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Aminoglycoside Accumulation Kinetics in Rat Renal Parenchyma

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To test the hypotheses that the renal parenchymal accumulation kinetics of aminoglycosides can predict nephrotoxicity, we measured renal parenchymal concentrations in rats receiving gentamicin and tobramycin. In addition to comparing the drugs as single daily injections, we also examined the effect of multiple doses versus a single daily dose. Gentamicin accumulated to much greater concentrations in the kidney than did tobramycin. Gentamicin given twice daily accumulated more rapidly and to greater concentrations than did the same total dose given once daily. We conclude that aminoglycoside accumulation in the kidney depends on the drug and dose regimen. These differences may explain relative nephrotoxicities.

The aminoglycosides, a class of nephrotoxic antibiotics, bind to kidney tissue and exhibit long half-lives in renal parenchyma (7). The nephrotoxic potential of these agents has been extensively evaluated in rats. The degree of nephrotoxicity differs among the aminoglycosides and is related to the dose interval (1, 4, 6, 8). Gentamicin is more toxic to the kidney than is tobramycin, and gentamicin given by frequent injections or by continuous infusion is more toxic than is the same total gentamicin dose given as a single injection (10–12). Among the factors which are known to influence the development of toxicity, renal parenchymal aminoglycoside kinetics may offer a valuable predictive clue.

Whelton (13) has suggested that, to correlate renal drug concentrations with resultant toxicity, the drug dose, the duration of therapy, the time relationship of renal tissue removal for drug analysis versus the concomitant serum drug concentration, and the kinetic status of the serum drug concentration must be precisely defined. Moreover, frequent measurements of parenchymal concentrations must be made, and the duration of the study should be at least four times the tissue accumulation half-life to insure that steady-state-plateau tissue concentrations have been achieved. Appropriate statistical analyses must be applied to compare regimens. Such a detailed analysis has not previously been applied in the study of renal parenchymal aminoglycoside kinetics as related to nephrotoxicity. Therefore, we measured the accumulation kinetics of gentamicin and tobramycin in rat kidneys to test the hypotheses that (i) the renal accumulation of gentamicin differs from tobramycin accumulation and (ii) aminoglycoside accumulation is dependent on dose interval. Unlike other studies of these two well-established toxicity models, we chose doses which would cause neither a decrease in glomerular filtration rate nor tubular necrosis. Thus, we established a kinetic model of tissue accumulation unaffected by proximal tubular injury or cellular regeneration.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats weighing 250 to 300 g were randomly assigned to six groups of 120 rats each. They were allowed free access to water and fed a standard Wayne rat diet ad libitum. Rats received subcutaneous injections of aminoglycoside diluted to 1 ml of 0.9% saline according to the regimens outlined in Table 1.

Four rats from each group were sacrificed every day for 30 days. On the day before sacrifice, those animals were housed singly in metabolism cages. Renal function was determined by the measurement of a 24-h endogenous creatinine clearance. Renal tissue was obtained at sacrifice for the measurement of parenchymal aminoglycoside concentrations. The kidneys were weighed, homogenized, and diluted with phosphate buffer (pH 7). Aminoglycoside concentrations were measured microbiologically by agar well diffusion, with *Bacillus subtilis* as the reference organism (2).

On days 1, 14, and 28, additional kidneys were removed at sacrifice and prepared for light microscopy. The kidneys were halved through the hilus along the long axis, fixed in buffered 10% Formalin, embedded in paraffin, and stained with hematoxylin and eosin. The histological sections were coded and read

TABLE 1. Model parameters

Group	Drug	A^a (g^{-1})	Alpha[a] ($days^{-1}$)	C_RSS ($\mu g/g$)	$t_{1/2\ accum}$ (days)
1	Tobramycin (5 mg/kg once daily)	1.93 (0.32)	0.17 (0.04)	57	4.08
2	Gentamicin (5 mg/kg once daily)	3.54 (0.35)	0.10 (0.02)	175	6.90
3	Gentamicin (2.5 mg/kg twice daily)	6.17 (0.86)	0.17 (0.03)	178	4.01
4	Tobramycin (20 mg/kg once daily)	1.20 (0.18)	0.13 (0.03)	180	5.20
5	Gentamicin (20 mg/kg once daily)	2.20 (0.22)	0.14 (0.02)	304	4.79
6	Gentamicin (10 mg/kg twice daily)	4.18 (0.56)	0.23 (0.04)	371	3.08

[a] The standard error of the estimate is given in parentheses.

by one of us (M.D.G.), who was unaware of the regimens used.

To assess the histological extent of renal injury, we evaluated six parameters. The extents of tubular degeneration, tubular inflammation, tubular necrosis, tubular dilatation, tubular basophilia, and interstitial fibrosis were graded from 0 to 4 where 0 was normal. In grade 1, less than 10% of the nephrons were involved; in grade 2, 10 to 50% of the nephrons were involved; in grade 3, 51 to 90% of the nephrons were involved; and in grade 4, >90% of the nephrons were involved.

Parenchymal aminoglycoside accumulation kinetics were assessed by two independent techniques. We performed a pharmacokinetic analysis of the renal concentration time data, using the interactive modeling and graphics program M-LAB (5). An accumulation model was used, described by the general form C_R = (daily dose)(A/alpha)$(1 - e^{-\text{alpha} \times t})$, where C_R is the renal parenchymal aminoglycoside concentration, t is the time after the first dose, and A and alpha are constants. The steady-state renal aminoglycoside concentration (C_RSS) was predicted from these parameters, using the relationship C_RSS = (daily dose)(A/alpha), and the accumulation half-life ($t_{1/2\ accum}$) was predicted from the expression $t_{1/2\ accum}$ = ln 2/alpha, which is the time required to accumulate half of the steady-state renal parenchymal aminoglycoside concentration.

A statistical evaluation of the model was performed by measuring the standard error of the estimate for the model parameters, A and alpha, and by plotting the residual difference between the model-predicted renal aminoglycoside concentrations and the measured concentrations, with the time after the first dose as the independent variable.

Independently of the kinetic model, renal parenchymal aminoglycoside concentrations, creatinine clearances, and pathological scores were compared by two-way analysis of variance (ANOVA). Values for groups I and III were compared with those for group II. Similarly, values for groups IV and VI were compared with those of group V. A statistical evaluation of parenchymal concentrations from days 1 through 15 and days 16 through 30 was performed separately and from days 1 through 30 as a continuum.

RESULTS

Mean renal parenchymal aminoglycoside concentrations are shown in Fig. 1 and 2 along with the concentrations predicted by the kinetic model. The model parameters are listed in Table 1. Gentamicin (5 mg/kg given once daily) accumulated to three times the renal parenchymal concentrations of the same dose of tobramycin ($P < 0.001$). Similarly, gentamicin (20 mg/kg given once daily) accumulated to over 1.5 times the renal parenchymal concentrations of the same dose of tobramycin ($P < 0.001$). By analysis of variance, gentamicin accumulation was greater than that of tobramycin from days 1 to 15 and from days 16 to 30, as well as from days 1 to 30 ($P < 0.001$). From these data, the kinetic model predicted that steady-state parenchymal concentrations would not be achieved until after 10 to 21 days of therapy.

Gentamicin at a dosage of 10 mg/kg given twice daily accumulated more rapidly and to a greater parenchymal concentration than it did at a dosage of 20 mg/kg given once daily ($P < 0.001$). Analysis of variance confirmed these observations over the entire course of therapy.

Nephrotoxicity was not observed in any group. Neither tubular necrosis nor interstitial fibrosis was seen in any kidney. With a maximum possible pathological score of 24, the total score never exceeded 3. No statistical interaction of the pathological score occurred with time or among the regimens. Creatinine clearance did not change over time, nor were there any differences among the regimens. Representative data are shown in Table 2.

DISCUSSION

The comparative nephrotoxic potential of the aminoglycosides has been extensively evaluated. In rats, several investigators have shown that gentamicin is more toxic to kidneys than is

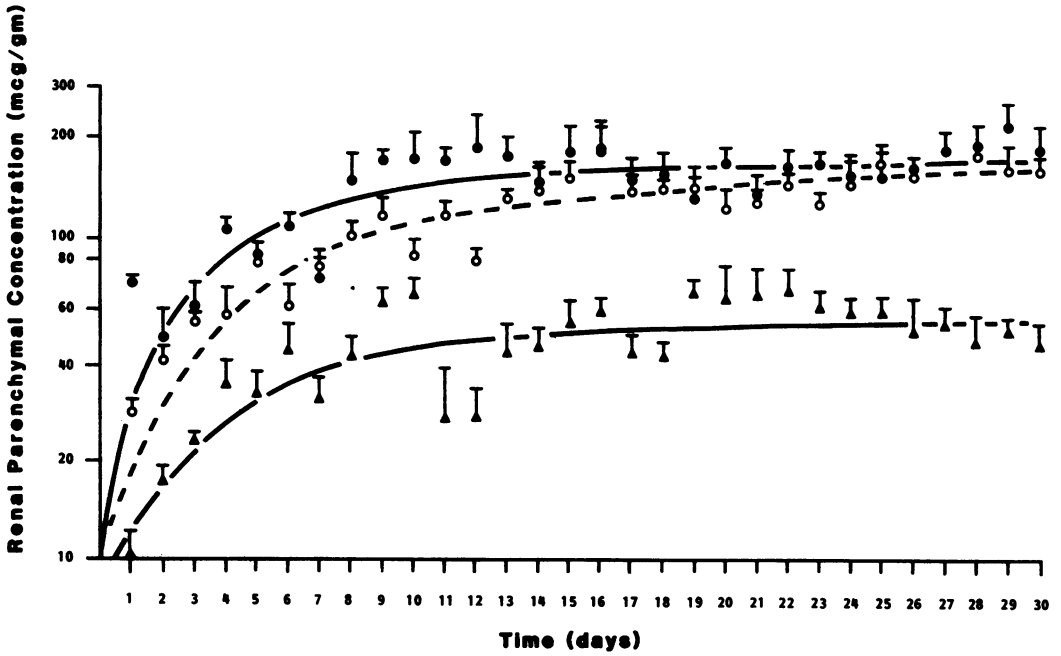

FIG. 1. Mean renal parenchymal aminoglycoside concentration (± standard deviation). Lines represent concentrations predicted by the kinetic model at the lower dose. ●, Gentamicin (2.5 mg/kg twice daily); ○, gentamicin (5 mg/kg once daily); △, tobramycin (5 mg/kg once daily).

tobramycin (4, 6, 12). More recently, in a prospective, double-blind, and randomized study, Smith et al. (11) made similar observations in humans. The relationship between the aminoglycoside dose interval and the development of nephrotoxicity has been established in rats, rab-

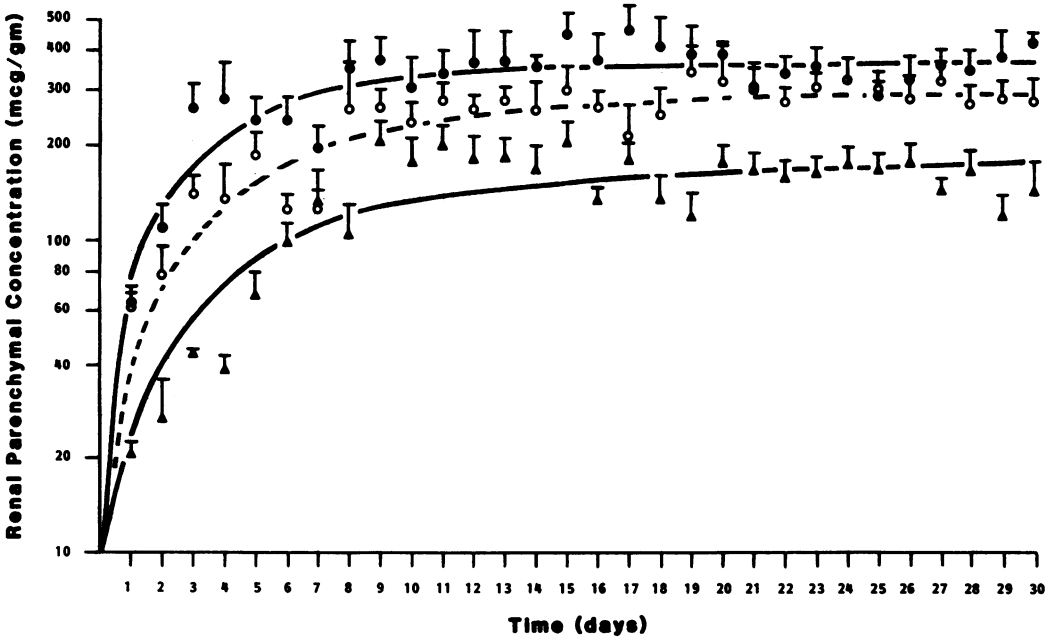

FIG. 2. Mean renal parenchymal aminoglycoside concentration (± standard deviation). Lines represent concentrations predicted by the kinetic model at the higher dose. ●, Gentamicin (10 mg/kg twice daily); ○, gentamicin (20 mg/kg once daily); △, tobramycin (20 mg/kg once daily).

TABLE 2. Creatinine clearance

Group	Creatinine clearance (ml/min)[a] at (day):			ANOVA
	1	14	28	
1	1.49 (0.67)	1.62 (0.20)	2.35 (0.48)	NS[b]
2	2.28 (0.76)	2.61 (1.27)	2.89 (1.07)	NS
3	2.16 (0.39)	2.35 (1.19)	2.16 (1.18)	NS
4	1.42 (0.33)	1.75 (0.27)	1.61 (0.79)	NS
5	1.80 (0.38)	2.63 (1.07)	2.59 (0.53)	NS
6	1.73 (0.57)	2.22 (0.88)	1.46 (0.63)	NS
ANOVA	NS	NS	NS	

[a] ± The standard deviation.
[b] NS, Not significant.

bits, and dogs (1, 3, 8). Gentamicin given by frequent injections or by continuous infusion was consistently more toxic than the same total dose given as a single injection.

Many factors are known to influence the nephrotoxicity of aminoglycosides. The precise mechanisms of this injury are unknown; however, these agents accumulate in renal tissue, where they have long half-lives (7). Therefore, renal cortical kinetics may be of value in predicting nephrotoxic potential. Experiments have been performed to correlate toxicity with parenchymal drug concentration. These experiments have not regularly shown an association between drug accumulation and toxicity (6, 12). Because most of these investigations primarily addressed the issue of relative toxicity, large doses were used to induce renal injury. Decreasing glomerular filtration rate, tubular necrosis, and cellular regeneration may have prevented the demonstration of a consistent correlation between renal parenchymal aminoglycoside concentration and resultant toxicity.

We studied the renal parenchymal accumulation kinetics of two well-established models of aminoglycoside nephrotoxicity. Doses were chosen which did not cause a decrease in glomerular filtration rate or tubular necrosis. Thus, the differences in accumulation were not the result of proximal tubular injury or cellular regeneration. Our data corroborate those of others who have shown that gentamicin, at subtoxic doses, accumulates to much greater renal concentrations in rats than does tobramycin. This observation supports the notion that gentamicin has a greater affinity for proximal tubular cells than does tobramycin and that this increased accumulation may be related to its greater nephrotoxicity. The data also demonstrate that gentamicin given frequently accumulates more rapidly and to higher renal levels than does the same dose given at a longer dose interval. Gentamicin uptake by tubular cells seems to depend more on the persistant exposure of the tubule to the drug than to the magnitude of the peak serum level.

Schentag et al. (9) have described a model of total body aminoglycoside accumulation and the subsequent development of renal insufficiency in humans; however, a kinetic model of renal tissue accumulation has not previously been reported. If the accumulation of aminoglycosides in kidney tissue is related to nephrotoxicity, our model predicts that gentamicin will be more nephrotoxic than tobramycin and that gentamicin given as multiple doses will be more toxic than a single daily dose. Furthermore, the lengthy accumulation half-life predicts the time course of toxicity. These observations are consistent with the results of toxicological studies.

ACKNOWLEDGMENTS

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