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PHARMACOLOGY

Substantial Targeting Advantage Achieved by Pulmonary Administration of Colistin Methanesulfonate in a Large-Animal Model

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ABSTRACT Colistin, administered as its inactive prodrug colistin methanesulfonate (CMS), is often used in multidrug-resistant Gram-negative pulmonary infections. The CMS and colistin pharmacokinetics in plasma and epithelial lining fluid (ELF) following intravenous and pulmonary dosing have not been evaluated in a large-animal model with pulmonary architecture similar to that of humans. Six merino sheep (34 to 43 kg body weight) received an intravenous or pulmonary dose of 4 to 8 mg/kg CMS (sodium) or 2 to 3 mg/kg colistin (sulfate) in a 4-way crossover study. Pulmonary dosing was achieved via jet nebulization through an endotracheal tube cuff. CMS and colistin were quantified in plasma and bronchoalveolar lavage fluid (BALF) samples by high-performance liquid chromatography (HPLC). ELF concentrations were calculated via the urea method. CMS and colistin were comodeled in S-ADAPT. Following intravenous CMS or colistin administration, no concentrations were quantifiable in BALF samples. Elimination clearance was 1.97 liters/h (4% interindividual variability) for CMS (other than conversion to colistin) and 1.08 liters/h (25%) for colistin. On average, 18% of a CMS dose was converted to colistin. Following pulmonary delivery, colistin was not quantifiable in plasma and CMS was detected in only one sheep. Average ELF concentrations (standard deviations [SD]) of formed colistin were 400 (243), 384 (187), and 184 (190) mg/liter at 1, 4, and 24 h after pulmonary CMS administration. The population pharmacokinetic model described well CMS and colistin in plasma and ELF following intravenous and pulmonary administration. Pulmonary dosing provided high ELF and low plasma colistin concentrations, representing a substantial targeting advantage over intravenous administration. Predictions from the pharmacokinetic model indicate that sheep are an advantageous model for translational research.

KEYWORDS colistin, intravenous administration, pulmonary administration, pulmonary pharmacokinetics, sheep, systemic pharmacokinetics

Colistin, a cationic lipopeptide antibiotic, is active against many multidrug-resistant (MDR) Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* (1). Colistin is marketed as the inactive prodrug colistimethate (CMS), as the latter is substantially less nephrotoxic (2–4). Following approval of CMS for clinical use in the 1950s, concerns over cases of nephrotoxicity in patients (5) and the development of other antibiotic classes considered at the time to be less toxic (6, 7) resulted in limited usage for several decades. However, the increasing

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prevalence of MDR Gram-negative infections in recent years has necessitated the reintroduction of CMS into the clinic, especially in intensive care units, where current frontline antimicrobial treatments are failing (8).

For the treatment of MDR Gram-negative lung infections (e.g., cystic fibrosis exacerbations, ventilator-associated pneumonia), CMS is administered intravenously (i.v.) or by nebulization or by both routes (9–11). The increasing clinical use of CMS in serious lung infections makes essential an understanding of the time courses of concentrations of CMS and, in particular, colistin in lung fluid and in systemic circulation, following i.v. administration and nebulization. The colistin concentration in lung fluid is a critical determinant of the antibacterial effect and the potential for emergence of resistance (12, 13), while the plasma exposure influences the risk of development of concentration-related nephrotoxicity (14, 15). Thus, the relative levels of colistin exposure in these two body regions determine the therapeutic window following administration by each route.

Recent studies have provided very useful information on the plasma and lung fluid concentrations of CMS and formed colistin following i.v. and nebulized administration of the prodrug to patients in a crossover design (13, 16). Such studies are inherently invasive due to the need for repetitive sampling of lung fluid and plasma and can be difficult to control because of the potential for other factors to influence pulmonary and systemic pharmacokinetics (PK), e.g., interpatient variability in clinical presentation, disease fluctuations within each patient, and other drug therapy. A thorough investigation of the pulmonary and systemic disposition of the prodrug and the active drug formed from it following i.v. and pulmonary administration requires both CMS and colistin to be administered by each route, i.e., using a four-way crossover design. Such an investigation would be extremely difficult to perform in a clinical study, especially because colistin is not approved for direct parenteral administration to patients.

Aspects of the pulmonary and systemic disposition of CMS and colistin following direct delivery to the lungs and i.v. administration have been investigated in rats (17–19) and baboon monkeys (20). Bronchoalveolar fluid (BALF) was collected in addition to plasma in three of the studies (17–19). Two studies involved administration of only colistin (19) or only CMS (20) by both routes. Only one of the studies (18) involved i.v. and pulmonary administration of both CMS and colistin, albeit to separate groups of animals; i.e., it was not a crossover study. It is important that significant differences in the anatomical structure of the rodent (monopodial) and human (dichotomous) respiratory systems (21, 22) may lead to differences in drug disposition within the lungs and in absorption into the systemic circulation. Clinically relevant delivery systems cannot be easily replicated in rodents, which may cause differences in drug disposition (23). Moreover, collection of multiple BALF samples per animal is not possible in rodents (17–19).

The ready availability and placid nature of sheep have long made them a useful preclinical model to assess therapies for chronic pulmonary diseases, lung injury, and vaccine delivery (24, 25). Furthermore, the sheep lung exhibits a dichotomous structure, lobation, tidal volume, and alveolar diameter similar to those of the human lung and a breathing rate only slightly higher (26–29). The relative weight of sheep (up to 50 kg) also allows more clinically relevant dose alignment. Thus, the aim of this study was to use the sheep model to assess the pulmonary and systemic disposition of CMS and formed colistin after inhaled and i.v. administration of CMS. Importantly, this 4-way crossover study also involved administration of preformed colistin by both routes to allow thorough examination of the global disposition of CMS and colistin.

RESULTS

Pharmacokinetics following i.v. administration. Minor adverse reactions (including rapid breathing) that resolved without requiring any interventions were observed in one sheep at 45 min after the i.v. CMS dose and in another sheep at 2 h after i.v. colistin. It was uncertain whether these responses were related to the study medication. Plasma concentration-time profiles of colistin following administration of colistin and of

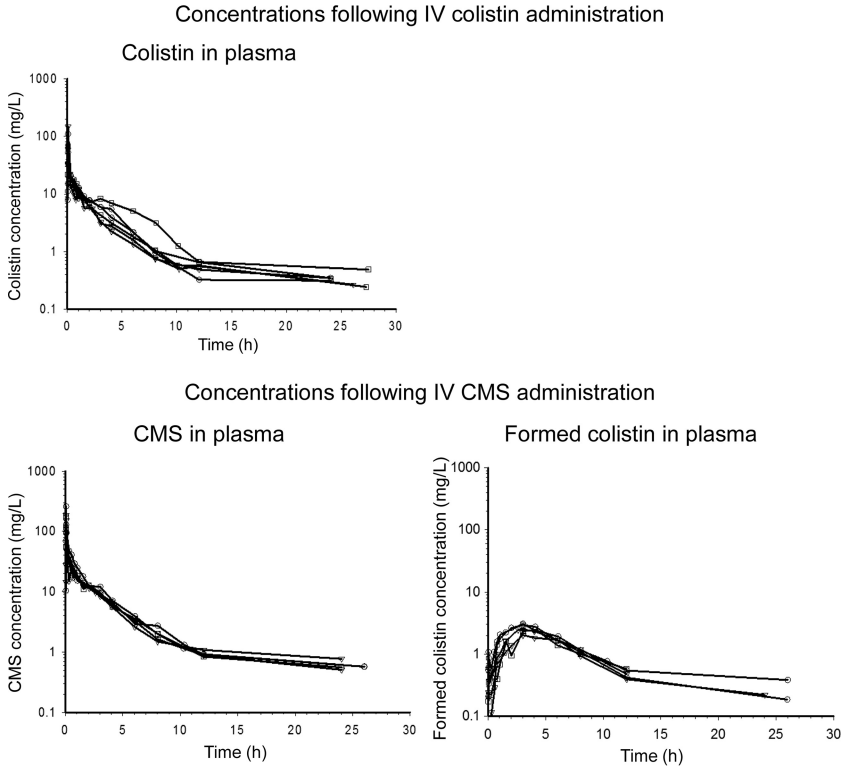


FIG 1 Observed plasma concentrations of CMS and colistin following i.v. administration of 83 mg colistin (expressed as colistin base [upper panel]) and 250 mg CMS (expressed as CMS base [lower panels]).

CMS and formed colistin after administration of CMS are presented in Fig. 1, and the corresponding noncompartmental PK parameters are summarized in Table 1. The fraction of an i.v. CMS dose converted to colistin in the systemic circulation (fm) was on average 0.174 (range, 0.10 to 0.26) (Table 1). No colistin or CMS was detected in BALF after i.v. administration of either CMS or colistin.

Pharmacokinetics following pulmonary administration. No adverse response was observed after pulmonary administration of colistin or CMS. After accounting for the residual dosing solution retained in the nebulizer, 84% ± 15% of the colistin and 63% ± 11% of the CMS were actually administered. CMS and colistin were generally not quantifiable in plasma following nebulization of either compound. One sheep had a single CMS plasma concentration of 1.18 mg/liter, which was only slightly above the lower limit of quantitation (LLOQ) of the assay (1.0 mg/liter), at 10 h after administration. Following nebulization of colistin, colistin concentrations in epithelial lining fluid (ELF) remained stable at 104 ± 83 and 104 ± 61 mg/liter at 1 and 4 h, respectively, and decreased to 25 ± 17 mg/liter at 24 h (Fig. 2). CMS concentrations in ELF in the first 4

TABLE 1 Noncompartmental parameters for colistin following i.v. dosing of colistin and for CMS and formed colistin following i.v. dosing of CMS

Pharmacokinetic parameter[a]	Values (avg ± SD)		
	Colistin	CMS	Colistin formed from CMS
T_{max} (h)	0.0339 ± 0.0014	0.0200 ± 0.0075	3.13 ± 0.55
C_{max} (mg · liter^{-1})	78.2 ± 42	179 ± 49	2.60 ± 0.42
AUC_{0-inf} (mg · liter^{-1} · h)	64.9 ± 13	111 ± 16	23.9 ± 7.5
$t_{1/2}$ (h)	14.3 ± 2.3	14.2 ± 3.8	9.06 ± 6.1
CL (liters · h^{-1})	1.32 ± 0.23	2.29 ± 0.33	7.92 ± 1.9[b]
fm			0.174 ± 0.056

[a] T_{max}, time to maximum concentration of drug in plasma; C_{max}, maximum concentration of drug in plasma.
[b] Apparent clearance (CL/fm) for formed colistin.

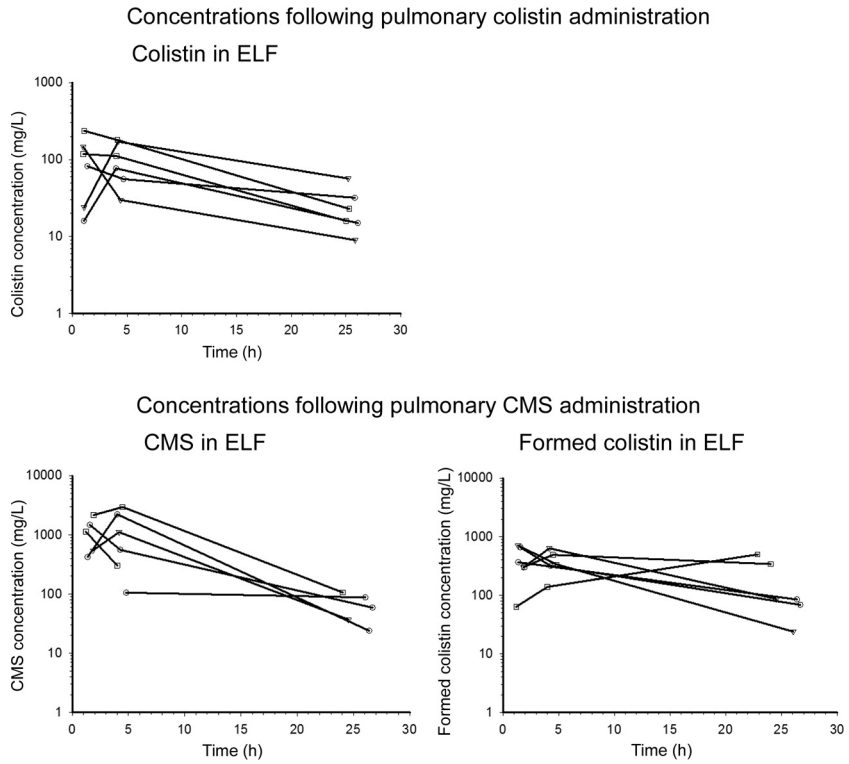
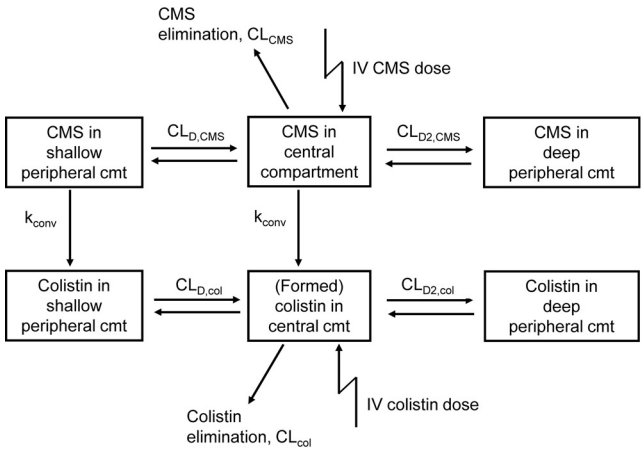


FIG 2 Observed ELF concentrations of CMS and colistin following pulmonary administration of (on average) 70 mg colistin (expressed as colistin base [upper panel]) and 158 mg CMS (expressed as CMS base [lower panels]).

h after pulmonary administration also remained relatively stable at 1,147 ± 710 and 1,209 ± 1,143 mg/liter at 1 and 4 h, respectively, and decreased to 63 ± 34 mg/liter at 24 h. Following nebulization of CMS, the average concentration of formed colistin in ELF was 400 ± 243 mg/liter at 1 h and 384 ± 187 mg/liter at 4 h and decreased to 184 ± 190 mg/liter at 24 h (Fig. 2). The therapeutic availability (TA) and drug targeting index (DTI) values associated with nebulization of colistin into the lungs were 1.4 and 7.7. Following nebulization of CMS, the respective TA and DTI values were 3.5 and 8.9 for CMS and 15.5 and 21.2 for formed colistin.

Population pharmacokinetics. The plasma concentration-time profiles of colistin and CMS following i.v. dosing of colistin and CMS were well described by the developed population PK model (Fig. 3A). The final model included three equilibrating kinetic compartments for both colistin and CMS. A first-order process of conversion of CMS to formed colistin in the central and the shallow peripheral compartments best described the increase of formed colistin concentrations in plasma. The profiles could be well fitted with the same conversion rate constant (k_{conv}) for both compartments. A conversion rate constant for the deep peripheral compartment was estimated to be very small and was not required to describe the profiles; therefore, it was not included in the final model. The clearances of CMS after conversion to colistin, calculated based on k_{conv} and the relevant volumes of distribution, were 0.0437 liters/h for the central and 0.382 liters/h for the shallow peripheral compartment, while the elimination and clearance of CMS via other pathways was 1.97 liters/h (Table 2). Based on these estimates, the population mean fraction of a CMS dose converted to colistin was 0.178. The PK parameters for sheep following i.v. dosing were scaled allometrically to 59 kg, the median weight of patients in a study by Garonzik et al. (30), and a "normal" renal function for humans (creatinine clearance [CL_{CR}], 100 ml/min/1.73 m^2), to evaluate translation. This resulted in a total body clearance of colistin (CL_{col}) value of 1.63 liters/h/59 $kg^{0.75}$ and a volume of distribution at steady state for colistin (Vss_{col}) of 37.2

Panel A: Colistin, CMS and formed colistin following IV dosing of colistin and CMS.



Panel B: Colistin, CMS and formed colistin following pulmonary dosing of colistin and CMS.

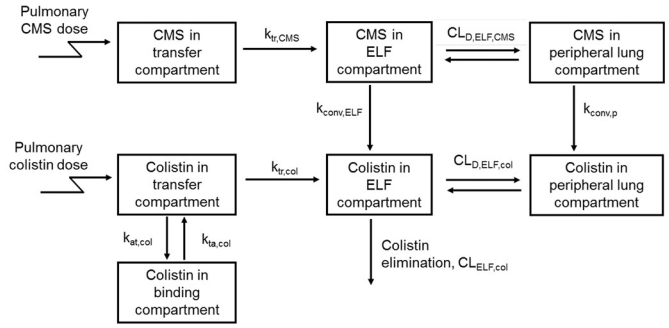


FIG 3 Model diagram. (A) Colistin, CMS, and formed colistin following i.v. dosing of colistin and CMS. cmt, compartment. (B) Colistin, CMS, and formed colistin following pulmonary dosing of colistin and CMS. (See Tables 1, 2, and 3 for definitions of abbreviations.)

liters/59 kg. The model had very good predictive performance for all three output variables modeled simultaneously (Fig. 4).

The colistin and CMS concentration-time profiles in ELF following pulmonary dosing were described by a model with an ELF and a peripheral lung compartment for both compounds, with first-order formation of colistin in both regions (Fig. 3B and Table 3). An equilibrating binding compartment for colistin following nebulization of colistin was required to successfully comodel the profiles of colistin, formed colistin, and CMS. The model achieved very good predictive performance (Fig. 5).

In order to evaluate the translation of the sheep model to colistin PK in humans, the CMS dosage regimen predicted to achieve an average concentration at steady state ($C_{ss,avg}$) of formed colistin of 2.5 mg/liter (136 mg colistin base activity [CBA] every 12 h) (30) in patients with a CL_{CR} similar to that reported in sheep (53 ml/min/1.73 m^2) was simulated from the final i.v. model. The sheep model predicted a $C_{ss,avg}$ of 2.7 mg/liter for the first of the two allometric scaling approaches described in Materials and Methods. The second allometric scaling approach predicted a $C_{ss,avg}$ of 2.6 mg/liter (Fig. 6).

DISCUSSION

This is the first four-way crossover study to quantitatively characterize the disposition of CMS and colistin in plasma and ELF following i.v. and pulmonary administration. Furthermore, it utilized a large-animal model to evaluate the potential targeting advantage of dosing by inhalation. Administration of colistin in addition to its prodrug is required to accurately quantify fm and estimate the actual instead of apparent

TABLE 2 Population pharmacokinetic parameter estimates and IIV for CMS and (formed) colistin following i.v. dosing of CMS and colistin[a]

Parameter	Mean (% CV)
CMS	
CL_{CMS} (liters/h)	1.97 (4.0)
$V1_{CMS}$ (liters)	0.763 (12)
$V2_{CMS}$ (liters)	6.66 (6.1)
$V3_{CMS}$ (liters)	12.3 (4.9)
$CL_{D,CMS}$ (liters/h)	19.4 (41)
$CL_{D2,CMS}$ (liters/h)	0.643 (7.0)
k_{conv} (h^{-1})	0.0573 (13)
$CL_{conv,1}$ (liters/h)	0.0437
$CL_{conv,2}$ (liters/h)	0.382
$CVCP_{CMS}$ (%)	32.8
$SDCP_{CMS}$ (mg/liter)	0.0865
Colistin	
CL_{col} (liters/h)	1.08 (25)
$V1_{col}$ (liters)	0.214 (112)
$V2_{col}$ (liters)	3.33 (8.4)
$V3_{col}$ (liters)	21.5 (5.5)
$CL_{D,col}$ (liters/h)	5.93 (4.6)
$CL_{D2,col}$ (liters/h)	0.529 (18)
$CVCP_{col}$ (%)	21.1
$SDCP_{col}$ (mg/liter)	0.0365

[a]IIV, interindividual variability; CL_{CMS}, clearance of CMS by pathways other than conversion to formed colistin; $V1_{CMS}$, central volume of distribution of CMS; $V2_{CMS}$, shallow peripheral volume of distribution of CMS; $V3_{CMS}$, deep peripheral volume of distribution of CMS; $CL_{D,CMS}$, intercompartmental clearance of CMS between central and shallow peripheral compartment; $CL_{D2,CMS}$, intercompartmental clearance of CMS between central and deep peripheral compartment; k_{conv}, rate constant for conversion of CMS to formed colistin; $CL_{conv,1}$, clearance of CMS by conversion to formed colistin in central compartment, calculated as $k_{conv} \times V1_{CMS}$; $CL_{conv,2}$, clearance of CMS by conversion to formed colistin in shallow peripheral compartment, calculated as $k_{conv} \times V2_{CMS}$; $CVCP_{CMS}$, proportional residual unexplained variability for CMS concentrations; $SDCP_{CMS}$, additive residual unexplained variability for CMS concentrations; CL_{col}, total body clearance of colistin; $V1_{col}$, central volume of distribution of colistin; $V2_{col}$, shallow peripheral volume of distribution of colistin; $V3_{col}$, deep peripheral volume of distribution of colistin; $CL_{D,col}$, intercompartmental clearance of colistin between central and shallow peripheral compartment; $CL_{D2,col}$, intercompartmental clearance of colistin between central and deep peripheral compartment; $CVCP_{col}$, proportional residual unexplained variability for colistin concentrations; $SDCP_{col}$, additive residual unexplained variability for colistin concentrations.

colistin clearances and volumes, as well as to thoroughly investigate the PK following pulmonary dosing. Due to safety concerns, however, colistin is not directly administered to humans (31, 32). While studies in smaller animals have provided invaluable insights into the PK of colistin-based therapies in humans, large differences in anatomical size and physiology, especially in relation to pulmonary architecture, suggest that a larger-animal model may provide additional utility.

Following i.v. CMS administration to sheep (Fig. 1), the average terminal half-life ($t_{1/2}$) of formed colistin (9.1 h [Table 1]) was within the range of values previously reported for critically ill patients (30, 33–36) and for CF patients in a clinical study (13) that used the same brand of CMS and similar i.v. doses (2.1 mg/kg of CBA) as the current sheep study (2.6 mg/kg of CBA). The $t_{1/2}$ (0.5 to 1.0 h) for formed colistin previously observed in rats (17, 37, 38) is lower than that observed in sheep and humans (30, 33–36, 39), in agreement with allometry.

CMS and formed colistin in BALF were not quantifiable following i.v. CMS dosing to sheep, which is consistent with a study on i.v. administration of 2.2 mg/kg CMS (0.82 mg/kg CBA) to critically ill patients (40). In contrast, similar colistin concentrations in ELF and plasma at steady state following i.v. CMS administration of 60 mg of CBA (0.76 mg/kg CBA) and mini-BALF sampling have been reported (16), although carryover from earlier administration of nebulized CMS remains a possibility. Low (<1 mg/liter) concentrations of formed colistin in sputum of cystic fibrosis patients have been quantified after a single i.v. CMS dose of 150 mg CBA (2.1 mg/kg CBA), but it was considered most likely that this was the result of carryover from nebulized doses administered several

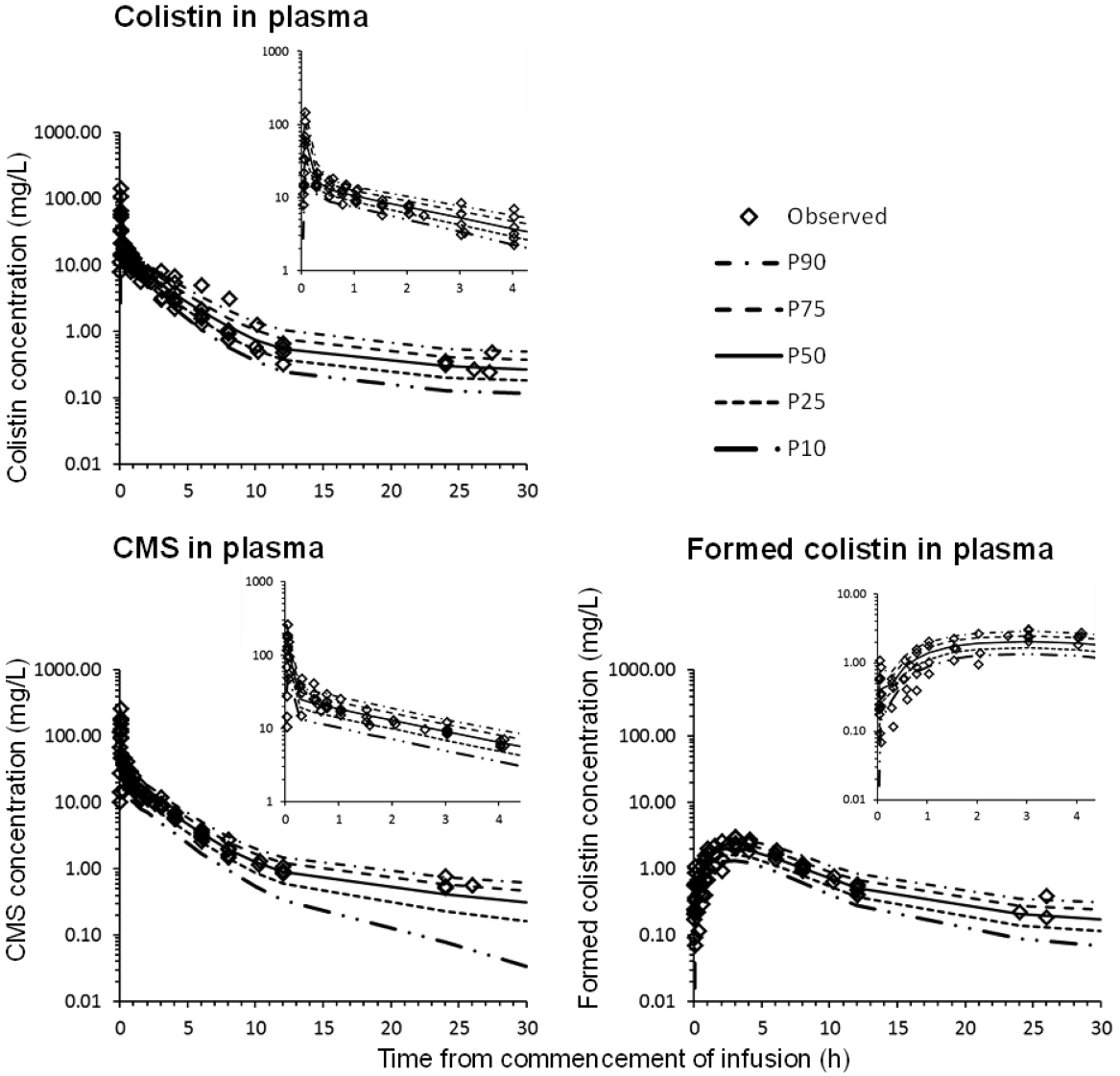


FIG 4 Visual predictive checks for colistin, CMS, and formed colistin in plasma following i.v. administration. Data represent colistin concentrations following colistin dosing (upper panel) and CMS and formed colistin concentrations following CMS dosing (lower panels). The diamonds represent the observations. The lines represent the model-predicted 10th percentile (lower broken line and dotted line), 25th percentile (lower broken line), median (solid line), 75th percentile (upper broken line), and 90th percentile (upper broken and dotted line).The LLOQ was 0.375 mg/liter for colistin and 1.0 mg/liter for CMS.

days earlier (13). Unquantifiable concentrations of formed colistin in ELF following i.v. CMS in the sheep (Fig. 1) would not be unexpected given the LLOQ for colistin in BALF and the dilution factor for interconversion of BALF and ELF concentrations.

Upon pulmonary delivery, the CMS ELF concentrations in sheep (Fig. 2 [dose 1.6 mg/kg CBA]) were ~2-fold to ~10-fold higher than the CMS concentrations in sputum of cystic fibrosis patients (13) following a dose of 1.7 mg/kg CBA; formed colistin concentrations were ~50-fold higher in sheep. CMS and formed colistin ELF concentrations in critically ill patients on treatment with nebulized CMS (0.76 mg/kg CBA) were overall slightly lower than those in sheep during the first 4 to 5 h and were highly variable (16). A previous study in critically ill patients who received inhaled CMS (~30 mg CBA [body weights not reported]) found formed colistin ELF concentrations ~10-fold to ~50-fold lower than those observed in the sheep during the first 4 h, with the doses being also considerably lower (41). However, such comparisons are fraught with difficulties as CMS and colistin concentrations are influenced by the widely differing efficiencies of the nebulizers used; in the sheep, nebulization was performed via an

TABLE 3 Population pharmacokinetic parameter estimates and IIV for CMS and (formed) colistin following pulmonary dosing of CMS and colistin

Parameter[a]	Mean (% CV)
CMS	
$k_{tr,CMS}$ (h^{-1})	0.426 (82)
$CL_{D,ELF,CMS}$ (liters/h)	0.0124 (87)
$V_{ELF,CMS,col}$ (liters)	0.0139 (13)
$V_{p,CMS}$ (liters)	1.19 (7.8)
$k_{conv,ELF}$ (h^{-1})	0.285 (48)
$k_{conv,p}$ (h^{-1})	0.00878 (217)
$CL_{conv,ELF}$ (liters/h)	0.00396
$CL_{conv,p}$ (liters/h)	0.01046
$CVCP_{CMS,ELF}$ (%)	47.2
Colistin formed from CMS	
$k_{tr,col}$ (h^{-1})	0.044 (8.2)
$CL_{D,ELF,col}$ (liters/h)	0.00067 (8.4)
$V_{p,col}$ (liters)	0.00652 (7.8)
$CL_{ELF,col}$ (liters/h)	0.00744 (5.7)
$k_{at,col}$ (h^{-1})	0.574 (32)
$k_{ta,col}$ (h^{-1})	0.0416 (66)
$CVCP_{col,ELF}$ (%)	39.9

[a] $k_{tr,CMS}$, rate constant for transfer of CMS into ELF; $CL_{D,ELF,CMS}$, intercompartmental clearance of CMS between ELF and peripheral lung compartment; $V_{ELF,CMS,col}$, volume of distribution in ELF compartment; $V_{p,CMS}$, peripheral volume of distribution of CMS in lung; $k_{conv,ELF}$, rate constant for conversion of CMS to formed colistin in ELF compartment; $k_{conv,p}$, rate constant for conversion of CMS to formed colistin in peripheral lung compartment; $CL_{conv,ELF}$, clearance of CMS by conversion to formed colistin in ELF compartment, calculated as $k_{conv,ELF} \times V_{ELF,CMS,col}$; $CL_{conv,p}$, clearance of CMS by conversion to formed colistin in peripheral lung compartment, calculated as $k_{conv,p} \times V_{p,CMS}$; $CVCP_{CMS,ELF}$, proportional residual unexplained variability for CMS concentrations; $k_{tr,col}$, rate constant for transfer of colistin into ELF; $CL_{D,ELF,col}$, intercompartmental clearance of colistin between ELF and peripheral lung compartment; $V_{p,col}$, peripheral volume of distribution of colistin in lung; $CL_{ELF,col}$, clearance of colistin from ELF; $k_{at,col}$, equilibration rate constant for colistin distribution to a binding compartment following nebulized colistin administration; $k_{ta,col}$, equilibration rate constant for colistin redistribution from a binding compartment following nebulized colistin administration; $CVCP_{col,ELF}$, proportional residual unexplained variability for colistin concentrations.

endotracheal (ET) tube and used a controlled, closed ventilation loop. Furthermore, most reported studies did not determine the residual dose left in the nebulizer.

In sheep, ELF colistin concentrations following administration of nebulized colistin sulfate were lower than those for formed colistin following administration of nebulized CMS, particularly at 1 and 4 h, even after accounting for differences in doses and molecular weights (Fig. 2). Colistin is a polycation with 5 free amino groups that may, in part, be responsible for its ability to bind electrostatically to negatively charged tissue phospholipids (42, 43). Animal studies have shown that colistin and polymyxin B accumulate in tissues, including the lungs (44–46). Unlike colistin, CMS has poor tissue binding properties which may be attributed to the amine side chains protected by sulfomethyl groups (2, 43, 45). Thus, colistin may bind extensively to various lung tissues shortly after administration, as assumed in the population PK model (Fig. 3B), whereas CMS may be more restricted to the ELF along with colistin immediately after its formation. Furthermore, colistin has been shown to bind strongly to mucin in lung fluids, thus limiting its antimicrobial activity (47).

CMS and formed colistin were not quantifiable in plasma following pulmonary dosing, despite very high concentrations in ELF. This suggests very low absorption from the lungs into the systemic circulation, an important finding given the systemic adverse effects of colistin that are dose-limiting in the clinical use of CMS. Similarly, following pulmonary CMS administration of 60 to 120 mg CBA to patients, low concentrations of formed colistin in plasma (≤0.7 mg/liter) were observed (1, 13, 16, 48). These concentrations were substantially lower than the corresponding concentrations in ELF (up to 1,100 mg/liter) or sputum (up to 20 to 40 mg/liter) (1, 13, 16) and were lower than the plasma concentrations (up to ~5 mg/liter) following i.v. administration of a similar dose of CMS (16, 30, 33). In rats, ELF concentrations after intratracheal administration of

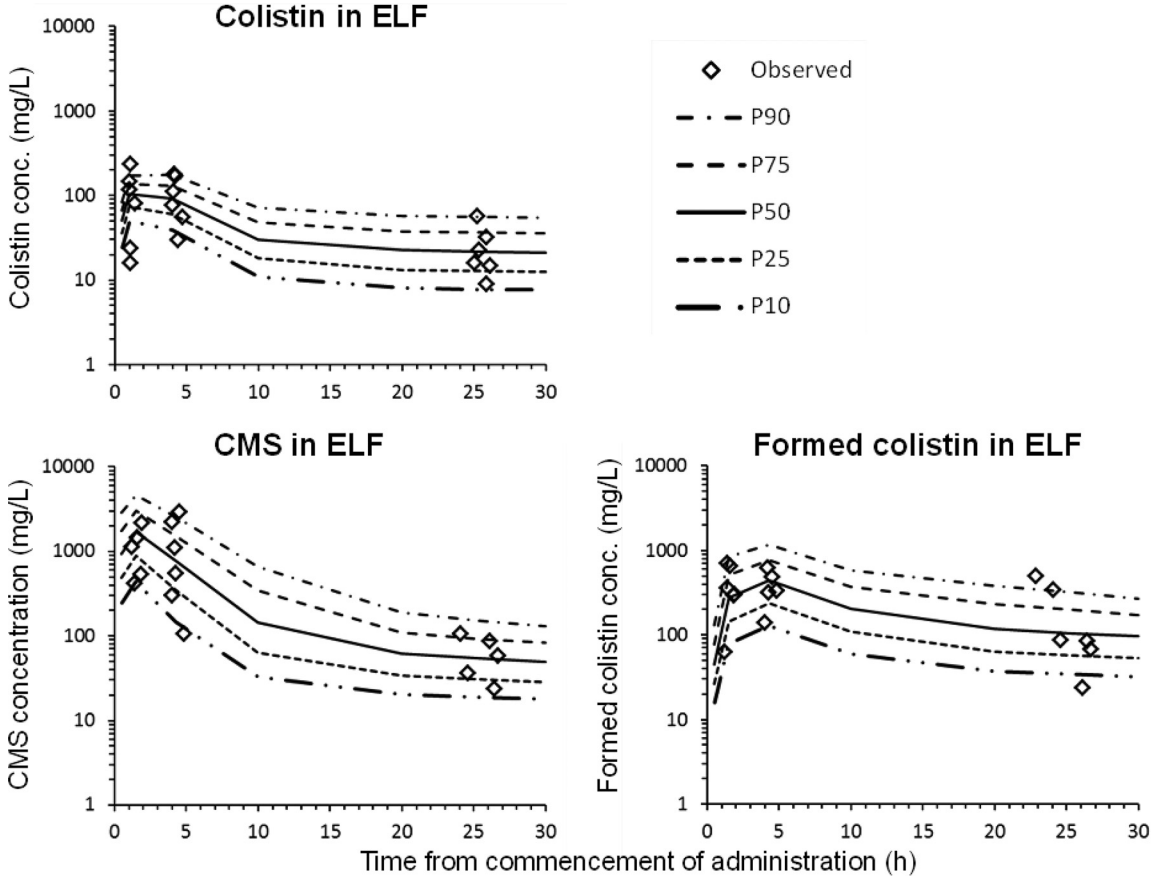


FIG 5 Visual predictive checks for colistin, CMS, and formed colistin in ELF following pulmonary administration. Data represent colistin concentrations following colistin dosing (upper panel) and CMS and formed colistin concentrations following CMS dosing (lower panels). The diamonds represent the observed concentrations. The lines represent the model-predicted 10th percentile (lower broken and dotted line), 25th percentile (lower broken line), median (solid line), 75th percentile (upper broken line), and 90th percentile (upper broken and dotted line).

nebulized colistin sulfate were 1,800-fold higher than unbound concentrations in plasma and were attributed in part to localized drug deposition in the lung and a nonlinear absorption process (19). However, in contrast to sheep and humans, the level of systemic exposure to formed colistin in rats after pulmonary dosing was found to be

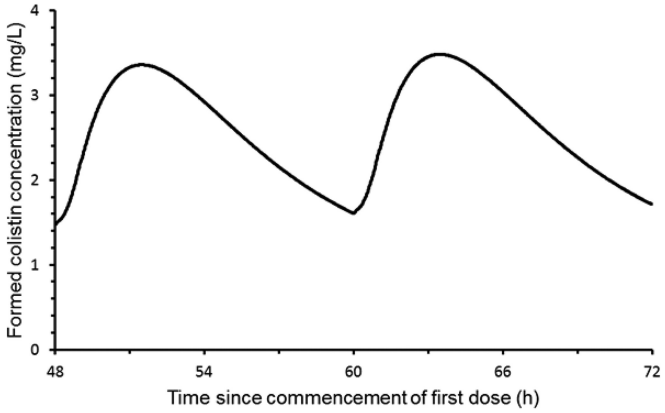


FIG 6 Simulated population average plasma concentration-versus-time profile of formed colistin at steady state which would be expected in a patient with a CL_{CR} of ~53 ml/min/1.73 m^2 and a body weight of 59 kg following an i.v. CMS dose of 136 mg CBA given every 12 h, based on predictions from the model developed for sheep. Allometric scaling was applied to nonrenal clearances, distribution clearances, and volumes of distribution of CMS and colistin.

"virtually identical" to (19) or even 2-fold to 4-fold higher than (17, 18) that seen after i.v. administration of the same dose of CMS or colistin. Overall, the sheep large-animal model in which a clinically used nebulizer was utilized appeared to more closely reflect the PK processes in patients than did a rodent model.

The potential benefits of pulmonary delivery over i.v. administration were evaluated via the TA and DTI. The TA quantifies the availability of CMS and colistin in ELF and was >1 for both CMS (TA = 3.5) and formed colistin (TA = 16). This indicated that, for a given CMS dose, the ELF exposures for CMS and formed colistin were considerably higher for the pulmonary route than the i.v. route. The degree of targeting to the ELF achieved via pulmonary administration compared to i.v. dosing was estimated by the DTI. The DTI values for both CMS (DTI = 8.9) and formed colistin (DTI = 21) were substantially >1. Therefore, substantial targeting to the lungs was achieved via pulmonary dosing compared to i.v. administration. The TA and DTI values for the active moiety, formed colistin, found in sheep were similar to the values previously reported for CF patients based on sputum concentrations (TA = 24; DTI = 35) (13). In contrast, the TA and DTI values for CMS were higher in CF patients (TA = 387; DTI = 15,952) than in sheep. The calculated TA and DTI values underestimate the true targeting advantage of pulmonary administration, as the LLOQ was used when concentrations were below the limit of quantification. Effective targeting to the lungs enables high colistin concentrations at the site of pulmonary infections, thus maximizing antibacterial efficacy and minimizing the potential for emergence of resistance, which is particularly important for a last-line antibiotic. In mice, lung infections were substantially more resilient with respect to systemic colistin treatment than thigh infections (49). At the same time, targeting to the lungs minimizes systemic concentrations and thus the potential for nephrotoxicity, an important consideration given the dose-limiting nature of this adverse effect (50).

Our population PK comodeling of colistin, CMS, and formed colistin within plasma and ELF successfully characterized the concentration-time courses. The population mean fm of an i.v. CMS dose in sheep was 0.178, which agreed very well with the estimate from noncompartmental analysis (NCA) and was close to the range of 0.20 to 0.25 estimated for patients with normal renal function (51). The population estimated rate constant of conversion of CMS to colistin was greater in ELF ($k_{conv,ELF}$ [Table 3]) than in plasma (k_{conv} [Table 2]). Similarly, the fm has been reported to be higher in ELF than in plasma in rats (17, 18). Greater fractional conversion of CMS to colistin in ELF probably occurs because CMS in lungs is not subjected to renal elimination.

The CL_{col} from sheep scaled by body weight and CL_{CR} (1.63 liters/h/59 $kg^{0.75}$) matched very well with the estimate of 1.56 liters/h/59 $kg^{0.75}$ in patients (30), assuming that the unknown fm (predicated on total CMS dose) was the same in patients and sheep. The scaled Vss_{col} values determined for sheep (37.2 liters/59 kg) and patients (25.9 liters/59 kg) (for fm = 0.178) were also similar (30). The allometrically scaled sheep model predicted a formed colistin $C_{ss,avg}$ that was in close agreement with that for patients with the same dosage regimen and a CL_{CR} similar to that reported in sheep. The excellent translation is remarkable considering the many factors that can affect PK in critically ill patients (52).

This is the first report of a 4-way crossover study characterizing the systemic and pulmonary PK of CMS and colistin following i.v. and pulmonary administration. The sheep model allowed nebulized delivery that is more similar to that used in clinical settings than to the pulmonary administration to smaller animals. Similarities in the disposition of colistin and CMS between sheep and humans and successful allometric scaling suggest that sheep may represent a clinically relevant PK model. Pulmonary dosing provides a substantial targeting advantage compared to i.v. administration due to high colistin ELF concentrations that may be beneficial for treatment of pulmonary infections and low colistin plasma concentrations that would be expected to decrease the risk of nephrotoxicity.

MATERIALS AND METHODS

Colistin sulfate (Sigma-Aldrich, St. Louis, MO, USA) (activity, ≥15,000 units/mg) was used to prepare i.v. and pulmonary dosing solutions for studies involving direct administration of the active entity. For CMS, two clinically available formulations were utilized: colistimethate sodium (Colistin Link; Link Pharmaceuticals, Auckland, New Zealand) for i.v. administration and colistimethate sodium (Tadim; Phebra, NSW, Australia) for pulmonary administration. Heparin sodium injection BP (35,000 IU/35 ml) was obtained from Hospira (Victoria, Australia). FMOC chloride (9-fluorenylmethyl chloroformate), sodium bicarbonate, sodium hydroxide, boric acid, sulfuric acid, and trifluoroacetic acid (TFA) were all of analytical reagent grade (Sigma-Aldrich, St. Louis, MO, USA). A commercial urea assay kit (Bioassay Systems, Hayward, CA, USA) was used to determine urea concentrations in plasma and BALF. High-performance liquid chromatography (HPLC)-grade acetone, methanol, acetonitrile, and tetrahydrofuran were from Merck (Darmstadt, Germany). All water came from a Milli-Q purification/filtration system (Millipore, MA, USA).

Preparation of colistin sulfate and CMS formulations. Colistin sulfate was prepared at 20 mg/ml in 0.9% saline solution for a nominal dose of 100 mg per sheep (equivalent to 83 mg colistin base) for both i.v. and pulmonary administration. The solution was filtered through a sterile 0.22-μm-pore-size syringe filter into a sterile tube and stored at 4°C for up to 30 min before use. CMS was prepared for i.v. and pulmonary administration at a dose of 267 mg CMS sodium (equivalent to ~250 mg CMS base, corresponding to microbiological activity of ~3.3 million IU of CMS and ~110 mg colistin base activity [CBA]) in 5 ml saline solution according to the instructions of the manufacturers. Given the potential for conversion to colistin (53), the CMS formulations were prepared, filtered through a 0.22-μm-pore-size sterile filter, and transferred to a sterile 10-ml tube immediately prior to administration.

Pharmacokinetic studies. (i) Study design. Six merino sheep (5 ewes, 1 wether, 1 to 2 years old, 34 to 43 kg body weight) were obtained from a commercial supplier. The study was approved by and conducted in accordance with the guidelines of the Monash University Animal Ethics Committee. Prior to the study, each sheep underwent surgery for cannulation of the jugular vein to facilitate i.v. administration of CMS and colistin and of the carotid artery for collection of blood samples. Animals were allowed to recover for 5 to 7 days prior to the commencement of the study. On 4 separate occasions, each sheep was administered CMS and colistin by the pulmonary and i.v. routes, according to a randomized 4-way crossover design with a 7-day washout period between treatments. In each of the 4 study periods, both blood and BALF were collected for quantification of CMS and colistin.

(ii) i.v. administration and collection of blood samples. Both colistin and CMS were administered i.v. via the indwelling jugular vein cannula. In each case, the dose (5 ml) was infused at 1 ml/min using an infusion pump (Harvard Apparatus, NSW, Australia). Upon completion of the 5-min infusion, heparinized saline solution (5 ml, 35 IU/ml) was infused to flush the cannula. Blood samples (3 ml) were collected via the carotid artery cannula prior to i.v. administration and at 1, 2, 15, 30, and 45 min as well as 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after the start of the infusion. The cannula was flushed with heparinized saline solution (10 ml, 35 IU/ml) after each sample was collected to maintain patency. At the collection of the subsequent sample, the volume in the cannula since the previous flush was not retained as part of the sample. Blood samples were immediately centrifuged at 3,500 × *g* for 10 min at 4°C, and plasma samples were collected and stored at −80°C until analysis.

(iii) Pulmonary administration and collection of blood samples. To ensure maximal delivery to the lung, the colistin and CMS formulations were nebulized via an endotracheal (ET) tube directly into the trachea, in a manner similar to that previously reported (54). Prior to administration, each animal was restrained and intubated with an ET tube (Smiths Medical, Bella Vista, NSW, Australia) (inner diameter [ID], 7.5 to 8 mm) via the nasal cavity using a fiber-optic endoscope (model FG-16X; Pentax, Montvale, NJ, USA). Lignocaine (2% [wt/vol] gel) was applied to the nasal passage to minimize any temporary discomfort that occurred as a result of the procedure. After insertion, the ET tube cuff was inflated to facilitate artificial ventilation and ensure controlled drug administration via the trachea and was connected to a Harvard apparatus ventilator (model 55-0723; Harvard Apparatus, MA) with the inspiratory/expiratory rates set to 50/50 with 20 breaths/min. The formulations (5 ml) were administered as an aerosol via a Nomad NExGen (EBOS Group Pty Ltd., Australia) travel nebulizer system with a NebuTech HDN jet nebulizer (Salter Labs, USA) connected to an enclosed ventilator system. The formulations were nebulized over 25 min, after which the ET tube was removed and the sheep were allowed to breathe independently. The exact doses delivered were determined by the actual volume administered (5 ml minus the volume remaining in the sample chamber of the nebulizer after 25 min of administration) and assay of the formulation dosed. All PK calculations and modeling used the actual doses. Blood samples (3 ml) were collected prior to administration and at 5, 10, 25, 35, and 45 min as well as 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after the commencement of dose administration. Procedures for blood collection, harvesting of plasma, and storage of samples were as described above.

Collection of bronchoalveolar lavage fluid. To assess the local concentration of colistin and CMS in the epithelial lining fluid (ELF) of the alveolar and bronchial spaces after i.v. and pulmonary administration, BALF was collected from a defined region or lobe of the lung (24). A fiber-optic endoscope coated with lignocaine (2% [wt/vol] gel) was inserted through the nasal passage and directed deep into the right caudal lung. Approximately 10 ml of a 0.9% saline solution was infused through the biopsy port of the endoscope into the lung section, mixing with the ELF. The ELF/saline solution mixture (BALF) was gently collected into a syringe through the same port, with 5 to 6 ml recovered from the 10 ml infused. BALF samples were collected from each sheep prior to i.v. and pulmonary administration of colistin and CMS formulations and at 1, 4, and 24 h postadministration. Samples were collected, centrifuged, and stored as described for the plasma samples.

Determination of CMS and colistin concentrations. The concentrations of CMS and colistin in plasma and BALF were determined via a modified assay described by Li et al. (55, 56) using solid-phase extraction, precolumn derivatization, and fluorescence detection. Briefly, for the assay of plasma samples, standards were prepared in blank sheep plasma within the range of 0.375 to 8.0 mg/liter for colistin and 1.0 to 20 mg/liter for CMS. For CMS, a separate aliquot of each sample was pretreated with acid to hydrolyze CMS to colistin and the concentration of the prodrug was then determined as the difference between the concentrations measured with and without the acid hydrolysis, accounting for the differences in molecular weights of CMS and colistin. Samples containing >8 mg/liter colistin or >20 mg/liter CMS were diluted with blank sheep plasma as required, reprocessed, and analyzed. Quality control samples were prepared for each HPLC run, and the assay run was deemed acceptable when replicates ($n = 3$) at three concentrations were within 15% of the target (±20% at the lower limit of quantification [LLOQ], which was 0.375 mg/liter for colistin and 1.0 mg/liter for CMS). For BALF samples, colistin and CMS standards were prepared in 50:50 acetonitrile/blank BALF across the range of 0.125 to 4 mg/liter (LLOQ = 0.125 mg/liter for both) and processed in a manner similar to that described for the plasma samples. Quality control criteria for BALF sample analysis were in accordance with those for plasma.

The urea concentrations in plasma and BALF were quantified via the use of a commercial kit (Bioassay Systems, Hayward, CA, USA). Urea was used as an endogenous marker to determine the apparent volume of ELF from BALF relative to urea concentrations in plasma (18, 57). Briefly, the apparent volume of ELF in each sample ($V_{ELF,sample}$) was calculated as follows: $V_{ELF,sample} = \{[\text{urea (in milligrams per deciliter)}]_{BALF}/[\text{urea (in milligrams per deciliter)}]_{plasma}\} \times V_{BALF,sample}$. The $V_{ELF,sample}$ was used to calculate the concentration of colistin or CMS in ELF from BALF as follows: $[\text{colistin or CMS}]_{ELF} = [\text{colistin or CMS}]_{BALF} \times (V_{BALF,sample}/V_{ELF,sample})$.

Noncompartmental pharmacokinetic analysis. NCA was performed using WinNonLin (version 5.3; Pharsight Corp., Mountain View, CA). The area under the plasma concentration versus time curve to the last observation time point (AUC_{0-t}), generally 24 h (AUC_{0-24}), was calculated using linear interpolation for increasing or constant concentrations and logarithmic interpolation for decreasing concentrations. For colistin and CMS in plasma, the AUC extrapolated to infinity (AUC_{0-inf}) was also calculated. The fraction of an i.v. CMS dose converted to colistin in the systemic circulation (fm) was calculated for each sheep as follows:

$$\text{fm} = \frac{\text{formed colistin AUC}_{0-\text{inf}}/D_{\text{CMS}}}{\text{colistin AUC}_{0-\text{inf}}/D_{\text{colistin}}} \quad (1)$$

where the formed colistin AUC_{0-inf} is the AUC_{0-inf} of formed colistin in plasma following i.v. CMS administration, the colistin AUC_{0-inf} is the AUC_{0-inf} of colistin in plasma following i.v. colistin administration, D_{CMS} is the CMS i.v. dose, and $D_{colistin}$ is the colistin i.v. dose. The molecular weights of CMS and colistin were used to normalize the doses to colistin equivalents.

To assess the targeting advantage of administering colistin and CMS to the lung compared to the i.v. route, the therapeutic availability (TA) (58) and drug targeting index (DTI) (58, 59) were calculated for both colistin and CMS as follows:

$$\text{TA} = \frac{(\text{mean ELF AUC}_{0-t}/D)_{\text{nebulized}}}{(\text{mean ELF AUC}_{0-t}/D)_{\text{i.v.}}} \quad (2)$$

$$\text{DTI} = \frac{\left(\dfrac{\text{mean ELF AUC}_{0-t}/D}{\text{mean plasma AUC}_{0-t}/D}\right)_{\text{nebulized}}}{\left(\dfrac{\text{mean ELF AUC}_{0-t}/D}{\text{mean plasma AUC}_{0-t}/D}\right)_{\text{i.v.}}} \quad (3)$$

where the ELF AUC_{0-t} and plasma AUC_{0-t} denote the AUC_{0-t} in ELF and plasma, respectively, and D denotes the dose for the respective compound, colistin or CMS. The terms in the numerator refer to areas and doses for nebulized administration and the terms in the denominator to i.v. administration. In all cases where no quantifiable concentration of colistin or CMS was measured, the relevant LLOQ was used in the calculation. Group data are presented as averages ± standard deviations (SD). Statistical analyses were performed using SPSS 17.0 for Windows.

Population pharmacokinetic modeling. Population PK modeling of colistin, CMS, and colistin formed from CMS in plasma and ELF following i.v. and pulmonary administration was performed in S-ADAPT (version 1.57) with the Monte Carlo parametric expectation maximization algorithm (MC-PEM [importance sampling; pmethod = 4]) (60, 61). SADAPT-TRAN was utilized for pre- and postprocessing (62). Models with one, two, or three disposition compartments for CMS and one or two disposition compartments for formed colistin were evaluated to fit the plasma concentrations following i.v. administration. For conversion of CMS to colistin, first-order, mixed-order (saturable), and zero-order processes were explored, as well as combinations of multiple processes and transit compartment models. The plasma concentrations of colistin, CMS, and colistin formed from CMS following separate i.v. administrations of colistin and CMS were comodeled. Similarly, the concentrations in ELF of colistin, CMS, and colistin formed from CMS following pulmonary colistin and CMS dosing were simultaneously modeled. The values corresponding to the interindividual variability (IIV) of the PK parameters were assumed to be log-normally distributed. Proportional and combined additive and proportional error models were explored to describe the residual unidentified variability. Observed versus individually fitted and observed versus population-fitted concentration plots, visual predictive checks, the normalized prediction distribution error, and the objective function in S-ADAPT were utilized to evaluate model performance.

To evaluate translation between sheep and humans, the final i.v. model for sheep was used in simulations based upon a population PK model and dosing algorithm developed for i.v. CMS administration to critically ill patients (30). A creatinine clearance (CL_{CR}) value of 53 ml/min/1.73 m^2 was used for sheep (63, 64). Allometric scaling by body weight was applied to either (i) only the central volume of distribution of CMS according to the covariate model in patients (30) or (ii) the nonrenal clearances, distribution clearances, and volumes of distribution of CMS and colistin (as the body weight of the sheep was at the lower range of body weights in patients). For approach ii, the nonrenal clearances of CMS and colistin were assumed to account for 37% and 74% of the respective total clearances, according to the covariate model in patients (30) and CL_{CR} in sheep. An i.v. CMS dose of 136 mg CBA (equivalent to ~330 mg CMS [sodium]) given every 12 h was simulated. This is the recommended regimen to achieve a target average plasma colistin concentration at steady-state ($C_{ss,avg}$) of 2.5 mg/liter in patients with a CL_{CR} similar to that in the sheep (30). Simulations were performed in Berkeley Madonna (version 8.3.18).

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