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N-acetylcysteine ameliorates liver injury in a rat model of intestinal ischemia reperfusion

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ABSTRACT

Background: N-acetylcysteine (NAC) is an antioxidant with direct and indirect antioxidant actions used in the clinical setting. Oxidative stress is known to play a pivotal role in the intestinal ischemia reperfusion (IIR). Therefore, we studied the effect of different pretreatment regimens with NAC on the IIR injury in rats.

Materials and methods: Thirty-five male Wistar rats were randomly assigned to five groups. In group sham, only laparotomy was performed. Group control underwent IIR without NAC. In the other groups, NAC was administered intraperitoneally with different regimens: 150 mg/kg before ischemia (NAC 150), 300 mg/kg before ischemia (NAC 300), and 150 mg/kg before ischemia plus 150 mg/kg 5 min before reperfusion (NAC 150 + 150). Measurements in tissues and blood were conducted at 4 h of reperfusion following exsanguination.

Results: Histological score of the liver was significantly improved in NAC 300 compared with control (1.7 ± 0.5 versus 2.9 ± 1.1, respectively, $P = 0.05$). In addition, NAC treatment significantly reduced liver transaminases in all groups of treatment, mostly in group NAC 300. Plasma malondialdehyde levels were lower with NAC treatment, although not statistically significant. Lung glutathione peroxidase was significantly increased in group NAC 300 ($P = 0.04$), while the other oxidation biomarkers showed no significant differences.

Conclusions: NAC exerts a significant protective role in liver injury following IIR, which seems to be independent of an intestinal protective effect. Additional administration of NAC before reperfusion was of no further benefit. The most effective regimen among the compared regimens was that of 300 mg/kg before ischemia.

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Introduction

Apart from the direct damage to the intestine, the intestinal ischemia reperfusion (IIR) can also lead to multiple organ dysfunction, with the lungs being frequently involved.[1] Another severely affected by IIR major organ is the liver.[2,3] The oxidative stress and the release of many inflammatory mediators following ischemia reperfusion injury are proposed as the main causes of remote liver damage.[4] It seems that lipid and protein oxidation characterizes liver injury after IIR, as malondialdehyde (MDA) and protein carbonyls increase in liver after ischemia-reperfusion injury.[5]

On this basis, many antioxidant agents have been tried as prevention with variable success, such as superoxide dismutase,[6] catalase,[7] mannitol,[8] and N-acetylcysteine (NAC).[9] NAC promotes the reduction of disulfide bonds in proteins, thus disrupting their ligand bonding and altering their structures as in the case of mucous proteins. Moreover, NAC exerts its antioxidant action directly by reacting with highly oxidizing radicals such as $^{\bullet}OH$, $^{\bullet}NO_2$, and $CO_3^{\bullet-}$, but more importantly by serving as a precursor of cysteine for glutathione (GSH) synthesis.[10,11] Intracellular GSH increases directly to cysteine elevations in the cell, even if these are mild.[12] GSH is a peptide that contains cysteine and its antioxidant properties are of paramount importance in human, as it is the most abundant antioxidant. The significance of GSH is pronounced even more in the liver, where it acts as the main thiol in the defense under oxidative conditions.[13,14] The importance of this mechanism is particularly evident in the effective use of NAC against paracetamol overdosage-induced liver injury which causes an abrupt depletion of GSH levels in the liver.[15-18]

In IIR, NAC has been found to attenuate oxidative damage alone or in combination with propofol.[19,20] However, neither the optimal dosage nor the optimal administration protocol of NAC is clear. Furthermore, the eventually protective effect of NAC on remote organs after IIR has not been clearly studied. We therefore aimed in this study to examine if NAC has a protective effect in IIR injury and the remote injury to the liver and the lung. In addition, we studied whether this effect depends on the dosage and the timing of administration.

Materials and methods

Animals

Adult male Wistar rats ($n = 35$) weighing 250 to 350 g were acclimatized to our laboratory conditions for 1 wk prior to use. They were housed individually in stainless steel cages at a constant temperature (25°C) and a 12-h day/night cycle. The day before the experiment the animals were fasted overnight and were allowed free access to water. Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) in accordance with the Greek Law 160, A-64, May 1991 were followed.[21]

Experimental design

Rats were randomly allocated into five groups: sham group ($n = 5$), control group with IIR ($n = 8$) and three groups with IIR who were given NAC in different dosages: 150 mg/kg intraperitoneally 5 min before ischemia ($n = 8$, group NAC 150), 300 mg/kg i.p 5 min before ischemia ($n = 7$, group NAC 300), and 150 mg/kg i.p 5 min before ischemia plus 150 mg/kg 5 min before reperfusion ($n = 7$, group NAC 150 + 150). After 4 h of reperfusion, the animals were euthanized by exsanguination from the abdominal aorta.

IIR model

Operative procedures were performed under general anesthesia, on warming pads at a temperature of 37°C. All rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. If the animal reacted after a painful stimulus on the tail, it was given additionally 25% of the initial dose of the anesthetics. Through a 5-cm-long midline laparotomy, the superior mesenteric artery was identified and occluded with a microvascular clamp. The laparotomy incision was covered with sterile moist swabs during the ischemia period. After 45 min, the vascular clamp was removed and the intestinal reperfusion was confirmed by the return of pulsation to the mesenteric arcade. The abdomen was sutured, and the rats were allowed to recover.[21]

In the control groups (sham), the mesenteric artery was exposed but not occluded and animals were followed for 45 min to simulate the ischemic interval. The reperfusion interval was simulated, and the animals were then subjected to the same procedures.

Blood and tissue collection

Blood was collected by the aorta (exsanguination) in heparinized syringes and was centrifuged at 2000 rpm for 20 min at 4°C. The plasma was then separated and stored at −80°C for further biochemical analysis. Intestine, liver, and lung tissue samples were collected and divided into two samples each. One sample was immersed into 10% formaldehyde solution for fixation and further processing for histological assessment. The other sample was stored at −80°C for biochemical analysis.

Bronchoalveolar lavage fluid (BALF)

After exsanguination and median sternotomy, the trachea was isolated and the left main bronchus was clamped. By a 14-G catheter, which was inserted to the right main bronchus, 6 mL of cold (4°C) normal saline was infused into the right lung and then directly aspirated back into the syringe. This procedure was repeated three times for each animal. The BALF that was collected was then filtered through sterile gauze and centrifuged at 500 g for 15 min at 4°C in order to remove mucus and cells. The supernatant was sent to the laboratory to measure the biochemical parameters.[21]

Cell counts in BALF

A Neubauer hemocytometer was used to count BALF total cells. As for the differential counts, they were made by