

Renal Adaptation to Gentamicin-Induced Mineral Loss

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Key Words

Renal adaptation · Calcium · Magnesium · Gentamicin

Abstract

Background: Gentamicin, a well-known nephrotoxic drug, affects calcium and magnesium homeostasis. Although gentamicin induces urinary calcium and magnesium wasting immediately, it rarely causes significant hypocalcemia or hypomagnesemia clinically. **Methods:** We conducted an animal study to investigate the renal adaptation in calcium and magnesium handling after gentamicin treatment and effects on the expression of calcium and magnesium transport molecules in distal tubule. Gentamicin (40 mg/kg) was injected daily in male Sprague-Dawley rats (220–250 g) for up to 7 days. **Results:** This treatment did not affect serum creatinine, calcium, or magnesium levels. Gentamicin induced significant hypercalciuria (14-fold) and hypermagnesiuria (10-fold) in 6 h, which was associated with upregulation of TRPV5 (175 ± 3%), TRPV6 (170 ± 4%), TRPM6 (156 ± 4%) and calbindin-D28k (174 ± 3%; all $p < 0.05$ vs. control). This gene upregulation was maintained with daily injection of gentamicin for 7 days. The gentamicin-induced urinary calcium loss was reduced by 80% at days 3 and 7, while magnesium loss was reduced by 52 and 57% at days 3 and 7, respectively. On the other hand, urinary loss of potassium became worse

on day 7 (2-fold), and phosphorus loss worse from day 3 to day 7 (3-fold). **Conclusion:** There is a rapid adaptation to gentamicin-induced hypercalciuria and hypermagnesiuria. The upregulation of distal tubule transport molecules, TRPV5, TRPV6, TRPM6 and calbindin-D28k occurs within 6 h of gentamicin treatment. This renal adaptation prevents further mineral loss due to gentamicin treatment.

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Introduction

Aminoglycosides are commonly used for Gram-negative bacterial infection of respiratory and urinary tracts. However, nephrotoxicity has significantly limited the use of aminoglycosides [1]. In addition to direct tubular damage especially in the proximal tubule, renal vasoconstriction and mesangial contraction also play important roles in aminoglycoside nephrotoxicity, which leads to reduced glomerular filtration rate and elevated serum creatinine levels as recently reviewed by Lopez-Novoa et al. [2]. Aminoglycosides also have specific effects on mineral metab-

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olism causing renal calcium and magnesium wasting. Unlike acute tubular necrosis which usually occurs after a few days of administration, hypercalciuria and hypermagnesiuria occur immediately after aminoglycosides are administered as well as during chronic therapy [3, 4].

The mechanisms responsible for renal divalent cation loss have not been fully elucidated yet. Gentamicin is known as a type I calcium-sensing receptor (CSR) agonist which activates CSR without the presence of calcium [5]. Activation of CSR by calcium has been shown to reduce sodium-potassium-chloride cotransporter (NKCC2) transport activities [5] and downregulate NKCC2 expression in the kidney [6]. It is likely that gentamicin induces calcium and magnesium wasting via a furosemide-like effect by inhibiting paracellular transport of calcium and magnesium in the thick ascending limb (TAL). On the other hand, in vivo micropuncture study conducted by Parsons et al. [7] indicated that early distal tubule rather than the proximal tubule or loop of Henle was the responsible segment for gentamicin-induced calcium wasting.

The distal nephron constitutes an important part for both water and electrolyte handling. Most of the filtered magnesium is reabsorbed in the TAL of Henle, and this segment along with distal convoluted tubule (DCT) is also a critical site for calcium transport [8]. In recent years, renal epithelial calcium and magnesium channels distributed in distal nephron TRPV5 and TRPM6 have been identified and found to play important roles in a variety of physiological and pathological processes [9]. More recently, new modulators such as klotho and WNKs that regulate these cation channels were identified, and these molecules exhibit unique upstream regulation mechanism [10, 11].

Although gentamicin induces urinary calcium and magnesium loss immediately, in the majority of patients who receive gentamicin, calcium and magnesium loss does not cause significant hypocalcemia or hypomagnesemia. Only sporadically has a syndrome similar to Bartter syndrome type 5 (gain of function mutation of CSR) with hypokalemia, hypomagnesemia and hypercalciuria been reported in gentamicin-treated patients [12, 13]. In the present study, we conducted an animal study to investigate the renal adaptation of calcium and magnesium handling during gentamicin administration, and gentamicin effects on the gene expression of calcium and magnesium transport molecules in the DCT. Aminoglycosides differ slightly or considerably in terms of tubulotoxicity [14, 15]. We selected gentamicin for our study because it is the most commonly used aminoglycoside and has been investigated most intensively.

Table 1. Lists of primers used for real-time PCR

Gene	Forward primer	Reverse primer
β-Actin	agtacccattgaacacggc	ttttcacggtggccttagg
CBD-28k	ggagctgcagaacttgatcc	gcagcaggaatctcttcg
TRPV5	tgttaccgccccctcaag	cacagcccaatgactgtca
TRPV6	atccgccgatgcaca	agttttctcctgagcttttcca
TRPM6	aaagccatgcgagttatcagc	cttcacaatgaaaacctgccc
NCC	gaacggcacaccattgtaga	gccttgactcccactccat
PCLN-1	ggaattcgtacctgcatgag	cacgcagtaagccaagga
CSR	cagatgcgagtgctgtga	agcaccgcaaagagagtgaga
Klotho	gtgacagccaatggaatcga	aatacgaagtagccaaaagg
WNK1	ggttagaactgcgacgactac	atcggaccttgctgccttgc
WNK4	cagcgaagcgggagaag	ggcgaagaggaaggattgg

CBD-28k = Calbindin-D28k; TRPV5, TRPV6 and TRPM6 = transient receptor potential V5, V6 and M6; PCLN-1 = paracellular-1; WNK1 and WNK4 = with no lysine kinase 1 and 4.

Methods

Animals

Male Sprague-Dawley rats (220–250 g) were purchased from animal center, National Science Council, Taiwan, Republic of China. All animals were maintained at 21–23°C and exposed to a normal 12-hour light cycle. They were allowed free access to selected food and water. The food contained sodium (0.2%), calcium (1.0%), and magnesium (0.16%). This study was approved by Institutional Animal Care and Use Committee of the Chang-Gung Memorial Hospital.

Experimental Protocol (6–8 Animals in Each Experiment)

Male Sprague-Dawley rats (220–250 g) were injected with gentamicin (40 mg/kg) daily for up to 7 days. We selected this dose because it has been shown to cause urinary calcium and magnesium wasting without raising serum creatinine levels or causing any systemic effects [16, 17]. Blood and urine samples were collected at 6 h, 3 days, and 7 days for biochemistry studies. Urine samples were collected using individual metabolic cages. Kidneys were harvested at 6 h, 3 days and 7 days after treatment, and processed for measuring mRNA and protein abundance of molecules involved in calcium and magnesium metabolisms using real-time reverse transcription polymerase chain reaction (RT-PCR), and immunoblot and immunohistochemistry, respectively.

Biochemical Measurement

Measurements of urine and serum levels of sodium, potassium, calcium, magnesium, and phosphorus were performed as previously described [18]. Serum intact parathyroid hormone (iPTH) levels were measured using Rat Intact PTH ELISA Kit (Immutopics Inc., San Clemente, Calif., USA).

Molecular Studies

RNA Isolation and Complementary DNA Synthesis

Renal cortex tissue was dissected, and total RNA was isolated using TRIZOL reagent (Invitrogen, Carlsbad, Calif., USA). Re-

Table 2. Biochemical data after gentamicin treatment

	Control	3 days	7 days
Serum creatinine, mg/dl	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Serum Ca, mg/dl	9.2 ± 0.3	9.5 ± 0.4	9.1 ± 0.5
Serum Mg, mg/dl	1.8 ± 0.3	2.1 ± 0.4	1.9 ± 0.4
Urine Ca excretion, mg/100 g/day	0.40 ± 0.05	1.00 ± 0.13*	1.05 ± 0.12*
Urine Mg excretion, mg/100 g/day	0.36 ± 0.14	1.69 ± 0.17*	1.51 ± 0.13*
Urine Na excretion, mmol/100 g/day	1.14 ± 0.1	1.17 ± 0.05	1.24 ± 0.05
Urine K excretion, mmol/100 g/day	1.09 ± 0.31	1.31 ± 0.09	2.3 ± 0.40*
Urine P excretion, mg/100 g/day	0.16 ± 0.05	0.51 ± 0.20	0.49 ± 0.09*

* p < 0.05 vs. control.

verse transcription for cDNA synthesis was performed using a reverse transcription system (Promega, Madison, Wisc., USA).

Real-Time Polymerase Chain Reaction

We analyzed gene expression using real-time RT-PCR. The molecules involved in calcium and magnesium transport, including TRPV5, TRPV6, calbindin-D28k, TRPM6, and paracellin-1, and molecules involved in regulation of these transporters, including CSR, vitamin D receptor (VDR), klotho, WNK1, WNK4, and sodium chloride cotransporter (NCC) were studied. β -Actin was used as the internal reference for each gene investigated. The synthesized cDNA was then subject to real-time PCR using the ABI prism 7900 HT Sequence Detection System (ABI, Foster City, Calif., USA). The emission signal was assessed using the fluorescent dye SYBR Green (ABI). The primer sequences of the studied genes are listed in table 1. To determine the gene expression, genes investigated in the present study were calculated as $2^{(\beta\text{-actin Ct} - \text{target gene Ct})}$, where Ct represents the first cycle at which the output signal exceeds the threshold signal [18]. PCR reaction of each gene was performed in triplicate to obtain a mean value. The changes in gene expression are presented as percentages (%) of control group animal values.

Immunoblotting Study

Renal cortex sections were frozen at -80°C and then homogenized at 4°C in protein lysis buffer solution containing 20 mM Tris-HCL (pH 7.4), 0.1% sodium dodecyl sulfate, 5 mM EDTA, 1% Triton X-100, and a protease inhibitor cocktail tablet (Roche, Penzberg, Germany). After centrifugation at 12,000 g for 10 min at 4°C , the protein concentrations were determined using bicinchoninic BCA protein assays. The protein samples were then run on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. β -Actin was used as the internal control in this study. For calbindin-D28k immunoblotting, membranes were incubated with goat anti-mouse calbindin-D28k monoclonal antibody (1:5,000, Sigma) first, and subsequently incubated with goat anti-rabbit antibody conjugated with horseradish peroxidase (1:30,000). The abundance of calbindin-D28k was then quantified by densitometric analyses. The changes in protein abundance are presented as percentages of control animal values.

Immunofluorescence Microscopy

Frozen kidney cortex tissue was used for calbindin-D28k, TRPV5 and TRPM6 protein assessment. Slices of 5 μm thickness were fixed with 4% paraformaldehyde for 15 min and incubated with primary antibody (goat anti-mouse calbindin-D28k monoclonal antibody 1:500, Sigma; rabbit anti-rat ECaC1 1:100, Alpha Diagnostic International, San Antonio, Tex., USA; Guinea pig anti-TRPM6 polyclonal antibody 1:50, Neuromics, Edina, Minn., USA) for 16 h, and then with FITC-conjugated secondary antibody for calbindin-D28k (Jackson ImmunoResearch Laboratories Inc.), streptavidin/FITC-conjugated secondary antibody for TRPV5 (DakoCytomation, Dako Corporation, Carpinteria, Calif., USA), and CyTM3-conjugated AffiniPure F(ab')₂ for 30 min and Cy3-conjugated donkey anti-guinea pig for TRPM6 (1:1,000, Jackson ImmunoResearch, Laboratories Inc.). The immunofluorescence pictures were then taken using a Zeiss fluorescence microscope connected with a digital photo camera (Evolution VF, MediaCybernetics). Semiquantitative determination of the protein expression was performed with the Image-Pro Plus 5.0 image analysis software. Amount of protein was expressed as mean of integrated optimal density. The alternation is expressed as percentage of control animals.

Statistical Analyses

Data are presented as mean \pm SEM. Statistical analyses of the data were performed using SPSS-PC software. Unpaired Student's t tests were used to compare differences between two groups. To determine the significant difference among controls, and different time points of gentamicin treatment, one-way analysis of variance and Tukey's test were used. A p value of less than 0.05 was considered statistically significant for all tests.

Results

Gentamicin Does Not Affect Renal Function or Serum Calcium and Magnesium Levels

As shown in table 2, gentamicin administration at 40 mg/kg per day for 3 or 7 days did not affect serum cre-

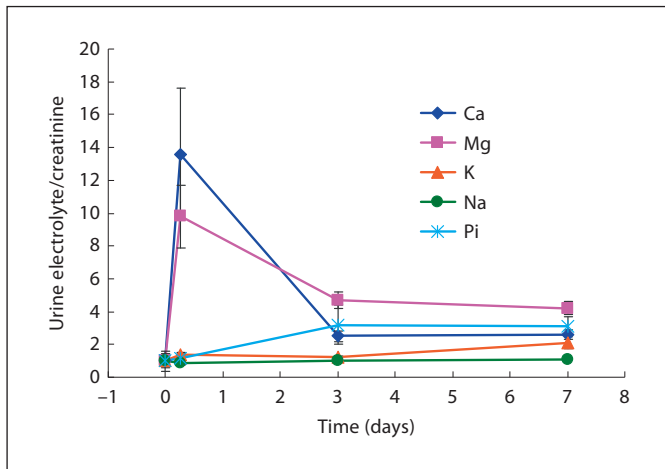


Fig. 1. Time course of changes in renal excretion of electrolytes after gentamicin treatment. Renal excretion of electrolytes is expressed as relative urine electrolyte/creatinine ratio with the time 0 value set as 1.0. $p < 0.05$ vs. time 0 for all time points for Ca and Mg, for K at 6 h and 7 days, and for Pi at 3 and 7 days. There were 6 animals for each time point.

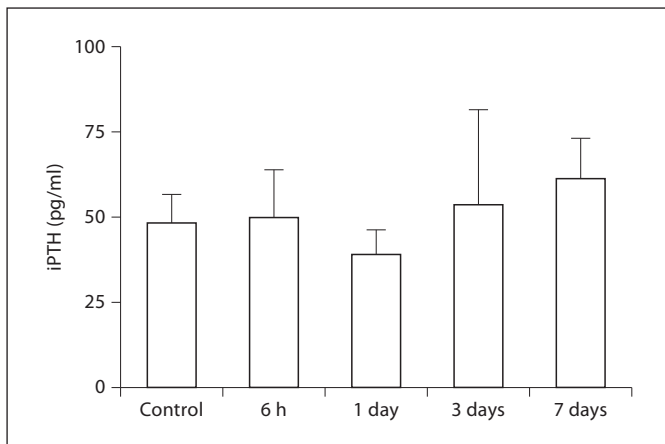


Fig. 2. Gentamicin treatment does not affect serum iPTH levels at various time points.

atinine level, indicating that at this dosage, renal function stayed unchanged. Furthermore, both serum calcium and magnesium levels were unchanged on both day 3 and day 7.

Effect of Gentamicin on Renal Excretion of Minerals and Electrolytes

Single-dose gentamicin administration induced significant calciuria and magnesiuria (urine calcium/creati-

nine: 0.11 ± 0.02 vs. 1.50 ± 0.44 , magnesium/creatinine: 0.12 ± 0.05 vs. 1.17 ± 0.23 , both $p < 0.05$). Urinary potassium excretion was also increased (potassium/creatinine: 3.38 ± 0.15 vs. 4.63 ± 0.17 , $p < 0.05$). There was no significant change in urinary sodium or phosphate excretion (sodium/creatinine: 2.21 ± 0.13 vs. 1.92 ± 0.23 ; phosphate/creatinine: 0.34 ± 0.21 vs. 0.39 ± 0.12). To evaluate renal adaptation to mineral loss, rats received daily injection of gentamicin, and urine electrolytes were collected on day 3 and day 7. The time course of changes in urine calcium, magnesium, sodium, potassium and phosphorus is shown in figure 1. A single gentamicin (40 mg/kg) induced significant hypercalciuria (14-fold) and hypermagnesiuria (10-fold) at 6 h. The gentamicin-induced urinary calcium loss was reduced by 80% on days 3 and 7, while magnesium loss was reduced by 52 and 57% on days 3 and 7, respectively. As comparison, urine sodium excretion was unaffected by gentamicin, while urinary potassium excretion was increased on day 7 (2-fold), and phosphorus was increased on days 3 and 7 (both 3-fold).

Effects of Gentamicin on Serum iPTH Level

Gentamicin exhibits calcimimetic effects and suppresses PTH release in dispersed parathyroid cells [19]. We measured serum iPTH levels at 6 h, 24 h, day 3 and day 7 after daily gentamicin injection to evaluate the role of iPTH on gentamicin-induced mineral loss. As shown in figure 2, gentamicin at 40 mg/kg did not have any effect on serum PTH levels in our studies.

Effects of Gentamicin on Gene Expression of DCT-Specific Calcium and Magnesium Transport Proteins

Our RT-PCR studies showed that mRNA abundance of DCT calcium and magnesium transport proteins was significantly increased 6 h after gentamicin administration: TRPV5 ($175 \pm 3\%$ of control), TRPV6 ($170 \pm 4\%$), calbindin-D28k ($174 \pm 3\%$) and TRPM6 ($156 \pm 4\%$). The upregulation of these proteins persisted at day 7 after daily injection of gentamicin: TRPV5 ($184 \pm 2\%$), TRPV6 ($198 \pm 2\%$), calbindin-D28k ($196 \pm 4\%$) and TRPM6 ($173 \pm 3\%$).

Other mineral transport-related proteins such as CSR, and paracellin-1, or key regulating proteins such as VDR, NCC, klotho, WNK1 and WNK4 were not affected by gentamicin treatment (table 3).

To assess whether protein expression was also increased by gentamicin, we performed immunoblotting and immunofluorescent staining studies to semiquantitate protein amount of calbindin-D28k, TRPV5 and

Table 3. Results of gene expression analysis during gentamicin treatment

Studied genes	Control	6 h (%)	7 days (%)
TRPV5	100 ± 1	175 ± 3*	184 ± 2*
TRPV6	100 ± 2	170 ± 4*	198 ± 2*
TRPM6	100 ± 1	156 ± 4*	173 ± 3*
CBD28k	100 ± 1	174 ± 3*	196 ± 4*
NCC	100 ± 1	92 ± 2	113 ± 3
CSR	100 ± 1	107 ± 2	104 ± 2
PCLN-1	100 ± 2	99 ± 3	112 ± 4
VDR	100 ± 1	108 ± 2	90 ± 4
Klotho	100 ± 2	92 ± 2	87 ± 4
WNK1	100 ± 1	114 ± 2	90 ± 4
WNK4	100 ± 1	96 ± 1	109 ± 2

* p < 0.05 vs. control.

TRPM6 in the kidney. Figure 3 shows that 7-day administration of gentamicin significantly increased calbindin-D28k protein as measured by immunoblotting ($202 \pm 5\%$ of control, $p < 0.05$). Similar increase was confirmed with immunofluorescent staining as shown in figure 4 ($213 \pm 5\%$ of control, $p < 0.05$). The immunofluorescent staining of TRPV5 and TRPM6 also revealed a significant increase in abundance of protein (TRPV5: $194 \pm 4\%$ of control; TRPM6: $178 \pm 4\%$ of control, both $p < 0.05$) as shown in figures 5 and 6, respectively.

Discussion

Our study has demonstrated that marked renal calcium and magnesium wasting occurs immediately after gentamicin injection and the wasting of these cations is reduced over time. The conservation of calcium and magnesium is associated with upregulation of DCT calcium and magnesium transport molecules such as TRPV5, TRPV6 and TRPM6 as well as calbindin-D28k. These findings suggest that there is adaptation of the kidney to counter the upstream loss of calcium and magnesium induced by gentamicin.

Both clinical observation and experimental animal studies have demonstrated that acute gentamicin administration produced significant urinary calcium and magnesium wasting [4, 20, 21]. As mentioned earlier, gentamicin can cause symptoms and signs identical to the Bartter syndrome type 5, which is due to a gain of function mutation of the CSR [22]. It has been shown

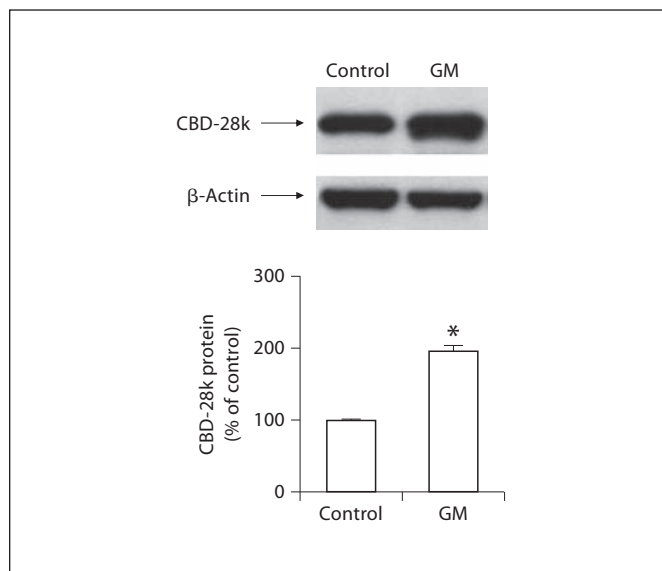


Fig. 3. Immunoblotting results for calbindin-D28k (CBD-28k) for control and gentamicin-treated (GM) animals. The top panel shows representative immunoblots of CBD-28k and β -actin. The molecular weight of CBD-28k is 28 kDa. The relative CBD-28k protein abundance is presented as the CBD-28k to β -actin ratio. The ratio of control animals is set as 100%. * $p < 0.05$ vs. controls.

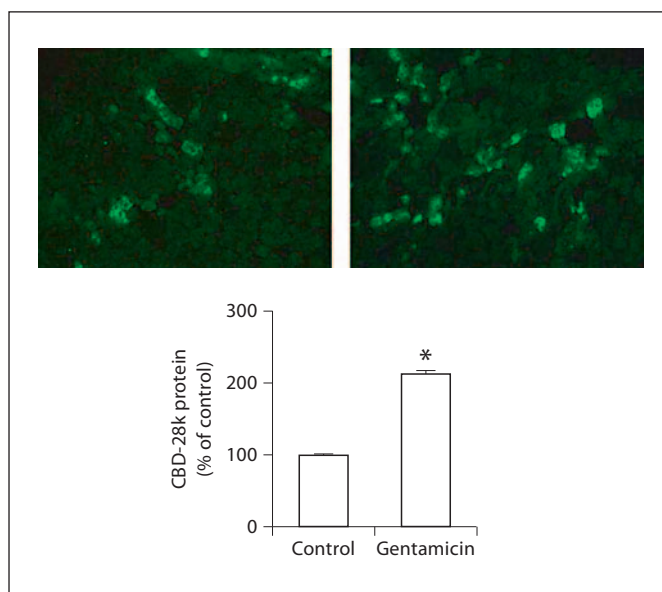


Fig. 4. Immunofluorescent staining of calbindin-D28k (CBD-28k). The top panel shows representative immunofluorescent staining in control (left) and gentamicin-treated (right) animals. CBD-28k is expressed in cytosol of the DCT. There are more positive-staining tubules after gentamicin treatment. The bottom panel shows results of semiquantitative studies. * $p < 0.05$ vs. controls.

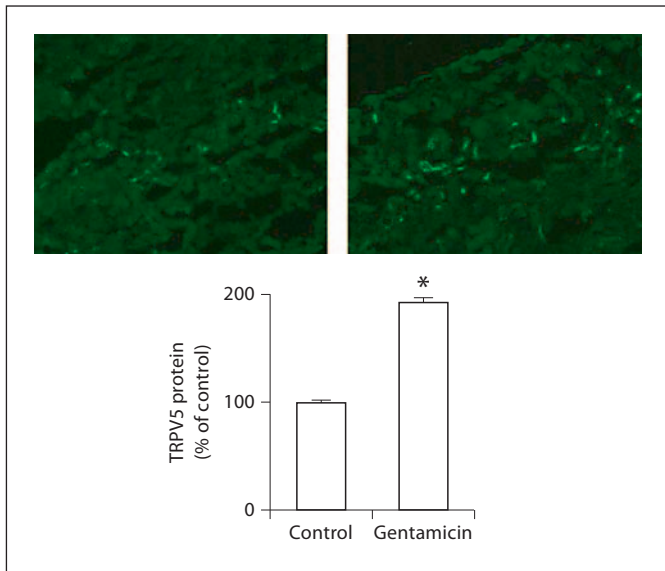


Fig. 5. Immunofluorescent staining of TRPV5. The top panel shows representative immunofluorescent staining in control (left) and gentamicin-treated (right) animals. TRPV5 is expressed in the apical membrane of the DCT. There are more positive staining tubules after gentamicin treatment. The bottom panel shows results of semiquantitative studies. * $p < 0.05$ vs. controls.

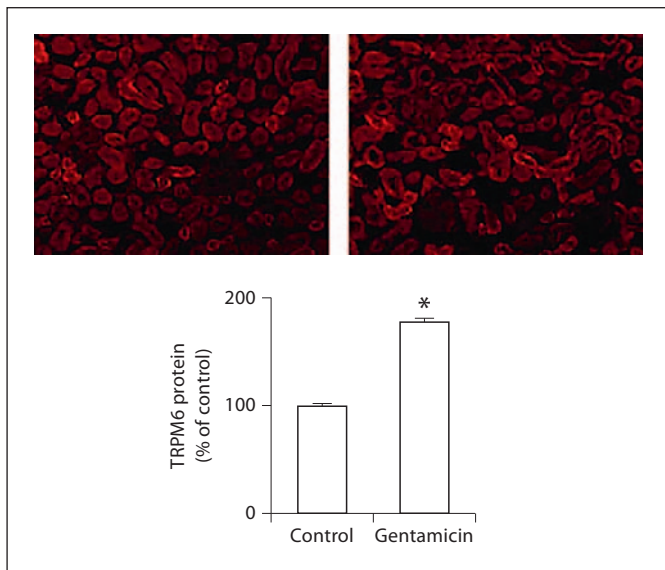


Fig. 6. Immunofluorescent staining of TRPM6. The top panel shows representative immunofluorescent staining in control (left) and gentamicin-treated (right) animals. TRPM6 is expressed in the apical membrane of loop of Henle and DCT. There are more positive-staining tubules after gentamicin treatment. The bottom panel shows results of semiquantitative studies. * $p < 0.05$ vs. controls.

that gentamicin activates CSR without the presence of calcium, and reduces transport activities and synthesis of NKCC2 in the kidney [5, 6, 23]. It is likely that gentamicin induces renal calcium and magnesium loss in the TAL via activation of CSR. The reabsorption of calcium and magnesium in the TAL is through a passive paracellular transport mediated by paracellin-1. We measured the paracellin-1 abundance in the kidney and did not find any changes after gentamicin treatment. This finding indicates that paracellin-1 synthesis is not affected by gentamicin. The driving force of this paracellular transport is the lumen-positive voltage. The decreased sodium reabsorption in TAL by the furosemide-like effect of gentamicin leads to reduced lumen-positive voltage and subsequent reduction in calcium and magnesium reabsorption in the TAL. Previously, Elliott and Patchin [4] demonstrated that coadministration of gentamicin and furosemide produced additive renal calcium wasting and argued that gentamicin and furosemide caused calciuria through different mechanisms. However, their data showed that the maximum urine calcium concentration after coadministration of gentamicin and furosemide was only significantly higher than in the group that received low dose of gentamicin alone (10 mg/kg), but was not different from that of furosemide alone. It is possible that gentamicin did not fully inhibit NKCC2; therefore, an additive effect of furosemide was demonstrated.

In our study, we found that gentamicin caused a profound calcium and magnesium wasting within 6 h after injection: urine calcium and magnesium excretion was 14- and 10-fold normal, respectively. However, over time, calcium and magnesium wasting is reduced to 2.5- and 4.2-fold on day 7 (fig. 1). We found that the reduction in urine calcium and magnesium excretion was associated with upregulation of DCT-specific calcium and magnesium transport proteins including TRPV5, TRPV6, TRPM6 and calbindin-D28k, which occurred within 6 h of gentamicin administration and persisted for 7 days. It is likely that renal adaptation results in increased calcium and magnesium reabsorption in DCT by upregulation of DCT-specific calcium and magnesium transport molecules.

To further explore the mechanisms underlying upregulation of TRPV5 by gentamicin, we examined the probable involvement of recently identified regulatory molecules of TRPV5 such as VDR, klotho, WNK1 and WNK4. We did not detect any changes in the mRNA abundance in these molecules, indicating that their synthesis may not be involved in gentamicin-induced TRPV5 upregula-

tion. Our negative data on serum iPTH after gentamicin treatment also suggest that PTH does not play a role in TRPV5 upregulation by gentamicin. Since calcium has been shown to be a major regulator of TRPV5 [24], it appears that high urine calcium and magnesium concentration in the DCT due to TAL calcium and magnesium wasting stimulates synthesis of TRPV5/6 and TRPM6. Similar mechanisms have been reported in furosemide and high-salt treatment, and in experimental diabetes [18, 25].

The gentamicin-induced renal adaptation may have important clinical significance. This adaptation may be one of reasons why hypocalcemia and hypomagnesemia are uncommon complications of gentamicin and other aminoglycosides [13]. There are some suggestions that hypocalcemia may be more common in neonates after aminoglycoside therapy. Chiruvolu et al. [26] reported that after gentamicin treatment (4 mg/kg/day), 15% of neonates had hypocalcemia (calcium <8.0 mg/dl) and the incidence increased to 24% in neonates with gestation age less than 37 weeks. There are several other factors that may affect serum calcium during the early period after birth. Persistent urinary calcium and magnesium wasting after gentamicin treatment (2.5 mg/kg/12 h) in neonates has been reported by Giapros et al. [21], although in their study, serum electrolyte levels were normal. Whether the differences in serum calcium levels between these two reports are related to different doses of gentamicin remains unclear. Interestingly, TRPV5 and calbindin-D28k increased 4- and 10-fold in the first 3 weeks of life in weaning mice [27]. Further studies are needed to determine whether in neonates, particularly preterm neonates, the renal adaptation to calcium and magnesium wasting in the distal tubule is fully developed.

The effects of gentamicin on sodium, potassium and phosphorus are different from calcium and magnesium. Renal wasting of these electrolytes by aminoglycosides has been reported previously in animal and clinical studies [23, 28, 29]. In our study, gentamicin did not affect renal sodium excretion, but significant renal potassium and phosphorus wasting was detected at day 7 and day 3, respectively. It is possible that sodium wasting is compensated by increased sodium transport activity in DCT and collecting ducts. Upregulation of epithelial sodium channel in outer medulla after gentamicin treatment has been reported [23]. We found that there was a 2-fold increase in urinary potassium excretion on day 7 after gentamicin administration, which is similar to the results from a previous rat study [23]. Our findings of late onset of phos-

phorus wasting induced by gentamicin are also consistent with an earlier neonate study in which phosphorus wasting was only demonstrated on day 7 [29]. Sorribas et al. [30] have demonstrated that gentamicin treatment decreased the expression of sodium phosphate cotransporter (Na/Pi II) protein in the apical membrane of the proximal convoluted tubule, with progressive intracellular accumulation of Na/Pi protein. It is possible that phosphouria is due to direct toxic effect of gentamicin on membrane lipid [31]. It should be mentioned that the gentamicin dosage used in this study is much higher than those used clinically; thus, it may not reflect precisely the pathophysiology of gentamicin-induced nephrotoxicity in human.

In conclusion, there is a rapid renal adaptation to gentamicin-induced urinary calcium and magnesium loss. This renal adaptation is achieved by upregulation of TRPV5, TRPV6, TRPM6 and calbindin-D28k in the DCT, and is likely to prevent further mineral loss due to gentamicin treatment.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

References

- 1 Swan SK: Aminoglycoside nephrotoxicity. *Semin Nephrol* 1997;17:27–33.
- 2 Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ: New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney Int* 2011;79:33–45.
- 3 Shah GM, Kirschenbaum MA: Renal magnesium wasting associated with therapeutic agents. *Miner Electrolyte Metab* 1991;17:58–64.
- 4 Elliott WC, Patchin DS: Effects and interactions of gentamicin, polyaspartic acid and diuretics on urine calcium concentration. *J Pharmacol Exp Ther* 1995;273:280–284.
- 5 Gamba G, Friedman PA: Thick ascending limb: the Na⁺:K⁺:2Cl⁻ co-transporter, NKCC2, and the calcium-sensing receptor, CaSR. *Pflügers Arch* 2009;458:61–76.

- 6 Wang W, Li C, Kwon TH, Miller RT, Knepper MA, Frøkiaer J, Nielsen S: Reduced expression of renal Na⁺ transporters in rats with PTH-induced hypercalcemia. *Am J Physiol Renal Physiol* 2004;286:F534–F545.
- 7 Parsons PP, Garland HO, Harpur ES: Localization of the nephron site of gentamicin-induced hypercalciuria in the rat: a micro-puncture study. *Br J Pharmacol* 2000;130:441–449.
- 8 Biner HL, Arpin-Bott MP, Loffing J, Wang X, Knepper M, Hebert SC, Kaissling B: Human cortical distal nephron: distribution of electrolyte and water transport pathways. *J Am Soc Nephrol* 2002;13:836–847.
- 9 Hoenderop JG, Bindels RJ: Epithelial Ca²⁺ and Mg²⁺ channels in health and disease. *J Am Soc Nephrol* 2005;16:15–26.
- 10 Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG: The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science* 2005;310:490–493.
- 11 Peng JB, Warnock DG: WNK4-mediated regulation of renal ion transport proteins. *Am J Physiol Renal Physiol* 2007;293:F961–F973.
- 12 Landau D, Kher KK: Gentamicin-induced Bartter-like syndrome. *Pediatr Nephrol* 1997;11:737–740.
- 13 Chou CL, Chen YH, Chau T, Lin SH: Acquired Bartter-like syndrome associated with gentamicin administration. *Am J Med Sci* 2005;329:144–149.
- 14 Brasseur R, Carlier M, Laurent G, Claes PJ, Vanderhaeghe HJ, Tulkens PM, Ruysschaert JM: Interactions of streptomycin and streptomycylamine derivatives with negatively charged lipid layers. Correlation between binding, conformation of complexes and inhibition of lysosomal phospholipase activities. *Biochem Pharmacol* 1985;34:1035–1047.
- 15 Tulkens P: Experimental studies on nephrotoxicity of aminoglycosides at low doses. *Am J Med* 1986;80:105–114.
- 16 Chahwala SB, Harpur ES: Gentamicin-induced hypercalciuria in the rat. *Acta Pharmacol Toxicol* 1983;53:358–362.
- 17 Phipps DJ, Spencer AJ, Harpur ES: Comparison of the renal response of male Fischer 344, Sprague-Dawley and Wistar rats to administration of gentamicin. *Toxicol Lett* 1990;53:203–205.
- 18 Lee CT, Lien YH, Lai LW, Chen JB, Lin CR, Chen HC: Increased renal calcium and magnesium transporter abundance in streptozotocin-induced diabetes mellitus. *Kidney Int* 2006;69:1786–1791.
- 19 Katz CL, Butters RR, Chen CJ, Brown EM: Structure-function relationships for the effects of various aminoglycoside antibiotics on dispersed bovine parathyroid cells. *Endocrinology* 1992;131:903–910.
- 20 Parsons PP, Garland HO, Harpur ES, Old S: Acute gentamicin-induced hypercalciuria and hypermagnesiuria in the rat: dose-response relationship and role of renal tubular injury. *Br J Pharmacol* 1997;122:570–576.
- 21 Giapros VI, Cholevas VI, Andronikou SK: Acute effects of gentamicin on urinary electrolyte excretion in neonates. *Pediatr Nephrol* 2004;19:322–325.
- 22 Hung CC, Guh JY, Kuo MC, Lai YH, Chen HC: Gentamicin-induced diffuse renal tubular dysfunction. *Nephrol Dial Transplant* 2006;21:547–548.
- 23 Sassen MC, Kim SW, Kwon TH, Knepper MA, Miller RT, Frøkiaer J, Nielsen S: Dysregulation of renal sodium transporters in gentamicin-treated rats. *Kidney Int* 2006;70:1026–1037.
- 24 van de Graaf SF, Bindels RJ, Hoenderop JG: Physiology of epithelial Ca²⁺ and Mg²⁺ transport. *Rev Physiol Biochem Pharmacol* 2006;158:77–160.
- 25 Lee CT, Chen HC, Lien YH, Lai LW: Effects of furosemide on renal distal tubule calcium handling. *Am J Physiol Renal Physiol* 2007;293:F1231–F1237.
- 26 Chiruvolu A, Engle WD, Sendelbach D, Manning MD, Jackson GL: Serum calcium values in term and late-preterm neonates receiving gentamicin. *Pediatr Nephrol* 2008;23:569–574.
- 27 Song Y, Peng X, Porta A, Takanaga H, Peng JB, Hediger MA, Fleet JC, Christakos S: Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1,25 dihydroxyvitamin D3 in the intestine and kidney of mice. *Endocrinology* 2003;144:3885–3894.
- 28 Cowan RH, Jukkola AF, Arant BS Jr: Pathophysiologic evidence of gentamicin nephrotoxicity in neonatal puppies. *Pediatr Res* 1980;14:1204–1211.
- 29 Andronikou S, Giapros VI, Cholevas VI, Papadopoulou ZL: Effect of aminoglycoside therapy on renal function in full-term infants. *Pediatr Nephrol* 1996;10:766–768.
- 30 Sorribas V, Halaihel N, Puttaparthi K, Rogers T, Cronin RE, Alcalde AI, Aramayona J, Sarasa M, Wang H, Wilson P, Zajicek H, Levi M: Gentamicin causes endocytosis of Na/Pi cotransporter protein (NaPi-2). *Kidney Int* 2001;59:1024–1036.
- 31 Levi M, Cronin RE: Early selective effects of gentamicin on renal brush-border membrane Na-Pi cotransport and Na-H exchange. *Am J Physiol Renal Physiol* 1990;258:F1379–F1387.