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The Antioxidative Property of Green Tea against Iron-induced Oxidative Stress in Rat Brain

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Abstract

The antioxidative property of green tea against iron-induced oxidative stress was investigated in the rat brain both *in vivo* and *in vivo*. Incubation of brain homogenates at 37 °C for 4 hours *in vitro* increased the formation of Schiff base fluorescent products of malonaldehyde, an indicator of lipid peroxidation. Auto-oxidation (without exogenous iron) of brain homogenates was inhibited by green tea extract in a concentration-dependent manner. Moreover, incubation with iron (1 μM) elevated lipid peroxidation of brain homogenates after 4-hour incubation at 37 °C. Co-incubation with green tea extract dose-dependently inhibited the iron-induced elevation in lipid peroxidation. For the *in vivo* studies: ferrous citrate (iron, 4.2 nmoles) was infused intranigrally and induced degeneration of the nigrostriatal dopaminergic system of rat brain. An increase in lipid peroxidation in substantia nigra as well as a decrease in dopamine content in striatum was observed seven days after the iron infusion. Intranigral infusion of green tea extract alone did not increase, and in some cases, even decreased lipid peroxidation in substantia nigra. Co-infusion of green tea extract prevented oxidative injury induced by iron. Both iron-induced elevation in lipid peroxidation in substantia nigra and iron-induced decrease in dopamine content in striatum were suppressed. Oral administration of green tea extract for two weeks did not prevent the iron-induced oxidative injury in nigrostriatal dopaminergic system. Our results suggest that intranigral infusion of green tea extract appears to be nontoxic to the nigrostriatal dopaminergic system. Furthermore, the potent antioxidative action of green tea extract protects the nigrostriatal dopaminergic system from the iron-induced oxidative injury.

Key Words: green tea, iron, oxidative stress, antioxidant, nigrostriatal system, lipid peroxidation

Introduction

Tea is one of the most commonly consumed beverages in the world. In addition to its use as a beverage, green tea has been proposed to prevent cardiovascular diseases (13, 29) , cancers and etc. (15, 32). Indeed, tea flavonoids have been reported to protect low density lipoprotein (LDL) from oxidative stress (13). Furthermore, oral administration of green tea reduced aortic lesion formation and prolonged the lag phase of LDL oxidation, indicating an antiatherosclerotic property of green tea (30). Regarding anticancer actions, several studies have been shown that green tea prevents tumor initiation and promotion as well as tumor progression (12, 15). One of the possible mechanisms for these actions of green tea is to prevent oxidative stress. Several investigations have demonstrated that green tea scavenges free radicals (8, 20, 35). In addition, green tea polyphenols reportedly protected DNA molecules from oxidative stress in cultured lung cells (17). Moreover, green tea catechin has been shown to inhibit free radical-induced lysis of red blood cells (34).

So far, the antioxidative effect of green tea has not been investigated in CNS degenerative diseases which are proposed to be resulted from oxidative

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stress, including parkinson's disease. (1, 4, 10, 14). In parkinson's patients, lipid peroxidation, a biological marker of oxidative stress, is reportedly higher than that of normal controls (5, 11, 27). Furthermore, several studies have found a diminished antioxidant defensive system, including decreased levels of superoxide dismutase, catalase and glutathione (23, 28, 31, 33) as well as an accumulation of iron in substantia nigra of parkinsonian patients (6, 27). Iron, a transitional metal, is known to produce oxygen radicals *in situ* via the Fenton's reaction (9) which may, in turn, result in deterioration of the nigrostriatal dopaminergic system. Accordingly, iron chelators, free radical scavengers as well as up-regulation of antioxidative defense systems may hold promise for the therapeutic treatment for parkinsonism (7).

In the present study, the effect of green tea extract was investigated for the ability to prevent iron-induced oxidative stress both in vivo and in vitro. First, the antioxidative property of green tea was tested using brain homogenates incubated with or without iron which induces lipid peroxidation, a biological marker of oxidative stress. Secondly, the potency of green tea extract was compared to that of vitamin E in inhibiting iron-induced elevation of lipid peroxidation. Both auto-oxidation and iron-induced lipid peroxidation of cortical homogenates from rats fed with green tea extract for two weeks was compared to those of the control group. Our previous studies and others have shown that infusion of iron in substantia nigra induced neurodegeneration of striatal dopaminergic transmission, including decreases in K^+-evoked dopamine release, a depletion of striatal dopamine content, and an increase in lipid peroxidation in iron-lesioned substantia nigra (2, 18, 24, 25, 26). In the present study, green tea extract was infused directly into the substantia nigra and dopamine content in striatum and lipid peroxidation in substantia nigra were analyzed using a high-performance liquid chromatography (HPLC) coupled with electrochemical (EC) detector and a fluorescent method, respectively. The effect of oral administration of green tea extract on iron-induced oxidative stress was also evaluated.

Methods

In Vitro Study

1.1. Cortical Brain Homogenates

Male Sprague-Dawley (SD) rats (200-300 g) were decapitated and the cortical samples were dissected. Brain tissues were homogenized in ice cold Ringer's solution using a homogenizer. Brain homogenates (50 mg/ml, 400 µl) were incubated at 37±1°C for 4 h following the addition of iron (ferrous ammonium sulfate and sodium citrate, 1 µM) with or without green tea extract.

1.2 Fluorometric Measurement of Lipid Peroxidation

Fluorescent Schiff base products of malonaldehydes which become cross-linked with amino acids representing a selective marker for lipid peroxidation were determined (16, 19). After incubation at 37°C for 4 h, a 400 µl sample was transferred to a tube containing 300 µl chloroform and 100 µl methanol. The slurry was mixed and then kept in ice for 15 minutes. After centrifugation 8000g for 5 min, an aliquot (400 µl) of chloroform extract was transferred to another tube containing 100 µl methanol. The relative fluorescent intensities of samples in a cuvette were measurement by a fluorescent spectrofluorometer (Aminco Bowman-2, U.S.A.). Lipid peroxidation was determined by measuring the levels of malondialdehyde (MDA) and its dihydropyridine polymers which emit fluorescence at 426 nm when activated by UVa at 356 nm. Lipid peroxidation induced by a mixture of iron and green tea extract was compared with that by iron alone.

2.1. In Vivo Study

Adult, male SD rats were used. These animals were maintained according to the guidelines established in "GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, U.S.A. (1985).

2.2. Chronic Surgery and Drug Infusion

To initiate oxidative stress in the nigrostriatal system, rats were anesthetized with chloral hydrate (450 mg/kg, i.p., Sigma, St. Louis, MO) and placed in a stereotaxic instrument (David Kopf Instruments, Palo Alto, CA). Rats were placed on a homeothermic blanket (Harvard Instruments, Southnatick, MA) to maintain a rectal temperature at 37±1°C. After skin incision and exposure of the parietal bone, holes were drilled above the cortical surface for intranigral infusion of drugs. One microliter Kreb's Ringer solution of ferrous citrate (4.2 nmoles) with or without green tea extract (50 µg/ml) was infused stereotaxically and unilaterally into substantia nigra of each hemispheres coordinates (22): 3.2 mm anterior and 2 mm above to the interaural zero; 2.1 mm lateral to the midline; -3.5 mm mouth bar. Drug solutions were

infused at a rate of 0.2 µl/min through a 30 gauge stainless steel needle. The injection needle was held in place for an additional five minutes following drug infusion. After the surgery, rats recovered from anesthesia and were placed in home cages for seven days.

2.3. Oral Administration of Green Tea Extract

Water or green tea extract was administered to SD rats to drink *ad libitum* as their sole source of drinking water. Body weight and food intake of both groups were monitored. Two weeks after oral administration, rats received an intranigral infusion of iron. One week after infusion, rats were sacrificed. Both lipid peroxidation in substantia nigra and striatal dopamine level were analyzed.

2.4. Striatal Dopamine Level by HPLC Coupled with EC Detection

Seven days after an intranigral infusion of iron, rats were sacrificed by decapitation. Brains were removed and striata were dissected. Striatal dopamine levels were determined using a high-performance liquid chromatography (HPLC) coupled with EC detection (3).

2.5. Lipid Peroxidation of Substantia Nigra

Seven days after an intranigral infusion of iron, rats were sacrificed by decapitation. Brains were removed and substantia nigra was dissected from both hemispheres. A fluorescence assay procedure was modified to measure lipid peroxidation in substantia nigra (16, 19). The dissected substantia nigra was homogenized in chilled 400 µl chloroform and 200 µl methanol. After centrifugation, an aliquot of chloroform and methanol layer was scanned using a spectrofluorometer (Aminco Bowman-2, U.S.A.). The lipid peroxidation was determined by measuring the levels of malondialdehyde and its dihydropyridine polymers which emit fluorescence at 426 nm when activated by UVa at 356 nm. Statistical comparisons were made using one way ANOVA and a Bonferroni t-test as a post test.

Preparation of Green Tea Extract

Powder of green tea (500 mg), a gift from Taiwan Tea Experiment Station (T.T.E.S. no.12) was soaked in 100 ml distilled water (100°C) for a duration of 5 minutes. The solution was then cooled in ice water (0°C) and filtered. The concentration of green tea extract was expressed as 5 mg/ml.

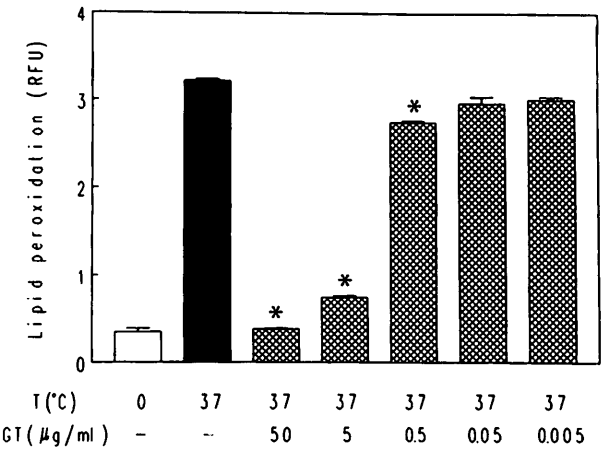

Fig. 1. Effects of green tea extract on auto-oxidation of brain homogenates. The brain lipid peroxidation (LP) was reported as relative fluorescence unit (RFU). Values are the mean±S.E.M. (n=4–5) from a representative experiment that was replicated with similar results. *$p<0.05$ in saline group compared to green tea group by one-way ANOVA followed by post-hoc analyses.

Results

In vitro Effects of Green Tea Extract

Incubation of brain homogenates at 37°C for 4 hours significantly increased the formation of peroxidized lipids assayed fluorometrically. A 9-fold increase in peroxidation of brain lipids was obtained compared to those incubated at 0°C (as basal level). Co-incubation with green tea extract suppressed the elevation of lipid peroxidation in a concentration-dependent manner (Fig. 1). Addition of iron (1 µM) profoundly increased lipid peroxidation in brain homogenates following a 4-h incubation at 37°C. Co-incubation with green tea extract concentration-dependently prevented iron-induced increase in lipid peroxidation (Fig. 2). The antioxidative property of green tea extract was compared that of vitamin E (Fig. 3). The green tea extract was more potent than that of vitamin E in inhibiting the iron-induced elevation of lipid peroxidation of brain homogenates (Fig. 3).

Effects of Green Tea Extract on Iron-induced Degeneration of Nigrostriatal System

Rats received a unilateral infusion of iron with or without green tea extract in substantia nigra; the contralateral striatum served as a control group. Seven days after iron infusion, HPLC analysis showed that dopamine level was depleted in striatum ipsilateral to the iron-infused substantia nigra. At this time, lipid peroxidation of the lesioned substantia nigra was

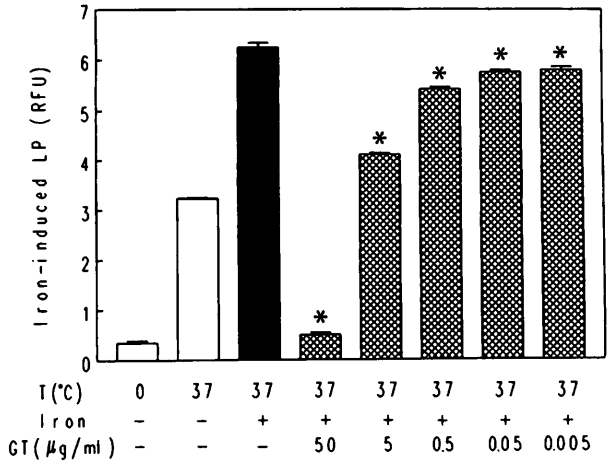

Fig. 2. Effects of green tea extract on iron-induced lipid peroxidation (LP) in cortical homogenates. The brain LP was reported as relative fluorescence unit (RFU). Values are the mean±S.E.M. (n=4–5) from a representative experiment that was replicated with similar results. *p<0.05 in iron group compared to green tea + iron group by one way ANOVA followed by post-hoc analyses.

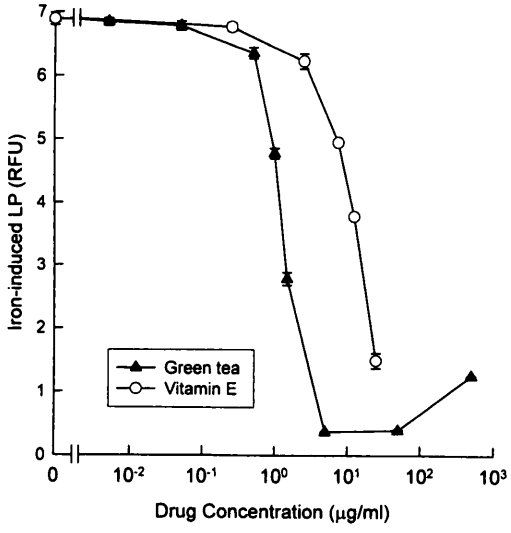

Fig. 3. Dose response curve for green tea extract and vitamin E on iron-induced elevation of lipid peroxidation (LP) of cortical homogenates. The brain LP was reported as relative fluorescence unit (RFU). Values are the mean±S.E.M. (n=4–5) from a representative experiment that was replicated with similar results.

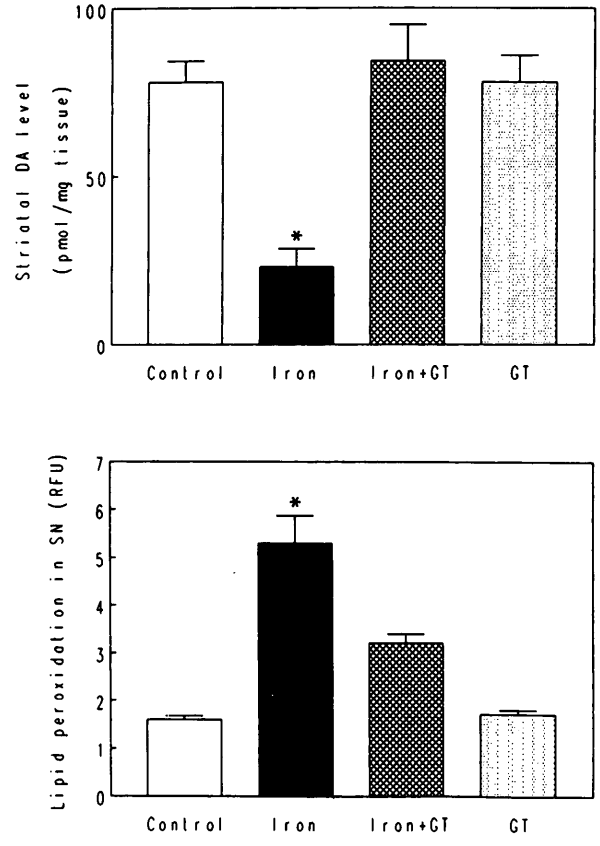

Fig. 4. Effects of green tea extract on iron-induced oxidative injury in nigrostriatal dopaminergic system seven days after an intranigral infusion of ferrous citrate. The lipid peroxidation (LP) in the microdissected substantia nigra was measured and reported as relative fluorescence units (RFU). Values are the mean±S.E.M. (n=6–12). *p<0.05 in iron group compared to intact controls and green tea + iron group by one-way ANOVA followed by post-hoc analyses.

increased compared to that of the intact substantia nigra (Fig. 4). Co-infusion of green tea extract suppressed the iron-induced elevation in lipid peroxidation. Furthermore, iron-induced depletion of dopamine content was recovered to 90% of intact control (Fig. 4). Intranigral infusion of green tea extract alone did not increase, and in some cases, even decreased lipid peroxidation in substantia nigra (Fig. 4).

After oral administration of green tea for two weeks, auto-oxidation (data not shown), iron-induced lipid peroxidation of brain homogenates (data not shown) and the dopamine content in striatum with intact substantia nigra were unaltered (81.1±4.2 pmole / mg tissue of the control versus 78.5±2.9 pmole / mg tissue of green tea group, n=9 / each group). At this time, the effect of green tea extract on iron-induced neurodegeneration was evaluated in vivo. Seven days after an intranigral infusion of iron, neither the iron-induced elevation in lipid peroxidation in substantia nigra nor the iron-induced reduction in dopamine content was prevented. The lipid peroxidation was increased from 2.84±0.07 to 4.88±0.61 URF in control group while in green tea group, the lipid peroxidation was increased from 2.90±0.20 to 4.58±0.43 RFU (n=8 / each group). The iron-induced reduction in dopamine content in striatum was 53.9±12.3 pmole / mg tissue in green tea group compared to 57.5±9.2 pmole / mg tissue in the control (n=8 each group).

Discussion

In the present study, the antioxidative effect of green tea extract was investigated to inhibit iron-induced oxidative stress in CNS. Our in vitro study showed that green tea extract dose-dependently inhibited auto-oxidation and iron-induced elevation of lipid peroxidation of brain homogenates. Co-infusion of green tea extract prevented iron-induced oxidative injury, including an elevation in lipid peroxidation in substantia nigra and a reduction in dopamine content in striatum. However, oral administration of green tea did not inhibit iron-induced oxidative stress in nigrostriatal dopaminergic system. Our study shows that green tea extract possesses an antioxidative property in preventing iron-induced oxidative stress.

Four main water-soluble phenol fractions analyzed by HPLC from various sources of green tea leaves include (-)-epigallocatechin gallate, 49-55%; (-)-epicatechin gallate, 8-18%; (-)-epigallocatechin, 3-12%; (-)-epicatechin, 3-7% (12). The antioxidative property of the green tea polyphenols has been reported in several contexts. First, the phenol components of green tea have been shown to be antioxidative in protecting auto-oxidation of lard (12). Furthermore, the green tea polyphenols have been demonstrated to inhibit iron-induced oxidation of synaptosomes by scavenging hydroxyl radicals from photolysis of hydrogen peroxides and lipid free radicals generated in the lecithin / peroxidase system (8). In the present study, our in vitro data support the notion that green tea extract is antioxidative in preventing iron-induced oxidative stress, including suppression of auto-oxidation and iron-induced lipid peroxidation. Several possible mechanisms have been proposed for the antioxidative action of green tea extract. It is possible that the chelating effect of green tea results in a reduction of free form of iron (8, 21). Furthermore, it is known that lipid peroxidation is a chain reaction (10), therefore, the scavenging property of polyphenols may decrease the concentration of hydroxyl radicals and lipid free radicals and thus terminates the initiation and propagation of the lipid peroxidation. The antioxidative potency of green tea extract was compared to that of vitamin E, a well-known antioxidant, in suppressing iron-induced oxidative stress. The IC_{50} for green tea extract is 10 fold more potent than that of vitamin E.

An intranigral infusion of iron was employed in the present study to establish an animal model of parkinson's disease (18). Our in vivo study showed that local application of exclusive green tea extract did not alter the function of nigrostriatal dopaminergic system, co-infusion of green tea extract prevented iron-induced degeneration of this system. In contrast, the data from oral administration of green tea failed to support the antioxidative action in CNS. Iron-induced oxidative injury was not ameliorated in rats which received a duration of total three weeks of oral administration of green tea extract. Therefore, it is possible that green tea can prevent the neurodegeneration in parkinsonism but only if oral administration of green tea reaches to an effective concentration in CNS.

In conclusion, the antioxidative property of green tea seems propitious in preventing iron-induced oxidative injury in CNS. However, further studies should be done to improve the bio-availability of green tea extract which may be useful for the therapeutic treatment for the damages including trauma, stroke and neurodegenerative diseases, in CNS.

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