Supplementation of green tea attenuates protein carbonyls formation in aged mice

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Abstract: To investigate the protective effect of green tea on the progression of protein carbonyls formation mediated by aged. The young and aged animals (8 week old and 52 week old, respectively) received distilled water, another set of aged animals received green tea extract (500 mg/kg) dissolved in distilled water for a period of 4 weeks. Body weight of all animals were measured once a week for a period of 4 weeks. The results showed that Protein carbonyl levels were significantly higher in the heart, liver and kidney of aged control group than in the young control group ($p < 0.05$). On the contrary, green tea extracts significantly decreased senescence mediated protein oxidative damage in target organs. Treatment with green tea extract was significantly decreased the cardiac, hepatic and renal percentage of protein carbonyls by 42%, 29% and 37%, respectively than that of the aged control group ($p < 0.05$). Therefore, the studies demonstrate that green tea exhibits potent protective effects on aged-mediated oxidative of protein in mice.
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1. Introduction

Aging is defined as an tremendously complex biological process related with a progressive functional decline in the performance of most organs (1,2). One major rationale of aging is the concept of oxidative stress, which increased production of reactive oxygen species (ROS), lipid peroxidation and oxidant-related reactions occur that result in oxidative damage (3).

Arising under normal aerobic metabolism of ROS can be induced protein oxidative damage by oxidation of glutamyl side chains and resulting in production of protein carbonyl groups. Therefore, protein carbonyl content is fundamentally the most universal biomarker of protein oxidation (4). Many studies have reported that dietary supplementation with extra natural antioxidants are efficacious to assist endogenous defense system to prevent aged-related oxidative stress on various organs due to particular interactions and synergisms for reducing the production of reactive metabolites (5,6).

Green tea (*Camellia sinensis*, Theaceae) is one of the most fashionable beverages in the world, particularly in Asian society. Many study of green tea extract has reported that daily consumption of green tea is safe and has no adverse effects for human health (7,8). Because green tea contains abundant bioactive substances, it has been shown to have beneficial biological effects. Most of the beneficial effects of green tea are attributed to its polyphenols, mainly catechins and catechin derivatives (9,10). Several studies have demonstrated that green tea catechins have been possess various physiological and medicinal properties (11,12). Many medicinal effects of green tea catechins, such as, anti-oxidant, anti-bacterial, anti-inflammatory and anti-tumor activities have been reported (13-15). Moreover, Tsai et al. demonstrated that green tea played a protective role in the reduction of oxidative stress and restored the activities of enzymes in the antioxidant defense system (16).

Therefore, the aim of the present study has been to investigate the protective effect of green tea on the aged mediated oxidative of protein in the heart, liver and kidney of aged mice.

2. Material and Methods

Material

Green tea extract made from the leaves of *Camellia sinensis* was obtained from commercially available preparations.

Animals

Thirty male ICR mice (young mice were 8 weeks old and aged mice were 52 weeks old) were obtained from a the Animal Department of BioLASCO Taiwan Company. Animals were quarantined and allowed to acclimate for one week prior to the beginning of experimentation. Animals were housed in 10 per cage under standard laboratory conditions with a 12 h light/dark cycle. The temperature of animal room was maintained at 25 ± 2ºC with a relative humidity of 55 ± 5%. Air handling units in the animal rooms were set to provide approximately 12 fresh air changes per hour. Food and water were available *ad libitum*. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee and the animals were cared for in accordance with the institutional ethical guidelines.

Experimental Design

Group I, used young mice, served as young control (n=10) and was orally administered distilled water (vehicle) daily for 4 weeks. The experimental groups (Group II and III) were used aged mice and the animals were randomly divided into two groups consisting of 10 animals in each group. Group II served as the aged control and was orally administered distilled water daily. Groups IIIwas orally administered green tea extract dissolved in distilled water at doses of 500 mg/kg daily for 4 weeks. Furthermore, food intake, water intake (daily) and body weights (weekly) were recorded throughout the study period.

Measurement of protein carbonyls

Oxidative damage to proteins was quantified by the carbonyl protein assay, which is based on the reaction with dinitrophenylhydrazine according to the method reported by Tsai et al (16). Briefly, proteins were precipitated by adding 20% trichloroacetic acid and redissolved in 10 mM dinitrophenylhydrazine to give a final protein concentration of 1-2 mg/ml, with 2 N hydrogen chloride added to the corresponding sample aliquot reagent blanks. The absorbance was measured at 370 nm with an ELISA plate reader (Quant, BioTek, VT, USA). The data were expressed as nmol of carbonyls/mg protein.

Statistical analysis

All values are expressed as the mean ± SD. Comparisons between groups were performed using a one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison tests using the statistical software SPSS (Drmarketing Co., Ltd. New Taipei City, Taiwan). Statistically significant differences between groups were defined as $p < 0.05$.

3. Results and discussion

We measured body weight, food intake and water intake over the 4 weeks of experimentation and there were no significantly difference in statistics among the test groups (data not shown).

Protein carbonyl is the most frequently used indicator of oxidative modification of proteins. To evaluate the effects of green tea extract treatment on aged mediated oxidative damage in heart, liver and kidney, protein carbonyl levels were determined in this study and the results are shown in Fig.1, Fig. 2 and Fig.3. Protein carbonyl levels were significantly higher in the all test organs of aged control group than in the young control group ($p < 0.05$). By contrast, green tea extract administration significantly decreased aged mediated oxidative damage of protein in heart, liver and kidney. Treatment with green tea extract was significantly decreased the percentage of protein carbonyls by 42%, 29% and 37% in heart, liver and kidney, respectively, than that of the aged control group ($p < 0.05$).

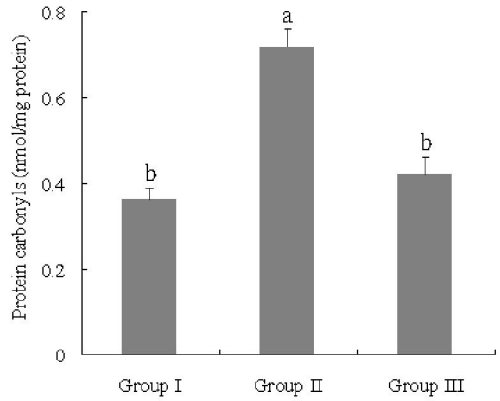

Fig. 1. Effects of green tea extract on protein carbonyls (nmol/mg protein) in cardiac tissue of aged mice. Values are means ± SDs for ten mice in each group. [a] compared with group I; [b] compared with group II. Group I, young control group; group II, aged control group; group III, aged mice treatment with green tea extract.

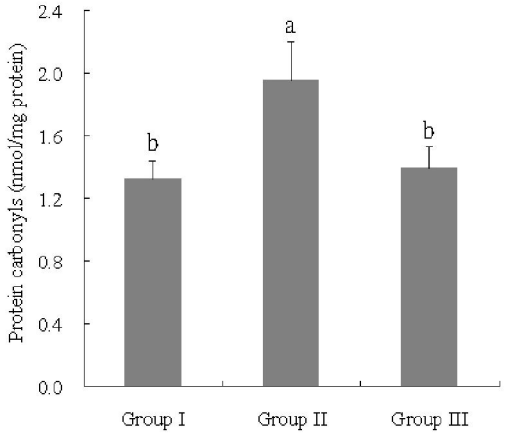

Fig. 2. Effects of green tea extract on protein carbonyls (nmol/mg protein) in hepatic tissue of aged mice. Values are means ± SDs for ten mice in each group. [a] compared with group I; [b] compared with group II. Group I, young control group; group II, aged control group; group III, aged mice treatment with green tea extract.

Protein carbonyl groups are an important biomarker of oxidative modification of proteins. Accumulation of protein carbonyls has been found in several human age-related degenerative diseases, including Alzheimer's disease, diabetes and Parkinson's diseases (17). Protein carbonyl groups can be induced by almost all types of ROS include radical

species such as superoxide, hydroxyl, peroxyl, alkoxyl and hydroperoxyl, and nonradical species such as H_2O_2, hypochlorous acid, ozone, singlet oxygen and peroxynitrite. Carbonyl groups are produced on protein side chains when they are oxidized. Therefore, protein carbonyl content is essentially the most general indicator of protein oxidation (16). In the present study, protein carbonyl contents significantly increased in the aged mice, otherwise, administration of green tea extract significantly decreased protein carbonyl levels relative to their age-matched group. Similar results from previous research confirmed that habitual green tea consumption can protect cells and tissues from oxidative damage by scavenging oxygen free radicals and significantly reduce the levels of protein carbonyl groups caused by ethanol in aged rats (18).

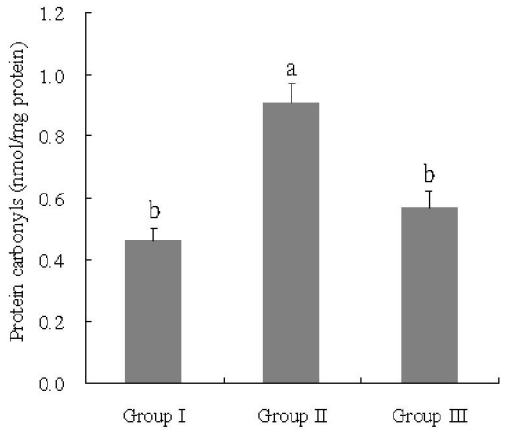

Fig. 3. Effects of green tea extract on protein carbonyls (nmol/mg protein) in renal tissue of aged mice. Values are means ± SDs for ten mice in each group. [a] compared with group I; [b] compared with group II. Group I, young control group; group II, aged control group; group III, aged mice treatment with green tea extract.

This study demonstrated that green tea extract exhibited significant inhibition of oxidative damage of protein in aged mice as evidence of decreased the protein carbonyl in heart, liver and kidney of aged mice. This result may be attributed to the ability of green tea polyphenols, mainly catechins and catechin derivatives, to extinguish excited sensitizer molecules and discontinue oxidative of protein (13). Moreover, several studies have demonstrated that dietary green tea extract supplementation of aged animals resulted in a significant reduction of protein oxidiation and improvement of the antioxidant defense system (11,18). Therefore, it can be concluded that green tea is an available source of natural antioxidants that provides the expected health benefits during aged process.

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