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RESEARCH PAPER

Pharmacokinetics of Colistin Methansulphonate (CMS) and Colistin after CMS Nebulisation in Baboon Monkeys

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ABSTRACT

Purpose The objective of this study was to compare two different nebulizers: Eflow rapid® and Pari LC star® by scintigraphy and PK modeling to simulate epithelial lining fluid concentrations from measured plasma concentrations, after nebulization of CMS in baboons.

Methods Three baboons received CMS by IV infusion and by 2 types of aerosols generators and colistin by subcutaneous infusion. Gamma imaging was performed after nebulisation to determine colistin distribution in lungs. Blood samples were collected during 9 h and colistin and CMS plasma concentrations were measured by LC-MS/MS. A population pharmacokinetic analysis was conducted and simulations were performed to predict lung concentrations after nebulization.

Results Higher aerosol distribution into lungs was observed by scintigraphy, when CMS was nebulized with Pari LC® star than with Eflow Rapid® nebulizer. This observation was confirmed by the fraction of CMS deposited into the lung (respectively 3.5% *versus* 1.3%).CMS and colistin simulated concentrations in epithelial lining fluid were higher after using the Pari LC star® than the Eflow rapid® system.

Conclusions A limited fraction of CMS reaches lungs after nebulization, but higher colistin plasma concentrations were measured and higher intrapulmonary colistin concentrations were simulated with the Pari LC Star® than with the Eflow Rapid® system.

KEY WORDS colistin · nebulization · pharmacokinetic modelling · scintigraphy

ABBREVIATIONS

99m Tc-DTPA	99m technetium-diethylene triamino pentaacetic acid
AUC_{ELF}	Area under the ELF concentrations-time curve
BAL	Broncho-alveolar lavage
C	Central lung
CBA	Colistin base activity
CF	Cystic fibrosis
CMS	Colistin methansulphonate
ELF	Epithelial lining fluid
ET	Extrathoracic
GDS	Geometric standard deviation
HPLC	High-performance liquid chromatography
IIV	Inter-individual variability
IM	Intramuscular
IOV	Inter-occasion variability
IV	Intravenous
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
LLOQ	Lower limit of quantification
MMAD	Mass median aerodynamic diameter
NLME	Non-linear mixed effects
OFV	Objective function value
P	Peripheral lung

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PK	Pharmacokinetics
ROIs	Regions of interest
SC	Subcutaneous
T	Lung
TH	Thoracic
VAP	Ventilator-associated pneumonia
VPC	Visual predictive checks

INTRODUCTION

Colonization and infection of the respiratory tract due to multidrug resistant *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients is a key issue in the natural history of this disease (1). Colistin has been extensively used in the form of aerosols to prevent and cure pulmonary infections due to *P. aeruginosa* in patients with CF over the 20 last years (2). In critical care patients, colistin aerosols are also used as an adjunctive treatment of nosocomial (3–6) or ventilator-associated pneumonia (VAP) (7, 8) due to multidrug-resistant Gram-negative such as *P. aeruginosa* and *A. baumannii*. Colistin is a multicomponent cationic polypeptide mainly constituted by colistin A (polymyxin E1) and colistin B (polymyxin E2). It was developed in the 1950s and used for parenteral administration and inhalation as colistin methansulphonate or CMS, acting as a prodrug, less toxic than colistin but with no antimicrobial activity (9). Yet CMS systemic administration has been limited because of neurotoxicity and nephrotoxicity (10). Therefore the expected advantage with the aerosol route is that high local and therefore efficient antibacterial concentrations should be obtained, while minimizing systemic exposure and therefore toxicity (11), which is supported by recent studies in animals (12, 13). It is also an obvious advantage for the treatment of ambulatory patients such as CF patients. Combination of nebulized colistin with oral ciprofloxacin is considered as a reference treatment in multidrug-resistant pneumonia in CF patients (7, 11, 14). Yet in order to be active CMS must be converted into colistin within the lung before leaving the pulmonary tractus by mucociliary clearance mechanisms with expectoration or swallowing of pulmonary secretions, or by systemic absorption (15). However, CMS and colistin PK data after inhalation (16, 17) and nebulization (18–20) in patients are limited, and most of these results (16–19) should be considered with caution due to inadequate analytical methods (21, 22). Reliable CMS and colistin PK in plasma and lung (sputum) after CMS nebulization in CF patients has only recently been described (20).

Antibiotics can also be aerosolized in children and drug delivery by aerosol is known to be dramatically reduced in children in comparison with adults (23). Moreover, different types of nebulizers can be used in CF patients that may have a major effect on CMS delivery and eventually on intrapulmonary concentrations of colistin. Baboon monkeys present an upper airway system anatomy close to the human system (24, 25), and morphometric comparison of baboon airways with the respiratory geometry of a 2-years-old child suggests functional interspecific relationship between nasal structure, cross-sectional area and tracheobronchial region (26–28). Tidal volume, breathing rate and inspiratory/expiratory ratio have been previously measured at 54 mL, 35 breaths/min and 0.69 respectively (29) and correspond to infant ventilation (30). Therefore experiments in baboon monkeys may be predictive of drug deposition in children after nebulization.

The objective of this study was to compare in baboons the ability of two types of nebulizers, one with vibrating mesh: Eflow rapid® and one using compressor: Pari LC star®, to induce proper intra-pulmonary concentrations of colistin, by combining imaging techniques with pharmacokinetic modelling approaches.

MATERIALS AND METHODS

Chemicals

Colistimethate sodium (Colymicine®, 1 M UI, Sanofi-Aventis, Paris, France) was provided by the pharmacy of Poitiers University Hospital and was used to prepare CMS solutions for intravenous (IV) administration and nebulisation. Colistin sulfate was purchased from Sigma (Saint Quentin Fallavier, France). All chemicals used were of analytical grade and solvents were of high-performance liquid chromatography (HPLC) grade.

99mTc-DPTA Labelling (99 m Technetium Diethylenetriamine Pentaacetate Labelling)

The 99 m technetium-diethylene triamino pentaacetic acid (99mTc-DPTA) labelling was prepared from a commercially available kit (Pentacis, CIS Bio International, France) as previously described (31). In order to determine the lung dose by scintigraphy, the nebulization solution was obtained by reconstituting the CMS sulfate powder in 3 mL of 99mTc-DTPA (74 MBq) dissolved in 0.9% NaCl for a final concentration of 0.33 million IU/mL corresponding to 26.6 mg/mL of CMS sulfate or 9.9 mg/mL of colistin base activity (CBA) (32). We have previously verified that the addition of this radioactive tracer does not change the normal distribution and dynamics of the medication within the aerosol and that radioactive reflects the mass of drug (33, 34).

Animals

Three female baboons (*Papio papio*) weighing between 10 and 13 kg were used for this study. Protocol was approved by the

local ethics committee (Commission d'Ethique en expérimentation animale Val de Loire N°19, Université de Tours; N°2011/02/4). Animals were housed under conventional conditions and maintained in accordance with the Guide of Principles of Laboratory Animal Care. Four dosing groups corresponding to different drugs administered and/or ways of administration (CMS by IV infusion, colistin by subcutaneous (SC) infusion and CMS for aerosol administration by two different types of nebulizer) were performed in the three baboons. There was 1 week of washout between drugs administration.

Anaesthesia

When CMS and colistin were administered intravenously and subcutaneously, baboons were anaesthetized approximately 5 h, which allowed administration and blood sampling 4 h post infusion. First, baboons were lightly sedated by an intramuscular (IM) injection of ketamine hydrochloride 10 mg/kg (Centravet Plancoet, Dinan, France). This first sedation was used to prepare baboons to general anaesthesia but also to allow aerosol administration under spontaneous ventilation. Then, baboons were weighed, placed in dorsal decubitus and intubated with a 4 or 5 mm internal diameter Portex tracheal tube and ventilated with a mixture of air-oxygen 21% charged with isoflurane 4% (Forene, Abbot, Rungis, France) at the beginning of anaesthesia. The percentage of isoflurane was decreased from 1.5 to 1% after intubation and kept constant for the rest of the anaesthesia (Siemens Servo Ventilator 900D, Saint Denis,France). The ventilation flow rate was ranging between 2.5 and 3.0 L/min with a frequency of 30 min^{-1}. Throughout the anaesthesia, animals were placed under a thermostated carpet set at 37°C and perfused at 4 mL/h/kg with polyionic solute (Glucidion g 10, B Braun Medical, Boulogne Billancourt, France), electrocardiogram and monitoring of oxygen saturation by oxymetry (Nellcor Puritan Bennett, Hertogenbosch, The Netherlands) were also performed. At the end of the 4 h period of sampling, baboons were awakened by stopping isoflurane and increasing percentage of oxygen to 100%. When first signs of spontaneous ventilation were observed, animals were extubated and housed into their cages. For last blood samples at 6 and 9 h, baboons was re-anesthetized in their cages by IM injection of ketamine hydrochloride 10 mg/kg.

Preparation of Solutions and Drugs Administration

CMS IV Infusion ($n=3$)

1 M UI of Colymicine® corresponding to 80 mg of sodium CMS (approximately 30 mg of colistin base activity (CBA)) was diluted before administration in 5 mL of sterile physiological serum 0.9%. This solution was infused in the left tibial vein at a flow rate of 0.2 ml/min for 10 min (PHD 2000 infusion pump, Harvard Apparatus, Les Ulis, France) corresponding to a dose of 32 mg of sodium CMS (12 mg CBA) per monkey.

CMS Nebulisation ($n=3$ per system)

Before aerosol administration, baboons were sedated with ketamine hydrochloride (10 mg/kg) and placed on contention chair. CMS aerosol was prepared as previously described (paragraph 99mTc-DPTA labelling) and was administered at a dose of 26.6 mg of CMS sulfate per monkey, using a conical mask directly connected to the nebuliser. With the Eflow rapid® (Pari, Starnberg, Germany) nebulizer, time of nebulization was between 2 and 3 min and was automatically stopped by the device detecting the end of the nebulization. With the Pari LC star® (Pari, Starnberg, Germany), the aerosolisation was stopped manually after 10 min of nebulisation as recommended by the pharmaceutical company commercializing the colistin. The particle size of CMS aerosol produced by Pari LC Star® nebulizer and Eflow rapid® were determined by a laser diffraction method (Spraytec, Malvern, UK) (35). They are characterized by a Mass Median Aerodynamic Diameter (MMAD) of 3.2 ± 0.2 and 4.0 ± 0.2 μm respectively and a Geometric standard Deviation (GSD) of 2.5 ± 0.1 and 1.6 ± 0.05 respectively. The CMS output produced by Pari LC Star® nebulizer and Eflow rapid® were determined by the residual gravimetric method (36). They are characterized respectively by an output of 33 ± 2 and 31 ± 3% expressed in term of the nebulizer charge (1 MU/3 mL).

Subcutaneous Infusion of Colistin ($n=3$)

Ten milligrams of colistin sulfate were dissolved into 2 mL sterile physiological serum 0.9% under laminar flow (5 mg/mL). The solution was then introduced in a sterile flask by sterile filtration (0.22 μm Filter Unit, Merck Millipore, Darmstadt, Germany,) and was frozen at −20°C until use. At the time of administration, the solution was warmed up at room temperature and infused in the left tibial vein at a flow rate of 0.1 mL/min during 10 min (PHD 2000 infusion pump, Harvard Apparatus, Les Ulis, France) corresponding to a dose of 5 mg colistin sulfate per monkey.

Sampling

Blood samples were collected in heparinised vacutainers before (0 min) and 10 min after the beginning of administrations (corresponding to the end of CMS IV administration and Pari LC® aerosolisation), and then 0.5, 1, 2, 3, 4, 6, and 9 h post dosing via the right saphenous vein. Plasma was immediately

separated by centrifugation and frozen at −20°C until analysis.

Gamma Camera Imaging

Tissue attenuation correction factors and lung oulines were determined from lung perfusion imaging of each baboon using pertechnetate-macroaggregated albumin (Covidien, Dublin, Ireland). Although the attenuation coefficient determined by perfusion scan is different between lung and the other organs, especially the stomach (37), the same attenuation coefficient was applied for every regions of interest (ie lung attenuation coefficient). Comparable attenuation coefficients may not affect comparison in lung deposition of the two nebulizers but may affect their extra thoracic deposition. The nebulizer charge was measured by counting the radioactivity in the syringe using a gamma counter (Capintec, New Jersey, USA) before and after charging the nebulizer. The aerosol was delivered to the baboon. Immediately after aerosol delivery, the animals were scanned using a gamma camera (Ecam, Siemens, Erlangen, Germany). A 120-s posterior static view was acquired on a 128×128 matrix. The regions of interest (ROIs) were determined manually, delimiting two main regions: the thoracic (TH), the extrathoracic (ET) including the stomach, the upper airways and the trachea (38). Furthermore, the peripheral lung (P) and the central lung (C) were also determined manually as follows. The central lung (C) was defined as the 1/3 of the central area of the (TH) and (P) was defined as the difference between the (TH) area and the (C) area (39). Background noise was subtracted to measured counts of radioactivity within ROIs. Furthermore, corrections for physical decay of ^{99m}Tc were made on all measurements. Tissue attenuation coefficients were also taken into account. The amount of ^{99m}Tc-DTPA deposited in the ROIs was then determined from the digitized images. C/P ratio reflecting the aerosol distribution into the lung was calculated.

CMS and Colistin Analysis in Plasma

Determination of colistin and CMS concentrations in plasma was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method as previously described (40). Reversed-phase chromatography was performed on a C18 Xbridge™ column (5.0 µm, 150×2.1 mm ID, Waters, St-Quentin en Yvelines, France). The mobile phase was 0.1% (v/v) formic acid in acetonitrile: 0.1% formic acid in water (20:80, v/v). The LC-MS/MS system consisted of a Waters Alliance 2695 separations module equipped with a binary pump and an autosampler thermostated et 4°C and of a Waters Micromass® Quattro micro API tandem mass spectrometer. The mass spectrometer was operated in the positive/ion mode. Ions were analysed by multiple reactions monitoring (MRM). Transition ions were m/z 585.5/101.2 for colistin A, 578.5/101.2 for colistin B and 602.5/241.2 for polymyxin B1, the internal standard. For CMS concentrations, eight point calibration standard curves in plasma were prepared at concentrations between 0.039 and 10 mg/L. Three control levels were carried out: 0.156, 0.625 and 3.75 mg/L. For colistin, eight point calibration standard curves in plasma were constructed at concentrations between 0.0195 and 10 mg/L. Three control levels (0.156, 0.625 and 3.75 mg/L) were also performed. The between-day variability for colistin was characterized at 3.75, 0.625 and 0.156 mg/L with coefficients of variation respectively equal to 11.71, 8.73 and 4.64% (n=13) and a bias equal to −3.27, −0.30 and 1.67% (n=13). For CMS, the between-day variability was characterized at 3.75, 0.625 and 0.156 mg/L with coefficients of variation respectively equal to 6.55, 8.92 and 10.47% (n=11) and a bias equal to −0.08, −0.65 and 1.67% (n=11).

Population Pharmacokinetic (PK) Modelling

The population pharmacokinetics analysis of CMS and colistin was conducted with a non-linear mixed effects (NLME) model. This modelling approach is characterised by a structural model comprising the fixed effects and a stochastic model accounting for the random effects. The stochastic part of the model is subdivided in first, the variability assigned to specific parameters and second, the residual error mostly describing the experimental variability and assigned to the observations. During the analysis, several structural models were tested and evaluated. One or two compartments were assessed to describe CMS PK in plasma. Dynamic transit compartments (41) were also tested to mimic the time delay needed for CMS to reach the exchange surface between the epithelial lining fluid (ELF) and the plasma. Considering our previous estimates of ELF volume at 21.7 µL/Kg in rats (12) and 21.4 µL/Kg in human (42), the typical value of V_{ELF} was set at 21.5 µL/Kg in baboons and corrected for body weight.

The interindividual variability (IIV) and interoccasion variability (IOV) were assumed to follow a log normal distribution as shown in Eq. 1 where θ_i is the individual parameter value, θ is the typical population value and η is the patient (i) - or occasion - specific random effect following a normal distribution with mean 0 and variance ω^2. The typical values of θ and ω^2 were estimated.

$$\theta_i = \theta.\exp(_i) \quad (1)$$

The study was subdivided in four occasions in order to determine the interoccasion variability parameter. The four occasions were representing the four different routes of administration (OCC1: IV infusion, OCC2: nebulisation with Pari LC®, OCC3: nebulisation with Eflow rapid® and

OCC4: SCinfusion). Different residual error models with additive, proportional and heteroscedastic error (additive + proportional) structures were evaluated for both CMS and colistin.

As many CMS and colistin plasma concentrations were below the lower limit of quantification (LLOQ), the M3 method for handling censored data described by Beal (43) was applied. The LLOQ was 0.039 mg/L for CMS and 0.0195 mg/L for colistin. The two different nebulisation systems were investigated as covariates in a stepwise fashion. The model selection was based on physiological and biological plausibility as well as on maximum likelihood statistics which is quantified by the objective function value (OFV). The OFV is defined as minus two times the log-likelihood with a 5% significance level applied for statistical tests. The difference in OFV (dOFV) was used in order to discriminate between nested models; it had to be at least −3.84.Covariates were included if they induced a reduction in IIV and if they were physiologically relevant. The relative standard errors were obtained from the covariance matrix.

Precision in parameter estimates and graphical analysis of goodness of fit plots (individual plots representing simultaneously observed concentrations with the population predictions and individual predictions *versus* time after dose) and visual predictive checks (VPC; simulations = 1000) were used as graphical evaluation of the model.

Software

The data analysis was performed with a nonlinear mixed-effects approach implemented in S-ADAPT (version 1.57). Importance sampling Monte Carlo expectation-maximization estimation algorithm (pmethod 4) was applied for parameter estimation and to obtain the standard errors for parameter estimates (covariance variance matrix). The modelling work was facilitated by S-ADAPT-TRAN (44) as modelling environment for automation of a diverse range of processes and for graphical evaluation. Monte Carlo simulations were run using Berkeley Madonna version 8.3.18 (Berkeley Madonna Inc., University of California, Berkeley, CA, USA). Goodness of fit plots and simulation graphics were plotted using the graphical visualisation R package ggplot2 (45).

RESULTS

Scintigraphy

Images are presented on Fig. 1 and show greater aerosol distribution into the baboon lungs when CMS is nebulized with Pari LC® star nebulizer than with Eflow Rapid® nebulizer. The mean fraction of aerosol deposited into the lung (T) was higher with Pari LC® Star than Eflow rapid® (3.5% *vs* 1.3%). The ratio between the aerosol deposited into the extrathoracic region (ET) and the lung was higher with Eflow® (5.5 *vs* 1.6) attesting for higher deposition distribution in the upper part of the airways. Similar aerosol distribution was obtained between central lung and peripheral lung for both nebulizers (mean of C/P ratio = 1.0 *vs* 0.8 for respectively Pari LC Star® and Eflow rapid®). Aerosol deposition results are presented in Table I.

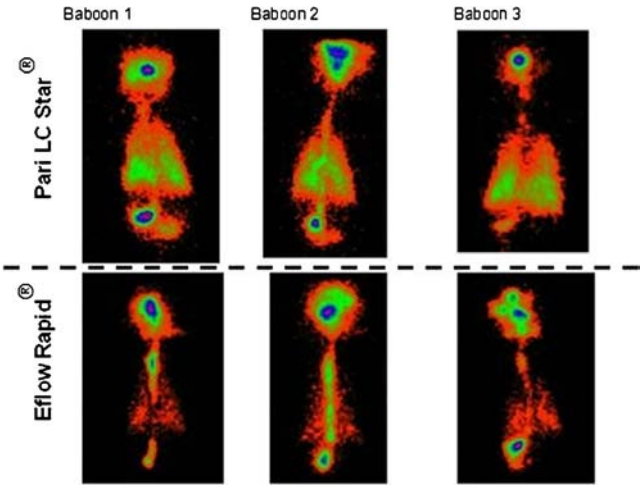

Fig. 1 Scintigraphy images in 3 baboons obtained after colistin nebulisation with Pari LC Star® jet nebulizer and Eflow rapid® mesh nebulizer.

Population Pharmacokinetic Modelling

The total data included 171 observations with 48 plasma concentrations below the lower limit of quantification (LLOQ). The pharmacokinetics of CMS was adequately described by a one-compartment model both in plasma and in the ELF. Similarly, a one-compartment model was best to describe colistin pharmacokinetics in plasma and ELF. A depot compartment was added to the model to mimic the subcutaneous administration of colistin. In plasma, CMS was cleared both via renal excretion (k_{r_CMS}) and via hydrolysis into colistin (k_{hyd_plasma}) whereas colistin was eliminated non-renally (CL_{Coli}/V_{Coli}). A hydrolysis rate (k_{hyd_ELF}) constant was also estimated in the model in order to describe the hydrolysis of CMS to colistin in the ELF (Fig. 2). The addition of dynamic transit compartments did not help to better fit

Table I Aerosol Deposition Results Expressed in Term of Nebulizer Charge for TH and ET, and in Term of Ratio Between the Aerosol Deposited into the Central Lung and the Peripheral Lung (C/P). Results are Expressed in Term of Min and Max

Nebulizers	TH	ET	C/P
Pari LC Star®	[3.3–3.6%]	[1.1–8.7%]	[0.86–1.35]
Eflow rapid®	[0.9–1.5%]	[4.8–8.1%]	[0.77–0.79]

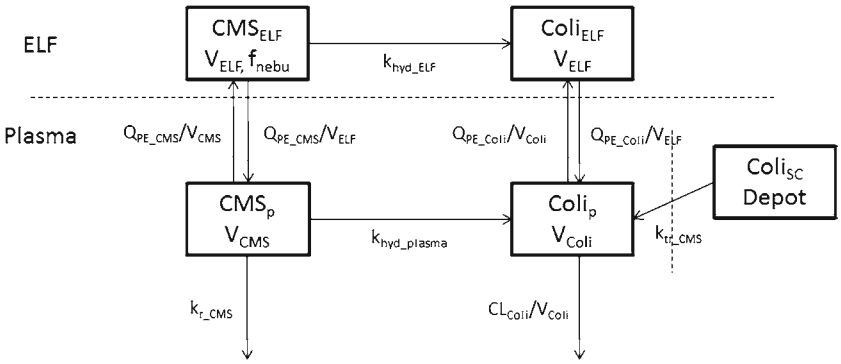

Fig. 2 Schematic representation of CMS and colistin pharmacokinetic model in plasma and ELF.

the data. f_{nebu_1} and f_{nebu_2} represent the fractions of CMS available at the exchange surface between the ELF and plasma, obtained with the Pari LC star® and Eflow rapid® nebulizers respectively.

Due to the limited number of animals, it was not possible to estimate an IIV on the different parameters. The IOV appeared to be non-significant on any of the parameters. A proportional residual error model was applied to CMS plasma concentrations and a heteroscedatic residual error model was applied to colistin plasma concentrations. Therefore, the type analysis used in this work was not a proper population pharmacokinetic analysis but a "Naïve pooling" approach. The typical population pharmacokinetic parameter values were estimated (fixed effects) together with the unexplained residual variability that accounted for the interindividual and interoccasion variability.

Parameter estimates with relative standard errors from the final model are presented in Table II. The Figs. 3 and 4 (a and b) show goodness of fit plots and stratified VPCs. In Table II, the model estimated a higher f_{nebu_1} than f_{nebu_2} indicating a higher plasma exposure of CMS when is administered through nebulisation with the Pari LC star® system than Eflow rapid® nebulizer. Consistently with imaging data (Fig. 1), it is graphically demonstrated that CMS and colistin exposure in ELF (lung) is higher for the nebulisation through the Pari LC star® system than Eflow rapid® nebulizer (Fig. 5). Moreover, the simulations of the typical PK profiles (Fig. 5) illustrate that CMS and more importantly colistin concentrations in ELF should be higher when administered through nebulisation than IV infusion.

DISCUSSION

Pharmacokinetic studies most often rely on systemic concentrations determinations. However for most drugs, the site of effect which corresponds to the infection site for antibiotics, is located within the extravascular space, and drug concentrations in plasma or serum are not always a good reflect of concentrations at the infection site. Furthermore patients disease may have an effect on antibiotics distribution, for example by altering protein binding or tissue blood flow or/and permeability. Peripheral degradation (46, 47) and the presence of efflux transport systems within tissue (48) are other complicating factors. It is therefore important to estimate extravascular antibiotic concentrations, which may be accomplished experimentally by microdialysis in a variety of tissues both in animals or human (49–53) or by

Table II Parameter Estimates with Relative Standard Errors from the Final Model

Fixed effects (θ)	Value (RSE, %)	Residual error	
CL_{coli}, L/h	1.48 (0.23)	Prop error CMS Value, % CV (RSE, %)	33.4 (9.51)
Q_{PE_CMS}, L/h	0.000131 (1.19)	Prop error Coli Value, % CV (RSE, %)	51.7 (10.4)
Q_{PE_coli}, L/h	0.0000972(0.925)	Add error Coli	0.00946 (19.4)
k_{r_CMS}, h^{-1}	0.225 (0.596)	Value (RSE, %)	
k_{hyd_plasma}, h^{-1}	0.964 (0.366)		
k_{hyd_ELF}, h^{-1}	0.441 (0.402)		
k_{tr_coli}, h^{-1}	0.264 (0.796)		
V_{c_CMS}, L	1.35 (0.691)		
V_{coli}, L	3.55 (0.500)		
f_{nebu_1}	0.0241 (1.03)		
f_{nebu_2}	0.00809 (1.34)		

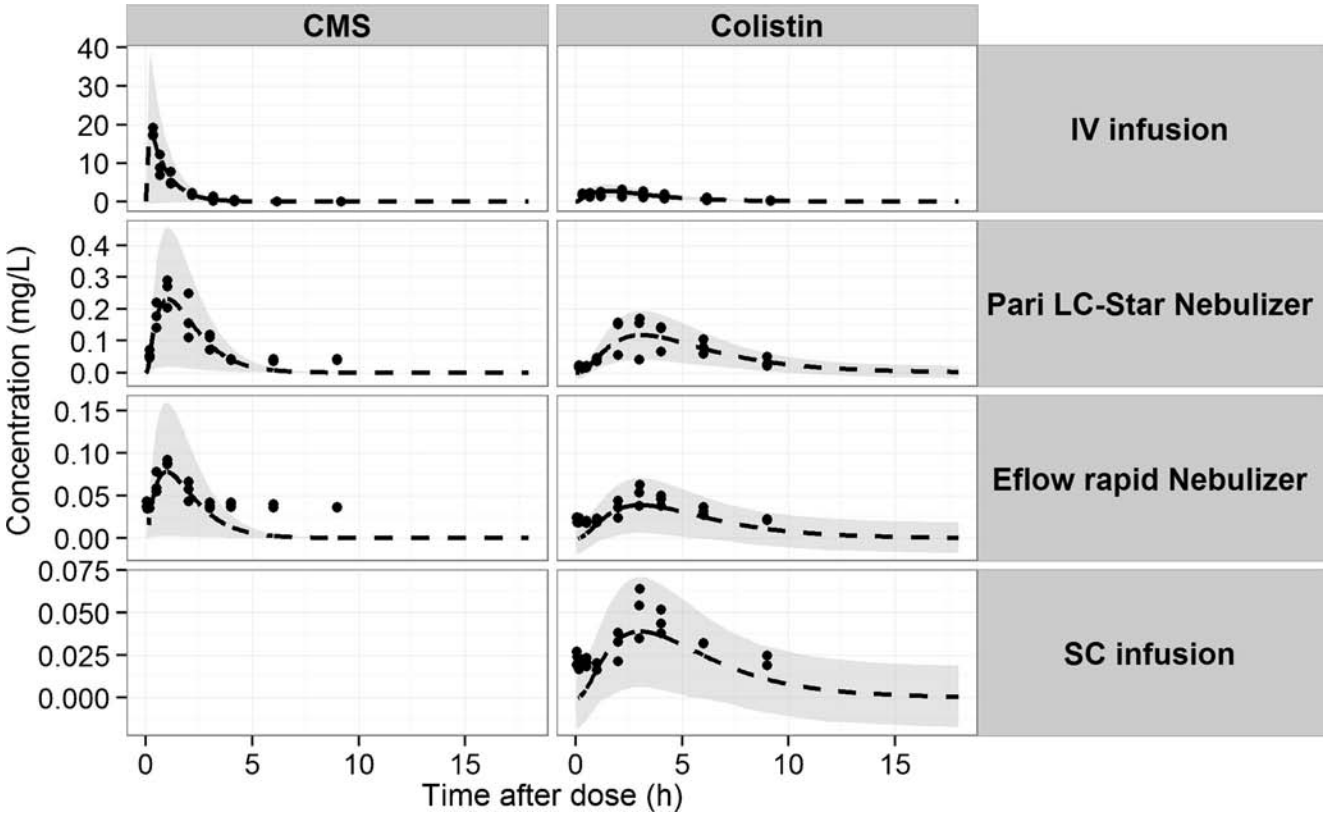

Fig. 3 Visual predictive checks showing the observed CMS and colistin plasma concentrations data (*dots*) and the median of the model simulated data (*dashed line*) and the 95% prediction interval following the different routes of administration.

broncho-alveolar lavage (BAL) when lung distribution is concerned. It may then be concluded that direct antibiotic delivery at the infection site should constitute an efficient way to increase local concentrations and therefore efficacy and at the same time reduce systemic exposure and consequently toxicity. Pulmonary infections could then be treated more efficiently by nebulization than by using any other route of administration, at least for some (12, 54) but not all (55) antibiotics. Furthermore intrapulmonary antibiotics concentrations may also vary widely with the type of nebulizer (56). Obviously experiments in rodents do not allow comparisons between nebulizers used in patients. Pig has been chosen to assess intrapulmonary colistin PK after nebulisation (57). However only a vibrating plate nebulizer (Aeronen Pro®, Aerogen Ltd, Galway; Ireland) was used in this study, which was mostly descriptive and relied on whole tissue homogenate concentrations determinations. This procedure is not ideal, not only because it requires animal sacrifice, limiting to one the number of time points per animal, and therefore the potential obtainable information, but also because concentrations measurements in whole tissue homogenates is not recommended for reasons previously discussed (58). Consequently, instead of pigs we have chosen to conduct a study in baboons, which is considered as an animal model to predict the aerosol deposition in children, due to its anatomy and respiratory parameters (25) and to combine scintigraphy and PK modeling to estimate ELF concentrations after colistin nebulization. After aerosol administration, baboons were intubated and ventilated. Intubation limits drug transport into the stomach due to mucociliary clearance. But negligible absorption of oral doses of CMS and colistin sulphate were described (21), limiting the effect of intubation on pulmonary fate of the deposited drug.

Two types of nebulizers were compared during this study, leading to different results in terms of deposition distribution characteristics in the upper part of the airways as illustrated on Fig. 1. These differences were confirmed by the mean fraction of aerosol deposited into the lung (T), that was about 2.5 times higher with Pari LC Star® (3.5%) than with Eflow rapid® (1.3%), when at the same time the ratio between the aerosol deposited into the extrathoracic region (ET) and the lung was about 3.5 times higher with Eflow® (5.5) than with Pari LC Star® (1.6) (Table I). The low deposition of colistin into the lungs for both nebulizers can be explained by the use of a conical mask directly connected to nebulizers in baboons which mimics the face mask used in children. With such a dispositive, a large part of particle was trapped in the nasal cavity and was deposited into the head of animals. However, our results of lung deposition (1.3% for Eflow rapid® *vs* 3.5% for LC Pari Star®) are consistent with Chua *et al.* study which

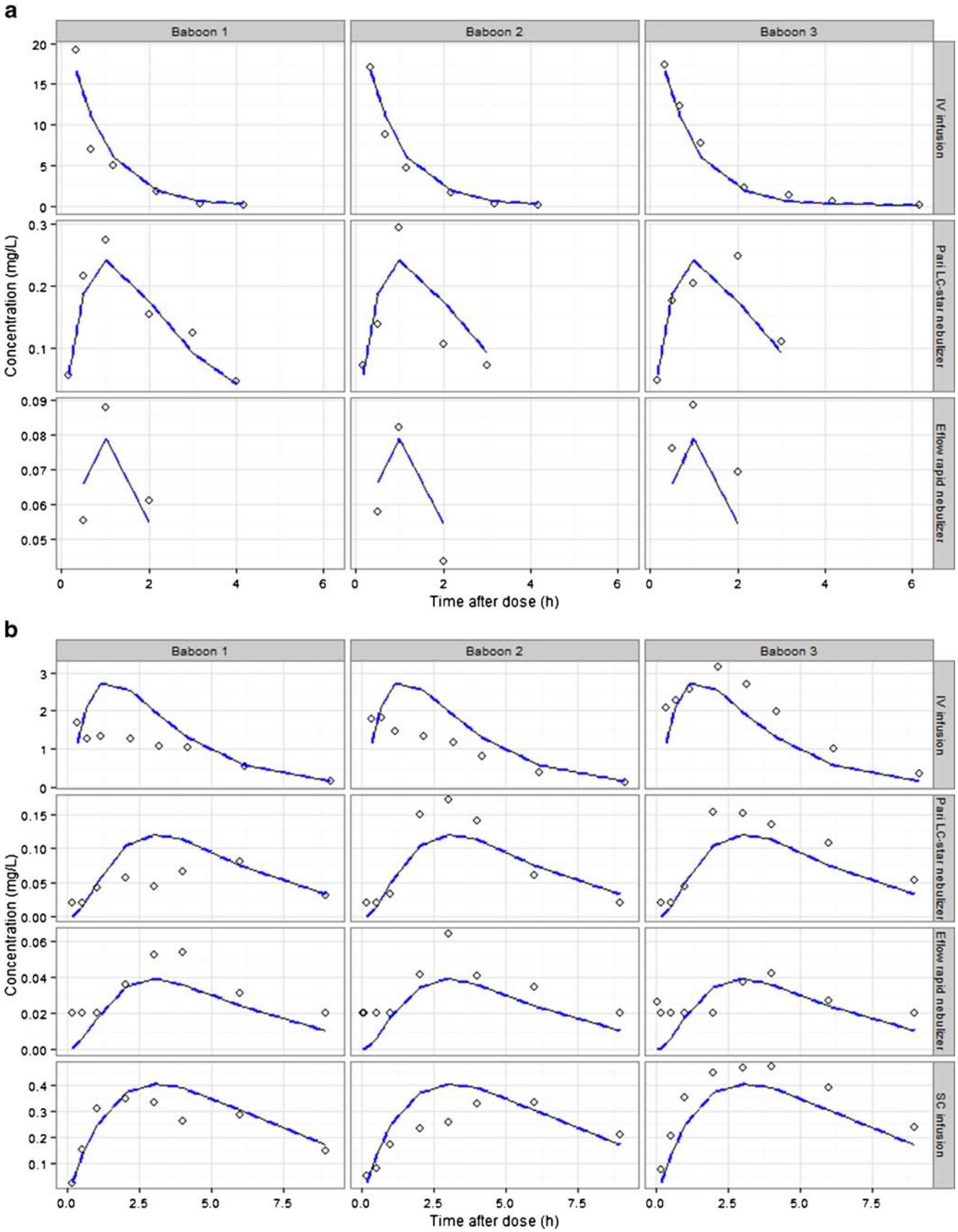

Fig. 4 Goodness of fit plots: (**a**) and (**b**) show the individual plasma PK profiles for CMS and colistin respectively following the four different routes of administration. In these graphs, the *black dots* represent the observed data and the *solid line* the population predictions.

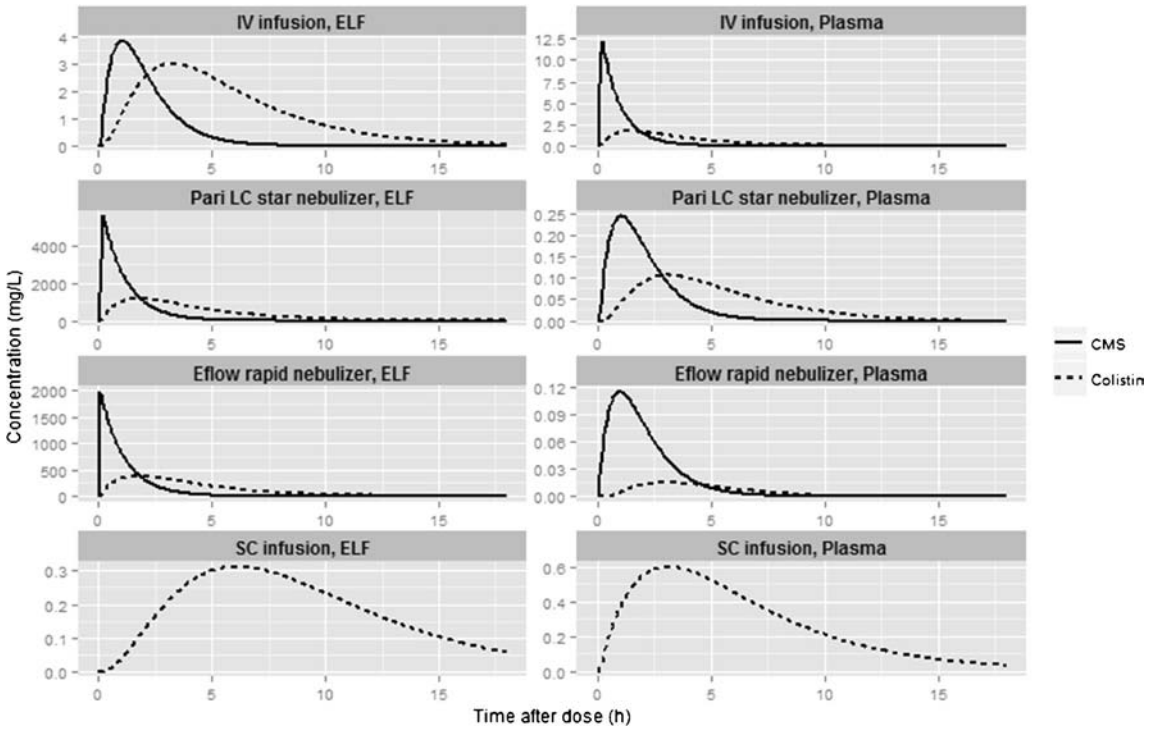

Fig. 5 Simulated concentrations in plasma and ELF for a typical animal following an IV infusion of 74.8 mg CMS sodium (1 M UI), a nebulisation of 30 mg of CMS (0.4 M UI) with both Pari LC Star® and Eflow rapid® nebulizers and an IV infusion of 5 mg of colistin sulphate.

measured a lung deposition of radiolabelled saline in children between 0.3 and 4.4% (23). Morever, The difference in lung deposition of the two nebulizers can probably be explained by the difference in terms of particle size produced. Eflow rapid® nebulizer is characterized by a mass median aerodynamic diameter (MMAD) of 4 μm whereas the Pari LC Star® nebulizer is characterized by a smaller MMAD of 3.2 μm predicting a lower ET deposition.

Interestingly, the PK modelling analysis confirmed almost exactly the differences between nebulizers performances assessed with scintigraphy imaging. This analysis characterized the typical PK profiles in plasma only accounting for the residual unexplained variability. The estimated typical fractions of the dose reaching the systemic circulation were at 2.4% with Pari LC Star® and 0.8% with Eflow®, corresponding to a 3 fold ratio (Table II). The PK modelling approach takes into consideration the difference in nebulizers by estimating the fractions of the dose reaching the plasma for each nebulizer but also the specificities of CMS and colistin PK. Ideally CMS and colistin should have been assayed within lung. As previously reminded tissue homogenate concentrations require animal sacrifice and should not be used as they are not informative (58). For antibiotics acting within the extracellular space, such as colistin, ELF concentrations obtained after broncho-alveolar lavage (BAL) and corrections for dilution by comparing urea concentration in plasma and BAL should be preferred. Yet BAL presents a number of potential drawbacks (59) but has been used to characterize CMS and colistin intrapulmonary disposition by several groups including ours (12, 13, 54). Administering colistin as a prodrug (CMS) that must be converted into the active moiety pre-systemically after nebulization, but which is also converted systemically, complicates the pharmacokinetic analysis (12). Relatively complex PK models with several compartments (13) or non-linear transfers (54) had to be developed in order to describe intrapulmonary colistin disposition in rats. Yet for practical reasons BAL was not done in monkeys and ELF concentrations were simulated instead of being determined experimentally, using CMS and colistin plasma concentrations determined after CMS nebulization and intravenous administration. Subcutaneous injection of colistin was useful to estimate its clearance, the fraction of CMS converted in colistin, and then the bioavailability after nebulization. Ideally colistin should have been administered IV but because of a potential toxicity even after a low dose, sub-cutaneous administration was preferred under the assumption that bioavailability would then been complete as previously considered (12). The final model used for simulating colistin ELF concentrations in baboons (Fig. 5) is simpler than model previously described in rats (13) and also derived from experimental data, which may constitute a limit for the interpretation of the results. Other limits such as the use of healthy animals would prevent direct extrapolation to the clinics since in particular biofilm formation in CF patients may have an effect on CMS and colistin absorption after nebulization. In these patients sputum and nor ELF concentrations are usually

reported (20). Simulated intrapulmonary concentrations of colistin in baboons should be considered with great caution in absolute terms and should not be used to predict efficacy. Yet they are informative in relative terms and allow comparisons between nebulizers and between routes of administration.

The simulated CMS and colistin concentrations appear to be higher when CMS is nebulized as compared to the IV administration of CMS or the SC administration of colistin. Also, the nebulizer Pari LC star® system leads to higher CMS and colistin in ELF than Eflow rapid®.

The concept of bioavailability is quite complex and may be misleading when a pro-drug is administered to deliver high local pre-systemic concentrations of the active moiety to increase efficacy and low systemic concentrations to decrease toxicity. It should therefore be reminded that f_{nebu} terms reported in Table II correspond to the fractions of CMS reaching the ELF compartment, with values consistent with gamma imaging data as already discussed. But interestingly the model predicts that whatever the nebulizer, typically 44% of the CMS that reaches the ELF compartment is converted into colistin pre-systemically (k_{hyd_ELF}) to provide antimicrobial efficacy (Table II), which is relatively close to the average value (39%) obtained by non-compartmental analysis of data, including ELF concentrations, obtained after nebulization in rats (12). Noticeably the model also predicts that 96.4% of the CMS is converted into colistin at a systemic level, which is different with values reported in rats (between 6.8 and 12.5%) (12, 60, 61) and human 30% (62). But by multiplying the f_{nebu} terms by 44%, the fraction of CMS that is converted into colistin pre-systemically, one can estimate the fraction of the CMS dose nebulized that becomes eventually available as colistin within the ELF compartment, with values equal to 1% with Pari LC Star® and 0.4% with Eflow rapid®. The relative colistin ELF exposure after nebulization of CMS with Pari LC Star® and Eflow® (characterized by area under the ELF concentrations-time curve; AUC_{ELF}) which is likely to be related to antimicrobial efficacy (63), is respectively equal to 6238 and 2014 mg.h/L, which is 296 and 96 times higher than colistin ELF exposure after intravenous administration of CMS (21 mg.h/L). The PK model predicts that the systemic colistin exposure (AUC_{syst}) would be respectively equal to 0.551 and 0.082 mg.h/L after nebulization with Pari LC Star® and Eflow rapid® which is respectively 25 and 170 folds lower than after colistin plasma exposure after CMS IV administration (13 mg.h/L).

CONCLUSION

In conclusion, the comparison between two nebulizers in their ability to deliver an antibiotic within the lung for the treatment of pulmonary infections has been addressed for the first time, at least to our knowledge, by using two different and complementary approaches. It appeared that gamma camera imaging of nebulized 99mTc-DPTA and PK modelling from measured systemic colistin concentrations, lead to similar results.

Although only a relatively limited fraction of the nebulized dose of CMS can reach the infected sites within the lung, measured plasma and estimated ELF concentrations of this prodrug and its active moiety colistin, are likely to be higher after nebulization with the Pari LC Star® than with the Eflow Rapid® sytem.

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