

Pharmacokinetics of inhaled colistin in patients with cystic fibrosis

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Objectives: Inhaled colistin is commonly used in patients with cystic fibrosis (CF), but only limited data are available to define its pharmacokinetic profile.

Patients and methods: We performed a multicentre study in 30 CF patients to assess sputum, serum and urine concentrations after a single dose of 2 million units of colistin administered by inhalation. In a subgroup of patients we also compared the efficacy of two different nebulizers for administration of inhaled colistin.

Results: Serum concentrations of colistin reached their maximum 1.5 h after inhalation and decreased thereafter. Serum concentrations were well below those previously reported for systemic application in all patients. A mean $4.3 \pm 1.3\%$ of the inhaled dose was detected in urine. Elimination characteristics did not differ significantly from those previously reported for systemic application. A positive correlation was found between forced expiratory volume in 1 s (FEV₁) in per cent predicted and both AUC and maximal colistin concentrations in serum (C_{max}). Maximum sputum concentrations were at least 10 times higher than the MIC breakpoint for *Pseudomonas aeruginosa* proposed by the British Society for Antimicrobial Chemotherapy. Although sputum drug concentrations decreased after a peak at 1 h, the mean colistin concentrations were still above 4 mg/L after 12 h. No differences were seen in polymyxin E sputum concentrations, for CF patients between the two nebulizer systems.

Conclusions: The low systemic and high local concentrations of colistin support the use of inhaled colistin in CF patients infected with *P. aeruginosa*.

Keywords: *Pseudomonas*, antimicrobial therapy, airways

Introduction

Cystic fibrosis (CF) is characterized by chronic airway infection and inflammation that leads to permanent lung damage and ultimately pulmonary insufficiency.¹ *Pseudomonas aeruginosa* is the main pathogen in CF lung disease and chronic infection with mucoid strains is associated with deterioration in both lung function and clinical status.²⁻⁵ Most adolescent and adult patients with CF have chronic *P. aeruginosa* infection and multiple studies have demonstrated a positive effect of antibiotic treatment on the subsequent course of lung disease.⁶ Inhaled antibiotics have the advantage of achieving high concentrations in the respiratory tract while avoiding systemic side effects. Inhaled tobramycin and

colistin have been used for decades in Europe for both early and chronic infection and evidence from large controlled trials with inhaled tobramycin in the United States would support their use.⁷⁻¹³ While limited data are available on the pharmacokinetics of inhaled tobramycin and intravenously administered colistin in CF patients, insufficient evidence exists for inhaled colistin making recommendations for both dosages and dosing intervals difficult.^{14,15} The efficacy of inhaled drug therapy can be affected by the interaction of the substance and the inhalation drug delivery system.¹⁶ This may be particularly important for surface tension lowering and foaming drugs, such as colistin, which can decrease the droplet size and the mass median diameter (MMD) compared with isotonic saline. New and potentially more effective

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inhalation devices have been recently developed and definition of the adequate dose that should be administered to the patient is important to ensure efficacy and avoid side effects. Hence, we performed a multicentre pharmacokinetic study in CF patients to assess sputum, serum and urine concentrations after a single dose of inhaled colistin administered by a commonly used jet nebulizer. In a subgroup of patients we also compared the pharmacokinetic profile of two different nebulizers in their efficacy for administration of inhaled colistin.

Patients and methods

Study population

The study population consisted of 30 CF patients aged 12–48 years (mean age \pm SD: 25 \pm 9 years) from the CF centres at the Children's Hospital of Essen, the Department of Pulmonary Medicine at the Ruhrlandklinik Essen, and the Children's Hospital at the University of Cologne. Baseline characteristics of the study population are shown in Table 1. All patients in this study were admitted to one of the participating centres for treatment of a pulmonary exacerbation with intravenous antibiotics for 14–21 days. Studies were performed in the second week of treatment when patients had stabilized as reflected by improvement of clinical symptoms. *P. aeruginosa* was isolated from sputum in all patients. Inhaled antibiotics were stopped at least 5 days prior to initiation of the study. None of the patients received inhaled or systemic colistin at the time of study. The study was approved by the ethic committees of the participating institutions, and written informed consent was obtained from all patients and/or their parents.

Table 1. Characteristics of the study population

<i>n</i> (female:male)	30 (12:18)
Age in years; mean \pm SD (range)	25 \pm 9 (12–48)
CFTR genotype	
Δ F508 homozygous	15
Δ F508 compound heterozygous	7
others	4
unknown	4
FEV ₁ % predicted; mean \pm SD (range)	46 \pm 16 (28–88)

CFTR, cystic fibrosis transmembrane regulator.

Study protocol

On the day of the study baseline blood, urine and sputum samples were obtained in the morning prior to inhalation. All patients received two puffs of albuterol prior to inhalation of study medication. Subsequently, patients inhaled 2 million international units of colistin (Grünenthal, Aachen, Germany), which is equal to 158 mg of colistimethate-Na (or 66 mg of colistin) dissolved in 6 mL of isotonic saline via a PARI LC Star nebulizer and a PARI Master compressor (PARI, Starnberg, Germany). Subsequently, blood samples were taken at 0.25, 0.5, 1, 2, 3, 6, 8 and 12 h after inhalation. Up to 12 h after administration urine was sampled at intervals of 4 h. Sputum samples were obtained in those patients capable of spontaneously expectorating adequate amounts of sputum after 1, 4 and 12 h. Serum, urine and sputum samples were stored at -20°C prior to analysis.

In a subgroup of eight patients inhalation of the same dose of colistin was repeated on a separate day (at least 3 days apart) using a customized PARI eFlow electronic nebulizer from pilot series 03. This device makes use of a vibrating membrane aerosol generation principle,^{16,17} which delivers a target aerosol at a high output rate (0.73 mL/min) with a small geometric standard deviation. Two million units of colistin, dissolved in a mixture of 1.5 mL of purified water and 1.0 mL of isotonic saline, were nebulized. The physicochemical properties of the two formulations and their corresponding aerosol characteristics analysed *in vitro* are summarized in Tables 2 and 3. The sampling intervals were identical to those used for the initial study in these patients.

Determination of colistin

Colistin is a multicomponent antibiotic drug consisting of a mixture of several closely related decapeptides (polymyxins E). Thirteen different components have been identified. As polymyxin E1 is the main component of colistin, it was used as a marker for the quantification. For the determination of polymyxin E1 in human serum, urine and sputum, a HPLC method was developed and validated with respect to linearity, intra- and interday accuracy, intra- and interday precision, specificity of the assay in the presence of common co-administered drugs (azithromycin, ceftazidime, prednisone, ibuprofen, naproxen, phosphomycin, ipratropium, meropenem, salbutamol and tobramycin), carryover effect, extraction recovery, dilution and stability at different storage and processing conditions, which were of importance for sample collection and processing. The international guidelines and requirements of the International Conference on Harmonisation (ICH) and the Food and Drug Administration (FDA)^{18–20} have been followed for the validation of the method. For all assays, precision and accuracy

Table 2. Physicochemical properties of isotonic saline compared with colistin solutions as used for nebulization via the PARI LC star and eFlow

Physicochemical properties	Isotonic saline NaCl (9 mg/1 mL)	Colistimethate-Na 158 mg/6 mL saline ^a	Colistimethate-Na 158 mg/2.5 mL ^b
Surface tension (mN/m)	72.9	48.3	44.2
Dynamic viscosity (mP/s)	1.0	1.1	1.3
Osmolality (mosmol/kg)	291	400	334
pH	5.2	7.1	6.7

^aThe medication was prepared for the PARI LC star by dissolving the powdered colistin with 3 mL of saline, followed by the transfer of the content of two vials into the nebulizer cup.

^bFor eFlow pilot series 03 the powder of one vial was dissolved in 1.5 mL of purified water and the resulting liquid was transferred into a second vial. Then 1 mL of saline was added and, after mixing, the entire content was transferred into the eFlow medication cup.

Inhaled colistin in cystic fibrosis

Table 3. Aerosol characteristics of the LC star and eFlow pilot series 03 upon nebulization of 2 million units of colistin in 6 and 2.5 mL, respectively

	PARI LC Star	eFlow pilot series 03
Percentage of droplets <5 µm	80.8	79.2
MMD (µm)	3.0	3.4
GSD	2.1	1.6
Total output rate (mg/min)	428	150

MMD, mass median diameter; GSD, geometric standard deviation.

were calculated as the mean coefficient of variation (CV) of all samples used for validation and the mean relative deviation of validation samples from their nominal values, respectively. The CV for measurements in serum ranged from -5.3 to 11.8%, from -3.6 to 17.3% in sputum and from -11.0 to 13.4% in urine.

Determination of polymyxin E1 in human serum

The bioanalytical method used for the determination of polymyxin E1 in human serum consisted of a protein precipitation, followed by derivatization with 9-fluorenylmethyl chloroformate on a solid-phase extraction C18 cartridge, and subsequent reversed-phase HPLC with fluorescence detection. Thawed serum samples were shaken on a vortex shaker and centrifuged at 5°C (5 min, 2000 g). To 250 µL of the sample, 50 µL of aqueous internal standard solution (10 µg/mL netilmicin sulphate) and 50 µL of trifluoroacetic acid (50%) were added. After shaking the samples for 30 s on a vortex shaker and centrifugation (5°C, 3000 g, 10 min), the supernatant was transferred to a polypropylene tube for derivatization and solid-phase extraction, which was performed using VARIAN solid-phase extraction cartridges (C18 Bond Elut 100 mg cm³). After preparation of the C18 cartridges with 1 mL of methanol and 1 mL of a buffer solution (sodium hydrogen carbonate buffer, pH 10) and addition of the supernatant and further 1 mL of the buffer solution, the cartridges were dried under vacuum. Then, 60 µL of a 9-fluorenylmethyl chloroformate solution (50 mM) was added and left to react for 15 min. After washing with 2 mL of acetonitrile (70%) and an additional drying step, samples were eluted with 1 mL of acetone. The eluates were evaporated to dryness in a stream of nitrogen at ~30°C, redissolved in 200 µL of dimethyl sulphoxide and 300 µL of a boric acid solution (0.2 M) and transferred into vials for injection into the HPLC. Chromatography was carried out by injecting a 4 µL aliquot of the processed samples onto a Luna (Phenomenex) C18 column (5.0 µm, 150 × 4.6 mm). Column temperature was maintained at 30°C. Samples were eluted with a mobile phase of acetonitrile, methanol, water and tetrahydrofuran (820/100/80/40, v/v/v/v).

A fluorescence detector (excitation wavelength, 260 nm; emission wavelength, 315 nm) was used for detection.

A linear concentration range from 30.0 to 2000 ng/mL for colistin was used for validation. As for all assays, the lowest calibration point represents the validated lower limit of quantification.

Determination of polymyxin E1 in human urine

In outline, the bioanalytical method used for the determination of polymyxin E1 in human urine included addition of 20 µL of blank serum and 50 µL of aqueous internal standard solution (10 µg/mL netilmicin sulphate) to 250 µL of the human urine sample prior to

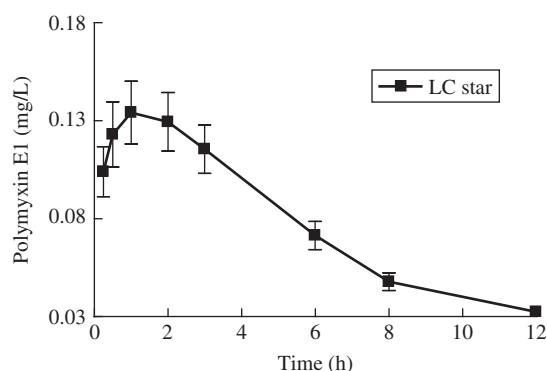


Figure 1. Serum concentrations of polymyxin E1 after a single dose of 2 million units of colistin (equal to 66 mg of colistin) administered with a PARI LC Star jet nebulizer in 30 patients with cystic fibrosis. Data represent means ± SEM.

Table 4. Pharmacokinetic serum parameters for inhaled colistin

	PARI LC Star	eFlow pilot series 03
AUC _{0-t} (mg·h/L)	0.913 ± 0.095	0.865 ± 0.187
AUC (mg·h/L)	1.190 ± 0.095	1.167 ± 0.186
T _{max} (h)	1.47 ± 0.16	1.5 ± 0.3
C _{max} (mg/L)	0.178 ± 0.018	0.170 ± 0.036
t _{1/2} (h)	4.09 ± 0.31	4.51 ± 0.57
MRT (h)	6.44 ± 0.43	7.02 ± 0.9
CL/f (mL/min)	787 ± 65.9	813 ± 175.5

AUC, area under the concentration–time curve; T_{max}, time to attain maximum concentration; C_{max}, maximum observed concentration; t_{1/2}, half-life; MRT, mean residence time; CL/f, total clearance of the drug. Data are shown as means ± SEM.

protein precipitation, derivatization and HPLC analysis as described for the serum assay. The analytical procedure in human urine was shown to be linear from 100 to 1000 ng/mL using a sample volume of 250 µL.

Determination of polymyxin E1 in human sputum

For the determination of polymyxin E1 in human sputum, the assay included addition of 10 µL of blank serum and 50 µL of aqueous internal standard solution to 100 µL of the human sputum sample prior to protein precipitation, derivatization and HPLC analysis as described for the serum assay. The analytical procedure in human sputum was validated within the calibration range of 300–3000 ng/mL.

For all assays, seven calibration standards in duplicate were used for external calibration. The performance of the methods obtained during the analysis of the study samples fulfilled all criteria stipulated by the international regulatory guidelines for validation.^{18–20}

Statistical analysis

Data were tested for normal distribution by the Kolmogorov–Smirnov test and expressed as means ± SEM. Comparisons were performed with paired *t*-test for changes within groups and by unpaired *t*-test for comparisons between groups.

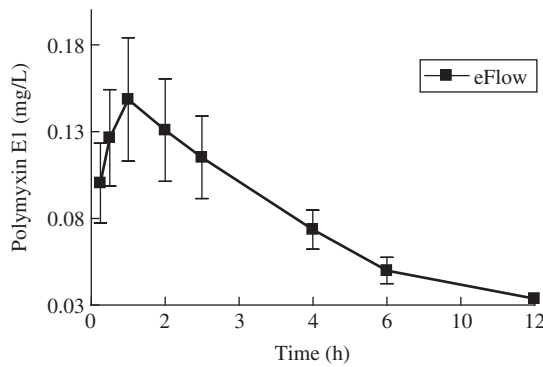


Figure 2. Serum concentrations of polymyxin E1 after a single dose of 2 million units of colistin (equal to 66 mg of colistin) administered with a customized PARI eFlow pilot-series 03 nebulizer in 8 patients with cystic fibrosis. Data represent means \pm SEM.

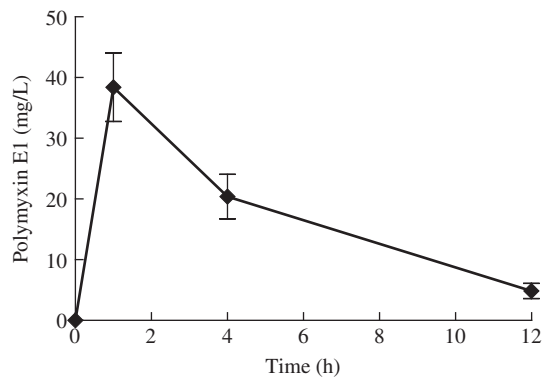


Figure 3. Sputum concentrations of polymyxin E1 after a single dose of 2 million units of colistin (equal to 66 mg of colistin) administered with a PARI LC Star jet nebulizer powered by a PARI Master compressor in patients with cystic fibrosis. Data represent means \pm SEM.

Results

The inhalation of study medication was well tolerated in all individuals as demonstrated by a lack of change in forced expiratory volume in 1 s (FEV_1) 5 and 20 min after completion of the inhalation. Serum concentrations of colistin reached their maximum at a mean of 1.5 h after inhalation and decreased thereafter (Figure 1). Pharmacokinetic serum parameters are summarized in Table 4. Serum concentrations were well below those previously reported for systemic application in all patients. Cumulative concentrations of colistin methate in urine ranged from 0.18 to 16.13 mg, which demonstrates that a mean $4.3 \pm 1.3\%$ (range 0.3–24.2%) of the inhaled dose was detected in urine. Elimination characteristics did not differ significantly from those previously reported for systemic application.^{21,22} Both maximum concentrations in serum and the pharmacokinetic profile were similar when reassessed in a subgroup of patients with the PARI eFlow nebulizer (Table 4 and Figure 2).

Sputum concentrations peaked at 1 h after inhalation (Figure 3). Maximum concentrations were at least 10 times higher than the MIC breakpoint for *P. aeruginosa* proposed by the British Society for Antimicrobial Chemotherapy (BSAC).²³ Absolute peak sputum concentrations have to be interpreted with caution, since five patients had peak concentrations above the linear

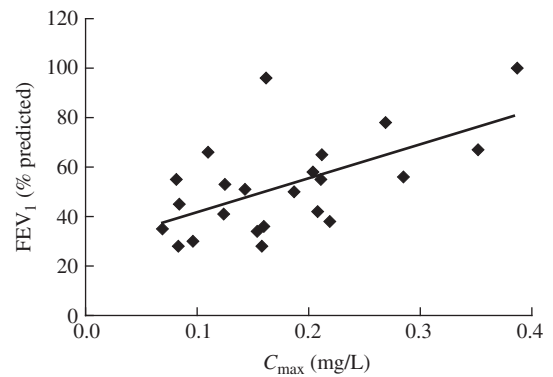


Figure 4. Relationship between FEV_1 in per cent predicted and maximal serum concentrations (C_{max}) of polymyxin E1. Each diamond represents one individual. The line represents the line of linear correlation. C_{max} was positively correlated to FEV_1 ($P = 0.003$, $r = 0.59$).

range of the assay. Although sputum drug concentrations decreased after the 1 h peak, the mean colistin concentrations were still above 4 mg/L after 12 h (Figure 3). No differences in polymyxin E sputum concentrations were seen for CF patients between the two nebulizer systems (LC Star and eFlow).

There was no age dependency in systemic deposition in the study population (data not shown). A positive correlation was found between FEV_1 in per cent predicted and both AUC and maximal colistin concentrations in serum (C_{max}) (Figure 4). A similar relationship could not be defined for sputum colistin concentrations or the fraction of colistin being excreted in urine (data not shown).

Discussion

In this study we defined the pharmacokinetics of a single dose of inhaled colistin in CF patients. High drug concentrations were achieved in sputum and were maintained for up to 12 h in the majority of patients. Systemic colistin concentrations were low with a systemic half-life of 4 h. Therefore, our data indicate that colistin can safely and effectively be delivered via inhalation to patients with CF.

The pharmacodynamics of inhaled medications are incompletely understood. Unlike systemic exposure inhaled drugs will be trapped in respiratory secretions where the elimination rate may be delayed and difficult to predict. The absorption rate will depend on many factors including the location of deposition (centrally versus peripherally) as well as the volume and mechanical properties of airway secretions. For patients with chronic respiratory infections, high concentrations in respiratory secretions may actually be beneficial, since recent evidence suggests that bacteria are mainly found in CF mucus rather than on the epithelial surface and delayed clearance of antibiotics from secretions may lengthen the exposure time to antibiotics.²⁴ Because of the difficulties in obtaining material from the lower airways limited data are available on antibiotic absorption into the circulation from the lower respiratory tract. Our data would suggest that high concentrations of the inhaled antibiotic are maintained over at least 8 h in CF patients. Evidence for prolonged clearance of inhaled antibiotics compared with systemic application also comes from a recent study where significant concentrations of

tobramycin were detected in bronchoalveolar lavage fluid of young children with CF 8 h after the last inhalation.²⁵

These results bear some relevance for the dosing intervals used in the treatment of CF patients. Based on the data after intravenous administration a two or three time daily dosing regimen is usually used in the CF population. Our results would favour the use of a twice daily regimen, since adequate concentrations were maintained for 12 h. We did not follow sputum concentrations beyond the 12 h time point, but these data raise the interesting question of whether a single high dose once daily may be sufficient for maintenance treatment in CF patients. This may be more compatible with patients' lifestyles and may therefore positively affect compliance. A pharmacokinetic profile over a longer time period than the one used in this study is needed to address this question.

There is some limitation in the interpretation of the sputum data obtained in this study. Sputum is inhomogeneous in origin and may not reflect the overall drug concentrations in the lower respiratory tract. Therefore, reliable pharmacokinetic data are usually based on assessing serum and urinary concentrations of a drug. However, these may also poorly reflect the pulmonary exposure to the drug, as the target region for inhaled antibiotics is the bronchi rather than the alveoli whereas systemic absorption is highest for drugs deposited in the alveoli. Another factor that needs to be considered is that colistin is a pro-drug that is converted into its active component polymyxin E1. We have elected to study the active component, which was found in high concentration in CF sputum. Other components or metabolites of colistin may differ in their half-life characteristics and may accumulate in the respiratory tract. Some studies have questioned whether MIC cut-off levels for colistin are reliable.^{26–29} Nevertheless, our results suggest concentrations well above MIC breakpoints of 4 mg/L that have been proposed by the BSAC.²³ Indeed, mean sputum levels were above this breakpoint even 12 h after the inhalation. Therefore, these data indicate that colistin can be administered in sufficient quantities to the lower respiratory tract of CF patients.

We did not see any age dependency in drug deposition, but the majority of patients included in this study were adults and age dependency may be seen in younger children. However, we observed a significant correlation between lung function and maximal serum concentrations, suggesting that lower concentrations reached the periphery of the lung in patients with advanced lung disease. A similar relationship was not seen for peak sputum concentrations, but these data have to be interpreted with caution, since patients had peak concentrations above the linear range of the assay. Nevertheless, the serum colistin data are compatible with the concept that more of the drug is trapped in respiratory secretions in patients with poorer lung function. Based on these results one could speculate that higher antibiotic doses could potentially be used in patients with advanced disease compared with those with limited disease, but this requires confirmation in a larger cohort of patients.

We did not observe any differences in pharmacokinetics between the two types of nebulizers, whereas their *in vitro* delivery rates for tobramycin have been reported to be quite different.³⁰ Although the output rate of the eFlow pilot series 03 was smaller than for the LC Star (Table 3), when determined *in vitro* by continuous nebulization (Table 3), the eFlow delivered the same dose in approximately a third of the time interval, which is a positive factor for patients that receive multiple medications via inhalation. However, the shorter nebulization time is most probably due to

the lower fill volume (2.5 mL instead of 6 mL). It should be considered in this context that selection of the solvent (water and saline) and volume (2.5 mL versus 6 mL) can affect physico-chemical and nebulization properties (Tables 2 and 3). These findings stress the importance of testing the characteristics of each drug individually in all nebulizer systems.³¹

In summary, we report low systemic and high local concentrations after a single dose of 2 million units of colistin administered via inhalation in CF patients. In view of the low incidence of resistance of *P. aeruginosa* against colistin^{32–34} and its proven tolerability in the majority of patients, these data would favour the use of inhaled colistin in CF patients chronically infected with *P. aeruginosa*.

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Transparency declarations

No declarations were made by the authors of this paper.

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