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Combination of chlorogenic acid and salvianolic acid B protects against polychlorinated biphenyls-induced oxidative stress through Nrf2

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ABSTRACT

Caffeic acid derivatives (CADs) are well-known phytochemicals with multiple physiological and pharmacological activities. This study aimed to investigate the combined protective effects of CADs on PCB126-induced liver damages and oxidative stress in mice. Here, we used chemiluminescence and chose chlorogenic acid (CGA), salvianolic acid B (Sal B) as the best antioxidants. Then, mice were intragastrically administered with 60 mg/kg/d CGA, Sal B, and CGA plus Sal B (1:1) for 3 weeks before exposing to 0.05 mg/kg/d PCB126 for 2 weeks. We found that pretreatment with CGA, Sal B, and CGA plus Sal B effectively attenuated liver injury and cytotoxicity caused by PCB126, but improved the expressions of superoxide dismutase (SOD), glutathione reduced (GSH), heme oxygenase-1 (HO-1) and nuclear factor E2-related factor 2 (Nrf2), CGA plus Sal B especially, was found to have the best effects that indicated a synergetic protective effect. Taken together, as the Nrf2 regulates the cyto-protective response by up-regulating the expression of antioxidant genes, we suggested that CGA plus Sal B had a combined protection on PCB126-induced tissue damages and that the Nrf2 signaling might be involved.

1. Introduction

With the deterioration of human living environment, the environmental pollutant caused by emissions has raised increasing concerns (Hemming et al., 2015). Once they enter into human body through respiratory tract inhalation, skin contact or digestive tract intake (Alvarez et al., 2006), they react with proteins, cells, or tissue fluid which causes diseases. Polychlorinated biphenyls (PCBs) are main environmental pollutant (Lovato et al., 2015), comprised by 209 individual congeners due to the difference of number and position of chlorine atoms. Although the industrial application of the PCBs was discontinued in the late 1970s, their environmental existence raises long-term toxicological concerns, as bringing about damage to the living tissues of organism, in which oxidative stress is involved (Qian et al., 2015). Among the 209 PCBs, 3, 3′, 4′, 4′, 5-pentachlorobiphenyl (PCB126) has similar structure and biological effects to those of 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and thus is considered to be the most toxic PCB congener (Kopec et al., 2008; Rigaud et al., 2013). Therefore, more and more attention has been paid on how to reduce intakes and the damage caused by PCB126 in internal body, and we would like to figure out that whether the intake of dietary plays an important role on harm reduction?

The plant-based diet (Lazzarotto et al., 2015), a vital part of dietary in east and south Asia, has been accepted as a healthy life style all over the world. Phytochemicals (Inanc Horuz and Maskan, 2015), especially flavonoids, phytosterols, saponins, glucosinolates, and polyphenols in fruits and vegetables are thought to be the major bioactive compounds for health benefits (Tuorkey, 2015). Caffeic acid (3, 4-dihydroxycinnamic acid, CaA) and its derivatives (Caffeic acid derviatives, CADs), a naturally occurring phytochemicals, are widely spread in the labiatae, asteraceae, rosaceae, brassicaceae and other plant foods/diet (Murthy et al., 2014). Extensive research shows that, CADs exhibit the notable anti-viral, anti-oxidant, anti-inflammatory and anti-tumor effects (Jin et al., 2015; Li et al., 2015a,b). Especially, in rat models of ischemia/reperfusion injury to the liver, CADs, has been discovered to have a variety of pharmacological activities, including antioxidant and anti-inflammatory.

These phytonutrients alone or its combination with other nutrients (or other phytonutrients) have attracted significant attention on its importantly useful effects and pivotal role in physiolog-

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ical and pathological activities, especially the combined effects (Cojocneanu Petric et al., 2015; Li et al., 2015c). For the reasons that: the chemical substances in the living environment are not single but very complex, there is a mixed expose to those ingredients at low dosage; Also, in daily life and dietary, there are many kinds of chemicals, they are not a single function on the crowd, more and more show a composite role, many of which show a synergistic effect (Cedergreen, 2014; Li et al., 2014). The chemical substances in the living environment are not single but very complex, for this reason, there is a mixed expose to those ingredients at low dosage. Also, in daily life and dietary, there are many kinds of chemicals, they are not a single function on the crowd, more and more of them show a composite role in which many exhibit a synergistic effect. In previous research, we have studied the effects of CADs on 3, 3′, 4, 4′, 5, 5′-hexachlorobiphenyl (PCB169) induced hepatotoxicity in male mice, we found that ferulic acid, rosmarinic acid and CGA had synergistic protective effects on PCB169-induced liver damage. However, the reasons for choosing these CADs compounds and the molecular mechanisms of CADs on PCBs caused damage underlying in remain unclear (Li et al., 2014). Besides, PCB169 was the main pollutant in Huaihe River Basin in Jiangsu Province, China. How about PCB126? The most representative and toxic of PCB co-planar congener (Rigaud et al., 2013). Here, we used ultra-weak chemiluminescence and found that in a total of 12 CADs (CaA, tanshinol, chlorogenic acid, L-epicatechin, ferulic acid, protocatechualdehyde, rosmarinic acid, epicatechin gallate, salvianolic acid A, lithospermic acid, salvianolic acid C and salvianolic acid B), the chlorogenic acid (CGA) and salvianolic acid B (Sal B) had the best antioxidant activities. We further found that the combined treatment of CGA plus Sal B could inhibit the liver injury caused by polychlorinated biphenyls (PCB 126), and that the Nrf2 signaling might play a part in this effect.

2. Materials and methods

2.1. Materials

CGA, Sal B, CaA, L-epicatechin, epicatechin gallate, ferulic acid, rosmarinic acid, protocatechu aldehyde, Sal A, PCB126, 1–10-Phenanthroline and calf thymus DNA were obtained from Sigma (St. Louis, MO, USA). The details of phytochemicals are the followings: CGA (purity ≥ 95.0%), Sal B (purity ≥ 94.0%), CaA (purity ≥ 98.0%), L-epicatechin (purity ≥ 90.0%), epicatechin gallate (purity ≥ 98.0%), ferulic acid (purity ≥ 99.0%), rosmarinic acid (purity ≥ 98.0%), protocatechu aldehyde (purity ≥ 97.0%), Sal A (purity ≥ 98.0%). Other phytochemicals (purity ≥ 98.0%) were obtained from Zelang Bio. Co (Nanjing, China). The other reagents were of the highest analytical grade and were bought from Jiancheng Bio. Co (Nanjing, China).

2.2. Protective effects of CADs on the oxidative DNA damage induced by •OH

A total of 20 μL H_2O_2 was mixed with $CuSO_4$-Phen-VitC-DNA system (1.5×10^{-4} mol/L $CuSO_4$, 3.5×10^{-3} mol/L Phen, 2.8×10^{-3} mol/L VitC, 150 μg/mL DNA) and 60 μL CADs (50, 100 and 200 μg/mL) or vehicle in 100 μL, 0.1 mol/L sodium acetate-acetic acid (pH 5.5) buffer. The ultra-weak chemiluminescence was determined immediately at room temperature.

2.3. Animals and in vivo study

This study was performed according to a protocol approved by the Nanjing Medical University Institutional Animal Care and Use Committee. ICR male mice, weighing approximately 26 ± 2 g were provided by the Animal Center of Nanjing Medical University. They were acclimatized under laboratory condition for 2 weeks prior to the experiments. All mice were maintained under standard conditions of temperature (23 ± 2 °C) and humidity (50 ± 10%) with an alternating 12 h light/dark cycles. All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee. The animals were randomly divided into five groups (A–E). Each group consisted of eight mice. PCB126 was dissolved in sodium carboxymethyl cellulose (1% CMC) with reference to the previous studies. PCB126 was administered orally at a dose of 0.05 mg/kg body weight/day to group B for 2 weeks after CMC was administered orally for first 3 weeks. At the same time, group C, D and E were treated intragastrically with CGA, Sal B, CGA plus Sal B (1:1) respectively at a dose of 60 mg/kg body weight/day for 3 weeks. Then PCB126 was administered orally at a dose of 0.05 mg/kg body weight/day to these three groups for 2 weeks. Group A as the vehicle control group was treated with CMC at the same volume all the time. The experimental design and treatment schedule are as follows (n = 8):

i Group A, mice received only CMC (normal healthy control).
ii Group B, mice treated with CMC followed by PCB126.
iii Group C, mice treated with CGA followed by PCB126.
iv Group D, mice treated with Sal B followed by PCB126.
v Group E, mice treated with CGA plus Sal B (1:1) followed by PCB126.

At the end of the experimental period, mice in the different groups were sacrificed and liver tissues were collected. Blood samples were collected from the inferior vena cava and centrifuged (1500 r/min for 15 min at room temperature) to obtain the serum that stored at −80 °C until analysis. Liver tissue was isolated and stored at −80 °C until analysis, except for the left lobe, which was used for histological studies.

2.4. Hematoxylin and eosin stain (HE) and masson staining

After fixation in 10% formalin for 24 h, samples were embedded in paraffin, sectioned at 4 mm by microtome (Leica, Germany). Samples were then stained with haematoxylin-eosin (HE) and Masson's trichrome staining to investigate liver histological and fibrotic changes.

2.5. Assay of oxidative biochemical parameters in liver tissue

The activities of the antioxidant enzymes Superoxide Dismutase (SOD) and Glutathione peroxidase (GPx) were estimated according to the manufacturer instructions. Briefly, the resulting absorbance of each sample was measured at 550 and 340 nm and total SOD, GPx activity expressed as U/mg protein. The levels of Malon-dialdehyde (MDA) and Glutathione (GSH) in the kidney tissue homogenates of the experimental animals were determined following the methods as follows. MDA content in each homogenate was measured using a thiobarbituric acid (TBA) method, and the results were expressed as nmol MDA/mg protein. The level of GSH was measured using the GSH assay kit, which is based on the non-enzymatic reaction of thiols with a quinolinium chromogen. Crystalline GSH was used as standard, and the GSH content was expressed as mg GSH/g protein. The GSSG level was analyzed using an oxidized glutathione (GSSG) assay kit (Abcam, Cambridge, MA, USA) according to the manufacturer's instruction, and the GSSG content was expressed as mg GSSG/g protein.

2.6. ELISA for HO-1 in blood

The activity of Heme oxygenase-1 (HO-1) in blood were detected with an assay kit according to the manufacturer's instructions (Nanjing Jiancheng Co., China). Briefly, 200 mM solution of HO-1 was