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Acetyl-L-carnitine partially prevents benzene-induced hematotoxicity and oxidative stress in C3H/He mice

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ABSTRACT

Benzene is an environmental pollutant and occupational toxicant which induces hematotoxicity. Our previous metabonomics study suggested that acetyl-L-carnitine (ALCAR) decreased in the mouse plasma and bone marrow (BM) cells due to benzene exposure. In the present study, the topic on whether ALCAR influences hematotoxicity caused by benzene exposure was explored. Thirty-two male C3H/He mice were divided into four groups: control group (C: vehicle, oil), benzene group (150 mg/kg body weight (b.w.) benzene), benzene + A1 group (150 mg/kg b.w. benzene + 100 mg/kg b.w. ALCAR), and benzene + A2 group (150 mg/kg b.w. benzene + 200 mg/kg b.w. ALCAR). Benzene was injected subcutaneously, and ALCAR was orally administrated via gavage once daily for 4 weeks consecutively. After the experimental period, the blood routine, BM cell number and frequency of hematopoietic stem/progenitor cell (HS/PC) were assessed. The mitochondrial membrane potential and ATP level were determined to evaluate the mitochondrial function. Reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels were also examined, and the comet assay was performed to measure oxidative stress. Results showed that ALCAR intervention can partially reduce the benzene-induced damage on BM and HS/PCs and can simultaneously alleviate the DNA damage by reducing benzene-induced H_2O_2, ROS, and MDA.

1. Introduction

Benzene is a widely used industrial chemical and a ubiquitous airborne environmental pollutant (Carbonari et al., 2016; Gao et al., 2014; Mitri et al., 2015). It is classified as a carcinogen (Baan et al., 2009). Benzene exposure induces hematotoxicity, including aplastic anemia, myelodysplastic syndrome, leukemia, and lymphoma (Irons et al., 2013; Lagorio et al., 2013; Snyder, 2012). Although occupational benzene exposure has declined, and environmental benzene concentration has been controlled (Carbonari et al., 2014; Fustinoni et al., 2005), benzene still presents a significant potential risk to both public health and environment. Thus, exploring a new and effective prevention or intervention measure is necessary.

Our previous metabonomics study (Sun et al., 2014) suggested that acetyl-L-carnitine (ALCAR) decreased in the mouse plasma and bone marrow (BM) cells due to benzene exposure. ALCAR participates in oxidation of fatty acid metabolic pathway by facilitating the entry and exit of essential fatty acids from the mitochondria (Jia et al., 2014; Liu et al., 2014). Studies (Folmes et al., 2012; Yusuf and Scadden, 2012) demonstrated that fatty acid oxidation plays a crucial role in determining whether hematopoietic stem cells (HSCs) will undergo self-renewal or differentiation. Benzene and its metabolites target hematopoietic cells at different stages of differentiation through multiple mechanisms. Therefore, ALCAR may play a role in hematotoxicity caused by benzene exposure.

Reactive oxygen species (ROS)-induced oxidative damage plays a major role in the toxicological effects of environmental pollutants (Smith et al., 2016). Oxygen radicals are produced during benzene metabolism and can induce direct toxic effects (Winn, 2003). The imbalance between ROS and antioxidant defense mechanisms can induce oxidative stress, which then causes structural and functional changes in the mitochondria (Murphy, 2013; Rose et al., 2014). Fenga et al. (2016) found that the signal transduction pathways activated by oxidative stress represent a possible mech-

Abbreviations: ALCAR, acetyl-L-carnitine; BM, bone marrow; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; HSCs, hematopoietic stem cells; HPCs, hematopoietic progenitor cells; LSKs, lin$^-$ c-kit$^+$ sca-1$^+$ cells; WBC, white blood cells; RBC, red blood cells; Pit, platelet; Hgb, hemoglobin.

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anism of carcinogenic action caused by chronic benzene exposure. Carugno et al. (2012) reported that increased blood mitochondrial DNA copy number (MtDNAcn) in individuals exposed to low benzene levels can reflect mitochondrial DNA damage and dysfunction. Studies indicated that ALCAR exerts protective effects against multiple toxicities and diseases through its direct or indirect antioxidant activities (Altun et al., 2014; Dhitavat et al., 2002; Di Cesare Mannelli et al., 2007). Cuevas et al. (2013) found that ALCAR protects both motor and sensory neurons from ketamine-induced neurotoxicity. Li et al. (2016) reported that ALCAR treatment might remedy toxicity and mitochondrial dysfunction caused by aluminum oxide (Al_2O_3).

However, to date, little evidence is available about the effects of ALCAR against benzene-induced hematotoxicity. Therefore, in the present study, we investigate the potential effects of ALCAR on hematotoxicity, mitochondrial dysfunction and oxidative stress induced by benzene.

2. Materials and methods

2.1. Chemicals

Benzene and ALCAR were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animal treatment

Male C3H/He mice aged 4–5 weeks were purchased from Wei Tong Li Hua Laboratory Animal Co., Ltd. (Beijing, China) and maintained as previously described (Sun et al., 2014). Thirty-two mice were randomly divided into four groups ($n = 8$/group): control group (C: vehicle, oil), benzene group (150 mg/kg body weight (b.w.) benzene), benzene + A1 group (150 mg/kg b.w. benzene + 100 mg/kg b.w. ALCAR), and benzene + A2 group (150 mg/kg b.w. benzene + 200 mg/kg b.w. ALCAR). Because benzene is miscible with oil, corn oil or corn oil-benzene mixture was injected subcutaneously once daily for 4 weeks consecutively. Significant hematotoxicity could be caused in C3H/He mice exposed to 150 mg/kg b.w. for four weeks based on our previous study (Sun et al., 2012). At the same time, the ALCAR dissolved in buffered saline solution was orally administered through gavage. The two ALCAR doses were chosen based on Li et al. (2016). The Committee on Animal Use and Care of Southeast University (Approval No: 20130027) approved all the procedures.

2.3. Body weight and blood routine

The body weight of each mouse was recorded once a week during and after the period of benzene exposure. At the end of the 4-week exposure, 180 μl of peripheral blood from each mouse was collected and gently mixed with 20 μl of EDTA–2 K to prevent clotting. A Sysmex XE-2100 fully automatic hematology analyzer (Sysmex, Kobe, Japan) was used to examine the samples.

2.4. Flow cytometric analysis of HS/PCs

The mouse BM cells were collected from the mouse tibias and femurs and filtered through a 70 μm cell strainer (BD Pharmingen, CA, USA) to obtain a single-cell suspension. Afterward, the cells were stained with specific antibodies (APC-conjugated lineage cocktail, PE-conjugated c-Kit, and PE/Cy7-conjugated Sca-1) and incubated in the dark for 45 min at 4 °C. HSCs were defined as lin^- $c\text{-}kit^+$ $sca\text{-}1^+$ (LSKs), whereas hematopoietic progenitor cells (HPCs) were defined as lin^- $c\text{-}kit^+$ $sca\text{-}1^-$ and lin^- $c\text{-}kit^-$ $sca\text{-}1^+$. FACSAria™ II flow cytometer (Becton Dickinson (BD) Bioscience, CA, USA) was used for flow cytometric analysis.

2.5. Mitochondrial ATP measurement

The ATP amount in the BM cells was measured using a luciferase ATP assay kit (Beyotime, Nantong, China). Every 10^6 BM cells were lysed with 200 μl of lysis buffer and centrifuged at 12000 rpm for 5 min at 4 °C. Subsequently, 10 μl of supernatant was mixed with 100 μl of working solution, and the mixture was assayed using a luminometer (Berthold Detection System, Pforzheim, Germany).

2.6. Mitochondrial membrane potential measurement

The mitochondrial membrane potential was detected using cationic dye JC-1 (Beyotime, Nantong, China). Cells were stained with 2 μM JC-1, and data were acquired using BD FACSAria™ II flow cytometer. The results were expressed as the red/green fluorescence ratios.

2.7. Detection of ROS

The mouse BM cells were acquired and washed with PBS, and then the cells were incubated in the dark with 2 ml of dichloro-dihydro-fluorescein diacetate (DCFH-DA) working solution (10 μM) at 37 °C for 30 min. Fluorescence intensity was examined by flow cytometry to determine the intracellular production of ROS.

2.8. Detection of H_2O_2

The mitochondrial H_2O_2 production was determined using the H_2O_2 assay kit (Beyotime, Nantong, China) according to the manufacturer's instructions. Briefly, cells were lysed, and the supernatants were gathered by centrifugation. Subsequently, the supernatants were mixed with test solutions and incubated at room temperature for 20 min. Finally, the absorbance at 560 nm was recorded to calculate the H_2O_2 concentration.

2.9. Detection of malondialdehyde (MDA)

MDA concentration was measured using the lipid peroxidation MDA assay kit (Beyotime, Nantong, China). Cells were lysed and reacted with thiobarbituric-acid (TBA). The optical density (OD) was determined at 532 nm. The MDA level was calculated using a standard curve and expressed as μmol MDA/mg protein. The protein content was measured using Bradford method.

2.10. Comet assay

The comet assay was performed using an OxiSelect™ comet assay kit (Cell Biolabs Inc., CA, USA) according to the manufacturer's protocol. Briefly, the cells were mixed with agarose at 37 °C and treated with lysis buffer and alkaline solution. Afterward, electrophoresis was performed for 15 min at 1 V/cm. Finally, the cells were stained with DNA dye and visualized on a fluorescence microscope (Olympus, Tokyo, Japan) with the FITC filter. Fifty cells were analyzed per slide.

2.11. Statistical analysis

Data were represented as mean ± SD. Statistically significant differences were determined by one-way ANOVA, followed by Dunnett's multiple comparison tests, which were performed using SPSS