Dose-ranging pharmacokinetics of colistin methanesulphonate (CMS) and colistin in rats following single intravenous CMS doses

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Objectives: The aim of this study was to evaluate the effect of colistin methanesulphonate (CMS) dose on CMS and colistin pharmacokinetics in rats.

Methods: Three rats per group received an intravenous bolus of CMS at a dose of 5, 15, 30, 60 or 120 mg/kg. Arterial blood samples were drawn at 0, 5, 15, 30, 60, 90, 120, 150 and 180 min. CMS and colistin plasma concentrations were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The pharmacokinetic parameters of CMS and colistin were calculated by non-compartmental analysis.

Results: Linear relationships were observed between CMS and colistin AUCs to infinity and CMS doses, as well as between CMS and colistin $C_{\text{max}}$ and CMS doses.

Conclusions: CMS and colistin pharmacokinetics were linear for a range of colistin concentrations covering the range of values encountered and recommended in patients even during treatment with higher doses.

Keywords: nosocomial infections, linearity, prodrug

Introduction

Colistin is a re-emerging agent due to the increased incidence of infections caused by multidrug-resistant Gram-negative pathogens and the absence of antimicrobial agents with activity against these bacteria.1,2 Although colistin has been commercially available since the 1960s, it was virtually abandoned because of its potential systemic toxicity including neurotoxicity and nephrotoxicity.3 Therefore although 50 years have elapsed since its discovery and introduction into clinical use, colistin has not been subjected to modern drug development processes, and in particular accurate pharmacokinetic data are missing.1 Colistin is a cationic polypeptide antibiotic corresponding to a complex mixture of at least 30 different components, with two major compounds, colistin A (polymyxin E1) and colistin B (polymyxin E2), differing only in their fatty acyl side chain, and colistin is actually administered as a prodrug, colistin methanesulphonate (CMS). Initial pharmacokinetic studies of colistin or CMS in animals were obtained by using microbiological methods, leading to inaccurate results because of uncontrolled conversion of CMS into colistin during incubation.3 Development of specific analytical methods was therefore a prerequisite for pharmacokinetic studies, and several methods including HPLC assays with fluorimetric detection following post-column derivatization,3 or more recently liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS),4,5 are now available.

The first pharmacokinetic study conducted in critically ill patients concluded that currently recommended doses of CMS lead to quite low colistin concentrations and should probably be increased to gain antimicrobial efficacy.6 It is therefore important to test the linearity of CMS and colistin pharmacokinetics over a large range of CMS doses. Although data obtained in rats may not predict what would happen in humans, we have decided to conduct this initial dose-ranging study in rats in order to investigate pharmacokinetic linearity in the largest possible range of doses administered and to determine which disposition step could be first affected by dose in humans.

Materials and methods

Animals

Experiments were done in accordance with Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985). Fifteen male Sprague–Dawley rats from Janvier Laboratories (Le Genest-St-Isle, France), weighing between 265 and 335 g, were divided into five
groups (n = 3) corresponding to CMS administration at different doses (5, 15, 30, 60 and 120 mg/kg). All animals were acclimatized in wire cages in a 12 h light–dark cycle for a minimum of 5 days before the beginning of the experiment to allow them to adjust to the new environment. During this period, they had free access to food (A03, Safe, Villemoisson-sur-Orge, France) and water. Ethical approval was obtained from the local Animals Ethics Committee.

Implantation of femoral vein and artery catheters
The day before the experiment, rats were anaesthetized by isoflurane inhalation (forene®, Abbot, Rungis, France) and equipped with vein and artery femoral catheters for CMS administration and blood sampling, respectively, as previously described.7

CMS intravenous bolus administrations for dose-ranging experiments
On the day of the experiment, rats received CMS as a short (20–30 s) intravenous infusion, called as a bolus, via the left femoral vein, at a dose of 5, 15, 30, 60 or 120 mg/kg. The CMS solution was freshly prepared by dissolving an adequate amount of CMS sodium salt [Colymicine®; 1 million international units (MIU) corresponding to 80 mg of CMS sodium salt; Sanofi-Aventis, Paris, France] in 0.9% NaCl. A 1 mL injection volume was chosen.

Sampling protocol for dose-ranging experiments
Blood samples (300–400 μL) were drawn at 0, 5, 15, 30, 60, 90, 120, 150 and 180 min via the left femoral artery. Plasma was separated immediately by centrifugation (3000 rpm, 10 min, 4°C) and frozen at −20°C until analysis, which was undertaken within 2 weeks.

LC-MS/MS analysis
Determination of colistin and CMS concentrations in plasma was performed by a new in-house LC-MS/MS method recently published.5 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). All chemicals used were of analytical grade and solvents were HPLC grade. The LC-MS/MS system consisted of a Waters Alliance 2695 separation module, equipped with a binary pump and 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 analysed by multiple reaction monitoring (MRM). Transition ions were m/z 585.5/1754 for colistin A, 578.5/101.2 for colistin B and 602.5/241.2 for polymyxin B1, the internal standard.

Plasma samples were assayed as soon as they had thawed. Seven-point calibration standard curves in rat plasma for CMS and colistin concentrations between 20 and 0.078 μg/mL and three levels of control (0.156, 0.625 and 2.5 μg/mL) were produced. For colistin, calibration standards, controls and samples, 25 μL of internal standard solution (6.25 μg/mL) and 450 μL of water were added to 50 μL of spiked plasma or samples. Mixtures were loaded onto SPE columns (Oasis® HLB, 1 mL, Waters). After washing, analytes were eluted with 1 mL of 0.1% (v/v) formic acid in methanol. Eluates were then evaporated under a nitrogen stream at 45°C and re-dissolved in 200 μL of 0.1% (v/v) formic acid in water. CMS was analysed indirectly by hydrolysing CMS to colistin (1 M sulphuric acid for 1 h) before pre-treatment. Samples with higher concentrations outside the calibration curve were appropriately diluted with drug-free rat plasma. CMS concentrations were determined by subtraction of the colistin molar concentrations measured before and after hydrolysis. The between-day variabilities for colistin and CMS were characterized at the three levels of concentrations with a precision and accuracy always <20%.

Non-compartmental pharmacokinetic analysis
Pharmacokinetic parameters were determined in each individual rat by a non-compartmental approach according to standard procedures and with the software WinNonLin version 3.3 (Pharsight Corporation, Mountain View, CA, USA). Total area under the plasma concentration versus time curves from zero to infinity for CMS and colistin (AUCcoli and AUCCMS) were calculated using the linear trapezoidal rule. The area remaining after the last measured concentration (Clast) was determined from Cull/Cull. The CMS and colistin rate constant k2 and corresponding half-lives (t1/2,CMS and t1/2,coli) were estimated by least squares fit of data points (log concentration time), in the terminal phase of the decline. Total body clearance of CMS (CLcoli) was calculated as the ratio between the intravenous dose of CMS (DoseCMS) and the corresponding AUCcoli. The CMS steady-state volume of distribution (VSS,CMS) was obtained from (DoseCMS×AUMCCMS/AUCCMS)2 where AUMCCMS is the total area under the first moment curve calculated after intravenous bolus administration of CMS. CMS mean residence time was estimated as MRTCMS = AUCCMS/AUMC CMS and colistin mean residence time was estimated as MRTcoli = (AUMCcoli/AUCcoli) − MRTCMS.

Treatment of data and statistics
Concentrations were expressed as means±SD. AUCCMS/AUCcoli and CMSS/CMS ratios were calculated on a molar basis. For both CMS and colistin, linear regressions were conducted between AUC and CMS doses and between concentrations obtained after 5 min of administration called CMSmax and CMS doses. Goodness of fit was estimated by coefficients of determination (r2). Between-dose comparisons were conducted on VSS,CMS, CLcoli, t1/2,CMS and MTRcoli. On t1/2,coli and MTRcoli on CMS and colistin AUCs arbitrarily normalized to the dose 15 mg/kg and on AUCCMS/AUCcoli and CMSS/CMS in CMSmax/CMS ratios. All these between-dose comparisons were done using the non-parametric Kruskal–Wallis test with a significance level at P<0.05. A Dunn’s multiple comparison test was performed thereafter when necessary (P<0.05). All statistics were conducted using GraphPad Prism version 5 for Windows.

Results
Mean ± SD CMS and colistin plasma concentration versus time curves following the various CMS doses are shown in Figure 1. Corresponding pharmacokinetic parameters are presented in Tables 1 and 2. The colistin peak was most often already reached at the first sampling time (5 min). Early (5 min) CMS concentrations (CMSmax/CMS) were higher than colistin concentrations (by ~20-fold on average), but decayed more rapidly in such a way that CMS and colistin concentrations became comparable after 150–180 min post-dosing (Figure 1). Accordingly, the CMS elimination half-life was shorter than the colistin half-life whatever the dose (Tables 1 and 2). Linear relationships were observed between AUCs and CMS doses, as well as between CMSmax and CMS doses, for both compounds (Figure 2). Accordingly, normalized AUCs and CMSmax of CMS and colistin did not vary significantly with the dose (Tables 1 and 2). No significant dose effect was observed on VSS,CMS and CLcoli on CMS or CMS and colistin half-lives (Tables 1 and 2).
Figure 1. Mean ± SD (n=3) total plasma concentration versus time profiles for CMS and colistin following intravenous administration of CMS at different doses; 5, 15, 30, 60 and 120 mg/kg.

Table 1. Pharmacokinetic parameters characteristic of CMS in rats following its intravenous administration at doses ranging between 5 and 120 mg/kg (mean ± SD, n=3 rats per dose)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>$C_{\text{max, CMS}}$ (µg/mL)</th>
<th>$V_{\text{SS, CMS}}$ (mL/kg)</th>
<th>$CL_{\text{CMS}}$ (mL/min/kg)</th>
<th>$t_{1/2, CMS}$ (min)</th>
<th>$\text{MRT}_{\text{CMS}}$ (min)</th>
<th>Normalized AUC$_{\text{CMS}}$ (µg·min/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>15.3 ± 4.1</td>
<td>301 ± 87</td>
<td>12.5 ± 3.7</td>
<td>25.2 ± 2.8</td>
<td>24.0 ± 0.3</td>
<td>2526 ± 687</td>
</tr>
<tr>
<td>15</td>
<td>38.6 ± 3.6</td>
<td>333 ± 42</td>
<td>12.9 ± 2.5</td>
<td>24.7 ± 3.7</td>
<td>26.1 ± 3.0</td>
<td>2383 ± 512</td>
</tr>
<tr>
<td>30</td>
<td>100.0 ± 18.2</td>
<td>224 ± 27</td>
<td>9.5 ± 0.8</td>
<td>20.6 ± 1.0</td>
<td>23.9 ± 1.5</td>
<td>3184 ± 297</td>
</tr>
<tr>
<td>60</td>
<td>175.0 ± 36.7</td>
<td>334 ± 32</td>
<td>14.9 ± 1.7</td>
<td>21.5 ± 0.5</td>
<td>22.5 ± 0.6</td>
<td>2032 ± 213</td>
</tr>
<tr>
<td>120</td>
<td>291.4 ± 59.6</td>
<td>380 ± 107</td>
<td>14.6 ± 4.9</td>
<td>25.5 ± 2.1</td>
<td>26.5 ± 3.6</td>
<td>2230 ± 816</td>
</tr>
</tbody>
</table>

*aNot significantly different between doses after Kruskal–Wallis test.
Discussion

CMS and colistin plasma concentrations versus time profiles were consistent with those of a previous study in rats after CMS intravenous bolus administration at a dose at 15 mg/kg. In particular, colistin elimination half-life ($t_{1/2,\text{coli}}$) was longer than that of CMS ($t_{1/2,\text{CMS}}$), suggesting that colistin elimination was not rate limited by its formation, i.e. CMS hydrolysis, which was confirmed by the estimated colistin MRT value. Most pharmacokinetic parameter values estimated in the present study are consistent with those previously published in rats after CMS administration. In the case of formation rate-limited elimination, MRT$_{\text{coli}}$ would be close to zero, but this was not the case since MRT$_{\text{coli}}$ was estimated to be between $17.6\pm 5.1$ and $36.8\pm 3.1$ min (Table 2). Yet colistin did not build-up extensively in the body as would be

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>$C_{\text{max,coli}}$ (µg/mL)</th>
<th>$t_{1/2,\text{coli}}$ (min)</th>
<th>MRT$_{\text{coli}}$ (min)</th>
<th>Normalized AUC$_{\text{coli}}$ (µg·min/mL)</th>
<th>$C_{\text{max,coli}}/C_{\text{max,CMS}}$</th>
<th>AUC$<em>{\text{coli}}$/AUC$</em>{\text{CMS}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.71±0.16</td>
<td>37.5±5.9</td>
<td>24.9±7.0</td>
<td>219±15</td>
<td>0.072±0.038</td>
<td>0.127±0.032</td>
</tr>
<tr>
<td>15</td>
<td>3.17±0.24</td>
<td>32.4±5.0</td>
<td>17.6±5.1$^c$</td>
<td>318±58</td>
<td>0.115±0.009</td>
<td>0.189±0.026</td>
</tr>
<tr>
<td>30</td>
<td>3.54±1.39</td>
<td>38.2±2.2</td>
<td>29.0±5.2</td>
<td>226±88</td>
<td>0.049±0.014</td>
<td>0.100±0.042</td>
</tr>
<tr>
<td>60</td>
<td>9.77±0.45</td>
<td>34.8±4.5</td>
<td>22.8±1.7$^c$</td>
<td>275±17</td>
<td>0.081±0.018</td>
<td>0.191±0.031</td>
</tr>
<tr>
<td>120</td>
<td>16.7±1.0</td>
<td>45.2±5.5</td>
<td>36.8±3.1$^c$</td>
<td>251±20</td>
<td>0.083±0.022</td>
<td>0.169±0.048</td>
</tr>
</tbody>
</table>

*Not significantly different between doses after Kruskal–Wallis test.

*Estimations of AUC and $C_{\text{max}}$ ratios were done on a molar basis.

*Significantly different between doses ($P<0.05$).
Colistin pharmacokinetic linearity in rats

expected when metabolite elimination is the slower step.\(^9\) Furthermore, in that situation most of the parent drug (CMS) would have been eliminated before the peak metabolite (colistin) level was reached,\(^9\) as we observed in humans\(^6\) but as was not the case in rats (Figure 1). Yet comparable profiles with early peak and longer elimination half-life for the metabolite have already been observed in the past, in particular with methylprednisolone and its water-soluble hemisuccinate ester administered intravenously as a bolus.\(^10\) In order to analyse these data one should consider the colistin to CMS AUC ratio, which is equal to the fraction of the CMS converted into colistin (fm) multiplied by the CMS to colistin clearance ratio (Eqn 1).\(^9\)

\[
\frac{AUC_{coli}}{AUC_{CMS}} = fm \times \left(\frac{CL_{CMS}}{CL_{coli}}\right)
\]

This area ratio was found to be independent of the dose of CMS administered and was estimated to be \(15.5 \pm 4.8\%\) on average in the 15 rats treated with CMS, independent of dose. This value would be difficult to interpret without information on fm, which, however, may vary with dose. Yet the absence of a dose effect on \(V_{1/2, coli}\) and MRT\(_{coli}\) most probably suggests that colistin clearance (CL\(_{coli}\) as well as its volume terms (\(V_P\) or \(V_{SS, coli}\)) should also be independent of dose. Then according to Eqn 1, since \(AUC_{coli}/AUC_{CMS}\) as well as CL\(_{CMS}\) and CL\(_{coli}\) did not vary with dose, one should conclude that fm is also dose independent. An estimate was previously obtained for this parameter in rats (fm=6.8%).\(^6\) The relatively low value of colistin AUC is therefore partly due to this low fm value. Furthermore, considering that \(AUC_{coli}/AUC_{CMS} (15.5 \pm 4.8\%)\) is roughly twice as high as fm (6.8%), one could conclude that colistin clearance should be about half that of CMS, and in turn responsible for the longer elimination half-life. This conclusion is consistent with previous estimates of colistin and CMS clearances, equal to 5.2±0.4 and 11.7±1.8 mL/min/kg, respectively.\(^3,8\) However, a more precise estimate of fm, conducted under the same experimental conditions as for other parameters, in particular using the same rats and the same analytical assay, would be required to confirm this conclusion.

CMS and colistin pharmacokinetics were linear in the range of doses investigated, corresponding to maximum colistin concentrations between <1 µg/mL for the lowest dose and >15 µg/mL for the highest dose (Figure 2). A mean steady-state colistin concentration of 2.3 µg/mL was recently reported in critically ill patients treated with CMS at a dose of 3 MIU (corresponding to 240 mg of CMS) every 8 h.\(^6\) Therefore, the range of concentrations most likely to be encountered in clinical practice should have been covered by this dose-ranging study. The lowest dose (5 mg/kg) was actually selected from analytical sensitivity constraints, and 120 mg/kg was the highest dose that could be safely administered to rats. Under these conditions no sign of toxicity or discomfort was observed whatever the dose administered.

The absence of signs of non-linearity observed during this study would favour safe use of CMS at doses higher than those currently used, as recently recommended by Plachouras et al.\(^6\) However, the important question remains as to whether the absence of non-linearity can be extrapolated to patients in a clinical setting. Two major issues should then be considered. One is that colistin disposition may differ between rats and humans as suggested by the early peak of colistin occurring in rats but not in humans. The other issue has to do with a possible effect of the disease on colistin pharmacokinetics, possibly due to altered protein binding. Colistin protein binding in rat spiked plasma at concentrations within the range of those encountered during this dose-ranging study (4–12 µg/mL) was found to be linear and roughly close to 65% on average.\(^3\) However, this plasma protein binding was shown to be highly concentration dependent in neutropenic infected mice, with fraction unbound values ranging from only 8% to 45% when total colistin concentrations increased from 0.97 to 30 µg/mL.\(^11\) Furthermore, colistin was reported to bind not only to albumin, but also to α1-glycoprotein, and therefore disease-related altered protein binding would not be surprising.\(^11\) Yet CMS protein binding is never mentioned as it would be difficult to determine, but this should also be considered since competition for protein binding between CMS and colistin should not be excluded. Therefore, complicating factors not considered during this study cannot be excluded in critically ill patients.

In conclusion, no sign of non-linearity could be observed in the pharmacokinetics of CMS or colistin in rats treated intravenously with CMS for a range of doses leading to colistin concentrations covering the likely range of values encountered in patients even during treatments with doses higher than those currently recommended. Yet complicating factors such as interspecies pharmacokinetic differences or an effect of disease state on CMS and/or colistin disposition cannot be excluded before extrapolating these data to the clinical setting.

Acknowledgements

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Transparency declarations

None to declare.

References

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