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ScienceDirect
European Journal of Pharmacology 575 (2007) 142–148
www.elsevier.com/locate/ejphar

Antioxidant *N*-acetylcysteine attenuates the acute liver injury caused by X-ray in mice

Yang Liu [a], Hong Zhang [a,*], Luwei Zhang [b], Qingming Zhou [a], Xiaohu Wang [c], Jing Long [a], Tao Dong [a], Weiping Zhao [a]

[a] *Department of Medical Physics, Institute of Modern physics, Chinese Academy of Sciences, Lanzhou 730000, China*
[b] *Gansu Agricultural University, Lanzhou 730070, China*
[c] *Institute of Medicine Science inGansu Province, Lanzhou 73000, China*

Received 11 December 2006; received in revised form 5 July 2007; accepted 16 July 2007
Available online 21 July 2007

Abstract

The aim of this study was to evaluate the protective effects of different doses and administration modes of *N*-acetylcysteine (NAC) against X-ray -induced liver damage in mice. Kun-Ming mice were divided into four groups, each composed of six animals: two control groups and two NAC-treated groups. An acute study was carried out to determine alterations in lipid peroxidation (determined by measuring malondiadehyde (MDA) level), glutathione (GSH) content and superoxide dismutase (SOD) activity (assayed by colorimetric method), and DNA damage (characterized by DNA-single strand break using with comet assay) as well as cell apoptosis (measured by flow cytometry) at 12 h after irradiation. The results showed that there were dose-related decreases in MDA level, DNA damage and cell apoptosis, and dose-dependent increases in GSH content and SOD activity in all NAC-treated groups compared to control groups, indicating that pre-treatment or post-treatment with NAC significantly attenuates the acute liver damage caused by X-ray. In addition, significant positive correlations were observed between MDA level and DNA damage or cell apoptosis, implying that lipid peroxidation plays a major role in X-ray-induced liver injury. The data suggest that NAC exerts its radioprotective effect by counteracting accumulated reactive oxygen species in the liver through its properties as a direct antioxidant and a GSH precursor, when administered before or after X-ray irradiation.

Keywords: *N*-acetylcysteine; Reactive oxygen species; X-ray; Mouse liver

1. Introduction

Ionizing radiation (X, γ-ray) can produce reactive oxygen species through the decomposition of cellular water (Poli and Parola, 1996; Rugo et al., 2002; Tominaqa et al., 2004; Takenshita et al., 2004), such as superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), nitric oxide (NO), and nitrogen dioxide ($\cdot NO_2$). These reactive oxygen species play a critical role in cell damage by causing DNA strand breaks, lipid peroxidation, and protein modification, and also by initiating a variety of cellular single transduction pathways (Barzilai and Yamamoto, 2004; Min et al., 2003; Calini et al., 2002; Bacsi et al., 2005; Well et al., 2005), and eventually result in physical and chemical damage to tissues if not scavenged.

The response to oxidative damage depends on inherent antioxidation and detoxification systems. Exogenous antioxidants also may effectively counteract the oxidative-stress state. NAC, a power antioxidant, is a glutathione (GSH) precursor and directly eliminates reactive oxygen species (Aruoma et al., 1989). Importantly, clinical NAC supplementation has been shown to suppress human immunodeficiency virus (HIV) replication and to successfully cure respiration distress syndrome, lung, heart diseases and hepatitis (Nakamura et al., 2002; Laurent et al., 1996; Behr et al., 1997; Pan et al., 2003;

* Corresponding author. Tel.: +86 931 4969344; fax: +86 931 8272100.
E-mail address: zhangh@impcas.ac.cn (H. Zhang).

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doi:10.1016/j.ejphar.2007.07.026

Sminia et al., 1996; Sridharan and Shyamaladevi, 2002). However, there are different views regarding the therapeutic properties of NAC. For example, Sprong et al. (1998) found that high-dose NAC (900 mg/kg, in 24 h) increased lipopolysaccharide-induced lung injury. Leehey et al. (2005) proved that pre-treatment with NAC had no effect on sodium ferric gluconate caused-oxidative stress. Okiawa et al. (1999) observed that NAC caused oxidative damage, and Xu et al. (2005) as well as Wang et al. (2006) reported that post-treatment with NAC aggravated lipopolysaccharide-induced preterm labor and acute ethanol-induced liver damage in mice, suggesting that inappropriate NAC usage in different doses or routes of administration may cause adverse effects. Hence, it is important to choose optimized doses and routes of administration for NAC application.

With regard to the radioprotective effects of NAC, the majority of studies have been performed in vitro (Sminia et al., 1996; Abt et al., 1997; Grootveld et al., 1999). In order to investigate the application of NAC in vivo, mice were used as model animals because they have common or similar responses, at least in part, to ionizing irradiation to those seen in human (Vitral et al., 2006). The present study was designed to evaluate the radioprotective effects of NAC in different doses and administration modes on the liver damage in mice induced by X-ray under acute conditions. Furthermore, the mechanisms by which NAC protects against irradiation damage were also investigated. NAC (50, 100 or 200 mg/kg) in this study was administered 1 h before or after exposure to X-ray irradiation, respectively, and the activity of antioxidant enzymes, the levels of malondialdenyde (MDA) and DNA-single strand break, and the content of GSH (glutathione) as well as the number of apoptotic cells were measured.

2. Materials and methods

2.1. Chemicals

NAC, Sodium lauroyl sarcosine and Triton X-100 were purchased from Amresco (USA). Low and normal melting point agaroses were obtained from Sigma (USA). MDA, superoxide dismutase (SOD) and GSH reagent kits were supplied by Nanjing Jiancheng Bioengineering (China). Ethidium bromide, propidium iodide and RNase A were provided by Shanhai Sangon Bioengineering (China). All other chemicals were of analytical purity.

2.2. Animals

Female Kun-Ming mice (3 weeks) obtained from Lanzhou Medical College (Lanzhou, China) were used. All animal studies were performed according to the requirements of the Animal Care Committee at the Institute.

2.3. Irradiation procedure

A mouse was restrained in a chamber which was fixed to the SIMENS Prims high-energy electron linear accelerator operated at 200 mv. The source-to-surface distance was 100 cm, and the dose rate was approximately 2 Gy/min. The dose for whole-body radiation was 4 Gy.

2.4. NAC supplement assays

Mice were divided into four groups each containing six individuals. The first group (control I) was injected intraperitoneally (i.p.) with physiologic saline solution (0.85% NaCl) 1 h prior to exposure to X-ray. The second group (control II) was irradiated with X-ray 1 h before injection of physiologic saline. The third group (pre-treatment with NAC group) received i.p. 50, 100 or 200 mg/kg doses of NAC (dissolved in physiologic saline solution) 1 h before X-ray exposure. The last group (post-treatment with NAC group) was irradiated with X-ray, and 1 h later was injected with NAC (50, 100 or 200 mg/kg). The mice were killed by cervical dislocation 12 h after exposure. The livers were excised immediately on an ice-cold plate and washed with physiologic saline solution. A small piece of the fresh liver was used for analysis of DNA damage, and residual samples were stored and frozen at −80 °C until biochemical determinations. The livers of three animals were pooled in all experiments.

2.5. Comet assay

2.5.1. Preparation of single cell suspension and microscope slides

The isolated liver was repeatedly rinsed in ice-cold phosphate-buffered saline (PBS), and then minced into fine pieces to obtain a cell suspension. Samples were diluted so that three or four cells could be seen in a single field at 400× magnification. A three-layer procedure with a slight modification was performed (Klaude et al., 1996). Slides were cleaned with 100% ethanol and flame dried. Two solutions containing 0.8% normal melting agarose (NMA) and 0.6% low melting agarose (LMA) were prepared in-Ca^{2+}–Mg^{2+}-free phosphate-buffered saline. The first layer was laid by dipping the slide in molten 0.8% NMA and allowing the gel to dry at room temperature. Then the diluted cell suspension was mixed with 0.6% LMA and kept in a 37 °C water bath. Freshly prepared LMA suspension was pipetted onto the first layer and immediately covered with a 24×24 mm coverslip. After the coverslip was gently removed, the third layer of 0.6% LMA was laid on the slide and again covered with coverslip, and the agarose was allowed to solidify for 20 min on chilled metal tray. Finally, the coverslip was removed from slide.

2.5.2. The alkaline comet assay

The standard procedure originally described by Singh et al. (1998) with modifications (Hartmann et al., 2004) was used. Briefly, the slides with solidified agarose were carefully submersed in ice-cold freshly prepared lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 1% sodium lauroyl sarcosine, 10% dimethyl sulfoxide, 1% Triton X-100 added just before use, 4 °C) for least 1 h. Then the slides were placed in electrophoresis solution (300 mM NaOH, 1 mM