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Neuroscience Letters 434 (2008) 139–143
Neuroscience Letters
www.elsevier.com/locate/neulet

Inhibition of brain creatine kinase activity after renal ischemia is attenuated by *N*-acetylcysteine and deferoxamine administration

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Received 9 October 2007; received in revised form 28 November 2007; accepted 24 January 2008

Abstract

Encephalopathy may accompany acute or chronic renal failure, and the mechanisms responsible for neurological complications in patients with renal failure are poorly known. Considering that creatine kinase (CK) is important for brain energy homeostasis and is inhibited by free radicals, and that oxidative stress is probably involved in the pathogenesis of uremic encephalopathy, we measured CK activity (hippocampus, striatum, cerebellum, cerebral cortex and prefrontal cortex) in brain if rats submitted to renal ischemia and the effect of administration of antioxidants (*N*-acetylcysteine, NAC and deferoxamine, DFX) on this enzyme. We verified that CK activity was not altered in cerebellum and striatum of rats. CK activity was inhibited in prefrontal cortex and hippocampus of rats 12 h after renal ischemia. The treatment with antioxidants prevented such effect. Cerebral cortex was also affected, but in this area CK activity was inhibited 6 and 12 h after renal ischemia. Moreover, only NAC or NAC plus DFX were able to prevent the inhibition on the enzyme. Although it is difficult to extrapolate our findings to the human condition, the inhibition of brain CK activity after renal failure may be associated to neuronal loss and may be involved in the pathogenesis of uremic encephalopathy.

Keywords: Renal failure; Creatine kinase; *N*-Acetylcysteine; Deferoxamine

Acute renal failure (ARF) is defined by a loss of renal function over a period of hours to days, resulting in accumulation of nitrogenous waste products and unbalance in the maintenance of fluid and electrolyte homeostasis. The major causes of ARF are: (i) decreased renal perfusion without cellular injury; (ii) an ischemic, toxic, or obstructive insult to the renal tubule; (iii) a tubulointerstitial process with inflammation and edema; (iv) or a primary reduction in the filtering capacity of the glomerulus [25,29]. These patients often present signs and symptoms related to fluid and electrolyte disturbances.

Altered mental status reflects the toxic effect of uremia in the brain, and contributes largely to the morbidity and mortality in patients with renal failure [4]. Encephalopathy may accompany acute or chronic renal failure, but in patients with ARF the symptoms are generally more pronounced and progress more rapidly. In general, uremic encephalopathy presents with a symptom complex progressing from mild sensorial clouding to delirium and coma [4,5]. The pathophysiologic mechanisms that underlie renal dysfunction have come to be understood over the last decades [25,29], but the mechanisms responsible for neurological complications in patients with ARF are still poorly known [3].

Creatine kinase (CK, E.C. 2.7.3.2) is a crucial enzyme for high energy consuming tissues like the brain. This enzyme works as a buffering system of cellular ATP levels, playing a central role in energy metabolism [2,22,31]. It is also known that a decrease in CK activity is associated with neurodegenerative pathways that result in neuronal death in brain ischemia [30], neurodegenerative diseases [1,8], bipolar disorder (unpublished results) and other pathological states [10,11,23].

Oxidative stress is an important event that has been related to the pathogenesis of diseases affecting the central nervous system. This is understandable since this tissue is highly sensitive to oxidative stress due to its high oxygen consumption, its high iron and lipid contents, especially polyunsaturated fatty acids, and the low activity of antioxidant defenses [6]. Moreover, the accumulation of toxic metabolites in renal failure may lead to excessive production of free radicals or depletion of antioxidant

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doi:10.1016/j.neulet.2008.01.051

capacity [29,24,3]. It is also known that CK is very sensitive to free radicals, especially by the oxidation of thiol groups of its structure [32]. In this context, we believe that the administration of antioxidants may be important for the treatment of neurological alteration in patients affected by ARF.

Considering that there is a lack of literature information regarding the pathophysiological mechanisms underlying neurological complications in patients with renal failure, that CK is important for brain energy homeostasis and is inhibited by free radicals, and that oxidative stress is probably involved in the pathogenesis of uremic encephalopathy, we measured brain CK activity (hippocampus, striatum, cerebellum, cerebral cortex and prefrontal cortex) in an animal model of ARF (ischemia) and the effect of administration of antioxidants (*N*-acetylcysteine, NAC and deferoxamine, DFX) on this enzyme.

Animals. Adult male Wistar rats (250–300 g) obtained from Central Animal House of Universidade do Extremo Sul Catarinense were caged in groups of five with free access to food and water and maintained on a 12-h light–dark cycle (lights on 7:00 a.m.) at a temperature of 22 ± 1 °C. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of local Ethics Committee.

Animal model of renal ischemia and treatment with antioxidants. Sixty-day-old rats were divided into the following groups: sham-operated group (control), or renal ischemia treated with saline, NAC 20 mg/kg, DFX 20 mg/kg or both antioxidants. Under anesthesia (ketamine 70 mg/kg and xylazine 15 mg/kg; i.p.) and aseptic conditions, via a dorsal incision, ischemia/reperfusion injury was induced by clamping both renal vascular pedicles for 45 min, and the kidney was subsequently reperfused [13]. NAC and DFX were administered in the abdominal aorta 10 min before ischemia [18–20]. Rats were killed 1, 6 or 12 h after renal ischemia. The brain was removed and hippocampus, striatum, cerebellum, cortex and prefrontal cortex were isolated. Moreover, blood was collected for measurement of urea nitrogen and creatinine.

Tissue and homogenate preparation. These brain areas were homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin). The homogenates were centrifuged at 800 × *g* for 10 min and the supernatants kept at −70 °C until used for CK activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. Protein content was determined by the method described by Lowry et al. [17] using bovine serum albumin as standard.

Creatine kinase (*CK*) *activity assay*. Creatine kinase activity was measured in brain homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM $MgSO_4$ and approximately 0.4–1.2 μg protein in a final volume of 100 μL. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 0.3 μmol of ADP plus 0.08 μmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 μmol of *p*-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes [12]. The color was developed by the addition of 100 μL 2% α-naphthol and 100 μL 0.05% diacetyl in a final volume of 1 mL and read spectrophotometrically after 20 min at 540 nm. Results were expressed as units/min × mg protein.

Statistical analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test when F was significant and are expressed as mean ± standard deviation. All analyses were performed using the Statistical Package for the Social Science (SPSS) software.

In the present study we submitted rats to renal ischemia, with previous treatment with NAC and DFX. After that, brain CK was measured. Our findings show that the model used in our work caused renal injury, since blood urea nitrogen and creatinine levels were increased in all groups submitted to renal ischemia, treated with saline or antioxidants (Fig. 1). Moreover, CK activity was inhibited in prefrontal cortex and hippocampus of rats 12 h after renal ischemia and the treatment with NAC and DFX alone or in combination prevented such effect (Fig. 2A and C). Cerebral cortex was also affected, but in this area CK activity was inhibited 6 and 12 h after renal ischemia. In this area, only NAC or NAC plus DFX were able to prevent the inhibition on

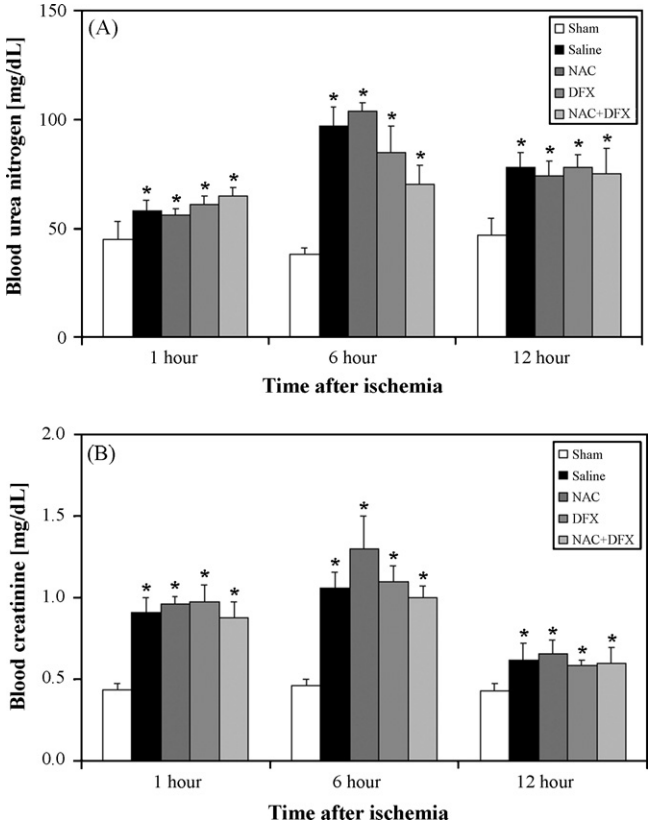

Fig. 1. Effect of antioxidants on blood urea nitrogen (A) and creatinine (B) levels after renal ischemia. Rats were sham-operated (control group) or submitted to renal ischemia and treated with *N*-acetylcysteine (NAC), deferoxamine (DFX), both or only saline. Blood samples were collected 1, 6 and 12 h after renal ischemia. Values are expressed as mean ± S.D. for five-independent experiments. Different from control (sham group); $^{*}p < 0.05$ (one-way ANOVA followed by Tukey).

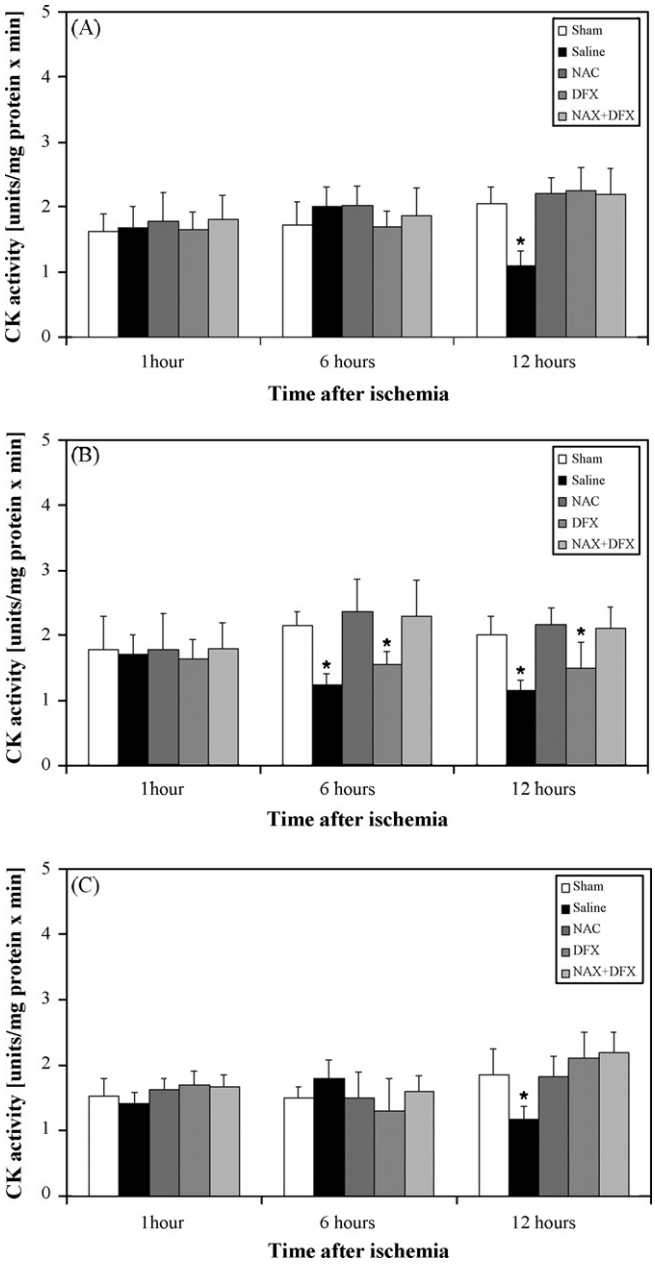

Fig. 2. Effect of antioxidants on creatine kinase activity in prefrontal cortex (A), cerebral cortex (B) and hippocampus (C) of rats after renal ischemia. Rats were sham-operated (control group) or submitted to renal ischemia and treated with *N*-acetylcysteine (NAC), deferoxamine (DFX), both or only saline. Creatine kinase activity was assayed in brain of rats 1, 6 and 12 h after renal ischemia. Values are expressed as mean ± S.D. for five-independent experiments. Different from control (sham group); $*p < 0.05$ (one-way ANOVA followed by Tukey).

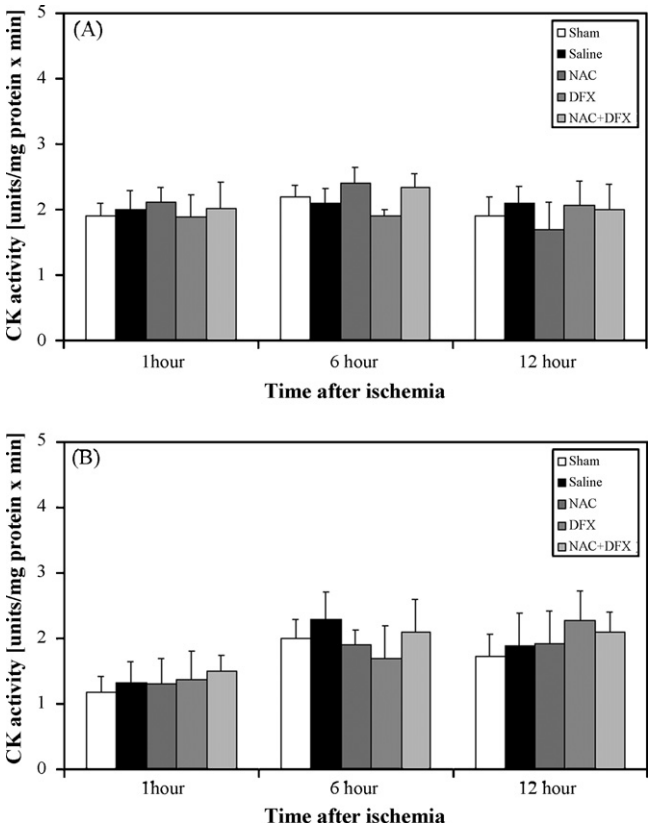

Fig. 3. Effect of antioxidants on creatine kinase activity in cerebellum (A) and striatum (B) of rats after renal ischemia. Rats were sham-operated (control group) or submitted to renal ischemia and treated with *N*-acetylcysteine (NAC), deferoxamine (DFX), both or only saline. Creatine kinase activity was assayed in brain of rats 1, 6 and 12 h after renal ischemia. Values are expressed as mean ± S.D. for five-independent experiments.

the enzyme (Fig. 2B). Our results also show that CK activity was not altered in cerebellum and striatum (Fig. 3).

CK catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to ADP, regenerating ATP. This system is important for normal energy homeostasis by exerting several integrated functions, such as temporary energy buffering, metabolic capacity, energy transfer and metabolic control. The brain of adult rats, like other tissues with high and variable rates of ATP metabolism, presents high phosphocreatine concentration and CK activity. It is well described that inhibition of this enzyme is implicated in the pathogenesis of a number of diseases, especially in brain [15,21]. In the present work, we showed that CK is affected in prefrontal cortex, cerebral cortex and hippocampus of rats, 6 and 12 h after renal ischemia. Moreover, we showed that antioxidants were able to prevent the inhibition of this enzyme.

In this model of ARF, the initial insult is caused by hypoxia to the tissue followed by altered microcirculation. Inflammation and reactive oxygen species formation are also involved in the progression of tubular injury. Moreover, there are several similarities between this model and human ischemic acute tubular necrosis. A number of studies have demonstrated the antioxidant role of NAC. Thus, NAC supplementation was found to reduce oxidative stress by improving thiol redox status, to inhibit oxidative metabolism and to scavenge superoxide, hydrogen peroxide and hydroxyl radicals. It has been demonstrated that isolated administration of NAC could produce pro-oxidant effects. The oxidative metabolism of NAC can generate thiyl free radicals and NAC can reduce Fe^{3+} ions to participate in the generation of hydroxyl radical via Fenton reaction. In this context, we have demonstrated that the combination of NAC and DFX, but not their isolated use, is an effective treatment for several inflammatory diseases [7,18–20].

Uremic encephalopathy is characterized by a mix of clinical features and is often associated with headache, visual abnormalities, tremor, asterixis and seizures. The pathophysiology of uremic encephalopathy is complex and still poorly understood. The main contributing factors are accumulation of metabolites, hormonal disturbance, disturbance of the intermediary metabolism and imbalance in excitatory and inhibitory neurotransmitters. Besides the general symptom complex of encephalopathy, focal motor signs and the "uremic twitchconvulsive" syndrome may be present. Impaired cognitive has also been reported in these patients [4].

In this context, it has also been demonstrated that the creatine/phosphocreatine/CK circuit is involved in processes that involve habituation, spatial learning and seizure susceptibility [14,26,27]. In the present work we showed that CK was inhibited in prefrontal cortex, cerebral cortex and hippocampus, brain areas that are crucial for cognitive processes [9,16,28]. So, we speculate that diminished CK may be involved in the cognitive impairment reported in these patients. Moreover, we showed that inhibition of CK was prevented by antioxidants. In this context, we also speculate that oxidative stress may be involved in the mechanism of CK activity inhibition. This hypothesis is reinforced by findings from Sener et al. [24], which show increased malondialdehyde and diminished glutathione levels in brain of rats submitted to a model of chronic renal failure.

Although it is difficult to extrapolate our findings to the human condition, the inhibition of brain CK activity after renal failure may be involved in the pathogenesis of uremic encephalopathy. We also showed that antioxidants prevented the inhibition of CK, and we believe that this protocol could be used as an adjuvant therapy for the treatment of these neurological complications. Moreover, other important steps of energy metabolism must also be studied.

Acknowledgements

This research was supported by grants from Programa de Pós-graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense (UNESC) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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