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Protective effect of ALDH2 against cyclophosphamide-induced acute hepatotoxicity via attenuating oxidative stress and reactive aldehydes

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ABSTRACT

Cyclophosphamide (CY) is a widely used chemotherapeutic agent that is associated with severe side effects, such as hepatotoxicity and nephrotoxicity. However, the extent, mechanisms and potential prevention and treatment strategies of CY-induced acute hepatotoxicity and nephrotoxicity are largely unknown. In this study, we determined the existence and extent of CY-induced acute hepatotoxicity and nephrotoxicity, and demonstrated the effect of ALDH2 on CY-induced acute tissue toxicity and related mechanisms. Adult male C57BL/6J (wide-type, WT) and ALDH2$^{-/-}$ (KO) mice were divided into four groups: WT, WT + CY, KO + CY and WT + CY + Alda-1. Biochemical analysis showed that plasma ALT was increased by 35.8% in KO + CY group and decreased by 21.1% in WT + CY + Alda-1 group compared to WT + CY group ($P < 0.05$, respectively). However, there was no significant difference among WT, WT + CY and KO + CY groups regarding plasma renal marker enzymes, including blood urea nitrogen (BUN), creatinine and cystatin C (CysC). Levels of reactive oxygen species (ROS) and toxic aldehydes (acrolein, 4-hydroxynonenol and malondialdehyde) were increased significantly in KO + CY group and decreased significantly in WT + CY + Alda-1 group compared to WT + CY group ($P < 0.05$, respectively). These findings demonstrate that CY could induce acute hepatotoxicity without nephrotoxicity, and ALDH2 plays a protective role in CY-induced acute hepatotoxicity. The underlying mechanisms are associated with attenuating oxidative stress and detoxifying reactive aldehydes.

1. Introduction

Cyclophosphamide (CY) is a widely used chemotherapeutic agent in the treatment of both neoplastic diseases (e.g. solid tumors and lymhpomas) and nonneoplastic diseases (e.g. rheumatoid arthritis and systemic lupus erythematosus) [1]. Several antineoplastic protocols require high doses of CY that are associated with severe side effects, such as urotoxicity, cardiotoxicity, immunotoxicity, hepatotoxicity and nephrotoxicity [2–5]. Among them, hepatotoxicity and nephrotoxicity are theoretically considered as two major adverse effects, because liver and kidney are key organs responsible for metabolism and excretion of CY and its reactive metabolites. Although CY-induced chronic hepatotoxicity and nephrotoxicity have been observed, the extent, mechanisms and potential prevention and treatment strategies of CY-induced acute hepatotoxicity and nephrotoxicity are largely unknown.

Previous studies have demonstrated that chemotherapeutic drugs including CY could generate substantial reactive oxygen species (ROS) and contribute to succeeding elevated levels of highly cytotoxic oxidative stress-derived lipid peroxidation aldehydes,

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such as 4-hydroxynonenol (4-HNE), malondialdehyde (MDA) and acrolein [6–8]. Additionally, acrolein, one of CY metabolites, initiates oxidative stress directly as well as be generated through lipid peroxidation, and thus contributes to a vicious circle [9]. However, whether the oxidative stress and toxic aldehydes play key roles in CY-induced acute tissue toxicity and the specific reagents that could antagonize them need to be elucidated.

Aldehyde dehydrogenase 2 (ALDH2) has recently been identified as an important protective enzyme, which could metabolize a variety of oxidative stress-derived lipid peroxidation aldehydes in physiological process of diseases, as well as oxidize acetaldehyde to acetic acid after ethanol consumption [10]. Previous evidence showed that ALDH2 was beneficial in reducing tissue injury induced by oxidative stress and aldehydes [11–14]. However, whether ALDH2 plays a protective role in CY-induced acute tissue toxicity remains elusive.

Here, our study was designed to (1) determine the existence and extent of CY-induced acute hepatotoxicity and nephrotoxicity, and (2) demonstrate the effect of ALDH2 on CY-induced acute tissue toxicity and related mechanisms.

2. Materials and methods

2.1. Animals and experimental procedures

Male C57BL/6J (wild-type, WT) mice were purchased from the Department of Experimental Animals of Shandong University (Jinan, China). ALDH2$^{-/-}$ mice were provided by the University of Occupational and Environmental Health (Fukuoka, Japan).

All mice were housed at constant temperature with a 12 h light/dark cycle and allowed free access to standard diet plus water. The mice were assigned to 4 groups:

(i) WT group, wild-type mice were treated with a single dose of saline via intraperitoneal injection (i.p.); (ii) WT + CY group, wild-type mice were treated with a single dose of CY (150 mg/kg, i.p.); (iii) KO + CY group, ALDH2$^{-/-}$ mice were treated with a single dose of CY (150 mg/kg, i.p.); (iv) WT + CY + Alda-1 group, wild-type mice were pretreated with Alda-1 (20 mg/kg, i.p.) for 3 consecutive days before CY administration. Animals were sacrificed 24 h after CY administration and blood, livers and kidneys were collected. All animal procedures were in accordance with the National Institutes of Health Guidelines and were approved by the Animal Use and Care Committee of Shandong University.

2.2. Blood analysis

Blood was collected from the apex of the mice, placed at room temperature, allowed to be coagulated and then centrifuged at 2500 g for 10 min. The resulting plasma was stored at −80 °C until analysis. Biochemical parameters, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (Cr) and cystatin C (CysC) were determined by separate enzyme linked immunosorbent assay (ELISA) kits (Jingkang, Shanghai, China).

2.3. Histological analysis

After sacrifice, livers and kidneys were immediately excised and fixed in 4% paraformaldehyde at room temperature for 24 h. The hepatic and renal tissues were embedded in paraffin, cut into 3 μm sections, stained with hematoxylin and eosin (H&E), and examined using an optical microscope.

2.4. Immunohistochemistry

Acrolein and 4-HNE levels were determined using immunohistochemistry methods [15]. Briefly, after routinely dewaxing and hydration, sections were stained with primary antibody directed against acrolein (Abcam, UK) or 4-HNE (Abcam, UK). And then sections were incubated in biotinylated goat anti-rabbit antibody, followed by incubation with DAB as chromogenic substrate. The stained sections were counterstained with hematoxylin. Brown immunoreactivity color indicated positive results under light microscope then quantified using Image-Pro Plus 6.0.

2.5. Measurement of MDA

Frozen hepatic tissue (20–30 mg) was melt and made into 10% homogenate with double-distilled water according to MDA assay kit instructions (Jiancheng, Nanjing, China). MDA levels were specified by measuring thiobarbituric acid reactive substances (TBARS) [16]. The absorbance of red TBARS was measured using a spectrophotometer at 532 nm.

2.6. Measurement of ROS

ROS was evaluated using dihydroethidium (DHE) (Beyotime, Nanjing, China). Tissues were embedded in Tissue-Tek OCT (Sakura, USA) and sectioned. The sections were incubated with DHE at 37 °C and protected from light for 30 min. Then DAPI (4′,6-diamidino-2-phenylindole), a cell-permeable fluorescent nuclear staining was applied to the tissue section and incubated for 10 min. The sections were washed and observed under fluorescence microscope then quantified using ImageJ 1.8.0.

2.7. Quantitative real-time PCR

Total RNA was extracted from frozen hepatic tissue (30 mg) using Trizol reagent (Thermo Fisher Scientific, USA). First-strand cDNA was synthesized from total RNA using the high capacity reverse transcription kit (Thermo Fisher Scientific, USA). Quantitative RT-PCR assay was performed using SYBR green (Thermo Fisher Scientific, USA). Primers were as follows: tumor necrosis factor α (TNF-α), forward 5′- TCCAGGCGGTGCCTATGT-3′ and reverse 5′-CGATCACCCCGAAGTTCAGTA-3′; monocyte chemoattractant protein-1 (MCP-1), forward 5′-GGTGTCCCAAAGAAGCTGTA-3′ and reverse 5′-TGTATGTCTGGACCCATTCC-3′; 18S rRNA, forward 5′-GCAATTATTCCCCATGAACG-3′ and reverse 5′-GGCCTCACTAAACCATCCAA-3′.

2.8. Statistical analysis

Data were expressed as mean ± SEM. A one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used for statistical significance of multiple treatments as appropriate. Two sided $P < 0.05$ was considered statistically significant. Statistical analysis was performed with GraphPad Prism 7 (GraphPad Software, San Diego).

3. Results

3.1. ALDH2 deficiency exacerbated CY-induced acute hepatotoxicity

The effects of CY on hepatic injury in terms of plasma ALT, AST and LDH and micromorphology of the liver were examined. The levels of plasma ALT were increased by 37.7% in WT + CY group compared with WT group ($P < 0.05$, Fig. 1A). AST and LDH levels were also slightly increased in WT + CY group compared to WT