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Protective effect of oral L-arginine administration on gentamicin-induced renal failure in rats

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Abstract

We investigated the effects of orally supplemented L-arginine, the substrate of nitric oxide (NO) and N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide-synthase inhibitor in gentamicin-induced renal failure. Rats were given gentamicin (100 mg/kg/day s.c.), gentamicin and L-arginine (2 g/l, drinking water), gentamicin and L-NAME (100 mg/l, drinking water) or gentamicin plus L-arginine and L-NAME. After 8 days, the gentamicin group developed marked renal failure, characterized by a significantly decreased creatinine clearance and increased blood creatinine, fractional excretion of sodium, fractional excretion of lithium, urine gamma glutamyl transferase, systolic blood pressure and daily urine volume when compared to controls. Renal histological analysis confirmed tubular necrosis. L-arginine administration caused normalization of these parameters, whereas L-NAME led to aggravation of the failure. Concomittant administration of L-NAME and L-arginine to gentamicin-treated rats caused no significant changes when compared to the rats receiving gentamicin alone. We conclude that L-arginine supplementation has beneficial effects in gentamicin-induced renal failure in rats and that these effects are reversed by the NO-synthase inhibitor, L-NAME.

Keywords: Renal failure; Gentamicin; Nitric oxide (NO); L-arginine; L-NAME (N^{ω}-nitro-L-arginine methyl ester); (Rat)

1. Introduction

The amino acid, L-arginine, plays an important role in the regulation of renal function in pathological conditions. Beneficial effects of dietary intervention with L-arginine have been reported in experimental models of kidney diseases. L-arginine supplementation has been shown to improve renal hemodynamics and protect renal tissue from inflammatory injury by decreasing macrophage infiltration and increasing endogenous corticoid production (Reyes et al., 1994). L-arginine also contributes to the processes which are involved in cell growth and tissue repair in the kidney (Ketteler et al., 1994). Most of these beneficial effects of L-arginine in renal pathologies are considered to be caused by the end-products of L-arginine metabolism, such as polyamines, L-proline and nitric oxide (NO). Data from recent studies have provided convincing evidence that the L-arginine-NO pathway is of major importance with regard to the role of L-arginine supplementation in renal disease.

NO is a biological mediator which is synthesized from L-arginine by a family of enzymes called NO synthases, all which are expressed in the kidney (Moncada, 1997). This shortlived molecule has been shown to play an important role in the regulation of renal function in physiological and pathological conditions (Zatz and De Nucci, 1991; Raij, 1993; Schramm et al., 1994, 1996; Arese et al., 1995). Recent evidence suggest that activation of the L-arginine-NO pathway plays a protective role against renal injury in experimental models of renal failure, whereas administration of nitric oxide-synthase inhibitors leads to aggravation of the impairment in renal function (Chintala et al., 1993; Maree et al., 1994; Ito et al., 1995; Wakabayashi and Kikawada, 1996; Yang et al., 1998; Jerkic et al., 1999).

The aminoglycoside antibiotic, gentamicin, is well-known to cause renal failure, which is seen in 10–20% of patients receiving the drug. Gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly to the proximal tubule (Bennett, 1986).

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The possible involvement of the L-arginine-NO pathway in gentamicin-induced nephrotoxicity has been suggested by Rivas-Cabanero and associates. These investigators reported increased glomerular synthesis of NO in rats with gentamicin-induced renal failure (Rivas-Cabanero et al., 1994). Furthermore, the same group demonstrated that inhibition of NO synthesis by a nitric oxide-synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), was associated with a more marked renal impairment, suggesting a protective role for the increased renal NO production against gentamicin-induced renal damage (Rivas-Cabanero et al., 1995).

Based on these observations, we postulated that in vivo activation of the L-arginine- NO pathway might have a beneficial effect on gentamicin-induced renal failure. Thus, we examined the effects of oral L-arginine administration on renal function and morphology in rats treated with gentamicin. In addition, the effects of a nitric oxide synthesis inhibitor, L-NAME, in this model were studied.

2. Materials and methods

The present study was approved by the ''Animal Care and Ethics Commitee'' of the Faculty of Medicine, Ege University, İzmir.

2.1. Animals

A total of 50 male albino rats (Ege University Animal Center, İzmir, Turkey) weighing 230–300 g were housed individually in metabolic cages under controlled environmental conditions (24 ± 2°C and a 12-h light/dark cycle) and had free access to pulverized standard rat chow and tap water.

2.2. Experimental protocol

Rats were randomly assigned to 5 groups of 10 rats each:

Group 1 (Gentamicin): daily subcutaneous injections of gentamicin (100 mg/kg) for 8 consecutive days (Walker and Shah, 1988).
Group 2 (Gentamicin + L-arginine): daily injections of gentamicin as described above and L-arginine administration in drinking water (2 g/l) (Ono et al., 1996).
Group 3 (Gentamicin + L-NAME): daily injections of gentamicin and L-NAME in drinking water (100 mg/l) (Huang et al., 1995).
Group 4 (Gentamicin + L-arginine + L-NAME): daily injections of gentamicin and L-arginine (2 g/l) plus L-NAME (100 mg/l) in drinking water.
Group 5 (Controls): daily subcutaneous injections of isotonic saline for 8 consecutive days.

Rats were killed 24 h after the last injection. On the day prior to killing urine samples were collected over a 24-h period for clearance and electrolyte studies. At the time of killing blood samples were obtained by direct intracardiac puncture under ether anesthesia and the kidneys were removed for histological examination.

2.3. Laboratory investigations

At 48 h prior to killing lithium chloride (LiCl) was added to the drinking water (20 mM) for the calculation of fractional excretion of Li^+, which has been validated as an indicator of proximal tubular reabsorption (Dieperink et al., 1987). [*This dosage of Li^+ was reported to produce detectable serum levels without causing toxicity in rats* (English et al., 1987)]. Na^+, K^+ and Li^+ levels in plasma

Table 1
Serum creatinine, creatinine clearance, fractional excretion of Na^+ and fractional excretion of Li^+, urine volume and urinary gamma glutamyl transferase excretion of the groups
L-NAME, N^{ω}-nitro-L-arginine methyl ester; C_{Cr}, creatinine clearance; FE_{Li}%, fractional excretion of Li^+; FE_{Na}%, fractional excretion of Na^+; GGT, gamma glutamyl transferase.
Data are expressed as means ± S.E.M. for the groups; $n = 10$ in each group.
Statistical analysis was performed by Kruskal–Wallis one-way ANOVA followed by Mann–Whitney *U*-test.

	Controls	Gentamicin	Gentamicin + L-arginine	Gentamicin + L-NAME	Gentamicin + L-arginine + L-NAME
Serum creatinine (mg/dl)	0.41 ± 0.02	0.82 ± 0.05[a]	0.56 ± 0.02[b]	0.85 ± 0.04	0.84 ± 0.06
C_{Cr} (ml/min/100 g BW)	0.77 ± 0.06	0.34 ± 0.01[a]	0.65 ± 0.05[b]	0.27 ± 0.02[b]	0.40 ± 0.04
FE_{Li}%	22.55 ± 2.91	56.46 ± 5.16[a]	27.92 ± 2.97[b]	63.89 ± 6.03	46.30 ± 7.6
FE_{Na}%	0.59 ± 0.06	1.14 ± 0.15[a]	0.56 ± 0.06[b]	1.27 ± 0.11	1.18 ± 0.09
Urinary GGT excretion (U/day)	1.06 ± 0.11	10.54 ± 1.88[a]	7.87 ± 0.86	9.35 ± 1.35	10.58 ± 2.07
Urine volume (ml/day)	8.74 ± 0.45	13.66 ± 1.17[a]	11.50 ± 0.54	13.50 ± 1.54	12.50 ± 1.03

[a] $P < 0.01$ (compared to controls).
[b] $P < 0.01$ (compared to the rats receiving gentamicin alone).

Table 2
Plasma Na^+, plasma K^+ and urine K^+ values of the groups
L-NAME, N^{ω}-nitro-L-arginine methyl ester.
Data are expressed as means ± S.E.M. for the groups; $n = 10$ in each group.
Statistical analysis was performed by Kruskal–Wallis one-way ANOVA followed by Mann–Whitney *U*-test.

	Controls	Gentamicin	Gentamicin + L-arginine	Gentamicin + L-NAME	Gentamicin + L-arginine + L-NAME
Plasma Na^+ (mEq/l)	139.5 ± 3.4	143.8 ± 1.1	140.5 ± 2.0	141.5 ± 1.47	140.8 ± 1.5
Plasma K^+ (mEq/l)	5.17 ± 0.29	4.35 ± 0.18[a]	4.18 ± 0.26	4.35 ± 0.2	4.16 ± 0.2
Urine K^+ (mEq/day)	3.26 ± 0.24	3.59 ± 0.35	2.94 ± 0.18	3.17 ± 0.44	3.19 ± 0.21

[a] $P < 0.01$ (compared to controls).

and urine samples were measured with a flame photometer (JENWAY). Urine and serum creatinine assays were performed by Enzyme Multiplied Immune Technology (EMIT) (Cobas-MIRA, Roche Diagnostics). Urine levels of gamma glutamyl transferase were determined by EMIT, to evaluate the enzymuria, which was considered as an early finding of tubular injury. Creatinine clearance, fractional excretion of Li^+ and fractional excretion of Na^+ were calculated from these values by using standard methods (Preuss et al., 1991).

2.4. Blood pressure

Systolic blood pressure was measured in awake rats by using a tail-cuff method (MAY-COM-9610, BPHR, Commat İletişim, Ankara, Turkey). Measurements were performed at the baseline and before killing. Each blood pressure measurement was the average of 5–6 readings.

2.5. Kidney histology

The kidneys were excised after killing. Tissue was fixed in mercury formalin (B5) and 4% formalin, embedded in paraffin and 3 to 5-μm slices were stained with hematoxylin–eosin. Light microscopy was used to evaluate the following: (1) tubular necrosis; (2) tubular regenerative changes; (3) myeloid bodies (4) tubulointerstitial mononuclear cell infiltration.

Tubular necrosis was graded as follows:

Mild: areas of subcapsular tubular necrosis in small foci.
Moderate: tubular necrosis at different foci throughout the cortex.
Severe: extensive and marked tubular necrosis throughout the cortex.

Tubular regeneration, presence of tubular myeloid bodies and tubulointerstitial mononuclear cell infiltration were graded similarly.

2.6. Data analysis

Data are expressed as means ± S.E.M. for the groups. One-way analysis of variance (ANOVA) followed by the Wilcoxon matched pair test was used to compare time-related parameters within each group. Comparisons of parameters between different groups were evaluated by Kruskal–Wallis one-way ANOVA with a subsequent Mann–Whitney *U*-test. Results were considered statistically significant when $P < 0.05$.

2.7. Reagents

L-NAME and L-arginine were purchased from Sigma (USA), LCl from Riedel-de Haen (Germany) and gentamicin sulphate from Schering-Plough (İstanbul,Turkey).

3. Results

3.1. Group 1 (Gentamicin)

Serum creatinine, fractional excretion of Na^+, fractional excretion of L^+, urinary excretion of gamma glutamyl

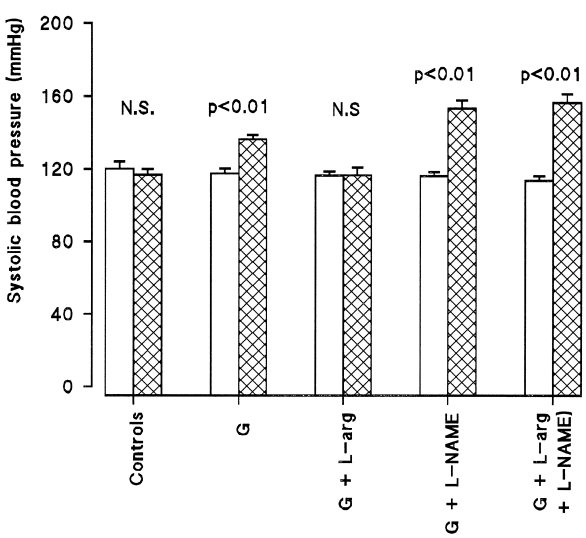

Fig. 1. Systolic blood pressure of the groups. Data are expressed as means ± S.E.M. for the groups; $n = 10$ in each group. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Wilcoxon matched pair test. Empty columns show baseline values, whereas full columns show the values on the ninth day of the study. L-arg, L-arginine; L-NAME, N^{ω}-nitro-L-arginine-methyl ester.

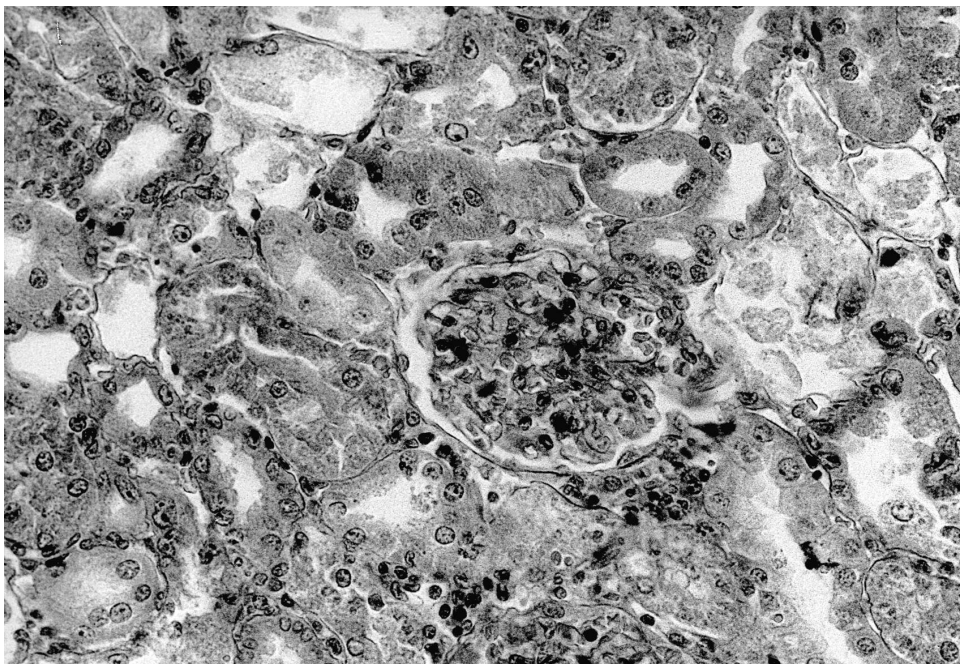

Fig. 2. Light microscopic section showing renal cortex from rats receiving gentamicin alone. Tubules show moderate necrosis and luminal debris (hematoxylin and eosin × 40).

transferase and daily urine volume increased significantly after 8 days of gentamicin administration compared to the controls (Table 1). This group was also characterized by a significantly lower creatinine clearance (Table 1) and plasma K^+ level (Table 2). Blood pressure increased significantly at the end of the study as compared to its initial value (Fig. 1). Histological examination of the kidney sections demonstrated different degrees of tubular

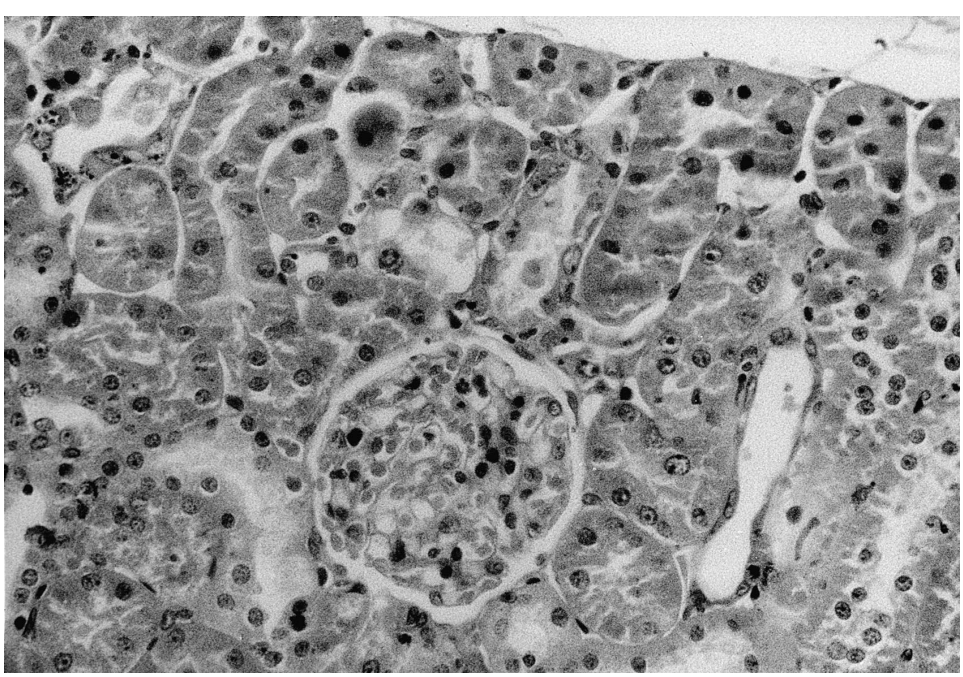

Fig. 3. Light photomicrograph of a kidney from rats receiving gentamicin and L-arginine. The morphology of the tubules reveals only mild tubular necrosis and slight degenerative changes (hematoxylin and eosin × 40).

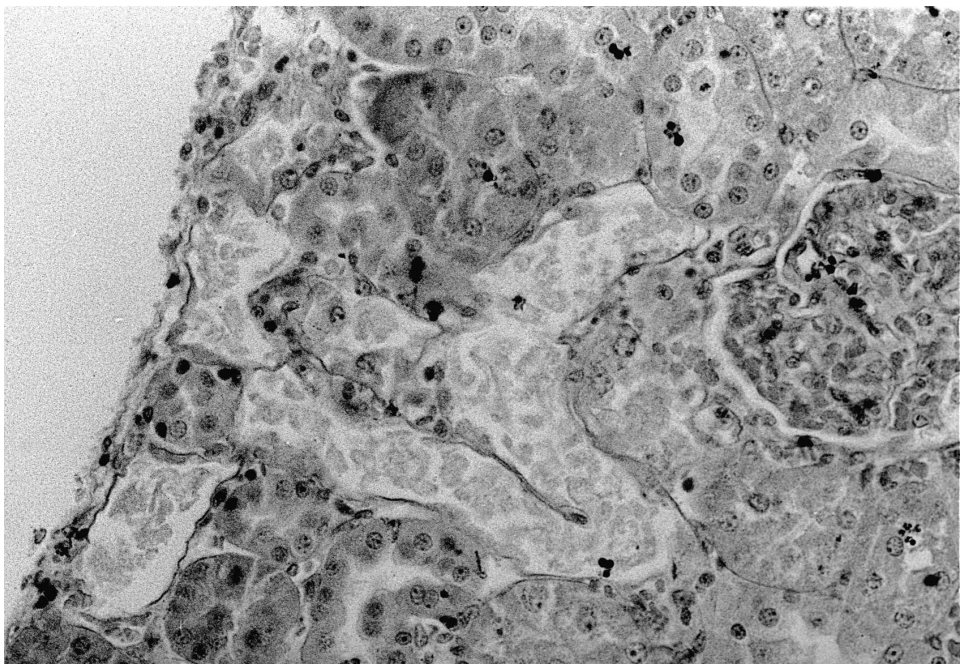

Fig. 4. Light microscopy of renal tissue from rats receiving gentamicin and L-NAME. Severe tubular necrosis and luminal debris are evident (hematoxylin and eosin × 40).

regeneration, mononuclear cell infiltration and myeloid bodies in all rats. Moderate tubular necrosis was seen in six the preparations (Fig. 2).

3.2. Group 2 (Gentamicin + L-arginine)

L-arginine administration provided marked protection manifested as significantly decreased serum creatinine, fractional excretion of Na^+, fractional excretion of Li^+ and significantly increased creatinine clearance compared to those in the animals treated with gentamicin alone (Table 1). In addition, normal systolic blood pressure was maintained in this group after the treatment period (Fig. 1). Urinary gamma glutamyl transferase excretion did not change compared to that in the gentamicin group

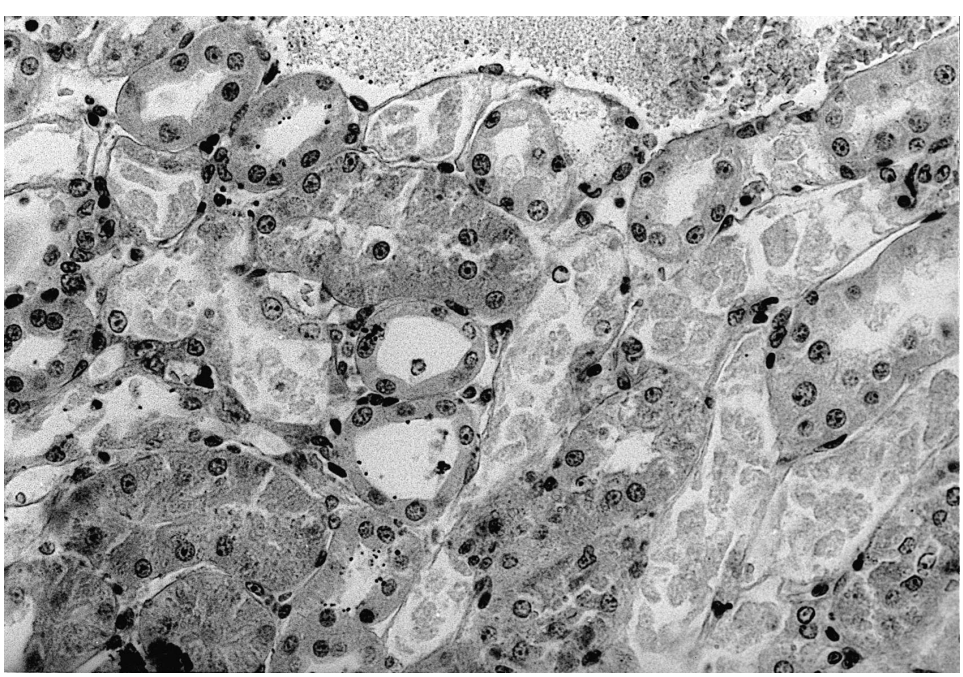

Fig. 5. Light photomicrograph of kidney from a rat receiving gentamicin, L-arginine and L-NAME together. Severe diffuse tubular necrosis and regenerative changes are present with excessive luminal debris (hematoxylin and eosin × 40).

(Table 1). Histological examination of the kidney sections revealed tubular regeneration and mononuclear cell infiltration in nine rats; six kidneys showed different amounts of myeloid bodies. However, tubular necrosis was conspicuously absent in this group, except in one kidney which showed mild necrosis and slight degenerative changes (Fig. 3).

3.3. Group 3 (Gentamicin + L-name)

Creatinine clearance significantly decreased in this group compared to that in the rats treated with gentamicin alone (Table 1). Furthermore, the increase in blood pressure was more evident in this group. (Fig. 1). Light microscopic examination of kidneys revealed mild to moderate degrees of tubular regeneration, mononuclear cell infiltration and tubular myeloid bodies in all sections. Different degrees of tubular necrosis were seen in 8 of the kidneys (Fig. 4).

3.4. Group 4 (Gentamicin + L-arginine + L-NAME)

No significant differences were observed in functional parameters in this group when compared to the rats treated with gentamicin alone. The final blood pressure increased significantly compared to the baseline value (Fig. 1). Microscopic appearance of the kidney sections from this group revealed marked morphologic deterioration. Mild to moderate degrees of tubular regeneration and numerous myeloid bodies were present in all kidneys. Mononuclear cell infiltration was observed in eight kidneys; five kidneys presented with moderate tubular necrosis, whereas severe necrosis was seen in four preparations (Fig. 5).

4. Discussion

We now showed that administration of gentamicin to rats induced a reduction in glomerular filtration rate as shown by a reduced creatinine clearance and increased serum creatinine. This impairment in glomerular function was accompanied by increased fractional excretion of Na^+ and Li^+, indicating proximal tubular dysfunction. The presence of tubular damage was further confirmed by the increased urinary excretion of the brush border marker, gamma glutamyl transferase, which indicates direct toxic injury. These findings correlated well with the renal morphologic examination, which revealed tubular necrosis and myeloid bodies on kidney sections. Considering the increased daily urine output, these data confirmed the well-known pattern of aminoglycoside nephrotoxicity characterized by decreased glomerular filtration rate and direct tubular damage associated with a well-maintained urinary output (Bennett, 1986). However, the increased systolic blood pressure in this group was not consistent with the findings of others (Rivas-Cabanero et al., 1995), who reported unchanged blood pressure after 5 days of gentamicin administration to rats. Assuming that this discrepancy might result from the different duration of gentamicin exposure in the two studies, we measured blood pressure daily in an additional group of 6 rats receiving gentamicin and L-arginine together. Table 3 presents the individual data obtained from these rats on days 0, 2, 5 and 9. As seen on the table, none of these rats demonstrated increased blood pressure before day 5, whereas the final values on day 9 had increased significantly. Thus, these results suggested a possible progression into chronic failure in our model, most probably due to the longer duration of gentamicin exposure.

Oral administration of L-arginine to gentamicin-treated rats resulted in almost complete normalization of the creatinine clearance and serum creatinine levels, indicating an increase in the glomerular filtration rate. These data support the findings of others who have previously reported recovery of glomerular function on L-arginine administration in different models of experimental renal failure (Maree et al., 1994; Schramm et al., 1994; Wakabayashi and Kikawada, 1996; Jerkic et al., 1999). The reduction of glomerular filtration rate in gentamicin nephrotoxicity is thought to be secondary to the tubular obstruction due to luminal debris (Neugarten et al., 1983), or to the decline in the glomerular capillary ultrafiltration coefficient, which is mediated by the contractile effect of angiotensin-II on glomerular mesengial cells (Schor et al., 1981). Rivas-Cabanero et al. (1997) demonstrated that the contractile effect of gentamicin on mesengial cells could be decreased in vitro by L-arginine. Based on these findings, they suggested that the protection of glomerular function by L-arginine might result from the reversal of the decreased glomerular capillary ultrafiltration coefficient. Collectively, these observations support the concept that activation of the L-arginine-NO pathway antagonizes the effects of endogenous vasoconstrictors on glomerular hemodynamics (Raij, 1993; Ono et al., 1998). The hypothesis that NO behaves like a natural antagonist of vasoconstrictor

Table 3
Systolic blood pressure values (mmHg) of the rats in the additional group receiving gentamicin and L-arginine together
Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Wilcoxon matched pair test.
(Each blood pressure measurement is the average of 5–6 readings).

	Day 0 (Baseline)	Day 2	Day 5	Day 9 (Final)
Rat 1	118	121	140	143
Rat 2	112	118	115.5	138
Rat 3	114	108	114	118
Rat 4	129	115	143.5	149
Rat 5	120	117	121	137.5
Rat 6	102	92	103.5	110
Mean ± S.E.M.	115.8 ± 3.6	111.8 ± 4.3	122.9 ± 6.4	132.6 ± 6.2[a]

[a] $P < 0.05$ (compared to the baseline value).

agents such as angiotensin-II and endothelin-1 is also consistent with our finding that normal blood pressure was strikingly maintained in rats treated with gentamicin and L-arginine together.

Besides the marked protection of glomerular function, our data also suggested a significant protection of tubular function by L-arginine, which was manifested as decreased fractional excretion of Na^+ and fractional excretion of Li^+. The histological demonstration of the absence of tubular necrosis on most of the kidneys confirmed the preserved tubular function. However, the presence of the degenerative changes on tubular epithelium, combined with the slightly decreased urinary excretion of gamma glutamyl transferase compared to the result of gentamicin treatment alone suggests that the protection against tubular injury was partial. In fact, the role of NO in tubular function is controversial. Recognizing that gentamicin-induced nephrotoxicity is a model of oxidant-induced renal failure, one might easily suggest that NO, because of its free radical nature might contribute to tubular damage. Previous studies have demonstrated that NO and its metabolite, peroxynitrite, may play an important role in renal tubular injury in vitro (Yu et al., 1994; Wangisiripaisan et al., 1999) and in vivo (Noiri et al., 1996; Yanagisawa et al., 1998). However, these findings are contradicted by the data of others which show that either L-arginine or exogenous NO donors attenuate ischemia-induced tubular injury in vivo (Matsamura et al., 1998; Jerkic et al., 1999). Our findings are consistent with these recent reports suggesting a protective effect of NO on tubular function. On the other hand, the data from our study do not exclude the possibility of the involvement of other L-arginine metabolites in this protective effect. Since it is known that polyamines are mediators of cell growth and that L-proline is involved in collagen synthesis, and both of these L-arginine metabolites are known to play roles in tissue repair processes (Ketteler et al., 1994), it is not unreasonable to suggest that these metabolites might also contribute to the beneficial effect of L-arginine on renal injury. Furthermore, it may also be speculated that L-arginine might have protected tubular function simply by decreasing gentamicin reabsorption in the proximal tubules. Thus, further investigation is required to elucidate the mechanisms underlying this possible beneficial effect.

The data obtained from the gentamicin-treated animals receiving the NO synthesis inhibitor, L-NAME evidenced further deterioration in renal function, which was characterized by a lower creatinine clearance and higher blood pressure when compared to those of the rats receiving gentamicin alone. The histological evaluation of the kidney preparations in this group also revealed a more severe tubular necrosis.

The effect of nitric oxide synthase inhibition on renal failure has been investigated by many authors. NO-synthase inhibitors have been found to aggravate renal dysfunction in in vivo studies, whereas in vitro experiments reveal their protective role on renal function. This paradoxical effect has been attributed to the existence of different isoforms of nitric oxide synthase in mammalian kidney. Recent data provided evidence that endothelial NOS leads to restoration of renal function after injury, while activation of inducible NOS, leading to excessive NO production, causes tubular cytotoxicity and aggravates renal failure (Noiri et al., 1996). Accordingly, it is suggested that, when the activity of all isoforms of NOS is inhibited with a non-selective inhibitor such as L-NAME, the deleterious consequences of inhibiting endothelial NOS prevail over the beneficial effects of inhibiting inducible NOS. At present, possible effects of selective NOS inhibitors in gentamicin nephrotoxicity remain to be investigated, but our data support the findings of others who have demonstrated that non-selective inhibition of NO synthesis is related with subsequent potentiation of the renal insufficiency in this model (Rivas-Cabanero et al., 1995). These authors reported increased arterial blood pressure and serum creatinine levels and decreased creatinine clearance after oral administration of L-NAME in rats with gentamicin nephrotoxicity. However, at variance with our results, they found decreased urinary excretion of Na^+ and K^+ in L-NAME-treated rats. The role of NO in renal Na^+ handling is controversial, especially in pathological conditions, and interpretation of the results is quite difficult as the studies are complicated by many factors including the complexity of the mechanisms involved in tubular Na^+ handling and the differences in study protocols (Raij, 1993). Although it is not possible to explain the exact role of NO in renal tubular sodium handling based on the present data, our findings makes it is plausible to speculate that protection of tubular integrity by L-arginine administration leads to increased reabsorption of Na^+ in the proximal tubules.

Finally, concomittant administration of L-NAME to gentamicin-treated rats almost totally abolished the beneficial effect of L-arginine on renal function. In a previous report Ashab et al. (1995) demonstrated a similar abolition of the recovery of renal function when a nitric oxide synthase inhibitor was given in combination with L-arginine to rats with chronic renal failure. It is accepted that the accumulation of endogenous inhibitors of nitric oxide synthase, leading to impaired NO synthesis is one of the key factors in renal failure (Vallance et al., 1992; Mendes-Ribeiro et al., 1996) and that the deleterious effects of these inhibitors can be overcome by exogenous administration of L-arginine (MacAllister et al., 1994). Therefore, the loss of recovery of renal function in this group is most likely due to the additive effect of exogenously administered L-NAME on the increased amount of endogenously produced NO synthesis inhibitors, which outweighed the beneficial effect of L-arginine on renal hemodynamics. However, the interesting, but still unexplained finding that the most severe tubular necrosis was observed in this group needs further investigation.

5. Conclusion

Our data suggest that administration of L-arginine has beneficial effects on both glomerular and tubular function in rats with gentamicin-induced renal failure and that these effects are reversed by the NO-synthase inhibitor L-NAME.

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