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N-acetylcysteine and deferoxamine protect against acute renal failure induced by ischemia/reperfusion in rats

N-acetilcisteína e deferoxamina protegem contra insuficiência renal aguda induzida por isquemia/reperfusão em ratos

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ABSTRACT

Objective: Antioxidants are widely used in animal models to prevent renal injury after ischemia/reperfusion, but it is unknown if the benefits of antioxidants are additive. In this study, we aimed to investigate the protective effects of N-acetylcysteine plus deferoxamine in an animal model of kidney ischemia/reperfusion injury.

Methods: Bilateral kidney ischemia was mastintained for 45 minutes. N-acetylcysteine, deferoxamine or both were administered into the aorta above the renal arteries immediately prior to induction of ischemia. Five rats from each group were sacrificed 1, 6 or 12 hours after reperfusion for the determination of blood creatinine, kidney oxidative damage parameters and myeloperoxidase activity.

Results: The combination of N-acetylcysteine and deferoxamine, but not their isolated use, prevented the increase in creatinine after ischemia/reperfusion. This prevention was followed by a consistent decrease in myeloperoxidase activity and oxidative damage parameters both in the kidney cortex and medulla.

Conclusion: Treatment with N-acetylcysteine and deferoxamine was superior to the isolated use of either compound in an animal model of kidney ischemia/reperfusion.

Keywords: Deferoxamine; Reperfusion injury; Renal insufficiency; Acetylcysteine; Reactive oxygen species; Rats

INTRODUCTION

Renal ischemia is observed in a variety of clinical situations, such as cardiac arrest with recovery, liver and kidney transplantation and partial nephrectomy. The acute renal failure (ARF) observed after ischemia is characterized by a decreased glomerular filtration rate, tubular necrosis and increased renal vascular resistance.[1,2]

The ischemia/reperfusion process (I/R) involves multiple pathophysiologic mechanisms, such as a disturbance in calcium homeostasis,[3] reactive oxygen species (ROS) production,[4] mitochondrial dysfunction,[5] and neutrophilic infiltration.[6] ROS have been implicated as a major pathophysiologic component of acute renal failure during I/R in the kidney,[4,7] and a component that may contribute to ROS generation is iron. Unbound iron can catalyze the conversion of H_2O_2 to $OH^{\bullet}$ or form reactive ferryl or perferryl species.[8] In addition, after I/R, activation of the endothelium and the recruitment of neutrophil cells were observed.[9] The migration of neutrophils into the injured kidney following reperfusion leads to increased renal myeloperoxidase (MPO) activity, suggesting that chlorinated species could play a role in kidney damage.[10]

In an effort to minimize these events, studies have used antioxidants, such as N-acetylcysteine[11] and deferoxamine.[12] N-acetylcysteine (NAC) is an antioxidant that acts by increasing intracellular levels of glutathione, enhancing glutathione-S-transferase activity, and scavenging ROS.[13-15] In addition, preloading animals with iron[16] could accelerate oxidative damage; the use of deferoxamine (DFX) prevents this effect,[17-21] suggesting that DFX could have clinical applications in preventing oxidative damage in ARF. Because NAC and DFX may confer protection by different mechanisms, their beneficial effects may be additive or synergistic.[22,23]

In this study, we hypothesized that NAC and DFX could have synergistic effects when administered in an animal model of kidney I/R injury.

METHODS

All experiments were performed in accordance with the National Institutes of Health (NIH) guidelines and with the approval of the Ethics Committee from the Universidade do Extremo Sul Catarinense.

General procedures

Male Wistar rats, 2-3 months old and weighing 300-350 g, were divided into five treatment groups containing 15 animals each: (1) sham operated animals, (2) I/R plus saline, (3) I/R plus NAC (20 mg/kg), (4) I/R plus DFX (20 mg/kg) and (5) I/R plus NAC and DFX (same doses as in groups 3 and 4). The drugs were administrated as a single dose immediately before the induction of ischemia. NAC and DFX doses were based on previous studies from our group.[24] For the I/R procedure, the rats were anaesthetized with ketamine (75 mg/kg). A midline incision was made, and the aorta and both renal arteries were identified. Drugs were administered into the aorta above the renal arteries, and then both pedicles were clamped with non-traumatic microvascular clamps. Ischemia was maintained for 45 min. After this time, fluid losses were replaced by the administration of 5 mL of warm isotonic saline solution, and the clamps were removed. Five rats from each group were sacrificed 1, 6 and 12 hours after reperfusion, and the blood and kidneys were removed and stored at -80°C.

Plasma creatinine

Creatinine was determined using an enzymatic assay. In brief, serum was exposed to 2% naphthol and 0.05% diacetyl in a final volume of 1 mL and measured spectrophotometrically at 540 nm after 20 min. The results were expressed as milligrams per deciliter.

Myeloperoxidase activity

Myeloperoxidase (MPO) activity, an index of leukocyte infiltration, was measured 1, 6 and 12 hours after reperfusion, as previously described.[25] Briefly, the kidneys were homogenized in 0.5% hexadecyltrimethylammonium bromide and centrifuged at 15,000 *g* for 40 minutes. An aliquot of supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM H_2O_2. The activity was measured spectrophotometrically as the change in absorbance at 650 nm and 37ºC.

Thiobarbituric acid reactive substances

The tissue TBARS levels were determined by a method based on the reaction with thiobarbituric acid (TBA) at 90-100ºC.[26] In the test, malondialdehyde (MDA) or MDA-like substances react with TBA to produce a pink pigment with a maximum absorption at 532 nm.

Protein oxidative damage

The oxidative damage to proteins was assessed by the determination of carbonyl group content based on the reaction with dinitrophenylhydrazine (DNPH), as previously described.[27] Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH, and the absorbance was monitored at 370 nm.

Statistical analyses

The difference between groups was evaluated by a one-way analysis of variance (ANOVA). When the value of *F* was significant, post hoc comparisons were performed by an SNK test.

RESULTS

Creatinine levels did not differ among groups 1 h after reperfusion but increased from 6 to 12 h when compared to the sham group (Figure 1). The combination of NAC and DFX, but not their isolated use, prevented this increase (Figure 1).

The rats subjected to renal I/R exhibited a substantial increase in MPO activity in the kidney (Figure 2), both in the cortex and medulla. Treatment with NAC plus DFX produced a higher attenuation in MPO activity both in the cortex (Figure 2A) and medulla (Figure 2B) when compared to their isolated use. Oxidative damage to the kidney was assessed (Figures 3 and 4), and the TBARS levels were generally lower when NAC and DFX were administered in combination when compared to NAC or DFX use alone (Figure 3). This differential effect of NAC and DFX was less pronounced when oxidative damage was assessed using protein carbonyls (Figure 4).

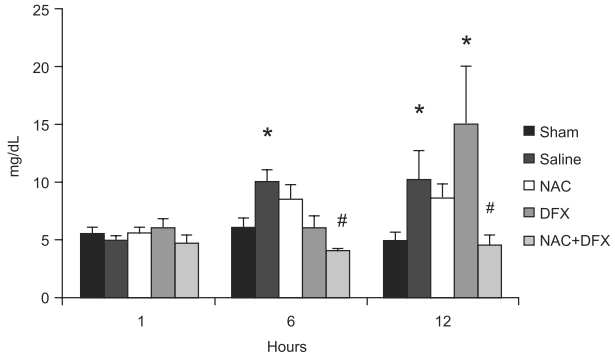

Figure 1 - Blood creatinine levels 1, 6 and 12 h after reperfusion. NAC - N-acetylcysteine; DFX -deferoxamine. The data are expressed as the mean ± SD. n = 5 in each group. *p<0.05 in relation to the sham group. #p<0.05 in relation to the saline group.

DISCUSSION

In this study, we demonstrated that the combination of NAC and DFX was able to decrease kidney oxidative and inflammatory damage after I/R injury in an animal model. A differential response was expected in the renal cortex and medulla, secondary to the fact that the medulla is physiologically hypoxemic; however, we were unable to demonstrate this finding in the present study.

Hydrogen peroxide is present at high levels following kidney reperfusion and is associated with the generation of MPO-derived oxidants and the Fenton reaction. Superoxide

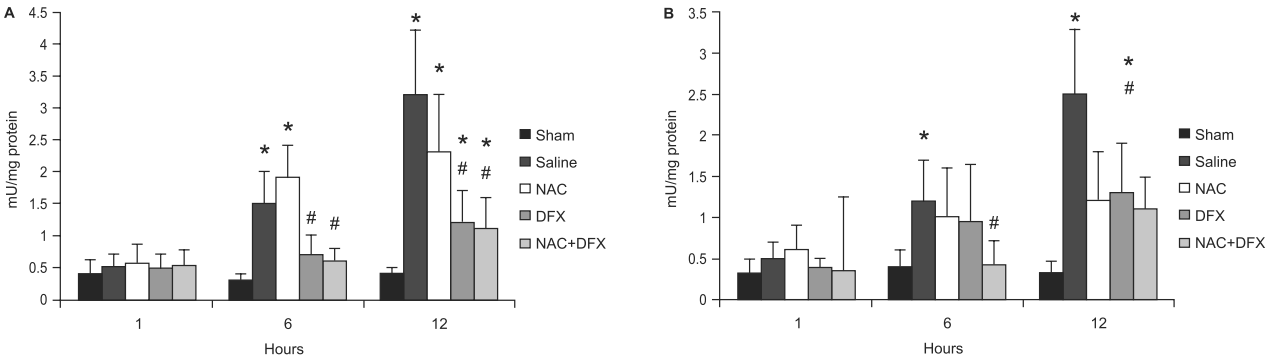

Figure 2 - Myeloperoxidase activity in the kidney cortex **(A)** and medulla **(B)** 1, 6 and 12 h after reperfusion. NAC - N-acetylcysteine; DFX - deferoxamine. The data are expressed as the mean ± SD. n = 5 in each group. *p<0.05 in relation to the sham group. #p<0.05 in relation to the saline group.

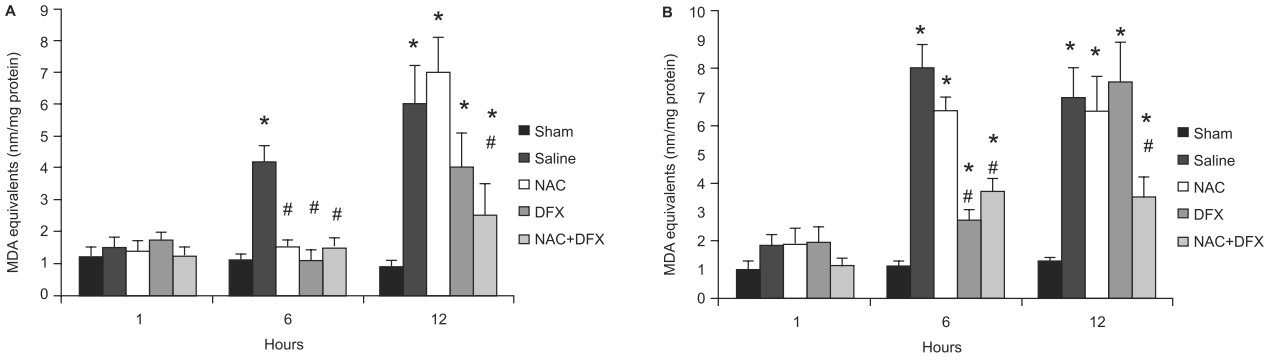

Figure 3 - Thiobarbituric acid reactive species levels in the kidney cortex **(A)** and medulla **(B)** 1, 6 and 12 h after reperfusion. MDA - malondialdehyde; NAC - N-acetylcysteine; DFX - deferoxamine. The data are expressed as the mean ± SD. n = 5 in each group. *p<0.05 in relation to the sham group. #p<0.05 in relation to the saline group.

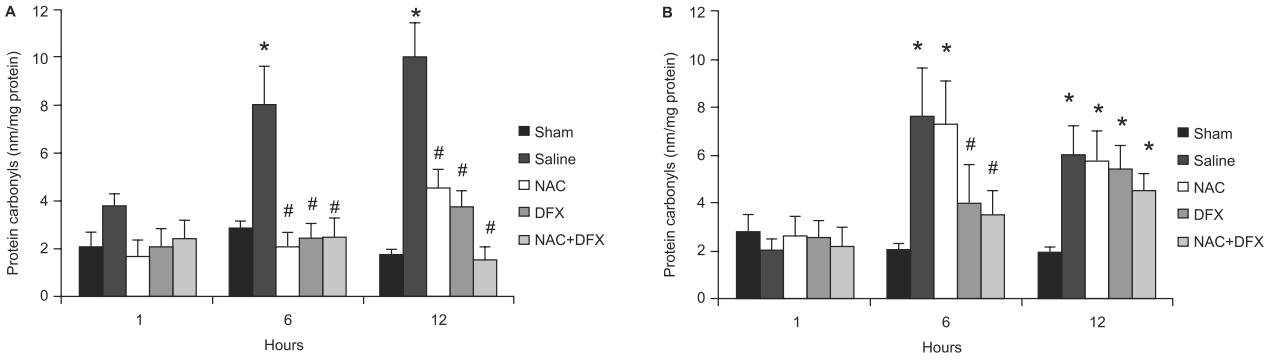

Figure 4 - Protein carbonyls levels in the kidney cortex **(A)** and medulla **(B)** 1, 6 and 12 h after reperfusion. NAC - N-acetylcysteine; DFX - deferoxamine. The data are expressed as the mean ± SD. n = 5 in each group. *p<0.05 in relation to the sham group. #p<0.05 in relation to the saline group.

anion radicals and hydrogen peroxide may be generated via membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidases or produced by the mitochondrial NADPH dehydrogenase complex in phagocytic (neutrophils) or nonphagocytic cells.[28-30] Hydrogen peroxide formation by the elevated activity of xanthine oxidase has not been proven to be relevant in human kidney reperfusion injury. However, in rodent kidneys, I/R induces the conversion of xanthine dehydrogenase, which uses oxidized nicotinamide adenine dinucleotide (NAD) as an electron acceptor, into xanthine oxidase, which, in contrast, uses oxygen as a substrate.[31-33] Because adenosine triphosphate (ATP) is consumed during ischemia, xanthine and hypoxanthine may accumulate; in the presence of oxygen, the superoxide anion radical and hydrogen peroxide could be generated during reperfusion.[34] Xanthine oxidase localized in renal endothelial cells could contribute in part to the microvascular oxidative injury that occurs following reperfusion.[35,36] In this context, antioxidants have been widely used in experimental models that attempt to prevent these alterations, but few studies have considered the role of antioxidant combinations. Shokeir et al. demonstrated that the combination of L-arginine and alpha-tocopherol has a more protective and synergistic antioxidant effect in an animal model of transplantation ischemia/reperfusion injury.[37] Furthermore, the combination of NAC and ebselen prevents kidney damage more extensively than when each drug is used alone,[38] and the same pattern of protection was observed using erdosteine and alpha-tocopherol.[39] In different models of inflammatory diseases, we had previously demonstrated that the combination of NAC and DFX is superior to the isolated use of the antioxidant.[24,40-42] Here we can confirm these previous results in a model of kidney ischemia/reperfusion. These synergistic actions are likely related to the ability to scavenge more than one radical species or to prevent the possible generation of antioxidant-derived free radicals. In fact, this finding is of major relevance to NAC, which can generate thiyl radicals in the presence of iron, thus the iron chelator effect of DFX can prevent NAC-induced oxidative stress.

CONCLUSION

Treatment with the combination of NAC and DFX was superior to the isolated use of these compounds in an animal model of kidney I/R, suggesting that the availability of iron can play a relevant role in the disease process or in the effectiveness of NAC.

RESUMO

Objetivo: Os antioxidantes são largamente utilizados em modelos animal para prevenir lesão renal após isquemia/reperfusão. Uma questão importante é se os benefícios dos antioxidantes são aditivos ou não. O objetivo deste estudo foi investigar os efeitos protetores da N-acetilcisteína com deferoxamina, em modelo animal, de isquemia renal/traumatismo por reperfusão.

Métodos: A isquemia renal bilateral foi mantida por 45 minutos. N-acetilcisteína, deferoxamina ou ambas foram administradas na aorta, acima das artérias renais, antes da isquemia. Cinco ratos de cada grupo foram sacrificados, entre 1, 6 ou 12 horas após reperfusão, para determinar a creatinina no sangue, os parâmetros de danos oxidativos no rim e a atividade da mieloperoxidase.

Resultados: A associação de N-acetilcisteína e deferoxamina, mas não o uso isolado de cada uma, evitou o aumento da creatinina após isquemia/reperfusão. Tal evento foi seguido de diminuição consistente da atividade da mieloperoxidase e dos parâmetros de danos oxidativos, tanto no córtex como na medula renais.

Conclusão: O tratamento com N-acetilcisteína e deferoxamina mostrou-se superior ao uso de cada substância isoladamente em modelo animal de isquemia/reperfusão renal.

Descritores: Desferroxamina; Traumatismo por reperfusão; Insuficiência renal; Acetilcisteína; Espécies de oxigênio reativas; Ratos

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