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A role for superoxide in gentamicin-mediated nephropathy in rats

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Abstract

Gentamicin is an antibiotic effective against Gram-negative infection, whose clinical use is limited by its nephrotoxicity. Oxygen free radicals are considered to be important mediators of gentamicin-mediated nephrotoxicity, but the exact nature of the radical in question is not known with certainty. We have investigated the potential role of superoxide in gentamicin-induced renal toxicity by using M40403, a low molecular weight synthetic manganese containing superoxide dismutase mimetic, which selectively removes superoxide. Administration of gentamicin at 100 mg/kg, s.c. for 5 days to rats induced a marked renal failure, characterised by a significant decrease in creatinine clearance and increased plasma creatinine levels, fractional excretion of sodium, lithium, urine γ glutamyl transferase (γGT) and daily urine volume. A significant increase in kidney myeloperoxidase activity and lipid peroxidation was also observed in gentamicin-treated rats. M40403 (10 mg/kg, i.p. for 5 days) attenuated all these parameters of damage. Immunohistochemical localisation demonstrated nitrotyrosine formation and poly(ADP-ribose) synthetase (PARS) activation in the proximal tubule of gentamicin-treated rats. Renal histology examination confirmed tubular necrosis. M40403 significantly prevented gentamicin-induced nitrotyrosine formation, poly(ADP-ribose) synthetase activation and tubular necrosis. These results confirm our hypothesis that superoxide anions play an important role in gentamicin-mediated nephropathy and support the possible clinical use of low molecular weight synthetic superoxide dismutase mimetics in those conditions that are associated with over production of superoxide.

Keywords: Gentamicin; Superoxide anion; Superoxide dismutase mimetic; Compound M40403

1. Introduction

Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans (Kahlmeter and Dahlager, 1984), and use of this class of antibiotics is known as one of the most common causes of acute renal failure, accounting for 10–20% of all cases (Walker and Shah, 1988). In fact, renal function is usually closely monitored in the in-patient setting, during therapy. Gentamicin-induced kidney damage is characterised by tubular necrosis, primarily localized to the proximal tubule. This might be explained by an increased kidney uptake of the antibiotic, mainly at the proximal tubular level (Beauchamp et al., 1985). Although gentamicin's effect on biological membranes appears to be critical in the pathogenetic events of gentamicin toxicity (Humes et al., 1982), the exact mechanisms of gentamicin nephrotoxicity are unknown: a possible mechanism is given by Walker et al. (1999).

Reactive oxygen species have been proposed as a causative agent of cell death in many different pathological states (Fantone and Ward, 1982, 1985; Weiss and LoBuglio, 1982; Halliwell and Gutteridge, 1990) as well as, in glomerular diseases (Shah, 1984; Shah et al., 1987), in renal ischemia and reperfusion injury (McCord et al., 1985; Kadkhondaee et al., 1995; Grune et al., 1995), and in various models of toxic renal failure (Baliga et al., 1999). Reactive oxygen species produce cellular injury and necrosis via several mechanisms including peroxidation of

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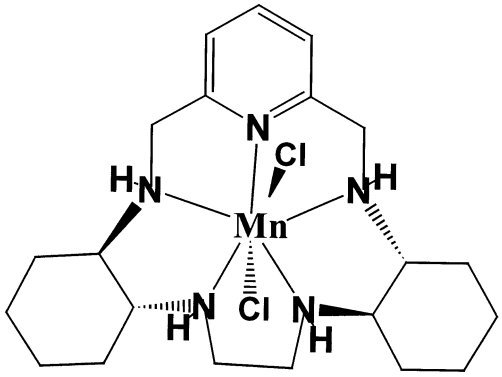
membrane lipids, protein denaturation and DNA damage (Dean et al., 1991). Evidence from studies utilising cultured cells have demonstrated that reactive oxygen species produce strand breaks in DNA which trigger the activation of the nuclear enzyme poly(ADP-ribose) synthetase (Szabo et al., 1997). There is recent evidence that the activation of poly(ADP-ribose) synthetase may also be implicated in the pathogenesis of shock and renal injury (Chatterjee et al., 1999, 2000a; Cuzzocrea et al., 2001a,b).

In animal models of free radical-induced nephrotoxicity, previous workers have demonstrated the protective effect of various therapeutic approaches aimed at a reduction in the formation of reactive oxygen species (Ramsammy et al., 1987; Walker and Shah, 1988; Nakajima et al., 1994). M40403 (Fig. 1), a selective superoxide dismutase mimetic, is a stable low molecular weight, manganese-containing, non-peptidic molecule possessing the function and catalytic rate of native superoxide dismutase enzymes (Salvemini et al., 1999). M40403 is not only a highly active catalyst for the dismutation of O_2^-, but it is also highly selective for superoxide. M40403 does not react with hydrogen peroxide, nor does it directly react with other biologically relevant oxidants such as nitric oxide or peroxynitrite (Riley et al., 1996, 1997; Salvemini et al., 1999). Thus, superoxide dismutase mimetic drugs such as M40403 can be regarded as selective probes for evaluating the role of superoxide in pathological situations where other oxidants could be present.

The aim of the present study was to evaluate the role of superoxide in gentamicin-mediated nephrotoxicity in rats and highlight the protective effect of M40403 on gentamicin-induced renal damage. We have evaluated the following endpoints of renal damage: (1) renal hemodynamics, (2) lipid peroxidation, (3) nitrotyrosine formation (immunohistochemistry), (4) activation of the nuclear enzyme poly(ADP-ribose) synthetase, (5) neutrophil infiltration and (6) kidney histopathology. The results reported in this study confirm the role of reactive oxygen species in gentamicin-induced renal injury and unravel a key role for superoxide in this disorder.



M40403

Fig. 1. Structure of M40403.

2. Materials and methods

2.1. Animals

Forty male Sprague–Dawley rats (Charles River, Milan, Italy) weighing 250 to 300 g were used. These were housed in a controlled environment and provided with standard rodent chow (Rodentia, Bergamo, Italy) and water ad libitum. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purpose (D.M. 116192), as well as with the EEC regulations (O.J. of E.C.L. 358/1 12/18/1986).

2.2. Experimental protocol

Animals were placed into individual metabolic cages and divided into 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.
- M40403 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 M40403. The dose of 10 mg/kg was based on one used in previous studies (Salvemini et al., 2001a,b).
- 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+ M40403 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 M40403.

The fifth day after starting gentamicin treatment, urine free of food and faeces was collected into ice-cold graduate cylinders containing mineral oil to prevent evaporation, and sodium azide 0.1% to minimize bacterial growth. These samples were used to determine enzymatic activity, electrolyte excretion and creatinine clearance.

2.3. Light microscopy

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

Morphological changes were analysed in a blind way by a pathologist and scored with a semiquantitative scale evaluating the changes more 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 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. The sections were then incubated overnight with 1:1000 dilution of primary anti-nitrotyrosine antibody or with control solutions. Controls included buffer alone or non specific purified rabbit immunoglobulin G. Some sections were also incubated with the primary antibody (anti-nitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding specificity. Specific labelling was detected with a biotin-conjugated goat anti-rabbit immunoglobulin G and avidin–biotin peroxidase complex. Diaminobenzidine was used as a cromogen (DBA, Milan, Italy).

2.5. Immunohistochemical localisation of poly(ADP-ribose) synthase

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). The sections were then incubated overnight with 1:500 dilution of primary anti-poly(ADP-ribose) antibody (DBA) or with control solutions. Controls included buffer alone or nonspecific purified rabbit immunoglobulin G. Specific labelling was detected with a biotin-conjugated goat anti-rabbit immunoglobulin G and avidin–biotin peroxidase complex. Diaminobenzidine was used as a cromogen (DBA).

2.6. Laboratory investigation

Na^+, K^+ and Li^+ in plasma and urine were determined by a flame photometer. Urine and serum creatinine assays were performed by a clinical laboratory. Urine was also assayed for the excretion of γ glutamyl transferase (γGT) using a clinical laboratory method. Creatinine clearance (C_{cr}), fractional excretion of lithium (FE_{Li}) and fractional excretion of sodium (FE_{Na}) were calculated from these values by using standard methods.

2.7. Myeloperoxidase activity

Myeloperoxidase activity, an index of polymorphonuclear leukocyte accumulation, was determined as previously described (Mullane et al., 1985). At the specified time following the gentamicin treatment, kidneys were obtained and weighed. Each piece of tissue was homogenised in a solution containing 0.5% hexadecyltrimethyl-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-methylbenzidine (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 of hydrogen 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 index of lipid peroxidation (Ohkawa et al., 1979). Kidney tissue, collected at the specified time, was homo-

Table 1
Serum creatinine, creatinine clearance (C_{cr}), percent of fractional excretion of lithium (FE_{li}), sodium (FE_{Na}), urine volume and urine gamma glutamyl transferase (γGT) levels of the three studied groups

	Serum creatinine (mg/dl)	C_{cr} (ml/dl in 100 g BW)	FE_{li} (%)	FE_{Na} (%)	Urine γGT (U/l)	Urine volume (ml)
Sham + vehicle	0.30 ± 0.06	0.70 ± 0.08	21.05 ± 1.98	0.55 ± 0.08	120.56 ± 13.8	8.01 ± 0.18
Sham + M40403	0.28 ± 0.08	0.66 ± 0.09	22.1 ± 1.85	0.57 ± 0.1	115.56 ± 12.4	7.95 ± 0.21
GM + vehicle	0.90 ± 0.08 *	0.25 ± 0.04 *	59.64 ± 5.16 *	1.10 ± 0.11 *	821.5 ± 35.4 *	12.23 ± 1.24 *
GM + M40403	0.35 ± 0.02°	0.60 ± 0.05°	29.21 ± 2.67°	0.60 ± 0.06°	410.4 ± 33.2°	8.0 ± 0.32°

Data are means ± S.E.M of 10 rats for each group.
GM was injected at the dose of 100 mg/kg, s.c. in 0.5 ml of saline for 5 days.
M40403 was injected at the dose of 10 mg/kg, i.p. with 0.5 ml s.c. of isotonic saline for 5 days.
GM + M40403 were injected at the dose of 100 mg/kg, s.c. in 0.5 ml of saline for 5 days and 10 mg/kg, i.p. of M40403.
* $P < 0.01$ versus sham.
° $P < 0.01$ versus gemtamicin (GM).

genised in 1.15% KCl solution. An aliquot (100 μl) of the homogenate was added to a reaction mixture containing 200 μl of 8.1% sodium dodecyl sulphate, 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. The absorbance of the organic phase was measured by spectrophotometry at 650 nm.

2.9. Materials

Biotin blocking kit, biotin-conjugated goat anti-rabbit immunoglobulin G. Primary anti-nitrotyrosine antibody was from Upstate Biotech (DBA). 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 mean ± standard error of the mean (S.E.M.) 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 analysis of variance 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 M40403 treatment on renal function

The first set of experiments studied the effects of gentamicin (100 mg/kg, s.c. in 0.5 ml of 0.5 ml of saline) on kidney function. Serum creatinine, fractional excretion of sodium, lithium, urinary excretion of γ glutamyl transferase and daily urine volume were significantly increased after administration of gentamicin for 5 consecutive days (Table 1). Gentamicin-treated animals were also characterised by having a significantly lower creatinine clearance (Table 1) and plasma K^+ levels (Table 2) and higher plasma Na^+ levels when compared with saline-treated rats. M40403 treatment (10 mg/kg, i.p.) provided a marked protective effect with significantly decreased serum creatinine, fractional excretion of sodium and lithium as well as a significant increased creatinine clearance and plasma K^+ levels (Tables 1 and 2). M40403 alone did not modify any of the parameters studied.

Table 2
Plasma Na^+, K^+ and urine K^+ levels of the three studied groups

	Plasma Na^+ (mEq/l)	Plasma K^+ (mEq/l)	Urine K^+ (mEq/day)
Sham + vehicle	111 ± 2.8	5.01 ± 0.18	3.16 ± 0.26
Sham + M40403	106 ± 3.8	4.89 ± 0.22	3.07 ± 0.16
GM + vehicle	153 ± 1.3	3.82 ± 0.1 *	3.25 ± 0.34
GM + M450403	128 ± 1.5	4.98 ± 0.11°	2.94 ± 0.21

Data are means ± S.E.M. of 10 rats for each group.
See Table 1 for the legend.
* $P < 0.01$ versus sham.
° $P < 0.01$ versus gemtamicin (GM).

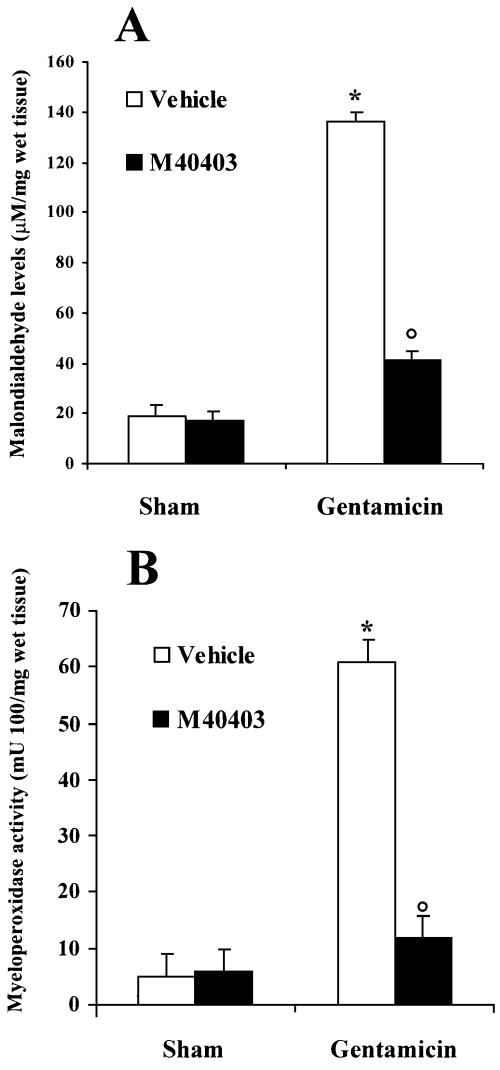


Fig. 2. Malondialdehyde levels (A), myeloperoxidase activity (B) at 5 days after gentamicin administration. Malondialdehyde levels and myeloperoxidase activity were significantly increased in the kidney of the gentamicin-treated rats (* $P < 0.01$). M40403 reduced the gentamicin-induced increase in tissue malondialdehyde levels and myeloperoxidase activity. Values are means ± S.E.M. of eight animals for each group. * $P < 0.01$ versus vehicle. ° $P < 0.01$ versus gentamicin.

3.2. Effect of M40403 treatment on malondialdehyde and myeloperoxidase activities

Gentamicin-treated rats showed an increase in kidney malondialdehyde levels, an index of lipid peroxidation.

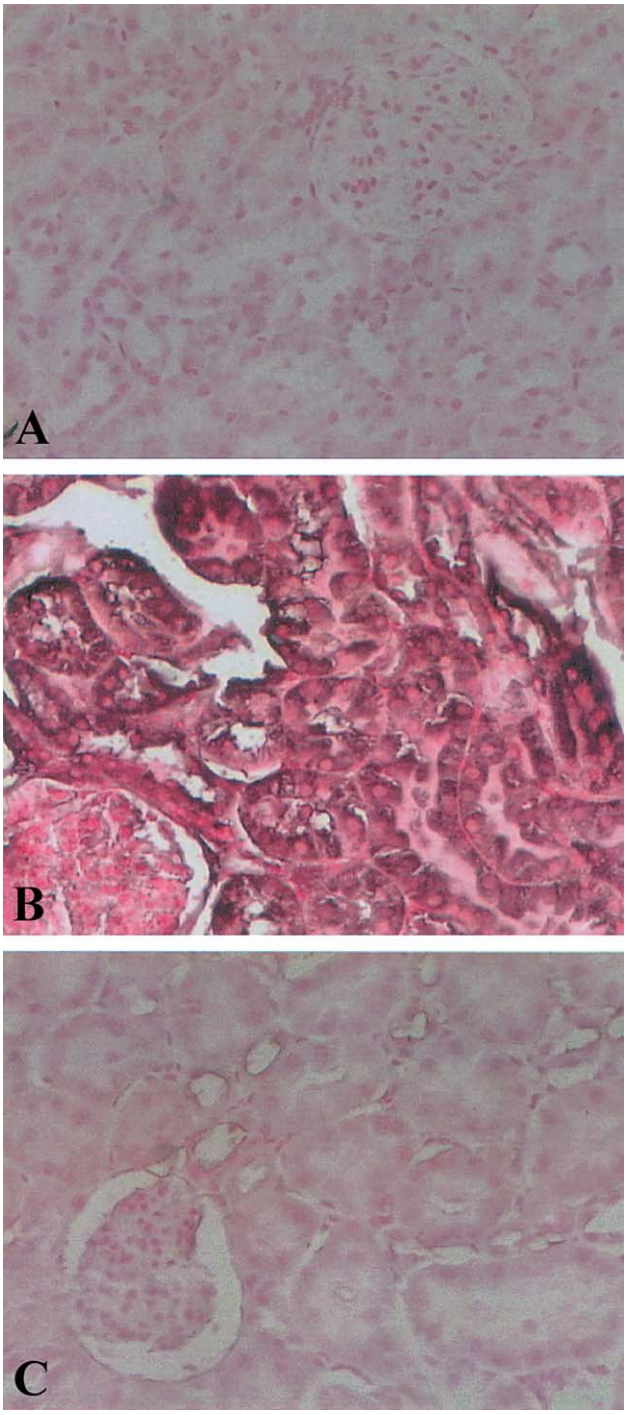


Fig. 3. Effect of M40403 on nitrotyrosine formation: no positive staining was found in sham-treated rats (A). Five days following gentamicin administration, positive staining for nitrotyrosine was observed in tubular epithelial cells (B). There was a marked reduction in the immunostaining in the kidney of gentamicin-treated rats treated with M40403 (C). Original magnification: × 250. Figure is representative of at least three experiments performed on different experimental days.

(Fig. 2A). 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. 2B). M40403 treatment significantly reduced ($P < 0.01$) myeloperoxi-

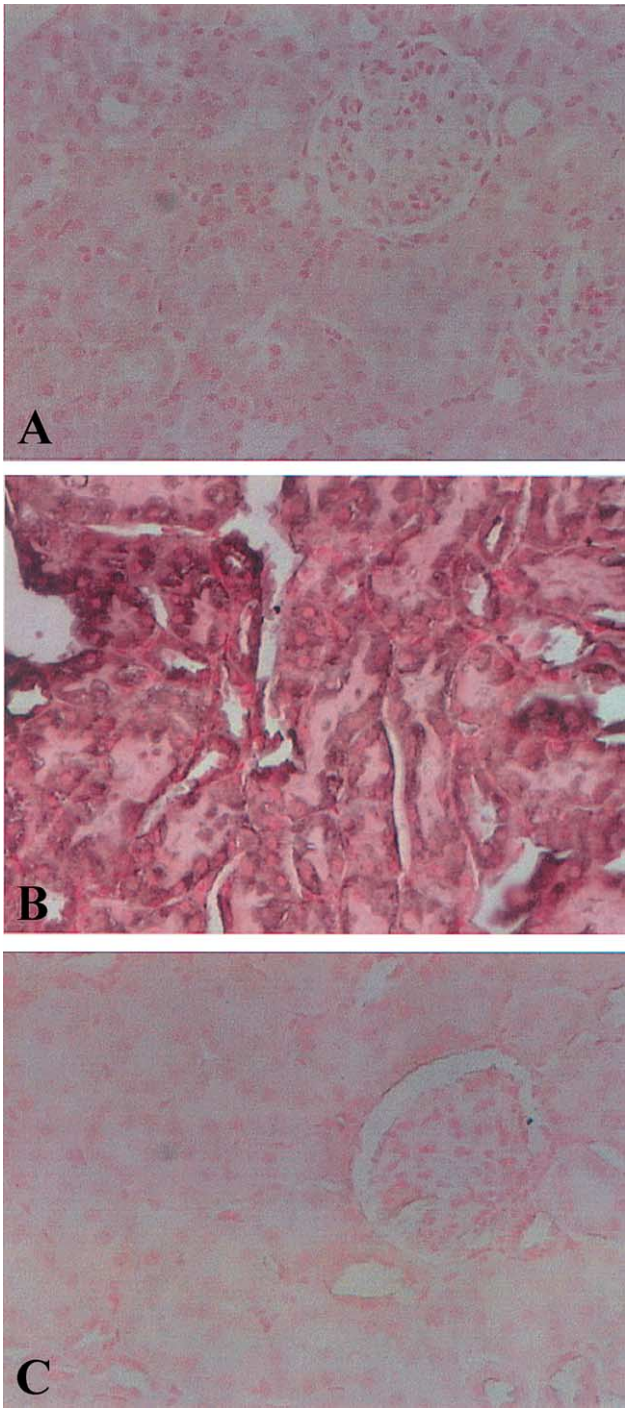


Fig. 4. Effect of M40403 on poly(ADP-ribose) synthase immunostaining: no positive staining were found in sham-treated rats (A). Five days following gentamicin administration, poly(ADP-ribose) synthase immunoreactivity was observed in tubular epithelial cells (B). In the kidney of gentamicin-treated rats with M40403 (C), no positive staining was found. Original magnification: × 250. Figure is representative of at least three experiments performed on different experimental days.

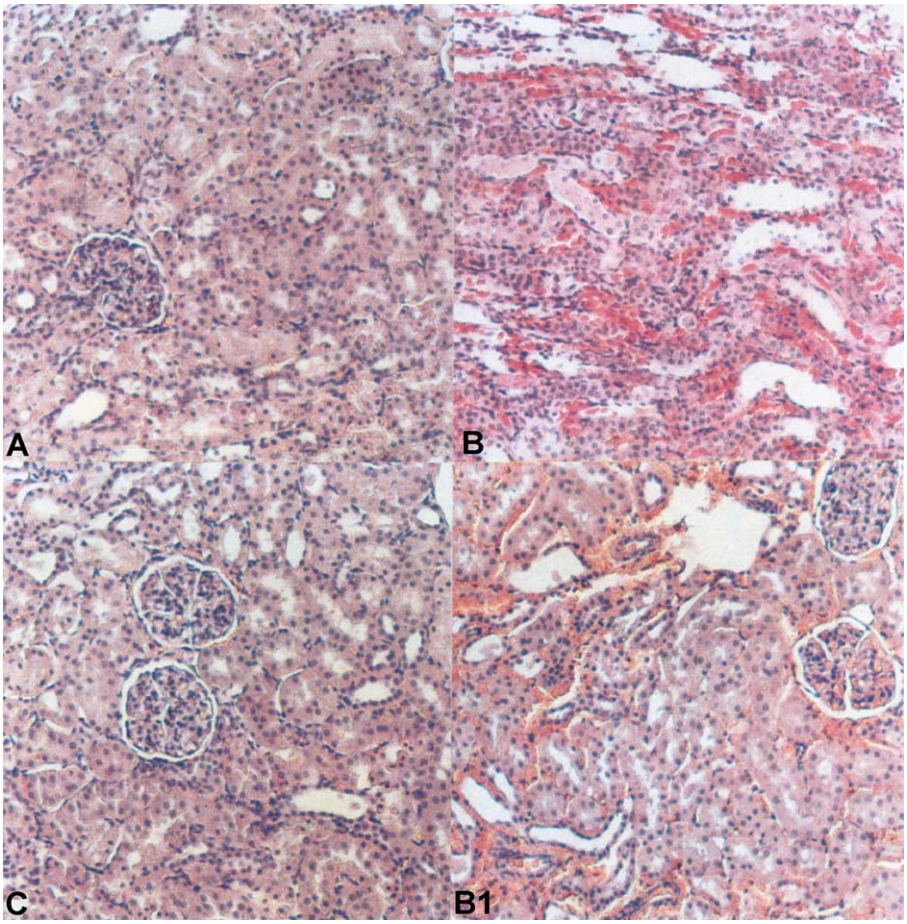


Fig. 5. Protective effects of M40403 on the gentamicin-induced nephrotoxicity. (A) Sham rat vehicle, (B) gentamicin-treated rats, (C) M40403-treated rats. Treatment with M40403 produced a significant attenuation of nephotoxicity as well as prevents the inflammatory cells infiltration. Original magnification: A, B, C, 125 ×; B1 150 ×. Figure is representative of at least three experiments performed on different experimental days.

dase activity as well as the malondialdehyde levels (Fig. 2A and B) in the kidney.

3.3. Effect of M40403 treatment on nitrotyrosine formation and poly(ADP-ribose) synthase activation

At the fifth day after gentamicin treatment, kidneys were taken in order to determine the immunohistological staining for nitrotyrosine. Immunohistochemical analysis, using a specific anti-nitrotyrosine antibody, revealed a positive staining in tubular epithelial cells from gentamicin-treated rats (Fig. 3B). In contrast, no positive nitrotyrosine staining was found in the kidney of control (untreated) rats and in the kidney from rats treated with M40403 together with gentamicin (Fig. 3C).

Immunohistochemical analysis, using a specific anti-poly(ADP-ribose) synthase antibody, revealed a positive staining in tubular epithelial cells from gentamicin-treated rats (Fig. 4B). M40403 treatment significantly reduced the degree of immunostaining for poly(ADP-ribose) in the kidney (Fig. 4C). There was no staining for poly(ADP-ribose) synthase in the kidney sections of sham animals (Figs. 3A and 4A).

3.4. Histological change

Light microscopic observations revealed tubular necrosis (Fig. 5A and B) and focal infiltration of inflammatory cells (e.g. neutrophils and monocytes) in the renal tubular in gentamicin-treated rats (Fig. 5B1). Treatment with M40403

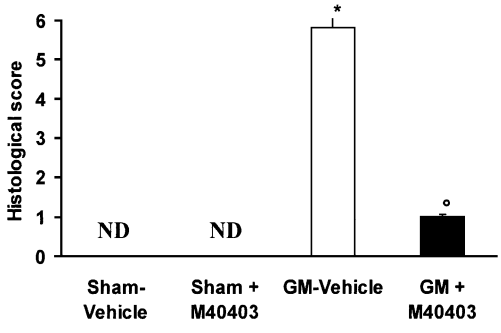


Fig. 6. Effects of M40403 on histological score obtained by the microscopic observation of kidney area of gentamicin-treated rats. Values are means ± S.E.M. of eight animals for each group. * $P < 0.01$ versus sham. ° $P < 0.01$ versus gentamicin.

significantly decreased the gentamicin-induced tubular necrosis and cast formation (Fig. 5C). The evaluation of sections from gentamicin-treated animals which received M40403 showed a significantly lesser score in comparison with that reached by gentamicin animals treated with vehicle (Fig. 6).

4. Discussion

In the present study, we have shown that the administration of gentamicin to rats induced a reduction in glomerular dysfunction which correlated with increased creatinine serum level. Glomerular dysfunction was correlated by increased fraction of sodium and lithium, indicating a proximal tubular injury (Dieperink et al., 1983). The tubular damage was also confirmed by increased urinary excretion of the γ glutamyl transferase (γGT), a brush border enzyme, which indicated a direct toxic effect. This observation correlates well with the renal histological evaluation which revealed tubular necrosis and a presence of myeloid bodies: lysosomes filled with phospholipid complex. All these data together with the increased daily urine output confirmed the well-known pattern of aminoglicosyde nephrotoxicity that is characterised by decreased glomerular filtration rate and direct tubular injury associated with a well-maintained urinary output (Bennett, 1986).

The interaction between cationic aminoglycoside with the membrane anionic phospholipid is considered the first step for the development of gentamicin cytotoxicity. The lysosomes cannot function properly and release their hydrolytic enzymes (Aubert-Tulkens et al., 1979).

Moreover, a recent study suggests that aminoglycoside antibiotics are a class of drug capable of stimulating the formation of reactive oxygen species (mechanism not known), which can be directly involved in gentamicin-induced acute renal failure; membrane lipid peroxidation could be a contributing factor in gentamicin nephrotoxicity (Ramsammy et al., 1985). It was observed that the level of unsaturated arachidonic acid decreased by 50% after two injections of gentamicin, and also reduced glutathione levels (Ramsammy et al., 1985).

The present results demonstrate that daily administration of M40403 to gentamicin-treated rats resulted in an almost complete normalization of kidney function as evidenced by creatinine clearance and serum creatinine level, and by decreased fractional excretion of sodium and lithium. Furthermore, morphological examination of the kidney samples revealed reduced tubular necrosis.

Free radicals have been implicated in a variety of pathologies and drug toxicity, and reactive oxygen species are implicated in renal ischemia–reperfusion injury (Paller et al., 1984; Greene and Paller, 1991). Reactive oxygen species produce strand breaks in the DNA of renal tissues (Richter, 1992, 1995), which in turn is responsible for the activation of poly(ADP-ribose) synthase (Thiemermann et al., 1997). The ability of reactive oxygen species to induce renal injury has been demonstrated in several studies (Paller et al., 1984) and several studies have reported on the beneficial effect of free radical scavengers in various models of renal injury (Walker and Shah, 1988; Nakajima et al. 1994).

In our study, the renal dysfunction produced by gentamicin appears to be secondary to the generation of superoxide anion as demonstrated by the protective effects observed with the selective superoxide dismutase mimetic, M40403. Free radical-mediated DNA damage may be of primary importance in renal injury as it is still unclear whether lipid peroxidation is a major contributor to the renal dysfunction associated with gentamicin treatment (Ramsammy et al., 1987).

In an attempt to relate the potential significance of our results to understanding gentamicin's nephrotoxicity, several observations are worthy of note. It has been reported that gentamicin enhanced, in a dose-dependent fashion, the generation of mitochondrial superoxide (Walker and Shah, 1988). Moreover, superoxide and peroxynitrite produce strand breaks in DNA (Inoue and Kawanishi, 1995; Salgo et al., 1998) 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 the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. This process has been termed 'the poly(ADP-ribose) synthase Suicide Hypothesis' (Szabo et al., 1997).

The importance of free radical-induced nephrotoxicity has been evaluated in animal models using various modalities. This, for instance, included the use of various antioxidants such as vitamin E and *N*-acetyl cysteine (Ramsammy et al., 1987; Walker and Shah, 1988; Nakajima et al., 1994; Mazzon et al., 2001). Furthermore, our results are supported by previous studies performed with the native SOD enzyme as shown by the study of Ali and Bashir (1996). Although this clearly points to a critical role of superoxide in this event, the native SOD enzyme suffers as viable therapeutic agents. In fact, there are drawbacks or problematic issues associated with the use of the native enzymes as therapeutic agents (e.g. solution instability, immunogenicity of nonhuman enzymes, bell-shaped dose response curves, high susceptibility to proteolytic digestion) and as pharmacological tools (e.g. they do not penetrate cells or cross the blood–brain barrier, limiting the dismutation of superoxide only to the extracellular space or compartments). Agents such as M40403, on the other hand, are viable drug candidates (Salvemini and Riley, 2000).

The results obtained in this study suggest that superoxide- (and possibly peroxynitrite) driven activation of poly(ADP-ribose) synthase contributes at least, in part, to the renal dysfunction and injury observed after gentamicin

treatment (Fig. 7). This hypothesis is supported by the following key findings: (i) M40403 significantly reduced plasma creatinine levels, reduced fractional excretion of sodium and lithium, (ii) decreased nitrosative stress and poly(ADP-ribose) synthase activation at the tubular level was demonstrated histochemically and (iii) M40403 reduced the renal dysfunction/injury caused by gentamicin administration. Thus, we propose that gentamicin administration causes an increase in oxidative stress and poly(ADP-ribose) synthase activity in the proximal tubular cells, which in turn contributes to the cellular injury and death. This hypothesis is reinforced by earlier findings that free radical scavengers and poly(ADP-ribose) synthase inhibitors protect primary cultures of rat proximal tubular cells against oxidant stress-mediated cell injury and death (Chatterjee et al., 1999, 2000b).

Taken together, our results strongly support a protective role of M40403 in nephrotoxicity associated with

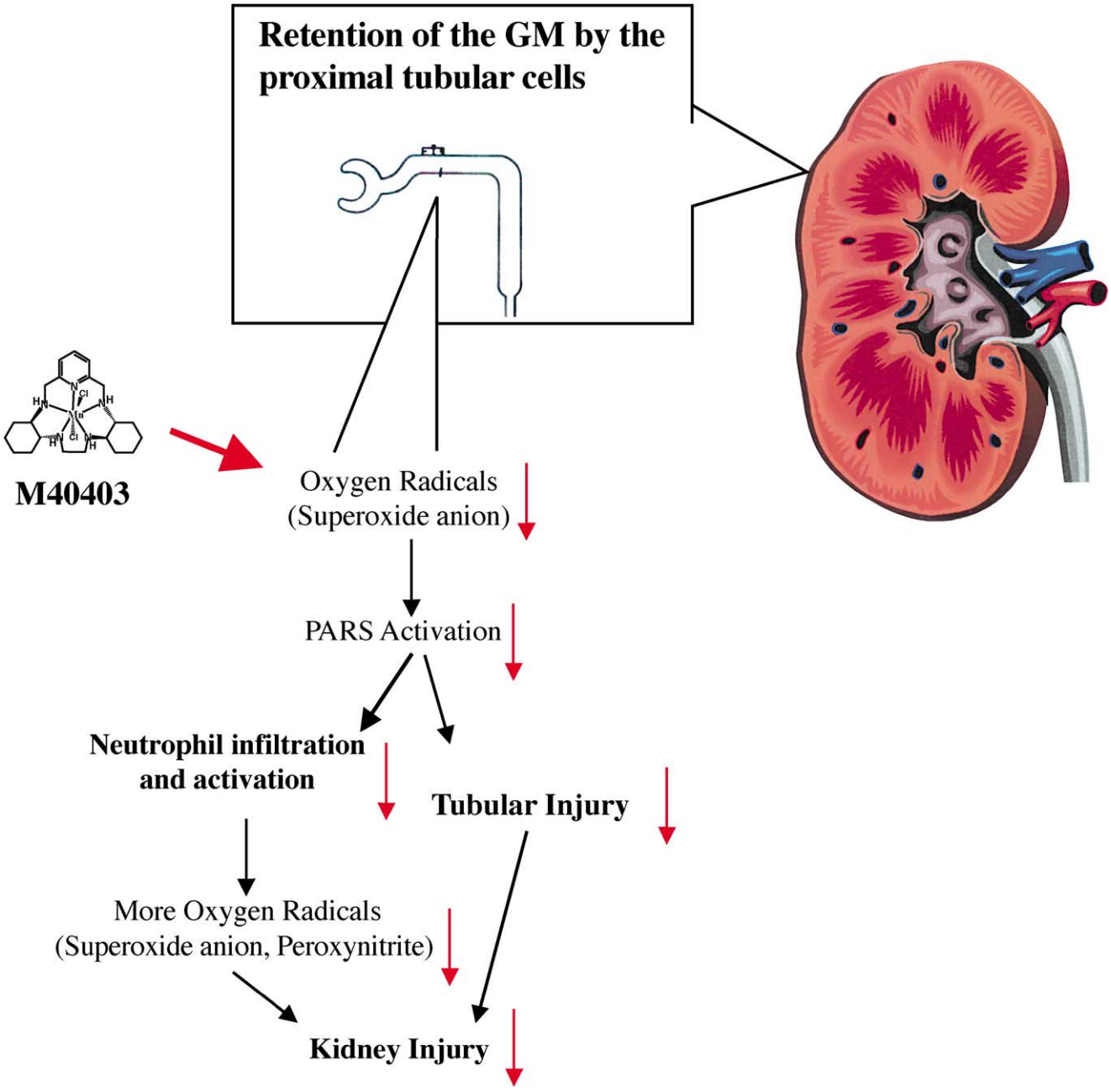


Fig. 7. Proposed scheme of some of the mechanisms of gentamicin-induced nephrotoxicity and potential sites of M40403 protective actions. Retention of gentamicin by the proximal tubular cells triggers the release and formation of oxygen radicals (e.g. superoxide anion, hydroxyl radical, peroxynitrite) ultimately leading to cellular injury. Part of the injury may be related to the development of DNA single strand breakage, with consequent activation of PARS, leading to cellular dysfunction. In this system, M40403's anti-inflammatory effects may include (1) inhibition of superoxide anion production (2) reduction of peroxynitrite formation and (3) inhibition of PARS activation (see discussion for further explanations).

gentamicin treatment. Our results suggest that the combination of M40403 and gentamicin might be a viable therapeutic avenue that can be used to attenuate gentamicin-induced side effects. Further work is necessary to evaluate such an opportunity.

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