Efficacy of the combination of N-acetylcysteine and desferrioxamine in the prevention and treatment of gentamicin-induced acute renal failure in male Wistar rats

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Abstract

Background. Oxidative stress and the formation of aminoglycoside–iron complexes through iron-dependent Fenton reaction have been proposed to be the major mechanisms in the development of GM-induced acute renal failure (ARF); however, the efficacy of the combination of N-acetylcysteine (NAC) and desferrioxamine (DFX) in the prevention and the treatment of GM-induced ARF has not previously been investigated.

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Methods. In the prevention protocol, adult male Wistar rats received gentamicin (GM) [70 mg/kg, intraperitoneally (i.p), each 12 h for 7 days], NAC (20 mg/kg, sc, each 8 h for 7 days) and/or DFX (20 mg/kg, sc, at first, fourth and seventh days). In the treatment protocol animals received GM for 7 days. Additionally, animals received NAC and or DFX starting in the fourth day after GM administration. Parameters of renal function had been evaluated 24 h, 4 and 8 days after the beginning of GM administration in the prevention protocol and in Days 5 and 8 in the treatment protocol. At the end of experiment, lipid peroxidation (TBARS assay) and protein oxidation (protein carbonyls levels) formation were evaluated in kidney tissue as oxidative damage parameters.

Results. In the prevention protocol, GM-induced ARF was prevented by the NAC and DFX association. Lipid peroxidation was attenuated by both antioxidant treatments, but the effects of NAC plus DFX were of greater magnitude. In the treatment protocol, plasma markers of renal injury were improved only in the NAC group, despite the similar antioxidant effect of both NAC, DFX and NAC plus DFX. Conclusion. Although the combination of NAC and DFX was more effective in the prevention protocol, the use of NAC alone seemed to be superior to NAC–DFX combination, in the treatment of GM-induced ARF in adult male Wistar rats.

Keywords: acute renal failure; desferrioxamine; gentamicin; N-acetylcysteine; oxidative stress

Introduction

Aminoglycoside antibiotics, as gentamicin (GM), are widely used in the clinical practice for the treatment of gram-negative infections. However, acute renal failure (ARF) is a major complication of GM treatment that limits its use [1]. GM-induced nephrotoxicity is characterized by direct tubular necrosis, without morphological changes in glomerular structures [2]. The mechanisms involved in GM-induced cell injury are not clearly understood; however, reactive oxygen species (ROS) are considered to be one of the important mediators [3]. Priuska and collaborators demonstrated that GM is an iron chelator [4], enhancing iron-mediated lipid peroxidation [5]. Probably GM reduces iron mediating the catalytic formation of superoxide from oxygen that initiates a chain reaction free radical generation [5–7]. In this context, iron chelators could suppress the GM-induced free radical generation [4], by competing to iron binding. Studies showed that GM induces superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical (HO•) production from renal mitochondria [8–10]. In addition, lipoxygenation [11–17], nitrotyrosine [11,18] and protein carbonyl content [1,19] are increased and reduced glutathione is diminished [15] in renal cortex from GM-treated rats, and the administration of N-acetylcysteine (NAC) is able to ameliorate the renal damage [1].

We had previously demonstrated that the use of an antioxidant in combination to an iron chelator is superior to its use in several different animal models of inflammatory disease [20–23]. Thus, since the generation of oxidative damage after GM administration seemed to be dependent on iron, it is reasonable to combine NAC and an iron chelator in the prevention and treatment of GM-induced ARF.

Methods

Animals

*In vivo* studies were performed in accordance with National Institute of Health guidelines and with approval of Universidade do Extremo Sul Catarinense Ethics Committee. Male Wistar rats (age, 2–3 months; weight, 250–320 g) were used in this study.

Drugs

N-Acetylcysteine (Zambon, Brazil), desferrioxamine (DFX) (Novartis, Brazil) and gentamicin sulfate (Schering-Plough, Brazil) were dissolved in saline (NaCl 0.9%). The solutions were prepared immediately before use.

Experimental design

Animals were randomly divided into five groups (n = 7 animals per group) as follows: (1) saline, (2) GM, (3) GM plus NAC, (4) GM plus DFX and (5) GM plus NAC plus DFX, subdivided in two protocols: prevention and treatment.

Prevention protocol

Animals received GM [70 mg/kg, intraperitoneally (i.p), each 12 h for 7 days] and starting at the same time animals received NAC (20 mg/kg, sc, each 8 h for 7 days) and/or DFX (20 mg/kg, sc, at first, fourth and seventh days). Blood samples were obtained from caudal vein at the Days 2, 4 and 8 to the determination of renal function. After this period, animals were killed and the kidneys were isolated and rapidly frozen and stored at −80°C until oxidative stress assessment.

Treatment protocol

In the treatment protocol animals received GM [70 mg/kg, intraperitoneally (i.p), each 12 h for 7 days]. In this protocol antioxidant administration initiated 4 days after GM, NAC [20 mg/kg, subcutaneously (sc), each 8 h in fourth to seventh days] and/or DFX (20 mg/kg, sc, at fourth and seventh days). Blood samples were obtained from caudal vein at fifth and eighth days for the evaluation of renal function. After this period, animals were killed and the kidneys were isolated and rapidly frozen and stored at −80°C until oxidative stress assessment.

Markers of kidney injury

Markers of kidney injury, creatinine and urea in serum were measured using commercially available kits (Labtest, Brazil).

Measurement of oxidative stress

As an index of oxidative damage in the kidney we used the formation of thiobarbituric acid reactive substances (TBARS) during an acid-heating reaction, which is widely adopted as a sensitive method for measurement of lipid peroxidation, as previously described [24]. Briefly, the samples were mixed with 1 ml of 10% trichloroacetic acid (TCA) and 1 ml of 0.67% thiobarbituric acid (TBA), then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm. Results were expressed as malondialdehyde (MDA) equivalents (nmol/mg protein).

The oxidative damage in proteins was assessed by determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) as previously described [25]. Briefly, proteins were precipitated by the addition of 20% TCA and redissolved in DNPH. The quantification of protein carbonyls in the samples was determined in the absorbance of 370 nm. Results were expressed as protein carbonyls (nmol/mg protein). The protein content was normalized by quantification according to the Lowry method [26].
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**Statistical analyses**

All data are presented as mean ± SEM. Differences among experimental groups were determined by one-way ANOVA, and multiple comparisons were performed by a Newman–Keuls test. All statistical analyses were performed using the statistical package SPSS 12.0 for Windows (SPSS, Inc., Chicago, IL, USA). In all experiments, P-values lower than 0.05 were considered to indicate statistical significance.

**Results**

**Prevention protocol**

GM induces alterations in renal function only at the eighth day of the experiment (visualized in Figure 1A and B as an increase in urea and creatinine levels). These alterations were attenuated using NAC + DFX, but not when these antioxidants were used alone (Figure 1A and B).

Instead of urea and creatinine being normal at the fourth day, we observed, at this time point, an increase in TBARS content in the kidney of animals treated with GM (Figure 2B), and this is prevented by all antioxidant regimes used. This pattern was similar to that observed in the eighth day. Increased protein carbonyl levels were observed as early as 24 h after GM administration, and oxidative protein damage persisted until the eighth day (Figure 3A–C). As demonstrated for TBARS levels, all antioxidant regimes were able to attenuate oxidative protein damage, but this effect was of greater magnitude with the use of NAC plus DFX (Figure 3A–C).

**Treatment protocol**

As oxidative damage was detected 4 days after GM, we started antioxidant treatment at this time point in the treatment protocol. GM induced an increase in urea and creatinine levels 5 days after its administration and this increase persisted until the eighth day (Figure 4A and B). The administration of DFX and NAC + DFX decrease urea and creatinine levels 5 days after GM. The administration of NAC could decrease urea and creatinine at 5 and 8 days after GM administration (Figure 4A and B). As demonstrated in the prevention protocol, GM induced an increase in kidney TBARS and protein carbonyl levels that were reversed using antioxidants (Figures 5A and B and 6A and B).
Fig. 3. Protein carbonyl levels in renal tissue (A) 24 h, (B) 4 and (C) 8 days after gentamicin administration in prevention protocol. Data are expressed as means ± SD. P < 0.05, * different from saline. SAL = saline; GM = gentamicin; NAC = N-acetylcysteine; DFX = desferrioxamine.

Discussion

The underlying mechanisms by which GM causes nephrotoxicity are not well understood. However, evidence suggests that ROS may be involved in GM-induced nephrotoxicity, since it has been found that the levels of $O_2^-$, $H_2O_2$ and HO increase with GM treatment [1–3], and this probably involves the reaction of gentamicin with iron [5]. Additional studies demonstrated that substances with antioxidant properties protect against kidney damage induced by GM [2,27,28]. In the present study, we demonstrated that GM-induced oxidative damage is an early event and occurs before any observed increase on urea and creatinine levels, suggesting that oxidative damage is related to GM-induced kidney injury. This inference was not completely supported by our results, since all antioxidant protocols attenuated kidney oxidative damage, but its effects on urea and creatinine levels were less consistent, mainly in the treatment protocol. Antioxidants that abolish oxidative damage did not improve urea and creatinine levels in the treatment protocol, suggesting that mechanisms not related to oxidative damage are involved in the nephroprotection provided by NAC. In this way, NAC exerts its effect both as a source of sulfhydryl groups and consequent repletion of intracellular glutathione pool and through a direct reaction with hydroxyl radicals [29]. On the other hand, Mazzon and colleagues [30] suggest that NAC protective effects on GM-mediated nephropathy are related to interference with peroxynitrite-related pathways. Additional protective effects of NAC may lie in the ability of this compound to decrease NF-kB activation, reduce kidney inflammation and improve renal function [27,31], and to improve microcirculation [32].

Several reports from our laboratory demonstrated that the combination of NAC and DFX is superior to their use alone in several animal models of inflammatory diseases [20–23]. We here demonstrated that in the prevention protocol this association is superior to their isolated use in preventing GM-induced kidney damage. This effect could be related to its superior effect as an antioxidant when compared to NAC or DFX alone, and this could be secondary...
to the competitive bind of DFX and iron impeding iron-GM binding, and free radical generation [33]. In addition, DFX could decrease NAC oxidative metabolism that depends on iron [7]. In contrast, the use of an iron chelator seemed to be detrimental in the treatment protocol, since, in this protocol, only the isolated use of NAC consistently decreased urea and creatinine levels, suggesting that iron ions are important in the regeneration of the damaged kidney, as previously demonstrated in vitro [33]. The protective effect seemed to be independent of antioxidant effect, since all antioxidants used presented a similar effect on TBARS and protein carbonyl levels. Thus, we suggest that avoiding GM-induced oxidative damage using NAC plus DFX is sufficient to prevent kidney failure, but once oxidative damage occurs its attenuation is not sufficient to prevent kidney failure.

The major limitation of our results is the fact that serum creatinine and the blood urea are poor markers of renal function and there is poor correlation between creatinine and glomerular filtration rate. Thus, besides the clinical relevance of serum creatinine measures, caution must be taken in the interpretation of the results.

We suggested that the association of NAC with DFX is effective in preventing GM-induced kidney damage, and this effect is not solely related to its antioxidant potential. In contrast, this combination attenuated kidney oxidative damage in the treatment protocol, but did not improve creatinine levels (differently from the isolate use of NAC), suggesting that iron ions must be relevant in the regeneration of previously damaged tubular cells.

Conflict of interest statement. Authors does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

9. Yang CL, Du XH, Han YX. Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. Ren Fail 1995; 17: 21–26

Fig. 5. TBARS levels in renal tissue (A) 5 and (B) 8 days after gentamicin administration in treatment protocol. Data are expressed as means ± SD. *P < 0.05, different from saline. SAL = saline; GM = gentamicin; NAC = N-acetylcysteine; DFX = desferrioxamine.

Fig. 6. Protein carbonyl levels in renal tissue (A) 5 and (B) 8 days after gentamicin administration in treatment protocol. Data are expressed as means ± SD. *P < 0.05, different from saline. SAL = saline; GM = gentamicin; NAC = N-acetylcysteine; DFX = desferrioxamine.
Haematopoietic stem cell migration to the ischemic damaged kidney is not altered by manipulating the SDF-1/CXCR4-axis

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Abstract

Background. Haematopoietic stem cells (HSC) have been shown to migrate to the ischemic kidney. The factors that regulate the trafficking of HSC to the ischemic damaged kidney are not fully understood. The stromal cell-derived factor-1 (SDF-1)/CXCR4-axis has been identified as the central signalling axis regulating trafficking of HSC to the bone marrow. Therefore, we hypothesized that SDF-1/CXCR4 interactions are implicated in the migration of HSC to the injured kidney.

Methods. HSC were isolated from mouse bone marrow and labelled with a cell tracker. Acceptor mice were subjected to unilateral ischemia and received HSC intravenously directly after reperfusion. In addition, in separate groups of acceptor

Received for publication: 10.9.08; Accepted in revised form: 23.12.08

doi: 10.1093/ndt/gfp050
Advance Access publication 16 February 2009