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Protective Effect of *trans*-Resveratrol on Gentamicin-Induced Nephrotoxicity

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ABSTRACT

Reactive oxygen species (ROS) have been involved in glomerular filtration rate (GFR) reduction observed after gentamicin treatment. *trans*-Resveratrol (TR), a natural hydroxystilbene, has been identified to be a potent inhibitor of ROS production. The aim of this work has been to study whether TR has a protective effect on gentamicin-induced nephrotoxicity *in vivo* and the effect of TR on lipid peroxidation and the oxidative stress induced by gentamicin. Animals that received a daily intraperitoneal injection of gentamicin (100 mg/kg body weight) showed lower GFR and renal blood flow (RBF) and higher urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG) than control rats. Rats receiving TR together with gentamicin showed higher GFR and RBF and lower NAG urinary excretion than rats receiving gentamicin alone. Moreover, renal lipid peroxidation increased in rats receiving gentamicin alone, and this increase was prevented by the administration of TR. The concentration in plasma of antioxidants was higher in the group that received TR with gentamicin than in the gentamicin and control groups. The activities of lactate dehydrogenase and alkaline phosphatase were higher in rats treated with gentamicin than in control rats and were reduced by the treatment with TR. This study demonstrates an improvement in renal function in response to the administration of TR in gentamicin-induced nephrotoxicity. At least a part of this effect of TR could be based on its antioxidant activity. *Antioxid. Redox Signal.* 4, 893–898.

INTRODUCTION

GENTAMICIN is an aminoglycoside antibiotic very effective in treating different gram negative infections (12). However, one of its main side effects is nephrotoxicity, even at the lower therapeutical doses (15). Although gentamicin-induced nephrotoxicity is mainly tubular, chronic treatment with gentamicin also modifies glomerular hemodynamics, *i.e.,* reduces renal blood flow (RBF) and glomerular filtration rate (GFR) without apparent glomerular damage (24).

Some *in vivo* experiments suggest that gentamicin-induced renal dysfunction is mediated by reactive oxygen species (ROS) production. Thus, administration of antioxidants such as superoxide dismutase (SOD) (2, 18, 36), selenium, vitamin E (1), and ascorbic acid (6) decrease the gentamicin-induced reduction in GFR. *In vitro* experiments in isolated mitochondria show that ROS production is enhanced by gentamicin (34). These ROS could be responsible for proximal tubular necrosis and acute renal failure caused by gentamicin *in vivo* (10). It has been also proposed that an elevated production of ROS may be responsible for the increased renal susceptibility to gentamicin observed in obstructive jaundice (27).

A possible role of natural antioxidant compounds in renal disease protection has been described (for review, see 29). *trans*-Resveratrol (*trans*-3,4´,5-trihydroxystilbene) is a natural hydroxystilbene present in grapes and other vegetables,

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with a strong antioxidant activity (17, 30). It has been reported that several stilbenes have therapeutic potential in some diseases, including ischemic heart disease, arteriosclerosis, and cancer (8, 13, 20). However, their effects on kidney disease have not been studied although a strong affinity of these substances for kidneys has been described (7).

Thus, the purpose of the present work is to study the effect of *trans*-resveratrol on renal function, lipid peroxidation, and oxidative stress in gentamicin-treated rats.

MATERIALS AND METHODS

Materials

trans-Resveratrol was obtained from Sigma–Aldrich–Química (Madrid, Spain). [^{14}C]Inulin and *p*-[^{3}H]aminohippuric acid (PAH) were purchased from Itisa Biomedica (Madrid, Spain). Gentamicin was a gift from Schering–Plough Laboratories (Madrid, Spain). All the other reagents were of the highest commercially available grade.

Experimental groups

Experiments were carried out in male Wistar rats weighing ~250 g, fed on a standard diet and water *ad libitum*. Lighting and temperature were controlled by a timer that permitted light between 0800 and 2000 h and temperature to 20 ± 1°C. Animals were treated according to the regulations of the following institutions: Conseil de l'Europe (published in the Official Daily no. L358/1–358/6, 18th December 1986), and Spanish Government (published in Boletín Oficial del Estado no. 67, pp. 8509–8512, 18th March 1988, and Boletín Oficial del Estado no. 256, pp. 31349–31362, 28th October 1990).

Animals were divided into four experimental groups: (1) control group (n = 5); (2) *trans*-resveratrol group (n = 8) that received a daily gavage dose of *trans*-resveratrol (120 mg/kg body weight); (3) gentamicin group (n = 11) that received a daily intraperitoneal injection of gentamicin (100 mg/kg body weight); and (4) *trans*-resveratrol and gentamicin group (n = 8) that received a daily gavage dose of *trans*-resveratrol (120 mg/kg body weight) and a daily intraperitoneal injection of gentamicin (100 mg/kg body weight). Rats were treated for 5 days.

The fifth day after starting treatment, urine free of food and feces was collected into graduated cylinders containing mineral oil to prevent evaporation and 0.1% sodium azide to minimize bacterial growth. These urine samples were used to determine electrolyte excretion and urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG). Urinary concentrations of sodium, potassium, and chloride ions were measured by selective ion electrodes. NAG was measured colorimetrically using a commercial reagent kit (Randox, Crumlin, U.K.).

Acute clearance studies

After treatment, animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and placed on a heated animal board. Rectal temperature was monitored and maintained at 37°C. Firstly, a tracheotomy was performed to facilitate breathing throughout the experiment. Animals were surgically prepared for clearance studies by inserting PE-50 polyethylene catheters in the femoral artery and vein and in the bladder. The femoral artery was connected to a pressure transducer and to a digital data recorder (Mac Lab, AD Instruments, Castle Hill, Australia) for the continuous recording of mean arterial pressure. Urine was collected from a bladder catheter into preweighed plastic vials containing 0.5 ml of water-stabilized mineral oil. An isotonic infusion containing [^{3}H]inulin and [^{14}C]PAH was started at 3 ml/h through the venous catheter to allow clearance determinations. After 30 min of equilibration, three 30-min urine collections were performed, with blood sampling (150 μl) at the beginning and the end of each clearance period. Packed cell volume was determined by the microcapillary method. ^{3}H and ^{14}C activities were measured in blood and urine samples using a two-channel liquid scintillation counter (Wallac 1409 DSA, Turku, Finland). Inulin and PAH clearances were calculated according to standard formulas.

Lipoperoxidation studies

At the end of study, animals were killed by exsanguination and kidneys were perfused with isotonic saline solution, removed, and homogenized (1/5, wt/vol) in 20 m*M* phosphate buffer, pH 7.4, 50 m*M* NaCl. The homogenate was treated with $HClO_4$ (7% final concentration) and centrifuged, and the supernatant was used to determine thiobarbituric acid reactive substances as an index of lipid peroxidation by the method described by Recknagel *et al.* (22).

Oxidative stress studies

The fifth day after treatment was started, samples of blood were collected and centrifuged. The plasma was used to determine total antioxidants (TAS), glutathione reductase (GR), glutathione peroxidase (GPX), alkaline phosphatase (AP), and lactate dehydrogenase (LDH). TAS, GR, and GPX were measured by a spectrophotometric method with commercial kits from Randox (Randox Laboratories Ltd., Crumlin, U.K.). AP and LDH were measured by commercial kits from Roche (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Data are expressed as means ± SEM. Comparison of means was performed by one- or two-way analysis of variance and Scheffé's multiple comparison test.

RESULTS

Acute clearance studies

The results of the effect of gentamicin treatment on renal function are shown in Table 1 and Fig. 1. The effect of gentamicin on mean arterial pressure was similar in all groups (Table 1). Gentamicin-treated rats had a lower GFR (Fig. 1A), RBF (Fig. 1B), and renal plasma flow (RPF) (Table 1) and a higher renal vascular resistance (RVR) (Table 1) than control rats. No significant differences in the parameters of renal function studied were observed between the animals receiv-

TABLE 1. EFFECT OF TREATMENT WITH *TRANS*-RESVERATROL ON RENAL FUNCTION IN RATS TREATED WITH GENTAMICIN FOR 5 DAYS

	Control (*n* = 5)	*Resveratrol* (*n* = 8)	*Gentamicin* (*n* = 11)	*Resveratrol + Gentamicin* (*n* = 8)
MAP (mm Hg)	127 ± 7	122 ± 15	126 ± 16	131 ± 17
UF (µl/min)	2.6 ± 0.15	2.1 ± 0.12	2.3 ± 0.02	2.4 ± 0.17
RPF (ml/min)	9.17 ± 1.09*	9.43 ± 1.04*	2.79 ± 0.69†	8.63 ± 1.22*
FF (ml/min)	35.40 ± 2.80	42.89 ± 7.72	34.97 ± 3.65	38.60 ± 3.02
RVR (mm Hg·min/ml)	16.30 ± 1.50*	10.70 ± 2.79*	411.0 ± 214.5†	10.99 ± 2.41*

The data are means ± SEM of three clearance periods in each rat. *Abbreviations:* MAP, mean arterial pressure; UF, urinary flow; RPF, renal plasma flow; FF, filtration fraction; RVR, renal vascular resistance.
*$p < 0.05$ vs. gentamicin group; †$p < 0.05$ vs. control group (one-way analysis of variance).

ing *trans*-resveratrol alone and the control group. The animals that received gentamicin and *trans*-resveratrol had higher values of GFR, RPF, RBF, and lower RVR than those that received gentamicin alone.

Urinary excretion studies

Gentamicin-treated rats showed a higher urinary fractional excretion of Na^+, K^+, and Cl^- than the control group. No significant differences in urinary ion excretion were observed between the animals receiving *trans*-resveratrol alone and the control group. When the gentamicin was administered together with *trans*-resveratrol, no significant differences in Na^+, K^+, and Cl^- excretion were found between these animals and the control group (Table 2). Urinary excretion of NAG was significantly higher in the gentamicin-treated group than in the control group (224.6 ± 24.2 vs. 29.0 ± 3.4 mU/24 h; $p < 0.01$). This increase was prevented by simultaneous administration of *trans*-resveratrol and gentamicin (62.0 ± 6.6 mU/24 h).

Lipoperoxidation studies

The level of renal lipid peroxidation was significantly higher in the group that received gentamicin than in the control one. Gentamicin-induced lipid peroxidation was reduced by simultaneous administration of *trans*-resveratrol (Fig. 2).

Oxidative stress studies

Plasma TAS concentrations were lower in the gentamicin group than in the control group. Rats treated with *trans*-resveratrol plus gentamicin show an increase in TAS that is even higher than in the control group (Table 3). No significant differences in GR were observed between the groups (Table 3). GPX activity decreased in plasma of the gentamicin-treated rats. This decrease in GPX activity was pre-

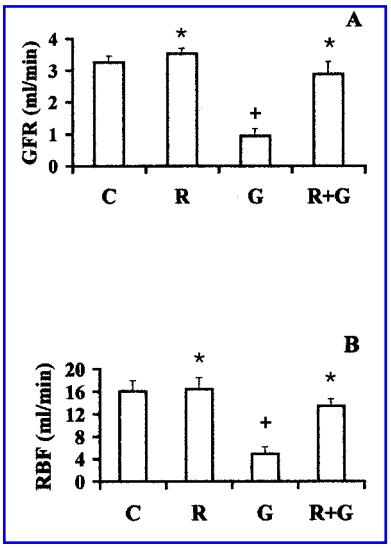

FIG. 1. Effect of treatment with *trans*-resveratrol, gentamicin and *trans*-resveratrol plus gentamicin on GFR (A) and RBF (B) in rats. Experimental groups: (1) control group (C) (*n* = 5), (2) resveratrol group (R) (*n* = 8) that received a daily oral dose of *trans*-resveratrol (120 mg/kg body weight), (3) gentamicin group (G) (*n* = 11) that received a daily intraperitoneal injection of gentamicin (100 mg/kg body weight), and (4) resveratrol and gentamicin group (R+G) (*n* = 8) that received a daily oral dose of resveratrol (120 mg/kg body weight) and a daily intraperitoneal injection of gentamicin (100 mg/kg body weight). Rats were treated for 5 days. Data are means ± SEM of three clearance periods in each rat. †$p < 0.05$ vs. C group; *$p < 0.05$ vs. G group (one-way analysis of variance).

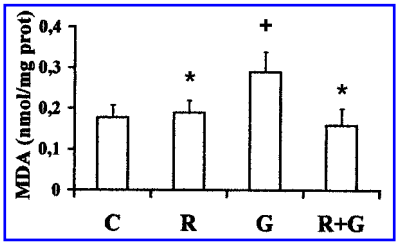

FIG. 2. Renal lipid peroxidation in the four groups of rats studied: (1) control group (C) (*n* = 5), (2) resveratrol group (R) (*n* = 8) that received a daily gavage dose of *trans*-resveratrol (120 mg/kg body weight), (3) gentamicin group (G) (*n* = 11) that received a daily intraperitoneal injection of gentamicin (100 mg/kg body weight), and (4) resveratrol and gentamicin group (R+G) (*n* = 8) that received a daily gavage dose of *trans*-resveratrol (120 mg/kg body weight) and a daily intraperitoneal injection of gentamicin (100 mg/kg body weight). Rats were treated for 5 days. Data are means ± SEM, †$p < 0.05$ vs. C group; *$p < 0.05$ vs. G group (one-way analysis of variance).

TABLE 2. EFFECT OF TREATMENT WITH *TRANS*-RESVERATROL ON ELECTROLYTE EXCRETION IN RATS TREATED WITH GENTAMICIN FOR 5 DAYS

	Control (n = 5)	*Resveratrol (n = 5)*	*Gentamicin (n = 11)*	*Resveratrol + Gentamicin (n = 7)*
FE Na^+	0.847 ± 0.006	0.655 ± 0.275	1.409 ± 0.150*	0.815 ± 0.216
FE K^+	44.54 ± 3.81†	33.29 ± 11.17†	95.25 ± 14.70*	37.42 ± 8.94†
FE Cl^-	1.335 ± 0.102	1.030 ± 0.153	1.842 ± 0.193	1.181 ± 0.383

The data are means ± SEM. *Abbreviations:* FE, urinary fractional excretion (%), FE Na^+, urinary fractional excretion of Na^+; FE K^+, urinary fractional excretion of K^+; FE Cl^-, urinary fractional excretion of Cl^-.

*$p < 0.05$ vs. control group; †$p < 0.05$ vs. gentamicin group (one-way analysis of variance).

vented partially in the *trans*-resveratrol plus gentamicin group (Table 3). The gentamicin-treated group showed higher plasma AP and LDH activities than control. The *trans*-resveratrol plus gentamicin group had lower values than rats treated with gentamicin alone (Table 3).

DISCUSSION

This study demonstrates that administration of *trans*-resveratrol markedly reduced the decrease in GFR and RBF induced by a nephrotoxic dose of gentamicin in rats. This protective effect of *trans*-resveratrol on the impairment of renal function induced by gentamicin was associated with its ability to prevent the increase in lipoperoxidation. Studies carried out by Ademuyiwa *et al.* (1), Yang *et al.* (33) and Baliga *et al.* (5) observed that preadministration of Zn^{2+} (10 mg/kg/day s.c. over 5 days) decreased the adverse effect of gentamicin. They suggested that Zn^{2+} could be involved in inducing the synthesis of metallothionein, which would act as a scavenger of the free radicals generated by gentamicin. Similar effects have been reported by Ademuyiwa *et al.* (1) using Se^{2+} and vitamin E and by Ben-Ismail *et al.* (6) using ascorbic acid. These substances act as antioxidants in the physiological processes of the degradation of superoxide and hydroxyl radicals to oxygen and water. Administered prior to aminoglycosides, all of them decreased the nephrotoxicity. Other works supporting the role of free radicals, in gentamicin-induced nephrotoxicity are those conducted with SOD. SOD is a key enzyme in the physiological process of the removal of free radicals, and its activity has been reported to be decreased during treatments with gentamicin. This would explain why the increase in the production of free radicals might contribute to the observed toxic renal effects. Indeed, the administration of SOD at least partially reverses aminoglycoside-induced nephrotoxicity (18, 28). These results suggest that increased ROS production could be responsible for proximal tubular necrosis and acute renal failure caused by gentamicin *in vivo* (10). As *trans*-resveratrol is a substance with a strong antioxidant activity (17, 30), a potent inhibitor of ROS production (14) and an inhibitor of lipid peroxidation (26), our results suggest that the protective effect of *trans*-resveratrol on renal function could be mediated by inhibiting the lipid peroxidation induced by ROS.

Another major result of this study is the correlation between the concentration of antioxidants in plasma and the activity of enzymes that serve as markers of cell damage. AP is a brush border-associated enzyme (23) and LDH is a marker of cell necrosis (25). *trans*-Resveratrol treatment reduces the increase in AP and LDH activities induced by gentamicin treatment and increases the levels of plasma antioxidants. This suggests that the increase in the antioxidant concentration in plasma can prevent the gentamicin-induced cell injury. These findings confirm that the formation of oxygen free radicals is one of the mechanisms involved in the gentamicin-induced nephrotoxicity. In addition, *trans*-resveratrol treatment also partially prevented the decrease in plasma GPX, which is synthesized almost exclusively in kidney proximal

TABLE 3. EFFECT OF TREATMENT WITH *TRANS*-RESVERATROL ON OXIDATIVE STRESS-RELATED SUBSTANCES AND ENZYMES ACTIVITIES IN RATS TREATED WITH GENTAMICIN FOR 5 DAYS

	Control (n = 5)	*Gentamicin (n = 5)*	*Resveratrol + Gentamicin (n = 4)*
TAS (μ*M*)	1.42 ± 0.08	1.11 ± 0.05*	2.07 ± 0.03*†
GR (U/L)	59.60 ± 2.67	55.60 ± 2.33	67.25 ± 1.75
GPX (U/L)	893.6 ± 85.0	431.4 ± 20.3*	563.0 ± 13.2*
AP (U/L)	553.7 ± 87.2	702.7 ± 31.7	506.0 ± 2.27†
LDH (U/L)	193.80 ± 6.79	370.25 ± 21.99*	182.25 ± 14.44†

The data are means ± SEM.

*$p < 0.05$ vs. control group; †$p < 0.05$ vs. gentamicin group (one-way analysis of variance).

tubular cells (4, 19, 35) and may be used as a marker of tubular damage (4, 31). Other authors describe similar results in studies of gentamicin nephrotoxicity using garlic as antioxidant treatment (21).

One of the early markers of kidney damage following the administration of aminoglycosides is an increase in the urinary excretion of several tubular enzymes (alanine-aminopeptidase, AP, and NAG). Our results show that resveratrol treatment prevents the gentamicin-induced increased urinary excretion of NAG. These results suggest that resveratrol is able to prevent the functional and structural tubular alterations.

Another symptom of kidney damage following the administration of gentamicin is an increase in urinary excretion of several ions. Na^+,K^+-ATPase is an enzyme located in the basolateral portion of the plasma membrane (16). In cortical homogenates from rats treated chronically with gentamicin, the activity of this enzyme decreases (3, 9, 32). These changes, together with the loss of the integrity of Na^+,K^+-ATPase, have been proposed to be also responsible for the nephrotoxic effect of gentamicin (11) because this enzyme regulates the transport of intracellular electrolytes and cell volume. In our work, this alteration in urinary fractional excretion of electrolytes is reverted by *trans*-resveratrol. These results confirm the protector effect of this antioxidant on tubular reabsorption and the secretion process.

In summary, the present study demonstrates that *trans*-resveratrol has a protective effect against gentamicin-induced reduction in renal function, lipid peroxidation, and cell damage. This effect seems to be mediated by its antioxidant properties. These results are in agreement with previous studies showing that the administration of antioxidant affords protective effects against renal damage caused by aminoglycosides.

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ABBREVIATIONS

AP, Alkaline phosphatase; GFR, glomerular filtration rate; GPX, glutathione peroxidase; GR, glutathione reductase; LDH, lactate dehydrogenase; NAG, *N*-acetyl-β-D-glucosaminidase; PAH, *p*-[^{3}H]aminohippuric acid; RBF, renal blood flow; ROS, reactive oxygen species; RPF, renal plasma flow; RVR, renal vascular resistance; SOD, superoxide dismutase; TAS, total antioxidants in plasma.

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