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Application of a Loading Dose of Colistin Methanesulfonate in Critically Ill Patients: Population Pharmacokinetics, Protein Binding, and Prediction of Bacterial Kill

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A previous pharmacokinetic study on dosing of colistin methanesulfonate (CMS) at 240 mg (3 million units [MU]) every 8 h indicated that colistin has a long half-life, resulting in insufficient concentrations for the first 12 to 48 h after initiation of treatment. A loading dose would therefore be beneficial. The aim of this study was to evaluate CMS and colistin pharmacokinetics following a 480-mg (6-MU) loading dose in critically ill patients and to explore the bacterial kill following the use of different dosing regimens obtained by predictions from a pharmacokinetic-pharmacodynamic model developed from an *in vitro* study on *Pseudomonas aeruginosa*. The unbound fractions of colistin A and colistin B were determined using equilibrium dialysis and considered in the predictions. Ten critically ill patients (6 males; mean age, 54 years; mean creatinine clearance, 82 ml/min) with infections caused by multidrug-resistant Gram-negative bacteria were enrolled in the study. The pharmacokinetic data collected after the first and eighth doses were analyzed simultaneously with the data from the previous study (total, 28 patients) in the NONMEM program. For CMS, a two-compartment model best described the pharmacokinetics, and the half-lives of the two phases were estimated to be 0.026 and 2.2 h, respectively. For colistin, a one-compartment model was sufficient and the estimated half-life was 18.5 h. The unbound fractions of colistin in the patients were 26 to 41% at clinical concentrations. Colistin A, but not colistin B, had a concentration-dependent binding. The predictions suggested that the time to 3-log-unit bacterial kill for a 480-mg loading dose was reduced to half of that for the dose of 240 mg.

Colistin, polymyxin E, is a cationic polypeptide antibiotic which consists of at least 30 components, the 2 major ones being colistin A and colistin B (33). The polymyxin antibiotic group was discovered in 1947 (1, 38), with colistin first reported in 1950 (23). Colistin was used clinically in the late 1950s but was shelved in the early 1970s due to reports of its nephrotoxicity and neurotoxicity and the availability of easier to use and less toxic antibiotics (14, 25). In face of the increasing frequency of infections caused by multidrug-resistant Gram-negative bacteria (MDR GNB), for example, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, colistin, administered as the inactive prodrug colistin methanesulfonate (CMS), has been used as a last-resort treatment for such infections in severely ill patients. Recent studies indicate that the toxicity of colistin may have been exaggerated and that the use of colistin in combinations with other antibiotics may lead to synergistic antibacterial effects (13, 26). However, the optimal dosing regimen of CMS was not established according to today's rigorous drug development procedure, and there has been a dearth of information on the pharmacokinetics (PK) of colistin and CMS.

The problems associated with proper colistin PK studies include the lack of a reliable assay with a workup procedure that avoids CMS degradation to colistin before quantification. The development of novel methods to measure both CMS and colistin (19, 24) has enabled several recent studies on PKs in different patient populations; for example, following a single CMS dose of 80 mg in healthy volunteers (5), in critically ill patients not on renal replacement therapy at delivery of first dose (34) and at steady state (15, 34), as well as in critically ill patients on renal replacement therapy (15, 18). The availability of these results has increased the understanding of the pharmacokinetics of both CMS and colistin at commonly utilized dosing regimens.

On the basis of the quantification methods developed, it has been demonstrated that colistin has a long half-life in critically ill patients (about 14 h). Following a dose regimen of 240 mg every 8 h, it takes approximately 12 to 48 h after initiation of CMS treatment to reach a total colistin concentration of 2 mg/liter (34), the MIC breakpoint suggested by EUCAST for *Acinetobacter baumannii* (12). The breakpoint for *Pseudomonas aeruginosa* has been suggested to be even higher, 4 mg/liter (12). It is likely that a low initial concentration would be suboptimal in killing the bacteria (2), especially in critically ill patients, where an immediate effect is important (16, 36). Subtherapeutic concentrations may also favor resistance development (26). Therefore, it was suggested that a higher initial dose should be given (34), and the current study is a follow-up on that recommendation.

In the establishment of dosing recommendations, it is also of importance to consider the protein binding since it is only the

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unbound fraction (f_u) of the antibiotic that exerts antibacterial activity (7, 9). There is limited knowledge on the protein binding of colistin, although there have been reports on concentration-dependent protein binding in mice as well as similar indications in humans over the concentration range observed at initiation of treatment (9, 11). Accurate determination of protein binding also relies on a reliable quantification method as well as procedures minimizing nonspecific binding of colistin to material. On the basis of a PK model, unbound fractions, and a pharmacokinetic-pharmacodynamic (PKPD) model for bacterial kill, predictions of different dosing regimens can be made to provide an understanding of the time course of bacterial kill (30, 31).

The aim of this study was to study the population PKs of CMS and colistin following a loading dose in critically ill patients and to predict the time course of bacterial kill for different CMS doses on the basis of the PK model developed, the unbound fraction of colistin, and a semimechanistic PKPD model developed from an *in vitro* study (29).

MATERIALS AND METHODS

Patients. A prospective study was conducted at the Critical Care Unit of Attikon University General Hospital, Athens, Greece, from July 2009 to January 2010. Ethics approval was obtained from the Ethics Committee of the hospital (registration no. 7/30-07-09). Critically ill patients who met the following inclusion criteria were enrolled: (i) age 18 years or above and (ii) receipt of colistin for suspected or proven multidrug-resistant Gram-negative bacterial infection. Patients receiving continuous venovenous hemodiafiltration as renal replacement therapy were excluded. Informed consent was obtained from all patients enrolled in the study. For each patient, detailed information was collected on the first day of colistin administration: gender, age, body weight (actual and ideal), serum creatinine concentration, serum albumin concentration, hemoglobin level, hematocrit levels, septicemic state, and APACHE II (Acute Physiology and Chronic Health Evaluation II) score. The serum creatinine level was measured on days 1, 7, 14, and 21, and creatinine clearance (CrCL) was calculated according to the Cockcroft-Gault formula (4).

Colistin administration. The loading dose of CMS (colistin; Norma, Greece) was administered at 480 mg (6 million units [MU]; approximately 180 mg of colistin base activity [CBA]), with subsequent maintenance doses of 80 to 240 mg of CMS (1 to 3 MU; 30 to 90 mg CBA) administered every 8 h. CMS was dissolved in 100 ml of normal saline (the same volume was used for all dose sizes) and administered as an intravenous infusion over 15 min.

Blood sampling. Serial venous blood collection was conducted for the loading dose and the eighth dose. For all patients, the samples were taken immediately prior to the start of the 15-min infusion and at 15, 30, 60, 120, 240, and 465 min after the end of the infusion. All blood samples were immediately chilled and centrifuged, and the plasma was stored at −70°C until assayed.

Analytical method. Plasma CMS and colistin concentrations were determined by a previously established method. Plasma concentrations of colistin A and colistin B were determined with a liquid chromatography-tandem mass spectrometry method (19), while CMS concentrations were determined from the difference of measured colistin concentrations in samples pre- and posthydrolysis (24), accounting for the difference in molecular weights (molar masses are, on average, 1,743 g/mol for CMS and 1,163 g/mol for colistin). The limits of quantification of the method for 100 μl of plasma were 0.019 mg/liter for colistin A and 0.010 mg/liter for colistin B. The coefficient of variation (CV) for the method was 6.2%. Seven CMS standards ranging from 0.12 to 18.45 mg/liter were analyzed, and the intraday CV was between 6.2 and 8.8% ($n = 6$). The interday CV ranged from 6.7 to 7.9%, based on a single analysis of the standards on 8 different days.

Plasma protein binding. The f_u of colistin was measured by equilibrium dialysis. Fresh human plasma (EDTA) from healthy volunteers was spiked with colistin purchased from Sigma Chemicals (St. Louis, MO) to concentrations of 0.25, 1, 4, 8, 16, and 24 mg/liter. Plasma (0.5 ml) was dialyzed across a semipermeable membrane (cutoff, 12 to 14,000 Da; Spectra/Por 4) against an equal volume of phosphate buffer (pH 7.4) containing sodium chloride. Triplicate samples were incubated for 24 h at 37°C. At sampling, the buffer was mixed with an equal volume of blank plasma to avoid unspecific binding of colistin to the tube material and thereby avoiding falsely low buffer concentrations. Colistins A and B were determined in both plasma and buffer with the same analytical method described above. The protein binding was also determined after adding α_1-acid glycoprotein (AAG) to plasma. In addition, the protein binding in thawed samples from each of the patients in the present and the previous (34) studies was determined by addition of colistin to pretreatment samples ($n = 9$), pretreatment samples mixed with posttreatment samples ($n = 18$), or posttreatment samples ($n = 3$) to result in total combined concentrations of colistin A and colistin B of 0.8 to 5.6 mg/liter in the equilibrium dialysis.

Population pharmacokinetic modeling. All data were log transformed and modeled simultaneously with the data from a previous study with a similar design (160 to 240 mg [2 to 3 MU] every 8 h in 18 patients from the same hospital; a similar sampling design with a total of 267 samples at two dosing occasions) where the initial PK model was developed (34). Concentrations (in molar units) were used to account for the fact that 1 molecule of CMS is hydrolyzed into 1 molecule of colistin.

The determined concentrations of colistin A and colistin B were added to form the total concentration of colistin in each sample, which was used in the modeling analysis. The ratio of colistin A to colistin B ranged from 2.5 to 4.8 among the patients and appeared to be constant within and between dosing intervals for an individual over time. Colistin was assumed to form from the prodrug CMS, and as the fraction of CMS that forms colistin cannot be determined without administering colistin itself, estimated colistin PK parameters were scaled to the unknown fraction of CMS metabolized to colistin (f_m). The structural model from the previous study with two CMS compartments and one colistin compartment was reevaluated with different combinations of one, two and three compartments, as well as linear and nonlinear elimination pathways.

The mean tendencies in the population, i.e., the typical parameter values, were estimated along with random effects described by the interindividual variability (IIV), interoccasion variability (IOV), and residual errors. The IIV and the IOV in the model parameters were assumed to be log-normally distributed. Correlation between individual parameters was also investigated. The residual error was modeled by using an additive, a proportional, or a combined additive and proportional error model. As the concentrations of CMS and colistin were analyzed at the same time point, the L2 method in the NONMEM program was used to investigate whether a correlation exists between their residual errors (21).

Covariate model building was performed in a stepwise fashion with forward inclusion and backward deletion. The possible covariates that were evaluated included gender, body weight, ideal body weight, age, serum creatinine concentration, CrCL, serum albumin concentration, septicemic state, APACHE II score, and hemoglobin and hematocrit levels. As CMS is partially eliminated renally and previous population PK analyses have reported that CrCL is a significant covariate for CMS and/or colistin (5, 15), CrCL and serum creatinine concentration were explored more extensively, including modeling of CrCL as a time-varying covariate (39). The units for CrCL were in liters/h in the modeling, and observed CrCL values above 7.8 liters/h (130 ml/min) were capped at 7.8 liters/h.

Model performance was assessed by evaluation of diagnostic plots and the objective function value (OFV). In order to discriminate between

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TABLE 1 Demographic and clinical data[a]

Patient no.	Gender	Age (yr)	Body wt (ideal body wt) (kg)	Total maintenance daily dose (mg [MU])	Serum creatinine concn at baseline, day 3 (mg/dl)	CrCL at baseline, day 3 (ml/min)	Serum albumin concn (g/dl)	APACHE II score	Diagnosis	Reason for colistin administration
19	M	46	80 (70)	720 (9)	1, 0.9	91.6, 101.7	2.3	8	Necrotizing fasciitis	Necrotizing fasciitis
20	F	51	60 (65)	720 (9)	0.8, 0.5	120.5, 134.8	3	17	Multiple sclerosis	VAP
21	M	59	140 (75)	720 (9)	1.1, 0.8	76.8, 105.7	2.7	9	Pneumonia	VAP
22	M	66	75 (75)	480 (6)	1.1, 0.8	70.2, 96.5	3.5	23	Cirrhosis-hepatic encephalopathy	VAP
23	M	32	80 (70)	720 (9)	0.6, 0.6	191.5, 191.5	1.9	17	Pneumonia	VAP
24	F	88	80 (70)	240 (3)	1.7, 1.7	24.9, 24.9	2.1	24	Acute mesenteric ischemia	VAP
25	M	60	85 (75)	720 (9)	1, 1	83.5, 83.5	3.2	7	Pneumonia	VAP
26	F	48	65 (65)	720 (9)	0.7, 0.5	99.5, 139.4	3.1	16	Trauma	VAP
27	M	52	80 (70)	720 (9)	0.4, 0.5	214.3, 171.4	3.8	18	NHL-pneumonia	VAP
28	F	52	65 (65)	720 (9)	0.7, 0.8	95.2, 83.3	2.5	15	H1N1 infection-pneumonia	Bacteremia

[a] F, female; M, male; NHL, non-Hodgkin's lymphoma; VAP, ventilator-associated pneumonia; the CrCL for patient 27 was fixed to 130 ml/min.

nested models, the difference in OFV (−2 log likelihood) was used. The more complex model was selected when the reduction in OFV (dOFV) was at least 10.83 (corresponding to a P value of <0.001 for 1 degree of freedom). Clinical relevance and a reduction in IIV were also requirements for covariate inclusion. The model was evaluated by use of a visual predictive check (VPC) (22), in which a total of 1,000 replicates were simulated by using the original data set as a template. The prediction-corrected VPC was utilized to account for the fact that some patients received a different dose (3). A bootstrap analysis (500 samples) was performed to obtain the confidence interval of the parameters.

Predictions of bacterial kill. Total and unbound colistin concentration-time profiles and *P. aeruginosa* counts for a wild-type strain (ATCC 27853; MIC, 1 mg/liter) were predicted for a typical individual on the basis of the final PK model developed here and a PKPD model developed from time-kill experiments. In the semimechanistic PKPD model for colistin (29), there were compartments for drug-susceptible, growing bacteria (S) and for nonsusceptible, resting bacteria (R) (32). The drug effect was described by a maximal kill rate constant (E_{max}) at 35 h^{-1} with an unbound colistin concentration that produces 50% of E_{max} (EC_{50}) of 2.9 mg/liter. Other model parameters included a rate constant of bacterial growth (0.99 h^{-1}), a rate constant of natural bacterial death (0.18 h^{-1}), a colistin concentration-dependent rate constant for apparent emergence of resistance (6.0×10^{-5} liter mg^{-1} h^{-1}), a rate constant for reversal of resistance (0.15 h^{-1}), and a maximal bacterial count in the stationary phase (1.8×10^8 CFU/ml).

In the predictions of bacterial killing in a typical patient following different dosing regimens, the predicted plasma concentration of total CA and CB was obtained from the PK model and the average ratio of observed CA to CB (3.6). The unbound concentrations of CA and CB were computed from the predicted total concentrations on the basis of the relationships identified for f_u and CA and CB. The total concentration of unbound colistin (i.e., the sum of unbound CA and unbound CB) was driving the bacterial kill, if it is assumed that CA and CB were the active components possessing similar potencies.

A starting inoculum of 4.5×10^5 CFU/ml (corresponding to the average value of the starting inocula in the previous *in vitro* experiments) was applied in all predictions. CMS was administered as an intravenous infusion over 15 min for the dosing schedule of 240 mg (3 MU) every 8 h with loading doses of CMS of 480, 720, and 960 mg (equivalent to 6, 9, and 12 MU, respectively) and with subsequent maintenance doses of 240 mg every 8 h or 360 mg every 12 h. Predictions were conducted for dosing intervals of 8, 12, and 24 h between the loading and maintenance doses. Predictions were also made for the individuals with the lowest and highest measured colistin concentrations at 4 h after the administration of the loading dose.

Software. Data analysis was conducted using the first-order conditional estimation method with interaction and ADVAN5 within the population analysis software NONMEM 7 (Icon Development Solutions, Ellicott City, MD). NONMEM was also used to predict total and unbound colistin concentration-versus-time and bacterial count-versus-time profiles. The Xpose program (version 4) (20) and R program (version 2.10; www.r-project.org) were used for data set review and graphical evaluation. Simulations and calculations for VPC, execution of stepwise covariate model (SCM) building, and bootstrap analysis were performed using the Perl speaks NONMEM (PsN) tool kit (27). The relationships between f_u and the total plasma concentration for CA and CB were obtained with nonlinear least-squares regression using the Microsoft Excel Solver program (17).

RESULTS

Patients. There were 10 patients (6 males, 4 females) enrolled in the study. The mean age was 55.4 years (age range, 32 to 88 years). Demographic and clinical data for each patient are shown in Table 1.

CMS and colistin concentrations. Samples were collected from all patients at all predetermined time points, and the observed individual plasma CMS and colistin concentration-versus-time profiles after the first and eighth doses are presented in Fig. 1. Colistin concentrations were, on average, 1.34 mg/liter (range, 0.374 to 2.59 mg/liter) at 8 h following the loading dose of 480 mg.

PK model. As in the previous study, a model with two compartments for CMS and one compartment for colistin was sufficient to describe the data. Parameter estimates are presented in Table 2. A one-compartment model for CMS resulted in an increase in OFV of 77 units. Combined additive and proportional residual error models were maintained for both CMS and colistin. There was no improvement in the model fit when CMS and colistin samples taken at the same time point were allowed to share a residual error. IIV was significant for CMS clearance (CL_{CMS}), colistin clearance (CL_{col}), intercompartmental clearance for CMS (Q), and the residual error of CMS. Inclusion of covariance between CL_{CMS} and CL_{col} improved the OFV further (27 units), and as the correlation was estimated to be 100%, the magnitude of the variability for CL_{col} was scaled from the estimated variability in CL_{CMS} with a factor estimated to be 1.8. IOV was significant for CL_{CMS}, the volume of distribution of CMS in the peripheral compartment (V_2), CL_{col}, and the volume of distribution of formed

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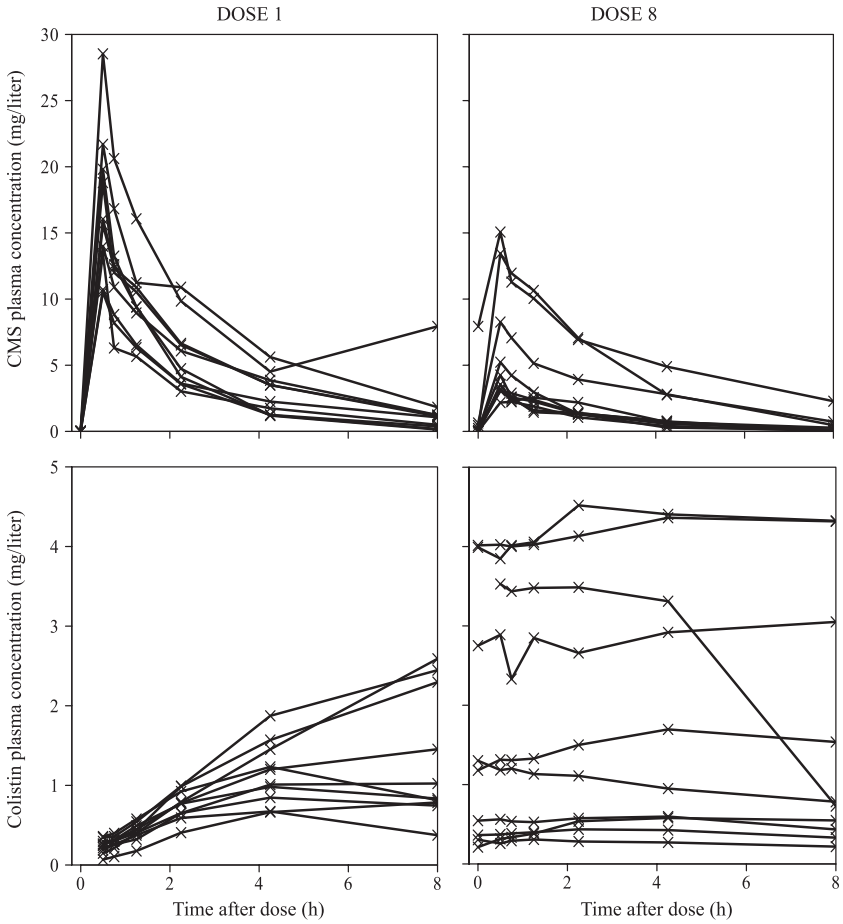

FIG 1 Observed individual plasma concentrations of CMS (top) and colistin (bottom) after administration of the first and eighth doses of CMS.

colistin (V_{col}). For a typical individual, the half-lives of the two phases of CMS were 0.026 and 2.2 h, respectively, and the half-life of colistin was 18.5 h.

No covariate relationship met the predefined statistical criteria when they were evaluated in the SCM. Although there was a wide variability in size and observed concentration, weight was not a significant covariate for any CMS or colistin parameter. CrCL was also separately evaluated as a covariate for clearance of CMS and clearance of colistin (CL_x; x representing either CMS or colistin) using different parameterizations:

TABLE 2 Estimated population pharmacokinetic parameters of CMS and colistin for the final model based on the combined data from the current and previous (34) studies

Parameter (units)	Explanation	CMS			Colistin		
		Typical value	IIV (%)	IOV (%)	Typical value	IIV (%)	IOV (%)
CL_{CMS} or CL_{col} (liters/h)	Clearance of CMS or formed colistin	13.1 (11.6–15.1)[a]	42 (21–42.3)	30 (21–31)	8.2 (6.0–11.2)	76[b] (52–76.1)	48 (34–51)
V_1 or V_{col} (liters)	Volume of distribution of central compartment for CMS or formed colistin	11.8 (7.6–12.5)			218 (192–252)		48 (34–51)
Q (liters/h)	Intercompartmental clearance for CMS	206 (93–641)	111 (52–197)				
V_2 (liters)	Volume of distribution in peripheral compartment for CMS	28.4 (22.4–33.5)		59 (51–85)			
Additive residual error (μmol/liter)		0.071 (0.063–0.25)			0.044 (0.018–0.053)		
Proportional error (%)		0.23 (0.12–0.21)	52 (31–61)		0.082 (0.065–0.12)		

[a] Data in parentheses are 90% CIs. The 90% CIs were obtained from a nonparametric bootstrap ($n = 500$).
[b] The value obtained when the variability of CL_{col} was scaled from the variability of CL_{CMS} by the estimated value of 1.8 (90% CI, 1.4 to 2.9).

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TABLE 3 Effects on OFV and variability by including CrCL as a covariate[a]

Model no.	Explanation	OFV	CL_{CMS}				CL_{col}			
			IIV	PPV_{modelx}	IIVP	$IIVP/PPV_{model1}$	IIV	PPV_{modelx}	IIVP	$IIVP/PPV_{model1}$
1	No covariate	−1,107.2	0.42	0.51			0.76	0.89		
2	CrCL on CL_{CMS}	−1,110.8	0.36	0.47	0.21	0.40	0.67	0.67	0.39	0.43
3	CrCL on CL_{Col}	−1,112.0	0.41	0.52	NA	NA	0.61	0.61	0.45	0.51
4	CrCL on f_m	−1,115.9	0.42	0.51	0.019	0.06	0.71	0.71	0.33	0.36

[a] Parameterization is as in equation 2. OFV, objective function value for the model; IIV, random, unexplained interindividual variability; IOV, interoccasion variability (values not shown in the table); PPV_{modelx}, total unexplained population parameter variability, where $PPV_{modelx} = \sqrt{(IIV^2 + IOV^2)}$; PPV_{model1}, PPV without covariate; IIVP, interindividual variability predictable from CrCL, calculated as $\sqrt{(PPV_{model1}{}^2 - PPV_{modelx}{}^2)}$; $IIVP/PPV_{model1}$, fraction of the total variability in the parameter that is predictable from CrCL; NA, value not possible to compute as PPV_{modelx} is higher than PPV_{model1}.

$$CL_x = \theta_1 \cdot (CrCL/CrCL_{median})^{\theta_2} \quad (1)$$

$$CL_x = \theta_1 + \theta_2 \cdot CrCL \quad (2)$$

where θ_1 is a typical value for clearance (nonrenal clearance in equation 2), and θ_2 is the power describing the change in clearance (equation 1) or the renal clearance proportional to CrCL (equation 2). Equivalent functions were also tested for the fraction of CMS metabolized to colistin (f_m) by simultaneously introducing the same relationship on CL_{col} and V_{col}. None of these relationships were, however, statistically significant (dOFV < 10.8), and consequently, the final model included no covariates. To give an overview of the explanatory value of CrCL when it was evaluated, the OFV, IIV, IOV, and population parameter variability (PPV) (28) estimates for the base model without covariates and the models, including equation 2, for CL_{CMS}, CL_{col}, and f_m are presented in Table 3. The drop in OFV was only 3.6 ($P > 0.05$) when CrCL was included for CL_{CMS} (with θ_1 and θ_2 estimated to be 6.80 liters/h and 0.0661, respectively), although CrCL was estimated to reduce PPV from 51% to 47% for CMS and from 76 to 67% for colistin.

The prediction-corrected visual predictive check (pcVPC) showed that the final model without covariates explained the observed data well (Fig. 2). The observed medians of the data were included within the model-predicted 95% confidence intervals of the medians for colistin and CMS at all doses, although there was a tendency for the observed medians of both the CMS and colistin data to be slightly overpredicted for the eighth dose. The bootstrap analysis indicated that all parameters were estimated with reasonable uncertainty, except for Q, where uncertainty was large (90% confidence interval [CI], 93 to 641 liters/h).

Plasma protein binding. The unbound fraction of colistin A (f_uA) and, consequently, the f_u based on the total colistin concentration (CA concentration plus CB concentration) were concentration dependent, whereas the unbound fraction of colistin B (f_uB) was found to be constant (average, 43%) across the concen-

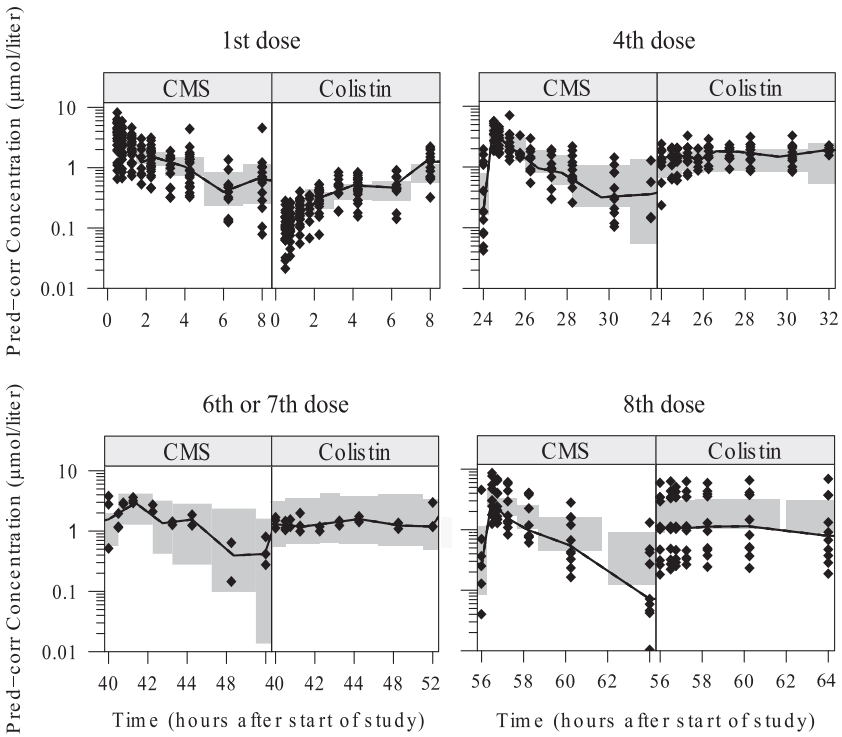

FIG 2 Visual predictive check of the final model stratified for the studied dosing occasions. Shown are the median (solid lines) of prediction-corrected observed concentrations (◆) and the 95% confidence intervals of the medians of prediction-corrected simulated concentrations (gray areas).

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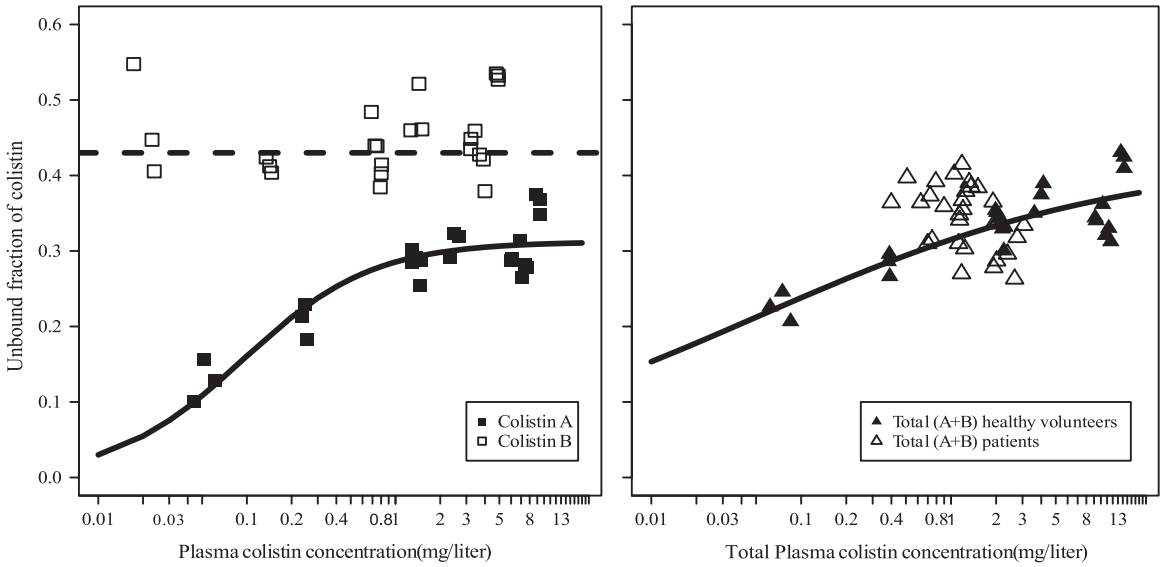

FIG 3 Unbound fraction of colistin A and colistin B in healthy volunteers (left) and total colistin in healthy volunteers and patients (right) versus total plasma concentrations of colistin A, colistin B, and total colistin at the end of dialysis in the equilibrium dialysis study. The solid lines represent the fits obtained by nonlinear least-squares regression for colistin A ($R^2 = 97\%$) and total colistin ($R^2 = 91\%$) for healthy volunteers. The fit for colistin B is a straight line at $y = 0.43$.

trations in the plasma of the healthy volunteers evaluated (Fig. 3). A nonlinear equation was fitted to the data for colistin A:

$$f_u\mathrm{A} = 31.2 \cdot \mathrm{CA}/(0.094 + \mathrm{CA}) \quad (3)$$

where CA is the total concentration of colistin A (in mg/liter), and the maximum f_uA was estimated to 31.2%. The predicted f_u were 15% for the total colistin concentration (CA plus CB) of 0.01 mg/liter and 34% for 2.5 mg/liter.

The measured unbound fractions of total colistin in the patients were 34% (median) and ranged from 26 to 41%. There was no obvious difference in plasma binding in the critically ill patients and the healthy volunteers over the concentration range studied (Fig. 3). It should, however, be noted that the unbound fraction is dependent on the ratio of colistin A and colistin B. There was no evident change in protein binding with the addition of AAG to plasma.

Predictions of bacterial kill. In Fig. 4A, the predicted time courses of total and unbound colistin concentrations and bacterial counts following a maintenance dose of 240 mg every 8 h or 360 mg every 12 h and loading doses of 480, 720, and 960 mg are shown for an individual with the typical population value. It was predicted that it takes approximately 12.5 h to achieve a 3-log-unit kill of wild-type *P. aeruginosa* following a loading dose of 480 mg, whereas a dose of 240 mg did not reach a 3-log-unit kill at all. For loading doses of 720 mg and 960 mg, the times to 3-log-unit kill decreased further and were estimated to be 6.5 and 5 h, respectively.

The predictions of bacterial kill following increased dosing intervals (Fig. 4D to F) show that the interval can be extended without a pronounced regrowth of bacteria. For the 960-mg loading dose with a subsequent 240-mg maintenance dose, the wild-type bacterial count stayed below 10 CFU/ml after 16 and 18 h for the time intervals of 12 and 24 h, respectively. The 960-mg loading dose with a subsequent 360-mg maintenance dose achieved a similar kill for the same time interval. For the lower loading doses, the time interval to the next dose could also be expanded to 12 h without a notable regrowth of bacteria, although the 240-mg and 480-mg loading doses did not result in a bacterial count lower than 10 CFU/ml at any time point.

For the patient with the lowest measured colistin concentration, only the highest loading dose, 960 mg, achieved a 3-log-unit kill (Fig. 4B). As expected, for the patient observed to have the highest measured colistin concentrations, the predicted bacterial kill occurred faster and the 240-mg dose achieved a 3-log-unit kill at approximately 9 h (Fig. 4C).

The nonlinear binding was predicted to have an impact on the initial bacterial kill, as for a loading dose of 720 mg (9 MU), the nonlinear f_u predicted a 3-log-unit bacterial kill at 6.5 h, while if f_u was constant and 34%, a 3-log-unit kill would be achieved 1 h earlier, i.e., at 5.5 h.

DISCUSSION

An efficient initial therapy may be critical to rapid clearance of an infection. The formation of colistin from CMS and its increase to the steady-state MICs are relatively slow following CMS administration. In this study, a loading dose of 480 mg CMS was successfully administered to critically ill patients. The total concentrations of CMS and colistin were as expected for drugs with linear PKs; i.e., the total concentrations doubled compared to those in our previous study, where the starting dose of CMS was 240 mg. The previously developed PK model, consisting of two compartments for CMS and one compartment for colistin (34), could well describe all data in this study with similar parameter estimates.

Predictions from the PK model developed in this study and the semimechanistic PKPD model developed previously (29) showed that a loading dose can indeed be of importance for rapid clearance of bacteria, as colistin concentrations increase slowly when therapy is initiated (Fig. 4A). In addition, the binding of colistin to plasma proteins was found to be nonlinear where lower colistin concentrations had a lower f_u and a higher colistin concentration had a higher f_u, with binding fractions being similar to what has been observed in neutropenic mice (10). As there is, to our knowl-

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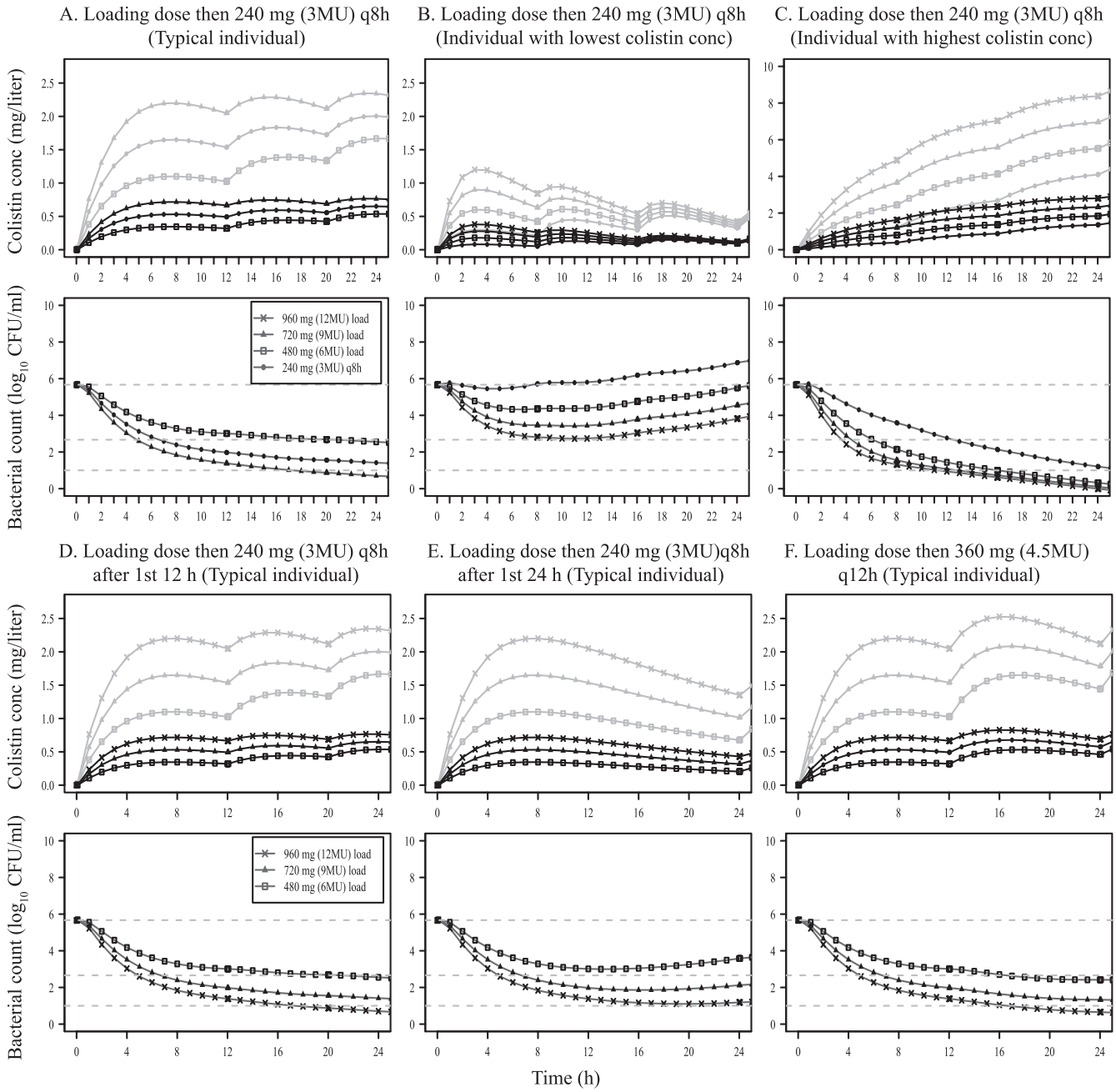

FIG 4 Model predictions of colistin concentration (top rows) with bacterial counts for a wild-type *P. aeruginosa* strain (bottom rows) for three individuals with typical population values (A, D, E, and F), lowest measured colistin concentration (B), and highest measured colistin concentration (C). The three individuals (A to C) initially received 480 mg, 720 mg, or 960 mg as a loading dose, followed by maintenance doses of 240 mg every 8 h (q8h) after the first 8 h (results for the commonly used 240-mg dose every 8 h are also shown). This is followed by model predictions for a typical individual receiving the loading doses followed by maintenance doses starting at different dosing intervals: 240 mg every 8 h after the first 12 h (D), 240 mg every 8 h after the first 24 h (E), and 360 mg every 12 h (q12h) (F). All doses were given as 15-min infusions. In the top rows, the black lines represent the unbound colistin concentration and the gray lines represent the total colistin concentration. In the bottom rows, the gray dashed lines represent the bacterial count at initiation of therapy, the bacterial count for 3-log-unit kill, and the bacterial count below the limit of detection at 10 CFU/ml.

edge, no reported difference in potency between colistin A and colistin B, the potencies were assumed to be similar. The difference in protein binding for colistin A and colistin B implies, however, that the binding resulting in bacterial kill may also possibly differ. As a consequence, when the initial dose was doubled from 240 mg to 480 mg, the unbound colistin concentrations more than doubled, leading to an even more efficient bacterial kill from higher doses. An increase in the dose from 240 mg to 480 mg was predicted to decrease the time to 3-log-unit kill from 20 to 8.5 h for the investigated bacteria, and an even shorter time, 5.5 h, was predicted for a 720-mg loading dose of CMS (Fig. 4A). Although the degree of bacterial kill depends on the bacterial strain and the

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site of infection, this example illustrates the importance of utilizing a loading dose of CMS, as a rapid bacterial kill will likely mean a faster resolution of the infection in a seriously ill patient.

Nephrotoxicity by colistin may limit the amount of the loading dose that can be administered. In the current study, there were no obvious signs of toxicity following the therapy with a 480-mg loading dose; for example, there were no obvious changes in the serum creatinine level on day 3 compared to that at the baseline (Table 1). However, the number of patients included in the study is insufficient to reach definite conclusions regarding safety. In an analysis of 26 studies on colistin in critically ill patients, large variations in the dosing regimens and the duration of treatment were reported (37). In many countries outside Europe, daily doses of >800 mg (10 MU) are not uncommon for critically ill patients (15). Two studies reported that during the course of colistin treatment 33% (8) and 43% (35) of the patients developed nephrotoxicity which was dose dependent. The total cumulative CMS dose was found to be associated with kidney damage, and thus, a short period of exposure would be advantageous (37). In similar settings as the current study where the patients are closely monitored and properly hydrated, the initial loading dose may be increased further with a longer interval of administration of the subsequent dose.

To limit the total dose of CMS during the first 24 h, the bacterial kill following different time intervals between the loading dose and the second dose was predicted (Fig. 4A and D to F). An extended dosing interval of 12 h had a limited impact on the bacterial kill for all individual PK profiles predicted, while longer dosing intervals resulted in pronounced regrowth for shorter exposures. For a typical individual, colistin appeared to be efficient in bacterial kill and suppression; i.e., it was efficient when the bacterial count was maintained below 3-log-unit kill from 17 h and onwards for the 480-mg loading dose, except when the interval to the maintenance dose was 24 h. The 720-mg and 960-mg loading doses resulted in 3-log-unit kill at 6.5 h and 5 h, respectively. Since there is a large interpatient variability in colistin concentrations (Fig. 1), the bacterial kill will vary widely in the population, even when the sensitivity of the bacteria is the same (Fig. 4B and C). However, a loading dose was shown to be beneficial for all concentration-time profiles predicted.

In the development of dosing guidelines, it is of importance to accurately characterize the PKs of both CMS and colistin. For this purpose, use of a selective analysis method with a workup procedure that minimizes the hydrolysis of CMS to colistin is crucial, especially when sampling times are included where CMS concentrations are higher than colistin concentrations, which is the case during the first dosing intervals. The data in the current study could well fit the same structural PK model suggested earlier (34). That model has also recently been applied in a study of healthy volunteers (5) and in a study of a large number (n = 105) of critically ill patients (15). As in the current study, when evaluated, CMS renal clearance was estimated to be similar to CrCL (5, 15). The lack of statistically significant covariate relationships in our analysis may be because of the relatively small number of patients and/or the lack of urine data to support the relationship. It is, however, not clear if the relationships between CrCL and CL_{CMS} proposed earlier explained parts of the large IIV and will thereby be of value for individual dose adjustments. For colistin, CrCL was, as expected, not a significant covariate for clearance. Renal clearance has earlier been estimated to be a negligible part of total colistin clearance in healthy volunteers (5), while its estimated value was higher than expected in critically ill patients (15). Weight has earlier been found to have a significant relationship with the central compartment volume for CMS (15) but was not a significant covariate for distribution volumes in the current analysis (dOFV, ≤0.2 units when tested with the volumes of distribution of CMS and colistin). As colistin is formed from CMS over time and concentrations increase over the dosing interval, a weight-based loading dose of CMS would have a limited impact on the initial rise in colistin concentrations. Therefore, only fixed loading doses were considered in this study.

In summary, a loading dose of 480 mg (6 MU) CMS was successfully administered to critically ill patients, and its potential value for fast bacterial eradication was illustrated. The observed PKs were as expected on the basis of a previously developed population PK model, and no significant covariates could be identified to explain the between-patient variability. The unbound fraction of colistin in plasma from healthy volunteers was determined and shown to be concentration dependent. Loading doses higher than the standard 160 to 240 mg CMS were shown to increase the initial bacterial kill, based on predictions of unbound colistin concentrations and a previously developed semimechanistic PKPD model describing bacterial kill. Based on these results, we recommend a loading dose of 480 to 720 mg (6 to 9 MU) in critically ill patients.

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