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Nonparallel Nephrotoxicity Dose-Response Curves of Aminoglycosides

G. H. HOTTENDORF,* D. BARNETT, L. L. GORDON, E. F. CHRISTENSEN, AND H. MADISSOO
Research Division, Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, New York 13201

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Nephrotoxicity comparisons of aminoglycosides in rats, utilizing large multiples of human doses, have indicated an advantage for netilmicin. However, no nephrotoxicity advantage of netilmicin has been demonstrated at the lower doses used in clinics. Some high-dose studies in rats have also suggested that the slope of the nephrotoxicity dose-response curve of netilmicin was less steep than the slopes of other aminoglycosides. Therefore, the slopes of the nephrotoxicity dose-response curves of gentamicin, amikacin, and netilmicin were compared in 200 rats at low multiples (one to five times) of human clinical doses. Histopathological evaluations of both kidneys from each rat revealed that netilmicin produced equivalent or greater nephrotoxicity as compared with gentamicin and amikacin and that the slope of the nephrotoxicity dose-response curve of netilmicin was approximately one-half as steep as the slopes of amikacin and gentamicin, which were parallel. The distribution of casts excreted in the urine after 2 weeks of dosing and the terminal gross observations corroborated the flatter dose-response slope of netilmicin. Nephrotoxicity advantages predicted by high-dose comparisons with netilmicin in rats are apparently a function of its less steep dose-response slope and therefore may have no relevance to lower doses.

When large multiples of the human dose of netilmicin are administered to rats, the drug is less nephrotoxic than gentamicin (2, 7, 10, 11, 13, 21), tobramycin (10, 16, 21), and amikacin (10), but netilmicin has appreciable nephrotoxicity in humans and cannot be differentiated from other aminoglycosides in clinics (1, 15, 20, 22). High-dose toxicity comparisons in animals may be inappropriate for extrapolations to lower doses unless the slopes of their respective dose-response curves are linear and parallel (9). Comparative nephrotoxicity results with netilmicin in rats have suggested a relatively flat dose response at high multiples of the human dose (10, 21), although these studies did not employ sufficient numbers of dose levels to describe a nephrotoxic dose-response curve. Utilizing the dose-response model recently described (5), the nephrotoxicity dose-response curves of netilmicin, gentamicin, and amikacin were compared in rats at low multiples of the human dose. (This paper was presented at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, 22 to 24 September 1980, New Orleans, La.)

MATERIALS AND METHODS

Animals. A total of 200 adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc.) weighing between 75 and 100 g upon arrival were acclimated for 14 days before the initiation of this study. The rats were housed in individual cages of appropriate type and size in an environmentally controlled room and were given Purina Laboratory Rodent Chow and fresh drinking water ad libitum. Subsequently, the animals were ranked by body weight and randomly divided into groups of 10 before being individually identified by a tag number attached to an ear.

Aminoglycoside administration. The doses (based on activity) were split and administered subcutaneously to the rats twice a day at approximately 9 a.m. and 3 p.m. Because of the differences in therapeutic doses, the daily doses of each aminoglycoside were normalized and expressed as multiples of therapeutic doses to facilitate comparisons. Six groups of rats received gentamicin doses varying from one to five times the human daily dose (4, 6, 8, 12, 16, and 20 mg/kg per day). Seven groups of rats received amikacin doses over the same range of multiples (15, 22.5, 30, 37.5, 45, 60, and 75 mg/kg per day), and five groups received netilmicin at one to five times (6, 12, 18, 24, and 30 mg/kg per day) the recommended human dose (10) subcutaneously for 28 days. Twenty saline-treated control rats were also included.

Antemortem observations. Clinical signs of toxicity, changes in general health and behavior, and body weights were recorded daily immediately before the administration of the first dose each day. Serum creatinine and blood urea nitrogen values were determined on individual blood samples obtained from all rats predose, after 2 weeks of dosing, and at the termination of dosing. Urinalyses were performed on

pooled 18-h urine samples collected from each treatment group and the control group at similar time points. Urine volume, specific gravity, and pH were determined for each sample, and protein, sugar, ketones, bilirubin, hemoglobin, erythrocytes, leukocytes, epithelial cells, bacteria, and casts were measured semiquantitatively.

Postmortem observations. All rats were sacrificed by an overdose of sodium pentobarbital 1 day after the last dose was administered. Both kidneys were removed and examined grossly by a single pathologist. The incidence of gross nephrotoxicity was recorded based on the appearance of pallor and mottling of the capsular or cut surfaces of the renal cortex and swelling and softening of the entire kidney. A longitudinal section of the left kidney and a cross section of the right kidney from each rat were preserved in 10% neutral buffered Formalin for histopathological evaluation. Tissue sections (6 μm each) were stained with hematoxylin and eosin, and the resulting tissue slides were randomized, coded, and examined by a single pathologist without knowledge of the treatment of the animals. Microscopic lesions were individually scored based on a previously described scheme which graded six different interrelated lesions on a scale from 0 to 4 (5). The incidence and severity of these lesions were equally weighted, and the lesion scores were summed to produce a single nephrotoxicity response for each animal, with a possible severity range of 0 to 24 (5).

Statistical analysis. Regression analyses (14) were performed for each aminoglycoside in which the nephrotoxicity response was related to the multiple of the therapeutic dose. Each analysis tested the significance of the linear dose response and the departure from linearity. The slopes of the dose-response curves were also tested for parallelism. An analysis of variance was then performed at each normalized dose multiple to determine whether the mean nephrotoxicity responses exhibited by the three aminoglycosides differed significantly from each other; if the overall analysis was significant, Duncan's test was used to test the significance between pairs of aminoglycosides (23).

RESULTS

Antemortem observations. All animals survived the 28-day dosing period, and no significant changes in general health, behavior, or body weight were observed. Individual serum creatinine values were all within normal (<1 mg/dl) limits, and all group averages were 0.5 to 0.6 mg/dl. Slightly increased blood urea nitrogen values (21 to 27 mg/dl) were observed in a few individuals in various treatment groups and in the control group. However, no significant differences from controls were discerned in group means which ranged from 13 to 19 mg/dl. Increased numbers of urinary casts were observed at 2 weeks in the gentamicin group with a dose five times that of the human dose and in the netilmicin groups with doses three, four, and five times that of the human dose. Significant numbers (>1 per high-power field) of casts were not observed in any group in the predose or termination pooled samples. Urinary casts may signal renal parenchymal disease in asymptomatic individuals (19) and are useful in identifying early renal tubular damage in patients given aminoglycosides (18).

Postmortem observations. An incidence of gross abnormalities greater than the control incidence was discerned at the four highest doses of netilmicin, the two highest doses of gentamicin, and only at the highest dose of amikacin (Table 1). The microscopic changes indicated in Table 1 are typical of aminoglycoside nephrotoxicity and have been illustrated previously (5).

Statistical analysis. When the mean microscopic nephrotoxicity score was plotted against the multiples of the human dose, a significant linear relationship was observed between the nephrotoxicity response and the dose multiple over the entire range (one to five times) of doses for amikacin and gentamicin and for the two- to fivefold doses of netilmicin (Fig. 1). The dose response to the netilmicin dose equivalent to the human dose was not on the linear portion of its curve, and the lack of fit was confirmed by statistical analysis. The nonlinearity of this dose is not improved statistically by excluding the dose two times the human equivalent, and the regression line described by the top four doses of netilmicin is an extremely close fit to the data points.

Log transformation of dose or nephrotoxicity response or both did not improve the analysis of the data. When plotted on an arithmetic scale, the netilmicin slope is about one-half as steep as the slopes for amikacin and gentamicin and significantly different from these two slopes. The slopes for amikacin and gentamicin are not significantly different and are therefore presumptively parallel. Since the slope of the netilmicin nephrotoxicity curve is not parallel to the other two, a mean relative toxicity comparison over the range of doses tested was not attempted. The slopes of the nephrotoxic dose response obtained with gentamicin and amikacin in this study were remarkably similar to those obtained in the previous study (5). Further statistical analysis of these nephrotoxicity responses revealed that (i) netilmicin was more nephrotoxic than gentamicin at two times the human therapeutic dose and equivalent at all other doses, (ii) netilmicin was equivalent to amikacin at the dose equivalent to the human dose but more nephrotoxic at all the other doses, and (iii) gentamicin was more nephrotoxic than amikacin at all doses. All doses of the three aminoglycosides were more toxic than the saline controls.

DISCUSSION

When comparisons of the nephrotoxic potentials of aminoglycosides are made in rats, utiliz-

TABLE 1. *Postmortem aminoglycoside nephrotoxicity responses in rats*

Group	Aminoglycoside[a]	Daily dose (mg/kg)[b]	Dose multiple	Mean nephrotoxicity score by type of lesion[c]						Total mean nephrotoxicity score (±SE)[d]	Incidence of gross lesions
				a	b	c	d	e	f		
1	Amikacin (n = 10 per dose)	15	1	0.2	0.9	0	0.3	1.0	0.2	2.6 (±0.4)	0
2		22.5	1.5	0.3	0.6	0	0.2	1.0	0.2	2.3 (±0.4)	1
3		30	2	0.1	0.7	0.1	0.3	0.9	0.3	2.4 (±0.3)	0
4		37.5	2.5	0.8	0.7	0	0.6	1.2	0.1	3.4 (±0.5)	1
5		45	3	1.1	0.8	0.2	0.8	1.2	0.4	4.5 (±0.6)	1
6		60	4	1.5	1.0	0.6	1.2	1.5	0.4	6.2 (±0.5)	1
7		75	5	2.2	1.4	0.4	1.4	1.9	0.6	7.9 (±1.1)	4
8	Gentamicin (n = 10 per dose)	4	1	0.9	1.2	0.3	0.1	1.2	0.2	3.9 (±0.4)	0
9		6	1.5	0.8	1.3	0.2	0.5	1.1	0.3	4.2 (±0.6)	0
10		8	2	1.3	1.6	0.2	0.9	1.4	0.5	5.9 (±0.6)	0
11		12	3	1.2	1.8	0.6	1.6	2.0	0.6	7.8 (±0.8)	1
12		16	4	1.7	2.3	0.8	2.1	2.6	1.1	10.6 (±0.9)	4
13		20	5	1.9	2.3	1.1	2.2	2.5	1.4	11.4 (±1.1)	7
14	Netilmicin (n = 10 per dose)	6	1	0.5	1.0	0.1	0.7	1.0	0	3.3 (±0.4)	0
15		12	2	1.5	1.8	0.2	2.0	2.3	0.8	8.6 (±1.0)	7
16		18	3	1.5	1.7	0.5	2.2	2.5	1.4	9.8 (±0.9)	8
17		24	4	1.6	1.9	0.6	2.1	2.5	1.8	10.5 (±1.0)	10
18		30	5	1.7	2.0	0.9	2.4	2.8	1.2	11.0 (±0.7)	10
19	Saline control (n = 20 per dose)	0		0	0.6	0	0	0.8	0	1.4 (±0.2)	2

[a] Administered twice a day for 28 days.
[b] Milligrams of activity.
[c] a, Tubular degeneration; b, peritubular inflammation; c, tubular necrosis; d, tubular dilatation; e, tubular basophilia; f, interstitial fibrosis.
[d] Group means of individual rat total scores. SE, Standard error.

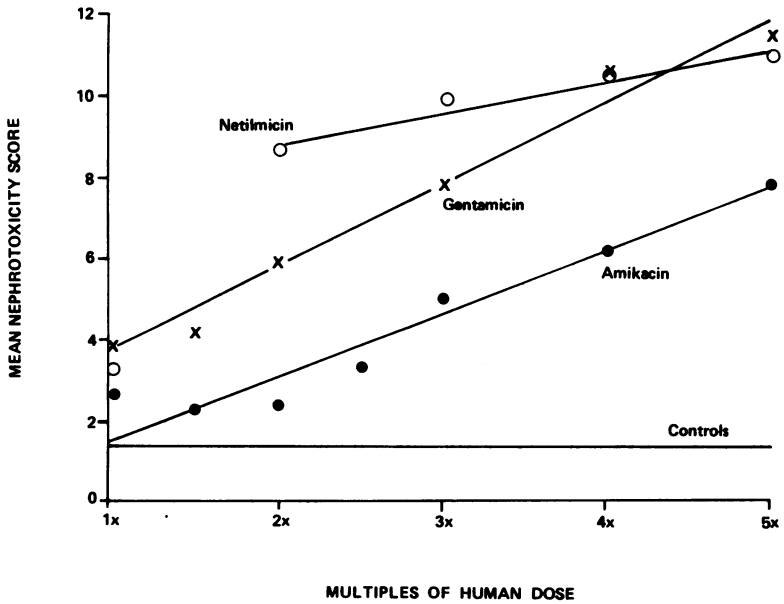

FIG. 1. *Histopathological nephrotoxicity dose-response curves for gentamicin* (×), *amikacin* (●), *and netilmicin* (○).

ing large multiples of human doses, a safety advantage for netilmicin is consistently observed (2, 7, 10, 11, 13, 16, 21). However, if more than one high-dose level is employed and renal histopathology is examined, a comparatively less steep dose-response slope has been suggested for netilmicin (10, 21). The safety advantage predicted by such high-dose comparisons in rats has not extrapolated to the lower doses used in clinics, in which the nephrotoxicity of netilmicin cannot be differentiated from other aminoglycosides (1, 15, 20, 22). Such a lack of extrapolation from high to low doses might be a function of a flatter dose-response slope.

In a new rat model the nephrotoxicity dose responses of aminoglycosides at low multiples of the human doses recently were compared, and it was concluded that the slopes of the nephrotoxicity dose responses of gentamicin, tobramycin, and amikacin were parallel (5). When the slope of the nephrotoxicity dose-response curve of netilmicin is compared with the slopes of gentamicin and amikacin in this low-dose model, the results obtained for gentamicin and amikacin are very similar to data obtained previously (5). Gentamicin was more nephrotoxic than amikacin, and these two aminoglycosides had parallel nephrotoxicity dose-response slopes. However, the results obtained with netilmicin indicate that the slope of its nephrotoxicity dose-response slope is not parallel to the other two aminoglycosides, confirming the suggestions of a flatter (nonparallel) dose-response slope for netilmicin (10, 21). Unlike the results reported at high doses, netilmicin was not less nephrotoxic than the other two aminoglycosides at these lower doses.

The new rat model employed placed equal importance on six different histopathological lesions involving primarily the proximal tubules of the nephron. The importance of evaluating six interrelated lesions that reflect the various degenerative and subsequent regenerative stages of nephrotoxicity cannot be overemphasized, especially when low doses are being assessed (5) and 28 days of dosing are utilized (21). The pathogenesis of aminoglycoside nephrotoxicity is reflected initially by degeneration of the epithelium of the proximal convoluted tubules which may progress to epithelial necrosis with or without a focal inflammatory reaction. Resolution of the necrosis is accomplished by basophilic regeneration of the tubular epithelium or, less successfully, by tubular dilatation (tubular atrophy) or focal fibrosis or both (5–8). After 28 days of dosing with aminoglycosides, tubular regeneration may be more prominent than necrosis in the kidneys of rats, as evidenced in this study (5, 8, 21). Since these lesions all represent different progressive stages of the reaction to the nephrotoxin and proximal tubular necrosis and tubular regeneration may occur simultaneously in adjacent nephrons (4–7, 11, 21), we believe that the lesions are all of equal importance in comparing the nephrotoxic potentials of aminoglycosides.

The shape of the dose-response curve for amikacin suggests an initial flat non-dose-dependent portion of the curve at the doses 1, 1.5, and 2 times the human therapeutic equivalent. A similar initial flat portion of the dose-response curve of gentamicin can also be envisioned in this and the previous study at 1 and 1.5 times the human equivalent (5). Toxic dose-response curves resembling hockey sticks have been described and interpreted as reflecting a threshold that must be exceeded before rising dose dependent responses can be detected (3). Fitting a sigmoid curve to the dose responses would provide another interpretation of the amikacin and gentamicin data. A sigmoid curve with a somewhat different shape could also accomodate the netilmicin responses. Whatever graphic is applied to the data, however, does not change the equivalency of the nephrotoxicity relationship between netilmicin and gentamicin at two, three, four, and five times their respective clinical doses nor the greater nephrotoxicity of netilmicin compared with amikacin at these dose multiples. The occurence of urinary casts and the incidence of gross renal changes observed in this study also corroborate these low-dose relationships. These comparative results at low doses in rats can be rationally reconciled with the reported reduced relative nephrotoxicity suggested for netilmicin at high doses when a comparatively less steep, nonparallel dose-response slope for netilmicin is considered.

Two other aminoglycosides may also have comparatively flatter nephrotoxicity dose responses in rats. Like netilmicin, streptomycin exhibited a relatively flat dose response with respect to histological renal damage (10). A new aminoglycoside, Sch 21420, had a nephrotoxic dose relationship interpreted as less steep than that of amikacin based on renal histopathology (17). Utilizing only renal function tests, the dose response of Sch 21420 was less steep than that of amikacin and gentamicin (12).

Despite the advantages predicted in high-dose comparisons in rats, appreciable nephrotoxicity has been observed with netilmicin in clinics (1, 15, 20, 22) and in rats at low multiples of the human dose. In addition, the slope of the nephrotoxicity dose-response curve of netilmicin was flatter and not parallel with the slopes of gentamicin and amikacin, confirming the suggestions made at high doses. Unless the slopes of

the dose-response curves are linear and parallel, it cannot be assumed that nephrotoxicity comparisons made at high doses can be extrapolated to lower doses in rats or to clinical doses in humans.

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