KIDNEY DISEASES

Original Paper

Role of Epidermal Growth Factor and Growth Hormone-releasing Peptide-6 in Acceleration of Renal Tissue Repair After Kanamycin Overdosing in Rats

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Introduction. Aminoglycosides nephrotoxicity limits their use in clinical practice. Growth hormone-releasing peptide-6 (GHRP6) and epidermal growth factor (EGF) have proven cytoprotective effects in various tissues, including the kidney. This study aimed to determine the cytoprotective effect of EGF and GHRP6 on glomerular, proximal tubular, and interstitial morphology in rats treated with an overdose of kanamycin.

Materials and Methods. Forty-four male Wistar adults rats were submitted to treatment for 20 days with sodium phosphate saline buffer (control group), kanamycin (kanamycin group), kanamycin and EGF (EGF group), kanamycin and GHRP6 (GHRP6 group), kanamycin, EGF, and GHRP6 (EGF-GHRP6 group). The kidneys were studied both during acute kidney injury (n = 19) and recovery phases (n = 25). The percentages of glomerular damage, tubular damage (reversible and irreversible changes), and interstitial damage were quantified in 10 histological fields per kidney using paraffin-embedded sections.

Results. The damage in the glomeruli, proximal tubules, and interstitium was less in the groups treated with the cytoprotective treatments than in kanamycin group during acute kidney injury. During the recovery phase, normal structure of several glomeruli and the interstitium was appreciated in the EGF and GHRP6 groups, although tissue repair was not as complete as it in the EGF-GHRP6 group. In the recovery phase, cytoprotective treatments accelerated the recovery of tubular damage and reversible tubular changes prevailed.

Conclusions. These results confirm the cytoprotective properties of EGF and GHRP6 alone and in combination and suggest the possibility of using these agents to accelerate kidney tissue repair after aminoglycoside-induced renal damage.

INTRODUCTION

Aminoglycosides are a family of antibiotics widely used in clinical practice due to their effective bactericidal activity against gram-negative microorganisms.[1] Eventually, aminoglycosides are the only therapeutic alternative against germs resistant to other antibiotics.[2] However, nephrotoxicity is one of their major negative side

effects and appears in 10% to 25% of patients during aminoglycoside treatment.[3] Several mechanisms have been involved in the nephrotoxicity of aminoglycosides.[4-6] Reactive oxygen species appear to be important mediators of its harmful effect since experimental studies have shown protection with antioxidant treatments.[7] The effect of aminoglycosides on the kidney follows 2 phases: the first is the acute kidney injury, which primarily affects epithelial cells of proximal tubules and consequently the interstitium and glomeruli.[8-11] In the second phase (recovery phase), tubular epithelial cells proliferate to replace lost cells of the epithelium, and usually, kidney function approaches again to normal values.[12]

Currently numerous studies have been conducted to prevent nephrotoxicity induced by aminoglycosides using various approaches, not only antioxidant therapy,[13-16] but also the use of growth factors.[17] However, no clinically efficient method has been established to counteract nephrotoxicity of aminoglycosides or accelerate recovery of damaged tissue.[18] Epidermal growth factor (EGF), also known as urogastrone, is a stable and resistant molecule, currently available as recombinant EGF. It is involved in the regulation of cell proliferation; thus, it is a potent stimulator of tissue repair, shown in in vitro and in vivo models.[19-21] Therapeutic potential of EGF in renal tubules reepithelization and reduction of oxidative stress after acute renal ischemic or nephrotoxic damage has been well documented in research.[17,22,23] Epidermal growth factor has shown a protective effect during acute kidney injury phase in rats treated with the aminoglycoside antibiotics gentamicin and kanamycin.[24-26] A preliminary study of this group showed that EGF accelerated regeneration of damaged renal tissue during the recovery phase in rats treated with kanamycin.[27]

Growth hormone-releasing peptide-6 (GHRP6) is a synthetic peptide derived from the intestinal metaenkephalin, used as stimulator of the secretion of growth hormone in various mammalian species, including humans.[28-30] Growth hormone-releasing peptide-6 has been shown to prevent necrotic or apoptotic cell death in various tissues,[31] including the kidney, in animals subjected to different insults such as episodes of hepatic ischemia-reperfusion with subsequent multiorgan failure or cytotoxicity by an antineoplastic agent.[32,33]

The combined administration of recombinant EGF and GHRP6 by our group has shown synergistic cytoprotective response to different insults in splanchnic organs, including the kidney and nervous tissue.[32,34-36] The effect of these agents in long-term recovery phase after acute kidney injury induced by aminoglycosides has not been evaluated yet. The objective of this study was to determine the effect of EGF, GHRP6, and a combination of both agents on morphology of the glomeruli, proximal tubules, and interstitium during acute kidney injury and long-term recovery phases induced by kanamycin in rats.

MATERIALS AND METHODS
Study Protocol

Forty-four male Wistar rats with a weight range of 300 g to 350 g, from the National Center for Laboratory Animal Production (Cenpalab, Havana, Cuba) were used. Rats were fed with a commercial standard diet and water ad libitum. The procedures were performed according to national and local ethics and regulatory standards for animal care.

The rats were divided into 5 groups. Rats in the control group (n = 5) were given 1 mL of 0.1-mol/L sodium phosphate saline buffer, pH 7.2 to 7.4, intraperitoneally. For the remaining four groups (kanamycin, n = 11; GHRP6, n = 10; EGF, n = 10; EGF-GHRP6, n = 8), 500 mg/kg body weight of kanamycin sulfate (AICA, Havana, Cuba) were administered subcutaneously once daily. Epidermal growth factor and GHRP6 were diluted in sterile phosphate saline buffer immediately before their intraperitoneal administration twice a day. The GHRP6 and EGF-GHRP6 groups received 600 μg/kg of GHRP6 per day. The EGF and EGF-GHRP6 groups received 100 μg/kg of EGF per day.

Epidermal growth factor is a polypeptide of 6-kDa molecular weight consisting of 53 aminoacids,[37] as recombinant human EGF1-52 (rhEGF1-52) expressed in *Saccharomyces cerevisiae*, was supplied in lyophilized form by Heber-Biotec, Cuba, consisting of a 60:40 mixture of EGF1-52 and EGF1-51 with similar biological activity to the EGF1-53 form. Growth hormone-releasing peptide-6 (sequence His-D-Trp-Ala-Trp-D-Phe-Lys-NH2), from BCN Peptides, Spain, was certified as free of pyrogens and contaminants in lyophilized form.

For the histological study, kidneys were taken from the groups Kanamycin, EGF, and EGF-GHRP6

(n = 19), which died on days 8 to 10 of treatment (acute kidney injury phase). With the 25 remaining animals, a cycle of 20 days of treatment was completed and euthanasia was performed 8 weeks after the last day of treatment (recovery phase).

Morphological Analysis

The kidneys were sagitally sectioned through the hilum. Hematoxylin-eosin- and periodic acid Schiff-stained sections (3 µm-thick) of paraffin-embedded kidneys were used. The sections were observed using a Nikon 50i light microscope with a high resolution digital camera DS-5M-U1. Ten cortical histological fields randomly taken from the superior to the inferior kidney pole were evaluated on 1 section per animal. Glomerular damage related to the presence of adhesions of the glomerular tuft to the Bowman capsule (synechiae), increased number of nuclei, and mesangial matrix were determined at × 200 magnification. Tubular damage was studied in transversely-sectioned proximal tubules at × 400 magnification. The presence of vacuolated cells and the absence of brush border were considered reversible changes. Denudation and thickening of tubular basement membrane, presence of necrotic cells in the epithelium and detached cells, and tubular casts in the lumen were considered irreversible changes.[38] Also, interstitial damage was assessed (inflammatory infiltrate and peritubular fibrosis) at × 200 magnification. For each variable, normal condition was classified as zero and pathological condition was considered 1 (software developed for .Net platform, using the integrated development environment Visual Studio 2008 and c# language).[39]

Statistical Analyses

The percentage of damaged tubules and glomeruli per histological field as well as the percentage of fields showing interstitial damage were compared between groups using the Graph Pad Prism software (version 5.00 for Windows, GraphPad Software, La Jolla, CA, USA). Differences between groups were analyzed using the nonparametric tests Kruskal-Wallis and test of multiple comparisons of Dunn. A *P* value less than .05 was considered significant.

RESULTS
Acute Kidney Injury Phase

In the kanamycin group, large areas with damaged glomeruli were found (thickening of the basement membrane of the Bowman capsule, synechiae, increased mesangial matrix at the expense of nuclei, and obliteration of capillary lumens). Tubules showed signs of necrosis with loss of brush border, epithelial flattening, and thickening of basement membranes. Also, detached cells and tubular casts were seen in tubular lumens. In areas of damaged tubules extensive inflammatory infiltration was found (Figure 1B). In the groups treated with kanamycin and any protective agent, large areas of damaged glomeruli, tubules, and interstitium were alternating with areas of normal structure. However, the damage was less than that in kanamycin group (Figure 2A).

Recovery Phase

In the control group, normal structure of glomeruli and tubules and absence of inflammatory infiltrate and fibrosis in the interstitium were observed (Figures 1A and 2B). In the kanamycin group, irregularly dilated Bowman space, mesangial widening with increased mesangial matrix and cellularity, obliteration of capillary lumens of some glomerular lobules, and synechiae were observed in the glomeruli. In the proximal tubules, there was a predominance of cell vacuolation; although necrotic cells, dilated tubular lumens with detached cells, and denudation of tubular basement membranes were observed in fewer amounts. Interstitial inflammatory infiltrates and fibrosis were found in the interstitium (Figures 1C and 2B). These changes were found in focal areas. Normal structure of most of the glomeruli and interstitium was noticed in the EGF and GHRP6 groups, although recovery was not complete as occurs in the EGF-GHRP6 group. Protective treatments accelerated the restoration of tubular damage and reversible tubular changes prevailed (Figures 1D to 1F and 2B).

DISCUSSION

In this study, a high dose of kanamycin was used to induce severe damage of the renal cortex. Patchy glomerular, tubular, and interstitial changes found in animals treated with kanamycin were due to the simultaneous occurrence of necrosis and tubular regeneration,[11] as occurs with other aminoglycosides.[40] Unlike other organs, such as the heart or brain, the kidney may completely recover after ischemic or toxic acute injury. First,
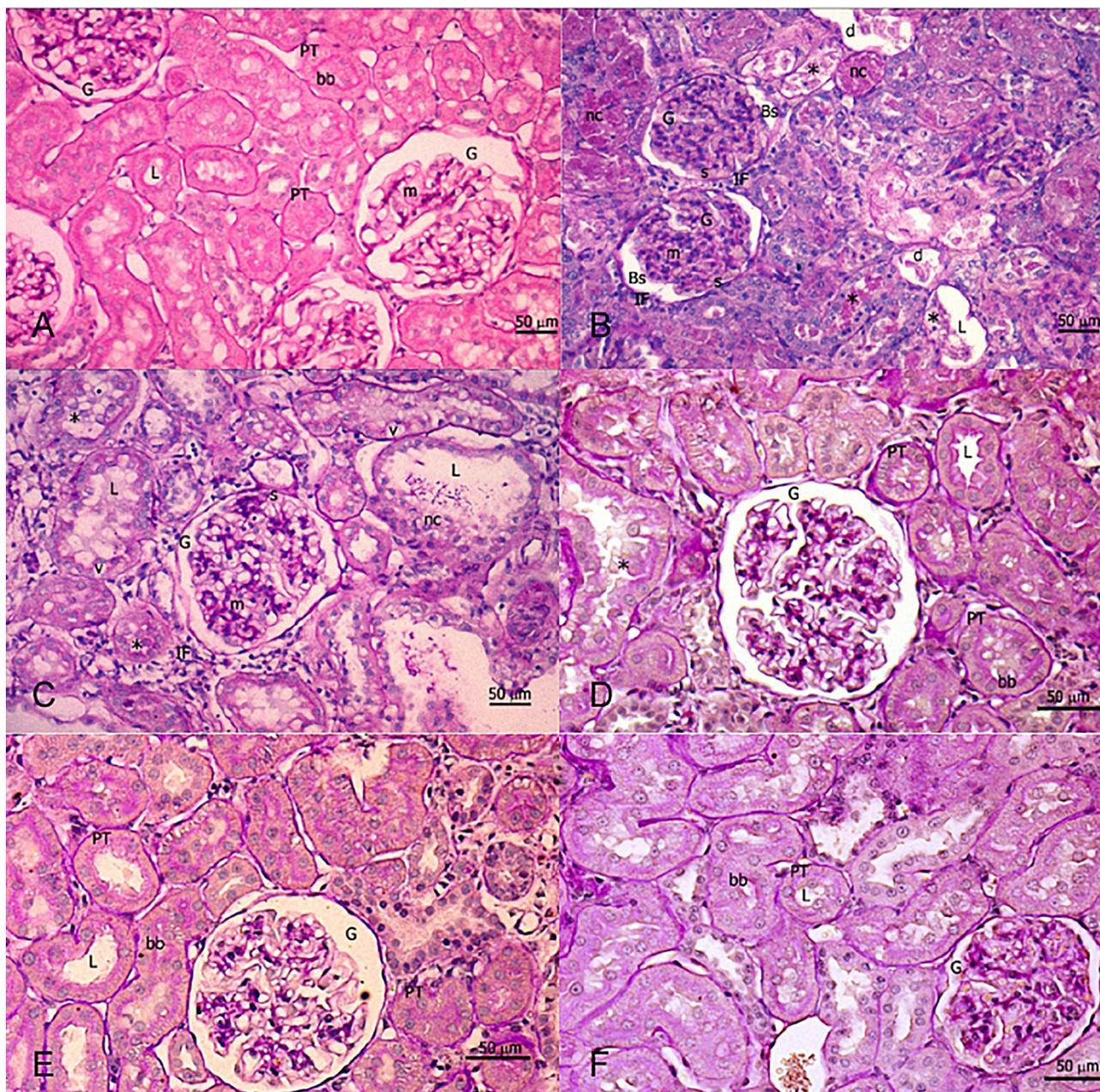
Figure 1. Rat renal cortex (periodic acid Schiff). **A**, Normal structure in the control group; **B**, Marked glomerular damage, tubular necrosis, and peritubular inflammatory infiltrate in the acute renal injury phase caused by kanamycin; **C**, Predominance of tubular cell vacuolization during the recovery phase in the kanamycin group; **D**, Recovery of glomerular, tubular, and interstitial structure in the growth hormone-releasing peptide-6 (GHRP6) group; **E**, Recovery of glomerular, tubular, and interstitial structure in the epidermal growth factor (EGF) group; **F**, Recovery of glomerular, tubular, and interstitial structure in the EGF-GHRP6 group. G indicates, glomerulus; s, synechiae; Bs, Bowman space; m, mesangium; PT, proximal tubule; L, tubular lumen; bb, brush border; asterisk, sloughed cells and casts; IF, inflammatory infiltrate; d, denuded tubular basement membrane; and nc, necrotic cell.

tubular epithelial cells are detached into the lumens, leaving flattened basement membranes. Recovering involves epithelial cell spreading and possibly migration to the naked areas of the basement membrane, proliferation, and dedifferentiation to restore cell number. Finally, differentiation of the cells takes place and functional integrity of the nephron is restored.[8,12] This is consistent with our morphological findings.

The aminoglycoside-induced kidney damage is associated with reactive oxygen species generation in tubular epithelial cells, capillary endothelial cells, and inflammatory cells in the cortex.[1] Cytotoxicity of proximal tubule epithelial cells is
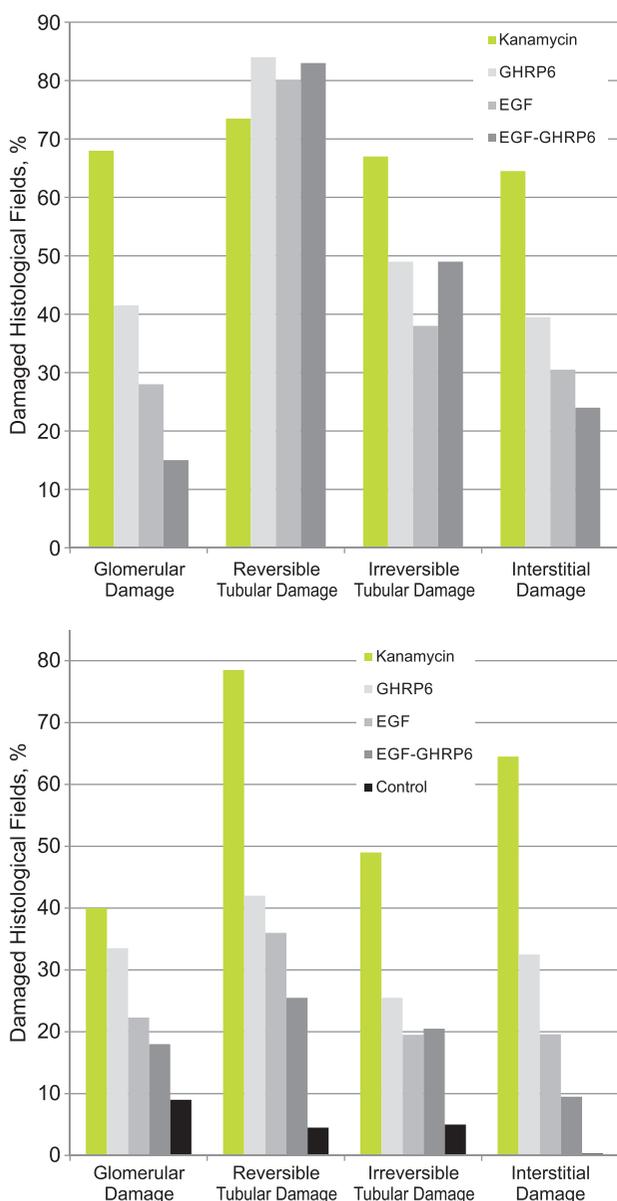
Figure 2. Evaluation of renal damage variables. **Top**, acute renal injury phase. Significant differences were seen between the four groups in all features except for reversible tubular damage. **Bottom**, recuperative phase. Significant differences were seen between the four groups in all features (control group not included in comparisons).

presumably because these cells take up and retain the drugs. In our work, brush border loss was one of the most striking findings during acute renal injury in the kanamycin group. This has been attributed to the direct effect of the drug on the apical membrane of proximal tubule cells in the early stages of aminoglycosides uptake.[3] Tubular damage leads to dysfunctional resorption process, which produces excessive water and electrolytes supply to the distal portion of the nephron, which

in turn activates the tubuloglomerular feedback mechanism.[5] Inflammatory cells are part of the regenerative process and their resorption occurs gradually after kanamycin administration ends.[5]

Reduction of kanamycin-induced damage exerted by EGF is in agreement with experimental results of protection using kanamycin and gentamicin nephrotoxicity.[24,26,27] This effect could be due to its reactive oxygen species scavenger property demonstrated in kidney and other tissues.[23] Growth factors play a key role in kidney recovery by means of highly specialized actions that promote tubular cells proliferation and differentiation.[42] Growth factors also have a useful role in the development, maintenance and remodeling of the renal microcirculation,[43] as well as other organs.[44]

Beneficial effect of EGF and growth hormone against renal ischemic damage, also caused by the release of reactive oxygen species, has been attributed to their property of being potent regulators of *IGF1* expression in the kidney.[45] Although further physiological studies are required, our results suggest the possibility of using EGF and GHRP6, administered separately or in combination, to accelerate renal tissue repair after aminoglycoside-induced renal damage. The relative low toxicity of EGF and GHRP6 is a relevant issue to consider to establishing a combined therapy between EGF and GHRP6 during aminoglycoside therapy in order to prevent nephrotoxicity.[46,47]

CONCLUSIONS

These results confirm the cytoprotective properties of EGF and GHRP6 alone or in combination and suggest the possibility of using these agents to accelerate renal tissue repair after aminoglycoside-induced kidney damage.

CONFLICT OF INTEREST

None declared.

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