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Resveratrol attenuates hepatotoxicity of rats exposed to arsenic trioxide

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ABSTRACT

Arsenic trioxide (As_2O_3) is an environmental pollutant and potent toxicant to humans. However, it also shows substantial anti-cancer activity in individuals with acute promyelocytic leukemia (APL). Unfortunately, As_2O_3-treated leukemia patients suffer hepatotoxicity. Resveratrol has been demonstrated to have efficient antioxidant and antineoplastic activities. The study that how As_2O_3 in combination with resveratrol affects hepatotoxicity and arsenic accumulation in the liver is lacking, and the present study tackles this question. Wistar rats were injected with 3 mg/kg As_2O_3 on alternate days; resveratrol (8 mg/kg) was administered 1 h before As_2O_3. Rats were killed on the 8th day to determine histological liver damage, the antioxidant enzymes in serum, the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG), and arsenic accumulation in the liver. In the resveratrol + As_2O_3 group, activities of superoxide dismutase, catalase in serum and GSH/GSSG were significantly increased, histopathological effects were reduced, and arsenic accumulation markedly decreased in the liver, compared with the As_2O_3-treated group. Thus, resveratrol attenuated As_2O_3-induced hepatotoxicity by decreasing oxidative stress and arsenic accumulation in the liver. These data suggest that use of resveratrol as post-remission therapy of APL and adjunctive therapy in patients with chronic exposure to arsenic may decrease arsenic hepatotoxicity.

1. Introduction

Arsenic and many of its compounds are potent poisons. Food is the main source of intake of arsenic in areas where there are no natural or anthropogenic problems of arsenic contamination (Devesa et al., 2008; Vieira et al., 2011). Inorganic trivalent arsenic (iAs(III)) is toxic, which increases in reactive oxygen species (ROS) by binding to vicinal thiols or biological ligands containing sulfur groups (Majhi et al., 2011; Patra et al., 2012). The toxicity of arsenic is mediated (at least in part) by redox-sensitive enzymes and proteins, which leads to oxidative stress. Cells normally can defend themselves against ROS damage through the use of endogenous oxidants and cellular antioxidant systems.

Arsenic causes toxicity but it can also play a protective role. Arsenic trioxide (As_2O_3) has been shown to have substantial efficacy in treating humans with relapsed or refractory acute promyelocytic leukemia (APL) (Diaz et al., 2005; Zhou et al., 2005). However, the therapeutic use of As_2O_3 has been limited by its dose-dependent toxicity. Paul et al. (2007) reported that As_2O_3-induced dysfunction of normal mitochondria in rat livers by generation of ROS, and respiration inhibition. Thus, synthetic scavengers of ROS and antioxidative agents could provide possible approaches to reduce the toxicity induced from the clinical use of As_2O_3 (Harris and Shi, 2003), and long term exposure to arsenic.

Resveratrol (tran-3,4,5-thihydroxystibene) is a phytoalexin that is present naturally in grapes, wine, and peanuts (Lucas-Abellán et al., 2011). Recently, there have been reported that resveratrol can effectively scavenge intracellular free radicals and other oxidants in various cell types (Almeida et al., 2011; Johnson et al., 2011). Resveratrol has also been shown to function as a cancer chemopreventive agent (Athar et al., 2007). Several studies reported that resveratrol protected acetaminophen-induced and pyrogallol-induced hepatotoxicity and modulated oxidative stress (Sener et al., 2006; Upadhyay et al., 2008). Resveratrol was also shown to protect hepatic antioxidant systems against ischemia reperfusion-induced oxidative stress (Sahar et al., 2008). Fan et al. (2009) reported that resveratrol treatment partially prevented carbon tetrachloride-induced acute liver damage.

Combining resveratrol with As_2O_3 has been postulated to protect As_2O_3-treated patients from the dose-dependent cardiotoxicity by increasing the activities of antioxidant enzymes in the heart and antiapoptotic activity in H9c2 cardiomyocytes (Zhao et al., 2008). The present study was undertaken to explore whether resveratrol can attenuate the As_2O_3-induced hepatotoxicity mediated by the decrease in arsenic accumulation and the increase in the activities of antioxidant enzymes in the liver.

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2. Materials and methods

2.1. Ethical approval of the study protocol and treatment conditions

The present study was carried out in accordance with the Guiding Principles in the Use of Animals in Toxicology adopted by the Chinese Society of Toxicology. The experimental protocol was approved by the Ethics Committee on the Use and Care of Animals, Northeast Agricultural University (Harbin, China). Housing and experimental treatments of animals were in accordance with National Institute of Health Guidelines (Institute of Laboratory Animal Resources, 1996).

2.2. Materials

As_2O_3 was purchased from Harbin Yida (Harbin, China). Resveratrol (purity, >99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Assay kits used to measure the activity of glutathione peroxidase (GPx), catalase and superoxide dismutase (SOD) were purchased from the Jiancheng Bio-engineering Institute (Nanjing, China). Unless otherwise stated, all other chemicals were purchased from Sigma–Aldrich.

2.3. Animals and treatments

Male Wistar rats (age, 6–8 weeks) were obtained from the Experimental Animal Centre of Harbin Medical University (Harbin, China). Rats were allowed to acclimatize to their environment for 1 week at 22 ± 1 °C under a 12-h light–dark cycle. Rats were fed with standard rodent chow and allowed free access to tap water.

Thirty-two rats were randomly divided into four groups: control, As_2O_3-treated, As_2O_3 + resveratrol, and resveratrol-treated. All treatments were given *via* the caudal vein on alternate days for 4 days (i.e., days 1, 3, 5, and 7) with measurements made on the 8th day. In the As_2O_3 group, rats were treated with As_2O_3 (3 mg/kg); in the As_2O_3 + tresveratrol group, rats were given resveratrol (8 mg/kg) 1 h before As_2O_3 administration; and the resveratrol group received 8 mg/kg body weight resveratrol. An equal amount of 0.9% normal saline was administered as a vehicle to control rats.

2.4. Plasma collection

On the 8th day, rats were given ether anesthesia and killed. Blood samples were selected by puncturing the retro-orbital venous sinus. Samples were centrifuged (4,000*g* for 20 min, 20 °C) and serum isolated for serological studies. Liver tissues were quickly removed. The activities of GPx, catalase and SOD in plasma were determined according to manufacturer instructions. These tests were undertaken using a spectrophotometer (T60 UV–VIS; Beijing Purkinje General Instruments, Beijing, China).

2.5. Analysis of reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio

Liver tissues were rapidly excised and homogenized in phosphate-buffered saline pH 7.4 using an Ultrathurax T25 Homogenisator, and centrifuged at 10,000*g* for 10 min at 4 °C. Supernatant glutathione was measured by high-performance liquid chromatography, with coulometric detection, after the addition of N-acetyl cysteine as an internal control. Two types of injection were performed for the assay of GSH and GSSG (Das et al., 2010), and calculated the ratio of GSH/GSSG.

2.6. Morphological studies

Small portions of the freshly removed livers were fixed by immersion in formaldehyde solution, dehydrated in an ethanol series, and embedded in paraffin wax for histological procedures. Paraffin sections (thickness, 5 μm) were stained with hematoxylin and eosin. Pathological examinations were conducted by experienced pathologists. Each slide was assessed for specific histological alterations under a light microscope (BX-FM; Olympus Corp, Tokyo, Japan).

2.7. Measurement of total arsenic in liver samples

Arsenic contents in the liver tissues of all rats were analyzed following the method of Csanaky and Gregus (2003). Briefly, a representative sample of liver (0.2 g) was digested three times with a mixture of deionized water, nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) until almost dry. The residual mass was dissolved in 1% HNO_3. The solution was used for the estimation of arsenic content using hydride generation system within an atomic fluorescence spectrometry system (AFS930, Beijing Jitian Instrument Co., Beijing, China).

2.8. Statistical analysis

Statistical analyses were done using the SPSS19.0 computer program (SPSS, Chicago, IL, USA). One-way analysis of variance was used for the determination of differences in measurements between groups. In all cases, $p < 0.05$ was considered significant.

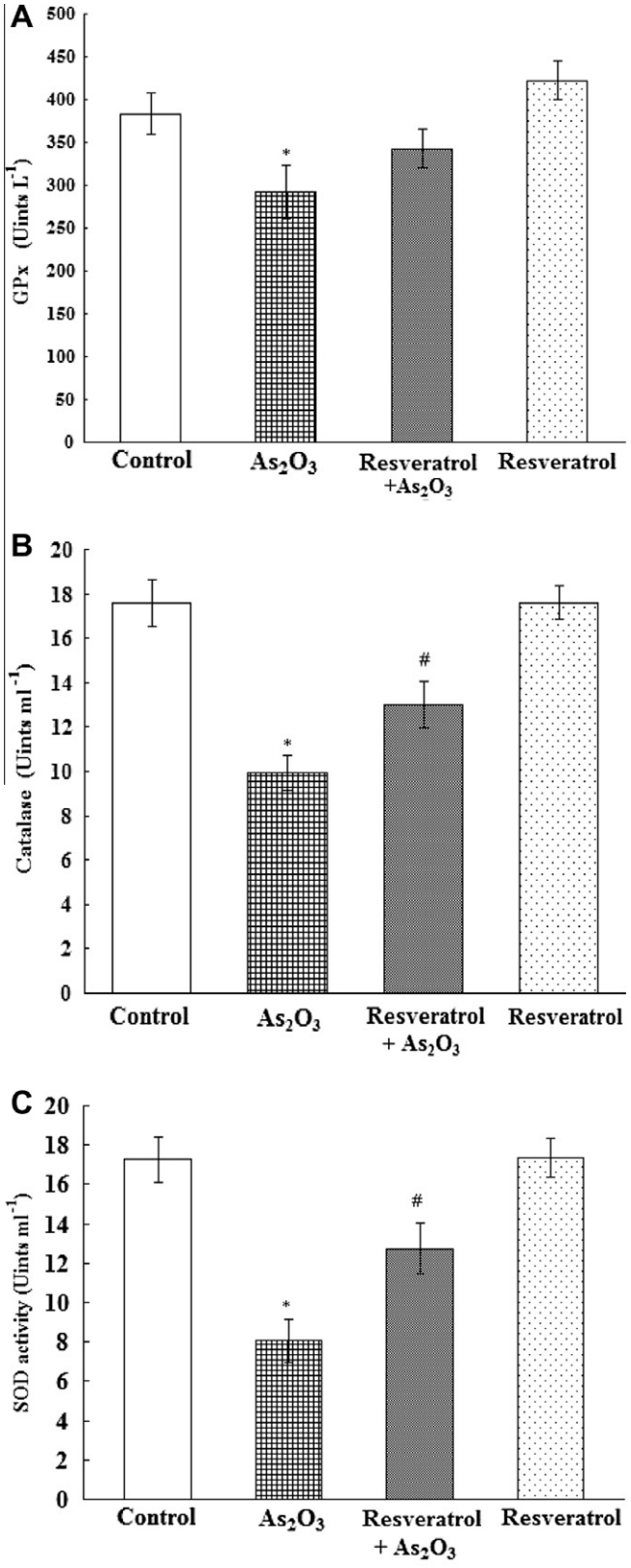

Fig. 1. Effect of As_2O_3 and resveratrol on activities of GPx (A), catalase (B), SOD (C). Values represent mean ± SE, for 8 rats in each group. $^{*}p < 0.05$ vs control group, $^{\#}p < 0.05$ vs As_2O_3-treated group. GPx, glutathione peroxidase; SOD, superoxide dismutase.