Kallikrein/kinin protects against gentamicin-induced nephrotoxicity by inhibition of inflammation and apoptosis

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Abstract

Background. Our previous study showed that kallikrein gene transfer protects against gentamicin-induced nephrotoxicity and enhances renal function. In this study, we investigated the effects and potential mechanisms of kallikrein/kinin on inflammation and apoptosis induced by gentamicin.

Methods. Rats were injected subcutaneously with gentamicin daily for 10 days and received an intravenous injection of adenovirus carrying the human tissue kallikrein gene or control virus on the first day of gentamicin administration.

Results. After 10 days of gentamicin treatment, kallikrein gene transfer significantly attenuated gentamicin-induced tubular dilatation and lumenal protein casts. Moreover, kallikrein gene transfer reduced monocyte/macrophage infiltration, monocyte chemoattractant peptide-1 expression and renal cell apoptosis. Kallikrein's protective effects were accompanied by increased nitric oxide formation, and reduced NADH oxidase activity and superoxide production. Suppression of oxidative stress was associated with diminished c-jun N-terminal kinase activation and intercellular adhesion molecule-1 and transforming growth factor-β protein levels. These biochemical effects were blocked by icatibant, indicating a kinin B2 receptor-mediated signalling event.

Conclusions. This study indicates that kallikrein/kinin protects against gentamicin-induced nephrotoxicity by inhibiting inflammatory cell recruitment and apoptosis through suppression of oxidative stress-mediated signalling pathways. These findings raise the potential of applying kallikrein therapy approaches in treating aminoglycoside-induced renal damage.

Keywords: apoptosis; gentamicin; inflammation; kidney; oxidative stress

Introduction

Gentamicin is an aminoglycoside antibiotic used in the treatment of Gram-negative bacterial infections. As gentamicin administration can induce severe nephrotoxicity [1], it has become a popular substance used to study drug-induced acute renal failure. Thus, a therapeutic approach to protect or reverse aminoglycoside-induced kidney injury would have significant clinical value. Rats treated with gentamicin have been shown to exhibit increased macrophage infiltration, which was associated with increases in transforming growth factor (TGF)-β, endothelin and angiotensin II levels, implying their contribution to the progression of tubulointerstitial nephritis [2]. Several other factors are believed to play a part in the pathogenesis of gentamicin nephrotoxicity either directly or indirectly; these include oxidative stress and reduced phospholipase activity, resulting in lysosomal phospholipidosis [1].

Several studies have indicated that inflammatory cells and agents play a major role in experimental tubulointerstitial nephritis and fibrosis. In fact, virtually every cell type in the kidney is capable of producing pro-inflammatory chemokines. Upon tissue injury, renal cells may express leukocyte adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, and the chemokine monocyte chemoattractant peptide (MCP)-1 [3]. The presence of ICAM-1 on the surface of tubular and endothelial cells facilitates the recruitment of monocytes and macrophages and their migration to the site of tissue damage. Apoptosis, or programmed cell death, has also been observed in rat proximal tubules after aminoglycoside administration [4]. The mitogen-activated protein (MAP) kinase c-jun
N-terminal kinase (JNK) has been shown to play a major role in the apoptotic signalling pathway [5]. TGF-β1 has also been implicated in apoptosis associated with glomerulonephritis [6], and may stimulate the apoptotic signalling pathway [7].

In the kallikrein/kinin system, vasoactive kinin peptides are formed from the cleavage of low molecular weight kininogen by tissue kallikrein [8]. Binding of kinins to the constitutively expressed B2 receptor results in increased intracellular Ca^{2+} mobilization as well as prostaglandin, cAMP, nitric oxide (NO) and cGMP formation [8]. It has been reported that gentamicin-treated rats exhibit significantly reduced levels of urinary kallikrein compared with untreated control rats [9]. This implies a potential role for kallikrein in the pathogenesis of acute renal failure. Therefore, restoration of kallikrein levels may offer protection against kidney damage. Indeed, we previously demonstrated that kallikrein gene delivery attenuates kidney damage and enhances renal function in gentamicin-treated rats [10]. In the present study, we further evaluated the protective actions of tissue kallikrein gene delivery on inflammation and apoptosis in gentamicin-induced nephrotoxicity.

Materials and methods

Adenovirus preparation

Adenoviruses were prepared as previously described [11]. Human tissue kallikrein plasmid cDNA (TK) along with cytomegalovirus (CMV) promoter was inserted at the XhoI and BglII sites of plasmid Kan-pShut. Subsequently, the 4F2 enhancer and bovine growth hormone poly(A) sequence were inserted at the XhoI site in the Kan-pShut vector. The Kan-pShut plasmid was modified from plasmid pHM4 (kindly provided by Dr. Mark A. Kay, Stanford University Medical Center) and engineered by insertion of a kanamycin-resistant gene expression unit. The expression cassettes of human tissue kallikrein cDNA along with the kanamycin-resistant gene were released with I-CeuI and PstI-SceI and inserted at the same sites of plasmid pAdHM4, a backbone vector of E1/E3-deleted adenovirus. The expression cassettes of human tissue kallikrein cDNA along with the kanamycin-resistant gene were released with I-CeuI and PstI-SceI and inserted at the same sites of plasmid pAdHM4, a backbone vector of E1/E3-deleted adenovirus. The expression cassettes of human tissue kallikrein cDNA along with the E1/E3-deleted adenoviral backbone DNA were released with PacI and transfected into human embryonic kidney 293 cells. The E1/E3-deleted adenoviruses Ad.Null (virus without reporter gene), Ad.CMV-LacZ (virus harbouring the LacZ gene) and Ad.CMV-TK (virus harbouring the human tissue kallikrein cDNA) were generated and amplified through six passages and purified by two rounds of CsCl ultracentrifugation.

Animal treatment

Male SD rats (Sprague-Dawley Harlan, Indianapolis, IN) (200–220 g) were used in this study. The rats were housed at a constant room temperature with a 12 h light/dark cycle and had free access to tap water and rat chow. All procedures complied with the standards for care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Resources, National Academy of Sciences, Bethesda, MD). Rats were randomly divided into two groups. Control rats received daily subcutaneous (s.c.) injections of saline. All other rats received s.c. injections of gentamicin (Sigma, St Louis, MO) daily for 10 days. Adenovirus delivery was performed on the first day of gentamicin treatment at a dosage of 4 × 10^{10} plaque-forming units per rat. On the tenth day, all rats were sacrificed.

Study 1. Rats (n = 5–6 per group) receiving a high dose (80 mg/kg body weight/day) (for evaluation of renal histopathology) of gentamicin were subdivided into two groups. Rats were injected intravenously (i.v.) with Ad.CMV-LacZ or Ad.CMV-TK.

Study 2. Rats (n = 5–7 per group) receiving a low dose (20 mg/kg body weight/day) (for investigation of cellular biochemistry and signalling) of gentamicin were subdivided into three groups. Rats were injected i.v. with control virus Ad.Null, Ad.CMV-TK or Ad.CMV-TK plus treatment with the B2 receptor antagonist icatibant administered via an osmotic mini-pump implanted s.c. at a dose of 0.65 μg/h (Durect Corporation, Cupertino, CA).

Morphological and histological investigations

Rats were sacrificed after 10 days of gentamicin treatment, at which time kidneys were removed and weighed. Kidneys were then fixed in 4% formaldehyde solution and placed in 70% ethanol for storage. After fixation, the kidneys were dehydrated and embedded. Sections 4 μm thick were obtained from each slice. Sections were stained with haematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) for morphometric analysis. Morphological damage was scored as follows: (1+) proximal tubule damage in parts of the outer cortex; (2+) proximal tubule damage throughout the outer cortical nephrons; (3+) proximal tubule damage throughout the outer and inner cortex; and (4+) proximal tubule damage throughout the cortex and extended into the outer medulla. DNA fragmentation was determined using terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) using an in situ cell death detection kit (Roche, Indianapolis, IN). Immunohistochemistry was performed using the Vectastain Universal Elite ABC Kit (Vector Laboratories, Burlingame, CA), following the supplied instructions. Kidney sections were incubated at 4°C overnight with ED-1 (a monocyte/macrophage marker) (Chemicon, Temecula, CA) or MCP-1 (Santa Cruz Biotechnology, Santa Cruz, CA) antibody.

Tissue homogenization and western blot analysis

Renal tissue was homogenized in lysis buffer (25 mM Tris–HCl, pH 7.4, 1% Triton X-100, 0.1% SDS, 2 mM EDTA) containing 1:100 protease inhibitor cocktail (Sigma) and centrifuged at 4°C for 30 min. The supernatants were stored at −80°C. Kidney homogenates were used for western blot analysis using specific antibodies for phospho-JNK and total JNK (Cell Signaling Technology, Beverly, MA), TGF-β1 and ICAM-1 (Santa Cruz Biotechnology). Protein concentrations were determined by Lowry’s method.
Blood and urine collection

For blood collection, unanaesthetized rats were placed in a 37°C incubator for 10–15 min. Rats were then transferred to a plastic holder and an insulin syringe was used to withdraw blood from the tail vein. After collection, blood samples were centrifuged at 1000 g for 20 min and sera were removed and stored at −20°C. Twenty-four hour urine samples were collected on day 6 using metabolic cages. Rats received only water in order to eliminate contamination of food particles in the urine samples. After collection, urine samples were centrifuged at 1000 g to remove particles. Urine volume was measured and the supernatant was used for further analysis.

Assays for renal gentamicin, blood urea nitrogen (BUN) and creatinine

Renal gentamicin concentrations were determined using a gentamicin one-step enzyme-linked immunosorbent assay (ELISA) kit, following the supplied instructions (International Diagnostic Systems Corporation, St Joseph, MI). BUN levels were measured on day 10 using a modified urease–indophenol method. Serum and urine creatinine levels, using blood and urine collected on day 6, were determined by a commercial kit (BioAssay Systems, Hayward, CA). Creatinine clearance was calculated using the data obtained from serum and urine creatinine measurements and urine volume.

Assays for human tissue kallikrein, kinin and nitrate/nitrite (NOx) levels

Immunoreactive human tissue kallikrein levels in rat sera and urine as well as urinary kallikrein levels were determined by an ELISA specific for human tissue kallikrein and radiomunnoassay, respectively, as previously described [10]. Urinary and renal NOx levels (expressed as nitrate/nitrite) were determined by fluorometric assay [12].

Measurement of NADH oxidase activity and superoxide formation

NADH oxidase activity was measured using a lucigenin chemiluminescence assay [13]. Kidney tissues were excised and then homogenized in lysis buffer. Superoxide production in the renal extract in the presence of the substrate NADH (100 μM) (Sigma) was measured by the lucigenin (Sigma)-derived chemiluminescence. The lucigenin concentration in the final reaction mixture was 75 μM. Light emission levels were expressed as relative light units (RLU)/min/mg of protein. Superoxide production in renal extracts was quantified by a spectrophotometric assay based on rapid reduction of ferricytochrome c to ferrocytochrome c according to a modified previous protocol [13]. Reduction of ferrocytochrome c independent of superoxide was corrected for by deducting the activity not inhibited by superoxide dismutase.

Statistical analysis

Data were analysed using standard statistical methods, analysis of variance (ANOVA) and unpaired Student’s t-test. Group data are expressed as the mean ± SEM. Values of all parameters were considered significantly different at a P-value of <0.05.

Results

Expression of recombinant human tissue kallikrein

Immunoreactive human tissue kallikrein levels were measured using a specific ELISA. High levels of recombinant human kallikrein in rat sera were detected at 3 and 7 days after injection with adenovirus containing the human tissue kallikrein gene (Figure 1A). Human tissue kallikrein was also measured in rat urine 6 days post-injection (Figure 1B). Recombinant human tissue kallikrein was not detected in serum or urine of rats receiving control virus.

Study 1

Effect of kallikrein gene delivery on gentamicin-induced kidney damage. After 10 days of gentamicin treatment, renal injury was analysed by histological examinations of kidney tissue sections stained with H&E and periodic acid-Schiff (PAS) (Figure 2). The saline-treated rats...
had well preserved kidneys. However, administration of gentamicin for 10 days resulted in notable damage in the cortex, including tubular necrosis, tubular dilatation and accumulation of protein casts, as well as a loss of brush border. In the medulla, gentamicin treatment caused an increase in tubular protein casts, although the structural integrity of the tubules appeared to be unaffected. Kallikrein gene delivery reduced the renal damage produced from gentamicin treatment as indicated by fewer necrotic cells, dilated tubules and protein casts, and attenuation of brush border loss. The extent of tubular damage was assessed in PAS-stained slides and quantitated by a scoring method. Rats receiving gentamicin had a higher index of proximal tubular damage compared with the saline-treated rats (3.50±0.29 vs 0.25±0.25, n=4, P<0.0001). Kallikrein gene transfer, however, significantly decreased the tubular damage score compared with rats receiving gentamicin alone (2.25±0.25 vs 3.50±0.29, n=4, P<0.01).

Effect of kallikrein gene delivery on gentamicin-induced inflammatory cell infiltration and MCP-1 expression. Gentamicin treatment resulted in the accumulation of inflammatory cells in the cortex and medulla of rats injected with control virus, as determined by immunostaining with antibody against ED-1, a specific marker for monocytes and macrophages (Figure 3A). These cells were primarily localized in the interstitium. Kallikrein gene delivery significantly reduced inflammatory cell infiltration in the kidney compared with rats receiving control virus. However, ED-1 immunostaining was barely detectable in the saline group. ED-1-positive cells were counted for the quantification of monocyte/macrophage number (Figure 3B). Gentamicin administration caused an increase in monocytes/macrophages compared with saline-treated rats (1307.4±60.0 vs 79.2±26.8 cells/mm², n=4, P<0.001), whereas kallikrein gene delivery significantly reduced gentamicin’s effect (807.6±31.6 vs 1307.4±60.0 cells/mm², n=4, P<0.001). Since MCP-1 is a monocyte chemotactic mediator, we investigated its potential involvement in gentamicin-induced renal injury. Gentamicin treatment increased MCP-1 immunostaining in both renal interstitium and glomeruli of rats injected with control virus compared with rats injected with saline (Figure 3C). Slight MCP-1 immunostaining was identified in the glomeruli and interstitium of kallikrein-treated rats, but markedly reduced overall compared with the control virus group.

Effect of kallikrein gene transfer on gentamicin-induced apoptosis. Renal apoptosis was evident in the cells of the cortex and medulla of rats injected with control virus and treated with gentamicin, as determined by TUNEL staining (Figure 4), whereas the saline-treated group had almost undetectable staining. Kallikrein gene transfer in gentamicin-treated rats was able to decrease the amount of TUNEL-positive cells in both the cortex and medulla to levels similar to that in rats injected with saline.

Study 2

Gentamicin levels in the kidney. Gentamicin levels in kidney extracts from rats receiving a low dose of gentamicin (20 mg/kg body weight/day) were determined by ELISA. No differences were observed.
between the three groups receiving gentamicin treatment (Gent./Null, 8.05 ± 0.82; Gent./TK, 8.97 ± 0.54; Gent./TK + icatibant, 9.20 ± 0.98 μg/mg protein; n = 6–8, P > 0.05). These data indicate that kallikrein gene delivery does not affect gentamicin uptake or excretion from the kidney.

**Effect of gentamicin on renal function.** BUN and serum creatinine levels, as well as creatinine clearance, were measured to evaluate the effect of gentamicin on renal function (Table 1). These parameters were unaltered in rats receiving gentamicin treatment, with or without kallikrein gene delivery, compared...
with saline-treated rats. However, daily urine volume increased upon kallikrein gene transfer, with or without co-administration of icatibant infusion.

Kallikrein gene delivery increases kinin and NOx levels. The effect of kallikrein gene transfer on kinin and NOx levels in gentamicin-treated rats is shown in Figure 5. Urinary kinin levels were elevated in rats receiving kallikrein gene transfer as compared with saline-treated rats (21.4 ± 4.3 vs 1.6 ± 0.6 ng/100 g body weight/day) and rats receiving control virus (21.4 ± 4.3 vs 4.6 ± 0.7 ng/100 g body weight/day, n = 5–6, P < 0.01) (Figure 5A). Icatibant infusion had no effect on kinin levels in rats receiving kallikrein gene transfer. The increase in urinary kinin levels after kallikrein gene delivery was accompanied by an increase in urinary NOx. Kallikrein gene delivery elevated NOx excretion as compared with rats injected with saline (145.1 ± 12.3 vs 106.7 ± 9.8 nmol/100 g body weight/day, n = 4–6, P < 0.01) and with control virus (145.1 ± 12.3 vs 117.3 ± 4.2 nmol/100 g body weight/day, n = 5–6, P < 0.05) (Figure 5B). Icatibant infusion, however, in conjunction with kallikrein gene delivery, partially blocked kallikrein’s effect on urinary NOx levels (126.9 ± 8.2 vs 145.1 ± 12.3 nmol/100 g body weight/day, n = 5, P > 0.05). Renal NOx production was also measured, as shown in Figure 5C. Administration of gentamicin reduced NOx production in the kidney as compared with saline-treated rats (533.5 ± 52.9 vs 726.7 ± 36.7 pmol/mg protein, n = 4–7, P < 0.01), whereas kallikrein gene delivery restored NOx levels, significantly above that of rats receiving gentamicin and control virus (778.9 ± 39.5 vs 533.5 ± 52.9 pmol/mg protein, n = 7, P < 0.01) (Figure 5C). NOx production by kallikrein gene transfer was blocked by co-administration with icatibant (778.9 ± 39.5 vs 626.5 ± 18.4 pmol/mg protein, n = 6–7, P < 0.05).

Kallikrein gene delivery reduces NADH oxidase activity and superoxide formation. Gentamicin significantly elevated NADH oxidase activity in renal extracts, but the effect was abolished by kallikrein (3.35 ± 0.76 vs 1.36 ± 0.17 RLU/min/mg protein, n = 7, P < 0.05) (Figure 6A). However, icatibant administration reversed the reduction in NADH oxidase activity caused by kallikrein gene delivery alone (2.67 ± 0.31 vs 1.36 ± 0.17 RLU/min/mg protein, n = 7, P < 0.05). In addition, gentamicin augmented superoxide formation.
formation, although kallikrein significantly blocked this increase (42.0 ± 10.1 vs 15.0 ± 2.5 nmol/min/mg protein, n = 4, P < 0.05) (Figure 6B). Kallikrein’s effect was abolished by icatibant (15.0 ± 2.5 vs 44.0 ± 9.3 nmol/min/mg protein, n = 4–5, P < 0.05). These observations implicate a role for a kinin B2 receptor signalling event in reducing oxidative stress.

Discussion

This is the first study to demonstrate that kallikrein, through the kinin B2 receptor, protects against gentamicin-induced nephrotoxicity by inhibiting inflammation and apoptosis. Enhanced tissue kallikrein levels, detected in the sera and urine, were achieved by adenovirus human kallikrein gene delivery. Renal injury induced by gentamicin treatment was markedly decreased by kallikrein gene transfer, as evidenced by a reduction in proximal tubular damage and protein cast formation, as well as attenuation of brush border loss. These beneficial effects of kallikrein on the kidney were accompanied by the significant decline in oxidative stress, monocyte/macrophage recruitment and renal cell apoptosis. In addition, our results showed that kallikrein gene delivery did not lower the gentamicin concentration in kidney tissues, signifying that kallikrein does not alter gentamicin uptake or excretion from the kidneys. Taken together, our results indicate that kallikrein/kinin could act as an anti-oxidant and anti-inflammatory agent in protection against gentamicin-induced nephrotoxicity.

We previously reported that a high dose of gentamicin (80 mg/kg/day) causes an elevation in BUN levels [10]. Yet, in the current study, we observed that BUN and serum creatinine levels as well as creatinine clearance were unaltered in rats administered a low dose of gentamicin (20 mg/kg/day), with or without kallikrein gene delivery, compared with saline-treated rats. These findings indicate that injection of gentamicin at the low dose is not as severely nephrotoxic as the high dose. These results are consistent with a previous report demonstrating that the same dose of gentamicin (20 mg/kg/day) did not significantly alter serum creatinine or BUN levels compared with saline-treated rats, and thus did not cause substantial renal dysfunction [4].
increase in monocyte/macrophage populations in the kidney. In this study, however, we showed that kallikrein gene delivery attenuated the inflammatory response by reducing inflammatory cell accumulation. Moreover, we demonstrated that gentamicin administration induced increased MCP-1 immunostaining compared with control rats, though this effect was attenuated by kallikrein gene delivery. TGF-β1, in addition to MCP-1, has also been shown to be chemotactic for monocytes [14]. Interestingly, Diamond et al. [15] demonstrated that the expression of both TGF-β1 and MCP-1 is increased in parallel with the interstitial macrophage number in unilateral ureteral obstruction. In our study, we observed that gentamicin significantly increased renal TGF-β1 protein levels compared with control rats. The increased protein levels of TGF-β1 induced by gentamicin may be indicative of its secretion from macrophages (or other cellular sources, such as fibroblasts) that have accumulated at the sites of tissue injury. However, kallikrein gene transfer markedly reduced TGF-β1 protein levels, and the effect was blocked by icatibant, indicating a kinin B2 receptor-mediated event. Furthermore, we showed that gentamicin treatment stimulated ICAM-1 protein expression significantly above levels in the saline group. Although kallikrein gene transfer normalized ICAM-1 to levels of the saline group, the effect was reversed by icatibant. Taken together, these data indicate that the inflammatory process has a significant role in the pathogenesis of gentamicin-induced nephrotoxicity, and that kallikrein gene delivery can attenuate the resultant kidney damage by suppression of MCP-1, ICAM-1 and TGF-β1 protein levels.

As it has been noted that gentamicin can cause apoptosis in renal proximal tubular cells [4], we investigated a possible protective effect of kallikrein against gentamicin-induced programmed cell death. Indeed, we observed a significant amount of TUNEL-positive staining in the renal cortex and medulla in rats receiving gentamicin treatment compared with rats receiving saline injections, and kallikrein gene transfer reduced TUNEL staining to levels similar to that of the saline group. In order to explore further the mechanism of apoptosis caused by gentamicin, we examined the role of JNK, a MAP kinase signalling molecule that has been implicated as a stimulator of apoptosis [5]. It is most likely that the increase in gentamicin-induced JNK phosphorylation is due to the elevation of superoxide levels generated by NADH oxidase, as reactive oxygen species (ROS) can stimulate JNK activation [16]. The decrease in phosphorylated JNK by kallikrein gene delivery, by reduction of ROS formation, may partly explain kallikrein’s protective effect against gentamicin-induced apoptosis. Moreover, TGF-β1 has been associated with apoptosis in kidney disease [6] and may stimulate the apoptotic signalling pathway [7]. Therefore, the reduction of TGF-β1 levels, together with decreased JNK activation, may account for the attenuation of apoptosis by kallikrein gene delivery.

However, we did find dramatic modifications in renal cellular biochemical events induced by gentamicin administration at the 20 mg/kg/day dose.

Kidney injury caused by gentamicin-induced tubular necrosis can stimulate an inflammatory response in which chemokines, such as MCP-1 [3], are secreted to attract leukocytes to damaged areas. The cell adhesion molecule ICAM-1 is upregulated during the inflammatory response and is expressed on the surface of certain cell types in order to facilitate the attachment of circulating monocytes and macrophages. The inflammatory cells then proceed to the site of injury and secrete various agents that contribute to the pathological condition. Therefore, we investigated the presence of pro-inflammatory agents and inflammatory cells in the kidney stimulated by aminoglycoside administration. Rats treated with gentamicin displayed a marked increase in monocyte/macrophage infiltration into the renal cortex and medulla, as indicated by the large number of ED-1-positive cells in the interstitium. This has been observed previously by Geleilete et al. [2], who found that gentamicin treatment caused an

Fig. 6. Kallikrein gene delivery reduced renal (A) NADH oxidase activity and (B) superoxide formation after 10 days of gentamicin treatment (20 mg/kg/day). Values are expressed as mean ± SEM (n = 4–7). *P < 0.05 vs Gent./Null; †P < 0.05 vs Gent./Null and Gent./TK + icatibant.

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<th>NADH Oxidase Activity (RLU/min/mg protein)</th>
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The involvement of oxidative stress in gentamicin-induced nephrotoxicity was also examined and verified by the detection of increased levels of renal NADH oxidase activity and superoxide formation. Kallikrein gene delivery attenuated the rise in NADH oxidase activity and superoxide levels, and these results were reversed by icatibant, indicating that kallikrein’s effects on ROS are mediated by the kinin B2 receptor. Elevated levels of NOx, which result from kinin B2 receptor activation [8], accompanied the suppression of oxidative stress by kallikrein. However, when icatibant was administered in conjunction with kallikrein gene delivery, NOx production was reduced, establishing the role of the kinin B2 receptor in kallikrein’s protective actions against gentamicin. ROS, generated primarily by NADH oxidase, can induce the expression of pro-inflammatory factors [17] and TGF-β1 [18], as well as activate JNK [16]. In contrast, NO is capable of abrogating the redox mechanisms of ROS. This may be accomplished through direct inhibition of the assembly of NAD(P)H oxidase subunits [19], thereby preventing the production of superoxide, or by the formation of peroxynitrite by scavenging superoxide anions. Consequently, the reduction of MCP-1, ICAM-1 and TGF-β1 levels; and the JNK activation we observed may be the result of NO interfering

Fig. 7. (A) Representative western blots show that kallikrein gene delivery reduced renal ICAM-1 and TGF-β1 levels as well as JNK phosphorylation. (B) Quantitative analyses were performed on the western blots by densitometry. Values are expressed as mean ± SEM (n = 3–5). † P < 0.05 vs Gent./Null and Gent./TK + icatibant.
with ROS-induced gene expression and the redox regulation of JNK.

In conclusion, by examining the mechanisms by which kallikrein attenuates gentamicin-induced kidney damage, we showed that the reduction of renal inflammation and apoptosis was accompanied by a decrease in oxidative stress, JNK activation, and ICAM-1 and TGF-β1 protein levels. Furthermore, we demonstrated that NO may play an important role in the protective effect of kallikrein. Alternatively, the protective effects of kallikrein may be due to other mechanisms. For instance, it has been shown that bradykinin can enhance the activity of phospholipase A2 in endothelial cells [20]. In this manner, kallikrein may prevent the accumulation of phospholipids, the primary mode of cellular damage caused by gentamicin, by increasing their metabolism in the lysosomes. However, the effect of kallikrein on phospholipase activity was not investigated in this study. Altogether, our data support the notion that kallikrein gene transfer, by increasing NO formation through kinin B2 receptor activation, modulates aminoglycoside-induced kidney damage, inflammation and apoptosis.

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Conflict of interest statement. None declared.

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