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Puerarin protects the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead

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ABSTRACT

Puerarin (PU), a natural flavonoid, has been reported to have many benefits and medicinal properties. In this study, we valuated the protective effect of puerarin against lead-induced oxidative DNA damage and apoptosis in rat liver. A total of forty male Wistar rats (8-week-old) was divided into 4 groups: control group; lead-treated group (500 mg Pb/l as the only drinking fluid); lead + puerarin treated group (500 mg Pb/l as the only drinking fluid plus 400 mg PU/kg bwt intra-gastrically once daily); and puerarin-treated group (400 mg PU/kg bwt intra-gastrically once daily). The experimental period was lasted for 75 successive days. Our data showed that puerarin significantly effectively improved the lead-induced histology changes in rat liver and decreased the serum ALT and AST activities in lead-treated rats. Puerarin markedly restored Cu/Zn-SOD, CAT and GPx activities and the GSH/GSSG ratio in the liver of lead-treated rat. Furthermore, the increase of 8-hydroxydeoxyguanosine induced by lead was effectively suppressed by puerarin. The enhanced caspase-3 activity in the rat liver induced by lead was also inhibited by puerarin. TUNEL assay showed that lead-induced apoptosis in rat liver was significantly inhibited by puerarin, which might be attributed to its antioxidant property. In conclusion, these results suggested that puerarin could protect the rat liver against lead-induced injury by reducing ROS production, renewing the activities of antioxidant enzymes and decreasing DNA oxidative damage.

1. Introduction

Puerarin, a major isoflavone compound isolated from *Pueraria lobata*, has a variety of biological actions in cardiovascular diseases, gynecology disease, osteoporosis, cognitive capability, diabetic nephropathy (Zhang et al., 2006; Yeung et al., 2006; Sun et al., 2007; Li et al., 2009). Many reports have demonstrated that puerarin possesses a lot of activities including anti-oxidative activity (Guerra et al., 2000; Yan et al., 2006; Xiong et al., 2006; Han et al., 2007; Wu et al., 2007; Chung et al., 2008; Chang et al., 2009; Zhao et al., 2010), anti-inflammation (Yang et al., 2010; Kim et al., 2010; Zheng et al., 2008, 2009; Singh et al., 2007a,b) and anti-apoptosis (Xiong et al., 2006; Cheng et al., 2009; Bo et al., 2005; Zheng and Xu, 2007; Mercer et al., 2005). Increasing evidence shows that puerarin can protect liver from injury induced by hepatoxins (Guerra et al., 2000; Chung et al., 2008; Wu et al., 2007; Zheng et al., 2008, 2009; Zhao et al., 2010; Hwang et al., 2007; Yang et al., 2009; Benlhabib et al., 2004; Zhang et al., 2006).

Lead (Pb) is one of the most widely used metals in industries and in many countries exposure to lead continues to be a widespread problem. Because it cannot be rendered harmless by chemical or biological remediation processes, lead is particularly worrisome among the environmental toxins (Xiang et al., 2010). Many investigations have indicated that lead exposure could induce a wide range of biochemical and physiological dysfunctions in humans and laboratory animals (Courtois et al., 2003). Mechanisms of lead-induced liver injury were to increase production of reactive oxygen species (ROS), and to induce oxidative stress, excitotoxicity, DNA damage and apoptosis (Sivaprasad et al., 2004; Jurczuk et al., 2007; Sieg and Billings, 1997; Xu et al., 2006, 2008; Franco et al., 2009; Pulido and Parrish, 2003). Reports from our laboratory and others have demonstrated that lead can alter Bcl-2/Bax ratio in liver, and these effects were shown to be associated with ROS formation resulting in caspase-3 dependent apoptosis (Liu et al., 2010; Pulido and Parrish, 2003; Gargioni et al., 2006; Xu et al., 2008; Franco et al., 2009).

However, the molecular mechanisms of lead-induced liver injury and hepatoprotective effects of puerarin are not yet completely understood. The aim of this study was to investigate the possible hepatoprotective mechanisms of puerarin against lead-induced oxidative DNA damage and apoptosis in rat liver.

Abbreviations: PU, puerarin; PbAc, lead-acetate; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; 8-OHdG, 8-hydroxy-2-deoxyguanosine; TUNEL, deoxyribonucleotidyl transferase (TdT)-mediated dUTP-fluorescein isothiocyanate (FITC) nick-end labeling.

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2. Materials and methods

2.1. Animals and treatment

Adult male Wistar rats (8-week-old weighing approximately 170 g) were purchased from the Branch of National Breeder Center of Rodents (Shanghai). The rats were maintained under constant conditions (23 ± 1 °C and 60% humidity) and had free access to rodent food and tap water under 12 h light/dark schedule (lights on from 08:30 to 20:30 h) (Liu et al., 2010).

After acclimatization to the laboratory conditions, the animals were randomly divided into four groups (ten rats in each).

(1) Control group, the rats received lead-free redistilled water and daily given physiological saline (0.9% NaCl) by oral gavage during the whole course of the experiment; (2) lead-treated group, animals received an aqueous solution of lead acetate ($Pb(CH_3COO)_2$) (Sigma–Aldrich, MO, USA) at a concentration of 500 mg Pb/l as the only drinking fluid (Jurczuk et al., 2007; Liu et al., 2010; Moniuszko-Jakoniuk et al., 2003); (3) lead + puerarin treated group, animals received an aqueous solution of lead acetate ($Pb(CH_3COO)_2$) (Sigma–Aldrich, MO, USA) (500 mg Pb/l in the drinking water) and received a daily oral gavage administration of puerarin (Sigma–Aldrich, MO, USA) at dose of 400 mg/((kg day) body weight) dissolved in distilled water (Zhang et al., 2006); (4) puerarin treated group, animals received a daily oral gavage administration of puerarin (Sigma–Aldrich, MO, USA) at dose of 400 mg/((kg day) body weight) dissolved in distilled water. Puerarin (400 mg/kg day) was administrated orally during the lead exposure.

The experiment lasted for 75 days. After the experiment termination, seven rats in each group were used for the biochemical analysis; the others were used for histological evaluations. Rats were sacrificed and 10 ml of blood was drawn from heart. The serum was collected after centrifugation at 5000 rpm for 10 min and stored at −70 °C freezer for further analysis. The liver was removed quickly and placed in ice-cold 0.9% NaCl solution, perfused with the physiological saline solution to remove blood cells, blotted on filter paper. And then the removed liver was immediately collected, respectively for experiments or stored at −70 °C for later use.

The dose of puerarin was selected on the basis of the previous studies (Zhang et al., 2006) and our preliminary experiment. The dose that rats consumed a solution of Pb (500 mg/l) as only drinking water exposed to lead in drinking water was selected on the basis of previous studies on the effect of lead exposure on hepatic peroxidative damage to simulate drinking water lead exposure (Jurczuk et al., 2007; Moniuszko-Jakoniuk et al., 2003; Liu et al., 2010). It had been reported that lead concentrations in the blood of rats continuously intoxicated with 500 mg Pb/l are within the range of values noted in lead workers (Jurczuk et al., 2007).

The present research reported in this paper was conducted in accordance with the Chinese legislation on the use and care of laboratory animals and were approved by the respective university committees for animal experiments.

2.2. Histological evaluations

The rats were perfused transcardially with 100 ml of normal saline (0.9%). The liver tissues were fixed in a fresh solution of 4% paraformaldehyde (pH 7.4) at 4 °C for 24 h, incubated overnight at 4 °C in 100 mM sodium phosphate buffer (pH 7.4) containing 30% sucrose; and embedded in optimal cutting temperature (OCT) compound (Leica, CA, Germany). Cryosections were collected on 3-aminopropyl-trimethoxysilane-coated slides (Sigma–Aldrich). The liver slices were stained with hematoxylin and eosin, and examined by an expert in liver pathology (S.M.) blinded to the type of treatment received by the animals.

2.3. Assay of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity

The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Reitman and Frankel (1957).

2.4. Assay of ROS level

ROS was measured as described previously, based on the oxidation of 2′7′-dichlorodihydrofluorescein diacetate to 2′7′-dichloro-fluorescein (Shinomol and Muralidhara, 2007). Briefly, the homogenate was diluted 1:20 times with ice-cold Locke's buffer (154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 10 mM D-glucose, and 5 mM HEPES, pH 7.4) to obtain a concentration of 5 mg tissue/ml. The reaction mixture (1 ml) containing Locke's buffer (pH 7.4), 0.2 ml homogenate and 10 ml of DCFH-DA (5 mM) was incubated for 15 min at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. After 30 min of further incubation, the conversion of DCFH-DA to the fluorescent product DCF was measured using a spectrofluorimeter with excitation at 484 nm and emission at 530 nm. Background fluorescence (conversion of DCFH-DA in the absence of homogenate) was corrected by the inclusion of parallel blanks. ROS formation was quantified from a DCF-standard curve and data are expressed as pmol DCF formed/min/mg protein.

2.5. Assay of lipid peroxidation level

Chemicals, including n-butanol, thiobarbutiric acid, 1,1,3,3-tetramethoxypropane and all other reagents, were purchased from Sigma Chemical Company (St. Louis, MO, USA). The level of malondialdehyde (MDA) concentrations (as a marker of lipid peroxidation) in liver tissue homogenates was determined using the method of Uchiyama and Mihara (1978). MDA levels were expressed as nmol/mg protein.

2.6. Assay of liver reduced glutathione (GSH) level and oxidized glutathione (GSSG) level

Liver GSH and GSSG levels were measured using the commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China) (Qu et al., 2009). GSH and GSSG content was expressed as nmol/mg protein.

2.7. Assay of Cu/Zn-SOD activity

Superoxide dismutase (SOD) kits were purchased from Jiancheng Institute of Biotechnology (Nanjing, China) (Qu et al., 2009). Data were expressed as units of SOD activity per milligram of protein.

2.8. Assay of CAT activity

Catalase (CAT) activity was assayed by the method of Aebi (1984). CAT activity was calculated as nM H_2O_2 consumed/min/mg of tissue protein.

2.9. Assay of GPx activity

The glutathione peroxidases (GPx) activity assay was based on the method of Paglia and Valentine (1967). Tert-butylhydroperoxide was used as substrate. GPx activity was computed using the molar extinction coefficient of 6.22 $mM^{-1}\,cm^{-1}$. One unit of GPx was defined as the amount