Kidney International, Vol. 33 (1988), pp. 1107–1112

Electrolyte composition of renal tubular cells in gentamicin nephrotoxicity

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Electrolyte composition of renal tubular cells in gentamicin nephrotoxicity. The effect of long-term gentamicin administration on sodium, potassium, chloride and phosphorus concentrations was studied in individual rat renal tubular cells using electron microprobe analysis. Histological damage was apparent only in proximal tubular cells. The extent of damage was only mild after 7 days of gentamicin administration (60 mg/kg body wt/day) but much more pronounced after 10 days. GFR showed a progressive decline during gentamicin treatment. In non-necrotic proximal tubular cells, sodium was increased from 14.6 ± 0.3 (mean ± SEM) in controls to 20.6 ± 0.4 after 7 and 22.0 ± 0.8 mmol/kg wet wt after 10 days of gentamicin administration. Chloride concentration was higher only after 10 days (20.6 ± 0.6 vs. 17.3 ± 0.2 mmol/kg wet wt). Both cell potassium and phosphorus concentrations were diminished by 6 and 15, and by 8 and 25 mmol/kg wet wt after 7 and 10 days of treatment, respectively. In contrast, no major alterations in distal tubular cell electrolyte concentrations could be observed after either 7 or 10 days of gentamicin administration. As in proximal tubular cells, distal tubular cell phosphorus concentrations were, however, lowered by gentamicin treatment. These results clearly indicate that gentamicin exerts its main effect on proximal tubular cells. Decreased potassium and increased sodium and chloride concentrations were observed in proximal tubular cells exhibiting only mild histological damage prior to the onset of advanced tissue injury. Necrotic cells, on the other hand, showed widely variable intracellular electrolyte concentration patterns.

The antibiotic gentamicin, an aminoglycoside, is widely used in the treatment of gram negative infections. Since, however, nephrotoxic side effects occur in 15% or more of patients receiving the drug, its therapeutic use, however, is associated with considerable risk [1–4]. Extensive morphological and functional studies [5–7] have provided increasing evidence that aminoglycosides and other nephrotoxins exert their detrimental effects by disturbing the membrane phase of tubular cells [8–14]. Among other effects, these alterations have been assumed to cause changes in intracellular electrolyte concentrations which could possibly result in disruption of the cell and finally in cell death [9].

During the last few years attention has been directed at gaining more information about the extent of alterations in cellular electrolyte concentrations during gentamicin treatment. Studies on renal tissue composition employing conventional chemical methods all agree that sodium, chloride and calcium contents rise, but results concerning changes in potassium, magnesium and phosphorus conflict [15–18]. The interpretation of all these data is, however, complicated by the fact that these chemical analyses only provide an average value for the electrolyte concentrations of the whole tissue, including both extra- and intracellular compartments. Since gentamicin is taken up preferentially by the cells of the proximal tubule [19, 20], and since aminoglycoside-induced tubular cell necrosis is confined almost exclusively to this nephron segment [21, 22], only very limited conclusions can be drawn from these results.

A more detailed insight into the mechanism of aminoglycoside-induced cell damage might be gained if the alterations in electrolyte concentrations of individual renal tubular cells were precisely known. A technique with which this goal can be realized is electron microprobe analysis of freeze-dried cryosections. This method allows the determination of element concentrations in individual, morphologically identified tubular cells [23–25], and was thus employed in the present investigation to determine sodium, potassium, magnesium, chloride and phosphorus concentrations in tubular cells of the rat renal cortex in controls and following 7 or 10 days of gentamicin treatment.

Methods

Treatment of rats

Studies were performed on 19 male Munich Wistar rats (SAVO, Kisslegg, Federal Republic of Germany) initially weighing between 200 and 250 g. Three groups of rats were studied:

Group I. Control rats received a daily subcutaneous injection of 0.20 to 0.25 ml isotonic saline for 7 ($N = 3$) or 10 ($N = 5$) days. Since the data obtained from both groups of control animals did not differ significantly, they will not be shown separately.

Group II. Six rats were injected gentamicin (Sulmycin, Byk-Essex, Munich, FRG) 60 mg/kg body wt/day subcutaneously for a period of 7 days.

Group III. This group followed the same procedure as for group II, but drug administration was continued for 10 days ($N = 5$).

Animals were housed in individual metabolic cages and had free access to food (Altromin, Lage, FRG) and water. Body weight was monitored every day.

Received for publication July 21, 1987
and in revised form January 11, 1988

Preparation of the animals

The animals were anesthetized 24 hours after the last saline or gentamicin injection by intraperitoneal administration (100 to 120 mg/kg body wt) of Inactin (Byk-Gulden, Konstanz, FRG). They were placed on a heated operating table and body temperature maintained between 37 and 38°C. After cannulating the trachea, both the right jugular vein and the left femoral artery were cannulated with polyethylene tubing for the infusion of saline and for monitoring blood pressure and periodic withdrawal of blood samples, respectively. The left kidney was approached via a flank incision, freed from fat and connective tissue, and placed into a plexiglass cup. The ureter was cannulated close to the renal pelvis for the timed collection of urine samples. Paraffin oil, heated to 38°C, was dripped continuously on the exposed kidney surface throughout the experiment to prevent heat and water loss. For the determination of glomerular filtration rate, polyfructosan (Inutest, Laevosan Gesellschaft, Linz, Austria) dissolved in isotonic saline (3 g/100 ml) was given via the jugular catheter initially as a priming injection (2 to 3 ml/kg body wt), followed by constant infusion (4 to 6 ml/kg body wt/hr). Urine samples were collected for 30 minute intervals and blood samples were taken at the midpoints of the urine collection periods.

At the end of the clearance period the kidney surface was thoroughly flushed with isotonic saline previously warmed to 38°C and carefully blotted free of oil and saline. Immediately thereafter the clean kidney capsule was covered with a thin layer of albumin Ringer's solution, which served as a standard for the quantification of the cellular X-ray spectra. The albumin standard solution consisted of 20 g bovine albumin dissolved in 100 ml of Ringer's solution and contained 145, 118, and 4.5 mmol/kg wet weight of sodium, chloride and potassium, respectively. The kidney was then removed rapidly from the animal and shock-frozen in a 1:3 (vol:vol) mixture of isopentane and propane at −196°C. The snap-frozen kidneys were subsequently stored in liquid nitrogen.

Preparation of freeze-dried cryosections and electron microprobe analysis

While immersed in liquid nitrogen the frozen tissue was fractured with a precooled scalpel blade. Small pieces from the kidney surface containing the adherent standard albumin layer were sandwiched between two pliable indium foils and then firmly mounted into a clamp-type holder, which was subsequently inserted into the cutting arm of a precooled ultracryomicrotome (modified Om U3, Reichert, Vienna, Austria). Cryosections about 1 μm thick were cut between −80 and −90°C and then freeze-dried at −80°C and 10^{-6} Torr.

Electron microprobe analysis was performed in a scanning electron microscope (S 150, Cambridge Ltd, Cambridge, UK) using an energy-dispersive detector system (Link Systems, High Wycombe, UK). Areas of about 1 μm^2 were scanned for 100 seconds and the emitted X-rays were analyzed in the energy range between 0 and 20 KeV. The acceleration voltage was 20.0 kV and the probe current 0.3 nA. Cellular measurements were confined to those tubular cells which were recognized as either proximal or distal convoluted tubular cells according to the presence or absence of brush border. The distal convoluted tubule is lined by one cell type only. Connecting and collecting tubules characterized by a mixture of light and dark appearing cells were omitted. Cellular analyses were restricted to the cell nuclei, to avoid contamination from extracellular compartments such as basolateral infoldings [23, 25]. In necrotic cells, where the nucleus had disappeared, the cytoplasm was also scanned. Proximal tubule cells with intact, continuous cell membranes were designated as "non-necrotic" cells, even if they exhibited rarefication of the brush border and slight clumping of nuclear chromatin. Cells in which rupture of the cell membrane was apparent were regarded as necrotic cells.

Quantification of the cell element concentrations and the cellular dry weight was achieved by comparing the element characteristic radiations and the white radiation of the cellular spectra with those of the "internal" albumin standard. The separation of peaks and continuum was performed by computer with a specially designed deconvolution program. Details of the preparation of freeze-dried cryosections for microanalysis and the quantification procedure have been described in previous publications [23–25].

Chemical analysis of fluid samples

Sodium and potassium concentrations in the albumin standard solution and the biological fluids were determined by flame photometry (Flame Photometer 534, Instrumentation Laboratory, Lexington, Massachusetts, USA), chloride concentrations by chloridometry (Eppendorf, Hamburg, FRG). Osmolality was measured by the depression of vapor pressure (5100 B, Wescor Inc, Logan, Utah, USA). The polyfructosan concentrations in plasma and urine were determined photometrically using the anthrone method. Dry weight of the standard solutions was measured by drying to constant weight at 80°C in an evacuated oven.

Presentation of data and statistical analysis

The cellular element concentrations are given in mmol/kg wet wt and the cell dry weight as g dry matter/100 g wet wt (g/100 g). Unless otherwise stated, the results are expressed as means ± SEM. Statistical analyses were performed by Student's *t*-test for unpaired data. Differences were regarded as statistically significant if the two-tailed probability was less than 0.05.

Results

In both control and gentamicin-treated rats, body weight increased by 20 ± 5 g over the initial 7 days. After 10 days, however, the control animals further gained body weight (change: 33 ± 4 g), whereas gentamicin animals lost weight, returning to their initial weight (net change: plus 3 ± 1 g).

As shown in Table 1, no significant differences in plasma osmolality, sodium, potassium, and chloride were observed between control and gentamicin treated rats. The progressive increase in urine flow rate seen in the course of continuing gentamicin administration was paralleled by a significant decrease in urine osmolality and urine/plasma inulin concentration ratios (U/P_{in}). Prolonged gentamicin administration resulted in a progressive decline in glomerular filtration rate from 0.41 ± 0.01 ml/min/100 g body wt in controls, to 0.30 ± 0.01 after 7 days and 0.12 ± 0.02 after 10 days. This was accompanied by a rise in fractional excretion rates of both Na and K from 0.04 and 10.5% in controls to 0.09 and 18.9% after 7 days, and to 0.66 and 50.8% after 10 days of gentamicin treatment, respectively.

Table 1. Body weight, plasma composition and renal function in control rats and after 7 or 10 days of gentamicin treatment

	Control	Gentamicin 7 days	Gentamicin 10 days
Body weight *g*	248 ± 4	259 ± 7	239 ± 4
Plasma sodium *mmol/liter*	142.0 ± 1.3	141.7 ± 2.5	142.5 ± 1.5
Plasma potassium *mmol/liter*	4.6 ± 0.1	4.4 ± 0.2	4.7 ± 0.3
Plasma chloride *mmol/liter*	100.6 ± 0.8	99.7 ± 1.4	98.7 ± 1.2
Plasma osmolality *mOsm/kg*	285 ± 3	285 ± 1.3	295 ± 4
Urine flow rate *μl/min/100 g body wt*	0.75 ± 0.04	1.29 ± 0.12	1.43 ± 0.17[a]
Urine osmolality *mOsm/kg*	2613 ± 117	1382 ± 59[a]	996 ± 100[a,b]
Urine/plasma inulin ratio	600.0 ± 39.6	220.2 ± 16.3[a]	84.3 ± 11.7[a,b]
GFR *ml/min/100 g body wt*	0.41 ± 0.01	0.30 ± 0.01[a]	0.12 ± 0.02[a,b]
Fractional sodium excretion %	0.040 ± 0.003	0.093 ± 0.023[a]	0.66 ± 0.10[a,b]
Fractional potassium excretion %	10.5 ± 1.8	18.9 ± 3.0[a]	50.8 ± 3.5[a,b]
No. of animals	8	6	5

Values are means ± SEM
[a] Significantly different from corresponding control value
[b] Significantly different from corresponding value obtained after 7 days of gentamicin treatment

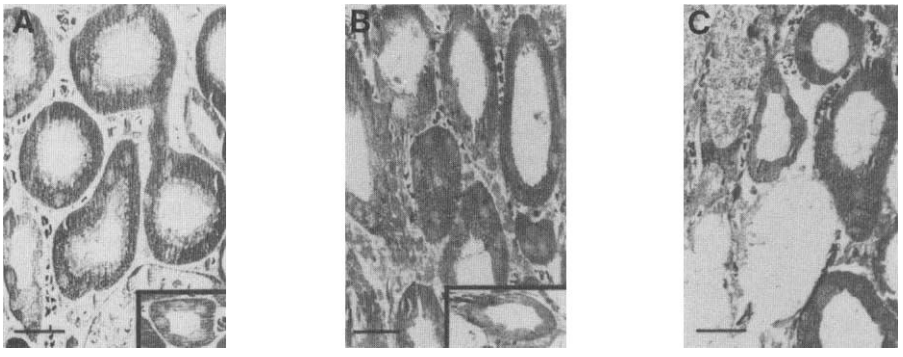

Fig. 1. *Scanning transmission electron micrographs of freeze-dried cryosections under control conditions (A) and following 7 (B) or 10 (C) days gentamicin administration.* In each panel a distal convoluted tubule is shown on the lower right corner (in A and B as inserts).

Table 2. Nuclear element concentrations and dry weights in proximal tubular cells under control conditions and following 7 or 10 days of gentamicin administration

	N	Na	K	Cl	P	Dry weight *g/100 g*
		mmol/kg wet wt				
Control	425	14.6 ± 0.3	136.4 ± 1.4	17.3 ± 0.2	138.1 ± 1.5	22.3 ± 0.2
Gentamicin *7 days*	442	20.6 ± 0.4[a]	130.2 ± 1.2[a]	16.8 ± 0.3	122.8 ± 1.4[a]	22.4 ± 0.2
Gentamicin *10 days*	302	22.0 ± 0.8[a]	128.2 ± 1.8[a]	20.6 ± 0.6[a,b]	113.0 ± 1.6[a,b]	22.9 ± 0.2

Values are means ± SEM
[a] Significantly different from corresponding control value
[b] Significantly different from corresponding value obtained after 7 days of gentamicin treatment

Examples of scanning transmission electron micrographs of freeze-dried cryosections obtained from controls and following a 7 or 10 days gentamicin treatment are shown in Figure 1. After 10 days on gentamicin, proximal tubules exhibit a variable pattern of morphological alterations ranging rarefication and loss of brush border up to frank cell necrosis and loss of cellular integrity. After 7 days of gentamicin administration proximal tubular cell necrosis was rarely encountered, whereas alterations and loss of brush border were common. Distal convoluted tubular cells did not show appreciable morphological changes after either 7 or 10 days.

Table 2 summarizes the effect of 7 or 10 days of gentamicin treatment on element concentrations and dry weight of non-necrotic proximal tubular cells. The gradual decline in cell potassium concentration during continuing gentamicin treatment was accompanied by an almost equivalent rise in cell sodium concentration. Chloride concentrations were not significantly altered in proximal tubular cells after 7 days gentamicin treatment, but were increased from 17.3 ± 0.2 mmol/kg wet wt in controls to 20.6 ± 0.6 after 10 days. The sodium, potassium and chloride concentrations of proximal tubular cells after 10 days gentamicin administration were not different from those observed after 7 days.

While the sodium concentration of distal convoluted tubular

Table 3. Nuclear element concentrations and dry weights in distal tubular cells under control conditions and following 7 or 10 days of gentamicin administration

	N	Na	K	Cl	P	Dry weight
		mmol/kg wet wt				*g/100 g*
Control	86	9.4 ± 0.6	144.8 ± 3.4	9.2 ± 0.4	191.5 ± 4.6	20.1 ± 0.4
Gentamicin *7 days*	103	10.2 ± 0.6	141.4 ± 2.7	9.5 ± 0.4	173.1 ± 3.8[a]	21.6 ± 0.4[a]
Gentamicin *10 days*	113	9.4 ± 0.7	149.1 ± 2.6[b]	13.0 ± 0.7[a,b]	168.4 ± 3.3[a]	23.4 ± 0.3[a,b]

Values are means ± SEM
[a] Significantly different from corresponding control value
[b] Significantly different from corresponding value obtained after 7 days of gentamicin treatment

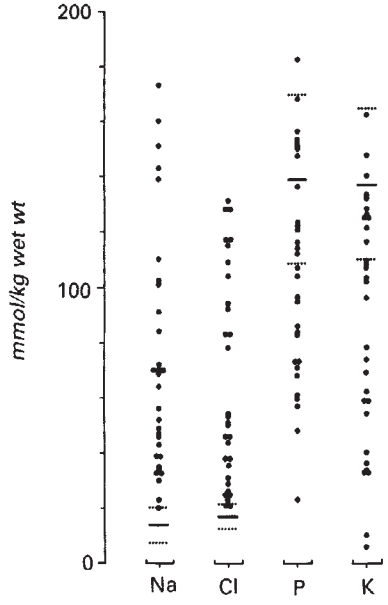

Fig. 2. *Distribution of element concentrations in necrotic cells following 10 days of gentamicin treatment.* Mean values and standard deviations obtained in control animals are given as solid and dotted lines, respectively.

cells was not significantly altered by gentamicin treatment (Table 3), chloride concentration was higher at 13.0 ± 0.7 mmol/kg wet wt after 10 days on gentamicin compared with 9.2 ± 0.4 in controls. Potassium concentration in these cells was slightly but significantly increased after 10 days of gentamicin treatment compared with the data obtained after 7 days on gentamicin.

In both proximal and distal convoluted tubular cells phosphorus concentrations declined progressively during gentamicin administration. In proximal tubular cells phosphorus concentrations were decreased from 138.1 ± 1.5 in controls to 122.8 ± 1.4 mmol/kg wet wt after 7 days and to 113.0 ± 1.6 after 10 days of gentamicin treatment. The corresponding values for the distal convoluted tubular cells were: 191.5 ± 4.6, 173.1 ± 3.8, and 168.4 ± 3.3 mmol/kg wet wt, respectively.

While the dry weight of proximal tubular cells was not significantly changed during the course of gentamicin treatment, the dry weight of distal convoluted tubular cells increased progressively from 20.1 ± 0.4 in controls to 21.6 ± 0.4 after 7 days and 23.4 ± 0.3 g% after 10 days of gentamicin administration.

As depicted in Figure 2, element concentrations in necrotic cells showed a considerable scatter, varying from values similar to controls to cellular electrolyte concentrations typical of extracellular compartments. While proximal cell magnesium concentration was not significantly affected by gentamicin treatment in non-necrotic cells, the concentration of this divalent cation was markedly lower in necrotic cells than in proximal tubule cells of control animals (6.6 ± 0.8 [N = 34] vs. 9.0 ± 0.2 [N = 425] mmol/kg wet wt).

Discussion

In the present study electron microprobe analysis was employed to monitor the effect of long-term gentamicin administration on the element composition of individual proximal and distal tubular cells. Several studies dealing with the effect of aminoglycoside treatment on renal tissue electrolyte contents are available in the literature [15–18]. These results, however, are firstly conflicting and secondly difficult to interpret, since the mean tissue element concentrations reported include both extra- and intracellular compartments. Hence, for instance, a moderate increase in tissue sodium content could indicate slight damage to all cells or severe injury of only a limited number of cells. These data thus are of limited value for the assessment of the impact of long-term gentamicin administration on cell ionic homeostasis.

The present results clearly demonstrate that this aminoglycoside antibiotic exerts its main, but not exclusive, effect on proximal tubular cells and is thus in agreement with previous studies. In addition, it could be shown that in distal tubular cells alterations in phosphorus, but not in sodium and potassium concentrations, occur during prolonged gentamicin treatment.

In proximal tubular cells exhibiting no signs of necrosis (Table 2) progressive increases in sodium and chloride concentrations and concomitant decreases in potassium and phosphorus concentrations were observed during the course of gentamicin administration. Even after seven days of gentamicin treatment, when cell morphology was only mildly affected, cell element composition was quite definitely altered. These changes in proximal tubular cell element pattern were accompanied by a deterioration of overall kidney function, such as diminished glomerular filtration rate and lower urine osmolalities. After 10 days of gentamicin administration the alteration in cell electrolyte and cell phosphorus concentrations were even further accentuated than after 7 days of exposure. The observation that long term administration of gentamicin induces increases in proximal cell sodium and chloride concentrations is in accord

with studies using chemical analysis on renal cortical tissue [15–18].

Conflicting results, however, have been reported with respect to changes in tissue potassium and phosphorus contents after long-term gentamicin administrations [15–18]. This agent has been reported to cause tissue potassium content to increase [16, 17], to decrease [15] or not to change [18]. Kidney phosphorus content has been claimed to decrease under gentamicin treatment [15, 18] or not to alter [16]. The present data obtained directly in individual renal tubular cells clearly demonstrate that both proximal tubular cell potassium and phosphorus concentrations are diminished by aminoglycoside treatment.

A rise in cell sodium concentration can be brought about, in principle, by a decrease in sodium efflux or by increased sodium influx or by a combination of both. These changes in the transmembrane fluxes of electrolytes need not necessarily be due to the direct action of gentamicin on proximal tubule cells, but could in principle be epiphenomena of gentamicin nephrotoxicity. This latter possibility, however, seems less likely, since there is convincing evidence demonstrating that Na-K/ATPase activity of renal tubular cells is impaired and membrane sodium permeability increased by gentamicin [9, 14–16, 18, 26–28]. The fall in cell potassium concentration could be due to either diminished potassium uptake via the Na-K/ATPase or to an enhanced efflux caused by increased potassium membrane permeability or depolarization of the cell electrical potential. Such a depolarization could also explain the observed rise in cell chloride concentration. The present data do not allow discrimination between these alternatives.

It is conceivable that these alterations in cell membrane transport properties are related to the effect of gentamicin on lysosomal enzymes and the consequent phospholipidosis [29]. Proposals concerning the impact of this derangement of lysosomal enzyme function on active and passive membrane transport pathways must, however, remain highly speculative, since the relation of the aminoglycoside-induced phospholipidosis to cellular dysfunction and necrosis is as yet unresolved [29].

The element pattern obtained in those cells exhibiting clear signs of necrosis was not uniformly characterized by low potassium and phosphorus and high sodium and chloride concentrations. Astonishingly enough, in these cells element concentrations were obtained quite often which deviated only modestly from control values (Fig. 2). This finding suggests that the mechanisms subserving the maintenance of ionic homeostasis may operate quite effectively in cells on the brink of disintegration.

In view of a possible pathogenetic role of cellular magnesium depletion in aminoglycoside nephrotoxicity [11], the observation of unaltered magnesium concentrations in intact and of diminished concentrations in necrotic cells deserves closer attention. This finding might indicate that perturbation of cell magnesium homeostasis is not an early pathogenetic event in gentamicin-mediated renal injury. It should be kept in mind, however, that magnesium concentrations were not assessed directly in the mitochondria—those cellular organelles where gentamicin is thought to interact with magnesium-sensitive cation permeabilities [30–36].

In distal convoluted tubular cells no significant histological alterations or perturbations in monovalent ion concentrations could be observed in any stage of gentamicin nephroptoxicity, except for a slight increase in cell chloride and potassium concentrations at day 10. It is conceivable that these changes as well as the increase in dry weight observed in these cells during prolonged gentamicin administration might reflect adaptive changes to alterations in solute load presented to the distal tubule [37]. Such an increase in distal tubule solute load can be envisaged to result from deterioration in proximal tubular function. As in proximal tubule cells, phosphorus concentrations of distal convoluted tubule cells showed a progressive decline during the course of gentamicin treatment. Thus loss of nuclear phosphorus due to aminoglycoside administrations is the sole common feature obtained for both proximal and distal tubular cells.

Acknowledgments

Parts of this work were published in abstract form in *Proc Int Congr Nephrol* 10: 474, 1987. Financial support was provided by the Deutsche Forschungsgemeinschaft. Osamu Matsuda was the recipient of a research fellowship from the Alexander von Humboldt-Stiftung. The technical assistance of M. Schramm, P. Jauernig, and I. Ötztürk is gratefully acknowledged. We are indebted to Dr. John Davis for his help in preparing the manuscript.

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