Effects of nitric oxide on gentamicin toxicity in isolated perfused rat kidneys

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ABSTRACT: Background: There have been many studies in recent years concerning the role of nitric oxide (NO) in acute renal failure (ARF). In this study, the effects of the inhibition or the induction of NO synthase (NOS) on gentamicin-induced ARF was investigated in isolated perfused rat kidneys.

Methods: Kidneys from male Sprague-Dawley rats were perfused in situ for 90 min. Perfusion was conducted in the presence of inulin (60 mg/dL in perfusion buffer) as a glomerular filtration rate (GFR) marker. Six groups (total: 42 rats) were studied: group 1, controls with no treatment; group 2, L-arginine (2 mM in perfusate); group 3, L-nitroarginine-methyl ester (L-NAME, 0.1 mM in perfusate); group 4, gentamicin (GM, 0.5 mg/mL in perfusate); group 5, GM + L-arginine (same dose as groups 2 and 4) and; group 6, GM + L-NAME (same dose as groups 3 and 4). Cell injury was assessed by measuring N-acetyl-β-D-glucosaminidase (NAG), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity in urine.

Results: L-arginine prevented, whereas L-NAME enhanced, GM-induced enzyme release and GFR reduction. Histological studies showed that GM-treated kidneys had clear signs of tubular damage and this damage was increased by simultaneous L-NAME and GM administration.

Conclusion: This study suggests that NO formation could prevent the GM-induced nephrotoxicity in this ARF model.

Key words: Gentamicin, Nephrotoxicity, Nitric oxide

INTRODUCTION

The role of nitric oxide (NO) in acute renal failure (ARF) is not yet completely understood. However, several studies have shown the beneficial effects of NO in different ARF models (1-4). On the other hand, there are some reports indicating toxic effects of NO in renal injury (3-5). The amino glycoside antibiotic “gentamicin” is known to cause renal failure in 10-20% of patients receiving the drug. Gentamicin-induced nephrotoxicity is mostly characterized by direct tubular necrosis. Gentamicin binds to the cell wall phospholipids, which blocks the chain reactions of the phosphatidylinositol pathway leading to the impairment of cell integrity (6). Reactive oxygen species (ROS) play an important role in gentamicin-mediated nephropathy (7). Some studies have reported that antioxidant administration ameliorates gentamicin-induced nephropathy (8, 9). Other possible mechanisms responsible for gentamicin-induced ARF are endothelin release (10), tubuloglomerular feedback activation and renal vascular resistance enhancement (6). The possible involvement of the L-arginine-NO pathway in gentamicin-induced nephrotoxicity was first suggested by Rivas Cabanero et al who reported increased glomerular NO synthesis in renal failure caused by gentamicin (11). This study was designed to assess the effects of altered NO production on gentamicin-induced ARF in isolated perfused rat kidneys.

SUBJECTS AND METHODS

Animals

Male Sprague-Dawley rats (Razi Institute, Iran) weighing 220-260 g were housed under controlled environmental conditions (24 ± 2°C and 12-h light/dark cycle) and allowed free access to standard rat chow and tap water. Animal care complied with the guidelines of the Animal and Human Ethical Committee of Tehran Medical Sciences University.
In situ isolated perfused kidneys

Rat kidneys were isolated and perfused with oxygenated Tyrode buffer at 37°C, at a rate of 8 ml/min (Peristaltic pump, IBS p803, EU) as described by Dehpour et al (12, 13). Mesenteric artery, right and left adrenal arteries, internal spermatic and iliolumbar vessels and abdominal aorta above and below the renal artery were ligated. The aorta was then cannulated and in situ perfusion was initiated after heparin injection. Ureters were cannulated and the end of the catheter was placed in 2 mL tubes for urine collection.

Study groups

Forty-two rats were randomly arranged in six groups of seven rats. Group 1 (controls) - kidneys perfused with Tyrode buffer without any treatment. Group 2 (L-arginine) - after 15 min of perfusion, L-arginine (Merck, Germany, 8 nM/min for 75 min) was added. Group 3 (L-nitro-arginine-methyl ester (L-NAME)) - after 15 min of perfusion, L-NAME (Sigma, Germany, 0.4 nM/min for 75 min) was added. Group 4 (gentamicin) - after 15 min of perfusion, gentamicin (Chemo, Switzerland, 0.8 mg/min for 75 min) was added. Group 5 (L-arginine + gentamicin) - after 15 min of perfusion, L-arginine and gentamicin were added (with the same doses as groups 2 and 4). Group 6 (L-NAME + gentamicin) - after 15 min of perfusion, L-NAME and gentamicin were added (with the same doses as groups 3 and 4).

In all groups, perfusion was performed for 90 min in which the first 15 min were considered as a stabilizing period. Urine samples were collected in 15 min periods in separate tubes. An adequate volume of each sample was kept at -30°C for inulin assays. N-acetyl-β-D-glucosaminidase (NAG), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity was measured in fresh samples. At the end of the perfusion, the kidneys were removed from the body, weighed and fixed in 10% formalin buffer and embedded in paraffin. Sections of the kidneys were stained with hematoxylin and eosin. Histology for all kidneys was scored per section in at least 10 randomly selected non-overlapping fields at x400 magnification. The results were scored as the percentage of the damaged tubules in the fields examined: no damage; mild damage: areas of tubular damage <25%; moderate damage: areas of tubular damage between 25 and 50%; severe damage: areas of tubular damage >50%. Presence of luminal debris, cellular vacuolation, reduction in tubular patency and pyknosis were used as evidence of tubular damage.

Biochemical assays

Samples were immediately analyzed for NAG, LDH and ALP activity. The assay for urinary NAG activity was measured based on the enzymatic hydrolysis of p-nitrophenyl-N-acetylglucosaminide at pH 4.4, and the subsequent detection of liberated p-nitrophenol in 405 nm by spectrophotometry (14). LDH activity was measured based on the DGKC method (conversion of piruvate to lactate) at 340 nm (15). ALP activity was assayed by the DGKC method (conversion of PNP-phosphate to PNP) at 405 nm (15). Inulin was measured in urine and perfusate by colorimetric analysis using anthrone complexation (16). GFR was then calculated by the standard formula.

Data analysis

Data are expressed as mean ± SEM and were analyzed by two-way analysis of variance followed by the Tukey tests.

RESULTS

GFR and NAG, ALP and LDH activity in the L-arginine and L-NAME groups were not significantly different from controls (data not shown). Urinary enzyme activity in the gentamicin group was increased (Fig. 1-3) compared to the controls (p<0.05). L-arginine administration prevented gentamicin-induced enzyme release. Co-administration of L-NAME and gentamicin caused marked increases in NAG, ALP and LDH activity compared to both controls (p<0.001) and the gentamicin group (p<0.05) (Fig. 1-3).

Gentamicin administration reduced GFR significantly (p<0.05). A reduction in GFR was more evident in the gentamicin + L-NAME group (p<0.001). L-arginine provided protection against gentamicin-induced GFR reduction (Fig. 4).

Histological studies of gentamicin-treated kidneys showed moderate degrees of tubular damage (Fig. 5b). Severe degrees of cell damage were demonstrated in L-NAME + gentamicin kidney sections (Fig. 5d). There was a trend of prevention from injury in the L-arginine-treated group, demonstrated by less vacuolation in cells and more preservation in tubular patency (mild degree of damage, Fig. 5c).

DISCUSSION

The role of NO in gentamicin-induced nephrotoxicity was assessed by measuring urinary NAG, LDH and ALP activity. In this study, tubular function was not examined, but changes in glomerular filtration (GFR alterations) and histological changes were investigated.
Effects of NO on gentamicin nephrotoxicity

It was demonstrated that gentamicin administration induces elevations in enzymes released in the urine. L-arginine prevented increases in urinary enzyme activities, suggesting a protective role for the L-arginine-NO pathway in gentamicin nephrotoxicity. Similarly, Can et al found that NO formation has a protective effect on tubular cell integrity in an \textit{in vivo} GM toxicity model (4). Since L-arginine metabolites including polyamines and L-proline are cell growth and collagen synthesis mediators, they are probably involved in the protective effects of L-arginine in tubular cells (4). Moreover, it has been demonstrated that nitric oxide synthase (NOS) induction can induce cytoprotective proteins such as stress proteins (17).

In this study, L-arginine prevented the gentamicin-induced GFR reduction. The same results are reported by others (4, 5, 18). NO can play a role in attenuating the action of vasoconstrictor substances such as endothelin, which is released in gentamicin nephrotoxicity (11). Another mechanism involved in gentamicin-induced GFR reduction is the modulation of tubuloglomerular feedback (TGF) activity (6). Since NO modulates TGF (18), it can antagonize gentamicin-induced TGF activation and normalize GFR.

ROS generation can be an important mechanism in gentamicin-induced tubular damage. Active oxygen...
metabolites play an important role in gentamicin-mediated nephropathy (7). Some studies have reported that antioxidant administration ameliorates gentamicin-induced nephropathy (8, 9). In addition, in the presence of high NO concentrations, ROS can react with it to produce the peroxynitrite ions, which are known to be highly damaging.

Gentamicin-induced cytotoxicity was investigated by measuring urinary NAG, LDH and ALP activity. These cellular enzymes exist in many tissues. NAG is a hydrolytic lysosomal enzyme (12). LDH is a key enzyme in energy metabolism, which is located in the cell cytoplasm (19). ALP, a phosphohydrolase enzyme, is attached to the cell wall by glycosil phosphatidylinositol anchors (20). The activity of these enzymes in urine is physiologically very low. Therefore, any increase in their activity suggests proximal tubular cell damage (14, 19, 20). Several human and animal studies (in vivo and ex vivo) have used these cellular enzymes as sensitive and early markers of tubular injury in different experimental ARF models (5, 6, 17-22).

Concurrent administration of gentamicin and L-NAME caused a marked increase in the enzyme activities and a reduction in GFR compared to both the gentamicin group and controls. Since L-NAME administration enhanced gentamicin-induced damage, it can be suggested that endogenously released NO, as well as the administration of NOS inducers, could have a protective effect on gentamicin nephrotoxicity. Rivas Cahanero et al reported increased glomerular NO synthesis in gentamicin-treated rats and suggested that enhanced NO formation plays a role in the amelioration of impaired endothelial function (11).

Despite this evidence, the exact role of NO in renal failure remains controversial. Some studies have reported deleterious effects for L-arginine and NO (5, 23). These ambiguous results could be attributed to differences in the dosage, duration of L-arginine administration or the animal model used in each study (24). There is an important correlation between NO levels and its pathophysiological behavior. At low concentrations (nM and µM), NO activates guanylate cyclase to generate cGMP, which could be involved in vascular homeostasis. At these concentrations, NO also induces cytoprotective proteins. Higher NO (mM and M) concentrations can cause DNA damage and apoptosis (17).

The duration of L-arginine administration can also be an important factor. It has been demonstrated that acute L-arginine infusion is beneficial in in vivo renal ischemia, while chronic L-arginine supplementation showed deleterious effects (5). This could be due to the activation of different NOS isoforms. Endothelial NOS (eNOS) is available in its catalytic form within the cell and produces NO immediately after a stimulus. eNOS activity is short and produces low NO concentrations, while a few hours are needed for catalytic protein synthesis of inducible NOS (iNOS). iNOS activity is prolonged and produces high NO amounts (17). Some data provide evidence that eNOS activity leads to the restoration of renal function after injury, while iNOS activation causes tubular cytotoxicity and aggravates renal failure (23).
administration is harmful in isolated hypoxic/reoxygenated proximal tubules, while it is beneficial in \textit{in vivo} renal ischemia. To explain this paradox, they suggested the opposite effects that NO has on tubules and glomeruli. NO could be harmful to tubular cells due to its direct cytotoxic effect or its reaction with superoxide generating the oxidant peroxynitrite, while it improves glomerular hemodynamics via endothelial protection and vasodilation. Therefore, the net effect of NO formation depends on the balance between its beneficial hemodynamic effects and cytotoxicity.

In conclusion, in this study, L-arginine infusion in intact isolated rat kidneys prevented gentamicin-induced renal damage, while NOS blockade enhanced renal injury. These findings are consistent with those reports suggesting a protective effect of NO on kidney function in ARF. More studies on the role of active oxygen metabolites are needed to reach a definite conclusion about the factors possibly involved in this ARF model.

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\textbf{REFERENCES}