a number of studies have highlighted potential shortcomings of colistin monotherapy when given following current dosing recommendations coupled with its high proclivity for nephrotoxicity, especially in critically ill patients (10–15).

Colistin is administered parenterally as colistin methanesulfonate (CMS), an inactive prodrug that undergoes hydrolysis *in vivo* to the active moiety colistin (16). Over the past 15 years with the resurgence of colistin, recent pharmacokinetic studies in critically ill patients demonstrated that the currently recommended dosage regimens of CMS result in suboptimal plasma concentrations of formed colistin (17, 18). Although these studies have provided significant insight into the disposition of CMS and formed colistin in the critically ill patient population, there has been little information to guide its clinical use in the setting of severe end-organ dysfunction. Haas et al. recently conducted the first pharmacokinetics study of CMS and formed colistin in defined groups of patients with stage 5 kidney disease or severe liver disease (Childs-Pugh class C) (19). We utilized this new information to evaluate pharmacokinetic/pharmacodynamic (PK/PD) relation-

ships for colistin and propose optimal dosage regimens in these patient populations.

The objective of the current study was to investigate “front-loading” as a potential dosing strategy to optimize the pharmacodynamics of colistin by maximizing killing and minimizing total drug exposure. Our hypothesis was that polymyxins are suitable candidates for front-loading (20–22), as they demonstrate rapid bactericidal activity which has been shown to be active against high bacterial density (7, 22–24). An *in vitro* pharmacodynamic model was employed to examine the impact of front-loaded colistin regimens against *P. aeruginosa* by simulating the pharmacokinetics in patients with severe liver and renal diseases.

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TABLE 1 Drug exposure by regimen simulating the pharmacokinetics in hepatic and renal failure patients

Proposed dosing regimen	Total daily colistin dose (mg)		fAUC (mg · h/liter)	
	0–24 h	24–48 h	0–24 h	24–48 h
Hepatic				
Traditional				
0.6 mg/liter every 24 h × 2 doses	0.162	0.162	2.76	2.77
1.0 mg/liter every 24 h × 2 doses	0.270	0.270	4.59	4.62
0.6 mg/liter every 12 h × 4 doses	0.324	0.324	5.32	5.54
1.0 mg/liter every 12 h × 4 doses	0.540	0.540	8.87	9.24
2.0 mg/liter every 24 h × 2 doses	0.540	0.540	9.18	9.24
2.0 mg/liter every 12 h × 4 doses	1.08	1.08	17.7	18.5
High dose				
4.0 mg/liter every 24 h × 2 doses	1.08	1.08	18.4	18.5
4.0 mg/liter every 12 h × 4 doses	2.16	2.16	35.5	36.9
8.0 mg/liter every 24 h × 2 doses	2.16	2.16	36.7	36.9
8.0 mg/liter every 12h × 4 doses	4.32	4.32	70.9	73.9
Front loaded				
6 mg/liter every 24 h × 1 dose followed by 2 mg/liter every 24 h × 1 dose	1.62	0.540	27.6	9.33
6 mg/liter every 12 h × 1 dose followed by 2 mg/liter every 12 h × 3 doses	2.16	0.540	36.1	18.6
10 mg/liter every 24 h × 1 dose followed by 2 mg/liter every 24 h × 1 dose	2.70	0.540	45.9	9.44
10 mg/liter every 12 h × 1 dose followed by 2 mg/liter every 12 h × 3 doses	3.24	0.540	54.5	18.7
Renal				
Traditional				
0.6 mg/liter every 24 h × 2 doses	0.162	0.162	8.65	11.5
1.0 mg/liter every 24 h × 2 doses	0.27	0.27	14.4	19.1
2.0 mg/liter every 24 h × 2 doses	0.54	0.54	28.8	38.2
High dose				
4.0 mg/liter every 24 h × 2 doses	1.08	1.08	57.7	76.4
8.0 mg/liter every 24 h × 2 doses	2.16	2.16	115	153
Front loaded				
6 mg/liter every 24 h × 1 dose followed by 2 mg/liter every 24 h × 1 dose	1.62	0.54	86.5	56.9

MATERIALS AND METHODS

Bacterial strains. Two clinical isolates of *P. aeruginosa*, URM C1 and URM C2, which were obtained from the University of Rochester Medical Center, Rochester, NY, were studied as previous described (23). The MICs were determined by broth microdilution in four replicates using colistin (sulfate) according to Clinical and Laboratory Standards Institute guidelines in cation-adjusted Mueller-Hinton broth (CAMHB). The MIC was 1.0 mg/liter for URM C1 and URM C2.

Medium and antibiotic. Colistin sulfate was purchased from Sigma-Aldrich, St. Louis, MO (lot number 011MZO62V). Fresh stock solutions of colistin (sulfate) were prepared and then sterilized by filtration with a 0.22- μ m Millex-GP filter (Millipore, Bedford, MA, USA). Mueller-Hinton broth (Difco Laboratories, Detroit, MI) (CAMHB) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) (SMHB) was utilized for susceptibility testing and *in vitro* models.

***In vitro* pharmacokinetic/pharmacodynamic model.** A one-compartment *in vitro* infection model (IVPM) was used to characterize the pharmacodynamics of colistin against clinical strains of *P. aeruginosa* as previously described (26). The IVPM experiments simulated the pharmacokinetics in critically ill patients with renal or hepatic impairment obtained from a recent pharmacokinetic study by Haas et al. (19). In brief, the experimental model consisted of sealed flasks representing compartments, each containing a working volume of 250 ml of CAMHB at 37°C with a magnetic stir bar to ensure adequate mixing. One of the flasks served as a control to characterize the growth dynamics of both clinical strains in the absence of colistin. Prior to each experiment, strains were subcultured onto Mueller-Hinton agar plates and incubated overnight at 37°C. Fresh bacterial colonies from this overnight growth were inoculated to SMHB and adjusted spectrophotometrically to a McFarland turbidity

of 1.0. Each compartment was inoculated with 1.0 ml of this log-phase bacterial suspension to yield a starting inoculum of $\sim 10^8$ CFU/ml.

Simulated colistin dosing regimens. The traditional and front-loaded regimens are described in Table 1. The simulated pharmacokinetic half-lives ($t_{1/2}$) of colistin selected for the experimental setup were 14.8 h in renal failure patients and 3.2 h in hepatic failure patients), based on a pharmacokinetic study by Haas et al. in adult patients with renal failure ($n = 10$) and hepatic failure ($n = 10$) (19). Patients with stage 5 renal disease, maintained on intermittent hemodialysis and of any race or gender, were included in the renal failure group. Patients with severe hepatic disease or those currently being evaluated for liver transplant with a Child-Pugh score of C (27) were included in the hepatic failure group. Lactating or pregnant women were excluded from both the renal and hepatic failure groups. Patients with hepatic disease were excluded from the kidney renal failure group, and patients with stage 4 or 5 kidney disease were excluded from the hepatic failure group. The dosage regimens simulated in the *in vitro* model included a traditional CMS dose of 2.5 mg/kg/day, those that were recommended in the patient package insert (28), and higher-exposure dosage regimens administered in a front-loading fashion as shown in Table 1. Free drug areas under the concentration-time curves (fAUCs) from 0 to 24 h and from 24 to 48 h, were computed for each regimen using numeric integration of the functions of concentration versus time (ADAPT 5, Biomedical Simulations Resource [BMSR]).

For both traditional and front-loaded regimens, serial samples were collected aseptically for determination of bacterial counts at 0, 0.5, 1, 2, 4, 6, 8, 24, 26, 28, 32, and 48 h for characterization of colistin pharmacodynamics. Serial dilutions with sterile saline were performed. Viable bacterial counts were determined by plating 50- μ l samples of each diluted sample on drug-free Mueller-Hinton agar plates using an automatic

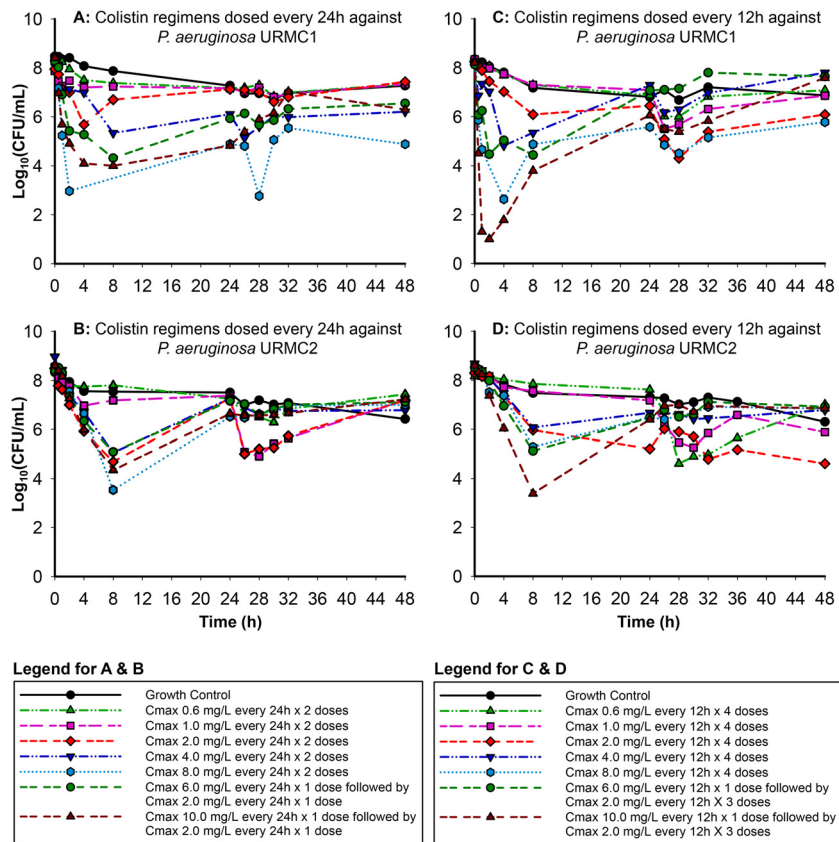


FIG 1 *In vitro* killing of two clinical *P. aeruginosa* isolates (URMC1 and URMC2) by colistin using pharmacokinetic profiles simulated for hepatic failure patients.

WASP spiral plater (Microbiology International, Rockville, MD). Plates were incubated for 24 h at 37°C before colony counts were determined. Colony counts (\log_{10} CFU/ml) were determined using an automated aCOLyte bacterial colony counter, (Symbiosis, Frederick, MD) with a limit of quantification of 2.0 CFU/ml (29). The colony count (\log_{10} CFU/ml) data were plotted as a function of time for all tested drug regimens for each clinical isolate (26). Bactericidal activity at 24 h was defined as a $\geq 99.9\%$ reduction (3 \log_{10} reduction) in colony counts compared to the starting inoculum at 0 h (predose).

Samples collected from the *in vitro* PK/PD experiments were placed in microcentrifuge tubes and immediately stored at -80°C until analysis. Colistin concentrations were determined by a standard agar diffusion bioassay using antibiotic medium 9 as the base seed agar and antibiotic medium 10 agar which was inoculated with *Escherichia coli* ATCC 35218 as an indicator organism. Each standard and sample were tested in triplicate using a cork borer to establish 5-mm holes in the agar, which were filled with 25 μl of the sample. Concentrations which were used as standard curves for colistin were 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/liter. The limit of detection for colistin was 0.25 mg/liter for control standards. The standard curves of the zone sizes versus the natural logarithm of the drug concentration were linear between 0.5 and 10 mg/liter when the standards were prepared in SMHB (r^2 of ≥ 0.90 , intraday coefficient of variation [CV] of $\leq 5.2\%$, and interday CV of $\leq 10.2\%$). Plates were incubated for 18 to 24 h at 37°C. The diameters of inhibition zones for samples and standards were measured to the nearest 0.1 mm with a vernier caliper. The observed colistin concentrations were within 10% of targeted concentrations.

PK/PD analysis. The initial rate of kill was quantified as the initial slope of the log CFU/ml versus time as well as the initial and maximum extents of killing relative to those for the growth control for both isolates

and all regimens. The colistin overall killing activity was quantified as the percent reduction in integrated bacterial burden. This was computed by first integrating the area under the CFU/ml curves ($\text{AUC}_{\text{CFU}_{\text{treatment}}}$ and $\text{AUC}_{\text{CFU}_{\text{growth control}}}$ were determined using the linear up, log down trapezoidal rule), for 24 h and 48 h, for all experiments. The percent reduction in the bacterial burden was then computed using equation 1:

$$\% \text{ reduction} = 100 \left(1 - \frac{\text{AUC}_{\text{CFU}_{\text{treatment}}}}{\text{AUC}_{\text{CFU}_{\text{Growth Control}}}} \right) \quad (1)$$

RESULTS

Regimens simulating the pharmacokinetics of colistin in hepatic failure. Colistin exhibited rapid concentration-dependent bactericidal initial activity against both clinical isolates (URMC1 and URMC2) (Fig. 1 and 2) for the higher-dosage regimens. The traditional regimens simulating hepatic failure against URMC1 dosed every 24 h (Fig. 1A) resulted in a reduction of up to 2.26 \log_{10} by 4 h. The high-dosage regimens resulted in a reduction of up to 3.32 \log_{10} by 8 h, while the front-loaded regimens resulted in a reduction of up to 4.40 \log_{10} in the same time period (Fig. 1A). All regimens resulted in regrowth starting at 24 h. The traditional regimens simulating hepatic failure against URMC2 dosed every 24 h (Fig. 1B) resulted in a reduction of up to 3.80 \log_{10} by 8 h which was not sustained beyond 24 h, with almost complete regrowth similar to that for the growth control by 48 h. The high-dosage regimens showed the greatest decrease in bacterial burden, with reductions of up to 5.05 \log_{10} by 8 h. The front-loaded regimens resulted in a reduction of up to 4.30 \log_{10} by 8 h.

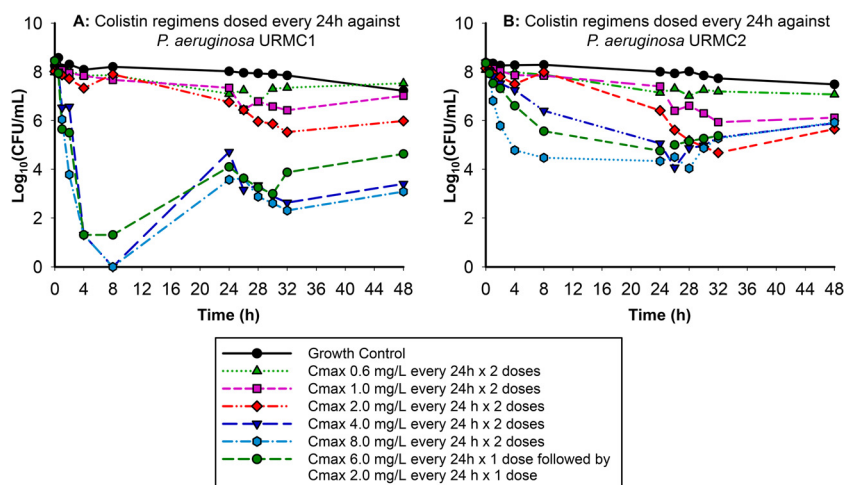


FIG 2 *In vitro* killing of two clinical *P. aeruginosa* isolates (URMC1 and URMC2) by colistin using pharmacokinetic profiles simulated for renal failure patients.

Traditional regimens simulating hepatic failure dosed every 12 h against URMC1 resulted in minimal reductions by 4 h (Fig. 1C). While the high-dosage regimens resulted in a reduction of up to 5.59 log₁₀ by 4 h, the reduction attained by the front-loaded regimens ranged from 3.67 to 7.19 log₁₀. The every-12-h (q12h) dosing regimens, with fAUCs similar to or higher than those of the q24h regimens, exhibited the most aggressive regrowth pattern. The regimens simulating hepatic failure dosed every 12 h against URMC2 (Fig. 1D) demonstrated bactericidal activity for the high-dosage and front-loaded regimens. The high-dosage regimens displayed aggressive regrowth compared to the traditional regimens, while the front-loaded regimens resulted in a reduction of up to 5.22 log₁₀ by 8 h, with considerable regrowth of ~6.84 log₁₀ by 48 h.

Regimens simulating the pharmacokinetics of colistin in renal failure. The regimens simulating renal failure against URMC1 dosed every 24 h (Fig. 2A) displayed a clear separation in the rate and extent of kill achieved by the higher-dose regimens and the front-loaded regimen (the ratio of initial rate of kill up to 2 h of these higher-dosage regimens compared to the lowest-dosage regimen, with a maximum concentration [C_{max}] of 0.6 mg/liter, ranged from 38.7 to 98.1). The regimens with a C_{max} of ≥4 mg/liter resulted in bacterial counts below the limit of quantification (i.e., 2 log₁₀ CFU/ml) by 4 h, and the bactericidal activity for these high-dosage regimens was sustained up to 48 h with minimal regrowth. The regimens simulating renal failure against URMC2 dosed every 24 h (Fig. 2B) demonstrated bactericidal killing activity for the higher-dosage regimens and the front-loaded regimen by 24 h. The antimicrobial pharmacodynamic activity observed against URMC2 was attenuated compared to the activity of these regimens against URMC1 (the ratio of the initial slope of the kill curves up to 2 h for these higher-dosage regimens compared to the lowest-dosage regimen, with a C_{max} of 0.6 mg/liter, ranged from 6.47 to 19.7) (Fig. 2A).

Pharmacodynamics of colistin exposure against *P. aeruginosa*. The regimens simulating renal and hepatic failure are described in Table 1 with the corresponding fAUCs at the two integrated endpoints of 24 h and 48 h. The analysis of these fAUCs based on equation 1 is presented in Table 2. The fAUCs (0 to 48 h) for the regimens simulating renal failure ranged from 20 to 268

mg · h/liter, while the fAUCs for the regimens simulating hepatic failure ranged between 6 and 73 mg · h/liter. Against URMC1, among regimens simulating hepatic failure, the front-loaded regimen with a C_{max} of 6 mg/liter × 1 dose followed by a C_{max} of 2 mg/liter × 1 dose outperformed the traditional regimens with a C_{max} of 2.0 mg/liter every 12 h × 4 doses and a C_{max} of 4.0 mg/liter every 24 h × 2 doses. Regimens with a C_{max} of 10 mg/liter every 12 h × 1 dose followed by a C_{max} of 2.0 mg/liter every 12 h × 3 doses, 4.0 mg/liter every 12 h × 4 doses, and 8.0 mg/liter every 24 h × 2 doses, with similar 0- to 48-h fAUCs, had very comparable percent reductions in bacterial burden during the first 24 h, but the performance of these regimens significantly diverged during the second 24 h. Regimens with a C_{max} of 8.0 mg/liter every 24 h × 2 doses, with identical fAUCs during 0 to 24 h and 24 to 48 h, had the highest reduction in bacterial burden among all the hepatic regimens. In contrast, even though the front-loaded regimen with a C_{max} of 10 mg/liter every 12 h × 1 dose followed by a C_{max} of 2.0 mg/liter every 12 h × 3 doses had the same 0- to 24-h and 24- to 48-h fAUCs, the increased dosing frequency of q12h did not perform as well as regimens with a q24h dosing frequency and similar fAUCs. The front-loaded regimen with a C_{max} of 10.0 mg/liter every 12 h × 1 dose followed by 2 mg/liter every 12 h × 3 doses had the highest fAUC of 54.5 during 0 to 24 h in this group, with an fAUC of 73.1 during 0 to 48 h, resulting in a 98.6% reduction during the first 24 h and only a 77.1% reduction over 48 h.

Against URMC2, the higher-dosage regimens simulating renal failure with the favorable pharmacokinetics and a longer t_{1/2} (14.8 h) performed well against URMC1 compared to the regimens simulating hepatic failure. The substantial increase in fAUC associated with the regimen of a C_{max} of 8.0 mg/liter every 24 h × 2 doses compared to those with a C_{max} of 4.0 mg/liter every 24 h × 2 doses and a C_{max} of 6 mg/liter every 24 h for 1 dose followed by 2 mg/liter every 24 h × 1 dose against both strains did not result in an increased reduction in the bacterial burden. Interestingly, against URMC2, even though the two *Pseudomonas aeruginosa* clinical isolates had the same MICs, the pharmacodynamic activity was markedly different. The regimens with a q12h dosing frequency did not perform as well as the regimens with q24h dosing despite the increase in drug exposure (fAUC). Overall, none of the

TABLE 2 Pharmacodynamic response against *P. aeruginosa* URMC1 and URMC2 for regimens simulating those for hepatic and renal failure patients

Dosage regimen	URMC1						URMC2			
	fAUC (mg · h/liter)		Log ₁₀ AUCCFU ^a (CFU · h/ml)		Reduction in bacterial burden (%) ^b		Log ₁₀ AUCCFU (CFU · h/ml)		Reduction in bacterial burden (%)	
	0–24 h	0–48 h	0–24 h	0–48 h	0–24 h	0–48 h	0–24 h	0–48 h	0–24 h	0–48 h
Hepatic										
Traditional										
0.6 mg/liter every 24 h × 2 doses	2.76	5.53	8.93	9.08	36.9	25.4	9.08	9.18	-8.07	12.0
1.0 mg/liter every 24 h × 2 doses	4.59	9.21	8.63	8.88	68.2	53.4	8.91	9.09	28.4	33.3
0.6 mg/liter every 12 h × 4 doses	5.32	10.9	8.89	8.97	42.5	41.6	9.11	9.17	-13.4	-3.24
1.0 mg/liter every 12 h × 4 doses	8.87	18.1	8.89	8.94	41.9	46.5	9.07	9.09	4.61	20.9
2.0 mg/liter every 24 h × 2 doses	9.18	18.4	8.36	8.76	82.7	64.7	8.50	8.66	72.2	68.1
2.0 mg/liter every 12 h × 4 doses	17.7	36.2	8.41	8.43	81.0	83.4	8.74	8.75	55.8	63.5
High dose										
4.0 mg/liter every 24 h × 2 doses	18.4	36.8	8.26	8.32	86.4	87.1	8.82	8.91	40.3	40.1
4.0 mg/liter every 12 h × 4 doses	35.5	72.4	8.32	8.92	84.4	49.0	8.88	8.94	36.6	40.9
8.0 mg/liter every 24 h × 2 doses	36.7	73.7	7.68	7.72	96.4	96.7	8.65	8.81	59.9	53.1
8.0 mg/liter every 12 h × 4 doses	70.9	144	7.65	7.71	94.5	95.2	8.75	8.85	54.9	53.7
Front loaded										
6 mg/liter every 24 h × 1 dose followed by 2 mg/liter every 24 h × 1 dose	27.6	36.9	8.05	8.22	91.6	89.7	8.72	8.89	52.6	43.0
6 mg/liter × 1 dose followed by 2mg/liter × 3 doses	36.1	54.7	8.04	9.07	91.8	28.0	8.77	8.90	47.8	45.7
10 mg/liter every 24 h × 1 dose followed by 2mg/liter every 24 h × 1 dose	45.9	55.4	7.83	8.27	94.9	88.5	8.56	8.75	67.3	58.6
10 mg/liter every 12 h × 1 dose followed by 2mg/liter every 12 h × 3 doses	54.5	73.1	7.27	8.53	98.6	77.1	8.60	8.77	68.2	60.5
Renal										
Traditional										
0.6 mg/liter every 24 h × 2 doses	8.65	20.1	9.13	9.28	61.0	60.4	9.17	9.26	62.2	65.3
1.0 mg/liter every 24 h × 2 doses	14.4	33.5	9.06	9.11	66.9	73.2	9.17	9.18	62.2	70.8
2.0 mg/liter every 24 h × 2 doses	28.8	67.0	9.04	9.05	68.1	76.7	9.13	9.13	65.4	74.2
High dose										
4.0 mg/liter every 24 h × 2 doses	57.7	134	8.10	8.10	96.3	97.4	8.47	8.49	92.3	94.2
8.0 mg/liter every 24 h × 2 doses	115	268	8.06	8.06	96.7	97.6	8.24	8.27	95.6	96.5
Front loaded										
6 mg/liter every 24 h × 1 dose followed by 2 mg/liter every 24 h × 1 dose	86.5	143	8.07	8.07	96.6	97.6	8.06	8.10	97.0	97.6

^a Log₁₀ AUCCFU, log of the area under the CFU/ml-versus-time curve.

^b Reduction in bacterial burden was computed as per equation 1.

regimens were able to achieve good pharmacodynamic activity against URMC2.

DISCUSSION

Colistin is a last-resort antibiotic increasingly used in critically ill patients infected with *P. aeruginosa* strains which are resistant to nearly all other commercially available antibiotics (6, 8, 30, 31). However, there is a paucity of clinical studies focused on the critically ill patient population to guide its optimal use (17, 18). As a result, dosing recommendations for CMS have substantial deficiencies, and resultant plasma concentrations in critically ill patients are suboptimal (32). In the present study, we investigated new front-loaded dosing schemes simulating concentration-time profiles of formed colistin in patients with severe liver and renal disease against two clinical *P. aeruginosa* isolates (19) from a recent study. This pharmacokinetic study (19) demonstrated that the disposition of formed colistin was significantly different for the hepatic failure ($t_{1/2}$ of 3.2 h) and the renal failure ($t_{1/2}$ of 14.8 h)

patients. Interestingly, the terminal half-life in renal failure patients from this study was similar to that in critically ill patients as determined by Plachorous et al. ($t_{1/2}$ of 14.4 h) and Garonzik et al. ($t_{1/2}$ of 13.0 h) (17, 18). Mohamed et al., using a previously developed PK/PD model based on *in vitro* time-kill studies describing the bactericidal activity of colistin against a resistant *P. aeruginosa* strain (33), found that a loading dose of 480 to 720 mg of CMS (6 to 9 million units [MU]) administered to critically ill patients was very effective in decreasing the bacterial load by 3 log₁₀ CFU/ml during the first 5 to 6 h of therapy. Colistin concentrations measured in these patients were, on average, 1.34 mg/liter at 8 h following the loading dose of 480 mg (34). Taken together, these new pharmacokinetic findings have vastly improved our understanding of the disposition of CMS and formed colistin in critically ill patients. Therefore, we hypothesized that front-loaded colistin regimens will result in a rapid reduction of a high bacterial density of *P. aeruginosa* and reduce cumulative drug exposure.

A number of observations emerged from the present investi-

gation. First, the current achievable plasma concentrations and dosing recommendations for colistin point to the fact that there is a need for a loading dose and a change in the current suggested CMS dosing strategy (17, 35). Clearly, CMS doses at the upper end of the product label recommendation have resulted in suboptimal concentrations. For example, the median of the average concentration at steady state (CSS_{avg}) determined by Garonzik et al. (18) was 2.36 mg/liter by 24 h, which barely exceeds the breakpoint of 2 mg/liter. Second, colistin-induced nephrotoxicity has been shown to be dose limiting and reversible upon discontinuation of treatment (36, 37). Pogue et al. reported an 8-fold increase in the propensity for developing nephrotoxicity in patients receiving CMS 4.0 mg/kg/day (colistin base activity [CBA]) or greater versus patients receiving 2.0 mg/kg/day (CBA) or less (38). A prospective study by Paul et al. (39) compared 200 patients treated with colistin (average colistin dose of 6 MU/day, equivalent to 180 mg of colistin base activity) with 295 patients treated with comparator antibiotics (imipenem, meropenem, or ampicillin-sulbactam). Although one of the confounding factors of this study was that the patient population was comprised of those already at increased risk for developing nephrotoxicity and the colistin arm was comprised of patients with worse prognostic factors, a major conclusion of the study was that there was an increased incidence of nephrotoxicity and a lower survival rate associated with the colistin treatment arm (the 30-day mortality for the colistin treatment arm was 39%, compared to the 28.8% for the comparator arm) (39). With increased and improved supportive care of critically ill patients, close monitoring of their renal function and avoidance of coadministration of nephrotoxic agents has led to colistin-related nephrotoxicity being less prominent (6). Recent reports support these claims, with lower rates of nephrotoxicity (10 to 30%) (40) compared to those found in older studies (50%) (41). Therefore, increasing the daily dose for colistin for a short duration seems a promising and clinically viable strategy to decrease the potential for nephrotoxicity while enhancing the antimicrobial killing activity. This strategy has been applied for other antimicrobials (42–44) and may be particularly promising for colistin, which can achieve rapid and concentration-dependent bactericidal activity (7). However, these findings should also be balanced with toxicodynamic evaluations of these new regimens, as it has been shown that the *f*AUC is the driver for nephrotoxicity for colistin (41, 45).

Third, in the current study, we determined that front-loaded regimens, with a high C_{max} for a short duration (for front-loaded regimens simulating both renal and hepatic failure), resulted in different killing activity than traditional regimens with similar *f*AUC values. The longer half-life seen in the renal failure population combined with a colistin front-loaded regimen provided a highly beneficial strategy to achieve high initial peak concentrations followed by de-escalation of therapy, resulting in lower exposures and at least ≥ 3 -log₁₀ reductions in the bacterial burden against both isolates. These IVPK experiments have illustrated that both front-loaded and higher-than-current traditional regimens selected to have maximal colistin drug exposure were unable to successfully eradicate the two clinical isolates. Although the front-loaded regimens showed initial promising bactericidal killing against both clinical isolates, when maximal levels of drug were administered, extensive regrowth occurred beyond 8 h.

Collectively, front-loading CMS/colistin combination regimens in the clinical setting may be considered as a strategy to treat

high-bacterial-density infections (46). At even higher bacterial inocula, as seen in bacterial ventilator-associated pneumonia (VAP) (47), achieving high colistin concentrations against more-difficult-to-treat polymyxin-heteroresistant strains may pose a formidable challenge. Bergen et al. investigated the combination of colistin plus doripenem against *P. aeruginosa* *in vitro*, simulating colistin pharmacokinetics obtained from critically ill patients (35). Their results suggested that the addition of doripenem to clinically achievable colistin regimens (e.g., C_{max} of 0.5 and 2.0 mg/liter) substantially improved antibacterial activity compared to colistin monotherapy. Hence, simply increasing the C_{max} of colistin monotherapy is not likely to provide complete bacterial reduction in immunocompromised patients. Therefore, the addition of a synergistic second antimicrobial agent may be a promising strategy to serve as a backbone of a combination regimen by sustaining the initial killing achieved by a colistin front-loaded regimen.

We acknowledge potential limitations of the current study, as bacterial eradication is multifactorial and depends on the choice of the antibiotic, the dose selected, the type of offending agent, the severity of the infection, and the immune status of the host. Our study utilized only two clinical isolates which may not be representative of all *P. aeruginosa* strains. Further *in vivo* studies are warranted to strengthen the translation of these *in vitro* findings before these results can guide the selection of optimal dosage regimens for critically ill patients. Overall, the results from the current study are promising, as they herald a new dosing strategy for polymyxins.

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