Pharmacokinetics of novel antimicrobial cationic peptides NAB 7061 and NAB 739 in rats following intravenous administration

Feda’ Emad Atta Ali1†, Guoying Cao1,2†, Anima Poudyal1, Timo Vaara3, Roger L. Nation1‡, Martti Vaara3,4 and Jian Li1*‡

1Facility for Anti-infective Drug Development and Innovation, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Victoria, Australia; 2Department of Pharmacy, Beijing Hospital, Beijing 100730, P. R. China; 3Northern Antibiotics Ltd, FI-00720 Helsinki, Finland; 4Division of Clinical Microbiology, Helsinki University Hospital, FI-00029 HUSLAB, Helsinki, Finland

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Objectives: To determine the disposition of novel antimicrobial cationic peptides NAB 7061 and NAB 739 following intravenous administration in rats.

Methods: Sprague-Dawley rats received a single intravenous bolus of 1.0 mg/kg NAB 7061 or NAB 739. Plasma concentrations of NAB 7061 or NAB 739 were determined by HPLC or liquid chromatography–mass spectrometry. The pharmacokinetic parameters of NAB 7061 and NAB 739 were calculated using non-compartmental analysis.

Results: Corresponding total body clearance, volume of distribution at steady state and terminal half-life of NAB 7061 and NAB 739 averaged 3.84 and 2.63 mL/min/kg, 339 and 222 mL/kg, and 66.2 and 69.0 min, respectively. Approximately 7.16% and 19.4% of the dose was eliminated in an unchanged form via the urine in 24 h for NAB 7061 and NAB 739, respectively.

Conclusions: While both compounds had generally similar pharmacokinetics to colistin, even minor alterations in the chemical structures appear to have an impact on their pharmacokinetics, especially on their clearance by the kidney. There are also substantial differences in relation to the relative contributions of renal and non-renal clearance to overall elimination from the body.

Keywords: polymyxin, new antibiotics, PK

Introduction

Gram-negative pathogens, such as Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae, which are resistant to all available antibiotics, are presenting a global medical challenge.1 Unfortunately, possibly due to lack of knowledge on their pharmacokinetics and pharmadynamics and suboptimal use,2,3 resistance to polymyxins, the last-line therapy against these difficult-to-treat pathogens, has been increasingly reported recently.4,5 Therefore, development of novel antibiotics against these extremely drug-resistant pathogens is urgently required. Very recently, novel polymyxin-like antibiotics have been developed.6 It is expected that these compounds (NAB 7061 and NAB 739; Figure 1) possess better toxicity and pharmacokinetic profiles than polymyxins.7 The objective of this study was to investigate the pharmacokinetics of the above-mentioned polymyxin-like peptides in rats after an intravenous bolus of 1 mg/kg.

Materials and methods

Animals

All experiments performed with rats were approved by the Faculty of Pharmacy and Pharmaceutical Sciences Animal Ethics Committee, Monash University (Victoria, Australia).

Pharmacokinetic studies

Pharmacokinetic studies were conducted as described previously.8 On the day of the experiment, NAB 7061 (n=5 rats, synthesized by...
Bachem AG, Bubendorf, Switzerland) or NAB 739 (n=4 rats, synthesized by Bachem AG) was injected (1 mg/kg in 300 μL of sterile saline) as a bolus into the jugular vein. Blood (~200 μL) was collected prior to administration of the compound and at 10, 20 and 30 min, and 1, 1.5, 2, 3 and 4 h thereafter, and plasma was harvested by centrifugation. Urine was collected from the metabolic cage prior to administration of the compound and over the intervals 0–4, 4–6 and 6–24 h thereafter. Plasma and urine samples were stored at −80°C until analysis.

Analysis of NAB 7061 and NAB 739 in plasma and urine
Concentrations of NAB 7061 in plasma and urine were determined by HPLC using a modified method for colistin.9 A 50×4.6 mm Onyx Monolithic C18 column (Phenomenex, Lane Cove, Australia) was employed. A mobile phase of acetonitrile/tetrahydrofuran/water (60/15/30 v/v) was pumped at 1.0 mL/min. Calibration curves were constructed using blank rat plasma or urine with six known concentrations of NAB 7061 (0.125–3.00 mg/L). Concentrations of NAB 7061 and NAB 739 in plasma as a function of time in rats. For both NAB compounds, there was a short initial distribution phase followed by all rats and there were no visual signs of toxicity. The administration of NAB 7061 and NAB 739 was well tolerated by all rats and there were no visual signs of toxicity. Figure 2 shows the mean (±SD) concentrations of NAB 7061 and NAB 739 in plasma as a function of time in rats. For both NAB compounds, there was a short initial distribution phase followed by a slower log-linear decline in the concentrations over approximately one order of magnitude. Following the 1 mg/kg dose of NAB 7061 and NAB 739, the mean extrapolated area under the plasma concentration–time curve represented 7.43% ± 3.11% and 7.87% ± 5.84%, respectively, of the total area from zero to infinite time. Key pharmacokinetic parameters for NAB 7061 and NAB 739 are presented in Table 1. During the first 24 h after dosing (a time equivalent to ~21 times their terminal half-lives), ~20% of the dose of NAB 739 was monitored at m/z = 538.8 [M+ 2H]+ and 359.6 [M+ 3H]+, and the compound eluted at 9.50 ± 0.03 min. The system (an LCMS-2010EV single quadrupole coupled with an ESI interface; Shimadzu) was controlled by LCMS Solutions (Version 3.4). Calibration curves were constructed using blank rat plasma or urine samples with 10 known concentrations of NAB 739, ranging from 0.010 to 10.0 mg/L, and from 0.050 to 30.0 mg/L, respectively. The LOQ was 0.010 mg/L (plasma assay) and 0.050 mg/L (urine assay). The assays for NAB 7061 and NAB 739 were sensitive, accurate and reproducible. Good linearity of calibration curves was achieved (r² ≥ 0.994 and r² ≥ 0.996, respectively). The accuracy and reproducibility were within 11%.

Pharmacokinetic analysis
Non-compartmental analyses of NAB 7061 and NAB 739 in plasma were conducted for their pharmacokinetics.

Results
The administration of NAB 7061 and NAB 739 was well tolerated by all rats and there were no visual signs of toxicity.
Pharmacokinetics of polymyxin-like compounds in rats

recovered in urine as unchanged drug, a value almost three times that of NAB 7061 (Table 1).

Discussion

NAB 7061 and NAB 739 are novel polymyxin analogues carrying three positive charges. While NAB 739 has in vitro antibacterial activity, comparable to polymyxin B, against Escherichia coli, K. pneumoniae and A. baumannii, its affinity for isolated rat kidney brush border membrane was only ~16% of that of polymyxin B, which may indicate low potential for nephrotoxicity. In this study, it is evident that the differences in the chemical structures across NAB 7061, NAB 739 and colistin (Figure 1) led to differences in their pharmacokinetics. There is only one amino acid difference between NAB 739 and NAB 7061 in the peptide side chain [d-serine and α-aminobutyric acid (Abu), respectively]. The mean half-lives of NAB 7061 and NAB 739 following the 1 mg/kg dose (Table 1) were similar to that of colistin. The volume of distribution of NAB 7061 was substantially lower than those observed for NAB 7061 and colistin. The total body clearance of NAB 7061 was considerably lower than that of NAB 7061 and colistin (Table 1). The low percentage of the dose that was recovered in urine in unchanged form indicates that non-renal clearance was the predominant clearance pathway for both NAB 7061 and NAB 739. A similar observation has been reported for colistin in rats, although the percentage urinary recovery of the unchanged colistin was substantially lower (0.18% ± 0.14% of the dose) than reported here for NAB 7061 and NAB 739. Thus, whereas the renal clearance values of NAB 7061 and NAB 739 were ~0.3 and 0.5 mL/min/kg, respectively (Table 1), that of colistin was very much lower (0.010 ± 0.008 mL/min/kg).

The relative magnitude of the differences in non-renal clearances across NAB 7061 (3.56 mL/min/kg), NAB 739 (2.10 mL/min/kg) and colistin (5.21 mL/min/kg) are not as great as those observed in the renal clearance values, as noted above. Comparison of the magnitude of the non-renal clearance values of NAB 7061 and NAB 739 with normal hepatic blood flow in the rat (72–95 mL/min/kg) indicates that both compounds must have a very low hepatic extraction ratio, similar to that of colistin. Further studies will be needed to determine the metabolic pathways for these polymyxin-like compounds.

A small difference in the chemical structure (Figure 1) leads to a lower total body clearance of NAB 739, which is due to a lower non-renal clearance compared with that of NAB 7061. While the renal clearances of both NAB compounds were a relatively low proportion of the corresponding total body clearances, the renal clearance of NAB 739 was about 2-fold that of NAB 7061, which indicates differences in their renal handling. It is important to note that the differences in the pharmacokinetics between the two NAB compounds were relatively modest. Arguably, the most important difference revealed by this study relates to the difference in renal clearance between the NAB compounds and colistin. Both NAB compounds contain fewer Dab residues than colistin (Figure 1). The renal clearance of colistin (and the urinary recovery of unchanged drug) is very much lower than observed for NAB 7061 and NAB 739. It appears, therefore, that the positively charged Dab residues in the peptide side chain play an important role in the renal elimination of polymyxins and their derivatives (e.g. the NAB compounds). It has been reported previously that colistin undergoes very extensive renal tubular reabsorption. Interestingly, it has been demonstrated that the affinity of NAB 7061 and NAB 739 for isolated rat kidney brush border membrane is lower than that of polymyxin B by a factor of six to seven. Whether that difference in affinity and/or the difference in renal handling between colistin and the NAB compounds observed in the present study impacts their potential to induce nephrotoxicity in vivo remains to be determined.

In conclusion, this is the first report of the pharmacokinetics of NAB 7061 and NAB 739 in rats. Even minor alterations in the chemical structures of NAB compounds appear to have an impact on their pharmacokinetics.

Table 1. Pharmacokinetic parameters for NAB 7061 and NAB 739 from the present study and colistin from a previous study, following intravenous administration (1 mg/kg) in rats (means ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NAB 7061</th>
<th>NAB 739</th>
<th>Colistin&lt;sup&gt;9&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life (min)</td>
<td>66.2 ± 12.3</td>
<td>69.0 ± 21.9</td>
<td>74.6 ± 13.2</td>
</tr>
<tr>
<td>Volume of distribution (mL/kg)</td>
<td>339 ± 96</td>
<td>222 ± 20.5</td>
<td>496 ± 60</td>
</tr>
<tr>
<td>Clearance (mL/min/kg)</td>
<td>3.84 ± 0.75</td>
<td>2.63 ± 0.54</td>
<td>5.22 ± 0.4</td>
</tr>
<tr>
<td>Urinary recovery (% of dose)</td>
<td>7.16 ± 3.70</td>
<td>19.4 ± 7.38</td>
<td>0.18 ± 0.14</td>
</tr>
<tr>
<td>Renal clearance (mL/min/kg)</td>
<td>0.28 ± 0.16</td>
<td>0.53 ± 0.30</td>
<td>0.010 ± 0.008</td>
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Transparency declarations
T. V. and M. V. are employees and shareholders of Northern Antibiotics Ltd. Other authors: none to declare.

References