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Gentamicin, Netilmicin, Dibekacin, and Amikacin Nephrotoxicity and Its Relationship to Tubular Reabsorption in Rabbits

NILS BRION,[1]* JANINE BARGE,[2] ISABELLE GODEFROY,[1] FRANÇOISE DROMER,[1] CATHERINE DUBOIS,[1] ALAIN CONTREPOIS,[1] AND CLAUDE CARBON[1]

Services de Médecine Interne[1] and Anatomie Pathologique,[2] Hôpital Louis Mourier, 92701 Colombes Cedex, France

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The role of the tubular reabsorption of aminoglycosides in nephrotoxicity was considered. The tubular reabsorption rate, fractional reabsorption, and net balance, expressed as the excreted to infused aminoglycoside ratio, were concomitantly studied in male rabbits by continuous infusion of gentamicin, netilmicin, dibekacin, and amikacin. Aminoglycoside nephrotoxicity was evaluated by creatinine levels in serum and pathological renal damage after 14 days of a low- or high-dose regimen, comprising either eight, hourly intramuscular injections of gentamicin, netilmicin, or dibekacin (4 mg/kg) or amikacin (16 mg/kg); twelve, hourly intramuscular injections of gentamicin, netilmicin, or dibekacin (15 mg/kg) or amikacin (60 mg/kg); or injections of saline for the control group. Aminoglycosides exhibited three degrees of tubular reabsorption: gentamicin had the highest, netilmicin had the lowest, and dibekacin and amikacin had intermediate degrees of reabsorption. Nephrotoxicity associated with alteration in renal histology was observed with gentamicin and, to a lesser extent, with dibekacin in the high-dose regimen. No nephrotoxicity was noted with netilmicin or amikacin compared with the control group. Concentrations of the aminoglycosides in renal cortex and serum were not predictive of renal toxicity. Except for amikacin, which appeared to exhibit the lowest intrinsic renal toxicity, nephrotoxicity was correlated with the tubular reabsorption of each aminoglycoside. It was concluded that aminoglycoside renal toxicity can be determined by two major factors: importance of transport into tubular cells and intrinsic intracellular toxicity.

Aminoglycoside nephrotoxicity has been the subject of many clinical, pharmacological, and biochemical investigations (16, 20, 28). Although the nephrotoxic potential of these drugs varies from one species to another, similar pathological patterns have generally been observed in both humans and animals. Morphological lesions, mainly of the proximal tubular cells (10, 25), have been demonstrated by light and electron microscopy. In animal models, differences in aminoglycoside nephrotoxicity were identified some time ago, particularly when, on a weight basis, the doses were 10 to 25 times those used in humans. Under these conditions, gentamicin exhibited greater nephrotoxicity than netilmicin, tobramycin, dibekacin, or amikacin (11, 14, 25, 27). The available evidence indicates that the nephrotoxic potential of aminoglycosides cannot be correlated with peak or trough levels in serum (3). Aminoglycoside accumulation in rat parenchyma was recently correlated with gentamicin and tobramycin nephrotoxicity (1). However, the importance of tissue accumulation per se in the pathogenesis of nephrotoxicity remains uncertain (14, 25, 27). By using a mathematically derived pharmacokinetic model, Schentag et al. (22) concluded that the extent of timed renal cortical accumulation correlated with clinical toxicity in humans. This renal cortical accumulation reflected the uptake of aminoglycosides exclusively inside proximal tubular cells. Gentamicin has been demonstrated by various techniques to undergo reabsorption from the lumens of the proximal tubules via the brush border (2, 8, 13, 26). There is no evidence to suggest that in the intact in vivo kidney, aminoglycosides enter the cells directly via the basolateral or antiluminal membranes (2, 18, 28).

In a previous study (4) in rabbits we demonstrated, through the effect of furosemide, that gentamicin undergoes bidirectional tubular transport. A significant degree of nephron heterogeneity has been reported for gentamicin transport in the rat kidney (23, 24). No study has directly examined renal handling of the other aminoglycosides.

The aim of the present work was to explore, in rabbits, the possibility of a relationship between net tubular reabsorption and experimental nephrotoxicity of gentamicin, netilmicin, dibekacin, and amikacin. To this end, we examined the effects of two dosage regimens on renal function and histology and the extent to which these effects were correlated with tubular reabsorption, net balance, and the cortical concentration of these antibiotics.

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MATERIALS AND METHODS

Animal model. The investigations were carried out in male rabbits (Fauve de Bourgogne; weight range 1.9 to 2.2 kg for the toxicity study and 2 to 4 kg for the renal handling study). They were allowed free access to water and fed a standard rabbit diet ad libitum. Each animal was used for one study only.

Renal handling. The renal handling of the four aminoglycosides was studied in four groups of seven rabbits each.

Animals were briefly anesthetized with ketamine hydrochloride, administered by intramuscular (i.m.) injection (10 mg/kg). Two catheters were inserted into the femoral veins for infusion and sampling. For urine collection, both ureters were catheterized through a suprapubic incision. The wounds were carefully closed around the catheters with surgical silk after local infiltration with lidocaine and then packed with gauze treated in saline. Two hours after ketamine injection, continuous isotonic saline infusion was start-

* Corresponding author.

ed at 0.5 ml/kg per min. Each aminoglycoside was infused at 1.5 mg/kg per h concomitantly with [^{125}I]iothalamate (0.1 μCi/min) (Amersham Corp., Versailles, France) over a period of 2 h for equilibration. Observations were made over two experimental clearance periods of 15 min each. Blood samples for antibiotic and iothalamate assays were collected at the end of each experimental period. Urine was collected throughout the two periods. For each period and each aminoglycoside, we calculated (i) the glomerular filtered load as the product of the iothalamate renal clearance and the unbound aminoglycoside blood concentrations; (ii) the tubular reabsorption rate as the filtered load of the antibiotic minus its urinary excretion; (iii) fractional reabsorption as the antibiotic reabsorption rate to antibiotic filtration rate ratio; and (iv) renal clearance of the antibiotic. [^{125}I]iothalamate was counted on an Auto-Gamma-Scintillation spectrometer, (model 3002; Packard Instrument Co., Inc., Downers Grove, Ill.).

Toxicity study. (i) Low-dose regimen. Four groups of six rabbits each received an i.m. injection of 4 mg of gentamicin, netilmicin, or dibekacin per kg or 16 mg of amikacin per kg every 8 h for 14 days. On days 1 and 14, insertion of a catheter into femoral veins allowed blood sampling for kinetic study of i.m. injected antibiotics and measurement of the glomerular filtration rate (GFR) with [^{125}I]iothalamate (10 μCi injected by intravenous (i.v.) bolus). The GFR was considered as the total body clearance of iothalamate calculated from serum levels. Renal function was also roughly estimated by measuring creatinine levels in serum (Jaffe's colorimetric method by Astra 8 Automat analyser system; Beckman Instruments, Inc., Fullerton, Calif.). Animals were killed by chloroform inhalation on day 14, 8 h after the last injection. The kidneys were immediately removed; one was used for histological study, and the other one was stored at −30°C for antibiotic assay in the cortex. A wedge of cortex was weighed, minced, and homogenized in 0.2 M Tris buffer (pH 7.4). The tissue homogenate was centrifuged at 3,500 rpm for 10 min and the eluate diluted 10-fold in buffer and used in the microbiological assay.

The other kidney removed for histological study was sliced into 2-mm thick sections which were fixed in Duboscq-Brazil liquid for 1 day, embedded in Paraplast, sectioned at 3 μm, and stained by hematoxylin-eosin-saffron or Masson trichrome for light microscopy. Sections were studied randomly by a pathologist (J. Barge) blinded with respect to the aminoglycoside that was injected. Histopathological changes were graded for the presence of tubular vacuolar degeneration, peritubular inflammation, tubular necrosis, and interstitial fibrosis by criteria set by Hottendorf and Gordon (11) and Luft et al. (15). Each section was scored as follows: 0, absence of lesions; +, lesions present in fewer than 10% of the nephrons; ++, lesions present in 10 to 50% of the nephrons; +++, lesions present in 50 to 90% of the nephrons; ++++, lesions present in more than 90% of the nephrons.

(ii) High-dose regimen. For 14 days, five groups, with five rabbits in each group, received an i.m. injection of 15 mg of gentamicin, netilmicin, or dibekacin per kg, 60 mg of amikacin per kg, or an equal volume of saline every 12 h; the latter group acted as the control. Renal function was evaluated by the levels of creatinine in serum at days 1 and 14. Histology was studied as described for the low-dose regimen.

Protein binding. Protein binding in serum was investigated by equilibrium dialysis for 6 h at 37°C in 0.15 M phosphate buffer (pH 7.4) with a Dianorm System (Diachema A.G., Rüchlikon, Switzerland) with 2-ml cells and cellulose dialysis membranes (Union Carbide Corp., Chicago, Ill.). Each antibiotic was tested at a concentration of 5 μg/ml in normal rabbit serum in which Ca^{2+} and Mg^{2+} concentrations were similar to those measured in the serum of experimental animals. Antibiotic concentrations were measured on each side of the dialysis membrane. Six hours of dialysis was enough to reach equilibrium.

Assays. Blood samples were allowed to clot before centrifugation at 3,500 rpm for 15 min. Serum and urine samples were stored at −30°C.

Antibiotic assays. Standards for the assays of serum samples were prepared in normal rabbit serum. Standards for the assay of urine samples were prepared in a 0.15 M phosphate buffer (pH 7.4). Each serum and urine sample was measured in duplicate. Antibiotic concentrations were determined by diffusion in nutrient agar, with *Bacillus cereus* (Institut Pasteur, 5832, Paris) as the test organism.

Statistical analysis. Statistical analysis was carried out by analysis of variance. The degree of significance between means was evaluated by Student's *t* test by using the residual variance and its degree of freedom.

RESULTS

Serum protein binding. Binding to serum proteins was nil for gentamicin, dibekacin, and amikacin, but it was 15% for netilmicin (range, 8 to 19%).

Renal handling. The serum concentrations and urinary excretion for each aminoglycoside are given in Table 1. During this experiment, a mean urinary flow rate of 0.45 ± 0.30 ml/min was noted. The mean GFR was 5.44 ± 1.36 ml/kg per min. Serum concentrations of aminoglycosides differed significantly in the following decreasing order: gentamicin, dibekacin, amikacin, and netilmicin. The filtered load and net tubular reabsorption rates were significantly greater for gentamicin than for the other three antibiotics. These parameters were similar for amikacin and dibekacin and significantly greater than those for netilmicin. Fractional reabsorption was significantly greater for gentamicin than for the other three aminoglycosides. Fractional reabsorption of netilmicin was lower than that of dibekacin and amikacin. However, these differences were not significant. Amikacin and dibekacin fractional reabsorptions were similar. Excreted/infused ratios were similar for gentamicin, dibekacin, and amikacin and significantly higher than those for netilmicin.

Toxicity. (i) Low-dose regimen. No animal died in any group during this study. The GFRs, determined by iothalamate clearance on days 1 and 14, are given in Table 2. No significant variations in GFR were observed within any group. Serum levels of each antibiotic, measured on days 1 and 14, are given in Table 3. No significant antibiotic accumulation was observed in the serum of any group. The following renal cortex concentrations of each aminoglycoside were measured on day 14 (mean ± standard deviation [SD], microgram per gram of cortex): gentamicin, 520 ± 168; netilmicin, 597 ± 109; dibekacin, 904 ± 267; and amikacin, 493 ± 187. The dibekacin concentration was significantly higher than that of the other three drugs.

Results of the histopathological study are given in Table 4. At this low-dose regimen, no tubular necrosis was observed. No animal receiving netilmicin displayed tubular vacuolar degeneration, and only one rabbit injected with amikacin had these cellular lesions in one tubule. Gentamicin and dibekacin exhibited similar tubular vacuolar degeneration in a few tubules in one-half of the animals of each group.

(ii) High-dose regimen. In the gentamicin group, two

TABLE 1. Concentrations in serum and urinary excretion of gentamicin, netilmicin, dibekacin, and amikacin in rabbits[a]

Antibiotic	Serum concn (total drug) (μg/ml)	Glomerular filtered load (μg/min per kg)	Absolute rate of net tubular reabsorption (μg/min per kg)	Fractional reabsorption (%)	Excreted/infused ratio (%)	Antibiotic renal clearance (ml/min per kg)
Gentamicin	5.41 ± 0.88[b]	35.52 ± 8.63[b]	20.48 ± 7.92[b]	56 ± 11[b]	73 ± 8	2.4 ± 0.6[b]
Netilmicin	2.44 ± 0.25[b]	10.25 ± 1.93[b]	1.85 ± 1.72[b]	16 ± 13	47 ± 15[b]	4.6 ± 0.6
Dibekacin	4.15 ± 0.25[b]	21.28 ± 7.27	6.40 ± 5.05	27 ± 15	68 ± 18	4.0 ± 0.8
Amikacin	3.65 ± 0.44[b]	18.41 ± 3.27	4.98 ± 2.52	26 ± 10	71 ± 11	4.0 ± 0.3

[a] For each antibiotic, seven animals were given a continuous i.v. infusion of saline (0.5 ml/kg per min) containing [^{125}I]iothalamate (0.1 μCi/min) and 1.5 mg/kg per h of antibiotic. Two hours were needed for equilibration. Two experimental periods of 15 min each were then observed. Each value represents mean ± SD for these two periods.

[b] Significantly different from the other three values ($P < 0.05$) (other significant differences are reported in the text).

animals died on days 6 and 9, respectively, probably of renal failure, as shown by the renal pathological study. One rabbit without pathological tubular necrosis died of an unknown cause on day 10 in the amikacin group. The creatinine concentrations in serum measured on days 1 and 14 in the 22 surviving animals are reported in Table 5. No significant variations occurred between days 1 and 14 in any group. The mean serum creatinine concentrations measured on day 14 were not different from those measured in the control group. No serum antibiotic accumulation was noted on day 14, since serum levels were nil 8 h after the last injection of each aminoglycoside in all but the gentamicin group, in which residual concentrations of 0.5 μg/ml were found in two of the three surviving animals.

Results of the histopathological study are given in Table 4. Vacuolar tubular degeneration was observed in all groups, but in the netilmicin, amikacin, and control groups, it was slight and limited to a few tubules in a few animals. These lesions were more extensive in all the rabbits of the dibekacin group than in those of the three groups mentioned above. Lastly, the gentamicin group exhibited the most severe vacuolar lesions, which affected all of the animals except the two who died during the study. No tubular necrosis was observed in any rabbit in the netilmicin, amikacin, or control groups. Four of the five animals in the dibekacin group had localized necrosis in the tubules. The most severe necrotic lesions were found in the gentamicin group, with total necrosis of several tubule groups in three rabbits (two of which died) and focal necrosis of tubule groups in the other two animals. No peritubular inflammation or interstitial fibrosis was observed.

DISCUSSION

In this study, we demonstrated that striking differences exist in the degree of intratubular cell transport of the four aminoglycosides tested and that the nephrotoxicity of gentamicin, netilmicin, and dibekacin, evaluated on the basis of pathological changes, correlates roughly with the degree of their tubular reabsorption, as expressed by the net tubular reabsorption rate and fractional reabsorption. In humans, i.v. bolus injection of the four aminoglycosides tested appeared to produce the same pharmacokinetics, expressed by the beta half-life, distribution volume, and total body clearance (17). In guinea pigs, however, netilmicin has been shown to exhibit a greater volume of distribution in the central compartment and a greater total body clearance than gentamicin (7). Values of aminoglycoside binding to serum proteins ranging from 0 to 20% have been reported in humans and animals (19). In our study, we found that under the same experimental conditions, the gentamicin, amikacin, and dibekacin binding was nil, and the binding of netilmicin was 15%. Therefore, we calculated the renal parameters of netilmicin on the basis of 85% of its serum concentrations, which was taken as the amount of unbound antibiotic able to undergo glomerular filtration. We found that these results were not significantly different when the basis was 100% of the serum concentrations of netilmicin. Surprisingly, the serum concentrations of each aminoglycoside were significantly different, although the infusion rates were the same. We have no explanation for these discrepancies; however,

TABLE 2. GFR in low-dose regimens[a]

Antibiotic	GFR on the following days (ml/kg per min):	
	Day 1	Day 14
Gentamicin	8.60 ± 2.41	8.09 ± 1.62
Netilmicin	6.95 ± 0.95	6.87 ± 1.95
Dibekacin	6.51 ± 1.55	7.97 ± 2.34
Amikacin	8.20 ± 2.10	8.76 ± 1.45

[a] Evolution of GFR from days 1 to 14. Each group of six animals received i.m. every 8 h 4 mg of gentamicin, dibekacin, or netilmicin per kg or 16 mg of amikacin per kg for 14 days. On days 1 and 14, GFR was determined by calculation of [^{125}I]iothalamate total body clearance from serum levels after a single i.v. bolus injection of 10 μCi. Results represent means ± SD (in milliliters per kilogram per minute) of six determinations.

TABLE 3. Drug levels in serum in low-dose regimens[a]

Antibiotics measured on the following days:	Concn (μg/ml) at the following times (min) after i.m. injection			
	30	60	120	240[b]
Gentamicin				
1	7.8 ± 2.5	8.6 ± 2.2	6.0 ± 1.2	0.9 ± 0.4
14	7.6 ± 2.4	9.2 ± 2.1	5.9 ± 1.2	0.7 ± 0.4
Netilmicin				
1	7.2 ± 2.2	9.8 ± 2.4	7.9 ± 1.7	1.0 ± 0.6
14	8.0 ± 2.3	9.9 ± 2.1	7.5 ± 2.0	0.9 ± 0.4
Dibekacin				
1	7.4 ± 2.4	8.5 ± 3.2	4.2 ± 1.6	1.2 ± 0.6
14	7.8 ± 2.2	8.8 ± 2.8	4.0 ± 1.1	0.9 ± 0.7
Amikacin				
1	15.8 ± 2.2	19.2 ± 5.3	9.7 ± 1.5	1.4 ± 0.8
14	16.3 ± 4.1	20.5 ± 6.0	8.4 ± 2.8	1.7 ± 0.7

[a] Comparison of levels of gentamicin, netilmicin, dibekacin, and amikacin in serum measured on days 1 and 14. In each group, six animals received i.m. every 8 h 4 mg of gentamicin, dibekacin, or netilmicin per kg or 16 mg of amikacin per kg. Each value represents mean ± SD (in micrograms per milliliter) of six determinations.

[b] Concentrations in serum at 360 and 480 min were not detectable by available assay procedures.

TABLE 4. Renal pathology on day 14

Antibiotic	Proximal tubular changes with the following dosage regimens:[a]			
	Low dose[b]		High dose[c]	
	Vacuolar degeneration	Necrosis	Vacuolar degeneration	Necrosis
Gentamicin	+ (3/6)	0 (6/6)	+++ (3/5)	++, +++ (5/5)
Netilmicin	0 (6/6)	0 (6/6)	+ (3/5)	0 (5/5)
Dibekacin	+ (3/6)	0 (6/6)	++, +++ (4/5)	+ (4/5)
Amikacin	+ (1/6)	0 (6/6)	++ (1/5)	0 (5/5)
Control	Not done	Not done	+ (2/5)	0 (5/5)

[a] 0, Absence of lesion; +, lesions present in fewer than 10% of the nephrons; ++, lesions present in 10 to 50% of the nephrons; +++, lesions present in 50 to 90% of the nephrons; ++++, lesions present in more than 90% of the nephrons. Number of animals affected per total number of animals is indicated in parentheses.

[b] In the low-dose regimen, six animals in each group were injected i.m. every 8 h with 4 mg of gentamicin, netilmicin, or dibekacin per kg or 16 mg of amikacin per kg.

[c] In the high-dose regimen, five animals in each group were injected i.m. every 12 h with 15 mg of gentamicin, netilmicin, or dibekacin per kg, 60 mg of amikacin per kg, or saline in the control group.

they may be due to various values of volume of distribution. That netilmicin exhibited the lowest excreted/infused antibiotic ratio, in spite of having the lowest fractional reabsorption, might be due to its low filtered load in relation to its low serum levels.

Our data clearly indicate that all of the aminoglycosides tested undergo net tubular reabsorption. These data are in agreement with those previously reported for gentamicin in the intact animal (4, 6), the isolated perfused kidney (8), or isolated tubules (18, 23), with a significant degree of nephron heterogeneity for gentamicin transport in isolated renal tubules (24). In a previous study (4), we described a gentamicin fractional reabsorption of 39 ± 5%. The discrepancy with the result obtained in the present study (56 ± 11%) could be due to differences in experimental conditions, i.e., series of rabbits and indicator of glomerular filtration (inulin versus iothalamate). With similar GFRs but different serum concentrations, the glomerular filtered load, taken as one for netilmicin, was two for amikacin and dibekacin and three for gentamicin (Table 1). Although the same increasing order applied to the net tubular reabsorption rates of all four aminoglycosides, the rate for gentamicin was 10 times that for netilmicin and about 3 or 4 times that for amikacin and dibekacin. These facts argue against a concentration-dependent reabsorption process. In such a process, fractional reabsorption would vary in inverse proportion to the filtered load. Therefore, aminoglycosides can be separated into three groups on the basis of renal handling: (i) gentamicin with both the highest filtered load and the highest reabsorption rate, (ii) netilmicin with the lowest filtered load and the lowest reabsorption rate, and (iii) amikacin and dibekacin which are intermediate between these extremes.

TABLE 5. Concentrations of creatinine in serum in high-dose nephrotoxicity study[a]

Antibiotic	Creatinine concn in serum on the following days (μmol/liter):	
	Day 1	Day 14
Gentamicin	101.6 ± 18.4 (5)	109 ± 23.6 (3)
Netilmicin	111.2 ± 22.3 (5)	107.2 ± 19.3 (5)
Dibekacin	97.2 ± 15.6 (5)	110.8 ± 32.5 (5)
Amikacin	97.2 ± 15.9 (5)	102.2 ± 20.5 (4)
Control	96.4 ± 12.4 (5)	89.6 ± 5.5 (5)

[a] Comparison of concentrations of creatinine in serum on days 1 and 14. In each group, five animals received i.m. every 12 h 15 mg of gentamicin, netilmicin, or dibekacin per kg or 60 mg of amikacin per kg. Each value represents means ± SD of five determinations (in micromoles per liter). Numbers in parentheses indicate the number of animals.

In our present study, there were no obvious correlations between the values of the renal parameters and the renal antibiotic concentrations. This might be due to possible antiluminal or basolateral transport (2, 18, 28). To evaluate renal toxicity, we used light microscopy, which in previous studies gave clear-cut results (11, 15). The low-dose regimen revealed no obvious differences among the nephrotoxic potentials of the four aminoglycosides tested. These results are in agreement with those obtained by Hottendorf and Gordon (11) or Luft et al. (15) but are at variance with those of Frame et al. (9) for similar doses of gentamicin in rabbits. In the high-dose regimen, gentamicin exhibited the greatest toxicity. The following decreasing order of toxicity was noted: gentamicin > dibekacin > netilmicin ≥ amikacin = control group. Obviously, amikacin exhibited a lower nephrotoxic potential than the other aminoglycosides. Although amikacin and dibekacin have similar patterns of tubular reabsorption, the pathological changes observed with amikacin, injected at doses four times higher than those of dibekacin, were identical to those that occurred in the control group. Amikacin was reabsorbed at a higher rate than netilmicin but caused slightly fewer pathological changes. The low intrinsic nephrotoxicity of amikacin reported here is in agreement with the results obtained by Viotte et al. (27), who, on the basis of the ultrastructural and biochemical alterations observed in rat kidney cortices, gave the following decreasing order of toxicity: gentamicin ≥ netilmicin > dibekacin = tobramycin > amikacin.

Aminoglycosides are thought to interact with the renal tubular cells by binding to acidic phospholipid receptors in the membrane before uptake by these cells (21). Knauss et al. have demonstrated that altered phospholipid metabolism, specifically involving the acidic phospholipids, is an early and constant feature of gentamicin nephrotoxicity (12). Carlier et al. (5) have found that the ability of aminoglycosides to inhibit lysosomal phospholipases in vitro is related to their degree of binding to liposomes rich in phosphatidylinositol. The number, nature, and respective position of the amino groups in the molecule seem to play a major role in the inhibition of phospholipases by aminoglycosides. Amikacin caused less inhibition than gentamicin, dibekacin, or netilmicin (5). Our data suggest that the aminoglycoside nephrotoxic potential is determined by two major factors: the extent to which the antibiotic is transported into proximal tubular cells and the tendency of the drug to damage intracellular organelles. Consequently, a new aminoglycoside should be evaluated both for the lesions it causes in subcellular structures and for its net tubular reabsorption in vivo. The relative variations in these two characteristics might explain the renal toxic potential of the drug. The relationship between the relative binding of aminoglycosides to the phospholipids of the tubular cell membranes and either the extent of intracellular transport or the capacity of the drug to evoke cellular damage by inhibiting phospholipid breakdown should be further investigated. Our results, showing the different degrees of tubular reabsorption of

aminoglycosides and the relationship between reabsorption and renal toxicity, were obtained in male rabbits. Cautious extrapolation to humans is warranted.

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