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# N-acetylcysteine reduces the renal oxidative stress and apoptosis induced by hemorrhagic shock

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ABSTRACT

*Background:* Renal ischemia/reperfusion injury induced by hemorrhagic shock (HS) and subsequent fluid resuscitation is a common cause of acute renal failure. The objective of this study was to evaluate the effect of combining N-acetylcysteine (NAC) with fluid resuscitation on renal injury in rats that underwent HS.

*Materials and methods:* Two groups of male Wistar rats were induced to controlled HS at 35 mm Hg mean arterial pressure for 60 min. After this period, the HS and fluid resuscitation (HS/R) group was resuscitated with lactate containing 50% of the blood that was withdrawn. The HS/R + NAC group was resuscitated with Ringer's lactate combined with 150 mg/kg of NAC and blood. The sham group animals were catheterized but were not subjected to shock. All animals were kept under anesthesia and euthanized after 120 min of fluid resuscitation or observation.

*Results:* Animals treated with NAC presented attenuation of histologic lesions, reduced oxidative stress, and apoptosis markers when compared with animals from the HS/R group. The serum creatinine was similar in all the groups.

*Conclusions:* NAC is a promising drug for combining with fluid resuscitation to attenuate the kidney injury associated with HS.



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## Introduction

Hemorrhagic shock (HS) causes local and systemic reductions in tissue perfusion. The kidneys are particularly prone to HS because they are directly dependent on the perfusion pressure and blood flow to maintain the balance between filtration and reabsorption.[1]

Hypoxia caused by renal hypoperfusion associated with HS is the primary cause of renal injury; however, reperfusion-induced secondary injury is established when the blood flow in the ischemic tissues is restored. The lesion progresses to prerenal azotemia, apoptotic cell death, and tubular cell necrosis, which characterize most of cases of acute renal failure (ARF).[2]

Reactive oxygen species (ROS) and the activation of the inflammatory cascade may be responsible for renal tubular cell injury. Ischemia/reperfusion (IR) injury, which is triggered by hemorrhage and fluid resuscitation, promotes an excessive production of ROS, which overwhelms the antioxidant defense system.[3]

N-acetylcysteine (NAC) is a low-cost compound with high availability and a low rate of the adverse effects that are prominent among the antioxidant drugs. Widely used in several areas of medical care, NAC was initially used as a mucolytic agent and then as an antidote to acetaminophen overdose. Although the data are still conflicting, this compound is currently used to prevent renal dysfunction induced by radiocontrast agents.[4]

The metabolism of NAC results in increased intracellular glutathione that produces sulfhydryl groups that directly eliminate ROS, such as superoxide radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), hypohydrochloric acid, and the hydroxyl radical.[5]

Lee *et al.*[6] demonstrated that continuous infusion of NAC initiated in the early stages after HS induction attenuated inflammatory responses in the lung and kidney. Moreover, our group has observed that the administration of NAC in the late stage of controlled HS in animal models reduces lung injury.[7,8] Thus, we hypothesize that the same dose of NAC combined with fluid resuscitation reduces oxidative stress and apoptosis, attenuating renal injury after HS. Clinically, we hypothesized that by reducing the oxidative stress and cellular death, we could prevent the evolution to multiple organ failure and therefore with decreased mortality.

## Materials and methods

### Animals

This study was conducted according to the International Guiding Principles for Biomedical Research Involving Animals, and the research project was approved by the research ethics committee of our institution (protocol no. 0218/12).

Adult male Wistar rats aged between 90 and 120 d and weighing between 300 and 350 g were kept for 6 d in 40 × 30 × 25-cm cages for evaluation and acclimation in controlled temperature and with free access to water and food appropriate for the species. The animals were randomly distributed into three experimental groups with 10 animals per group: sham group (surgical preparation, no shock, and no fluid resuscitation), HS and fluid resuscitation (HS/R) group, and HS/R + NAC group (HS/R and treatment with NAC).

### Experimental procedure

Animals were anesthetized by peritoneal injection of 40 mg/kg of sodium pentobarbital. Next, the right carotid artery was catheterized with a polyethylene catheter (P50) for HS induction, and the right femoral artery and vein were catheterized with polyethylene catheters (P50 and P10) for mean arterial pressure (MAP) monitoring and infusion of fluids for fluid resuscitation, respectively. The femoral artery catheter was connected to a pressure transducer coupled to a calibrated preamplifier (General Purpose Amplifier 4 model 2; Stemtech Inc, WI) and a computerized data acquisition system (Windaq/Dataq Instruments Inc, Akron, OH).

The animals were spontaneously breathing during the entire experiment and were placed on a heated platform to avoid hypothermia. After the surgical procedure, the animals received unfractionated sodium heparin (100 IU/rat) and were observed for 20 min for stabilization.

HS was induced by drawing blood through the arterial catheter for a period of 10 min using a preheparinized 10-mL syringe until reaching a MAP of 35 mm Hg, and maintained for 50 min, with blood removed or replaced in cases of changes in MAP (±5 mm Hg). After this period, animals from the HS/R group received 33 mL/kg of Ringer's lactate containing 50% of the blood withdrawn over a period of 15 min. The animals from the HS/R + NAC group received 33 mL/kg of Ringer's lactate containing 150 mg/kg of NAC and 50% of the blood withdrawn over a period of 15 min. After 120 min from the beginning of the fluid resuscitation, MAP was monitored along experiment and recorded at the end. The animals were killed by exsanguination, and blood samples and both the kidneys were collected and prepared for subsequent histologic and biochemical analyses.

### Plasma creatinine

Plasma creatinine was measured by the colorimetric method using Lloyd's reagent from a Creatinine Kit (Labtest, Brazil). The sample and picric acid was added in an alkaline solution, producing an orange–red color complex whose absorbance was read at 520 nm in an enzyme-linked immunosorbent assay reader (Asys Expert Plus; HI-TECH, Austria).

### Levels of thiobarbituric acid–reactive substances (TBARS) in the kidney

Lipid peroxidation was determined in the renal tissue by the TBARS method, and its value is expressed in nmol/mg protein. The frozen kidney sample (−80°C) was homogenized in 1 mL of 1.15% KCl using a sonicator (Polytron PT100; Lucerne, Switzerland). The aliquots were centrifuged at 10,000 rpm for 20 min at 4°C (Eppendorf Centrifuge 5804; Hamburg, Germany). Next, 100 μL of 8.1% sodium dodecyl sulfate, 750 μL of 20% acetic acid, and 750 μL of 0.8% thiobarbituric acid were