Sub-acute toxicity of colistin methanesulfonate in rats: comparison of various intravenous dosage regimens

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Abstract

The relative nephro- and neuro-toxicity of colistin methanesulfonate (CMS) was investigated in rats during 7 days of intravenous administration in regimens mimicking twice- and once-daily dosing of a clinically relevant dose in humans. Histological examination revealed more severe renal lesions with the regimen corresponding to once-daily dosing, indicating the potential for renal toxicity may be greater with extended-interval dosing.
Colistin, an old antibiotic, clinically available in the form of its prodrug, colistin methanesulphonate (CMS), is increasingly used to treat infections caused by MDR Gram-negative bacteria that are resistant to conventional antibiotics(5). Arguably, neuro- and nephro-toxicities are the potential adverse effects that are of greatest concern to physicians(12). CMS hydrolysates \textit{in vitro}(8) and \textit{in vivo}(11) to liberate the microbiologically active(3) and more toxic(2) parent compound, colistin. Currently, various dosage intervals for the total daily dose are being used despite the lack of evidence in support of their use. For patients with normal renal function, it is recommended that the daily intravenous dose of CMS be administered in 2 – 4 divided doses(2, 3), but some clinicians are administering the entire daily dose of CMS as a single dose(15). The selection of the most appropriate dosage regimen for CMS (which maximizes efficacy, minimizes toxicity and the development of bacterial resistance) requires the integration of pharmacokinetic, antimicrobial pharmacodynamic and toxicity data. This study aims to address some of the issues relating to the toxicity aspect of optimizing CMS dosing regimens. \textit{In vitro} data suggest that the toxicity of colistin is concentration and time dependent(6); therefore, the propensity for colistin-induced neuro- and nephro-toxicity may differ for extended-interval (24 h) dosing, relative to dosing at shorter intervals (6 -12 h). The relative toxicity of conventional and extended-interval dosing was investigated in a rat model, in studies that were approved by the institutional animal ethics committee.

CMS was administered to Sprague-Dawley rats (6-8 weeks old) for 7 days in different regimens via a jugular vein cannula: 20 mg/kg/8h (n = 9) (equivalent to 4.5 mg/kg/12h
of CMS in humans), 30 mg/kg/12h (n = 11) (equivalent to 9 mg/kg/24h of CMS in humans), 150 mg/kg/12h (n = 8) (high dose, no corresponding human regimen) or saline placebo (n = 8). The equivalent rat-to-human dosing regimens were calculated based on pharmacokinetic studies of CMS, and generated colistin, in rats and patients with cystic fibrosis(7, 11). Blood samples were collected before and after the first dose on days 1, 4 and 7 and prior to the second dose on day 1; post-dose samples were collected 5 – 20 min after administration. Plasma concentrations of colistin and CMS were quantified using HPLC(9, 10).

Cage-side observations of general well-being and behaviour were made daily (e.g. feet color, position in cage, sleeping patterns, respiration, body weight, food and water intake, response to stimuli and the incidence of piloerection and hunching). Rats were monitored for neurotoxicity daily using a functional screening battery(1). In the assessment of motor control, rats were required to walk for 5 minutes per day on a rat rota-rod treadmill (4). Forelimb grip-strength was assessed using a horizontal retort stand set-up(13). Rats were required to hang from the horizontal beam and hold their own bodyweight in order to pass this test. Locomotor activity was measured using a photobeam activity meter(14).

Monitoring for nephrotoxicity involved the measurement of plasma creatinine concentration using a commercial creatinine assay kit (BioAssay Systems, Hayward, CA, USA). In addition, a post-mortem renal histological examination was carried out. The examination assessed cellular degeneration that manifested as pallor of the cytoplasm of tubular epithelial cells and necrosis as indicated by a loss of cytoplasmic definition,
nuclear pyknosis, cellular desquamation and the presence of hyaline and cellular casts within tubular lumens. A scale of 1+, 2+ and 3+ corresponding to mild, moderate and severe changes, respectively, was used to grade lesion severity. The histologist was blinded as to which rats had received CMS treatment regimens or placebo.

Immediately after the first dose, the 150 mg/kg/12h group exhibited signs of overt neurotoxicity, notably muscular weakness, ataxia and laboured respiration. They also displayed signs of excessive thirst and blue discoloration of the lips and tongue. Blood samples were taken from this group immediately after the dose and at 6 and 12 hours post-dose. Significantly higher plasma creatinine concentrations were detected in this group 12 hours after the dose was given. It was considered unethical to continue dosing this group as significant toxicity had been identified; the animals were euthanized at 12 hours. Animals of this group exhibited severe renal pathological changes including severe proximal tubular necrosis (Table 1). While the dose administered to this group did not correspond to any clinically relevant human CMS regimen, the inclusion of this group substantiated the use of the rat as a model for colistin/CMS nephrotoxicity and neurotoxicity.

Regimens of 20 mg/kg/8h and 30 mg/kg/12h were intended to produce clinically relevant plasma concentrations of colistin and, indeed, concentrations (data not shown) were similar to those seen in cystic fibrosis patients(7). The sparse blood sampling approach used in this study was not intended to define the toxicokinetics or toxicodynamics of CMS/colistin. Average plasma concentrations of CMS and colistin would be expected to
be the same for rats in the 20 mg/kg/8h and 30 mg/kg/12h groups because the total daily
dose of CMS was the same.

Rats in the 20 mg/kg/8h CMS, 30 mg/kg/12h CMS and placebo groups showed no
indications of compromised well-being during the 7-day treatment; there were no
significant differences (ANOVA, $p > 0.05$) in the amount of food and water consumed, or
in body surface temperature. The motor control and forelimb grip-strength measurements
were not different ($p > 0.05$) in the rats that received 20 mg/kg/8h and 30 mg/kg/12h
CMS compared to the control group. The locomotor activity of rats that received 30
mg/kg/12h, however, was significantly lower than the control on days 2 ($p = 0.002$) and 6
($p = 0.044$) of treatment. While this finding was not consistent across all days of
treatment, it may be indicative of greater potential for neurotoxicity, as measured by
locomotor activity, when a larger dose of CMS is administered less frequently.

No significant change in plasma creatinine concentration (ANOVA, $p > 0.05$) was
observed from the respective baseline measurement for the 20 mg/kg/8h, 30 mg/kg/12h
or placebo groups. However, significant histological abnormalities, that were not present
in the control group, were detected in kidneys of the 20 mg/kg/8h and 30 mg/kg/12h
groups (Table 1). Five of the 9 rats in the group that received 20 mg/kg/8h CMS were
observed to have cellular casts in the proximal tubule, of low-grade severity. A similar
proportion of animals (7 of 11) in the 30 mg/kg/12h group showed lesions, however the
lesions were more severe and diverse in nature (Table 1).
Because of interspecies differences in the pharmacokinetics of CMS and generated colistin, achieving clinically relevant plasma concentrations of colistin in rats required that the corresponding plasma concentrations of CMS be substantially higher than those that occur in humans(7). It has been reported previously that CMS is cleared mainly via the kidneys, a process involving net tubular secretion(12). The relatively high plasma concentrations of CMS achieved in rats may have resulted in high concentrations of colistin within tubular cells as a consequence of intracellular hydrolysis of CMS(12). As colistin is a more toxic entity than CMS(2), high concentrations of CMS/colistin within renal tubular cells may potentiate nephrotoxicity in the rat, when compared to humans. Thus, care must be taken when interpreting histological results and extrapolating data from rats to humans.

That the range and severity of renal lesions was greater for the 30mg/kg/12h group is consistent with the reported concentration- and time-dependent toxicity of colistin(6). This group was intended to mimic the once-daily dosing of CMS to humans; the greater nephrotoxicity observed cautions against the use of extended-interval dosage regimens of CMS.

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Table 1: Results of renal histological examinations

<table>
<thead>
<tr>
<th>Type of abnormality</th>
<th>Control (n = 8)</th>
<th>20 mg/kg/8h (n = 9)</th>
<th>30 mg/kg/12h (n = 11)</th>
<th>150 mg/kg/12h (n = 8)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of animals showing lesion</td>
<td>Severity of lesion</td>
<td>Number of animals showing lesion</td>
<td>Severity of lesion</td>
</tr>
<tr>
<td>Protein/cellular casts in proximal tubule</td>
<td>0 n/a**</td>
<td>5 1+</td>
<td>3 1+, 2+, 3+</td>
<td>8 3+</td>
</tr>
<tr>
<td>Cortical proximal convoluted tubule necrosis</td>
<td>0 n/a</td>
<td>0 n/a</td>
<td>3 1+, 2+</td>
<td>7 3+</td>
</tr>
<tr>
<td>Pallor of outer stripe of proximal tubule</td>
<td>0 n/a</td>
<td>0 n/a</td>
<td>2</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Intracellular mineralisation</td>
<td>0 n/a</td>
<td>0 n/a</td>
<td>0</td>
<td>0 n/a</td>
</tr>
<tr>
<td>Nuclear pyknosis</td>
<td>0 n/a</td>
<td>0 n/a</td>
<td>2 1+</td>
<td>7 3+</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>0 n/a</td>
<td>0 n/a</td>
<td>2 1+</td>
<td>0 n/a</td>
</tr>
<tr>
<td><strong>Total number of animals showing lesions</strong></td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

* rats received one dose only and were euthanized on day 1; ** n/a = not applicable
References


