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Short communication

Effect of lyophilized prune extract on hyperhomocysteinemia in mice

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ABSTRACT

Altered homocysteine metabolism defined as hyperhomocysteinemia is implicated as pathogenic factor in several cardiovascular diseases and atherosclerosis. The purpose of this study was to investigate the efficacy of prune extract, a good source of phenolic antioxidants, on lowering plasma homocysteine level in male hyperhomocysteinemic mice from average weight of 28 g. The administration of lyophilized prune extract was carried out by intraperitoneal injection one day preceding and one hour before sacrifice of mice. Prune extract decreased significantly plasma homocysteine level, correlated with an increased activity of S-adenosylhomocysteine (SAH) hydrolase and NAD(P)H: quinone oxydoreductase-1 activities. Our results suggest a beneficial effect of prune extract on hyperhomocysteinemia with reduction of homocysteine level by its conversion on to SAH by S-adenosylhomocysteine hydrolase, which is activated by NAD^+, a by-product of NAD(P)H: quinone oxydo reductase-1.

1. Introduction

Homocysteine (Hcy), a metabolic by-product of methionine, can cause widespread hazards to human body if its level is increased in plasma, defined by hyperhomocysteinemia (Zhang et al., 2005). It can be remethylated into methionine by means of vitamin B12-dependent methionine synthase and 5-methyltetrahydrofolate as a methyl donor. Hcy can also be catabolized into cysteine by the transsulfuration pathway via cystathionine beta synthase (CBS) and cystathioninase, both enzymes being vitamin B6-dependent. A third way to remove Hcy is conversion to S-adenosylhomocysteine (SAH). The last reaction is mediated by SAH-hydrolase (SAHH) and favors the SAH formation in case of increased Hcy concentrations (Obeid and Herrmann, 2009). Hcy is the main factor implicated or linked to several metabolic diseases like atherosclerosis, thrombosis, diabetes, Alzheimer, cerebral, and cardiovascular diseases (Jacobsen, 1998; Lawrence de Koning et al., 2003; Buysschaert et al., 2007; Van Dam and Van Gool, 2009). Some phenolic compounds and plant extracts such as catechin, quercetin, chlorogenic acid, coffee, wine phenolics have been demonstrated to be effective in decreasing plasma Hcy level (Nygård et al., 1997; Noll et al., 2009a, 2011; Kim et al., 2012). Prune, dried fruits of plum (*Prunus domestica*) contain higher level of phenolic compounds. In according to the study of Donovan et al. (1998) the level of phenolic compounds in prunes (1840 mg/kg) surpasses the levels reported for many other popular fruits like as Red Flame seedless table grapes (<250 mg/kg of grape), white table varieties (<50 mg/kg of grape), apples (1200 mg/kg), oranges (830 mg/kg), pears (265 mg/kg), and cherries (850 mg/kg of fruit) (Donovan et al., 1998). Phenolic compounds in prunes have been found to inhibit human low-density lipoprotein oxidation *in vitro*, and thus might serve as preventive agents against chronic diseases, such as heart disease and cancer (Stacewicz-Sapuntzakis et al., 2001). Hyperhomocysteinemia is well defined as risk factor for cardiovascular diseases, therefore, the present study investigated the effects of polyphenolic prune extract, which contains notably chlorogenic acid and its isomer neochlorogenic acid, on plasma Hcy level in hyperhomocysteinemic mice and its implication on SAHH activity, a third way of Hcy metabolism.

2. Materials and methods

2.1. Extraction of prune phenolic compounds

The extraction of phenolic compounds from prune was carried

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out by microwave assisted extraction under optimized conditions (Haddadi-Guemghar et al., 2014). The fruit samples were prepared for extraction by cutting into small pieces by a knife. Two grams of sample were placed in a round-bottom flask with 40 mL of distilled water. The flask was placed in a microwave oven and connected to condenser. After irradiation (1mn at 500 W), the flask was taken out and cooled in ice water bath (4 °C). The solution was filtered through Wathman n°1 paper under vacuum. After concentrating using rotavapor at 40 °C, the extract was lyophilized.

2.2. Total phenols and hydrogen peroxide determination

Total phenols (TP) content was determined by the Folin-Ciocalteu method. 1 mL of Folin-Ciocalteu reagent (diluted 10 times by water) was mixed with 100 μL of the prune extract. After 5min, 1 mL of aqueous solution of sodium carbonate (6%) was added. The mixture was kept for 60 min at room temperature. Absorbance was measured at 755 nm. Ethanol solution of gallic acid was used as standard (Velioglu et al., 1998). Hydrogen peroxide content was determined with the hydrogen peroxide assay kit (Abcam, France) as recommended by the supplier's instructions.

2.3. Mice, genotyping and experimental protocol

Mice were maintained in a controlled environment with unlimited access to food and water on a 12-h light/dark cycle. All procedures were carried out in accordance with internal guidelines of the French Agriculture Ministry for animal handing. Number of mice and suffering were minimized as possible. Mice heterozygous for targeted disruption of the Cbs gene (Cbs +/−) were generously donated by Dr. N. Maeda (Department of Pathology, University of North Carolina, Chapel Hill, NC, USA) (Watanabe et al., 1995). Cbs ± mice, on a C57BL/6 background, were obtained by mating male Cbs ± mice with female wild-type C57BL/6 (Cbs +/+) mice. DNA isolated from 4-week-aged mice tail biopsies was subjected to genotyping of the targeted CBS allele using polymerase chain reaction assay (Watanabe et al., 1995). The study was carried out on male 2-month-old Cbs ± and Cbs +/+. The administration of prune extract was carried out by intraperitoneal injection of 100 μL of prune extract at concentration of 1 mg/mL (1 mg of lyophilized prune extract was resuspended in 1 mL of NaCl 0.9%) to Cbs ± and Cbs +/+ mice. The mice control, both Cbs ± and Cbs +/+, were treated by intraperitoneal injection of 100 μL of NaCl 0.9%. All mice were treated one day preceding and one hour before sacrifice. Two experimental protocols (one with five mice per group and the second with three mice per group) have been done, depending on the variability of the analyzed biomarker.

2.4. Preparation of serum samples, tissue collection, and plasma tHcy assay

At the time of sacrifice, blood samples were collected into tubes containing an 1/10 volume of 3.8% sodium citrate, placed on ice immediately. Plasma was isolated by centrifugation at 2500*g* for 15 min at 4 °C. Liver was harvested, snap-frozen and stored at −80 °C until use. Plasma tHcy was assayed by using the fluorimetric high-performance liquid chromatography method described by Fortin and Genest (1995).

2.5. Determination of SAHH activity

SAHH activity assay was performed on 200 μg of total proteins obtained from liver samples following the protocol described previously (Villanueva and Halsted, 2004).

2.6. Determination of NAD(P)H: quinone oxydo reductase-1 activity

Determination of NAD(P)H: quinone oxydo reductase-1 (NQO1) activity was assayed on 100 μg of total proteins obtained from liver samples following the protocol described by Ernster (1967), and modified by Benson and colleagues (Benson et al., 1980).

2.7. Data analysis

Statistical analysis was done with one-way ANOVA followed by Student's unpaired *t*-test using Statview software. The results are expressed as medians with interquartile ranges. Data were considered significant when $p < 0.05$. A p value of 0.06–0.10 was considered to indicate a strong statistical tendency due to the small sample size. Correlations between tHcy level, SAHH activity and NQO1 activity were determined by using Spearman's rank correlation as data were not normally distributed according to Shapiro – Wilk test. Data were analyzed using R software (http://www.R-project.org) and considered significant when $p < 0.05$.

3. Results and discussion

3.1. Effects of prune extract on plasma tHcy level in wild type and hyperhomocysteinemic mice

Previous study demonstrated that administration of wine polyphenols such as catechin, epicatechin or wine extract modifies significantly Hcy metabolism, this effect depending on quantity of polyphenols administered (Noll et al., 2009a). The concentration of prune exptract injected here to mice, in terms of total polyphenols, was measured by Folin ciochalteu method. It was 3 mg GAE (gallic acid equivalents)/mL per injection. The volume of prune extract injected was 100 μL, thereby the quantity of total phenols expressed as equivalent of gallic acid was 300 μg. The effects of prune extract administration on plasma tHcy level in wild type mice (Cbs +/+) and hyperhomocysteinemic mice (Cbs +/−) were shown in Fig. 1. The prune extract administration decreased significantly plasma tHcy level in both Cbs +/+ and Cbs ± mice, in comparison with mice on control diet respectively. Although the phenolic content of prune extract is different to phenolic content of

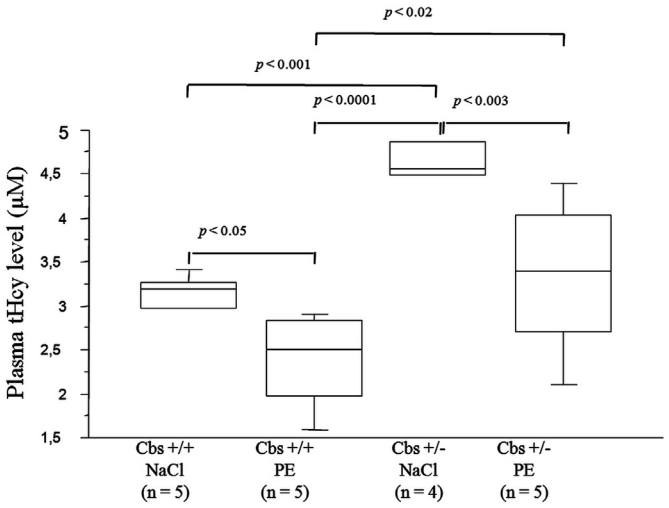

Fig. 1. Effects of prune extract on plasma tHcy levels in wild type (Cbs +/+) and hyperhomocysteinemic (Cbs +/−) mice fed the control diet supplemented (prune extract: PE) or not (NaCl). Data correspond to the medians with interquartile ranges. n = number of mice. Statistical analysis was done with one-way ANOVA followed by Student's unpaired t-tests.