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Effect of *N*-acetylcysteine on gentamicin-mediated nephropathy in rats

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Abstract

Studies were performed on the mechanisms of the protective effects of free-radical scavengers against gentamicin-mediated nephropathy. Administration of gentamicin, 100 mg/kg s.c., for 5 days to rats induced marked renal failure, characterised by a significantly decreased creatinine clearance and increased blood creatinine levels, fractional excretion of sodium Na^+, lithium Li^+, urine gamma glutamyl transferase and daily urine volume. A significant increase in kidney myeloperoxidase activity and lipid peroxidation was observed in gentamicin-treated rats. Immunohistochemical localisation demonstrated nitrotyrosine formation and poly(ADP-ribose)synthase activation in the proximal tubule from gentamicin-treated rats. Renal histology examination confirmed the tubular necrosis. *N*-acetylcysteine (10 mg/kg i.p. for 5 days) caused normalisation of the above biochemical parameters. In addition, *N*-acetylcysteine treatment significantly prevents the gentamicin-induced tubular necrosis. These results suggest that (1) *N*-acetylcysteine has protective effects on gentamicin-mediated nephropathy, and (2) the mechanisms of the protective effects can be, at least in part, related to interference with peroxynitrite-related pathways. © 2001 Published by Elsevier Science B.V.

Keywords: Nitric oxide (NO); Peroxynitrite; Poly(ADP ribose)synthetase; Gentamicin; Renal injury

1. Introduction

Aminoglycoside antibiotics induce a dose-dependent nephrotoxicity in 10–20% of therapeutic courses, even despite rigorous monitoring of serum drug concentration. Gentamicin-induced nephrotoxicity is characterised by tubular necrosis, without morphological changes in glomerular structures (Rodriguez-Barbero et al., 1992). Gentamicin treatment also causes marked decreases in glomerular filtration rate and alters intraglomerular dynamics (Schor et al., 1981).

The pathophysiological mechanisms responsible for the impairment of glomerular filtration rate in gentamicin-mediated nephropathy have been studied using experimental animal models (Baylis et al., 1977), but the mechanisms remain unclear. Both proximal tubular injury and the abnormalities of the renal circulation, such as the renal vasoconstriction (Baylis et al., 1977; Schor et al., 1981) and the reduction of glomerular capillary ultrafiltration coefficient (Kf) (Baylis et al., 1977; Schor et al., 1981; Chonko et al., 1979), contribute to the decrease in glomerular filtration rate in gentamicin-mediated nephropathy. Recent studies have demonstrated that suppression of the renin–angiotensin system by deoxycorticosterone acetate (DOCA) treatment plus isotonic saline drinking significantly attenuated the gentamicin-induced reduction in glomerular filtration rate but did not lessen tubular injury (Yamada et al., 1992). The beneficial effect of DOCA plus saline drinking was associated with a higher renal blood flow. Schor et al. (1981) also demonstrated that an angiotensin-converting enzyme inhibitor attenuated the gentamicin-induced decrease in glomerular filtration rate and renal blood flow but did not ameliorate the tubular injury.

Reactive oxygen metabolites have been implicated as mediators of tissue injury in several animal models of acute renal failure (Paller et al., 1984; Walker and Shah, 1988). Walker and Shah (1988) demonstrated that hy-

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droxyl radical scavengers and iron chelators lessen the GM-induced reduction in glomerular filtration rate as well as lessen the severity of the tubular damage.

Reactive oxygen species and peroxynitrite produce cellular injury and necrosis via several mechanisms, including peroxidation of membrane lipids, protein denaturation and DNA damage. Reactive oxygen species produce strand breaks in DNA, which triggers energy-consuming DNA repair mechanisms and activates the nuclear enzyme poly(ADP-ribose)synthase, resulting in the depletion of its substrate, NAD, in vitro and in a reduction in the rate of glycolysis. As NAD functions as a cofactor in glycolysis and the in tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. This process has been termed 'the PARS Suicide Hypothesis.' There is recent evidence that the activation of poly(ADP-ribose)synthase may be implicated in the pathogenesis of shock and renal injury (Cuzzocrea et al., 1997; Chatterjee et al., 1999, 2000).

N-acetylcyteine has antioxidant properties (Auroma et al., 1989) and, as a sulfhydryl donor, may contribute to the regeneration of endothelium-derived relaxing factor and glutathione (Harrison et al., 1991). Increasing evidence indicates that the action of *N*-acetylcysteine is pertinent to microcirculatory blood flow and tissue oxygenation. *N*-acetylcysteine was shown to enhance oxygen consumption via increased oxygen extraction in patients 18 h after the onset of fulminant liver failure (Harrison et al., 1991). It was speculated that *N*-acetylcysteine could also exert beneficial effects on impaired nutritive blood flow in patients with severe sepsis (Harrison et al., 1991).

In the present study, we examined the protective effect of *N*-acetylcysteine against gentamicin-mediated nephropathy based on both biochemical and morphological parameters.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (250 g; Charles River, Milan, Italy) were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with Italian regulations on protection of animals used for experimental and scientific purposes (D.M. 116192), as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986)

2.2. Experiments protocol

The animals were placed into individual metabolic cages and divided among four experimental groups:

Control group ($n = 10$): rats received a daily subcutaneous (s.c.) injection of 0.5-ml isotonic saline solution for 5 days.

Control + *N*-acetylcysteine group ($n = 10$): rats received a daily s.c. injection of 0.5-ml isotonic saline solution for 5 days and 10 mg/kg i.p. of *N*-acetylcysteine.

Gentamicin group ($n = 10$): this group was injected with gentamicin-sulfate 100 mg/kg s.c. in 0.5 ml of saline solution for 5 days.

Gentamicin + *N*-acetylcysteine group ($n = 10$): this group was injected with gentamicin-sulfate 100 mg/kg s.c. in 0.5 ml of saline solution for 5 days and 10 mg/kg i.p. of *N*-acetylcysteine.

The 5th day after the start of gentamicin treatment, urine, free of food and faeces, was collected into ice-cold graduated cylinders containing mineral oil to prevent evaporation, and sodium azide 0.1% to minimize bacterial growth. These urine samples were used to determine enzymatic activity, electrolyte excretion, and creatinine clearance. In another set of experiments, the rats were randomized to receive treatment regimens identical to those listed above ($n = 7$ for each group), but were killed 2 days after gentamicin or saline treatment in order to evaluate lipid peroxidation in the early phase.

2.3. Light microscopy

A kidney was removed after tying of the renal pedicle and cut, by saggital section, into halves which were then fixed by immersion in 10% formaldehyde for 1 day. After dehydration, the pieces were embedded in paraffin and cut into fine sections, mounted on glass slides and counterstained with hematoxylin-eosin for light-microscopic analyses.

Morphological changes were analysed blind by a pathologist and scored on a semiquantitative scale to evaluate the changes most frequently found in acute renal failure. Higher scores represent more severe damage (maximum score per tubule was 10) with points given for brush-border loss (1 point), cell membrane bleb formation (1 or 2 points), cell necrosis (1 or 2 points), cytoplasmic vacuolization (1 point), and tubular lumen obstruction (1 point).

2.4. Immunohistochemical localisation of nitrotyrosine

Tyrosine nitration, an index of the nitrosylation of proteins by peroxynitrite and/or oxygen-derived free radicals, was determined by immunohistochemistry as previously described (Cuzzocrea et al., 1997). At 5 days after gentamicin administration, the kidneys were fixed in 10% buffered formaldehyde and 8-μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in phosphate buffer solution

Table 1
Serum creatinine, creatinine clearance, fractional excretion of sodium and fractional excretion of lithium, urine volume and urine γ − glutamyl transferase levels

	Serum Creatinine (mg/dl)	C_{cr} (ml/dk/100 gr BW)	FE_{Li} (%)	FE_{Na} (%)	Urine GGT (U/l)	Urine volume (ml)
Sham + vehicle	0.33 ± 0.06	0.75 ± 0.08	21.4 ± 1.9	0.61 ± 0.08	127 ± 14	8.31 ± 0.18
Gentamicin + vehicle	0.85 ± 0.08[a]	0.31 ± 0.04[a]	58 ± 5[a]	1.22 ± 0.11[a]	830 ± 40[a]	15.4 ± 1.2[a]
Gentamicin + NAC	0.49 ± 0.02[b]	0.63 ± 0.05[b]	28 ± 3[b]	0.58 ± 0.06[b]	440 ± 30[b]	9.9 ± 0.3[b]

Data are means ± S.E.M. for 10 rats per group.
[a] $P < 0.01$ versus sham.
[b] $P < 0.01$ versus gentamicin (GEM).

(PBS) for 20 min. Nonspecific adsorption was minimised by incubating the section in 2% normal goat serum in phosphate-buffered saline for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin. The sections were then incubated overnight with a 1:1000 dilution of primary anti-nitrotyrosine antibody or with control solutions. Controls included buffer alone or nonspecific purified rabbit immunoglobulin G (IgG). Some sections were also incubated with the primary antibody (anti-nitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify binding specificity. Specific labelling was detected with a biotin-conjugated goat anti-rabbit IgG and avidin–biotin peroxidase complex. Diaminobenzidine was used as a chromogen (DBA, Milan, Italy).

2.5. Immunohistochemical localisation of PARS

At 5 days after gentamicin administration, the kidneys were fixed in 10% buffered formalin and 8-μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline for 20 min. Nonspecific adsorption was minimised by incubating the section in 2% normal goat serum in phosphate-buffered saline for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin (DBA, Milan, Italy). The sections were then incubated overnight with a 1:500 dilution of primary anti-poly(ADP-ribose) antibody (DBA, Milan, Italy) or with control solutions. Controls included buffer alone or nonspecific purified rabbit IgG. Specific labelling was detected with a biotin-conjugated goat anti-rabbit IgG and avidin–biotin peroxidase complex. Diaminobenzidine was used as a chromogen (DBA, Milan, Italy).

2.6. Laboratory investigation

Na^+, K^+ and Li^+ in plasma and urine were determined by flame photometry. Urine and serum creatinine assays were performed by a clinical laboratory. Urine was also assayed for the excretion of gamma glutamyl finding transferase by a clinical laboratory. Creatinine clearance, fractional excretion of lithium and fractional excretion of sodium were calculated from these value using standard methods.

2.7. Myeloperoxidase activity

Myeloperoxidase activity, an indicator of polymorphonuclear leukocyte accumulation, was determined as previously described (Mullane et al., 1985). At the specified time following gentamicin treatment, the kidneys were removed and weighed. Each piece of tissue was homogenised in a solution containing 0.5% hexa-decyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 × *g* at 4 °C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol peroxide min^{-1} at 37 °C and was expressed in milliunits per gram weight of wet tissue.

2.8. Malondialdehyde measurement

Malondialdehyde levels in the kidney were determined as an indicator of lipid peroxidation (Ohkawa et al., 1979). Kidney tissue, collected at the specified time, was homogenised in 1.15% KCl solution. An aliquot (100 μl) of the homogenate was added to a reaction mixture contain-

Table 2
Plasma Na^+, K^+ and urine K^+ levels

	Plasma Na^+ (mEq/l)	Plasma K^+ (mEq/l)	Urine K^+ (mEq/day)
Sham + vehicle	121 ± 3	5.2 ± 0.18	3.3 ± 0.3
Gentamicin + vehicle	143 ± 1.3	4 ± 0.1[a]	3.6 ± 0.3
Gentamicin + NAC	138 ± 1.5	4.9 ± 0.1[b]	2.9 ± 0.2

Data are means ± S.E.M. for 10 rats per group.
[a] $P < 0.01$ versus sham.
[b] $P < 0.01$ versus gentamicin.

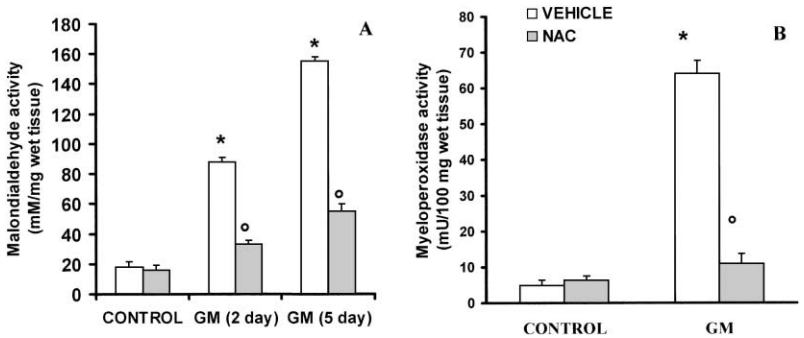

Fig. 1. Malondialdehyde levels (A), myeloperoxydase activity (B) after gentamicin administration. Myeloperoxydase activity and malondialdehyde levels were significantly increased in the kidney of the gentamicin-treated rats ($^{*}P < 0.01$). *N*-acetylcystein reduced the gentamicin-induced increase in malondialdehyde levels and myeloperoxydase activity. Values are means ± S.E.M. for eight animals in each group. $^{*}P < 0.01$ versus sham. $^{\circ}P < 0.01$ versus gentamicin.

ing 200 μl of 8.1% sodium dodecyl sulfate (SDS), 1500 μl of 20% acetic acid (pH 3.5), 1500 μl of 0.8% thiobarbituric acid and 700 μl distilled water. Samples were then boiled for 1 h at 95 °C and centrifuged at 3000 × *g* for 10 min. Absorbance of the supernatant was measured by spectrophotometry at 650 nm.

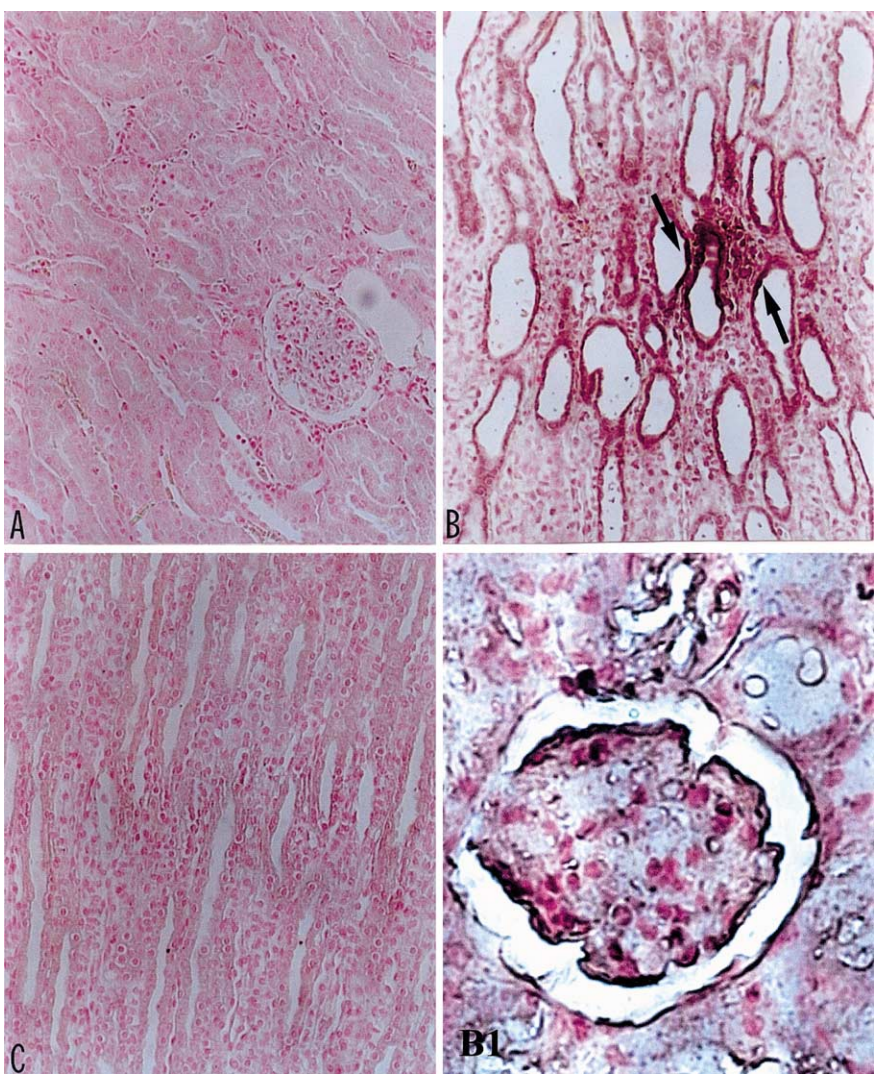

Fig. 2. Effect of *N*-acetylcysteine on nitrotyrosine formation: no positive staining was found in sham-treated rats (A). Five days following gentamicin administration, positive staining for nitrotyrosine was observed in tubular epithelial cells and in Bowan capsule (B, B1). There was a marked reduction in the immunostaining in the kidney of gentamicin-treated rats treated with *N*-acetylcysteine (C). Original magnification: A, B, C 125 ×; B1 200 ×. Figure is representative of at least three experiments performed on different experimental days.

2.9. Materials

Zambon Italia, Bresso, (MI), Italy supplied *N*-acetylcysteine, a biotin blocking kit, biotin-conjugated goat anti-rabbit IgG. Primary anti-nitrotyrosine antibody was from Upstate Biotech (DBA, Milan, Italy). All other reagents and compounds used were obtained from Sigma (Milan, Italy).

2.10. Data analysis

All values in the figures and text are expressed as means ± standard error (S.E.M.) of the mean of *n* observations. For the in vivo studies *n* represents the number of animals studied. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days. The results were analysed by one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons. A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Effect of NAC treatment on renal function

Serum creatinine, fractional excretion of sodium, lithium, urinary excretion of gamma glutamyl transferase and daily urine volume were significantly increased after administration of gentamicin for 5 consecutive days (Table 1). These gentamicin-treated animals also had a significantly lower creatinine clearance (Table 1) and plasma K^+ levels (Table 2) than did saline-treated rats. *N*-acetylcysteine treatment provided a marked protective effect with significantly decreased serum creatinine, fractional excretion of sodium and lithium as well as a significantly increased creatinine clearance (Tables 1 and 2).

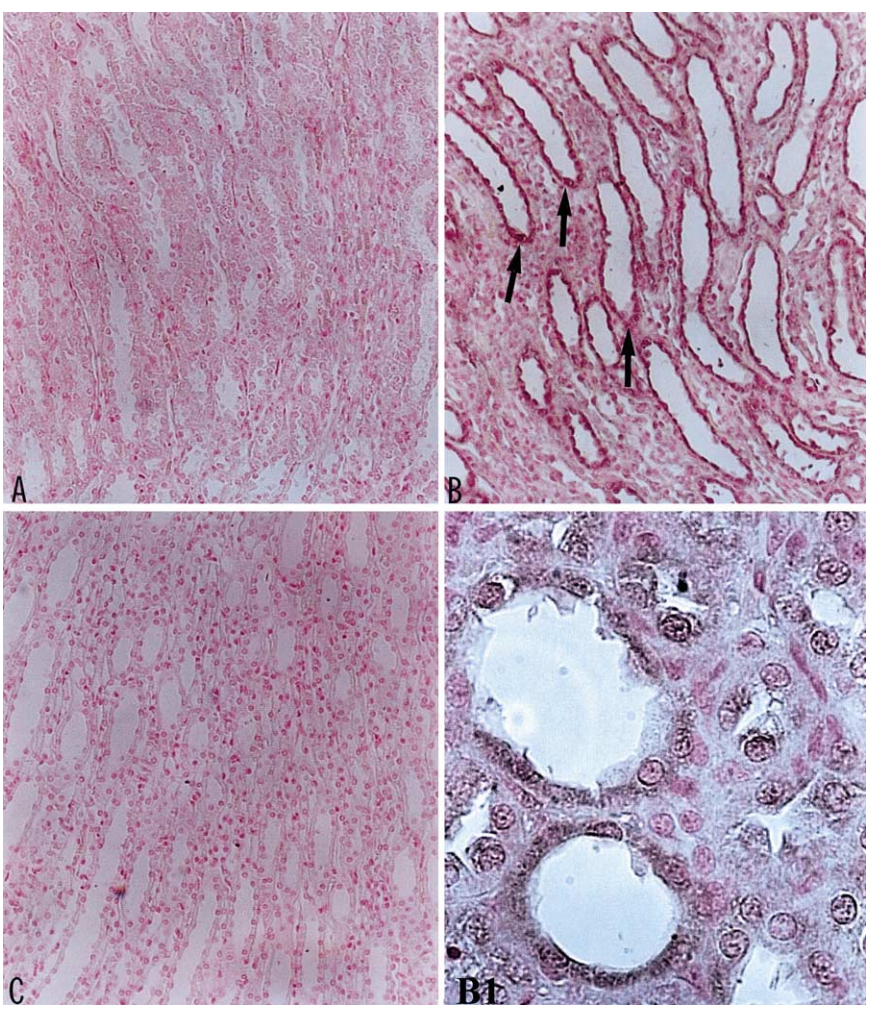

Fig. 3. Effect of *N*-acetylcysteine on poly (ADP-ribose) synthase immunostaining: No positive staining was found in sham-treated rats (A). Five days following gentamicin administration, poly(ADP-ribose) synthase immunoreactivity was observed in the nucleus of tubular epithelial cells (B, B1). No positive staining was found in the tissue obtained from *N*-acetylcysteine-treated rats (C). Original magnification: A, B, C 125×; B1 200×. Figure is representative of at least three experiments performed on different experimental days.

3.2. Effect of N-acetylcysteine treatment on malonaldehyde and myeloperoxidase activities

Gentamicin-treated rats showed a significant time-dependent increase in kidney malondialdehyde levels, indicative of lipid peroxidation (Fig. 1A). The accumulation of neutrophils was investigated by measuring myeloperoxidase activity in the kidney after administration of gentamicin for 5 consecutive days. Gentamicin-treated rats showed a significant increase in myeloperoxidase activity (Fig. 1B). *N*-acetylcysteine treatment significantly reduced ($P < 0.01$) myeloperoxidase activity as well as malondialdehyde levels (Fig. 1A,B).

3.3. Effect of N-acetylcysteine treatment on nitrotyrosine formation and poly(ADP-ribose)synthase activation

On the 5th day after gentamicin-treatment the kidneys were removed in order to evaluate the immunohistological staining for nitrotyrosine. Immunohistochemical analysis, using a specific anti-nitrotyrosine antibody, revealed positive staining in tubular epithelial cells (see arrows) from gentamicin-treated rats (Fig. 2B). In contrast, no positive nitrotyrosine staining was found in the kidneys of gentamicin-treated rats that had been treated with *N*-acetylcysteine (Fig. 2C).

Immunohistochemical analysis, using a specific anti-PARS antibody, revealed positive staining in tubular epithelial cells (see arrows) from gentamicin-treated rats (Fig. 3B). *N*-acetylcysteine treatment significantly reduced the degree of immunostaining for poly(ADP-ribose)synthase in the kidney (Fig. 3C). There was no staining for either nitrotyrosine or poly(ADP-ribose)synthase in the kidney sections of sham-treated animals (Figs. 2A and 3A). Note that there was positive staining for either nitrotyrosine or poly(ADP-ribose)synthase in kidneys obtained at 2 days after gentamicin administration (data not shown).

3.4. Histological change

Light microscopic observations revealed tubular necrosis (see arrows, Fig. 4B) and focal infiltration of inflammatory cells (e.g. neutrophils and monocytes) in the renal

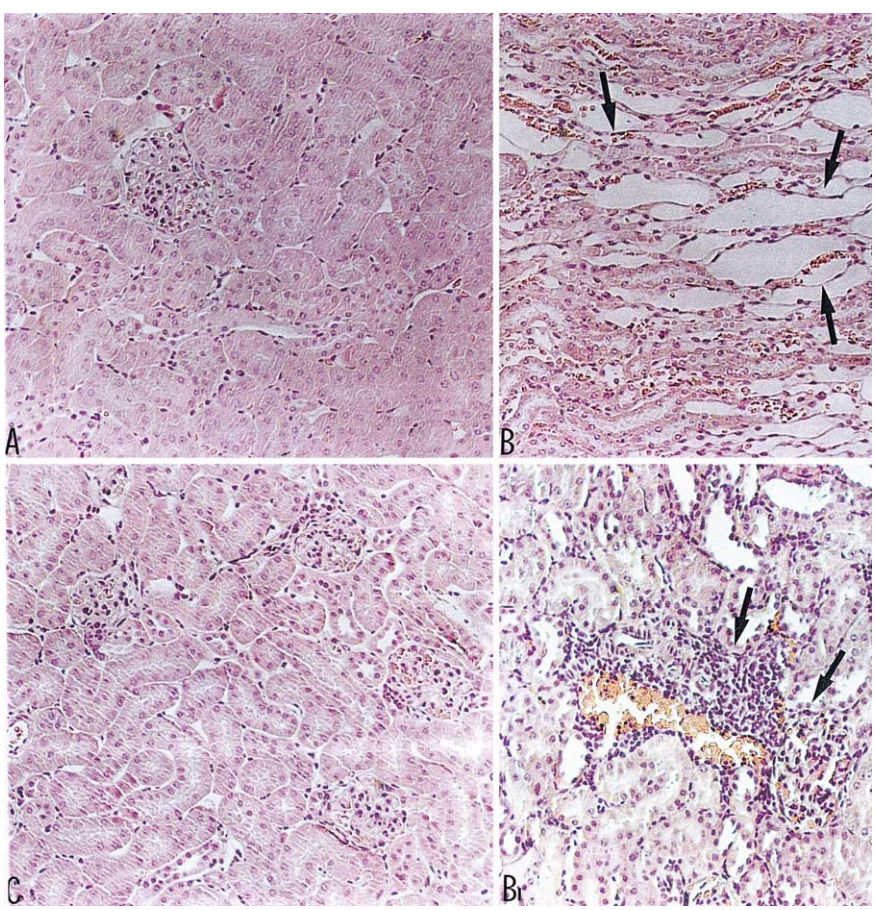

Fig. 4. Protective effects of *N*-acetylcysteine on gentamicin-induced nephotoxicity. (A) Sham rat, vehicle, (B) gentamicin-treated rats, (C) *N*-acetylcysteine-treated rats. Treatment with *N*-acetylcysteine produced a significant attenuation of nephrotoxicity as well as prevented the inflammatory cells infiltration. Original magnification: A, B, C, 125×; B1 150×. Figure is representative of at least three experiments performed on different experimental days.

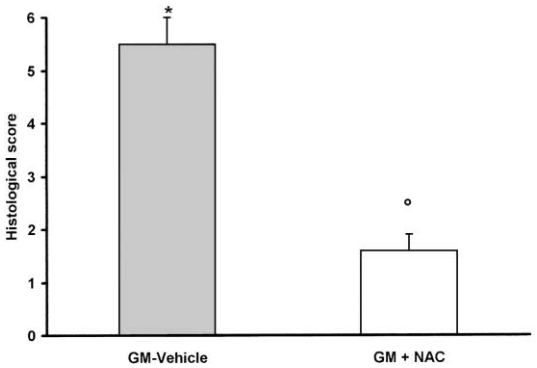

Fig. 5. Effects of *N*-acetylcysteine on histological score obtained from microscopic observation of kidney area of gentamicin-treated rats. Values are means ± S.E.M. for eight animals per each group. $^{*}P < 0.01$ versus sham. $^{\circ}P < 0.01$ versus gentamicin.

tubular in gentamicin-treated rats (see arrows, Fig. 4B1). Treatment with *N*-acetylcysteine significantly lessened the gentamicin-induced tubular necrosis and cast formation (Fig. 4C). The evaluation of sections from gentamicin-treated animals, which received *N*-acetylcysteine, yielded a significantly lower score in comparison with that for animals receiving gentamicin and treated with vehicle (Fig. 5).

4. Discussion

Acute renal failure, a serious complication in gram-negative bacterial infections, is caused by renal injury induced by endotoxemia (Wardle, 1982). These gram-negative infections are commonly treated with aminoglycosides (Appel and Neu, 1977). The aminoglycoside, gentamicin, which is widely used in clinical practice, can have a broad range of nephrotoxic side-effects such as proteinuria, enzymunuria and deterioration of the glomerular filtration rate (Humes et al., 1982). Although this drug has proven its usefulness, its nephrotoxic action limits the extent of its use. In fact, it has been estimated that approximately 10–20% of all courses of therapy with aminoglycosides are associated with renal dysfunction (Lerner et al., 1986).

Recent studies have demonstrated that systemic endotoxin administration increases gemtamicin nephrotoxicity (Beauchamp et al., 1985). This increase in the renal toxicity of aminoglycosides is partially explained by an enhanced uptake of aminoglycosides by the kidney, especially at the proximal tubules (Beauchamp et al., 1985). Although gentamicin's effect on biological membranes appears to be critical in pathogenetic sequences, the exact mechanisms of gentamicin nephrotoxicity are not yet clearly understood (Humes and Weinberg, 1986).

Several lines of evidence suggest that free oxygen radicals may be involved in gentamicin-induced acute renal failure. It has been suggested that lipid peroxidation may be a contributing factor in the development of gentamicin nephrotoxicity.

There is now substantial evidence that much of the cytotoxicity is due to a concerted action of oxygen- and nitrogen-derived free radicals and oxidants. Peroxynitrite, a cytotoxic oxidant species formed from the reaction of nitric oxide (NO) and superoxide (Beckman et al., 1990), may mediate part of the oxidative injury associated with simultaneous production of NO and oxyradicals. Peroxynitrite is cytotoxic via a number of independent mechanisms including (i) the initiation of lipid peroxidation, (ii) the inactivation of a variety of enzymes (most notably, mitochondrial respiratory enzymes and membrane pumps) (Crow and Beckman, 1995) and (iii) depletion of glutathione (Phelps et al., 1995). Moreover, peroxynitrite can also cause DNA damage (Inoue and Kawanishi, 1995).

In our study, we showed that administration of gentamicin to rats induced a reduction in glomerular filtration rate as demonstrated by a reduced creatinine clearance and increased serum creatinine. This impairment in glomerular function was accompanied by increased fractional excretion of sodium and lithium, indicating a proximal tubular dysfunction. The presence of tubular damage was additionally confirmed by the increased urinary excretion of the brush border marker gamma, glutamyl transferase, indicating a direct toxic injury. These findings correlated well with the renal morphologic examination which revealed tubular necrosis and presence of myeloid bodies. Taken together with the increased daily urine output, these data confirmed the well known pattern of aminoglycoside nephrotoxicity characterised by decreased glomerular filtration rate and direct tubular damage associated with a well-maintained urinary output.

N-acetylcysteine administration to gentamicin-treated rats reduces: (1) the development of nephrotoxicity; (2) morphological injury and neutrophil infiltration; (3) clearance dysfunction, (4) nitrotyrosine staining and (5) poly(ADP-ribose)synthase expression.

Therefore, this study confirmed in agreement with previous observations (Nakajima et al., 1994) that nephrotoxicity of gentamicin is mediated, at least in part, by the additive production of free radicals.

Increased lipid peroxidation has been reported in gentamicin-treated rats. Hydroxyl radical scavengers are able to protect against gentamicin nephrotoxicity (Walker and Shah, 1988). Treatment with vitamin E results in inhibition of lipid peroxidation in gentamicin-treated rats (Ramasammy et al., 1987). The gentamicin-induced enhancement of the production of hydrogen peroxide by mitochondria is dose-dependent (Wardle, 1982).

The most effective form of antioxidant repletion is likely to include the combination of antioxidants with known synergistic action. Vitamin E protects against lipid peroxidation, vitamin C is a powerful electron donor, reacting with superoxide and hydroxyl radicals.

N-acetylcysteine exerts its effect both as a source of sulfhydryl groups (repletion of intracellular reduced glutathione) and through a direct reaction with hydroxyl radicals (Galley et al., 1997). Recently, a number of studies in animals have suggested benefits from acetylcysteine in the context of the systemic inflammatory response syndrome caused in the severe sepsis model. In a pig gram-negative sepsis model, an infusion of acetylcysteine reduced pulmonary capillary leak without reducing mortality (Groenveld et al., 1990). Acetylcysteine also beneficially modulates inflammatory cell function in animals. Endotoxin-induced neutrophil activation in sheep lung is reduced (Lucht et al., 1987; Bernard et al., 1984).

There is a number of sites where *N*-acetylcysteine can interfere with gentamicin-induced nephrotoxicity. The results of the current study showed that *N*-acetylcysteine fully inhibited the appearance of nitrotyrosine staining in the gentamicin-treated kidney. This effect may be related to a direct scavenging effect of *N*-acetylcysteine on peroxynitrite.

There is, however, recent evidence that certain other reactions can also induce tyrosine nitration; e.g., the reaction of nitrite with hypochlorous acid and the reaction of myeloperoxidase with hydrogen peroxide can lead to the formation of nitrotyrosine (Eiserich et al., 1998). Increased nitrotyrosine staining is, therefore, considered an indication of "increased nitrosative stress" rather than a specific marker of the generation of peroxynitrite.

Additional protective effects of *N*-acetylcysteine may lie in the ability of this compound to reduce oxyradical-related oxidant processes by either directly interfering with the oxidants, or up-regulating antioxidant systems such as superoxide dismutase (Galley et al., 1997) or enhancing the catalytic activity of glutathione peroxidase (Schillier et al., 1993). Therefore, oxygen radical scavengers, administered before or at the onset of sepsis, were shown to improve survival in animal models of sepsis (Pouwell et al., 1991). *N*-acetylcysteine has antioxidant properties (Auroma et al., 1989) and, as a sulfhydryl donor, may contribute to the regeneration of endothelium-derived relaxing factor and glutathione (Harrison et al., 1991). Increasing evidence indicates that the action of *N*-acetylcysteine is pertinent to microcirculatory blood flow and tissue oxygenation. *N*-acetylcysteine was shown to enhance oxygen consumption via increased oxygen extraction in patients 18 h after the onset of fulminant liver failure (Harrison et al., 1991). It was speculated that *N*-acetylcysteine could also exert beneficial effects on impaired nutritive blood flow in patients with severe sepsis (Harrison et al., 1991).

Reactive oxygen species and peroxynitrite produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage. Reactive oxygen species produce strand breaks in DNA which trigger energy-consuming DNA repair mechanisms and activates the nuclear enzyme, poly(ADP-ribose)synthase, resulting in the depletion of its substrate, NAD, in vitro and a reduction in the rate of glycolysis. As NAD functions as a cofactor in glycolysis and in the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. This process has been termed 'the PARS Suicide Hypothesis' (see Section 1). There is recent evidence that the activation of poly(ADP-ribose)synthase may also play an important role in inflammation (Cuzzocrea et al., 1997; Chatterjee et al., 2000; Szabó et al., 1997; Thiemermann et al., 1997). We demonstrate here that *N*-acetylcysteine attenuates the increase in PARS activity caused by gentamicin treatment in the kidney.

Together, our results strongly support the claim that *N*-acetylcysteine can exert a potent protective effect on nephrotoxicity associated with gentamicin treatment. Based on the current results, we propose that the combination of *N*-acetylcysteine and gentamicin can prevent the gentamicin-mediated nephrotoxicity and the mode of *N*-acetylcysteine's action is, at least in part, related to interference with peroxynitrite-related pathways.

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