Pharmacokinetics of colistin methanesulphonate and colistin in rats following an intravenous dose of colistin methanesulphonate

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Objectives: To determine the disposition of colistin methanesulphonate (CMS) and colistin following intravenous (iv) administration of CMS in rats.

Methods: Five rats received a single iv bolus of 15 mg/kg CMS. Plasma concentrations of CMS and of colistin formed by the hydrolysis of CMS were determined by HPLC. The pharmacokinetic parameters of CMS and colistin were calculated using non-compartmental analysis.

Results: Total body clearance, volume of distribution at steady state and terminal half-life of CMS averaged 11.7 mL/min/kg, 299 mL/kg and 23.6 min, respectively. The mean terminal half-life of colistin was 55.7 min. Approximately 60% of the dose was eliminated via the urine in 24 h and presented as a mixture of CMS and colistin.

Conclusions: Colistin appeared in plasma soon after administration of CMS, indicating rapid conversion of CMS into colistin. CMS had a shorter terminal half-life than did colistin, indicating that the disposition of the colistin generated from CMS was rate-limited by its elimination. Most of the dose was recovered in urine, half in the form of colistin. The high percentage of colistin recovered in urine was believed to be formed by hydrolysis of CMS in the bladder and in the collection vessel, and/or conversion from CMS in the kidney.

Keywords: antibacterials, HPLC, Pseudomonas aeruginosa

Introduction

Colistin was the first antibiotic with significant in vitro activity against Pseudomonas aeruginosa and was released commercially in 1959.¹ There are two different forms of colistin available commercially: colistin sulphate used orally and topically, and sodium colistin methanesulphonate (CMS) used parenterally and by inhalation. CMS is less toxic and has fewer undesirable side effects than colistin. Unfortunately, CMS also has lower in vitro antibacterial potency.²⁻⁵ CMS has the potential to hydrolyse in aqueous solutions and form an extremely complex mixture of partially sulphomethylated derivatives plus colistin.⁶ The resultant solution exhibits increased antibacterial activity.⁴⁻⁵

Serious infections with the multidrug-resistant P. aeruginosa represent a significant clinical problem,⁷ particularly in immunocompromised hosts⁸ and patients with cystic fibrosis (CF).⁹ The availability of less toxic anti-pseudomonal antibiotics had relegated colistin to the status of a reserve agent. However, interest in its clinical value has been rekindled by the increasing prevalence of multidrug-resistant P. aeruginosa, and the advantages it offers of rapid bactericidal activity and slow development of resistance.¹⁰¹¹

Notwithstanding that CMS has been used for more than 40 years, its pharmacokinetics have not been studied comprehensively,¹² largely because of the lack of specific analytical methods. Almost all previous data on the pharmacokinetics of CMS were obtained by using microbiological assays to measure the concentrations of ‘CMS’ in plasma and urine.¹³⁻¹⁶ Unfortunately, microbiological assays lack the ability to differentiate CMS from colistin formed by hydrolysis. In a recent report of CMS pharmacokinetics in CF patients employing HPLC by Reed et al.,¹⁷ it is not clear which form was quantified—colistin, CMS or both; in addition, under the conditions described for sample preparation prior to HPLC, substantial in vitro hydrolysis of CMS to colistin may have occurred. Therefore, the limited pharmacokinetic information currently available on CMS

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is probably for a mixture of CMS, different sulphomethylated derivatives and colistin.

In the present study, two sensitive HPLC methods developed previously in our laboratory were used to quantify CMS and colistin separately in plasma and urine, allowing the pharmacokinetics of the administered CMS and the colistin generated from it to be determined. This study is complementary to a previous report, which described the pharmacokinetics of colistin in rats following an intravenous (iv) bolus of colistin. The combined findings from both studies will provide a more comprehensive description of the pharmacokinetic relationship between CMS and colistin. Such dual studies could not have been performed in humans because of the relatively greater toxicity of colistin and the lack of availability of a suitable dosage form, precluding its iv administration to humans.

Materials and methods

**Animals and treatment**

The study protocol was approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Sciences (Adelaide, SA, Australia). The treatment of animals and surgery for jugular vein cannulation were the same as described in a previous study.

**Pharmacokinetic experiment**

CMS (Sigma, St Louis, MO, USA) (15 mg/kg, freshly prepared in sterile saline) was administered in a volume of 300 µL via the jugular vein cannula in less than 20 s. The cannula was flushed immediately with 1 mL of heparinized saline (10 U/mL). Preliminary studies conducted in vitro indicated that this method of administration resulted in <0.01% of the dose remaining in the cannula after the flush. The content of colistin in the dosing solution of CMS was ∼0.11% (on a molar basis) as measured by HPLC. Blood (500 µL) was collected prior to the administration of CMS and at 5, 10, 20, 30, 60, 90, 120 and 180 min thereafter. During the sampling, 100 µL of blood was drawn into a syringe before collecting the actual sample with another syringe; subsequently, the 100 µL of blood was returned to the rat. The cannula was flushed with 500 µL of heparinized saline to maintain a constant intravascular volume. Each sample of blood was centrifuged immediately (1000g, 10 min) at 4°C. Urine was collected from the metabolic cage prior to administration of CMS, and over the intervals of 0–4, 4–8, 8–12 and 12–24 h thereafter. After collection of each urine sample, the metabolic cage was rinsed with Milli-Q water (5–10 mL) and the water kept separately for assay. Plasma and urine samples were stored immediately at −20°C pending assay.

**HPLC analysis of colistin and CMS in plasma and urine**

Concentrations of colistin in plasma and urine were determined by HPLC, with minor modifications. These included changes to the volume of plasma (100 µL) and urine (200 µL) samples, the amount of internal standard (10 µL, 2.5 mg/L netilmicin sulphate), the solid-phase extraction cartridge (Sep-Pak C18 Waters), HPLC injection volume (30 µL) and the use of a mobile phase of acetoni-tretrahydrofuran/water (50:30:20, v/v/v), which generated shortened retention times for derivatized netilmicin, colistin B and colistin A of ∼10, 12 and 14 min, respectively. The inter-day accuracy and reproducibility were within 7.0% for plasma and 6.7% for urine. Concentrations of CMS and partially sulphomethylated derivatives were determined by HPLC with subtraction of the concentration of colistin, after correcting for differences in the average molecular weights. The inter-day accuracy and reproducibility were within 7.9% for plasma and 9.0% for urine. When blank plasma and urine from a rat were spiked with CMS (26.7 mg/L) and stored for 7 days at −20°C, there was no measurable colistin at the quantification limit of 0.20 and 0.10 mg/L, respectively. All samples were assayed within 4 days.

**Pharmacokinetic analysis**

Non-compartmental analysis of the pharmacokinetics of CMS and colistin was performed using WinNonlin (model 201, Version 3.0, Pharsight Corp., Cary, NC, USA). The terminal half-life (t1/2) was calculated following linear least-squares regression analysis using the last five log-transformed plasma concentration–time points for CMS and the last three points for colistin. The following pharmacokinetic parameters of CMS were also calculated: area under the concentration–time curve to infinite time (AUC∞); volume of distribution at steady state (Vss); total body clearance (CL); fraction of the dose recovered in urine in 24 h (fD, determined as the summed amount of CMS and colistin recovered in urine in 24 h divided by the dose of CMS); and apparent value of the renal clearance for CMS (CLR).

The AUC∞ of colistin was calculated with the presumption of the initial concentration being zero. Assuming that the disposition of colistin—formed from CMS in vivo after the dose of CMS—is identical to that observed for colistin in a previous study following the administration of colistin, the fraction of CMS converted into colistin (fD) in the present study was calculated as: 

\[
 f_D = \frac{[\text{AUC}_\infty \text{of colistin after CMS}]}{[\text{AUC}_\infty \text{of CMS}]} \times [\text{dose of colistin}]
\]

where I403 and I743 are the average molecular weights for the A and B forms of colistin sulphate and sodium CMS, respectively.

**Stability of CMS in rat urine at room temperature and 37°C**

Blank rat urine was spiked with CMS at a concentration of 1.0 mg/mL using a freshly prepared stock solution of CMS. Aliquots (10 mL) of the urine were transferred into 50 mL centrifuge tubes (Greiner Labortecich, Austria); one aliquot was kept at room temperature (23 ± 2°C) and the other at 37 ± 1°C. Samples (0.5 mL) were taken at 0, 2, 4, 6, 12 and 24 h and stored immediately at −20°C pending analysis. Colistin in urine was quantified by HPLC (see above) and the percentage of CMS converted into colistin was calculated after correcting for differences in their average molecular weights.

**Statistical analysis**

Group data are reported as mean ± s.d. The difference between the t1/2 of CMS and colistin was evaluated with a paired Student’s t-test. A P value <0.05 was considered significant.

**Results**

Figure 1 shows the mean (±s.d.) concentrations of CMS and colistin in plasma as a function of time in five rats after an iv dose of 15 mg/kg CMS. The concentrations of CMS at 180 min in two of the five rats were below the limit of quantification; likewise for colistin in three of the five rats. There was a short distribution phase (∼20 min) for both CMS and colistin. In three of the five rats, maximum concentrations of colistin were observed at 5 min, and at 10 min in the remaining two rats. The mean (±s.d.) values of AUC∞ for CMS and colistin were 21.8 ± 3.0 mg·h/L and 2.6 ± 0.47 mg·h/L, respectively. The pharmacokinetic parameters for CMS are presented in Table 1. CMS had a significantly (P < 0.02) shorter terminal half-life (23.6 ± 3.9 min) than colistin (55.7 ± 19.3 min). The fraction of CMS converted into colistin (fD) was calculated as 0.068. Overall, 61.1% ± 14.4% of the dose was recovered in urine during the first 24 h after dosing, with 32.6% ± 15.1% present as colistin. The majority (>99%) of the urinary recovery occurred during the first 12 h. During storage of
Pharmacokinetics of colistin methanesulphonate in rats

Table 1. Pharmacokinetic parameters for CMS in rats (n = 5)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Mean estimates (±S.D.)</th>
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</thead>
<tbody>
<tr>
<td>CL (mL/min/kg)</td>
<td>11.7 ± 1.8</td>
</tr>
<tr>
<td>Vₐ (mL/kg)</td>
<td>299 ± 55</td>
</tr>
<tr>
<td>t₁/₂ (min)</td>
<td>23.6 ± 3.9</td>
</tr>
<tr>
<td>fᵦ</td>
<td>0.611 ± 0.144</td>
</tr>
<tr>
<td>CLₗ (mL/min/kg)</td>
<td>7.2 ± 2.2</td>
</tr>
</tbody>
</table>

CMS in rat urine at room temperature (23°C) and 37°C, 4.4% and 29.3%, respectively, was converted into colistin in 4 h.

Discussion

CMS and colistin differ in their chemistry, pharmacokinetics, antibiotic activity, and toxicity. Since CMS hydrolyses to colistin in aqueous media and the latter has been observed in the urine of humans following a dose of CMS, it is important to determine the concentrations of both CMS and colistin when examining the pharmacokinetics of CMS. Unfortunately, this is impossible with microbiological assays or the HPLC method for CMS reported by Reed et al. Therefore, the major goal of the current study was to characterize the pharmacokinetics of CMS and colistin in rats, after an iv bolus of CMS, with the concentrations of colistin and CMS measured separately in plasma and urine.

Interestingly, substantial concentrations of colistin were observed within 5 min after administration of CMS (Figure 1); maximum concentrations of colistin were achieved in all rats within 10 min. In the dosing solution, ~0.11% of CMS was present in the form of colistin, which indicated ~0.011 mg/kg colistin was co-administered with CMS to the rats. However, even in the 5 min and 10 min plasma samples, colistin measured 3.37 ± 1.31 mg/L and 2.76 ± 0.37 mg/L, respectively, and the AUCₘₙₙ of colistin was 2.6 ± 0.47 mg/L after the administration of CMS. From a previous pharmacokinetic study of colistin in rats, colistin measured 3.33 ± 0.16 mg/L in plasma 10 min after an iv bolus of 1 mg/kg, and the AUCₘₙₙ of colistin was 3.2 ± 0.23 mg h/L. Assuming that colistin displays linear pharmacokinetics in rats, the AUCₘₙₙ of colistin in plasma after administration of CMS was much higher than would have been predicted from the administration of 0.011 mg/kg colistin. Recent studies in our laboratory have shown that only 9.3% of CMS was hydrolysed to colistin in vitro when CMS (20 mg/L) was incubated in human plasma at 37°C for 2 h. In addition, in the present study, there was no measurable conversion of CMS into colistin during frozen storage and thawing of rat plasma prior to assay. Therefore, it would appear that there are mechanisms other than blood/plasma-mediated hydrolysis leading to the rapid in vivo formation of colistin.

The pharmacokinetic parameters for CMS may best be considered as hybrid parameters for CMS and the partially sulphomethylated derivatives. The Vₐ of CMS (299 ± 55 mL/kg) was ~60% of that for colistin (496 ± 62 mL/kg). The t₁/₂ of 55.7 ± 19.3 min for colistin in the current study compares favourably with the value of 74.6 ± 13.2 min after an iv bolus of colistin (1 mg/kg) in rats, but is approximately double that of CMS (23.6 ± 3.9 min, Table 1). The longer t₁/₂ of colistin indicated that its disposition after CMS administration was rate-limited by its elimination, not by its formation from the hydrolysis of CMS. The relativity in half-lives of CMS and colistin is similar to the reports in dogs and CF patients.

In contrast to the low urinary recovery of colistin (0.18% ± 0.14%, n = 5) in rats administered colistin, 61% of the dose of CMS was recovered in urine during the first 24 h, and almost all of this was recovered during the first 12 h. The high urinary recovery from rats of the dose of CMS is consistent with the reports from dogs and humans. For the treatment of urinary tract infections with CMS, the extremely high concentrations of colistin and CMS in urine in the current study, and of ‘CMS’ in humans, would be expected to be beneficial. The efficacy of CMS for treating infections of the urinary tract by Pseudomonas has been proven.

Given the low urinary recovery of colistin in rats administered colistin (and with similar plasma colistin levels to those in the present study), it is possible that the higher urinary recovery of colistin from the dose of CMS is an artefact of chemical hydrolysis of CMS in urine at 37°C in the bladder and/or at room temperature in the collection vessel. When CMS was stored in urine at 37°C and at room temperature, 29.3% and 4.4%, respectively, was converted into colistin in 4 h; 4 h being the longest time in the first 12 h that collected CMS would have remained in urine at these temperatures prior to being frozen. This suggests that a substantial component of the colistin measured in urine was the result of hydrolysis of CMS at physiological temperature. Furthermore, with the assumption that the clearance of colistin was the same irrespective of whether it was generated in vivo after an iv bolus of CMS (in the current study) or administered directly, a value for fᵦ of 0.068 shows that only a small fraction of CMS was converted into colistin systemically. Therefore, it is most likely that the high recovery of colistin in urine was the result of the following sources: intra-renal conversion of CMS into colistin within tubular cells of the kidney, with the majority of the colistin so-formed excreted directly into urine; and/or hydrolysis of CMS in the bladder and to a lesser degree in the collection vessel. The CLₗ of CMS (7.2 mL/min/kg, Table 1) was calculated using the summed amount of CMS and colistin in urine. A comparison of the non-renal clearance (4.9 mL/min/kg) with normal hepatic blood flow in rats (i.e. 72–95 mL/min/kg) indicates that the hepatic extraction ratio of CMS was very low. The fate of the remaining proportion (~40%) of the CMS dose not eliminated by the kidney remains unknown. There is little information on the metabolism of CMS. Following iv administration of CMS (100 mg/kg) to rabbits, colistin-N-glucuronide was identi-
fied tentatively, and accounted for 1.7% and 6.7% of the dose in urine and bile, respectively, collected for 24 h.30

The glomerular filtration rate (GFR) in rats is ∼5.2 mL/min/kg.30 While the exact unbound fraction (f u) of CMS in plasma remains unknown, even allowing for an f u at the upper limit of unity, the CL-R (7.2 mL/min/kg) is greater than the product of f u and GFR (renal filtration clearance). Therefore, net renal tubular secretion of CMS into the urine is apparent, which is substantially different from the very extensive tubular re-absorption observed for colistin.30 Clearly, derivatization of the free aminic groups of colistin with the methanesulphonate groups converts the molecule from one that undergoes very extensive net tubular re-absorption into one that undergoes modest net secretion.

In conclusion, the pharmacokinetics of CMS in rats following an iv bolus have been described after measuring the concentrations of CMS and colistin separately in plasma and urine by HPLC. The significant difference between the mechanisms of urinary excretion of CMS and colistin requires further study as this may provide some clues to their relative nephrotoxicities. Being able to quantify CMS and colistin separately in biological fluids, as demonstrated in the present study, will allow detailed pharmacokinetic and pharmacodynamic investigations in animals and humans.

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References