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Influence of Hydrocortisone on Gentamicin-Induced Nephrotoxicity in Rats

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Many risk factors associated with aminoglycoside nephrotoxicity have been identified in humans and experimental animals. They include an initial high rate of creatinine clearance, high initial peak levels in serum, age, sex, duration of therapy, liver disease, and renal infection. The concomitant administration of steroids has never been investigated. We evaluated the role of hydrocortisone on gentamicin-induced nephroxicity in a model of infused rats. We showed that hydrocortisone given over 3 days after the infusion did not modify the gentamicin half-life in the renal cortex, gentamicin-induced lysosomal phospholipidosis, or histopathology but did reduce significantly the ^{3}H/DNA ratio on day 4 after gentamicin infusion. We concluded that hydrocortisone interferes with the postnecrotic cellular regeneration process, an important step that is responsible for the recovery of normal kidney structure and function following toxic injuries associated with aminoglycoside therapy.

Eliminated essentially by glomerular filtration, aminoglycosides are partially reabsorbed by proximal tubular cells through absorptive endocytosis (pinocytosis) into small vesicles that fuse with primary lysosomes (11, 28). These primary lysosomes transfer the aminoglycoside to secondary lysosomes, where storage occurs independently of the duration of treatment (11). These drugs induce a lysosomal phospholipidosis characterized by inhibition of sphingomyelinase and phospholipase A_1 activity and by phospholipid accumulation into lysosomes (17). This lysosomal phospholipidosis is accompanied by cellular necrosis and postnecrotic cell regeneration (10, 18, 20).

Many risk factors associated with aminoglycoside nephrotoxicity have been identified in humans. They are an initial high rate of creatinine clearance, high initial peak levels in serum, age, sex, duration of treatment, and liver disease (23, 26). In animals, dose and frequency (4), age (21, 22), sex (3, 15), concomitant use of vancomycin (32), and renal infections (2) are clearly recognized as risk factors. The role of antiinflammatory agents like hydrocortisone on aminoglycoside nephrotoxicity is unknown. Steroids and nonsteroidal antiinflammatory agents are frequently administered with antibiotics to patients suffering from severe localized infections. In addition to their mineral and glucocorticoid effects, it has been shown that steroids suppress DNA synthesis in the livers of growing rats (6, 14) and in various nonlymphoid tissue of the weanling rats (19) and inhibit mitosis in various cells in tissue cultures (12).

The objective of the present study was to evaluate the role of hydrocortisone on the pathophysiology of gentamicin nephrotoxicity. Special attention was focused on the effect of hydrocortisone on renal tubular epithelial cells undergoing renewal and repair.

MATERIALS AND METHODS

Female Sprague-Dawley rats (weight, between 200 and 250 g) were used in this study. They were housed singly and had free access to food and water throughout the experiment. Gentamicin was infused over a 12-h period by the technique described by Giuliano et al. (10). Steady-state levels of approximately 25 μg/ml were achieved in serum. Control animals received normal saline solution through a similar infusion administration.

The day before infusion, animals were anesthetized with chloral hydrate (350 mg/kg, intraperitoneally; Fisher Scientific Co., Quebec, Quebec, Canada), and a polyethylene catheter (PE-20, Intramedic; Clay-Adams, Parsippany, N.J.) prefilled with normal saline was inserted into the right jugular vein. The other end of the catheter was guided subcutaneously, until it emerged through a hole made through the skin of the neck, and was secured in place. Animals were then returned to their cages until the beginning of the infusion.

Four gentamicin-treated rats and four control rats were killed 2 h after the end of the infusion and are referred to as day 0 rats. Twelve hours after the end of the infusion, animals of both groups were injected subcutaneously with either normal saline or hydrocortisone (12 mg/kg) every 12 h for 1 and 3 days, respectively. Twelve hours after the last injection, four to eight animals of each group were killed and are referred to as day 2 and day 4 rats.

The experiment was started on the day of the infusion by injecting a loading dose of gentamicin in a volume of 0.2 to 0.3 ml through the catheter. Control animals were injected with 0.25 ml of normal saline. The catheters were then connected to 20-ml plastic syringes (Becton-Dickinson and Co., Rutherford, N.J.) containing the appropriate solution and mouted for 12 h on a continuous-flow compact infusion pump (model 975, Harvard Apparatus; Ealing Scientific Ltd., St.-Laurent, Quebec, Canada). Animals remained quiet during the infusion, and therefore, no restraining device or anesthesia was needed. At the end of the infusion, the catheter was cut and heat clamped, and animals were returned to their cages until the time of sacrifice. Animals were killed 2 h (day 0) or 2 or 4 days after the end of the infusion. One hour before sacrifice all animals received an intraperitoneal injection of [^{3}H]thymidine (200 μCi).

The loading (LD) and infusion (ID) doses were adjusted on the basis of body weight and calculated by using the following parameters, which were previously determined in our

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laboratory: serum half-life, elimination constant (k_{el}), and volume of distribution (V_c) of gentamicin, where LD = desired serum levels × V_c and ID = (LD × k_{el})/rate of infusion.

Blood samples were drawn from the tip of the tail 4 and 10 h after the beginning of the infusion. At the time of sacrifice, animals were killed by decapitation, blood was collected, and both kidneys were rapidly removed and bissected. One half of the left kidney was immersed in formol (10%) fixative for conventional histology and histoautoradiography. The cortex of the other half was dissected and quickly frozen in dry ice for further determination of the [^{3}H]thymidine/DNA ratio. The cortex of both parts of the right kidneys was also dissected. Both parts were quickly frozen on dry ice, and one half was used for antibiotic determination and the other half was used for further biochemical analysis. Blood urea nitrogen and serum creatinine were measured on a blood urea nitrogen-creatinine analyzer (ABA-100; Abbott Laboratories, North Chicago, Ill.) from serum samples obtained at the time of sacrifice.

For conventional histopathology, samples were embedded in paraffin, cut to approximately 6-μm thick, and mounted onto glass slides. Two slices were made from each kidney. One slice was stained with hematoxylin-eosin and the other was prepared for autoradiography. In brief, slices were first coated with nuclear emulsion (NTB-2; Eastman Kodak Co., Rochester, N.Y.). After 2 weeks of exposure, slides were developed in Dektol and stained with thionine. The labeling index and the label distribution among the different histological structures of the cortex were performed on renal sections of animals that were killed on day 4 after the end of the infusion. Approximately 20,000 nuclei per rat were counted.

The measurement of DNA-specific radioactivity was performed on purified DNA obtained from the cortical tissue of the left kidney as described by Laurent et al. (18). Only one kidney was used per animal, since it has been shown that the rate of DNA synthesis is similar in both kidneys of the same animal (10, 18). Sphingomyelinase (EC 3.1.4.12) activity and total phospholipids were assayed in the cortex of each right kidney by previously published procedures (17).

Gentamicin concentrations in serum during the infusion and in renal tissue were measured by microbiological assay, with *Bacillus subtilis* used as the test organism. In brief, samples of the left kidney cortex were weighed, homogenized in distilled water with a Tissue-Tearor RTM (Biospec Products, Bartlesville, Okla.), sonicated with a sonicator (model W-375; Bionetics Ltd., Montreal, Quebec, Canada), and thereafter diluted to obtain a concentration in the range of the standard curve (0.4 to 25 μg/ml). The limit of assay sensitivity was 10 μg/g of tissue. Gentamicin standards were prepared in normal rat serum for serum assays and in blank rat cortex at the same tissue dilutions used for tissue assays. The levels of recovery of gentamicin after known amounts of drug-free homogenates were added were 98.0 ± 1.6%. Hydrocortisone did not affect bacterial growth and did not interfere with gentamicin during the assay.

Statistical analysis of the differences between groups was performed first by analysis of variance by a least-squares method. If the P value was <0.05, a group comparison was done by the Waller-Duncan multiple range test with the Kramer adjustment for unequal frequencies (16). A P value of <0.05 was considered significant. Calculations were made by using SAS software (SAS Institute Inc., Cary, N.C.).

Materials. Rats were purchased from Charles River Breeding Laboratories Inc., (Montreal, Quebec, Canada). Gentamicin was kindly donated by Schering Canada Inc. (Pointe-Claire, Quebec, Canada). Hydrocortisone was kindly donated by La Cie Upjohn du Canada (Don Mills, Ontario, Canada). *N*-[*methyl*-^{14}C]sphingomyelin (58 mCi/mmol) and [*methyl*-^{3}H]thymidine (49 Ci/mmol) came from Amersham Canada Ltd. (Oakville, Ontario, Canada). DNA (from salmon testes [type III]) and sphingomyelin (from bovine brain) came from Sigma Chemical Co. (St. Louis, Mo.). Other reagents were of analytical grade and were purchased from Fisher Scientific Ltd. (Quebec, Quebec, Canada) and Sigma Chemical Co.

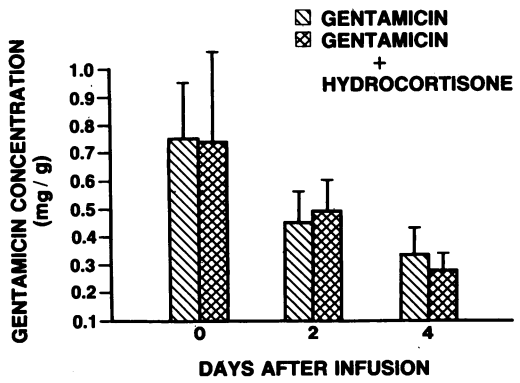

FIG. 1. Influence of hydrocortisone on the postinfusion accumulation and release of gentamicin from the renal cortex. Treatment with saline or hydrocortisone was started 12 h after the end of gentamicin infusion and was given for 1 and 3 days. Animals were killed 2 h and 2 and 4 days after the end of infusion. Bars represent standard deviations.

RESULTS

Gentamicin concentration in serum and kidney cortex. Gentamicin levels in serum measured after 4 and 10 h of infusion were 25.1 ± 3.8 μg/ml (n = 4) in animals killed 2 h after the end of the infusion (day 0), 26.3 ± 4.6 (n = 11) in animals selected to be treated with saline and 24.6 ± 4.5 (n = 12) in animals selected to be treated with hydrocortisone (i.e., subcutaneous treatment with saline and subcutaneous treatment with hydrocortisone was started 12 h after the end of infusion). There was no significant difference between the results at 4 and 10 h and between groups, indicating that all gentamicin-treated rats exhibited similar steady-state levels of drug in serum.

Levels of gentamicin in the kidney cortex measured 2 h (day 0) and 2 and 4 days after the end of the infusion are illustrated in Fig. 1. Two hours after the end of the infusion, gentamicin concentrations in the renal cortex were similar in both gentamicin-treated groups and reached levels of approximately 0.75 mg/g of tissue. Over the following days there was a decline in drug levels in tissue. On day 4, the concentrations of gentamicin were significantly lower in both groups as compared with their respective value on day 0 ($P < 0.01$). In fact, only half of the initial levels of gentamicin remained in the renal cortex. This decline, however, was not significant among groups showing that hydrocortisone had no effect on the further handling of gentamicin by the renal tissue.

Lysosomal phospholipidosis. The inhibition of sphingomyelinase activity in renal cortex expressed as a percentage of the value found in control animals is given in Fig. 2. Compared with the control, a severe inhibition of sphingomyelinase activity was induced by gentamicin at the end of the infusion ($P < 0.01$), which was only partially relieved on

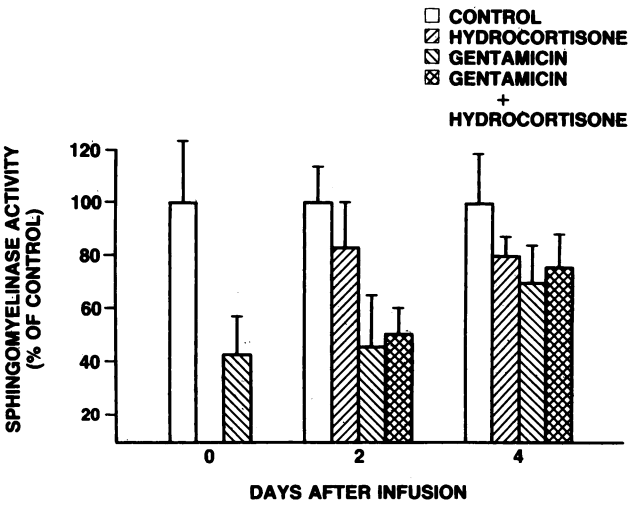

FIG. 2. Influence of hydrocortisone on the initial inhibition and relief of sphingomyelinase activity induced by gentamicin infusion. Treatment with saline and hydrocortisone was started 12 h after the end of gentamicin infusion and was given for 1 and 3 days. Animals were killed 2 h and 2 and 4 days after the end of infusion. Bars represent standard deviations. Control animals were infused with saline and then treated with saline.

day 4 ($P < 0.05$). Treatment with hydrocortisone did not modify the effect of gentamicin on this enzyme activity. Hydrocortisone injected into normal animals induced a slight but not significant inhibition of sphingomyelinase activity. Total phospholipids measured 2 h (day 0) and 2 and 4 days after the end of infusion showed no difference among groups (data not shown).

Renal function. Blood urea nitrogen and serum creatinine were in the normal range in all groups throughout the experiments.

Cellular proliferation in renal cortex. The specific radioactivity of kidney cortex DNA is illustrated in Fig. 3. In rats treated with normal saline, gentamicin induced a significant time-dependent increase in [^{3}H]thymidine incorporation into cortex DNA ($P < 0.01$) compared with that in control animals. This pattern is consistent with previous observations in animals treated with gentamicin administered by infusion (10). On day 4 [^{3}H]thymidine incorporation was significantly lower in animals treated with gentamicin and hydrocortisone compared with that in animals treated with gentamicin and saline ($P < 0.01$). Although gentamicin-infused animals treated with hydrocortisone showed a slight increase in [^{3}H]thymidine incorporation into DNA at either day 2 or 4 compared with that in normal saline-infused animals, it was not significant. Hydrocortisone given to control animals had no effect on [^{3}H]thymidine incorporation.

Morphological examination: light microscopy and histoautoradiography. The kidney cortex of animals infused with saline and injected over 3 days with either saline or hydrocortisone showed no histopathological modification on day 4. In contrast, kidney slices of gentamicin-infused animals and injected with either saline or hydrocortisone could be easily identified. They showed signs of gentamicin nephrotoxicity that mostly affected the proximal tubule (tubular degeneration, cell necrosis, and cellular debris in the tubular lumen). This pattern was similar in animals injected with either saline or hydrocortisone. In fact, it was impossible to differentiate the kidney slices that came from these two groups of animals when taken at random, on the basis of histopathological alterations.

Histoautoradiography showed labeled nuclei throughout the cortex, and their frequency was related to the rate of [^{3}H]thymidine incorporation into the DNA. In fact, the labeling index was significantly higher in gentamicin-infused rats treated with saline compared with that in all other rats ($P < 0.01$) (Table 1). Table 1 also shows the labeling distribution of [^{3}H]thymidine among the different cortical cell types: proximal tubules, interstitial cells, distal tubule, and glomerular cells. The presence of grains did not allow the differentiation of the cell types recorded in the interstitium. No figure was prepared to illustrate labeled nuclei since it has been previously published by Laurent et al. (18), and our results were consistent with those of Laurent et al. (18).

The distributions of labeled nuclei were similar in all groups, but the relative percentage of the labeling in distal tubules and glomeruli was lower in the renal cortexes of animals infused with gentamicin and treated with either saline or hydrocortisone, indicating that both proximal tubular and interstitial cells are, for the most part, responsible for the higher [^{3}H]thymidine/DNA ratio.

DISCUSSION

In the present study we showed that hydrocortisone does not affect gentamicin elimination from the renal cortex, gentamicin-induced lysosomal phospholipidosis, or histopathology but does reduce significantly the ^{3}H/DNA ratio on day 4 after gentamicin infusion compared with that in gentamicin-infused rats treated with normal saline.

Constant infusion of gentamicin was used since it resulted in a rapid and better incorporation of the drug in proximal tubular cells compared with that after the administration of the same amount in one or multiple daily injections (1, 25). It gave us the opportunity to analyze better the handling of this drug by kidney cells at steady state. Moreover, this model allowed the dissociation of drug accumulation (and the subsequent handling of the drug by tissue) from subcellular alterations subsequent to the administration of aminoglycosides (i.e., lysosomal phospholipidosis, cellular necrosis, and regeneration) (10). Giuliano et al. (10) have shown that

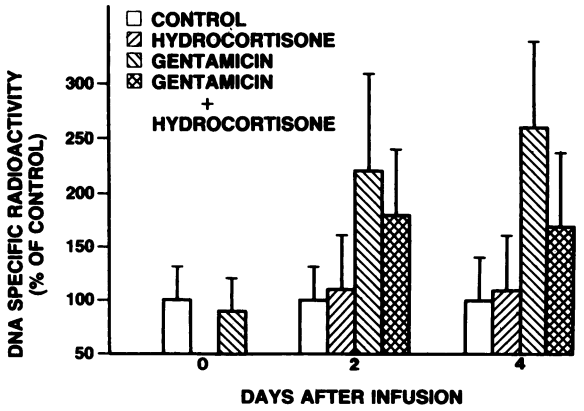

FIG. 3. Influence of hydrocortisone on the rate of DNA synthesis in the kidneys of gentamicin-infused rats. Treatment with saline and hydrocortisone was started 12 h after the end of gentamicin infusion and was given for 1 and 3 days. Animals were killed 2 h and 2 and 4 days after the end of infusion. Bars represent standard deviations. Control animals were infused with saline and then treated with saline.

TABLE 1. Morphological characterization of cellular regeneration 4 days after the end of infusion

Treatment	No. of animals	Labeling index[a]	% of labeled nuclei by histoautoradiography in:		
			Proximal tubule	Interstitial space	Others[b]
Normal saline	4	2.7 ± 0.9	60.7 ± 16.0	22.9 ± 8.7	16.4 ± 7.8
Normal saline and hydrocortisone	4	2.5 ± 1.1	63.2 ± 4.5	21.4 ± 1.4	15.4 ± 3.4
Gentamicin and normal saline	8	8.2 ± 1.2	67.9 ± 2.9	26.0 ± 4.3	6.1 ± 2.3
Gentamicin and hydrocortisone	8	2.7 ± 1.2	71.7 ± 7.8	22.8 ± 7.8	6.4 ± 1.1

[a] Number of labeled nuclei per 1,000 (± standard deviation). Approximately 20,000 nuclei were evaluated per animal (by using an objective of ×40, a mean of 40 nuclei were counted in each field observed). A total of 500 fields were observed for each kidney cortex.
[b] Distal tubular and glomerular cells were recorded together.

this technique allows the comparison of the nephrotoxicity between different aminoglycosides or the same aminoglycoside under different experimental conditions. The serum concentrations in animals were kept slightly higher than the usual toxic range for humans, but the conditions of the study are clinically relevant since the pharmacokinetics of gentamicin is different in rats (i.e., serum half-life, 24 min in rats and approximately 120 min in humans) (7, 13, 24, 29), and the cortical concentration of gentamicin recovered was just slightly higher than that found in human kidney cortex after the administration of standard doses of aminoglycoside (8, 27). When body mass is taken into consideration, it has also been shown previously that more drug is needed to induce similar aminoglycoside-induced biochemical and histopathological alteration in animals than is needed in humans. To evaluate as precisely as possible the drug-induced alterations with respect to their significance and predictive value for human nephrotoxicity, it is essential that cortical concentrations be adjusted to those concentrations obtained in human.

We chose hydrocortisone since it is a commonly used drug in patients who must be given aminoglycosides. In our previous study (5) on the influence of hydrocortisone on gentamicin cortical uptake in normal and endotoxin-treated rats, we observed a synergistic interaction between hydrocortisone and endotoxin on the intracortical uptake of gentamicin. We have shown that there is increased renal uptake of gentamicin in steroid-treated rats. These studies prompted us to undertake the present investigation in order to evaluate the effects of steroids on the pathophysiology of aminoglycoside nephrotoxicity. We were interested by the cell division regulation of hydrocortisone. Renal tubular regeneration and renewal is an important step in the recovery of aminoglycoside nephrotoxicity (18). We looked specifically at the influence of hydrocortisone on cell division regulation.

Hydrocortisone treatment was started 12 h after the end of gentamicin infusion in order to avoid any effect of the steroid on gentamicin uptake and accumulation. To ensure identical experimental conditions in all groups and to better delineate the role of steroids on the nephrotoxic effects of aminoglycosides, we started our experiment with levels of drugs in the cortex which were identical to those in both the control and hydrocortisone-treated groups. This was important since accumulation of the drug into lysosomes and development of lysosomal phospholipidosis are directly related phenomena (10, 17).

The most important observation made in the present study was that the rate of DNA synthesis was significantly diminished in gentamicin-infused rats treated with hydrocortisone compared with that in rats treated with normal saline, it spite of a similar initial toxicity induced by gentamicin. As shown by Laurent et al. (18), even low doses of aminoglycosides can induce tubular toxicity and a significant increase in thymidine incorporation in kidney cortex DNA can be observed.

The mechanism by which the rate of DNA synthesis was decreased in the presence of hydrocortisone in gentamicin-treated rats is unknown. We propose two hypotheses for this. First, hydrocortisone might have stabilized the lysosomal membrane, preserving the cell death which occurs when lysosomal enzyme from ruptured lysosome are liberated into the cytoplasm (30). In fact, as aminoglycosides accumulate within the lysosomes, it is believed, although not proven, that cell necrosis in aminoglycoside-treated animals and humans (31) results from the liberation of lysosomal enzyme into the cytoplasm of tubular cells. Stabilization of the lysosomal membrane, which preserves cell life, should have decreased gentamicin elimination from the renal cortex since cell necrosis is associated with the loss of drug from tissue (9, 10). This was not the case in our present study. In fact, gentamicin elimination from renal cortex was similar in gentamicin-infused groups of rats treated and not treated with steroids. The second hypothesis is that the postnecrotic cell proliferation might be delayed by hydrocortisone. Several observations favor this second hypothesis. In fact, it has been shown that hydrocortisone suppresses the DNA synthesis in the livers of growing rats (6, 14) and in tissue culture (12). However, this effect has never been investigated in the kidney after toxic injuries. Results of this study support this hypothesis and provide the first demonstration that hydrocortisone may interfere with the postnecrotic cellular regeneration induced by aminoglycosides. Kidney cell regeneration is a key factor for the restoration of normal kidney structure and function. If drugs like steroids can interfere with proximal tubular cell regeneration following aminoglycoside-related nephrotoxicity, then kidney repair and recovery may be delayed and signs of toxicity may be present for a longer period of time.

In conclusion, hydrocortisone has no effect on the intrinsic nephrotoxic potential of gentamicin. However, it may be considered as a risk factor associated with gentamicin therapy since it interferes with the postnecrotic cell regeneration in the kidneys. We are now in the process of investigating the impact of cellular regeneration inhibition following acute renal toxicity induced by aminoglycosides on the length of recovery by using the glomerular filtration rate as an index of renal toxicity.

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LITERATURE CITED

1. **Aronoff, G. R., S. T. Pottratz, M. E. Brier, N. E. Walker, N. S. Fineberg, M. D. Glant, and F. C. Luft.** 1983. Aminoglycoside accumulation kinetics in rat renal parenchyma. Antimicrob. Agents Chemother. **23**:74–78.
2. **Beauchamp, D., A. Poirier, and M. G. Bergeron.** 1985. Increased nephrotoxicity of gentamicin in pyelonephritic rats. Kidney Int. **28**:106–113.
3. **Bennett, W. M., R. A. Parker, W. C. Elliott, D. N. Gilbert, and D. C. Houghton.** 1982. Sex-related differences in the susceptibility of rats to gentamicin nephrotoxicity. J. Infect. Dis. **145**: 370–373.
4. **Bennett, W. M., C. E. Plamp, D. N. Gilbert, R. A. Parker, and G. A. Porter.** 1979. The influence of dosage regimen on experimental gentamicin nephrotoxicity: dissociation of peak serum levels from renal failure. J. Infect. Dis. **140**:576–580.
5. **Bergeron, M. G., Y. Bergeron, and D. Beauchamp.** 1987. Influence of hydrocortisone succinate on the intrarenal accumulation of gentamicin in endotoxemic rats. Antimicrob. Agents Chemother. **31**:1816–1821.
6. **Castellano, T. J., R. L. Schiffman, C. J. Matteethro, and J. N. Loeb.** 1978. Suppression of liver cell proliferation by glucorticoid hormone: a comparison of normally growing and regenerating tissue in the immature rat. Endocrinology **102**:1107–1112.
7. **Clarke, J. T., R. D. Libke, C. Regamey, and W. M. M. Kirby.** 1974. Comparative pharmacokinetics of amikacin and kanamycin. Clin. Pharmacol Ther. **15**:610–616.
8. **De Broe, M. E., R. A. Giuliano, and G. A. Verpooten.** 1986. Choice of drug and dosage regimen. Two important risk factors for aminoglycoside nephrotoxicity. Am. J. Med. **80**(Suppl. 6B): 115–118.
9. **Gilbert, D. N., D. C. Houghton, W. M. Bennett, C. E. Plamp, K. Reger, and G. A. Porter.** 1979. Reversibility of gentamicin nephrotoxicity in rats: recovery during continuous drug administration. Proc. Soc. Exp. Biol. Med. **160**:99–103.
10. **Giuliano, R. A., G. J. Paulus, G. A. Verpooten, V. M. Pattijn, D. E. Pollet, E. J. Nouwen, G. Laurent, M. B. Carlier, P. Maldague, P. M. Tulkens, and M. E. De Broe.** 1984. Recovery of cortical phospholipidosis and necrosis after acute gentamicin loading in rats. Kidney Int. **26**:54–63.
11. **Giurgea-Marion, L., G. Toubeau, G. Laurent, J. A. Henson-Stiennon, and P. M. Tulkens.** 1986. Impairment of lysosome-pinocytic vesicle fusion in rat kidney proximal tubules after treatment with gentamicin at low doses. Toxicol. Appl. Pharmacol. **86**:271–285.
12. **Grossfeld, H., and C. Ragan.** 1954. Action of hydrocortisone on cells in tissue culture. Proc. Soc. Exp. Biol. Med. **86**:63–68.
13. **Gyselynck, A. M., A. Forrey, and R. Cutler.** 1971. Pharmacokinetics of gentamicin: distribution and plasma and renal clearance. J. Infect. Dis. **124**(Suppl.):S70–S76.
14. **Henderson, C., R. E. Fischel, and J. N. Loeb.** 1971. Suppression of liver DNA synthesis by cortisone. Endocrinology **88**:1471–1476.
15. **Kourilsky, O., K. Soley, L., Morel-Maroger, A. Whelton, P. Duhoux, and J. D. Seaer.** 1982. The pathology of acute renal failure due to interstitial nephritis in man with comments on the role of interstitial inflammation and sex on gentamicin nephrotoxicity. Medicine **61**:258–268.
16. **Kramer, C. Y.** 1956. Extension of multiple range tests to group means with unequal numbers of replication. Biometrics **23**:307–310.
17. **Laurent, G., M. B. Carlier, B. Rollmann, F. Van Hoff, and P. Tulkens.** 1982. Mechanism of aminoglycoside-induced lysosomal phospholipidosis: in vitro and in vivo studies with gentamicin and amikacin. Biochem. Pharmacol **31**:3861–3870.
18. **Laurent, G., P. Maldague, M. B. Carlier, and P. M. Tulkens.** 1983. Increased renal DNA synthesis in vivo after administration of low doses of gentamicin to rats. Antimicrob. Agents Chemother. **24**:586–593.
19. **Loeb, J. M., and L. L. Yeung.** 1973. Effects of cortisone on thymidine incorporation by various non-lymphoid tissue of the weanling rat. Proc. Soc. Exp. Biol. Med. **143**:502–507.
20. **Luft, F. C., L. I. Rankin, R. S. Sloan, and M. N. Yum.** 1978. Recovery from aminoglycoside nephrotoxicity with continued drug administration. Antimicrob. Agents Chemother. **14**:284–287.
21. **Marre, R., N. Tarara, T. Louton, and K. Sack.** 1980. Age-dependent nephrotoxicity and the pharmacokinetics of gentamicin in rats. Eur. J. Pediatr. **133**:25–29.
22. **McMartin, D. M., and S. G. Engel.** 1982. Effect of aging on gentamicin nephrotoxicity and pharmacokinetics in rats. Res. Commun. Chem. Pathol. Pharmacol. **38**:193–207.
23. **Moore, R. D., C. R. Smith, J. J. Lepsky, E. D. Mellets, and P. S. Leitman.** 1984. Risk factors for nephrotoxicity in patients treated with aminoglycosides. Ann. Intern. Med. **100**:352–357.
24. **Regamey, C., R. C. Gordon, and W. M. M. Kirby.** 1973. Comparative pharmacokinetics of tobramycin and gentamicin. Clin. Pharmacol. Ther. **14**:396–403.
25. **Reiner, N. E., D. C. Bloxham, and W. L. Thompson.** 1978. Nephrotoxicity of gentamicin and tobramycin given once daily or continuously in dogs. J. Antimicrob. Chemother **4**(Suppl. A): 85–101.
26. **Sawyers, C. L., R. D. Moore, S. A. Lerner, and C. R. Smith.** 1986. A model for predicting nephrotoxicity in patients treated with aminoglycosides. J. Infect. Dis. **153**:1062–1068.
27. **Schentag, J. J., M. E. Plaut, and F. B. Cerro.** 1981. Comparative nephrotoxicity of gentamicin and tobramycin: pharmacokinetics and clinical studies in 201 patients. Antimicrob. Agents Chemother. **19**:859–866.
28. **Silverblatt, F. J., and C. Kuehn.** 1979. Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kidney Int. **15**:335–345.
29. **Simon, V. K., E. V. Mosinger, and V. Malerczy.** 1973. Pharmacokinetic studies of tobramycin and gentamicin. Antimicrob. Agents Chemother. **3**:445–450.
30. **Smith, R. J., S. Crawford, H. Gilchrest, and S. Williams.** 1976. Effects of anti-inflammatory drugs on lysosomes and lysosomal enzymes from rat liver. Biochem. Pharmacol. **25**:2171–2177.
31. **Tulkens, P. M.** 1986. Experimental studies on nephrotoxicity of aminoglycosides at low doses. Am. J. Med. **80**(Suppl. 6B):105–114.
32. **Wood, C. A., S. J. Kohlhepp, P. W. Kohnen, D. C. Houghton, and D. N. Gilbert.** 1986. Vancomycin enhancement of experimental tobramycin nephrotoxicity. Antimicrob. Agents Chemother. **30**:20–24.