Green tea extracts suppress oxidative stress induced by dietary lipid peroxidation products.

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Summary

Green tea and its components possess antioxidative effects *in vivo* and *in vitro*. In this study, suppressive effects of green tea extracts on oxidative stress induced by dietary lipid peroxidation products were investigated in the rat liver *in vivo*. Green tea extracts (10 mg/rat/day for 3 days) were orally administered to male Wistar rats (five-week-old) 10 minutes prior to the post-oral administration of secondary autoxidation products of linoleic acid (400 mg/rat/day for 3 days). Control rats were given saline solution instead of green tea extracts and/or the secondary products. Green tea extracts prevented body weight loss caused by the secondary products. The effects of green tea extracts on oxidative stress were investigated 24 hours after the third dose. Green tea extracts showed the significant decreases in the amounts of thiobarbituric acid reactive substances and the levels of 4-hydroxynonenal and n-hexanal in rats dosed with the secondary products. Moreover, the extracts protected hepatic dysfunction as estimated by measuring the activity of five enzymes, which were specifically inactivated by the secondary products. These results clearly demonstrated that green tea extracts suppress both oxidative stress and hepatic dysfunction induced by dietary lipid peroxidation products.

Key Words

Lipid peroxidation, Autoxidation products, Hydroxynonenal, Hepatic dysfunction, Rat

Introduction

Green tea has a potency to modulate many biological functions *in vivo* and *in vitro* [1,2] and drinking green tea may lead us to healthy. Among biological functions of green tea, antioxidative effects are important for prevention and/or improvement of various diseases relating to the evoking of oxidative stress, since oxidative stress, which is accompanied by the generation of reactive oxygen species such as hydroxyl radical, hydrogen peroxide, and superoxide anion, changes biological functions for the worse [3]. Thus, it is important to make clear the preventive effects of green tea and its components, catechins on oxidative stress from various points of views.

Since oxidative stress is closely associated with various diseases, many investigators have studied to make the disease models using pro-oxidative chemicals including drugs. After treatment with pro-oxidative chemicals to the animals or cultured cells, lipid peroxidation products were formed endogenously or intracellularly [5,6]. Dietary lipid peroxidation products in food also induce oxidative stress [7]. Although induction of oxidative stress by treatment with them is weaker than that by pro-oxidative chemicals, appreciable amounts of lipid peroxidation products are present in our daily diet [8-10].

Primary products, lipid hydroperoxides are easily broken down in the stomach and form decomposed secondary products, which are incorporated into the body from the intestines [9,10]. Secondary products induce not only oxidative stress but also hepatic dysfunctions. With regard to hepatic dysfunction, we have almost explained it, i.e., secondary products reduce the activities of glucokinase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, mitochondrial NAD-dependent aldehyde dehydrogenase, and succinate dehydrogenase, and a depletion of coenzyme A [7, 11-15]. Moreover, secondary products impaired specific functions in the liver such as ureogenesis, gluconeogenesis, and hormonal responses [16].

It is, however, unknown yet that protective and/or suppressive effects of green tea and its components on oxidative stress and hepatic dysfunction induced by dietary lipid peroxidation products *in vivo*. In the present study, we investigated whether pre-treatment with green tea extracts can prevent endogenous lipid peroxidation and hepatic dysfunction induced by oral administration of secondary products prepared from autoxidized linoleic acid in the liver of rats.
Materials and Methods

**Materials.** Green tea extracts (dried powder, Sunphenon™) were obtained from Taiyo Kagaku Co., Ltd. (Yokkaichi, Japan). Linoleic acid was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and was autoxidized in air at 37 °C for 14 days. The autoxidized linoleic acid was separated into linoleic acid, its mono-hydroperoxides, and secondary products by silica-gel column and thin-layer chromatographies [16]. Components in the secondary products fraction were analyzed by gas-chromatography-mass spectrometry as described elsewhere [16].

**Animals and treatments.** Male Wistar rats (4 week-old, ST, SPF: Japan SLC Inc.) were housed in stainless cages, with free access to tap water and freshly prepared diet as described previously [14]. Rats were maintained in a climate-controlled room (temperature 24 ± 1°C, and humidity 70%, and the 12-hour light cycle) and acclimated for 1 week before treatments. Food was withheld for 4 hours and then the rats were divided at random into 4 groups of 3 rats each. First group was given green tea extracts and secondary products, second was secondary products alone, third was green tea extract alone, and last was vehicle control. Green tea extracts (10 mg/rat/day for 3 days) were orally administered to rats 10 minutes prior to the post-oral administration of secondary products (400 mg/rat/day for 3 days). Control rats were given the same amounts of saline solution instead of green tea extracts and/or the secondary products. Rats were killed 24 hours after the third doses.

**Measurement of oxidative stress.** Oxidative stress was estimated by measuring the amounts of thiobarbituric acid reactive substances (TBARS) and aldehydes (4-hydroxy-2-nonenal and n-hexanal) in the liver. The liver was homogenized with 10 volumes of ice-cold 1.15% (w/v) KCl solution, and obtained homogenate was used for measurement of thiobarbituric acid (TBA) test [17] and the levels of aldehydes [18].

**Measurement of hepatic dysfunction.** Hepatic dysfunction was estimated by measuring the activities of five hepatic enzymes, which were specifically inactivated by administration of secondary products [11-15]. Subcellular fractions were prepared from the liver homogenate by centrifugations. The activities of glucokinase, phosphoglomutase, and glucose-6-phosphate dehydrogenase were measured using the cytosolic fraction, and the activities of aldehyde dehydrogenase and succinate dehydrogenase were in the mitochondrial fraction.

**Statistical Analysis.** Statistical significance was analyzed by Student’s t-test; p<0.05 was considered significant.

Results and Discussion

**Green tea extracts suppressed secondary products-caused body weight loss.**

As shown in Figure 1, green tea extracts did not affect growth of rats. On the other hand, secondary products decreased body weight of rats after second administration, and this result is coincide with our previous observation [14]. When green tea extracts was pre-treated to rats, secondary products-caused body weight loss was significantly suppressed. Secondary products cause not only hepatotoxicity but also damage to small intestine [19]. Intake of green tea extracts may prevent intestinal damage by dietary lipid peroxidation products. There is another possibility that green tea extracts may inhibit incorporation of secondary products by interacting with together in digestive tracts. Taken together these possibilities, green tea extracts may reduce oxidative stress and result in suppression of body weight loss caused by secondary products.

![Figure 1. Changes in body weight after treatment with green tea extracts and/or secondary products.](image-url)
Green tea extracts suppressed secondary products-induced oxidative stress.  

Secondary products increased the amounts of TBARS in the liver of rats compared with that in the liver of control animals (Figure 2). Pre-treatment with green tea significantly suppressed increased TBARS by secondary products. Since TBA reaction has broad spectrum, we also determined major aldehydes in the liver. After liver homogenate was reacted with dinitrophenyl-hydrazine (DNPH), DNPH-aldehydes were extracted and analyzed by gas chromatography. Among various DNPH-aldehydes detected in the liver, 4-hydroxy-2-nonenal and hexanal were markedly increased after administration of secondary products (data not shown). Thus, we examined the effects of green tea extracts on the increased 4-hydroxy-2-nonenal and hexanal, and found that pre-treatment with green tea extracts significantly decreased the levels of both aldehydes in the liver of rats dosed with secondary products. The reduction of both the amounts of TBARS and the levels of aldehydes by green tea extracts was also observed ex vivo experiments using primary cultured hepatocytes from rats dosed with secondary products (data not shown). Moreover, the results from ex vivo experiments showed that antioxidative effects of catechins were stronger than those of flavones and flavonols (data not shown). Thus, green tea extracts and catechins will be effective to prevent in vivo oxidative stress induced by intake of secondary products.

![Figure 2](image.png)

Figure 2. Effects of green tea extracts on hepatic oxidative stress induced by secondary products.  
Rats were treated with the combination of green tea (GTE) and secondary products (SP). The amounts of TBARS and the levels of 4-hydroxynonenal (HNE) and hexanal (HEX) were measured as described in Materials and Methods. Data are represented as % of corresponding control value (mean ± SE, n=3). Asterisks indicate significant difference from secondary products-dosed rats (green tea’s effect, p<0.05).

Green tea extracts suppressed secondary products-induced hepatic dysfunction.  

Oxidative stress induced by secondary products is accompanied by hepatic dysfunction in vivo. Thus, we, finally, investigated that the effects of pre-treatment with green tea on the activities of marker enzymes for hepatic dysfunction. All enzymes tested here were decreased their activities by administration of secondary products, and their decreased activities were suppressed by pre-treatment with green tea extracts (Figure 3). These results indicated that green tea extracts suppressed not only oxidative stress but also hepatic dysfunction after intake of dietary lipid peroxidation products. Low-molecular-weight aldehydes, such as 4-hydroxy-2-nonenal and malonaldehyde, directly interact with amino acids and inactivate enzymes [20]. Our previous results also demonstrated that aldehydes in the secondary products fraction inactivate certain enzymes [21]. It is, therefore, suggested that green tea can prevent impaired liver functions directly or indirectly. In conclusion, our findings clearly demonstrated that drinking green tea is effective to prevent both oxidative stress and hepatic dysfunction induced by dietary lipid peroxidation products.
Rats were treated with the combination of green tea and secondary products. The activities of glucokinase (GK), glucose-6-phosphate dehydrogenase (G6PDH), phosphoglucomutase (PGM), aldehyde dehydrogenase (ALDH), and succinate dehydrogenase (SDH) were measured as described in Materials and Methods. The activity in the livers of control- and secondary products-dosed rats were taken as 100% and 0%, respectively, and the activity in the liver of rats dosed with both green tea and secondary products was represented as suppressive %. Data are expressed as mean ± SE (n=3). Asterisks indicate significant difference from secondary products-dosed rats (green tea’s effect, p<0.05).

References