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Association of changes in ER stress-mediated signaling pathway with lead-induced insulin resistance and apoptosis in rats and their prevention by A-type dimeric epigallocatechin-3-gallate

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ABSTRACT

A-type dimeric epigallocatechin-3-gallate (A-type-EGCG-dimer, AEd), a new proanthocyanidins dimer from persimmon fruits, has been shown to have health benefit effects. However, A-type-EGCG-dimer affects gluose metabolism in the liver and the underlying mechanism is not clarified. The present study aims to examine the protective effects of A-type-EGCG-dimer on Pb-induced hepatic insulin resistance, endoplasmic reticulum (ER) stress and apoptosis in rats. Male wistar rats exposed to 0.05% w/v Pb acetate in the drinking water with or without A-type-EGCG-dimer coadministration (200 mg/kg body weight/day, intragastrically) for three months. We found that A-type-EGCG-dimer and pioglitazone supplementation significantly deceased glucose and insulin levels in plasma as compared with the Pb group. A-type-EGCG-dimer markedly prevents Pb-induced oxidative stress, ER stress and apoptosis in livers. A-type-EGCG-dimer and pioglitazone reduced the expression levels of the GRP78, PEPCK, G6Pase, p-PERK, p-IRE1, *p*-JNK, ATF4, CHOP and increased *p*-AKT in livers of the Pb group. Moreover, A-type-EGCG-dimer reduced ROS production and restored the activities of SOD and GPx in livers. A-type-EGCG-dimer decreased Bax, cytosolic cytochrome *c* and cleaved caspase-3 and increased Bcl-2 in livers of Pb-exposed rats. Our results suggest that A-type-EGCG-dimer might be a potential natural candidate for the prevention of hepatic insulin resistance and apoptosis induced by Pb.

1. Introduction

Persimmon (*Diospyros kaki* L.) is a nutritious fruit belonging to Ebenaceae family. Persimmon is native to China and cultivated in warm regions all around the world. Persimmon fruits have long been used by Chinese traditional medicine against hypercholesterolemia, diabetes mellitus, cancer, dermal disorders, diarrhea, cough, bleeding, general oxidative processes and hypertension (Butt et al., 2015). Persimmon used in the food industry to make juice, dried fruit and jam. The highest content of bioactive molecules like proanthocyanidin, carotenoids, tannins, flavonoids, anthocyanidin, catechin, etc. was found in persimmon (Direito et al., 2017). Epigallocatechin-3-gallate-(4β→2β→O→7)-eoigallocatechin-3-gallate (A-type EGCG dimer, AEd) is a new proanthocyanidins dimer in the fruits of Persimmon (*Diospyros kaki* L.). Previous research showed that A-type EGCG dimer was a potent anti-amyloidogenic substance because of its strong inhibition of amyloid fibril formation (Nie et al., 2016). A-type EGCG dimer could also inhibit preadipocytes 3T3-L1 cell differentiation, intracellular lipid accumulation and adipogenesis by down-regulating expression of adipogenic transcription factors (Zhu et al., 2015).

Lead (Pb), used by humans since the prehistoric era, is a toxic environmental pollutant in the air, water, soil and consequently, in food (Duruibe et al., 2007). Although Pb exposure in the US has been falling for decades, low levels persist in the environment and pose a hazard to health (Grandjean, 2010). Epidemiological studies had revealed that exposure to Pb could affect the development of many metabolic diseases such as hyperlipidemia, hyperglycemia and diabetes (Jones et al., 2008). The comparative research in industrial workers found a significant correlation between blood levels of Pb and blood glucose, lipid and a variety of risk factors for diabetes mellitus (Bener et al., 2001).

Abbreviations: AEd, A-type dimeric epigallocatechin-3-gallate; AKT, phosphorylated protein kinase B; CHOP, C/EBP homologous protein; EGCG, epigallocatechin-3-gallate; G6Pase, cytoplasmic glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase; GRP78, glucose-regulated protein 78; IRE1, inositol-requiring enzyme 1; JNK, c-jun N-terminal kinase; PEPCK, phosphoenolpyruvate carboxykinase; PERK, protein kinase RNA (PKR)-like ER kinase; ROS, reactive oxygen species; SOD, superoxide dismutase

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The clinical research revealed that the high levels of Pb in biological samples of diabetic patients and type 1 diabetic mothers played a role in the pathogenesis of diabetes mellitus and impacts on their neonates (Afridi et al., 2008; Kolachi et al., 2011). Our previous study and others also discovered that exposure to Pb could disrupt hepatic glucose metabolism and lead to hyperlipidemia, hyperglycemia and insulin resistance in rats (Liu et al., 2011; Mostafalou et al., 2015; Tyrrell et al., 2017). The endoplasmic reticulum (ER) is the central intracellular organelle that is responsible for the synthesis of lipids and the modification of proteins. A variety of stimuli can disturb protein-folding processes in ER, resulting in ER stress and triggering the unfolded protein response (UPR) (Sano and Reed, 2013; Lee, 2017). Chronic ER stress culminating in proteotoxicity contributes to the development of insulin resistance and apoptosis (Ozcan et al., 2004; Lee, 2017). Our previous research showed that Pb could also induce ER stress in livers (Liu et al., 2013).

In this study, we hypothesized that A-type EGCG dimer might ameliorate Pb-induced disorders of glucose by inhibiting ER stress in livers. We examined protective effects of A-type EGCG dimer and revealed the mechanisms focusing on these effects in inhibiting Pb-induced insulin resistance and apoptosis in rats through inhibiting ER stress in livers.

2. Materials and methods

2.1. Chemical reagents

Lead acetate (PbAc), pioglitazone (Pio) obtained from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies against phosphorylated-PERK (p-PERK), PERK (total), phospho-Akt (Ser473), Akt (total), phosphorylated JNK (*p*-JNK), JNK (total), ATF4, GRP78, CHOP, PEPCK, G6Pase, Bcl-2, Bax, cleaved caspase-3 and cytochrome *c* antibody from Santa Cruz Biotechnology (CA, USA) or Cell Signaling Technology (Beverly, MA, USA). All of the other solvents and reagents were purchased from Aladdin (Aladdin, Shanghai, China).

2.2. Sample preparation

The persimmon (*Diospyros kaki* L.) was collected in late October from an orchard in Xuzhou (Jiangsu province, China). A-type EGCG dimer was prepared as described previously (Dong et al., 2013). The purity of A-type EGCG dimer was determined to be 90.35% by RP-HPLC (Agilent 1100 series). The final product was stored at 4 °C in brown glass.

2.3. Animals and treatment

The entire experimental procedures were conducted in accordance with the Chinese legislation and NIH publication on the use and care of laboratory animals.

Male wistar rats (8-week-old) were randomly assigned to five groups (10 rats/group).

Control group Rats received equimolar acetate in drinking water in the form of Na acetate and daily given 0.9% NaCl by oral gavage.

Pb group Rats received 0.05% w/v PbAc in the drinking water and daily given 0.9% NaCl by oral gavage (Tyrrell et al., 2017).

Pb + AEd group Rats received 0.05% w/v PbAc in the drinking water and daily given A-type EGCG dimer (200 mg/kg) by oral gavages.

Pb + Pio group (positive control) Rats received 0.05% w/v PbAc in the drinking water and daily given pioglitazone (30 mg/kg) by oral gavage (Huang et al., 2012).

AEd group Rats received equimolar acetate in drinking water in the form of Na acetate (NaAc) and daily given A-type EGCG dimer (200 mg/kg) by oral gavage.

The experiment lasted for three months. At the end of treatment, rats were sacrificed and the liver tissue was immediately excised for experiments.

2.4. Intraperitoneal glucose tolerance test (IPGTT)

IPGTT was determined as described previously using a commercial kit (Sun et al., 2017). The blood glucose and insulin levels were characterized by a corresponding rat ELISA kit according to the manufacturer's instructions. Insulin resistance (IR) and the homeostatic index of insulin resistance (HOMA-IR) were calculated according to the formula: FBG × FINS/22.5.

2.5. Deoxyribonucleotidyl transferase (TdT)-mediated dUTP-uorescein isothiocyanate (FITC) nick-end labeling (TUNEL) assay

Apoptosis was assayed by TUNNEL staining using commercial diagnostic kits (BD Biosciences Clontech, Palo Alto, CA, USA) (Liu et al., 2012).

2.6. Measurement of the oxidative stress markers in livers

ROS was determined as described in our previous report, which based on the oxidation of 2′7′-dichlorodihydrofluorescein diacetate to 2′7′-dichloro-fluorescein (Liu et al., 2012). The activities of SOD and GPx in liver were determined using commercially assay kits (Jiancheng Biochemical, Inc., Nanjing, China).

2.7. Western blot analyses

To measure the effect of A-type EGCG dimer on gene expression in rat livers, western blot analysis was performed as previously described by us (Liu et al., 2011, 2012). Nuclear and cytoplasmic extracts for western blotting were obtained by using a nuclear/cytoplasmic isolation kit (Beyotime Institute of Biotechnology, Beijing, China). Total protein content in the supernatant was determined by BCA protein assay (Thermo Scientific Pierce, Rockford, IL, USA).

2.8. Statistical analysis

Results were expressed as mean ± standard error (SE). Statistical significance was analyzed by one-way analysis of variance (ANOVA) with post hoc Tukey's multi-comparison test ($P < 0.05$).

3. Results

3.1. Effect of A-type EGCG dimer on insulin resistance parameters

In order to determine whether A-type EGCG dimer can attenuate glucose homeostasis,we measured IPGTT at the 12th week of the study (Fig. 1). Pb induced the marked elevations of blood glucose (19.1%), plasma insulin (169.1%) and HOMA-IR (220.6%), while A-type EGCG dimer and pioglitazone (one drug with hypoglycemic action) treatment significantly lowered fasting plasma insulin, glucose and HOMA-IR relative to Pb exposure rats. No significant differences in blood glucose, plasma insulin and HOMA-IR were found between the A-type EGCG dimer group and the control group.

3.2. A-type EGCG dimer inhibited Pb-induced apoptosis in livers

To further determine whether A-type EGCG dimer protects against Pb-induced apoptosis in livers, we examined nuclear morphology in situ TUNEL assay for DNA fragmentation. As shown in Fig. 2, Pb exposure markedly increased the number of TUNEL-positive cells in the livers of rats ($P < 0.01$). However, A-type EGCG dimer and pioglitazone significantly inhibited Pb-induced apoptosis ($P < 0.01$). No significant differences in the number of TUNEL-positive cells in the livers of rats were found between the A-type EGCG dimer group and the control