QUANTITATIVE COMPARISON OF AMINOGLYCOSIDE NEPHROTOXICITY IN RATS FOR EFFECTIVE SCREENING AND EVALUATION OF NEW DERIVATIVES, AND DOSING RATIONALES THAT MINIMISE TOXICITY

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INTRODUCTION AND PURPOSE

Aminoglycosides (AGs) are a well-known class of antibiotics with an established record of efficacy. Their use has been limited due to concerns of nephrotoxicity. To support the development of neoglycosides, the next generation of AGs with both an improved antibacterial spectrum and clinical safety, we have refined a rat toxicity model to quantitate AG nephrotoxic potential. The model integrates extensive past research on AG nephrotoxicity[1-5] and allows for effective screening of novel AGs to search for and measure reduced nephrotoxicity.

METHODS

- Adult Sprague-Dawley rats were dosed once daily for 14 days. Rats were allowed full access to food and water, and AGs were dosed subcutaneously at 1 mL/kg dosing volume, formulated in water.
- Nephrotoxicity was assessed by monitoring changes in serum markers of glomerular filtration rate (GFR), namely blood urea nitrogen (BUN) and serum creatinine.
- Microscopic examination of kidney slices after fixation and hematoxylin and eosin (H&E) staining was also utilized, scoring for tubular dilation, cellular casts, interstitial inflammation, and regenerative changes to the tubules.
- Animal work was performed at the contract research laboratories of Murigenics (Berkeley, CA) and ITR (Montreal, Quebec).
- Neomycin (NEO), Gentamicin (GEN), Apramycin (APR), Tobramycin (TOB), Paromomycin (PAR), and Amikacin (AMK) were evaluated.

RESULTS

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

Relative Ranking of AG Nephrotoxicity

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

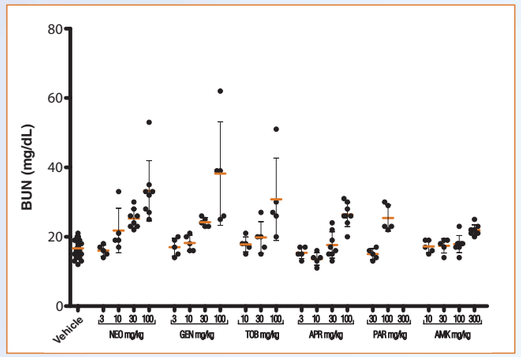
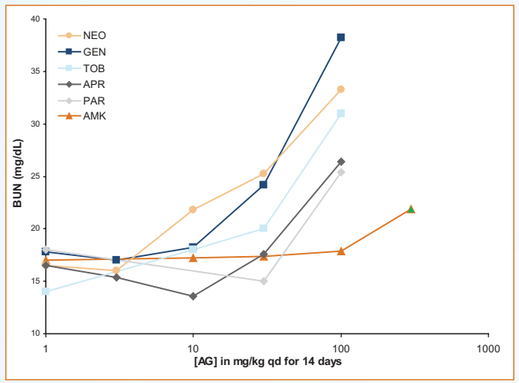


Table 1: Rat kidney histopathology analysis after 14 days of once-daily dosing of AGs

AG	Dose (mg/kg)	Average Histopathology Scores (0 to 5 Scale)				
		Total Score	*Dilation*	*Casts*	*Inflammation*	*Regeneration*
Vehicle	—	**0.2 ± 0.5**	0.2 ± 0.4	0.0 ± 0.2	0.3 ± 0.5	0.3 ± 0.6
NEO	3	**0.3 ± 0.5**	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4	0.4 ± 0.5
	10	**0.7 ± 0.6**	0.2 ± 0.4	0.2 ± 0.4	1.2 ± 0.4	1.0 ± 0.0
	30	**1.9 ± 0.7**	1.2 ± 0.4	1.6 ± 0.5	2.4 ± 0.5	2.4 ± 0.5
	100	**2.3 ± 0.9**	2.0 ± 1.4	2.0 ± 0.7	2.6 ± 0.5	2.6 ± 0.5
GEN	3	**0.6 ± 0.8**	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.5	0.8 ± 1.1
	10	**0.4 ± 0.6**	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.7	0.4 ± 0.5
	30	**2.8 ± 0.9**	3.0 ± 0.0	1.6 ± 0.9	3.0 ± 0.7	3.4 ± 0.5
	100	**3.4 ± 1.0**	2.6 ± 0.5	2.8 ± 0.4	3.6 ± 0.9	4.6 ± 0.5
TOB	10	**0.5 ± 0.9**	0.4 ± 0.9	0.0 ± 0.0	0.4 ± 0.9	1.0 ± 1.4
	30	**1.6 ± 0.9**	1.6 ± 1.1	0.8 ± 0.4	1.8 ± 0.8	2.2 ± 0.8
	100	**2.2 ± 1.0**	1.6 ± 1.1	1.8 ± 0.8	2.4 ± 0.9	3.0 ± 0.7
APR	3	**0.1 ± 0.3**	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	0.0 ± 0.0
	10	**0.3 ± 0.7**	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	0.6 ± 1.3
	30	**0.3 ± 0.5**	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.5	0.6 ± 0.5
	100	**1.7 ± 0.9**	0.6 ± 0.5	1.4 ± 0.5	2.4 ± 0.5	2.4 ± 0.5
PAR	30	**1.0 ± 0.9**	1.0 ± 1.0	0.0 ± 0.0	1.4 ± 0.9	1.4 ± 0.5
	100	**3.2 ± 0.6**	3.2 ± 0.4	2.6 ± 0.5	3.0 ± 0.0	4.0 ± 0.0
	300	**NA**	NA	NA	NA	NA
AMK	10	**0.2 ± 0.4**	0.2 ± 0.4	0.0 ± 0.0	0.4 ± 0.5	0.2 ± 0.4
	30	**0.2 ± 0.4**	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	0.2 ± 0.4
	100	**0.4 ± 0.5**	0.2 ± 0.4	0.0 ± 0.0	0.6 ± 0.5	0.6 ± 0.5
	300	**1.0 ± 0.8**	1.2 ± 0.8	0.0 ± 0.0	1.2 ± 0.4	1.4 ± 0.5

- As shown in Figure 1, each AG tested exhibited a dose-response effect on kidney function (BUN).
- As summarized in Table 1, a similar pattern of kidney histopathology changes was observed 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 deficit (e.g., 30x 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.[1,6,7]

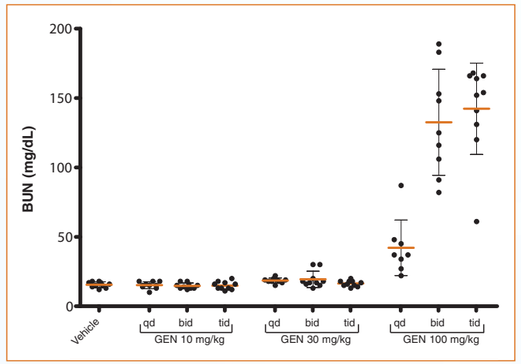
Figure 2: Average BUN after 14 days of once-daily dosing of AGs



- As illustrated in Figure 2, the apparent relative nephrotoxicity of AGs in this rat model is: NEO ≈ GEN > TOB > APR ≈ PAR > AMK.
- The nephrotoxic ranking of the AGs tested in this rat model correlates well with their relative clinical nephrotoxicity, where clinical data are available.

Once-daily Dosing of AGs Is Less Toxic

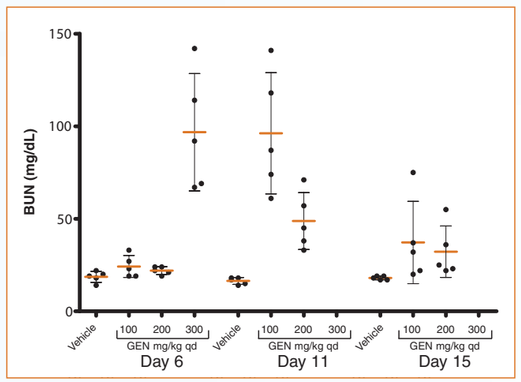
Figure 3: BUN of individual rats after 10 days of GEN dosing, equivalent total daily dose given either once daily, twice daily, or 3 times a day



- Consistent with prior work showing that kidney uptake of AGs is a saturable process,[8,9] once-daily dosing of GEN was significantly less toxic than twice- or three-times daily dosing of the same total daily dose (Figure 3).
- 100 mg/kg/day GEN results in significantly less impairment of kidney function when administered once daily instead of in 2 or 3 separate doses per day (p<0.0001).

Shorter Duration Allows Higher Dose Levels

Figure 4: BUN of individual rats after 5 days of once-daily GEN dosing. BUN sampled on days 6, 11, and 15

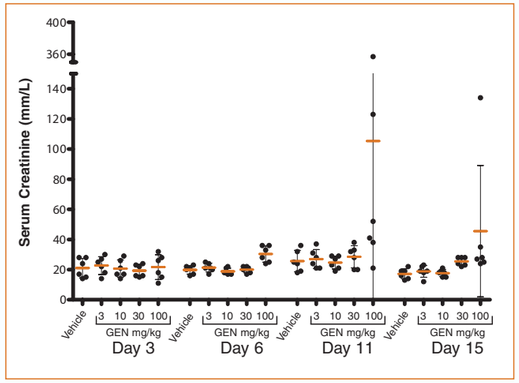


- Supporting the model that AG nephrotoxicity is correlated to the total duration of treatment, we found that limiting the duration of dosing to 5 days allows for doubling the dose of GEN without a significant increase in toxicity compared with 14 days of dosing (Figure 4).
- After 5 days of administration, the nephrotoxicity of 100 and 200 mg/kg/day GEN were indistinguishable from each other and from 100 mg/kg/day given longer (14 days).
- A tripling of the dose from 100 to 300 mg/kg/day did lead to a measurable increase in kidney function impairment on day 6 and subsequent mortality.

Time Course of GEN Nephrotoxicity

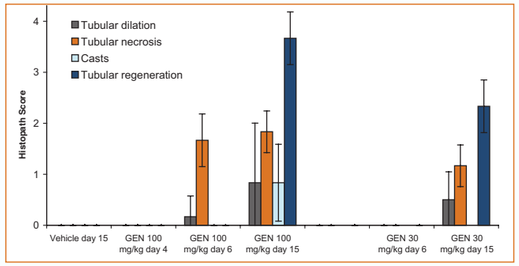
We studied the time course of GEN nephrotoxicity in detail in rats. Figure 5 shows the progression of serum creatinine changes after once-daily dosing of GEN for 14 days up to 100 mg/kg/day. Figure 6 presents the kidney histopathology results from the same study design.

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/day, creatinine peaks at day 11, recedes in most rats by day 15.
- At 30 mg/kg/day, creatinine rises slightly on day 15.
- Doses <30 mg/kg/day did not impair GFR over the 14 days of dosing.
- These results are consistent with prior investigations.[10]

Figure 6: Progression of kidney histopathology changes after 3, 5, or 14 days of once-daily GEN



- At 100mg/kg/day, signs of kidney necrosis appear after 5 days, and both ongoing damage and regeneration of kidney tubule epithelium are observed after 14 days.
- At 30 mg/kg/day, no microscopic changes are seen even after 5 days, but signs of damage and regeneration are evident after 14 days.
- Kidney histopathology findings are consistent with serum creatinine changes.
- At 100 mg/kg/day, no microscopic changes in kidneys are observed prior to day 6.
- At 30 mg/kg/day, no microscopic changes in kidneys are observed prior to day 15.
- Signs of tissue regeneration (recovery) are observed by day 15.

CONCLUSIONS

This 14-day rat model:

- Provides consistent and reliable evaluation of both functional and structural signs of nephrotoxicity of AGs,
- Allows screening of new AG derivatives and guides selection of less toxic neoglycosides for clinical development, and
- Confirms that less frequent once-daily dosing of AGs leads to reduced toxicity.

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

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