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Diallyl disulfide ameliorates gentamicin-induced oxidative stress and nephropathy in rats

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Abstract

Experimental evidences suggest a role of reactive oxygen species in gentamicin-induced nephropathy in rats. Therefore, we investigated if diallyl disulfide, a garlic-derived compound with antioxidant properties, has a renoprotective effect in this experimental model. Four groups of rats were studied: (1) control, (2) gentamicin treated subcutaneously with gentamicin (70 mg/kg/12 h/4 days), (3) diallyl disulfide treated intragastrically with diallyl disulfide (50 mg/kg/24 h/4 days), and (4) gentamicin + diallyl disulfide treated with gentamicin + diallyl disulfide. Gentamicin induced (a) nephrotoxicity, (b) increase in renal oxidative stress, and (c) decrease in the activity of manganese superoxide dismutase, glutathione peroxidase, and glutathione reductase. Diallyl disulfide ameliorated these changes induced by gentamicin. The mechanism by which diallyl disulfide has a renoprotective effect in gentamicin-induced acute renal failure in rats may be related, at least in part, to the amelioration in the oxidative stress and the preservation in the activity of the antioxidant enzymes in kidney.

Keywords: Garlic; Aminoglycoside; Superoxide dismutase; Glutathione peroxidase; Oxidative stress; Nephrotoxicity

1. Introduction

Gentamicin is an aminoglycoside antibiotic widely used for the treatment of life-threatening Gram-negative infections (Ali, 1995). However, the clinical usefulness of this drug is limited by the fact that 30% of the patients treated with gentamicin for more than seven days show some signs of nephrotoxicity (Lerner et al., 1986) which complicates and raises the cost of the treatment. Despite the introduction of newer and less toxic antibiotics, gentamicin continues serving a useful role in the treatment of serious enterococcal, mycobacterial, and Gram-negative infections, due to its low cost, and the low levels of resistance among enterobacteriaceae family bacterium (Edson and Terrell, 1999). Therefore, a potential therapeutic approach to protect or reverse gentamicin-induced renal damage would have very important clinical consequences. Rats with gentamicin-induced nephrotoxicity provides an excellent model of acute renal failure to test compounds, extracts or drugs which could be used to prevent side effects of gentamicin in humans (Ali, 1995). The exact mechanism by which gentamicin induces nephrotoxicity is unknown, however, reactive oxygen species have been involved (Cuzzocrea et al., 2002; Mazzon et al., 2001; Morales et al., 2002; Walker and Shah, 1987, 1988; Yang et al., 1995). It has been found that renal cortical lipoperoxidation (Abdel-Naim et al., 1999; Ali, 2002; Cuzzocrea et al., 2002; Mazzon et al., 2001; Nakajima et al., 1994; Walker and Shah, 1988) and in vivo renal hydrogen peroxide (H_2O_2) generation (Guidet and Shah, 1989) are increased and reduced glutathione is decreased (Ali, 2002; Sener et al., 2002) in gentamicin-treated rats. Walker and Shah (1988) demonstrated that the hydroxyl radical scavenger dimethylthiourea, and the iron chelator deferoxamine lessens the gentamicin-induced reduction in glomerular filtration rate and the severity of the tubular damage, and Nakajima et al. (1994) have found that superoxide dismutase (EC 1.15.1.1, superoxide: superoxide oxidoreductase) administration attenuates the fall in glomerular filtration rate and that dimethylthiourea ameliorated

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tubular damage in gentamicin-induced nephrotoxicity. Ademuyiwa et al. (1990) found that gentamicin-induced renal damage decreases by the dietary antioxidants vitamin E and selenium. Recently, our laboratory reported that a 2% garlic powder diet (Pedraza-Chaverrí et al., 2000) or aged garlic extract (Maldonado et al., 2003a) ameliorates the tubular and glomerular alterations induced by gentamicin administration. The protective effect of garlic on gentamicin nephrotoxicity was associated with the prevention in renal lipoperoxidation rise (Pedraza-Chaverrí et al., 2000) and protein carbonyl content (Maldonado et al., 2003a) and the preservation in manganese superoxide dismutase and glutathione peroxidase (EC 1.11.1.9, glutathione:H_2O_2 oxidoreductase) activities in rats.

Since the treatment with some antioxidants diminishes the gentamicin-induced nephropathy, in this work we studied the effect of diallyl disulfide, a garlic derived compound with antioxidant properties (Fanelli et al., 1998; Dwivedi et al., 1998; Yin et al., 2002), on the renal damage and oxidative stress induced by gentamicin. Diallyl disulfide is not present in garlic cloves but it is present in garlic oil (Block, 1996; Lawson, 1996, 1998). It is a product of allicin transformation (Lawson, 1996) which is produced when the enzyme alliinase acts on its substrate alliin during garlic cutting or crushing (Block, 1985; Lawson, 1996, 1998). Diallyl disulfide has been found in human breath after garlic consumption (Block et al., 1996; Cai et al., 1995; Laakso et al., 1989; Lawson, 1996; Rosen et al., 2000, 2001) which shows that this compound is a garlic metabolite in vivo.

2. Materials and methods

2.1. Reagents

Diallyl disulfide was from LKT Laboratories (St. Paul, MN, USA). Gentamicin (Garamicina G.U., 160 mg/2 ml) was from Schering-Plough (Mexico City, Mexico). Xanthine, xanthine oxidase, diethyldithiocarbamic acid, nitroblue tetrazolium, diaminobenzidine, and 2,4-dinitrophenylhydrazine were from Sigma (St. Louis, MO, USA). Rabbit anti-nitrotyrosine polyclonal antibodies were from Upstate (Charlottesville, VA, USA). Anti-rabbit immunoglobulin G horseradish peroxidase-linked antibodies were purchased from Amersham Life Sciences (Buckinghamshire, England). Commercial kits to measure creatinine and blood urea nitrogen were from Spinreact (Girona, Spain). All other chemicals were reagent grade.

2.2. Experimental design

Male Wistar rats (Harlan Teklad, Mexico City, Mexico) initially weighing 250–260 g were used. Experimental work was approved by CONACYT (34920-M) and followed the guidelines of Norma Oficial Mexicana (NOM-ECOL-087-1995). All animals had free access to water and commercial rodent diet (Harlan Teklad, catalog no. 2018S), and were randomly divided in four groups as follows: (1) Control, treated subcutaneously with isotonic saline solution and intragastrically with corn oil, (2) Gentamicin, treated subcutaneously with gentamicin (70 mg/kg/12 h/4 days); (3) Diallyl disulfide, treated intragastrically with diallyl disulfide dissolved in corn oil (50 mg/kg/24 h/4 days), and (4) Gentamicin + diallyl disulfide, treated with gentamicin and diallyl disulfide. During the study, rats were maintained in stainless steel metabolic cages with a 12-h light/dark cycle to collect 24 h urine at the end of the study. Urine was stored at −40 °C until *N*-acetyl-β-D-glucosaminidase activity, total protein and creatinine were measured. Animals were sacrificed by decapitation on day 5 and blood was collected to obtain serum which was stored at −80 °C until creatinine and blood urea nitrogen, markers of glomerular damage, and glutathione peroxidase activity, marker of tubular damage, were determined. One kidney was quickly removed to obtain cortex samples for measurement of gentamicin concentration, histological studies, and immunohistochemical localization of nitrotyrosine, an index of the nitrosylation of proteins by peroxynitrite and/or oxygen-derived free radicals. Peroxynitrite is readily produced from superoxide anion (O_2^-) and nitric oxide. The other kidney was removed to obtain 0.1 g of renal cortex which was homogenized in a Polytron for 10 s in cold 50 mM potassium phosphate and 0.1% Triton X-100, pH = 7.0. The homogenate was centrifuged at 19,000 × *g* and 4 °C for 30 min. Supernatant was separated to measure total protein, and the activity of total superoxide dismutase, manganese superoxide dismutase, catalase (EC 1.11.1.6, H_2O_2:H_2O_2, oxidoreductase), glutathione peroxidase, and glutathione reductase (EC 1.6.4.2, NAD[P]H:GSSG oxidoreductase). Another portion of this kidney was used to measure protein carbonyl content, a relatively stable marker of protein oxidation by reactive oxygen species.

2.3. Markers of glomerular and tubular damage

Blood urea nitrogen and creatinine in serum and creatinine in urine were measured using commercial kits and total protein in urine was measured by a turbidimetric method (Pedraza-Chaverrí et al., 1999). Creatinine clearance was calculated using the standard formula. Urinary *N*-acetyl-β-D-glucosaminidase activity was determined using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate (Pedraza-Chaverrí et al., 2000). Serum glutathione peroxidase activity was measured at 340 nm using glutathione reductase and nicotinamide adenine dinucleotide phosphate in a coupled reaction (Pedraza-Chaverrí et al., 2001).

2.4. Histological analysis

Kidney sections were fixed in 10% neutral buffered formaldehyde solution, dehydrated in graded anhydrous

absolute ethanol and xylol, and embedded in paraffin. Sections of 4 μm of thickness were obtained and stained with hematoxilin-eosin (Barrera et al., 2003). The histological profile of 20 proximal tubules randomly selected per rat (5–6 rats per experimental group) was recorded and number of normal tubules and tubules with histopathological alterations like swelling, cytoplasmic vacuolization, desquamation or necrosis was obtained. The percentage of tubules with histopathological alterations was calculated.

2.5. Oxidative stress markers

Protein carbonyl and nitrotyrosine content in renal cortex were used as markers of oxidative damage. Protein carbonyl groups were detected by its reactivity with 2,4-dinitrophenylhydrazine to form protein hidrazones (Barrera et al., 2003) which were detected at 370 nm. Nitrotyrosine was detected by immunohistochemical localization as previously described (Barrera et al., 2003). Kidney sections of 4 μm of thickness were obtained, deparaffined with xylol and rehydrated with ethanol. Endogenous peroxidase was quenched/inhibited with 4.5% H_2O_2 in methanol for 1.5 h at room temperature. Nonspecific adsorption was minimized by leaving the sections in 3% bovine albumin in phosphate buffer saline for 30 min. Sections were incubated overnight with a 1:700 dilution of anti-nitrotyrosine antibody. Samples were incubated with a 1:500 dilution of a peroxidase conjugated anti-rabbit Ig antibody for 1 h, and finally incubated with H_2O_2-diaminobenzidine for 1 min. Sections were counterstained with hematoxilin and observed under light microscopy. For quantitative image analysis approximately 50 tubular epithelial cells were randomly chosen and the intensity of immunohistochemistry staining was determined with a Zeiss KS 300 Imaging System 3.0 (Hallbergmoos, Germany). The standard for immunostaining was obtained from the control group. Data are expressed in arbitrary units. The sections from the four studied groups were incubated under the same conditions and with the same concentration of antibodies.

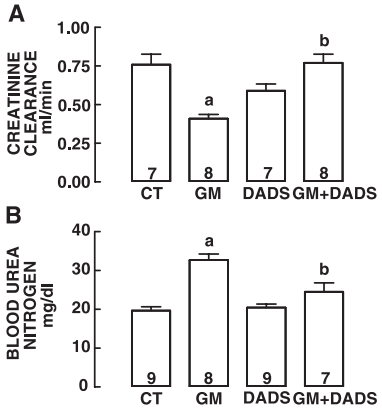

Fig. 2. Creatinine clearance (A) and blood urea nitrogen (B) on day 5 in the four groups of rats studied: CT: control group, GM: gentamicin group, DADS: diallyl disulfide group, and GM + DADS: gentamicin + diallyl disulfide group. The data represent the mean ± S.E.M. *n* for each group is printed in its respective bar. [a]$P < 0.001$ vs. control group, [b]$P < 0.01$ vs. gentamicin group.

2.6. Antioxidant enzymes in renal cortex

Total superoxide dismutase activity was assayed at 560 nm by a previously reported method using nitroblue tetrazolium as the indicator reagent (Pedraza-Chaverrí et al., 2001). To measure manganese superoxide dismutase activity, Cu^{2+}, Zn^{2+}-superoxide dismutase was inhibited with diethyldithiocarbamic acid (Pedraza-Chaverrí et al., 2001). Cu^{2+}, Zn^{2+}-superoxide dismutase activity was

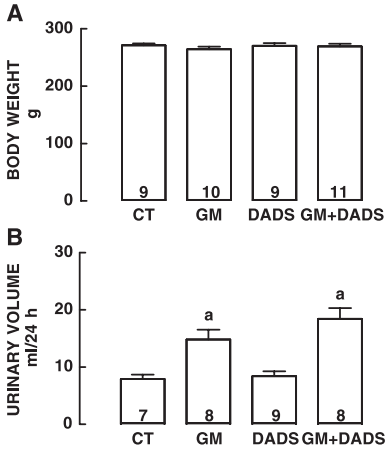

Fig. 1. Body weight (A) and urinary volume (B) on day 5 in the four groups of rats studied: CT: control group, GM: gentamicin group, DADS: diallyl disulfide group, and GM + DADS: gentamicin + diallyl disulfide group. The data represent the mean ± S.E.M. *n* for each group is printed in its respective bar. [a]$P < 0.05$ vs. control group.

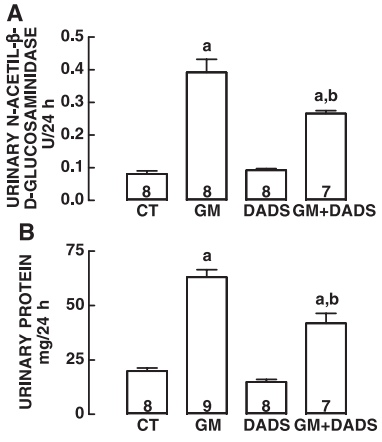

Fig. 3. Urinary excretion of *N*-acetyl-β-D-glucosaminidase (A) and total protein (B) on day 5 in the four groups of rats studied. CT: control group, GM: gentamicin group, DADS: diallyl disulfide group, and GM + DADS: gentamicin + diallyl disulfide group. The data represent the mean ± S.E.M. *n* for each group is printed in its respective bar. [a]$P < 0.001$ vs. control group, [b]$P < 0.01$ vs. gentamicin group.

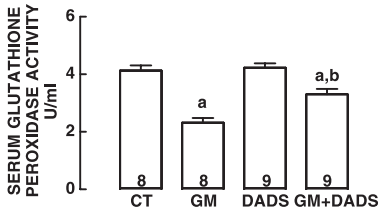

Fig. 4. Serum glutathione peroxidase activity on day 5 in the four groups of rats studied: CT: control group, GM: gentamicin group, DADS: diallyl disulfide group, and GM + DADS: gentamicin + diallyl disulfide group. The data represent the mean ± S.E.M. *n* for each group is printed in its respective bar. [a]$P < 0.05$ vs. control group, [b]$P < 0.01$ vs. gentamicin group.

obtained by subtracting the activity of the diethyldithiocarbamic acid-treated samples from that of total superoxide dismutase activity. Catalase activity was assayed by a method based on the disappearance of H_2O_2 at 240 nm (Pedraza-Chaverrí et al., 1999). Glutathione peroxidase activity in renal cortex was measured by a previously described method (Pedraza-Chaverrí et al., 2001). Glutathione reductase activity was assayed using oxidized glutathione as substrate and measuring the disappearance of nicotinamide adenine dinucleotide phosphate at 340 nm (Carlberg and Mannervik, 1975).

2.7. Renal gentamicin content

Gentamicin concentration was measured in renal cortical homogenates by the fluorescence polarization immunoassay technology, by using the AxSYM^R gentamicin assay (Abbot Laboratories, Abbot Park, IL, USA).

2.8. Statistics

Data are presented as mean ± S.E.M. and were analyzed by analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons, non-paired *t*-test and the Mann–Whitney *U*-test, as appropriate using the software Prism 3.02 (GraphPad, San Diego, CA, USA). $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Body weight and urinary volume

Body weight was not statistically different among the four groups along the study and on day of sacrifice (Fig. 1A). On day of sacrifice, urinary volume increased significantly in the gentamicin group and diallyl disulfide was

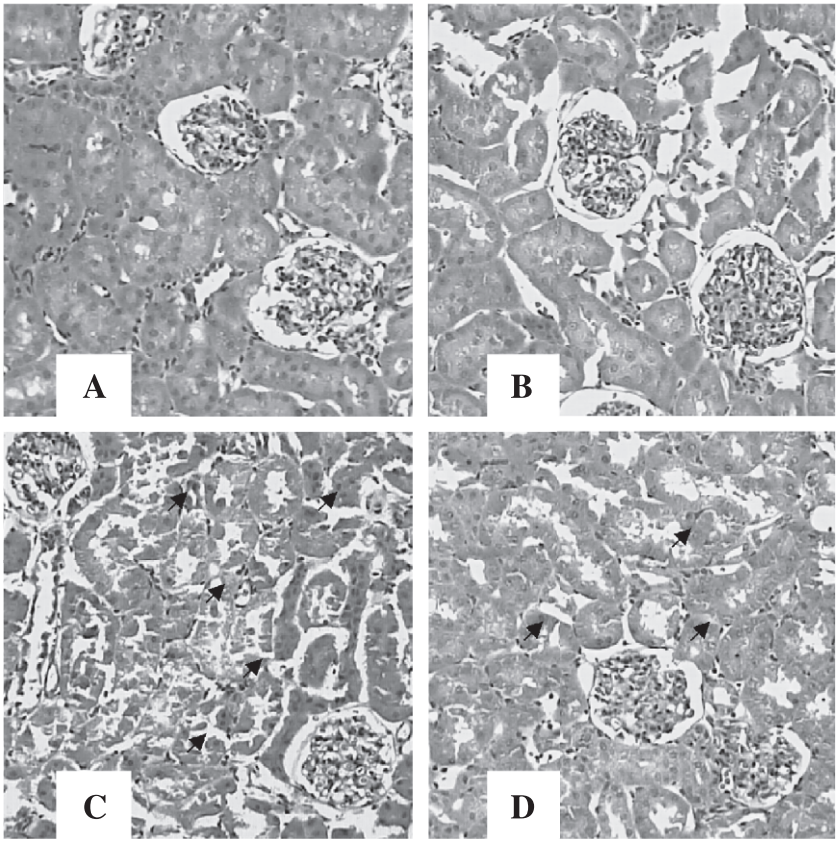

Fig. 5. Light microscopic findings in the renal cortex from control (A), diallyl disulfide (B), gentamicin (C) and gentamicin + diallyl disulfide (D) groups on day 5. Gentamicin-treated rats showed necrosis (arrows) in the proximal tubular epithelial cells. Histological damage decreased in gentamicin + diallyl disulfide group. Hematoxilin-eosin. 100 ×.

unable to prevent this increase in the gentamicin + diallyl disulfide group (Fig. 1B).

3.2. Markers of glomerular and tubular damage

Creatinine clearance decreased 46% and blood urea nitrogen increased 1.7 times, in gentamicin group compared to control one (Fig. 2A and B). Diallyl disulfide prevented the decrease in creatinine clearance and the increase in blood urea nitrogen levels in the gentamicin + diallyl disulfide group. Gentamicin increased urinary excretion of *N*-acetyl-β-D-glucosaminidase (4.8 times) and total protein (3.2 times) (Fig. 3A and B). The increase in both parameters was partially prevented by diallyl disulfide. Serum glutathione peroxidase activity diminished 44% in gentamicin group and the treatment with diallyl disulfide was able to partially prevent this reduction in gentamicin + diallyl disulfide group (Fig. 4). Urinary volume, creatinine clearance, blood urea nitrogen, serum glutathione peroxidase activity and urinary excretion of *N*-acetyl-β-D-glucosaminidase and total protein were similar in control and diallyl disulfide groups (Figs. 1–4).

3.3. Histological analysis

Rats treated with gentamicin showed vacuolization and necrosis in the proximal tubular epithelial cells (Fig. 5). The percentage of damaged tubules in gentamicin group was of 87 ± 2% and the treatment with diallyl disulfide significantly decreased this percentage to 52.4 ± 2% (gentamicin + diallyl disulfide group, $P < 0.0001$). There were no renal histological alterations in control and diallyl disulfide groups (Fig. 4).

3.4. Oxidative stress markers

Gentamicin-treatment induced a 40% increase in the protein carbonyl content in renal cortex compared to control group (Fig. 5). The increase induced by gentamicin was completely prevented by diallyl disulfide in the gentamicin + diallyl disulfide group. Nitrotyrosine was immunolocalized only in proximal tubules; it was absent from glomeruli (data not shown). Nitrotyrosine content, measured by immunohistochemistry, increased 3.6-fold in gentamicin group (Table 1). This increase was significantly ameliorated by diallyl disulfide in gentamicin + diallyl disulfide group but it

Table 1
Quantitative analysis of nitrotyrosine immunohistochemistry

Group	Nitrotyrosine (number times of control)
Control	1.00 ± 0.05
Gentamicin	3.59 ± 0.26[a]
Dially disulfide	1.01 ± 0.06
Gentamicin + diallyl disulfide	2.79 ± 0.49[a,b]

Mean ± S.E.M. $n = 5–10$ rats.
[a] $P < 0.001$ vs. control group.
[b] $P < 0.001$ vs. gentamicin group.

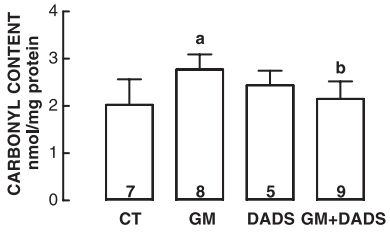

Fig. 6. Protein carbonyl content in the renal cortex on day 5 in the four groups of rats studied: CT: control group, GM: gentamicin group, DADS: diallyl disulfide group, and GM + DADS: gentamicin + diallyl disulfide group. The data represent the mean ± S.E.M. *n* for each group is printed in its respective bar. [a] $P < 0.01$ vs. control group, [b] $P < 0.05$ vs. gentamicin group.

remained significantly higher compared to control group (Table 1). Protein carbonyl and nitrotyrosine content in renal cortex in the diallyl disulfide and control groups were similar (Fig. 6 and Table 1).

3.5. Antioxidant enzymes in renal cortex

Total superoxide dismutase and manganese superoxide dismutase activities diminished and Cu^{2+}, Zn^{2+}-superoxide dismutase remained unaffected by gentamicin-treatment (Table 2). Diallyl disulfide completely prevented the de-

Table 2
Activity of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase in the renal cortex of the four groups of rats studied

	Control	Gentamicin	Dially disulfide	Gentamicin + diallyl disulfide
Total superoxide dismutase (U/mg protein)	19 ± 0.4	16 ± 0.3[a]	18 ± 0.6	19 ± 0.4[b]
Manganese superoxide dismutase (U/mg protein)	6 ± 0.3	5 ± 0.2[a]	7 ± 0.3	7 ± 0.4[b]
Cu^{2+}, Zn^{2+}-superoxide dismutase (U/mg protein)	12 ± 0.5	10 ± 0.3	11 ± 0.8	11 ± 0.5
Glutathione peroxidase (U/mg protein)	0.2 ± 0.01	0.1 ± 0.01[a]	0.2 ± 0.01	0.2 ± 0.01[b]
Glutathione reductase (U/mg protein)	0.05 ± 0.002	0.04 ± 0.001[a]	0.05 ± 0.002	0.05 ± 0.002[b]
Catalase (k/mg protein)	0.4 ± 0.03	0.2 ± 0.02[a]	0.3 ± 0.01	0.2 ± 0.02[a]

Values are mean ± S.E.M. $n = 6–10$ animals.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.001$ vs. control group.

crease in total superoxide dismutase and manganese superoxide dismutase activities in gentamicin + diallyl disulfide group. Glutathione peroxidase and glutathione reductase activities decreased in gentamicin-treated rats and these decreases were totally prevented by diallyl disulfide in the gentamicin + diallyl disulfide group (Table 2). Catalase activity decreased in rats treated with gentamicin and diallyl disulfide was unable to prevent this decrease in gentamicin + diallyl disulfide group (Table 2). Total superoxide dismutase, manganese superoxide dismutase, Cu^{2+}, Zn^{2+}-superoxide dismutase, glutathione peroxidase, and glutathione reductase activities were unchanged and catalase activity was significantly lowered in the diallyl disulfide group (Table 2).

3.6. Renal gentamicin content

Treatment with diallyl disulfide had no effect on renal cortical gentamicin content at the end of the study: 52.6 ± 1.0 in gentamicin group ($n = 8$) vs. 44.7 ± 2.5 μg/mg protein in gentamicin + diallyl disulfide group ($n = 11$, P = NS).

4. Discussion

Gentamicin is an aminoglycoside widely used in the clinical practice due to: (a) it possess a wide bactericide spectre specially against gramnegative bacteria, (b) it is effective against resistant β-lactamic microorganisms, (c) its low cost, and (d) the low levels of resistance that induces among enterobacteriaceae family bacterium (Edson and Terrell, 1999). Although this drug has a proven usefulness, its nephrotoxic effect limits widely its use. In fact, it has been estimated that approximately 30% of the patients treated with gentamicin are associated with renal dysfunction (Lerner et al., 1986). The exact mechanism by which gentamicin induces the renal damage is unknown, however, evidences suggest a role of reactive oxygen species in this damage; it has been found that O_2^-, H_2O_2 and hydroxyl radicals increase with gentamicin-treatment (Cuzzocrea et al., 2002; Guidet and Shah, 1989; Walker and Shah, 1987, 1988; Yang et al., 1995), and also several agents that scavenge or interfere with reactive oxygen species production ameliorate successfully gentamicin-induced nephropathy (Abdel-Naim et al., 1999; Ademuyiwa et al., 1990; Ali, 2002; Maldonado et al., 2003a; Mazzon et al., 2001; Morales et al., 2002; Nakajima et al., 1994; Pedraza-Chaverrí et al. 2000; Sener et al., 2002). Particularly, the protective effect of garlic powder and aged garlic extract on gentamicin nephrotoxicity was associated with the prevention in the rise of renal lipoperoxidation and protein carbonyl content, respectively, and the preservation in manganese superoxide dismutase and glutathione peroxidase activities (Maldonado et al., 2003a; Pedraza-Chaverrí et al., 2000). The garlic compound(s) responsible(s) for this renal protective effect is(are) not known and our laboratory is involved in the identification of it(them) at present. Therefore, in this work we studied the possible renoprotective effect of diallyl disulfide which is a garlic-derived compound with antioxidant properties (Fanelli et al., 1998; Dwivedi et al., 1996, 1998; Wu et al., 2001; Yin et al., 2002). Yin et al. (2002) showed that diallyl disulfide has antioxidant activity in a liposome system in a concentration dependent way, Dwivedi et al. (1998) found that diallyl disulfide inhibits liver microsomal lipid peroxidation induced by nicotinamide adenine dinucleotide phosphate, ascorbate and doxorubicin, and Fanelli et al. (1998) found that diallyl disulfide was able to (1) trap trichloromethyl and trichloromethylperoxyl free radicals, (2) inhibit carbon tetrachloride promoted liver microsomal lipid peroxidation, and (3) prevent carbon tetrachloride promoted oxidation of albumin in vitro. Diallyl disulfide is a major constituent of commercial garlic oil (Lawson, 1996, 1998) which also has antioxidant properties; in fact Iqbal and Athar (1998) found that garlic oil attenuates iron-nitrilotriacetate (Fe-NTA)-mediated renal oxidative stress and nephrotoxicity in rats.

In this work, it was found that diallyl disulfide ameliorated or prevented gentamicin-induced nephropathy which parallels the findings made with garlic powder (Pedraza-Chaverrí et al., 2000). The reduction in gentamicin-induced nephrotoxicity by diallyl disulfide was not due to the diminution of renal gentamicin concentration because diallyl disulfide was unable to modify this parameter. Diallyl disulfide prevented the increased in blood urea nitrogen and the decrease in creatinine clearance (glomerular damage markers) induced by gentamicin-treatment. Since glomeruli structure is normal in gentamicin-treated rats (Pedraza-Chaverrí et al., 2000), the decrease in glomerular function may not be attributed to structural damage. It has been found that the gentamicin-treatment increases H_2O_2 (Guidet and Shah, 1989; Walker and Shah, 1987; Yang et al., 1995) and O_2^- (Cuzzocrea et al., 2002) production, and it is known that H_2O_2 (Duque et al., 1992) and O_2^- (Martínez-Salgado et al., 2002) induces mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient, factors that decrease glomerular filtration rate. Moreover, the decrease in manganese superoxide dismutase activity favors the increase in O_2^- concentration, and this radical can react with nitric oxide (a vasodilator) to form peroxynitrite, a cytotoxic oxidant radical specie. The inactivation of nitric oxide by O_2^- also could lead to a decrease in the glomerular filtration rate (Rivas-Cabañero et al., 1997). These data indicate that H_2O_2 and O_2^- could be involved in the decrease of glomerular filtration rate in gentamicin-induced nephropathy, and that the protective effect of diallyl disulfide, could be related with its ability to preserve the activity of the antioxidant enzymes manganese superoxide dismutase and glutathione peroxidase which metabolizes O_2^- and H_2O_2, respectively.

Tubular function was evaluated by measuring serum glutathione peroxidase activity and the urinary excretion

of *N*-acetyl-β-D-glucosaminidase and total protein. In rats treated with gentamicin, the increase in urinary *N*-acetyl-β-D-glucosaminidase and total protein excretion, and the decrease in serum glutathione peroxidase activity, enzyme that is synthesized almost exclusively in proximal tubular cells (Avissar et al., 1994), could be associated with the necrosis of proximal tubules, the primary site of drug accumulation (Silverblatt and Kuehn, 1975). Diallyl disulfide partially prevented these alterations induced by gentamicin which could be associated with the ability of diallyl disulfide to ameliorate tubular necrosis. It is known that the increase in reactive oxygen species levels induces cytotoxicity due to a concerted action of oxygen- (Cuzzocrea et al., 2002) and nitrogen-derived free radicals (Augusto et al., 2002; Mazzon et al., 2001) and diallyl disulfide treatment was able to ameliorate the increase in renal protein carbonyl groups and nitrotyrosine content, markers of oxidative stress. Interestingly, it has been found previously that nitrotyrosine residues in proteins, which are indicative of peroxynitrite and/or oxygen-derived free radicals generation, are increased in gentamicin-induced nephropathy (Cuzzocrea et al., 2002; Mazzon et al., 2001). Diallyl disulfide was also able to prevent the fall in manganese superoxide dismutase, glutathione peroxidase, and glutathione reductase activities which significantly improves the antioxidant status of rat kidney. Interestingly, similar observations were made with garlic powder feeding (Pedraza-Chaverrí et al., 2000), aged garlic extract (Maldonado et al., 2003a) and *S*-allylcysteine (Maldonado et al., 2003b) which preserve manganese superoxide dismutase and glutathione peroxidase activities in gentamicin-induced nephropathy. Diallyl disulfide was unable to prevent the decrease of catalase activity, instead of it, diallyl disulfide itself decreased catalase activity. In previous works we found that the decrease in catalase activity by garlic powder is secondary to the decrease in catalase content and synthesis without changes in catalase mRNA (Pedraza-Chaverrí et al., 2000, 2001). At present, the mechanism by which diallyl disulfide decreases catalase activity is unknown. Interestingly diallyl sulfide, another garlic-derived compound which is structurally related to diallyl disulfide, decreases liver catalase activity (Chen et al., 1999), however neither aged garlic extract (Maldonado et al., 2003a) nor *S*-allylcysteine (Maldonado et al., 2003b) were able to decrease catalase activity.

Kidney reactive oxygen species levels increased by gentamicin (e.g. O_2^-, H_2O_2, and peroxynitrite) could induce the inactivation of manganese-superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase in renal cortex from gentamicin-treated rats. It is known that peroxynitrite anions impair manganese-superoxide dismutase (MacMillan-Crow and Thompson, 1999) and glutathione peroxidase (Padmaja et al., 1998) activity and O_2^- inactivates glutathione peroxidase and catalase (Rister and Baehner, 1976). Diallyl disulfide-treatment totally prevented the decrease in manganese-superoxide dismutase, glutathione peroxidase, and glutathione reductase activities. This protective effect could be a consequence of the antioxidant effect of diallyl disulfide which was evident in this work by the amelioration in protein carbonyl and nitrotyrosine content. Our results indicate that the increase in oxidative stress, and the decrease in antioxidant enzymes manganese-superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase activity, could be factors involved in the sequence of events leading to gentamicin-induced nephropathy, and that the protective effect of diallyl disulfide could be associated with its ability to prevent the increase in oxidative stress and the fall in the antioxidant enzymes activity (manganese-superoxide dismutase, glutathione peroxidase, and glutathione reductase), observed in renal cortex of rats treated with gentamicin.

In summary, our data shows that the in vivo antioxidant effect of diallyl disulfide may be involved, at least in part, in its protective effect in rats with gentamicin-induced nephrotoxicity. Diallyl disulfide may be a useful agent for the prevention of gentamicin-induced nephropathy.

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