Population pharmacokinetics of colistin methanesulfonate in rats: achieving sustained lung concentrations of colistin for targeting respiratory infections

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Abstract

Colistin methanesulfonate (CMS), the inactive prodrug of colistin, is administered by inhalation for the management of respiratory infections. However, limited pharmacokinetic data is available for CMS and colistin following pulmonary delivery. This study investigates the pharmacokinetics of CMS and colistin following intravenous (IV) and intratracheal (IT) administration in rats and determines the targeting advantage after direct delivery into the lungs. In addition to plasma, bronchoalveolar lavage (BAL) fluid was collected to quantify drug concentrations in lung epithelial lining fluid (ELF). The resulting data was analyzed using a population modeling approach in S-ADAPT. A three-compartment model described the disposition of both compounds in plasma following IV administration. The estimated mean clearance from the central compartment was 0.122 L/h for CMS and 0.0657 L/h for colistin. Conversion of CMS to colistin from all three compartments was required to fit the plasma data. The fraction of the IV dose converted to colistin in the systemic circulation was 0.0255. Two BAL fluid compartments were required to reflect drug kinetics in the ELF after IT dosing. A slow conversion of CMS (mean conversion time, MCTCMS = 3.48 h) in the lungs contributed to high and sustained concentrations of colistin in ELF. The fraction of the CMS dose converted to colistin in ELF (fm,ELF = 0.226) was higher than the corresponding fractional conversion in plasma after IV administration. In conclusion, pulmonary administration of CMS achieves high and sustained exposures of colistin in lungs for targeting respiratory infections.
Introduction

Pulmonary administration of antibiotics for the treatment of respiratory infections has gained significant interest over the last decade, due to the potential for achieving high local drug concentrations at the site of infection (1-4). Other favorable attributes for targeting antibiotics to the respiratory tract include a rapid onset, whilst minimizing systemic exposure and adverse effects (1-4). Several antibiotics are in clinical development for the treatment of lung infections, with tobramycin, aztreonam and colistin methanesulfonate (CMS) currently approved for inhalational administration in cystic fibrosis (CF) patients (5).

Colistin (also known as polymyxin E) was introduced into the market in the late 1950s for parenteral delivery but, due to the development of adverse effects including nephro- and neurotoxicity, was replaced by other antibiotics (6-8). In the last two decades, however, there has been resurgence in the use of colistin as last line therapy against multidrug-resistant (MDR) Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* (6, 7, 9). Clinically the inactive prodrug of colistin, CMS (10) is administered, with formation of colistin a pre-requisite for antibacterial activity (11). CMS is administered via the pulmonary route in CF patients for the treatment of initial colonization of *P. aeruginosa* in the airways (12), and as maintenance therapy in chronic infections (13, 14). Effective treatment with inhaled CMS is crucial for delaying lung function deterioration (12-14). More recently, inhaled CMS has been introduced as adjunctive therapy in critically-ill patients with ventilator-associated pneumonia (VAP) caused by *P. aeruginosa* and *A. baumannii* (15, 16).

Despite the increase in inhalational administration of CMS, there are limited pharmacokinetic data available for CMS and formed colistin after pulmonary dosing. Recently, pharmacokinetic evaluation in rats (17), CF patients (18) and critically-ill patients
have reported on CMS and/or formed colistin exposure in sputum or epithelial lining fluid (ELF) and plasma following inhalation of a single dose of CMS. Relative to plasma exposure, high CMS and/or formed colistin exposure in sputum/ELF were evident in all studies (17-19). However, none of these studies characterized the lung and systemic pharmacokinetics following administration of both CMS and colistin via the pulmonary and intravenous (IV) route. This information is required to accurately identify colistin absorption and disposition from the kinetics of formation following CMS conversion.

Therefore, the principal objective of the current study was to investigate the pharmacokinetics of CMS and colistin following IV and pulmonary administration of both compounds in rats. The development of a population pharmacokinetic model provided a better understanding of the CMS to colistin conversion kinetics and the disposition in plasma and ELF. Overall, the study identified the targeted advantage achieved following direct dosing of CMS into the lungs compared to IV administration.

**Materials and methods**

**Chemicals**

Colistin sulfate and sodium colistin methanesulfonate were from Sigma-Aldrich (Missouri, USA) and Link Pharmaceuticals Ltd (Auckland, New Zealand), respectively. All other chemicals were of analytical reagent grade and solvents were of at least high-performance liquid chromatography (HPLC) grade.

**Animals**

All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The study protocols were reviewed and approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee.
Male Sprague-Dawley rats (300-320 g) were acclimated for a minimum of seven days in the faculty animal house within a temperature range of 18-24°C, 40-70% relative humidity and 12 h light/dark cycles. Food and water were available ad libitum during the acclimation period and throughout the study. The day prior to the pharmacokinetic study, the right carotid artery of each rat was cannulated for collection of blood samples; the jugular vein was cannulated for IV dosing. Following surgery, rats were individually housed in metabolic cages and allowed 24 h for recovery.

**Drug formulations and administration**

Immediately prior to IV and pulmonary administration, CMS (sodium) and colistin (sulfate) dosing solutions were freshly prepared in sterile 0.9% sodium chloride (Baxter Healthcare Pty Ltd, New South Wales, Australia). For the IV studies, CMS or colistin solutions were administered by a bolus injection via the jugular vein cannula. Intratracheal (IT) instillation was utilized as the technique for pulmonary administration, as it delivers an accurate and reproducible volume of dosing solution into a localized region of the lungs (20). For IT instillation, rats were lightly anesthetized with gaseous isoflurane (Delvert Pty Ltd, New South Wales, Australia) and rested in a supine position against a restraining board angled at approximately 60-70° from the horizontal. The tongue of the rat was gently pulled outwards using forceps, and the blade of a small animal laryngoscope (PennCentury Inc, Pennsylvania, USA) positioned in the distal part of the mouth to enable visualization of the vocal cords. A 2.5 cm polyethylene tube (PE) (0.96 × 0.58 mm (o.d. × i.d.)) attached to a 23 gauge (G) needle and 1 mL syringe was then maneuvered past the vocal cords to the trachea-bronchus bifurcation. A 100 µL aliquot of dosing solution followed by a 200 µL bolus of air was then delivered into the rat lungs. The air was administered to ensure complete delivery of
the dosing solution from the syringe and cannula. Following IT instillation, rats were returned to metabolism cages where they rapidly recovered from the isoflurane anesthesia.

**Pharmacokinetic studies**

Animals were administered IV CMS at doses of 14 mg/kg, 28 mg/kg or 56 mg/kg (n=3 per dose). Blood samples (320 μL) were collected via the carotid artery prior to dosing and at 0.08, 0.25, 0.5, 1, 2, 3 and 4 h post-dosing. In an independent study, rats were administered IV colistin at doses of 0.21 mg/kg, 0.41 mg/kg or 0.62 mg/kg (n=3 per dose); blood samples (200 μL) were collected prior to dosing and at 0.02, 0.05, 0.08, 0.17, 0.33, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h post-dose. In the dose-ranging CMS study above, blood samples were immediately placed on ice prior to centrifugation (6,700 × g, 4°C, 10 min), to minimize any potential *in vitro* conversion of CMS to colistin. Additionally, any potential *in vitro* conversion was minimized by storage of plasma samples at -80°C, and subsequent HPLC analysis within 4 months of collection (21).

For the pulmonary dosing studies, two cohorts of animals were required to fully characterize systemic and lung exposure. Rats in the first cohort were administered IT CMS at doses of 14 mg/kg or 28 mg/kg (n=3 per dose). Blood samples (320 μL) were collected pre-dose and at 0.08, 0.5, 1, 2, 3, 4, 5, 6 and 8 h post-dose. In the second cohort, rats received a pulmonary dose of 14 mg/kg CMS, to allow for the collection of terminal bronchoalveolar lavage (BAL) fluid and a corresponding blood sample. Samples were collected at 0.08, 0.5, 2, 4, 6, 8 and 12 h post-dose (n=3 rats per time point). In a separate study, rats in the first cohort were administered colistin at IT doses of 0.41 mg/kg, 0.62 mg/kg, 0.99 mg/kg or 1.49 mg/kg (n=3 per dose). Blood samples were collected as described above following IV colistin dosing. A second cohort of rats received an IT dose of 0.62 mg/kg colistin; terminal BAL fluid and blood samples were collected as described for IT dosing of CMS. Blood samples
were processed as above and the plasma collected to quantify drug and urea concentrations (for the second cohort of rats). Bronchoalveolar lavage and processing of BAL fluid samples were undertaken as detailed below.

The lowest dosages in these studies were selected based on the quantification limits of the analytical methods in both plasma and in BAL fluid, whereas the higher dosages for IV CMS were defined on the basis of known tolerability in rats (22). IT administration of 56 mg/kg CMS was not well tolerated and therefore only two CMS IT dosages were evaluated. Colistin dosages were selected to achieve plasma concentrations known to be well tolerated by the animals.

**Bronchoalveolar lavage**

Rats were anesthetized with gaseous isoflurane and sacrificed via exsanguination. For BAL, the trachea was exposed and a small incision made to allow the insertion of a PE tube (1.70 × 1.20 mm (o.d. × i.d.)) attached to an 18G needle. The lungs were gently lavaged with 5 mL of phosphate buffered saline (PBS, pH 7.4, 4°C) for three cycles, using fresh PBS each time. The recovered lavage fluid was pooled and centrifuged (6,700 × g, 4°C, 10 min), and the resulting supernatant removed and stored at -80°C prior to HPLC analysis.

**Plasma and bronchoalveolar fluid analysis**

The concentrations of colistin and CMS in plasma and BAL fluid were determined using previously validated HPLC assays (23, 24) with minor modifications. Briefly, the analytical methods involved quantification of colistin before and after forced *in vitro* conversion from CMS; the concentration of CMS was calculated as the difference between the two assay results and adjusted for molecular weight (23, 24). For each of colistin and CMS assays, calibration standards and quality control (QC) samples were from independently
prepared stock solutions. For the plasma assay, colistin and CMS calibration standards were prepared in drug-free rat plasma, and ranged from 0.08 – 3.3 mg/L and 0.70 – 47 mg/L, respectively. For the analysis in BAL fluid, calibration standards were prepared in drug-free rat BAL fluid:acetonitrile (50:50, v/v), and ranged from 0.20 – 6.60 mg/L (colistin) and 0.70 – 47 mg/L (CMS). Rat plasma and BAL fluid samples with colistin and CMS concentrations above the upper end of the respective plasma and BAL fluid calibration curve were diluted to within the linear range with drug-free matrix; QC samples of colistin and CMS were treated similarly to confirm satisfactory assay performance. The limit of quantification (LOQ) was defined as the lowest concentration in the calibration curve, at which the accuracy and precision were within ± 20%; at all other concentrations, the accuracy and precision were within ± 15%.

Urea concentrations in plasma and BAL fluid were determined using a commercially available kit, QuantiChrom™ Urea Assay Kit (BioAssay Systems, California, USA). Urea is an endogenous marker of dilution, and was used to estimate the apparent volume of the ELF (V_{ELF}) (25). The apparent V_{ELF} was calculated as shown in Equation 1:

$$V_{ELF} = \frac{[\text{Urea}]_{\text{BAL fluid}}}{[\text{Urea}]_{\text{Plasma}}} \times V_{BALF}$$

(1)

where [Urea]_{BAL fluid}, [Urea]_{Plasma} and V_{BALF} are the urea concentration in BAL fluid (mg/dL), plasma (mg/dL) and the volume of recovered BAL fluid, respectively. The V_{ELF} was used to calculate the concentration of CMS or colistin in ELF ([CMS/Colistin]_{ELF}) using Equation 2:

$$[\text{CMS/Colistin}]_{ELF} = [\text{CMS/Colistin}]_{BALF} \times \frac{V_{BALF}}{V_{ELF}}$$

(2)

where [CMS/Colistin]_{BALF} is the concentration of CMS or colistin in the recovered BAL fluid.
Pharmacokinetic modeling

Initially, a non-compartmental analysis (NCA) of dose-normalized concentration-versus-time profiles was conducted using WinNonlin (version 5.3, Pharsight Corporation, USA) to guide model development. Population pharmacokinetic analysis of the observed (not dose-normalized) concentrations was then performed using nonlinear mixed-effects modeling in S-ADAPT (version 1.57) facilitated by SADAPT-TRAN (26). Initially, models were independently developed for CMS and colistin following their IV administration. One-, two-, three- and four-compartment models were tested to obtain key disposition parameters of CMS or colistin in plasma. The concentration-versus-time data of CMS and colistin in plasma, BAL fluid and ELF were then combined to simultaneously analyze the pharmacokinetic profiles after IV and IT administration. Two compartments (BAL fluid$_1$ and BAL fluid$_2$) were required to reflect drug kinetics in the BAL fluid. The total amount of drug in the BAL fluid was therefore represented by the sum of the predicted amounts in BAL fluid$_1$ and BAL fluid$_2$. These total drug amounts were divided by $V_{ELF}$, for the prediction of CMS or colistin concentrations in the ELF. This approach was used because direct estimation of ELF drug concentrations gave rise to model instability, probably due to the very low values derived for $V_{ELF}$.

First-order and mixed-order kinetics were tested to describe the conversion of CMS to colistin, and absorption of both compounds from the lungs into the systemic circulation. These models also explored whether a delay (represented by a series of transit (Tr) compartments) in conversion or absorption was required to adequately fit the data. Figure 1 illustrates the structure of the final composite model. The corresponding differential equations for the structural model are provided in the Appendix (Equations A1–A16).
The between-subject variability (BSV) in parameter estimates was assumed to follow a log-normal distribution, with the magnitude reported as a coefficient of variation (CV). Residual unexplained variability (RUV) in the plasma model was evaluated using a combined exponential and additive random error. For the BAL fluid and ELF model, the exponential and additive components were fixed to assay precision (15%) and LOQ (0.18 μmol/L for colistin and 0.45 μmol/L for CMS), respectively. This allowed for the estimation of BSV, since only a single observation was obtained from each rat due to terminal BAL sampling. Data below the LOQ were handled using a likelihood-based (M3) method (27).

Visual inspection of diagnostic scatter plots, the objective function value (OBJ, reported as \(-1 \times \log \text{ likelihood in S-ADAPT}\)) and the biological plausibility of the parameter estimates were used for model selection. Statistical comparison of the models was performed using a \(\chi^2\) test; a decrease in the OBJ of 1.92 units (\(\alpha = 0.05\)) was considered significant.

The final model was evaluated by performing a visual predictive check (VPC). For this, 1000 data sets were simulated from the final parameter estimates using the original data. The median and the 10th and 90th percentiles (25th and 75th percentiles for the ELF models) of the simulated predictions were computed and plotted against the observed values. All reported parameter estimates and VPCs were from the simultaneous modeling of CMS and colistin pharmacokinetics after IV and IT dosing. For clinical relevance, all concentrations are reported in mg/L units (although the model was developed in μmol/L units to facilitate accurate estimation of CMS to colistin conversion kinetics).

**Histopathology**

Histopathology examination was undertaken to determine whether cellular damage to the lung epithelium occurred following pulmonary dosing of CMS or colistin. Two control groups (n=3 rats per group), were dosed with 100 μL of blank saline via IT instillation; the
treatment groups (n=3 rats per group) received either 14 mg/kg CMS or 0.62 mg/kg colistin (doses corresponding to the studies where BAL fluid was collected) in a 100 μL aliquot. Following dosing, rats were anesthetized and sacrificed via exsanguination at 4 h (for CMS) or 0.75 h (for colistin); sample collection times reflect maximal concentrations in plasma. The trachea was exposed and an incision made to allow for the insertion of an 18G needle. The trachea/lungs were then immediately fixed with 5 mL of formalin solution (neutral buffered, 10%, Sigma-Aldrich), and stored in 10 mL of formalin solution. Histopathology examination (Cerberus Sciences, South Australia, Australia) was performed on the following lung tissues, 1) trachea in the lumen, mucosa, submucosa, submucosal glands, connective tissue and cartilage regions, and 2) lung lobes in the external/internal bronchi, terminal bronchioles/alveolar ducts, tracheal bifurcation (lumen, mucosa, submucosa, connective tissues), pleura, blood vessels and the bronchus-associated lymphoid tissues regions. BAL was not performed prior to tissue harvesting to ensure that the histopathology was not affected by excess fluid in the alveoli.

**Results**

**Pharmacokinetics following intravenous administration**

Following administration of IV CMS, average maximal plasma concentrations of CMS were 44 mg/L (lowest dose) and 201 mg/L (highest dose), and declined by approximately three-orders of magnitude over the study period (Figure 2A). The corresponding concentrations of formed colistin in plasma were quantifiable at the first sampling time (5 min), with mean peak concentrations of 0.20 mg/L (lowest dose) and 0.81 mg/L (highest dose) (Figure 2B). These maximal concentrations were achieved between 0.5 – 1 h after CMS administration, and declined relatively slowly during the sampling period (Figure 2B). Administration of the active antibacterial moiety, colistin, was undertaken to
characterize the disposition in plasma, with average maximal plasma concentrations ranging from 1.2 to 4.7 mg/L, and concentrations declining exponentially over the 4 h sampling period (Figure 2C).

Pharmacokinetics following intratracheal instillation

Pulmonary administration of CMS resulted in average ELF concentrations of 21,391 mg/L at 5 min post-dose, and thereafter concentrations declined over the 12 h sampling period (Figure 3A). Maximal concentrations of formed colistin in ELF were achieved at 4 h after CMS dosing, with relatively high concentrations maintained throughout the 12 h sampling period (Figure 3B). Measured concentrations of urea after IT CMS were 56 ± 13 mg/dL in plasma and 0.35 ± 0.13 mg/dL in BAL fluid, with an estimated apparent V_{ELF} of 0.084 ± 0.016 mL. Similar urea concentrations (50 ± 9.2 mg/dL and 0.41 ± 0.14 mg/dL in plasma and BAL fluid, respectively) and apparent V_{ELF} estimates (0.11 ± 0.023 mL) were obtained after IT administration of colistin.

Population pharmacokinetic model

In the full composite model, the disposition of both CMS and colistin in plasma was best described by a three-compartment model. Table 1 presents the parameter estimates from the final model. The estimated mean clearance from the central compartment was 0.122 L/h (BSV 10.7%) for CMS and 0.0657 L/h (BSV 24.7%) for colistin. Steady-state volumes of distribution were 0.292 L and 0.170 L for CMS and colistin, respectively. Conversion of CMS to colistin in the central compartment was described by a first-order process (\(CL_{pm} = 0.0016\) L/h; corresponding rate constant of 0.102 h\(^{-1}\)). Similar conversion clearances were incorporated from the shallow and deep peripheral compartments to fit the high concentrations of formed colistin at 2 h after IV administration of CMS. In the absence of these peripheral conversion terms, the model was statistically inferior (\(\Delta OBJ + 20.1\)).
rate constant for central conversion was more than three-fold faster than that estimated in shallow (0.0303 h⁻¹) and deep peripheral (0.00121 h⁻¹) compartments. The estimated fraction of the IV CMS dose converted to colistin ($f_{\text{m,systemic}}$) was 0.0255. Figure 2 presents the VPCs for both compounds after their IV administration.

Following IT instillation, the fractional dose ($F_{\text{pul}}$) available for exposure to the lungs was 40.9% for CMS and 48.5% for colistin. Two compartments (BAL fluid₁ and BAL fluid₂) described drug movement from the IT dosing site to the remaining regions of the respiratory airways (see Figure 1). For CMS the estimated mean time ($1/k_{\text{BALF}}$) for movement from BAL fluid₁ to BAL fluid₂ was 0.315 h, which was faster than that for colistin (2.52 h). Additional first-order terms were included to describe the distribution of both compounds from the lung lining fluid to the deep peripheral compartment in the plasma model.

The model required a slow rate of CMS conversion (mean conversion time, MCT$_{\text{CMS}}$ 3.48 h in BAL fluid₁) to describe the high concentrations of colistin maintained in the ELF (Figure 3B, Figure 3 provides the VPCs for both compounds in ELF and in plasma after IT dosing). This slow conversion adequately explained the subsequent delay in absorption of colistin into plasma (Figure 3E). In contrast, only a single first-order term ($k_{\text{pm}} = 0.0154$ h⁻¹) was required to describe the kinetics of CMS to colistin conversion in BAL fluid₂. The estimated fraction of the CMS dose converted to colistin in ELF ($f_{\text{m,ELF}}$) was 0.226, and was almost nine-fold higher than the corresponding fractional conversion in plasma (0.0255) after IV administration (Figure 4A).

First-order kinetics best described the absorption of both CMS ($k_{\text{acms}} = 0.936$ h⁻¹) and colistin ($k_{\text{acol}} = 2.15$ h⁻¹) from the IT dosing compartment (BAL fluid₁) to plasma. For CMS, an additional pathway incorporating zero-order with sequential delayed first-order absorption, was required to fit the high concentrations observed at 2-3 h in plasma (Figure 3D). This
route was a minor component of the model, and only contributed to 0.3% of total CMS absorption.

Exposure of formed colistin following intratracheal instillation

Following IT instillation of CMS (14 mg/kg), the AUC of formed colistin in ELF was 11,245 ± 7,568 mg·h/L. Of this, only 0.03% of formed colistin from ELF was absorbed into plasma (AUC = 3.01 ± 0.476 mg·h/L). After IV dosing of CMS, the AUC of formed colistin in plasma was 1.38 ± 0.0746 mg·h/L. Thus, IT dosing gave a formed colistin exposure in ELF that was 8000-fold higher than that in plasma after IV dosing (Figure 4B).

Histopathology examination

Histopathology examination of the trachea and lung lobes following IT administration of either CMS or colistin showed no significant difference between the control and treatment groups (data not shown).

Discussion

The current study evaluates the pharmacokinetics of CMS and colistin following IV and IT administration of both compounds in rats. The data showed that direct administration of CMS into the lungs achieved high ELF exposure to formed colistin, thus demonstrating the potential for local targeting of antibiotics for the treatment of respiratory infection. Population pharmacokinetic analysis was advantageous in this study, because it allowed for the simultaneous modeling of plasma sampled via serial bleeds in one cohort of animals and ELF data from a second cohort where terminal BAL samples were obtained. The pharmacokinetic model provided a better understanding of the conversion kinetics for CMS to colistin in ELF and in plasma. The population pharmacokinetic analysis identified that
high colistin concentrations in the ELF were obtained due to slow and sustained conversion kinetics of CMS in ELF after IT administration.

Intravenous dose-ranging studies demonstrated linear pharmacokinetics for CMS and colistin, consistent with the findings by Marchand et al. in Sprague-Dawley rats (28). The pharmacokinetic parameter estimates were broadly consistent with previously reported values arising from NCA after IV administration of CMS (17, 28, 29) and colistin (30) in rats.

There is limited knowledge on the mechanism by which colistin is formed from CMS in vitro and in vivo (21, 31, 32). Conversion of CMS generates a complex mixture of partially sulfomethylated derivatives and colistin, formation of the latter being a pre-requisite for antibacterial activity (10, 32). While previous studies in rats have reported on the fraction of an IV CMS dose that is converted to colistin systemically (17, 29), the kinetics of this conversion is currently unknown. Li et al. (29) and Marchand et al. (17), estimated a fractional systemic conversion of ~6% and ~13%, respectively, which is higher than that estimated in the present study (3%). The population pharmacokinetic model required conversion in all three disposition compartments (central, shallow and deep peripheral), to characterize the gradual appearance of formed colistin in plasma following IV administration of CMS. Maximal plasma concentrations of formed colistin were achieved at 0.5 – 1 h post-CMS administration, which was in contrast to studies by Li et al. (29) and Marchand et al. (17, 28) where peak concentrations of formed colistin were observed at the initial sampling time of 5 min. The inter-study variation in the conversion kinetics is potentially the result of the use of different brands of sodium CMS which can have varying ratios of fully and partially sulfomethylated CMS derivatives and yield different plasma concentration-versus-time courses for formed colistin (33). Importantly, in all of the above pre-clinical evaluations, a low proportion (~3 - 13%) of the IV dose of CMS was converted to colistin.
This study demonstrates that direct pulmonary administration of CMS achieves high ELF exposure to formed colistin compared to the exposure in plasma. The concentrations of colistin in ELF were maintained, over the 12 h sampling period, above the minimum inhibitory concentration required for 50 to 90% inhibition of bacterial growth based on clinical isolates of *P. aeruginosa* and *Acinetobacter* spp. (MIC$_{50-90}$) of 1.0 mg/L (34). The high concentrations of CMS and formed colistin in ELF described here are consistent with the findings by Marchand *et al.*, following IT nebulization to rats at a dose equivalent to 14 mg/kg CMS (17).

Population pharmacokinetic analysis identified slower conversion kinetics in the lung following IT instillation, with a greater fraction of the CMS dose (23%) converted to colistin in ELF compared to conversion in plasma after IV administration (3%) (Figure 4A). This slow and sustained conversion (in BAL fluid,) contributed to the high colistin concentrations in ELF and the corresponding delay in its plasma appearance after IT administration of CMS. With limited information available about the conversion mechanism of CMS to colistin, one potential explanation for the slower, but ultimately more extensive conversion observed in the lungs is that while CMS resides within the lung it is not available for renal clearance, which is the major systemic clearance mechanism (11). A further contributing factor to the increased ELF exposure is that CMS will be dissolved within a relatively small volume of lung lining fluid (ELF volume estimated to be 0.084 ± 0.016 mL) and thereby creating a reservoir of CMS available for ongoing conversion to colistin, which will be reflected in the high exposure of formed colistin in ELF and a greater fractional conversion of CMS in the lungs. Marchand *et al.* proposed similar explanations for observations of high CMS concentrations in ELF (slow conversion and absorption), with the authors reporting a slightly higher fraction of the IT CMS dose converted to colistin (39%) in the lungs (17).
Two BAL fluid compartments were included in the model to reflect the site of IT dosing and the movement of drug over time to other regions of the lungs. Thus, BAL fluid 1 potentially represents localized antibiotic concentrations in the trachea, upper airways and to some extent the peripheral airways, followed by mono-directional transfer into the remaining peripheral lung regions (represented as BAL fluid 2). Relative to CMS, a slower movement of colistin into the peripheral airways may have occurred due to colistin binding to lung tissues (35-37). Several sites have been proposed for the binding of lipophilic basic amines (pKa > 8.5) in the lungs (38-41). Further antibiotic distribution from the ELF to the deep peripheral compartment suggests that this compartment could equate to the lung tissue, although additional studies are required to confirm this hypothesis.

In the current model, the absorption of CMS and colistin from the lungs into the systemic circulation was best described by first-order kinetics, with an additional minor (<1%) delayed pathway for the former compound. Appearance of colistin in plasma following IT administration of colistin was more rapid (plasma concentrations quantifiable at 1–10 min post-dose, Figure 3F) than for formed colistin (1 h post-dose) after IT dosing of CMS (Figure 3E). For formed colistin this is likely to be due to the ongoing conversion from CMS in the lungs. Given the physicochemical properties of CMS (average Mw 1633 Da, polar surface area (PSA) 741 Å, Log P -12.09) and colistin (average Mw 1163 Da, PSA 490 Å, Log P 3.42), the proposed mechanism of absorption is passive diffusion via paracellular transport mechanisms. This is supported by Schanker and colleagues, who reported that the rat lung epithelium is characteristic of the classic lipoid-pore of biological membrane (42-44). Tronde et al. have also illustrated passive diffusion as the dominant transport mechanism for drug absorption from the rat lungs (45, 46). In the present study, the slow absorption of CMS from the lungs was consistent with the low apparent permeability for CMS through Calu-3 cells (apical-to-basolateral direction) (17).
Notwithstanding the extensive lung exposure to formed colistin, its relative systemic exposure following IT administration of CMS (14 mg/kg) was two-fold higher than the systemic exposure after IV administration of the same dose of CMS. The greater fraction of CMS dose converted to colistin in the ELF (23%) and absorption of a proportion of this pre-systemically formed colistin is the most likely cause for these findings. These results are, in-part, consistent with findings by Marchand et al. who reported that 39% of the IT CMS dose was converted to colistin in the lungs prior to absorption into the systemic circulation which the authors speculated led to the increased exposure to formed colistin in plasma (17). The potential for lung epithelium damage and resultant increased drug permeability was considered as an alternative explanation for the increased plasma exposure to formed colistin in this study, however histopathological analysis of lung tissue following IT administration confirmed that this was not the case. This study in rats suggests that a reduction in the pulmonary CMS dose could achieve colistin concentrations in ELF above the MIC<sub>50-90</sub> of 1.0 mg/L (34) and still reduce total systemic exposure and hence reduce the likelihood of adverse effects.

Population pharmacokinetic analysis demonstrated good predictive performance of a model that simultaneously fits six dependent variables. While minor model misspecification is apparent in some profiles (e.g. colistin in ELF following IT administration of colistin, Figure 3C), these would not alter the pharmacological implications of the exposure achieved after direct (targeted) administration to the lungs. Future applications of the current model could test whether similar CMS to colistin conversion occurs with the use of different inhalational formulations (e.g. dry powder) in the pre-clinical setting. Furthermore, the model serves as a basis for the translation of CMS and colistin pharmacokinetics from rodents to larger animal models and humans. While the current structural model is developed in non-infected animals, it can be adapted to the estimation of known PK differences in infected...
murine models (47-49). Ultimately, these models could be usefully employed to develop inhalational dosing recommendations for CMS in CF patients that simultaneously consider target endpoints in respiratory infection whilst avoiding potential adverse effects.

In conclusion, we have for the first time developed a detailed model that describes the pharmacokinetics of CMS and colistin after both IV and IT administration. We demonstrate that high concentrations of colistin in rat ELF are achieved as a result of slow and sustained CMS conversion following IT instillation. Slow absorption of CMS into the systemic circulation and less competing clearance pathways for CMS in the lungs contribute to the greater fractional conversion in the lungs. The higher colistin systemic exposure after IT administration compared to exposure after IV dosing of CMS is a result of absorption of formed colistin from the lungs. This has the potential to increases systemic toxicity, but may be modulated by a dose reduction, since colistin exposures in the current study were well above the MICs of *P. aeruginosa*. The current population pharmacokinetic model can be utilized to further investigate the conversion kinetics of CMS and to characterize the disposition of CMS and formed colistin following inhalational administration in larger animals and in humans.

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Appendix

*Equations describing the structural PK model for CMS and colistin*
Changes in the amount of CMS in the bronchoalveolar lavage fluid \((A_{BALF,cms})\) were calculated using equations A1 and A2:

\[
\frac{dA_{BALF1,cms}}{dt} = Dose \cdot \left( F_{pul,cms} - k_{tr,gm} \cdot A_{BALF1,cms} - k_{BALF1,cms} \cdot A_{BALF1,cms} - k_{a,cms} \cdot F_{ka} \right) \cdot A_{BALF1,cms} - (1 - F_{ka}) \cdot D_{alve,cms} (A1)
\]

\[
\frac{dA_{BALF2,cms}}{dt} = k_{BALF,cms} \cdot A_{BALF1,cms} - k_{p,1} \cdot A_{BALF2,cms} - k_{B,P,cms} \cdot A_{BALF2,cms} + k_{p,P,cms} \cdot A_{peri,cms} (A2)
\]

where the absorption of CMS from \(BALF_1\) was partitioned into a fraction \((F_{ka})\) via first-order kinetics, with the remaining fraction \((1 - F_{ka})\) via a minor zero order component. \(A_{peri,cms}\) is the amount of CMS in the plasma deep peripheral compartment, and all other parameters are defined in Table 1.

The slow conversion kinetics of CMS to colistin through 3 transit \((Tr)\) compartments in \(BALF_1\) was described by equations A3 to A5:

\[
\frac{dA_{trpm,1}}{dt} = k_{tr,gm} \cdot A_{BALF1,cms} - k_{tr,gm} \cdot A_{trpm,1} (A3)
\]

\[
\frac{dA_{trpm,2}}{dt} = k_{tr,gm} \cdot A_{trpm,1} - k_{tr,gm} \cdot A_{trpm,2} (A4)
\]

\[
\frac{dA_{trpm,3}}{dt} = k_{tr,gm} \cdot A_{trpm,2} - k_{tr,gm} \cdot A_{trpm,3} (A5)
\]

The minor zero order absorption of CMS followed by first-order transit through 3 compartments was described by equations A6 to A8:
Changes in the amount of colistin in the bronchoalveolar lavage fluid (ABALF, col) were calculated by equations A9 and A10:

\[
\frac{dA_{\text{BALF,col}}}{dt} = D_{\text{ose,col}} \cdot F_{\text{al,col}} + k_{\text{tr,pm}} \cdot A_{\text{pm,3}} - k_{\text{BALF,col}} \cdot A_{\text{BALF1,col}} - k_{\text{CL},\text{col}} \cdot A_{\text{CL},\text{col}} \tag{A9}
\]

\[
\frac{dA_{\text{BALF2,col}}}{dt} = k_{\text{pm,2}} \cdot A_{\text{BALF2,col}} + k_{\text{BALF,col}} \cdot A_{\text{BALF1,col}} - k_{\text{B,P,col}} \cdot A_{\text{B,P,col}} + k_{\text{p,b,col}} \cdot A_{\text{p,b,col}} \tag{A10}
\]

The CMS model in plasma was described by equations A11 to A13:

\[
\frac{dA_{\text{CMS}}}{dt} = k_{\text{CL},\text{CMS}} \cdot A_{\text{cl},\text{CMS}} - CL_{\text{D},\text{CMS}} \cdot C_{\text{CMS}} + CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} \tag{A11}
\]

\[
\frac{dA_{\text{p,CMS}}}{dt} = CL_{\text{D},\text{CMS}} \cdot C_{\text{CMS}} - CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} = CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} - CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} \tag{A12}
\]

\[
\frac{dA_{\text{p,CMS}}}{dt} = CL_{\text{D},\text{CMS}} \cdot C_{\text{CMS}} - CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} = CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} - CL_{\text{D},\text{CMS}} \cdot C_{\text{p,b,CMS}} \tag{A13}
\]

The colistin model in plasma was described by equations A14 to A16:

\[
\frac{dA_{\text{CL},\text{col}}}{dt} = k_{\text{CL},\text{col}} \cdot A_{\text{CL},\text{col}} + CL_{\text{CL},\text{col}} \cdot C_{\text{CL},\text{col}} - CL_{\text{D},\text{col}} \cdot C_{\text{CL},\text{col}} + CL_{\text{D},\text{col}} \cdot C_{\text{p,b,CMS}} \tag{A14}
\]
where \( cent, speri \) and \( dperi \) represent the central, shallow peripheral and deep peripheral compartments in plasma, respectively and \( C \) is the concentration of CMS or colistin.

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### Transparency declaration

None to declare.

### References


FIGURE LEGENDS

Figure 1: Structure of the population pharmacokinetic model for CMS and colistin following intravenous and intratracheal dosing in rats. Abbreviations are defined in Table 1.

Figure 2: Visual predictive check for (A) CMS and (B) formed colistin plasma concentration following intravenous (IV) CMS dosing, and (C) colistin plasma concentration following IV colistin dosing in Sprague-Dawley rats. The model-predicted median and 10th and 90th percentiles closely match corresponding observed percentiles, indicating the suitability of the pharmacokinetic model. Closed circles represent actual observed data in rats.

Figure 3: Visual predictive check for (A) CMS, (B) formed colistin in ELF following intratracheal (IT) CMS dosing and (C) colistin in ELF following IT colistin dosing; (D) CMS, (E) formed colistin in plasma after IT CMS dosing and (F) colistin in plasma following IT colistin dosing in Sprague-Dawley rats. The model-predicted median and 25th/75th and 10th/90th percentiles broadly match corresponding observed percentiles, suggesting reasonably good predictive performance. Closed circles represent actual observed data in rats. The 25th and 75th percentiles are presented for the ELF profiles, due to the small number of observations obtained via this sampling route.

Figure 4: (A) Percentage of IT (14 mg/kg) and IV (dose-ranging) CMS dose converted to colistin in ELF (black) and plasma (grey) and (B) formed colistin exposure (AUC$_{0-\infty}$, $\mu$moL·h/L) in ELF and plasma following IT and IV administration of CMS 14 mg/kg, respectively. Formed colistin exposure was approximately 8000-fold higher in ELF after IT dosing when compared to in plasma after IV dosing. For panel B, note the different scale for the right y-axis.
Table 1: Population pharmacokinetic parameter estimates for CMS and colistin after intravenous and pulmonary dosing.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
<th>Estimate</th>
<th>BSV (CV%)</th>
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<tr>
<td><strong>Plasma</strong></td>
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<td></td>
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<td>Colistin</td>
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<td>3.17</td>
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<td>MCT&lt;sub&gt;cms,conv&lt;/sub&gt;</td>
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<td>1/h</td>
<td>0.0154</td>
</tr>
</tbody>
</table>

**Residual Error**

| | | |
| | SD<sub>col,plas</sub> | Exponential error for colistin in plasma | % | 18.1 | - |
| | SD<sub>cms,plas</sub> | Exponential error for CMS in plasma | % | 16.7 | - |

---

* Plasma k<sub>p,1</sub>, k<sub>p,2</sub> and k<sub>p,3</sub> were 0.102, 0.0303 and 0.00121 h<sup>-1</sup>, respectively.

* Residual error for the BAL fluid and ELF model was fixed to the assay precision and limit of quantification, since only a single observation was obtained from each rat due to terminal BAL sampling.