Aerosol Therapy with Colistin Methanesulfonate: a Biopharmaceutical Issue Illustrated in Rats

Sandrine Marchand,1,2,3 Patrice Gobin,1,3 Julien Brillault,1,2 Sara Baptista,1,2 Christophe Adier,1,3 Jean-Christophe Olivier,1,2 Olivier Mimoz,1,2,4 and William Couet1,2,3,*

INSERM ERI-23, 40 Avenue du Recteur Pineau, 86000 Poitiers, France; Université de Poitiers, Faculté de Médecine et de Pharmacie, 6 Rue de la Milétrie, 86000 Poitiers, France; CHU de Poitiers, Laboratoire de Toxicologie et de Pharmacocinétique, 2 Rue de la Milétrie, 86000 Poitiers, France; and CHU de Poitiers, Service de Rénovation Chirurgicale, 2 Rue de la Milétrie, 86000 Poitiers, France

Received 27 March 2010/Returned for modification 4 May 2010/Accepted 8 June 2010

The aim of this study was to evaluate the biopharmaceutical behavior of colistin methanesulfonate (CMS) with special focus on colistin presystemic formation after CMS nebulization in rats. CMS was administered (15 mg · kg−1 of body weight) either intravenously for systemic pharmacokinetic studies (n = 6) or as an intratracheal nebulization for systemic pharmacokinetic studies (n = 5) or for CMS and colistin concentration measurements in epithelial lining fluid (ELF) at 30, 120, and 240 min after nebulization (n = 14). CMS and colistin concentrations were determined by a new liquid chromatography (LC)-tandem mass spectrometry (MS/MS) assay. Pharmacokinetic parameters were estimated by noncompartmental analysis. CMS and colistin pharmacokinetic data were consistent with previously published values when comparisons were possible. The fraction of the CMS dose converted systematically into colistin after intravenous CMS administration was estimated to be 12.5% on average. After CMS nebulization it was estimated that about two-thirds of the dose was directly absorbed within the systemic circulation, whereas one-third was first converted into active colistin, which was eventually absorbed. As a consequence, the colistin area under curve (AUC) reflecting systemic availability was about 4-fold greater after CMS intratracheal nebulization (607 ± 240 μg · min · ml−1) than after CMS intravenous administration (160 ± 20 μg · min · ml−1). CMS concentrations in ELF at 30 min and 120 min postnebulization were very high (in the order of several mg/ml) due to the limited volume of ELF but were considerably reduced at 240 min. Although lower (15% ± 5% at 120 min) in relative terms, colistin concentrations in ELF could be high enough for being active against microorganisms following CMS nebulization.

In the last decade aerosol administration of antibiotics has gained increasing attention for the treatment of pulmonary infections (9). The main advantage with this route of administration is that high local concentrations and, therefore, efficient antibacterial killing can be obtained while minimizing systemic exposure and, therefore, toxicity (13). Among the various antibiotics, colistin presents several major characteristics as an ideal candidate for aerosol delivery. After being introduced on the market more than 5 decades ago, it has been practically abandoned because of reports of nephrotoxicity and neurotoxicity (10). However, it often presents bactericidal activity against multiresistant Gram-negative bacilli, including Pseudomonas aeruginosa, frequently associated with chronic pulmonary infection in cystic fibrosis patients. Therefore, inhaled colistin has been used successfully for over 20 years to prevent and cure pulmonary infections due to P. aeruginosa in patients with cystic fibrosis (29). Aerosolized colistin in combination with oral ciprofloxacin is a reference antimicrobial treatment of nosocomial pneumonia (2, 11, 12, 23, 26) or ventilator-associated pneumonia (VAP) (18, 25) due to multidrug-resistant Gram-negative bacteria such as P. aeruginosa and Acinetobacter baumannii. Colistin is a multicomponent cationic polypeptide constituted mainly by colistin A (polymyxin E1) and colistin B (polymyxin E2), which is used for parenteral administration and inhalation as colistin methanesulfonate (CMS), acting as a prodrug that is less toxic than colistin but with no antimicrobial activity (1). Therefore, in order to be active, CMS must be converted into colistin within the lung before leaving the pulmonary tract by mucociliary clearance mechanisms with expectoration or swallowing of pulmonary secretions or by systemic absorption (9). The objective of this study was to address these various issues in rats.

MATERIALS AND METHODS

Chemicals. Colistimethate sodium (Colymicine, 1 million IU; Sanofi-Aventis, Paris, France) was provided by the pharmacy of the Poitiers University Hospital and was used to prepare CMS solutions in 0.9% and 0.4% NaCl for intravenous (i.v.) administration and nebulization, respectively. 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.

Calu-3 cell culture experiments. Transport experiments were conducted on Calu-3 cells in an apical-to-basolateral (AP-BL) direction, as previously described (3, 4), at CMS or colistin concentrations equivalent to 100 μg/ml of colistin base. Apparent permeability (Papp) values were obtained according to equation 1:

\[ P_{\text{app}} = \frac{V_{\text{A}}}{C_{\text{in}} - C_{\text{out}}} \]

where \( V_{\text{A}} \) is the apical-to-basolateral flux, \( C_{\text{in}} \) is the concentration at the apical side, and \( C_{\text{out}} \) is the concentration at the basolateral side.

\[ \text{CMS} + \text{H}_{2}\text{O} \rightleftharpoons \text{Colistin} + \text{H}_{2}\text{O} \]

The equilibrium constant for the formation of CMS is 2.15 × 10^11, and the extinction coefficient of CMS is 495 M^−1 · cm^−1 at 510 nm. Colistin concentrations were determined by a new liquid chromatography (LC)-tandem mass spectrometry (MS/MS) assay. Pharmacokinetic parameters were estimated by noncompartmental analysis. CMS and colistin pharmacokinetic data were consistent with previously published values when comparisons were possible.
P_{\text{app}} = \frac{Q}{T \times A \times C_0}

where $Q$ is the amount of drug in $\mu g$ that appeared into the acceptor compartment, $T$ is the incubation time of 6 h, $A$ is the semipermeable membrane surface area of 4.67 cm$^2$, and $C_0$ is the initial antibiotic concentration in the donor compartment expressed in $\mu g/cm^2$. The experiment was also conducted with monoflexin as a comparator.

Animals. With animals was carried out in accordance with the Principles of Laboratory Animal Care (26a). Male Sprague-Dawley rats ($n = 31$) from Janvier Laboratories (Le Genest-St-Ise, France), weighing between 280 and 350 g, were used for these investigations. Animals were acclimatized in wire cages in a 12:12-light-dark cycle for a minimum of 5 days before the beginning of the experiment. During this period, they had free access to food (A03, Safe-Villenmoulin-sur-Orge, France) and water.

Implantation of femoral vein and artery catheters. For CMS and colistin plasma pharmacokinetic studies, venous and arterial catheters were implanted the day before the experiment for drug administration and plasma collection, respectively. Rats were anesthetized by isoflurane inhalation (Forene; Abbot, Rungis, France). They were placed into a hermetic enclosure, which was supplied with an air-isoflurane mixture (3.9%) at a flow rate of 500 ml/min (Anesthe- sia Unity, Univerit400, Phymep, Paris, France). When animals were asleep, a mask was placed onto the muzzle, and the concentration of isoflurane was decreased to 1.5 to 2% during the surgery. The animals were then placed in the dorsal position with the tail toward the experimenter. Polyethylene catheters constituted with the connection of a small-diameter catheter (inner diameter of 0.26 mm and outer diameter of 0.61 mm; Phymep, Paris, France) with a larger catheter (inner diameter of 0.58 mm and outer diameter of 0.96 mm; Harvard, Les Ulis, France) were implanted into the left femoral vein for rats in the renal group and in the left femoral vein and artery for rats in the control group. Following surgery, the rats were kept under a heating lamp, and after the first signs of movement, the animals were placed into individual cages. Food was withdrawn 12 h before the experiment, but the animals had free access to water until drug administration.

CMS and colistin administrations and sample collection for plasma pharmacokinetics studies. (i) Intravenous administration of CMS ($n = 6$). The iv. bolus administration of CMS (15 mg · kg$^{-1}$ of body weight) was performed via the left femoral vein. Arterial blood samples were collected at 0, 5, 15, 30, 60, 90, 120, and 150 min postdosing via the left femoral artery catheter. Plasma was separated by centrifugation and frozen at −20°C until analysis.

(ii) Subcutaneous administration of colistin ($n = 6$). Subcutaneous adminis- tration of colistin (1.5 mg · kg$^{-1}$) was performed in the right hind leg of the animals. Arterial blood samples were collected at 0, 5, 15, 30, 60, 120, 180, 240, and 300 min postdosing via the left femoral artery catheter. Plasma was handled as described above.

(iii) Intratracheal nebulization with CMS ($n = 5$). A solution of CMS (50 mg · ml$^{-1}$) was first prepared in 0.4% NaCl, corresponding to an osmolarity ranging between 280 and 300 mosmL · kg$^{-1}$, and then split into aliquots, which were nebulized from −20°C until the day of the experiment. The CMS dose was 15 mg · kg$^{-1}$, corresponding approximately to a volume of 100 μL. A MicroSprayer IA-1B system (Penn Century Inc., Philadelphia, PA), composed of an angled stainless steel tube mounted onto a syringe with an atomizer at the tip, was used for nebulization (5). Rats were anesthetized by isoflurane inhalation as described above and maintained by the upper incisors on a rodent work stand inclined at an angle of 45° (Tem, Lormont, France). Vocal cords were visualized with the help of an otoscope. The tube of the MicroSprayer IA-1B system was inclined at an angle of 45° (Tem, Lormont, France). The mobile phase was 0.1% (vol/vol) formic acid in acetonitrile–0.1% formic acid in water (20:80, vol/vol). The LC-MS/MS system consisted of a Waters Alliance 2695 separation module equipped with a binary pump, an autosampler thermostated at 4°C, and a Waters Micromass Quattro Micro API tandem mass spectrometer. The mass spectrometer was operated in the positive-ion mode. Ions were analyzed by multiple-reaction monitoring (MRM). Transition ions were m/z 585.5/101.2 for colistin A, 575.8/ 101.2 for colistin B, and 602.5/241.2 for polymyxin B1, the internal standard. Seven-point calibration standard curves for CMS and colistin concentrations in rat plasma between 20 and 0.078 μg · ml$^{-1}$ and three levels of control (0.156, 0.625, and 2.5 μg · ml$^{-1}$) were prepared. For colistin, calibration standards, controls, and samples, 25 μL of internal standard solution (6.25 mg · ml$^{-1}$) and 450 μL of water were added to 50 μL of spiked plasma or samples. Mixtures were loaded onto Oasis HLC 1-ml columns (Waters, St-Quentin en Yvelines, France). After washing (0.5 mL methanol–0.5 mL water–0.5 mL water), analytes were eluted with 1 mL of 0.5% (vol/vol) formic acid in methanol. Eluates were then evaporated under a nitrogen stream at 45°C and redissolved in 200 μL of 0.1% (vol/vol) formic acid in water. CMS was analyzed indirectly by hydrolyzing CMS to colistin (0.5 M sulfuric acid during 1 h) before purification. Samples with higher concentrations outside the calibration curve were appropriately diluted with drug-free rat plasma. The between-day variabilities for colistin and CMS were character- ized at the three levels of concentrations ($n = 10$), with a precision and accuracy of less than 15%.

(ii) Analysis of CMS and colistin in BAL fluid. For CMS and colistin, six-point calibration standard curves with concentrations between 20 and 0.078 μg · ml$^{-1}$ in plasma and three levels of control (0.312, 1.25, and 5 μg · ml$^{-1}$) were done. Ten to fifty microliters of BAL fluid samples was mixed with 200 μL of plasma, 25 μL of internal standard solution (6.25 mg · ml$^{-1}$), and water up to 1 mL. Mixtures were loaded onto Oasis HLC 1-ml columns (Waters, St-Quentin en Yvelines, France). After washing (0.5 mL methanol–0.5 mL water–0.5 mL water), analytes were eluted with 1 mL of 0.5% (vol/vol) formic acid in methanol. Eluates were then evaporated under a nitrogen stream at 45°C and redissolved in 200 μL of 0.1% (vol/vol) formic acid in water. CMS was analyzed indirectly by hydrolyzing CMS to colistin (0.5 M sulfuric acid during 1 h) before purification. The between-day variabilities for colistin and CMS were characterized at the three levels of concentrations ($n = 10$), with a precision and accuracy of less than 15%.

(iii) Analysis of urea in BAL fluid and plasma. Urea concentrations in BAL fluid were determined by LC-MS/MS. The mass spectrometer was operated in the positive-ion mode. Ions were analyzed by MRM. Mass pair detection for urea was at m/z 61/44. Reversed-phase chromatography was performed with a C18 Xterra MS column (5.0 μm, 150- by 4.6-mm ID; Waters, St-Quentin en Yvelines, France). The mobile phase was 0.1% (vol/vol) formic acid in acetonitrile–0.1% (vol/vol) formic acid in water (10:90, vol/vol). The LC-MS/MS conditions for CMS and colistin were performed with 0.9% NaCl at concentrations of 100 and 1.25 μg · ml$^{-1}$. Briefly, 10 μL of standard solutions or BAL fluid samples was diluted in 190 μL of water (1/20 dilution) and directly injected (30 μL). Four levels of control (75, 25, 2.5, and 1.25 μg · ml$^{-1}$) were done. The limit of quantification (LOQ) for the determination of the urea concentration in BAL fluid was estimated at 1.25 μg/mL. Intra- and inter-day variabilities were charac- terized at these four levels of concentrations, with precisions and accuracies of 15% for 75%, 25-, and 2.5-μg/ml concentrations and 20% for the LOQ. Urea concentrations in plasma were evaluated by photometric detection using an automatic analyzer (Modular automatic analyzer: Roche, France).

Determination of CMS and colistin concentrations in ELF. Actual ELF concentra- tions of CMS or colistin (C$_{\text{ELF}}$) were obtained from measured BAL fluid concentrations after correction for dilution, according to equation 2:

$$C_{\text{ELF}} = C_{\text{BAL}} \times \frac{\text{Urea}_{\text{BAL}} - \text{Urea}_{\text{ELF}}}{\text{Urea}_{\text{ELF}} - \text{Urea}_{\text{CSF}}}(2)$$

where $C_{\text{BAL}}$ corresponds to the CMS or colistin concentration measured in BAL fluid and $\text{Urea}_{\text{ELF}}$ and $\text{Urea}_{\text{CSF}}$ correspond to the concentrations of urea determined in BAL fluid and plasma, respectively.

The ELF volume was estimated from the aspirated BAL fluid volume after correction for dilution, according to equation 3 (17):

$$V_{\text{ELF}} = V_{\text{BAL}} \times \frac{\text{Urea}_{\text{ELF}} - \text{Urea}_{\text{CSF}}}{\text{Urea}_{\text{BAL}} - \text{Urea}_{\text{CSF}}}(3)$$

where $V_{\text{BAL}}$ corresponds to the aspirated BAL fluid volume.
The average fraction of CMS converted systematically into colistin after i.v. administration ($f_{m,syst}$) was estimated from equation 5:

$$f_{m,syst} = \frac{\text{AUC}_{coli} \times CL_{coli}}{\text{AUC}_{CMS} \times CL_{CMS}}$$  \hspace{1cm} (5)$$

After CMS intratracheal nebulization, a fraction of the dose is absorbed systematically ($F_{CMS,neb}$), another one is converted locally to colistin ($f_{m,lung}$), and the remaining $(1 - F_{CMS,neb} - f_{m,lung})$ is excreted by mucociliary clearance with expectoration or swallowing of pulmonary secretions (Fig. 1). Therefore, colistin appearing in plasma after intratracheal administration was formed either systematically from CMS ($f_{m,lung}$) or locally in the lung before being absorbed ($F_{coli,lung}$). Accordingly, the colistin AUC following the intratracheal nebulization of CMS can be estimated from equation 6:

$$\text{AUC}_{coli,neb} = \left[ \frac{\text{Dose}_{CMS,neb} \times (F_{CMS,neb} \times f_{m,lung})}{\text{CL}_{coli}} \right] + \left[ \frac{\text{Dose}_{CMS,neb} \times (f_{m,lung} \times F_{coli,lung})}{\text{CL}_{coli}} \right]$$  \hspace{1cm} (6)$$

By rearranging equation 3, the fraction of the nebulized dose of CMS eventually bioavailable as colistin can be estimated as follows (equation 7):

$$f_{m,neb} \times F_{coli,lung} = \left[ \frac{\text{AUC}_{coli,neb} \times CL_{coli}}{\text{Dose}_{CMS,neb}} \right] - F_{CMS,neb} \times f_{m,syst}$$  \hspace{1cm} (7)$$

where $F_{coli,lung}$ corresponds to the fraction of colistin that is absorbed systematically after being formed from CMS (Fig. 1).

CMS and colistin concentrations expressed in moles were used for the estimations of $f_{m,neb}$ and $f_{m,lung}$. Results are expressed as means ± standard deviations (SD).

**RESULTS**

The apparent permeabilities ($P_{app}$) through Calu-3 cells were estimated to be 0.042 ± 0.020 cm · s⁻¹ for colistin and 0.058 ± 0.008 cm · s⁻¹ for CMS, which is about 150-fold lower than the value found (8.86 ± 0.13 cm · s⁻¹) for moxifloxacin.

CMS and colistin plasma concentrations after intravenous administration of CMS are shown in Fig. 2a. The CMS peak measured 5 min after its bolus administration was equal to 44.1 ± 10.6 μg · ml⁻¹. The colistin peak was already reached at the first sampling time (5 min) and was equal to 3.5 ± 0.6 μg · ml⁻¹. CMS concentrations were always higher but de-

![Nebulized dose](image)

**FIG. 1.** Representation of CMS behavior after intratracheal nebulization. A fraction of the CMS dose is absorbed systematically ($F_{CMS,neb}$), which is then partially converted into colistin within the systemic circulation ($f_{m,lung}$). Another fraction of the CMS dose is converted into colistin presystematically ($f_{m,syst}$), which is then partially absorbed within the systemic circulation ($F_{coli,lung}$).

**Pharmacokinetic analysis.** Pharmacokinetic parameters were determined for each individual rat by a noncompartmental approach according to standard procedures and with WinNonLin software, version 3.3 (Pharsight Corporation, Mountain View, CA). Areas under plasma concentration-versus-time curves (AUCs) were calculated by using the linear trapezoidal rule. The area remaining after the last measured concentration ($C_{last}$) was determined from $C_{last}/ke$. The rate constant, $ke$, and the corresponding half-lives ($t_{1/2}$) were estimated by a least-squares fit of data points (log concentration time) in the terminal phase of the decline. The total body clearance of CMS ($CL_{CMS}$) was calculated as the ratio between the intravenous dose of CMS ($Dose_{CMS,i.v.}$) and the corresponding AUC ($\text{AUC}_{CMS,i.v.}$). The CMS steady-state volume of distribution ($V_{ss,CMS}$) was obtained from the following equation: ($Dose_{CMS} \times \text{AUMC}_{CMS,i.v.})/(\text{AUC}_{CMS,i.v.})$ 2, where AUMC CMS,i.v. was the total area under the first moment curve calculated after i.v. bolus administration of CMS. The total body clearance of colistin ($CL_{coli}$) was calculated as the ratio between the subcutaneous dose of colistin ($Dose_{coli}$) and the corresponding AUC ($\text{AUC}_{coli}$), assuming complete bioavailability after subcutaneous administration.

The hypothesized behavior of CMS following intratracheal nebulization is summarized in Fig. 1. The corresponding mean estimate of CMS bioavailability ($F_{CMS,neb}$) was obtained from the ratio between the mean CMS area under the curve after intratracheal nebulization ($\text{AUC}_{CMS,neb}$) and i.v. administration ($\text{AUC}_{CMS,i.v.}$) (equation 4):

$$F_{CMS,neb} = \frac{\text{AUC}_{CMS,neb}}{\text{AUC}_{CMS,i.v.}}$$  \hspace{1cm} (4)$$

![Mean total plasma concentration-versus-time profiles](image)

**FIG. 2.** Mean total plasma concentration-versus-time profiles (±SD) for CMS and/or colistin after i.v. administration of CMS (15 mg · kg⁻¹; $n = 6$) (a), intratracheal nebulization of CMS (15 mg · kg⁻¹; $n = 5$) (b), and subcutaneous administration of colistin (1.5 mg · kg⁻¹; $n = 6$) (c).
TABLE 1. Values of pharmacokinetic parameters for CMS and colistin in i.v. and nebulized groups after a CMS dose of 15 mg · kg⁻¹ in each group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD (min)</th>
<th>Mean ± SD (µg · ml⁻¹ · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>i.v.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL₁/₂,CMS (ml · min⁻¹ · kg⁻¹)</td>
<td>14.6 ± 3.6</td>
<td>1.079 ± 268</td>
</tr>
<tr>
<td>V₄₆,CMS (ml · kg⁻¹)</td>
<td>330 ± 75</td>
<td>160 ± 20</td>
</tr>
<tr>
<td>t₁/₂,CMS (min)</td>
<td>22.0 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>t₁/₂,coli (min)</td>
<td>35.5 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>AUCCMS (µg · ml⁻¹ · min)</td>
<td>5.6 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>AUCCMS,i.v. (µg · ml⁻¹ · min)</td>
<td>210 ± 56</td>
<td></td>
</tr>
<tr>
<td>AUCCMS,neb (µg · ml⁻¹ · min)</td>
<td>607 ± 240</td>
<td></td>
</tr>
</tbody>
</table>

**Nebulized**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD (min)</th>
<th>Mean ± SD (µg · ml⁻¹ · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁/₂,CMS,neb (min)</td>
<td>21.0 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>t₁/₂,coli,neb (min)</td>
<td>63.6 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>AUCCMS,neb (µg · ml⁻¹ · min)</td>
<td>756 ± 210</td>
<td></td>
</tr>
<tr>
<td>AUCCMS,neb (µg · ml⁻¹ · min)</td>
<td>607 ± 240</td>
<td></td>
</tr>
</tbody>
</table>

cayed more rapidly than those of colistin. Accordingly, the colistin elimination half-life (t₁/₂,coli = 35.5 ± 5.6 min) was longer than that of CMS (t₁/₂,CMS = 22.0 ± 3.4 min). Other pharmacokinetic parameters are presented in Table 1.

Concentrations of CMS and colistin after intratracheal nebulization of CMS are presented in Fig. 2b. Between rats, variability was higher after nebulization than after i.v. administration. Maximum concentrations of CMS were much lower than after i.v. bolus administration at the same dose (Cₘₐₓ,CMS,neb = 5.6 ± 1.1 µg · ml⁻¹). CMS concentrations peaked early and were then sustained at a fairly constant value for 2 to 3 h postdosing. A decay was eventually observed, with a half-life (t₁/₂,CMS) equal to 21.0 ± 7.7 min. Colistin concentrations increased gradually after CMS nebulization to reach a peak concentration equal to 3.5 ± 1.2 µg · ml⁻¹ after 163 ± 41 min on average, followed by a decay of concentrations, with a half-life (t₁/₂,coli) estimated to be 63.6 ± 8.2 min. The elimination half-life was estimated from at least three data points, and extrapolated CMS and colistin AUCs were always less than 20%. Other pharmacokinetic parameters are presented in Table 1. CMS bioavailability after intratracheal nebulization estimated from equation 1 (Fₘₐₓ,CMS,lung) was equal to 70% on average, and the mean colistin AUC after CMS nebulization was on average 4-fold higher than that after i.v. administration at the same dose (607 ± 240 versus 160 ± 20 µg · min · ml⁻¹).

The fraction of CMS converted into colistin in systemic circulation (fₘₐₓ,lung) was estimated to be 12.5% on average, and the fraction of the nebulized dose of CMS that was converted locally into colistin before being absorbed systematically as such (equal to the product fₘₐₓ,lung × Fₙₜ,lung) was estimated to be 39%.

Mean concentrations of colistin after subcutaneous administration at a dose of 1.5 mg · kg⁻¹ are presented on Fig. 2c. Corresponding pharmacokinetic parameters are listed in Table 2.

Mean urea plasma and BAL fluid concentrations were estimated to be 275 ± 41 and 5.5 ± 2.9 µg/ml (n = 14), respectively, corresponding to a dilution factor of 63 ± 31 (F₉₅,CMS,lung) (Fig. 3). However, colistin ELF concentrations were several-fold lower than those of CMS, with the highest concentration ratio (15% ± 5%) observed at 120 min. A value of 7.6 ± 5.8 µl was obtained as an ELF volume estimate.

**DISCUSSION**

Assessment of antibiotic concentrations in lung is a difficult task; there is no ideal experimental setting for that (17), and results previously obtained with colistin (29) have been suspected of being biased in particular due to analytical difficulties (22). Therefore, a thorough analysis of plasma data after CMS or colistin administrations through various routes may provide useful information, in particular to estimate the fraction of the CMS dose converted into active colistin within the lung after intratracheal nebulization.

The fraction of CMS converted into colistin within the lung after intratracheal nebulization can be assessed by comparing AUCs of CMS and colistin after intratracheal and i.v. administrations. In fact, CMS conversion into colistin within the lung would have the same pharmacokinetic consequences as a first-pass effect after oral administration. In particular, in the case of a significant conversion of CMS within the lung, the colistin AUC and the colistin-to-CMS AUC ratio should be higher after intratracheal than after intravenous administration. However, in order to estimate the fraction of CMS converted locally into colistin and then absorbed, it was first necessary to determine the systemic clearance of colistin. This parameter was previously estimated to be 5.2 ± 0.4 ml · min⁻¹ · kg⁻¹ in rats after i.v. administration of 1 mg · kg⁻¹ (21). However, it was reassessed in the present study, except that in order to minimize rat discomfort and increase tolerability, colistin was administered subcutaneously at a dose of 1.5 mg · kg⁻¹. Assuming complete bioavailability, an estimate of colistin clearance equal to 8.5 ± 1.0 ml · min⁻¹ · kg⁻¹ was then obtained, consistent with previous data (15). Other observations were also

![FIG. 3. Mean CMS and colistin concentrations (±SD) in ELF 30, 120, and 240 min after intratracheal nebulization of CMS (15 mg · kg⁻¹; n = 3 to 5 per time group).](http://example.com/figure3.png)
consistent with those previously made by Li et al. (20). In particular, it was confirmed that colistin concentrations peaked very early after CMS administrations in rats (Fig. 2a), which is a major difference from what was observed for humans, where colistin plasma concentrations appeared to rise progressively after CMS injection (15, 28). Another interesting observation was that our estimate of the fraction of the dose of CMS converted into colistin ($f_{m,syst} = 12.5\%$) after i.v. administration of CMS was quite low. This confirms that in rats, only a small fraction of CMS is hydrolyzed systematically into colistin, as previously suggested by Li et al., who reported that only 6.8% of the CMS dose was converted into colistin (20), which is not ideal for a produg.

Most pharmacokinetic parameter values estimated in the present study were consistent with those previously reported for rats (20, 21) and are therefore probably reliable. However, because rats received only one treatment (i.e., administration or intratracheal nebulization of CMS or subcutaneous injection of colistin), key parameters such as $F_{CMS,lung}$ or $f_{m,syst}$ could not be estimated individually and then expressed as means ± SD but are reported simply as average values (see Materials and Methods). However, our data show that although CMS systemic absorption was relatively slow after intratracheal nebulization, as clearly indicated by the CMS concentration-versus-time profiles (Fig. 2b) and consistent with the low apparent permeability ($P_{app}$) through Calu-3 cells, a majority of the CMS dose nebulized with the MicroSprayer IA-1B system was directly absorbed, with an $F_{CMS,lung}$ value estimated to be 70% on average. A smaller fraction was first converted into colistin within the lung before being absorbed, as indicated by the product of $f_{m,lung} \times F_{coli,lung}$ estimated to be 39% on average. This 39% value was derived from $f_{m,syst}$ and $CL_{coli,c^{-}}$ (equation 7), and a small error made during the initial estimation of these parameters may have had a dramatic effect on the $f_{m,lung} \times F_{coli,lung}$ product estimate. However, added together, these estimated fractions of the nebulized CMS dose reaching the systemic circulation either directly ($F_{CMS,lung}$) or after local conversion into colistin ($f_{m,lung} \times F_{coli,lung}$) end up to be 109%, which is relatively close to the maximum theoretical value of 100%. It can therefore be concluded that after the intratracheal administration of CMS in rats using the MicroSprayer IA-1B system, the drug is totally absorbed either directly (for approximately two-thirds of the nebulized dose) or after presystemic conversion into colistin (for the remaining one-third of the dose). These results also indicate that within this experimental setting, the presystemically formed colistin is totally absorbed.

This behavior has major consequences for colistin systemic impregnation (AUC), which is almost 4-fold greater after CMS intratracheal nebulization (607 ± 240 μg·min·ml$^{-1}$) than after i.v. CMS administration (160 ± 20 μg·min·ml$^{-1}$). This spectacular result is made possible because the fraction of the CMS dose converted systematically into colistin in rats is quite low ($f_{m,syst} = 12.5\%$ on average), and therefore, the 39% of the nebulized dose of CMS reaching the systemic circulation after presystemic hydrolysis into colistin is responsible for a major increase in the colistin AUC in relative terms (39% versus 12.5%). Therefore, in that particular situation, local prodrug administration does not mean reduced systemic exposure to the active but also potentially toxic moiety.

However, having about one-third of the CMS dose converted into colistin within the lung does not guarantee high and active colistin concentrations in this tissue. Colistin ELF concentrations are determined by the relative rates of colistin appearance (from CMS hydrolysis) and disappearance (absorption). Relatively high local (ELF) concentrations of CMS were maintained during the initial 120 min following its nebulization (Fig. 3), which can be explained in part by a slow absorption (rate out), consistent with the molecule physicochemical characteristics and the low $P_{app}$ value estimated for Calu-3 cells. However, this should also be due to slow hydrolysis into colistin (rate in). Although the exact mechanism for in vivo CMS conversion into colistin is not precisely known, CMS should be relatively stable in biological fluids; for example, it takes about 12 h to convert approximately 50% of the CMS into colistin at 37°C in human plasma (19). This relative stability of CMS also explains the low $f_{m,syst}$ values estimated for rats. Therefore, probably both rate in and rate out are relatively slow. However, because colistin ELF concentrations were relatively low compared with those of CMS (15% ± 5% at the most at 120 min postnebulization), colistin formation should be the rate-limiting step.

The colistin concentrations in ELF should be low compared with those of CMS. However, because the ELF volume is limited, colistin concentrations may be sufficiently high to be active against microorganisms after CMS nebulization. We could not find any value for rat ELF volume in the literature, but our estimate of 7.6 ± 5.8 μl for a lung volume of 1.34 ml (27) seems to be relatively consistent with an ELF volume close to 1 ml for humans (6–8) for a lung volume close to 600 ml (27). Complementary experiments are urgently needed to confirm these initial observations of patients or at least larger animal species allowing CMS nebulization with the same devices as those used for patients.

Indeed, it is obvious that these results obtained after the intratracheal administration of CMS using the MicroSprayer IA-1B system in rats cannot be extrapolated directly to the clinical setting, where patients are treated with sophisticated nebulizer systems. With the MicroSprayer IA-1B system, the liquid is pushed through the system with a syringe, resulting in the formation of an aerosol of droplets with a diameter of 25 to 30 μm (5), which may not be comparable to nebulization with systems used for patients. Furthermore, using this system, we have observed that 100% of the nebulized dose of CMS should eventually reach the systemic circulation, either directly or after being converted to colistin. However, in clinical practice, a nonnegligible fraction of the nebulized dose could leave the respiratory tract by mucociliary clearance mechanisms with expectoration or swallowing of pulmonary secretions before being absorbed. Another potential limitation of this study conducted with rats is that although the in vivo mechanism for CMS conversion into colistin is not known precisely, this hydrolysis is likely to be different in rats and humans, since as previously mentioned, the colistin plasma concentration peaks early after CMS injection in rats (Fig. 1a) but not in humans (15, 28).

In conclusion, this study demonstrates that the intratracheal nebulization of CMS may lead to much higher (4-fold) systemic concentrations of colistin than i.v. injection at the same dose and that although colistin formation in lung is likely to be
quite slow, sufficiently high concentrations of this active moiety should be obtained after CMS nebulization due to the limited ELF volume. Complementary experiments are therefore necessary to complete these data under conditions that could be better extrapolated to the clinical setting.

ACKNOWLEDGMENTS

We thank Pharsight Corporation for the free supply of WinNonLin throughout Pharsight’s Academic Licensing (PAL) program and Sophie Magreault for her assistance in this study.

REFERENCES


AEROSOL THERAPY WITH COLISTIN IN RATS 3707

Downloaded from http://aac.asm.org/ on November 11, 2017 by guest