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# Pharmacokinetics of inhaled colistimethate sodium (CMS) in mechanically ventilated critically ill patients

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**Abstract** *Purpose:* The purpose of this study was to describe inhaled colistin pharmacokinetics in patients with ventilator-associated tracheobronchitis (VAT) due to polymyxin-only susceptible Gram-negative bacteria (GNB). *Methods:* Inhaled colistimethate sodium (CMS) was administered at a dose of 80 mg every 8 h for 7 days. Mini bronchoalveolar lavage (BAL) was performed before and at 1, 4 and 8 h, while blood samples were collected before and at 0.16, 0.5, 1, 2, 4 and 8 h after the first dose. Colistin concentrations in BAL and serum were determined by high-performance liquid chromatography. *Results:* Our study population included 20 patients. At the end of treatment, cure was achieved in 16 patients and favorable microbiological response in 12 patients. Median (25–75 % interquartile range) colistin concentrations in epithelial lining fluid (ELF) were 6.7 (4.8–10.1), 3.9 (2.5–6.0) and 2.0 (1.0–3.8) μg/ml at 1, 4 and 8 h, respectively, and fivefold higher than those achieved in serum. Median ELF concentrations at 1 and 4 h were above the minimum inhibitory concentrations of all isolated pathogens; however, the 4-h median was below the European Committee on Antimicrobial Susceptibility Guidelines (EUCAST) breakpoints for *Pseudomonas aeruginosa* and the 8-h median was low relative to EUCAST breakpoints for all GNB. Colistin pharmacokinetic/pharmacodynamic parameters in ELF were associated with favorable microbiological response at the end of treatment. *Conclusion:* Inhaled colistin may achieve high drug concentrations in the lung. However, a dose of 80 mg of inhaled CMS every 8 h may not be adequate for the treatment of lower respiratory tract infections due to multi-drug resistant GNB.

**Keywords** Inhaled antibiotics · Colistin · Pharmacokinetics · Mechanical ventilation · Ventilator-associated pneumonia

## Introduction

Ventilator-associated pneumonia (VAP) is the most common severe hospital-acquired infection [1]. Inadequate initial antimicrobial treatment of VAP is associated with adverse outcomes [1, 2]. However, early adequate therapy is increasingly more difficult to achieve, due to the emergence of multidrug-resistant gram-negative bacteria (GNB) and the paucity of new effective antimicrobial agents [3, 4]. In this setting, colistin, an old antibiotic, has recently been considered as the last therapeutic option for the treatment of patients with these types of infection [5]. Colistin is administered in the form of colistimethate sodium (CMS), which is hydrolyzed to colistin.

Colistin has been used intravenously for the treatment of VAP [6]. More recently nebulized CMS/colistin has

been used as adjunctive therapy for the treatment of VAP [6–10]; however, results regarding its effectiveness were inconsistent. The use of inhaled colistin is theoretically appealing for VAP treatment because of the potential achievement of high drug concentrations at the target site. However, since colistin is administered in the form of CMS, achievement of therapeutic drug concentrations in the lung may be difficult because CMS is inactive and must first be metabolized locally to colistin, and also may be systemically absorbed before it is metabolized. Evaluation of the pharmacokinetics of inhaled colistin is important because studies relating to pulmonary penetration of intravenously administered colistin have shown conflicting outcomes [11, 12]. Moreover, treatment with nebulized colistin may have the benefit of decreased toxicity by limiting systemic drug exposure.

In a previous study in patients with cystic fibrosis [13], and also in animal studies [14–16], administration CMS by inhalation resulted in high colistin concentrations in the lung. To date, colistin pharmacokinetics in the critically ill have not been studied. Thus, our aim was to assess colistin pharmacokinetic properties in critically ill patients after administration of a single dose of CMS by inhalation.

## Materials and methods

### Subjects

Patients admitted to the ICU of the KAT University General Hospital in Athens, Greece, were eligible if they: (1) were aged 18 years and older, (2) were intubated and mechanically ventilated, (3) had symptoms and signs suggestive of ventilator-associated tracheobronchitis (VAT) (e.g., purulent secretions, fever, increased white blood cell count), (4) did not exhibit an evolving infiltrate on chest plain radiography, (5) had microbiological confirmation of VAT due to GNB susceptible only to polymyxin in quantitative cultures of endotracheal aspirates (ETA) with a diagnostic threshold of $\geq 10^5$ colony forming units (CFU) per milliliter [17], and (6) had not received colistin intravenously or by inhalation for at least 7 days prior to study onset. Patients were excluded if they had severe bronchoconstriction, refractory hypoxemia, were pregnant or had infections requiring systemic colistin administration.

The minimum inhibitory concentration (MIC) of colistin was determined using the Vitek automated system (bioMérieux, Marcy l'Etoile, France), and the susceptibility results were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [18].

Eligible patients received CMS by inhalation for 7 days for the treatment of VAT. The following data were collected at study onset: age, sex, body weight, diagnosis on admission, serum urea, serum creatinine, acute physiology and chronic health evaluation (APACHE) II score, sequential organ failure assessment (SOFA) score, isolated pathogens and the MIC for colistin. Serum creatinine values were also assessed at the end of treatment. Creatinine clearance was calculated using the Cockcroft-Gault formula [19]. Outcomes at the end of treatment were characterized as cure, defined as resolution of signs and symptoms and favorable microbiological response defined as eradication or bacterial growth decline (i.e., isolation of pathogens at a concentration of $\leq 10^2$ CFU/mL) in quantitative cultures of ETA.

The study protocol was approved by the institutional review board on human research and therefore was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki (Scientific Committee of KAT Hospital, Athens, Greece; approval number 5, 27-04-2009). In all patients informed consent was obtained from their nearest relatives prior to initiation of the study.

### Colistin administration by inhalation

Selected patients received CMS (Tadim, Allertec, Greece) by inhalation at a dose of 1 MU (80 mg) dissolved in 3 ml of half-normal saline every 8 h via a vibrating-mesh nebulizer (Aeroneb Pro, Aerogen, Galway, Ireland) over 30 min. The nebulizer was placed at the inspiratory limb of a non-humidified ventilator circuit, 15 cm from the Y-piece. All patients received sedation and were ventilated with assist volume controlled mode. During nebulization ventilation was performed at a constant flow targeting an inspiration to expiration time ratio of 0.5, the respiratory ratio was set from 12 to 14 breaths per minute, the tidal volume was set in the range 500 to 600 mL and the positive and expiratory pressure was set at 5 $cmH_2O$.

### Sampling procedures

Mini-bronchoalveolar lavage (mini-BAL) was performed as previously described [20] immediately before and at 1, 4 and 8 h after the first nebulization via a 65-cm long, 12F diameter sterile catheter (Kimberly-Clark, Roswell, GA) inserted through the endotracheal tube. The lavage was performed by infusing two 20-mL aliquots of sterile 0.9 % saline at room temperature. The liquid recovered from the first aliquot was considered a bronchial wash and was discarded. The time between the beginning of the mini-BAL and the total recovery of the BAL fluid was kept at <2 min to avoid falsely elevated concentrations of urea in the BAL fluid [21]. Endogenous levels of urea in the retrieved BAL fluid and in serum samples were used as a dilution factor and applied to interpret colistin concentrations in the epithelial lining fluid (ELF) [22]. Blood

samples (5 ml each) were collected from all patients through a catheterized peripheral arterial line immediately before and at 0.16, 0.5, 1, 2, 4 and 8 h after the end of the first inhalation. Lavage and blood samples were centrifuged for 10 min at 4,000 rpm at 4 °C. The supernatants were collected in plastic micro test-tubes (Safe-Lock; Eppendorf, Hamburg, Germany) and were immediately frozen at −70 °C until analysis.

## Colistin concentration determination

Colistin (i.e., colistin base) was determined in serum and BAL fluid using an isocratic high-performance liquid chromatography assay [13, 23]. No interferences in serum or BAL fluid were observed for colistin or the internal standards. The calibration curve was constructed from colistin sulfate standard solutions (ratio of colistin base to colistin sulfate 1,163/1,403) using the ratios of the summed peak areas of colistin A and B to that of netilmicin (internal standard). The assay response was linear, between 200 and 5,000 ng/mL for colistin sulfate in serum and between 200 and 3,000 ng/mL in BAL fluid. On a daily basis for the needs of the specific research protocol, the calibration range used for serum samples was 200–3,000 ng/mL and for BAL fluid was 200–3,000 ng/mL. For those serum samples found to contain lower concentrations of drug, colistin measurement was repeated using a calibration range which included the lower limit of quantification. Validation studies included determination of precision, accuracy and recovery.

Intraassay and interassay variabilities were below 7.9 and 6.2 % for serum and 3.5 and 2.2 % for BAL fluid, respectively. The lower limits of detection and quantification were 50 and 80 ng/mL, respectively, for serum and 75 and 82 ng/mL, respectively, for BAL fluid.

Urea concentrations in serum and BAL fluid samples were determined as previously described [24]. Colistin concentrations in ELF were estimated from measured colistin concentrations in BAL fluid and urea concentrations in BAL fluid and serum using the formula: colistin (ELF) = colistin (BAL fluid) × urea (serum)/urea (BAL fluid). The penetration ratio of colistin from ELF to blood through the alveolar capillary membrane barrier was calculated from the ratio of colistin concentrations in the serum to the corresponding ELF concentrations at each collection time point.

## Pharmacometric methods

The pharmacokinetic parameters of colistin in serum were estimated from the concentration–time data of each patient by noncompartmental analysis using the WinNonlin pharmacokinetic software package (Pharsight Corporation, Mountain View, CA). The maximum concentration reflected the maximum observed colistin concentration in ELF ($C\max_{ELF}$) and serum ($C\max_{serum}$), while the minimum concentration $C\min_{serum}$) reflected the lowest observed colistin serum concentration during the 8-h dosage interval. The elimination rate constant ($\lambda z$) for colistin in serum was estimated by log-linear regression of the terminal portion of the concentration–time curve (on the basis of the last three datum points), while the elimination half-life was calculated as $\ln 2/\lambda z$. The area under the serum concentration–time curve from the time of initiation of infusion to the time of the last observation (AUC) in ELF ($AUC_{ELF}$) and serum ($AUC_{serum}$) was calculated by the linear trapezoidal method. Clearance/$F$ and volume of distribution/$F$ in serum were roughly estimated as dose/$AUC_{inf}$ and dose/$\lambda z \times AUC_{inf}$, respectively, where $AUC_{inf}$ is the AUC from the time of dosing extrapolated to infinity and $F$ the fraction of dose absorbed. Finally, the pharmacokinetic/pharmacodynamic parameters of colistin in ELF were evaluated by calculation of $AUC_{ELF}$/MIC, $C\max_{ELF}$/MIC and $T$/MIC, where $T$ is the percentage of the dosing interval in which the ELF levels exceed the MIC.

For the pharmacokinetic evaluations the CMS dose was corrected to an equivalent dose of colistin according to the molecular weight of the two major components, i.e. CMS dose × 1,163/1,743 = CMS dose × 0.667, where 1,163 is the average molecular weight of colistin A and B and 1,743 is the average molecular weight of the respective sodium methanesulfonate salts. Since the precise percentage of colistin A and B in the batch of CMS administered was unknown, the precise dose of colistin base could not be estimated with greater accuracy.

## Statistical analysis

Data are expressed as the means ± standard deviation (SD), or as medians (25–75 % interquartile range, 25–75 % IQR). Prior to performing the analyses, datasets were tested for normality (Kolmogorov-Smirnov test) and equality of variances (Bartlett test). The one-way ANOVA test was used for parametric analyses and the Kruskal–Wallis (nonparametric ANOVA) test for nonparametric analyses. The Tukey-Kramer multiple comparisons post hoc test was used to compare pairs of group means and Dunn's multiple comparisons post hoc test to compare pairs of group medians. The median serum/ELF drug concentration ratios were calculated by averaging the ratios for all the study patients at each collection time point. $P < 0.05$ was considered statistically significant. Statistical processing and data analysis were performed using GraphPad Prism 4 software (GraphPad Software, San Diego, CA).

## Results

Our study population included 20 patients (13 men). Isolated pathogens were *Acinetobacter baumannii* (11 patients), *Pseudomonas aeruginosa* (8 patients) and *Klebsiella pneumoniae* (2 patients), with MICs ranging from 0.5 to 2 μg/mL. Demographic, clinical and microbiological data for each patient are shown in Table 1.

At the end of treatment with nebulized colistin, cure had been achieved in 16 of 20 patients, and a microbiological response achieved in 12, of whom 8 showed eradication and 4 bacterial growth decline. No significant differences were observed in creatinine clearance values before and after the end of treatment with nebulized CMS in all patients (Table 1).

Colistin concentrations in ELF after CMS nebulization are shown in Fig. 1. Median (25–75 % IQR) colistin concentrations in ELF were 6.73 μg/mL (4.8–10.1 μg/mL), 3.9 μg/mL (2.5–6.0 μg/mL) and 2.0 μg/mL (1.0–3.8 μg/mL) at 1, 4 and 8 h, respectively, after inhalation; the median concentration at 1 h was significantly greater than that at 8 h ($P < 0.001$). Median colistin concentrations in ELF remained well above the MIC values for all isolated pathogens at 1 and 4 h, but had declined at 8 h. Also, colistin concentrations in ELF varied substantially between patients with observed coefficients of variation of 56.4 %, 70.9 % and 89.4 % at 1, 4 and 8 h, respectively. The pharmacokinetic, and the pharmacodynamic/pharmacokinetic parameters of colistin in ELF are shown in Table 2. A significant negative correlation was observed between $AUC_{ELF}$/MIC, $T >$ MIC, $Cmax_{ELF}$/MIC and bacteriological growth in cultures of ETA after the end of treatment with nebulized colistin ($r = -0.46$, $P = 0.04$, $r = -0.48$; $P = 0.03$; and $r = -0.48$, $P = 0.03$. respectively).

Colistin concentrations in serum after inhalation of nebulized CMS are shown in Fig. 2. Median (25–75 % IQR) colistin concentrations in serum were 1.2 μg/mL (1.1–1.4 μg/mL), 1.6 μg/mL (1.5–1.9 μg/mL), 0.75 μg/mL (0.68–0.95 μg/mL), 0.31 μg/mL (0.29–0.5 μg/mL) at 1, 2, 4 and 8 h, respectively. All pharmacokinetic serum parameters are summarized in Table 2.

The median (25–75 % IQR) serum to ELF concentration ratios of colistin were 0.17 (0.13–0.26) at 1 h, 0.19 (0.15−0.25) at 4 h and 0.19 (0.11−0.29) at 8 h; the ratios were not significantly different at the three time periods.

## Discussion

In this study we evaluated the pharmacokinetics of nebulized CMS/colistin in patients with VAT. To our

**Table 1** Demographic, clinical and microbiological data of the enrolled patients

| Patient no. | Gender | Age (years) | APACHE II score | SOFA score | Creatinine clearance (ml/min) | | Diagnosis | Isolated pathogens | MIC colistin (μg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | End of treatment | | | |
| 1 | M | 46 | 19 | 9 | 300 | 250 | Trauma | *A. baumannii* | 1 |
| 2 | F | 74 | 18 | 9 | 60 | 76 | Trauma | *P. aeruginosa* | 1 |
| 3 | M | 59 | 14 | 4 | 115 | 110 | Subarachnoid hemorrhage | *K. pneumoniae*, *P. aeruginosa* | 2<br>1 |
| 4 | M | 74 | 22 | 8 | 54 | 48 | Cardiac arrest | *A. baumannii* | 0.5 |
| 5 | M | 44 | 15 | 5 | 186 | 172 | Trauma | *A. baumannii* | 0.5 |
| 6 | M | 79 | 24 | 9 | 98 | 106 | Subdural hematoma | *P. aeruginosa* | 1 |
| 7 | F | 72 | 23 | 9 | 80 | 78 | Aspiration pneumonia | *A. baumannii* | 0.5 |
| 8 | F | 59 | 8 | 5 | 136 | 125 | Neuromuscular weakness | *A. baumannii* | 0.5 |
| 9 | M | 44 | 8 | 4 | 184 | 142 | Trauma | *A. baumannii* | 0.5 |
| 10 | M | 80 | 9 | 7 | 102 | 87 | Postoperative respiratory failure | *A. baumannii* | 0.5 |
| 11 | F | 62 | 8 | 3 | 128 | 126 | Postoperative respiratory failure | *A. baumannii* | 0.5 |
| 12 | M | 76 | 27 | 12 | 37 | 46 | Cardiogenic shock | *P. aeruginosa* | 2 |
| 13 | M | 38 | 14 | 10 | 250 | 232 | Trauma | *P. aeruginosa* | 1 |
| 14 | F | 82 | 14 | 6 | 37 | 35 | Stroke | *A. baumannii* | 0.5 |
| 15 | M | 53 | 19 | 10 | 127 | 132 | Trauma | *K. pneumoniae* | 2 |
| 16 | F | 70 | 20 | 9 | 115 | 123 | Trauma | *P. aeruginosa* | 1 |
| 17 | F | 78 | 6 | 3 | 49 | 44 | Trauma | *P. aeruginosa* | 1 |
| 18 | M | 78 | 17 | 6 | 100 | 86 | Subdural hematoma | *P. aeruginosa* | 2 |
| 19 | M | 75 | 25 | 10 | 72 | 75 | Postoperative respiratory failure | *A. baumannii* | 0.5 |
| 20 | M | 36 | 8 | 1 | 204 | 178 | Trauma | *A. baumannii* | 0.5 |

*APACHE* acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment, *MIC* minimum inhibitory concentration

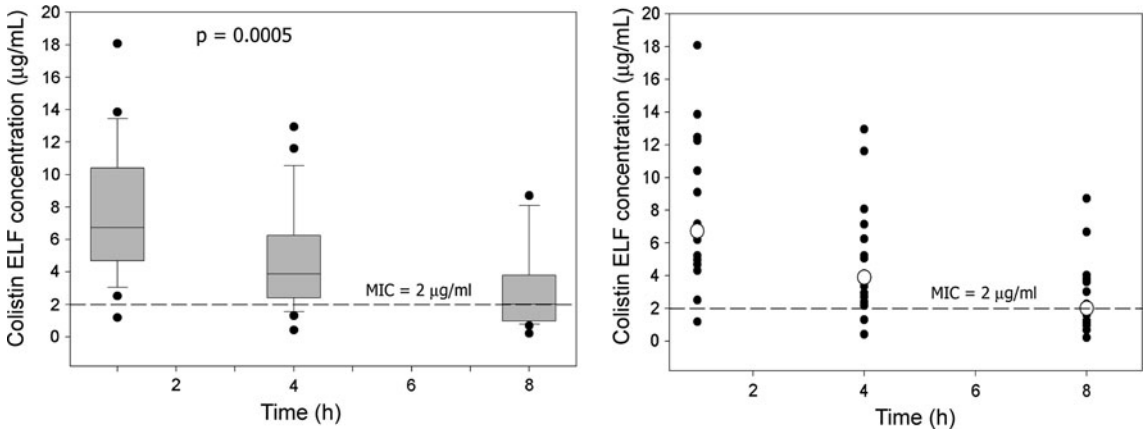


**Fig. 1** Colistin concentrations in ELF at 1, 4 and 8 h after administration of 80 mg of nebulized CMS. *Left panel* shows medians and 25–75 % IQRs; *right panel* shows individual values. *Dashed lines* represent MIC of colistin for *A. baumannii* and *K. pneumoniae* according to EUCAST susceptibility breakpoints. Variation among median concentrations at the different time points was significant ($P = 0.0005$); the median colistin concentration at 1 h was significantly higher than the concentration at 8 h ($P < 0.001$, Dunn's multiple comparisons post hoc test)

**Table 2** Pharmacokinetic and pharmacokinetic/pharmacodynamic ELF and serum parameters for inhaled colistin. Values are medians (25–75 % interquartile ranges)

| Parameter | ELF | Serum |
|---|---|---|
| $AUC_{0-8h}$ (µg/mL × h) | 29.8 (21.9–54.5) | 6.8 (6.2–8.2) |
| AUC/MIC | 40.0 (26.8–60.1) | 9.7 (6.0–13.7) |
| Maximum observed concentration (µg/mL) | 6.7 (4.8–10.1) | 1.6 (1.5–1.9) |
| Maximum observed concentration/MIC | 9.7 (5.2–21.8) | 2.4 (1.4–3.3) |
| Minimum observed concentration (µg/mL) | 2.0 (1.0–3.8) | 0.3 (0.3–0.5) |
| Half-life (h) | – | 2.7 (2.5–3.1) |
| Volume of distribution/fraction of dose absorbed (l) | – | 25.0 (21.7–29.4) |
| Clearance/fraction of dose absorbed (l/h) | – | 6.4 (4.8–6.8) |
| Time colistin concentration above MIC (h)[a] | 4.1 (2.0–6.0) | – |
| Serum/ELF ratio | | |
| At 1 h | 0.17 (0.13–0.26) | |
| At 4 h | 0.19 (0.15–0.25) | |
| At 8 h | 0.19 (0.11–0.29) | |

[a] Estimated from single-dose data

knowledge, this is the first study evaluating inhaled colistin pharmacokinetics in critically ill patients. A single dose of 80 mg of inhaled CMS resulted in relatively high colistin concentrations in ELF at 1 and 4 h and substantially lower concentrations at 8 h after inhalation. More specifically, median colistin concentrations in ELF at 1 h were significantly higher than those at 8 h ($P < 0.001$) but not significantly higher than those at 4 h ($P > 0.05$), while the median concentrations at 4 h did not differ significantly from those at 8 h. In addition, a significant negative correlation was observed between all pharmacokinetic/pharmacodynamic parameters in ELF and bacterial growth in ETA at the end of treatment. Colistin concentrations in serum were lower than those in ELF.

A main finding of this study was that the administration of nebulized colistin by inhalation resulted in high (relative to the MICs of isolated pathogens) median drug concentrations in ELF for up to 4 h after inhalation (6.7 and 3.9 µg/mL, respectively). These concentrations were above the colistin MIC values for all isolated pathogens, and also above the EUCAST MIC breakpoints for *A. baumannii* and *K. pneumoniae* (<2 µg/mL). However, median colistin concentrations in ELF at 4 h were below EUCAST MIC breakpoints for *P. aeruginosa* (<4 µg/mL) and at this time point two of the patients had concentrations below 2 µg/mL. The median colistin concentration in ELF was marginal at 8 h, while at this time point a substantial percentage of patients (8 of 20) had concentrations below 2 µg/mL. These findings suggest that higher doses of inhaled colistin are definitely required for *P. aeruginosa* and most probably for other GNB with lower MIC breakpoints (2 µg/mL). In some recent studies [7, 9] 2 MU of nebulized colistin have been administered. A comparison between colistin AUC values achieved in this study and those after higher doses would be useful especially in light of the fact that the effectiveness of colistin appears to correlate well with the AUC:MIC ratio [25].

An interesting finding of this study was that colistin concentrations in ELF varied substantially among patients. A possible explanation for this is that BAL was sampled by blind mini-BAL. This could have led to sampling of different lung segments in patients, thus

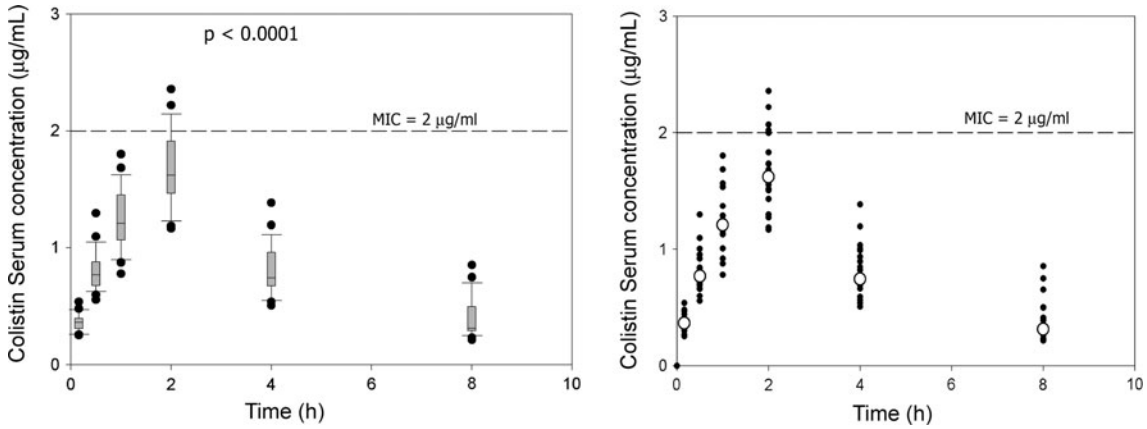


**Fig. 2** Colistin concentrations in serum at 0.16, 0.5, 1, 2, 4 and 8 h after administration of 80 mg of nebulized CMS. *Left panel* shows medians and 25–75 % IQRs; *right panel* shows individual values. *Dashed lines* represent MIC of colistin for *A. baumannii* and *K. pneumoniae* according to EUCAST susceptibility breakpoints. Variation among median concentrations at the different time points was significant ($P < 0.0001$); the median concentration at 2 h was significantly higher than the concentrations at other time points ($P < 0.001$, Dunn's multiple comparisons post hoc test)

accounting for the observed variation in concentrations in ELF [15]. Another potential explanation for this variation may be that our study population was not homogeneous with respect to reasons for ICU admission and comorbidities, and this may have affected the colistin pharmacokinetic parameters [26]. Also the finding that all ELF pharmacokinetic/pharmacodynamic parameters were negatively correlated with bacterial growth in ETA at the end of treatment with nebulized colistin, strongly suggests that the colistin concentration in ELF is a good indicator of colistin antimicrobial efficacy in patients with a lower respiratory tract infection.

Another important finding of our study was that colistin concentrations in serum were lower than in ELF, as observed in previous studies [13–15], and that the serum half-life of colistin was low. These findings suggest that inhaled colistin may be safe for the treatment of patients with nosocomial lower respiratory tract infections. However, since most of our patients had normal renal function, the findings of this study cannot be generalized to patients with renal impairment. Moreover, in this study nebulized colistin was administered at a dose of 1 MU every 8 h. The safety of nebulized colistin has been questioned when larger doses are used or when it is coadministered with intravenous colistin or other nephrotoxic antibiotics.

In this study, colistin concentrations in serum at 2 h were substantially higher than those reported after intravenous administration of 1 dose of 3 MU of CMS in a previous report [27]. A possible explanation for this may be that CMS is hydrolyzed in the alveoli more rapidly than in serum, thus leading to early penetration of preformed colistin into the lung and into the systemic circulation [14]. This finding suggests that coadministration of nebulized colistin could be useful for the treatment of severe extrapulmonary infections where early achievement of high systemic concentrations of antimicrobials is essential.

It should be emphasized that in the present study colistin was nebulized via a vibrating-mesh nebulizer which, according to recent data [28, 29], may result in increased delivery of inhaled drug to the lungs compared with jet or ultrasonic nebulizers. This is important because in mechanically ventilated patients deposition of inhaled drug may be suboptimal [30].

We acknowledge that our study had some limitations. Firstly, our patients did not have pneumonia, which may affect deposition of inhaled colistin in the alveoli. Secondly, colistin concentrations in ELF were assessed by mini-BAL. This may have led to sampling of different lung segments, and thus account for the observed variation in concentrations in ELF. However, previous studies have shown good agreement between mini-BAL and bronchoscopic BAL in the assessment of antibiotic concentrations in ELF [20]. Thirdly, since, according to previous studies [31], colistin can bind to lung tissue, assessment of colistin concentrations in ELF may have underestimated colistin concentrations in the lung. Fourthly, our estimation of colistin dose based on the molecular weights of CMS and colistin allowed only a rough estimation of certain pharmacokinetic parameters.

## Conclusions

Colistin administered via inhalation appears to achieve high drug concentrations at the infection site. However, according to our results, a dose of 80 mg of inhaled CMS

every 8 h may not be adequate for the treatment of patients with VAP due to multidrug-resistant GNB, because subtherapeutic concentrations in ELF were observed at the end of the dosing interval. Additional studies are required to determine whether larger doses of nebulized CMS may result in higher and sustained colistin concentrations at the target site in pulmonary infections.

**Conflicts of interest** None.

## References


1. American Thoracic Society/Infectious Diseases Society of America (2005) Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 171:388–416
2. Kollef KE, Schramm GE, Wills AR, Reichley RM, Micek ST, Kollef MH (2008) Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant Gram-negative bacteria. Chest 134:281–287
3. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB et al (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48(1):1–12
4. Chung DR, Song JH, Kim SH et al (2011) High prevalence of multidrug non-fermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med 184(12):1409–1417
5. Falagas ME, Kopterides P (2007) Old antibiotics for infections in critically ill patients. Curr Opin Crit Care 13(5):592–597
6. Linden PK, Paterson DL (2006) Parenteral and inhaled colistin for treatment of ventilator-associated pneumonia. Clin Infect Dis 43:S89–S94
7. Kofteridis DP, Alexopoulou C, Valachis A, Maraki S, Dimopoulou D, Georgopoulos D, Samonis G (2010) Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: a matched case-control study. Clin Infect Dis 51(11):1238–1244
8. Korbila IP, Michalopoulos A, Rafailidis PI, Nikita D, Samonis G, Falagas ME (2010) Inhaled colistin as adjunctive therapy to intravenous colistin for the treatment of microbiologically-documented ventilator-associated pneumonia: a comparative cohort study. Clin Microbiol Infect 16(8):1230–1236
9. Naesens R, Vlieghe E, Verbrugghe W, Jorens P, Ieven M (2011) A retrospective observational study on the efficacy of colistin by inhalation as compared to parenteral administration for the treatment of nosocomial pneumonia associated with multidrug-resistant Pseudomonas aeruginosa. BMC Infect Dis 11:317–324
10. Rattanaumpawan P, Lorsutthitham J, Angkasekwinaj N, Angasekwinai N, Thamlikitkul V (2010) Randomized controlled trial of nebulized colistimethate sodium as adjunctive therapy of ventilator-associated pneumonia caused by Gram-negative bacteria. J Antimicrob Chemother 65(12):2645–2649
11. Imberti R, Cusato M, Villani P, Carnevale L, Iotti GA, Langer M, Regazzi M (2010) Steady-state pharmacokinetics and BAL concentration of colistin in critically ill patients after IV colistin methanosulfate administration. Chest 138:1333–1339
12. Markou N, Fousteri M, Markantonis SL, Boutzouka E, Tsigou E, Baltopoulos G (2011) Colistin penetration in the alveolar lining fluid of critically ill patients treated with IV colistimethate sodium. Chest 139(1):232–233
13. Ratjen F, Rietschel E, Kasel D, Schwiertz R, Starke K, Beier H, van Koningsbruggen S, Grasemann H (2006) Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. J Antimicrob Chemother 57:306–311
14. Marchand S, Gobin P, Brillault J, Baptista S, Adier C, Olivier JC, Mimoz O, Couet W (2010) Aerosol therapy with colistin methanosulfate: a biopharmaceutical issue illustrated in rats. Antimicrob Agents Chemother 54(9):3702–3707
15. Lu Q, Girardi C, Zhang M, Bouhemad B, Louchahi K, Petitjean O, Wallet F, Becquemin MH, Le Naour G, Marquette CH, Rouby JJ (2010) Nebulized and intravenous colistin in experimental pneumonia caused by Pseudomonas aeruginosa. Intensive Care Med 36(7):1147–1155
16. Aoki N, Tateda K, Kikuchi Y, Kimura S, Miyazaki C, Ishii Y, Tanabe Y, Gejyo F, Yamaguchi K (2009) Efficacy of colistin combination therapy in a mouse model caused by multi-drug resistant Pseudomonas aeruginosa. J Antimicrob Chemother 63:534–542
17. Craven DE, Chroneou A, Zias N, Hjalmarson KI (2009) Ventilator-associated tracheobronchitis: the impact of targeted antibiotic therapy on patient outcomes. Chest 135:521–528
18. European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints. Available online at: http://www.eucast.org/clinical_breakpoints. Accessed 3 July 2012
19. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41
20. Boselli E, Breilh D, Djabarouti S, Guillaume C, Rimmelé T, Gordien JB, Xuereb F, Saux MC, Allaouchiche B (2007) Reliability of mini-bronchoalveolar lavage for the measurement of epithelial lining concentrations of tobramycin in critically-ill patients. Intensive Care Med 33:1519–1523
21. Baldwin DR, Honeybourne D, Wise R (1992) Pulmonary disposition of antimicrobial agents: methodological considerations. Antimicrob Agents Chemother 36:1171–1175
22. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, Crystal RG (1986) Estimation of the volume of epithelial lining fluid recovered by lavage using urea as a marker of dilution. J Appl Physiol 60:532–538
23. Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K, Johnson DW (2001) A simple method for the assay of colistin in human plasma, using pre-column derivatization with 9-fluorenylmethyl chloroformate in solid-phase extraction cartridges and reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 761(2):167–175

24. Panidis D, Markantonis SL, Boutzouka E, Karatzas S, Baltopoulos G (2005) Penetration of gentamicin into the alveolar lining fluid of critically ill patients with ventilator-associated pneumonia. Chest 128:545–552
25. Dudhani RV, Turnidge JD, Nation RL, Jian L (2010) fAUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against *Acinetobacter baumannii* in murine thigh and lung infection models. J Antimicrob Chemother 65:1984–1990
26. Boucher BA, Wood GC, Swanson JM (2006) Pharmacokinetic changes in critical illness. Crit Care Clin 22(2):255–271
27. Plachouras D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, Karaiskos I, Poulakou G, Kontopidou F, Armaganidis A, Cars O, Giamarellou H (2009) Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by Gram-negative bacteria. Antimicrob Agents Chemother 53(8):3430–3436
28. Ari A, Atalay OT, Harwood R, Sheard MM, Aljamhan EA, Fink JB (2010) Influence of nebulizer type, position and bias flow on aerosol drug delivery in simulated pediatric and adult lung models during mechanical ventilation. Respir Care 55(7):845–851
29. Ari A, Areabi H, Fink JB (2010) Evaluation of aerosol generator devices at 3 locations in humidified and non-humidified circuits during adult mechanical ventilation. Respir Care 55(7):837–844
30. Harvey CJ, O'Doherty MJ, Page CJ, Thomas SH, Nunan TO, Treacher DF (1997) Comparison of jet and ultrasonic nebulizer pulmonary aerosol deposition during mechanical ventilation. Eur Respir J 10:905–909
31. Kunin CM, Bugg A (1971) Binding of polymyxin antibiotics to tissues: the major determinant of distribution and persistence in the body. J Infect Dis 124(4):394–400