INTRODUCTION AND PURPOSE

Aminoglycosides (AGs) are a well-known class of antibiotics with an established record of efficacy. Their use has been linked to concerns of nephrotoxicity. To support the development of new-generation AGs, both on an empiric antibacterial spectrum and clinical safety, we have refined a test model to quantitate AG nephrotoxic potential. The model is a validated extension post research on AG nephrotoxicity and allows for the effective screening of new-generation AGs in search of reduced intrinsic nephrotoxicity.

METHODS

• Adult Sprague-Dawley rats were dosed twice daily for 14 days. Rats were allowed full access to food and water, and AGs were dosed intravenously at 1 mg/kg daily in 0.9% saline.
• Nephrotoxicity was assessed by monitoring changes in renal markers of glomerular filtration rate (GFR) summary blood chemistry analysis, and histology.
• Microscopic examination of kidney sections after H&E staining and en face (H&E) staining was also utilized, scoring for tubular dilatation, vaculolization, hyaline casts, interstitial inflammation, or regeneration changes for the tubules.
• Animal work was performed at the contract research laboratories of Eurofins Animal Health (Berkley, CA) and Ethical (Montreal, Quebec).
• Neomycin (NEO), Gentamicin (GEN), Amikacin (AMK), Tobramycin (Tob), Paromomycin (PMK), and Arabinoside (AraC) were evaluated.

RESULTS

This in vivo model provided a consistent measure of AG-induced impairment of renal function, as evidenced by the reliable dose-response relationships of several creative changes for GEN across a number of independent studies (no change at 10 mg/kg, mild elevation at 30 mg/kg, and ≥2x elevation or mortality at 100 mg/kg).

Relative Ranking of AG Nephrotoxicity

Table 1: BUN in individual rats after 14 days of once-daily dosing of AGs

<table>
<thead>
<tr>
<th>AG</th>
<th>Day</th>
<th>BUN (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>14</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td>GEN 30</td>
<td>14</td>
<td>22.5 ± 4.2</td>
</tr>
<tr>
<td>GEN 10</td>
<td>14</td>
<td>15.1 ± 1.6</td>
</tr>
<tr>
<td>GEN 1</td>
<td>14</td>
<td>19.6 ± 2.7</td>
</tr>
<tr>
<td>AMK 25</td>
<td>14</td>
<td>14.2 ± 0.6</td>
</tr>
<tr>
<td>AMK 10</td>
<td>14</td>
<td>16.7 ± 0.5</td>
</tr>
<tr>
<td>AMK 3</td>
<td>14</td>
<td>21.8 ± 1.9</td>
</tr>
</tbody>
</table>

As shown in Figure 1, each AG tested exhibited a dose-response effect on BUN levels. An average was calculated for each AG.

• Functional impairment and microscopic kidney changes occurred at different dose levels for the different AGs, revealing quantitatively different nephrotoxic potentials.
• AG induced kidney changes were detected by H&E staining at doses many multiples below those that cause a GFR functional decline (e.g., 200 mg/kg for GEN), illustrating the relative sensitivity of kidney histopathology to detect AG-induced changes, and the capacity of the kidney to respond effectively to those changes without detectable effects on kidney function.

• Results are in general agreement with prior investigations.

Figure 2: Average BUN after 14 days of once-daily dosing of AGs

As illustrated in Figure 2, the apparent relative nephrotoxicity of AGs in this rat model is: AMK > GEN > TOB > APR = NEO > PAR = NA = ND.

• The nephrotoxic ranking of the model is consistent with their relative clinical nephrotoxicity, with clinical data available.

Figure 3: Serum creatinine of individual rats after 2, 5, 10, or 14 days of once-daily GEN

- No changes in kidney function were observed at any dose level prior to day 6.
- At 100 mg/kg, creatinine peaks at day 11, recovers in mid range to day 15.
- At 200 mg/kg, creatinine rises slightly on day 10.
- Drug-related increase in GFR is not seen for 14 days of dosing.
- These results are consistent with prior investigations.

Figure 4: Progression of kidney histopathological changes after 2, 5, 10, or 14 days of once-daily GEN

- All 30 mg/kg/day, no histological changes are observed prior to day 15.
- At 30 mg/kg/day, significant changes in kidneys are observed prior to day 15.
- At 30 mg/kg/day, no changes in kidneys are observed prior to day 15.
- These results were confirmed prior investigations.

Figure 5: Serum creatinine of individual rats after 2, 5, 10, or 14 days of once-daily GEN

- No changes in kidney function were observed at any dose level prior to day 6.
- At 100 mg/kg, creatinine peaks at day 11, recovers in mid range to day 15.
- At 200 mg/kg, creatinine rises slightly on day 10.
- Drug-related increase in GFR is not seen for 14 days of dosing.
- These results are consistent with prior investigations.

CONCLUSIONS

This in vivo model:

• Provides consistent and reliable evaluation of both functional and structural signs of nephrotoxicity of AGs.
• Provides a consistent test for screening new derivatives and guides selection of less toxic neoglycosides for clinical development.
• Provides a consistent, reliable functional evaluation of both functional and structural signs of nephrotoxicity of AGs.

Shorter course of AG therapy may allow higher dosage for improved efficacy and/or activity against less susceptible strains without an increase in toxicity.

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