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Protective and prophylactic effects of chlorogenic acid on aluminum-induced acute hepatotoxicity and hematotoxicity in mice

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ABSTRACT

The possible health impact of the exposures to Al from environment would be inevitable for humans. Using chelating agents and natural antioxidants against Al-induced biotoxicity become a natural and modern way to prevent the adverse effects of Al in people. This study was undertaken to determine the effectiveness of chlorogenic acid (CGA, 5-*O*-caffeoylquinic acid) in preventing aluminum chloride ($AlCl_3$) induced hepatotoxicity and hematotoxicity in mice. Control, Al-treated (a single injection of 25 mg Al^{3+}/kg, i.p.), Al + CGA (2 h after, a single dose of 100 mg/kg, i.p.), CGA + Al (administered to mice daily for 5 days at 30 mg/kg before Al-treatment) and group of CGA per se (administered to mice daily for 5 days at 30 mg/kg) were used. The levels of Al in liver and blood, the activities of transaminases in serum and osmotic fragility were increased by comparison with the control, while the activities of superoxide dismutase and catalase decreased significantly in the Al-treated group. However, treating mice with CGA at either dosing regimens, post- or pre- Al administration alleviate Al oxidative damaging effects, stabilize cell membrane, prevent hepatocyte apoptosis. CGA supplementation may be favorable to avoid Al-induced hematotoxicity and hepatotoxicity for humans.

1. Introduction

Aluminum (Al) is one of the most abundant elements in the earth crust. The almost ubiquitous presence of Al has so heavily contaminated our environment that exposure is virtually inescapable [1]. In addition to environmental exposure from drinking water, kitchen utensils, inhaled fumes and particles, Al is a component of food additives, vaccines, antacids, parenteral fluids and renal dialysate [2,3]. Al accumulates in all tissues of animals, preferentially in kidneys, liver, heart, bones and brain [4]. This putative neurotoxic metal has been implicated in the etiology of Alzheimer's disease, Parkinsonism and dialysis encephalopathy [5]. Meanwhile, an increasing number of studies have focused on the Al induced liver dysfunction [6] and morphologic aberrations [7], possibly owing to oxidative stress and disturbance of the intracellular redox system [8,9]. The blood is a major determinant of the compartmentalization of the systemic body burden of Al and a vehicle for the transport of Al between compartments [10]. Al overload has been considered to a possible cause of anemia [11]. Al binding to erythrocytes presents much stronger than to serum proteins, half retention time in red blood cells ranging from 20 up to 40 days [12]. As a result, determination Al levels of red blood cell is considered a useful indicator of Al exposure and involve in studies on possible Al hematotoxicity.

Our interest focuses on using chelating agents and natural antioxidants which reduce the oxidative stress or induce the antioxidant environment against Al-induced biotoxicity. Our previous studies suggest the apple (*Ralls*) polyphenol extract (APE) [13] and jujube polyphenol [14] to be effective in reducing some of the biochemical parameters indicative of oxidative stress in the Al-treated rats. The chlorogenic acid (CGA, 5-*O*-caffeoylquinic acid), which is a major phenolic acid of the total polyphenol in APE, is shown to have antioxidant [15], anti-inflammatory [16], hepatic-protection [17], and erythrocyte protection properties [18]. CGA has reduced the toxic effects of arsenic [19], cadmium [20], and tetrachlorobenzoquinone [21] in mice. Furthermore, we have analyzed the stability constant for the CGA-Al (III) complex (log K = 10.51) is a high value considering its 1:1 stoichiometry. In

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addition, our previous result suggests that the dietary CGA could reduce Al absorption in rat [13].

Therefore, the present study was designed to investigate the protective and/or therapeutic effects of CGA against Al-induced hematotoxicity and hepatotoxicity in mice. Here, two dosing regimens of CGA were undertaken and the levels of cytosolic enzymes in serum, the extent of oxidative damage and levels of the key antioxidant enzymes in liver, and hepatic histopathology in mice were evaluated. The mechanism of action of CGA is indistinct, but maintain the red cell membrane integrity and induction of hepatocyte antioxidants in mice might suggested that CGA represent a safe, low-cost, natural way to prevent Al adverse effects in those people who are on acute exposure to Al.

2. Methods and material

2.1. Chemicals

Chlorogenic acid (CGA) supplied as 5-*O*-caffeoylquinic acid (CAS no 327-97-9), aluminum chloride ($AlCl_3$), acrylamide, NN'-methylenebisacrylamide, Brilliant Blue R-250, TEMED (NNN'N'-tetramethylethylenediamine) were bought from the Sigma–AldrichChemical Company (US). Other reagents used were of analytical grade. CGA was freshly dissolved in a 10 mM PBS (pH 7.4 at 37 °C in a water bath) and protected from light until time of use.

2.2. Animals and experimental design

Eight-week-old male Kunming mice, 30 ± 5 g, clean grade, were used. The experiments were performed according to the Animal Management Rules of the Ministry of Health of the People's Republic of China (documentation No. 55 (2001), Ministry of Health of P.R. China), with utilization permission from the Animal Department of the Academy of Military Medical Sciences, SCXK (Jun) 2007-004. All of the mice were placed in stainless steel cages and housed in a temperature-controlled room (25 ± 2 °C) at 60% ± 5% relative humidity, with a 12 h (dark) –12 h (light) cycle and allowed free access to deionized water and normal chow diet. After seven days for adjustment, mice were randomly distributed to 5 groups, 8 mice each, 4 four from one group were raised in one cage. Each of these groups was treated as follows: Group 1 was served as untreated control; each of the mouse was injected (intraperitoneally, i.p.) with saline (0.9% NaCl). Group 2 was served as $AlCl_3$ control; each of the mouse was injected (i.p.) with $AlCl_3$, equal to 25 mg Al/kg [22]. Group 3 was served as chlorogenic acid (CGA) protective group; each of the mouse was injected with $AlCl_3$ (25 mg Al/kg), after 2 h each of the mouse was injected (i.p.) with chlorogenic acid (CGA), equal to 100 mg CGA/kg. Mice in groups 4 and 5 were injected (i.p.) with CGA 30 mg/kg daily for 5 days [23]. One day later after the last dose of CGA, group 4 was injected (i.p.) with a single dose of 25 mg Al/kg, group 5 were injected (i.p.) with saline (0.9% NaCl).

After the last dose injection, mice were fasted for 24 h. All mice were weighed. The Blood samples were collected from jugular vein in anesthetized (pentobarbital sodium 35 mg/kg, i.p.) animals for measurement of Al content, isolation of erythrocytes and serum. Then all the mice were sacrificed by cervical decapitation. The liver tissues were excised immediately, washed in ice-cold isotonic saline, blotted with filter paper, and weighed. Each liver sample was divided into 5 parts for biochemical analysis, measurement of Al content, western blot, DNA fragmentation assay and light microscopy.

2.3. Isolation of erythrocytes from blood sample

The collected blood samples were allowed to stand (30–40 min), and centrifuged (1000 g for 20 min); harvested serum was used to assay the levels of cytosolic enzymes immediately. The sediments containing erythrocytes were suspended in phosphate buffer saline (PBS) (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4) and centrifuged as reported by Sinha et al. [24]. This process was repeated thrice. The cell pellet was washed and resuspended in PBS to give 10% (v/v) cell suspension (hematocrit). Heinz bodies were detected by all groups' erythrocytes suspension with 0.5% methyl violet (in 0.9% NaCl) for 45 min. Cells were then fixed on glass slides and observed under a light microscope at 100 × magnification [25].

2.4. Determination of osmotic fragility

Osmotic fragility was determined by using NaCl solutions at different concentrations [25]. An aliquot of 100 μL of erythrocytes suspension from all studied groups was added to tubes containing saline solutions (0.1–0.9% of NaCl). After 30 min of incubation at 37 °C, the erythrocytes suspensions were centrifuged at 1500 g for 10 min. The absorbance of released hemoglobin into the supernatant was detected at 540 nm according to Hitoshi and Hiroshi [26]. The absorbance of supernatants in each tube expressed in percent of hemolysis was compared with a 0.1% NaCl tube which served as a reference and represents 100% lysis.

2.5. Biochemical assessment

One portion of liver was homogenized in ice-cold saline (1:10 w/v), and the homogenate was centrifuged (10,000 g for 30 min at 4 °C) and the supernatant so formed was used for the estimation of various biochemical indices. The content of malondialdehyde (MDA) and the activities of alanine transaminase (ALT), aspartate transaminase (AST), superoxide dismutase (SOD) and catalase (CAT) were estimated by reagent kits (NanJing JianCheng Bio Inst, Nanjing, China). Protein content was determined by the method of Lowery et al. [27].

2.6. Histological study

Liver samples were sliced and fixed in a 10% formalin solution. The specimens were then embedded in paraffin and sliced into 5 μm thick sections, which were then stained with hematoxylin–eosin. The sections were examined by an experienced observer who was blind to the treatment under light microscope and then photomicrographs were taken.

2.7. DNA isolation for DNA fragmentation assay

One portion of liver was homogenized in digestion buffer (100 mM NaCl, 10 mM Tris–HCl, 25 mM EDTA, 0.5% SDS with proteinase K and RNase A) and incubated for 14–18 h at 55 °C in a shaking water bath. After digestion, samples were extracted three times with 10% saturated phenol/chloroform/isoamylalcohol (24:24:1) and precipitated with ethanol. The precipitate was washed thrice with 70% ethanol, air dried and resuspended in Tris–EDTA buffer [28]. The extracted DNA was separated on 1% of agarose gel. The gel was then stained with ethidium bromide and visualized on an UV-trans illuminator and photographed.

2.8. Western blotting analysis

Liver samples were homogenized in a buffer containing 8 M urea, 0.5% SDS, 2% b-mercaptoethanol, 1 mM phenylmethyl-