Subacute Toxicity of Colistin Methanesulfonate in Rats: Comparison of Various Intravenous Dosage Regimens

Stephanie J. Wallace, 1 Jian Li, 1 Roger L. Nation, 1* Craig R. Rayner, 1† David Taylor, 2 Deborah Middleton, 3 Robert W. Milne, 4 Kingsley Coulthard, 4, 5 and John D. Turnidge 6

Facility for Anti-infective Drug Development and Innovation 1 and Department of Pharmaceutical Biology, 2 Victorian College of Pharmacy, Monash University, Parkville, Melbourne, Victoria 3052, CSIRO Australian Animal Health Laboratory, East Geelong, Victoria 3220, 3 Sansom Institute, School of Pharmacy and Medical Science, University of South Australia, Adelaide, South Australia 5000, 4 and Department of Pharmacy 5 and Division of Laboratory Medicine, 6 Women’s and Children’s Hospital, North Adelaide, South Australia 5006, Australia

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The relative nephro- and neurotoxicity of colistin methanesulfonate (CMS) was investigated with rats during 7 days of intravenous administration in regimens mimicking twice- and once-daily dosing of a clinically relevant dose for humans. Histological examination revealed more-severe renal lesions with the regimen corresponding to once-daily dosing, indicating that 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 methanesulfonate (CMS), is increasingly used to treat infections caused by multidrug-resistant gram-negative bacteria that are resistant to conventional antibiotics (4). Arguably, neuro- and nephrotoxicities are the potential adverse effects that are of greatest concern to physicians (11). CMS hydrolyzes in vitro (7) and in vivo (10) to liberate the microbiologically active (2) and more toxic (1) 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 two to four divided doses (Coly-Mycin M parental package insert; Monarch Pharmaceuticals, Bristol, TN), but some clinicians are administering the entire daily dose of CMS as a single dose (14). The selection of the most appropriate dosage regimen for CMS (which maximizes efficacy and 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. In vitro data suggest that the toxicity of colistin is concentration and time dependent (5); therefore, the propensities for colistin-induced neuro- and nephrotoxicity may differ for extended-interval (24-h) dosing, relative to dosing at shorter intervals (6 to 12 h). The relative toxicities of conventional and extended-interval dosing were investigated with a rat model, in studies that were approved by the institutional animal ethics committee.

* Corresponding author. Mailing address: Facility for Anti-infective Drug Development and Innovation, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia. Phone: 61 3 9903 9061. Fax: 61 3 9903 9629. E-mail: Roger.Nation@vcp.monash.edu.au.
† Present address: F. Hoffman-La Roche Ltd., Pharmaceuticals Division, Basel, Switzerland.
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CMS was administered to Sprague-Dawley rats (6 to 8 weeks old) for 7 days in different regimens via a jugular vein cannula: 20 mg/kg of body weight/8 h (n = 9) (equivalent to 4.5 mg/kg/12 h of CMS in humans), 30 mg/kg/12 h (n = 11) (equivalent to 9 kg/kg/24 h of CMS in humans), 150 mg/kg/12 h (n = 8) (high dose, no corresponding human regimen), or saline placebo (n = 8). The equivalent rat and human dosing regimens were calculated based on pharmacokinetic studies of CMS and of generated colistin in rats and patients with cystic fibrosis (6, 10). 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; postdose samples were collected 5 to 20 min after administration. Plasma concentrations of colistin and CMS were quantified using high-performance liquid chromatography (8, 9).

Cage-side observations of general well-being and behavior (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) were made daily. Rats were monitored for neurotoxicity daily using a functional screening battery (15). In the assessment of motor control, rats were required to walk for 5 min per day on a rat rotorod treadmill (3). Forelimb grip strength was assessed using a horizontal retort stand setup (12). Rats were required to hang from the horizontal beam and hold their own body weight in order to pass this test. Locomotor activity was measured using a photobeam activity meter (13).

Monitoring for nephrotoxicity involved the measurement of plasma creatinine concentrations using a commercial creatinine assay kit (BioAssay Systems, Hayward, CA). In addition, a postmortem 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, nu-
TABLE 1. Results of renal histological examinations

<table>
<thead>
<tr>
<th>Type of abnormality</th>
<th>Control group (n = 8)</th>
<th>20-mg/kg/8-h CMS group (n = 9)</th>
<th>30-mg/kg/12-h CMS group (n = 11)</th>
<th>150-mg/kg/12-h CMS group (n = 8)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals</td>
<td>Severity of lesion</td>
<td>No. of animals</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 3+</td>
<td>0 N/A</td>
</tr>
<tr>
<td>Intracellular mineralization</td>
<td>0 N/A</td>
<td>0 N/A</td>
<td>0 N/A</td>
<td>7 3+</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>Total no. of animals showing lesions</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

* A scale of 1+, 2+, and 3+ corresponding to mild, moderate, and severe changes, respectively, was used to grade lesion severity. N/A, not applicable.

Rats received one dose only and were euthanized on day 1.

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Clear 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/12-h group exhibited signs of overt neurotoxicity, notably muscular weakness, ataxia, and labored 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 h postdose. Significantly higher plasma creatinine concentrations were detected in this group 12 h 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 h. 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/8 h and 30 mg/kg/12 h 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 (6). 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/8-h and 30-mg/kg/12-h groups, because the total daily doses of CMS were the same.

Rats in the 20-mg/kg/8-h CMS, 30-mg/kg/12-h CMS, and placebo groups showed no indications of compromised wellbeing during the 7-day treatment; there were no significant differences (analysis of variance, P > 0.05) in the amounts of food and water consumed or in body surface temperature. The motor control and forelimb grip strength measurements were not different (P > 0.05) for the rats that received 20 mg/kg/8-h and 30-mg/kg/12-h CMS compared to those for the control group. The locomotor activity of rats that received 30 mg/kg/12 h, however, was significantly lower than that of 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 a 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 concentrations (analysis of variance, P > 0.05) was observed from the respective baseline measurements for the 20-mg/kg/8-h, 30-mg/kg/12-h, or placebo groups. However, significant histological abnormalities that were not present in the control group were detected in kidneys of the 20-mg/kg/8-h and 30-mg/kg/12-h groups (Table 1). Five of the 9 rats in the group that received 20 mg/kg/8-h CMS were observed to have cellular casts of low-grade severity in the proximal tubule. A similar proportion of animals (7 of 11) in the 30-mg/kg/12-h 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 (6). It has previously been reported that CMS is cleared mainly via the kidneys, a process involving net tubular secretion (11). 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 the intracellular hydrolysis of CMS (11). As colistin is a more toxic entity than CMS (1), high concentrations of CMS/colistin within renal tubular cells may potentiate nephrotoxicity in the rat, 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 were greater for the 30-mg/kg/12-h group is consistent with the reported concentration- and time-dependent toxicity of colistin (5). This group was intended to mimic the once-daily dosing of CMS in humans; the greater nephrotoxicity observed cautions against the use of extended-interval dosage regimens of CMS.
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REFERENCES


