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Effects of Green Tea Catechin on Mixed Function Oxidase System and Antioxidative Defense System in Rat Lung Exposed to Microwave

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Abstract

The purpose of this study was to investigate the effects of green tea catechin on mixed function oxidase system (MFO), lipofuscin contents, carbonyl value, oxidative damage and the antioxidative defense system in lung of microwave exposed rats. Experimental groups were divided to normal group and microwave exposed group. The microwave exposed groups were subdivided into three groups: catechin free diet (MW-0C) group, 0.25% catechin (MW-0.25C) group and 0.5% catechin (MW-0.5C) group according to the levels of dietary catechin supplementation. The rats were irradiated with microwave at frequency of 2.45 GHz for 15 min. Experimental animals were sacrificed at 6th day after microwave irradiation. The contents of cytochrome P_{450} contents in MW-0C group was increased to 95%, compared with normal group. MW-0.25C and MW-0.5C groups were reduced to 16% and 31%, respectively, compared with MW-0C group. The activity of NADPH-cytochrome P_{450} reductase in MW-0C group was increased to 44%, compared with normal group. MW-0.25C and MW-0.5C groups were reduced to 12% and 17%, respectively, compared with MW-0C group. The activity of superoxide dismutase (SOD) in MW-0C group was decreased to 21%, compared with normal group. MW-0.25C and MW-0.5C group were significantly ($p<0.05$) increased, compared with MW-0C group. The activity of glutathione peroxidase (GSHpx) in MW-0C group was significantly decreased, compared with normal group. MW-0.25C and MW-0.5C groups were recovered to the level of normal group. The thiobarbituric acid reactive substances (TBARS) content in MW-0C group was increased to 34%, compared with normal group. Catechin supplementation groups were maintained the level of normal group. The levels of carbonyl value in MW-0C group was increased to 21%, compared with normal group. MW-0.25C and MW-0.5C groups were reduced to 14% and 12%, respectively, compared with MW-0C group. The lipofuscin contents in MW-0C group were increased to 23.4%, compared with normal group. That of MW-0.5C group was significantly reduced, compared with MW-0C group. In conclusion, MFO system was activated and the formation of oxidized protein, lipofuscin was increased and antioxidative defense system was weakened of lung tissue in microwave exposed rats, thus oxidative damage was increased. But it was rapidly recovered to normal level by green tea catechin supplementation.

Key words: microwave, green tea catechin, MFO system, antioxidative defense enzyme, oxidative damage

INTRODUCTION

A high frequency, such as a microwave, involves a substantial amount of energy. Thus, exposure to microwaves raises the body temperature and has a harmful effect on actively dividing cells, such as genital organs, cardiac and pulmonary tissue, and white blood corpuscels (1,2). As a result, the related development of cerebral cancer, leukemia, dementia, miscarriages, and male breast cancer has all been reported (3,4).

Based on the analysis of other studies and preceding basic researches (5-7) it is likely that the toxicity of electromagnetic wave is caused by the toxicity of active oxygen generated during oxidization. The previous researches (8,9) reported that mixed function oxidase (MFO) system was significantly increased and antioxidative defense enzyme activity was decreased and induced the changes of gene expression.

The pulmonary tissue, in particular, has high density of capillary and oxygen rate. Thus, the free radical is more likely to be generated and the possibility of damages from oxidization is high in pulmonary tissues. Giulivi and others (10) reported that the oxidative stress such as the generation of free radical increased when the pulmonary tissue was exposed to paraquat or hyperbarbic oxygen.

Free radicals were generated by MFO system and induced the lipid peroxidation and oxidative damage in

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tissue. On the other hand, free radicals such as superoxide radical and hydroxy radical in living bodies were protected by antioxidative defense enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHpx). They were also protected by physiological antioxidative materials such as vitamin E, β-carotene, ascorbic acid and glutathione. However, when living body was exposed to exogeneous stress, such as microwave irradiation and metal toxication and endogeneous stress, such as disease state, excessive free radical was accumulated, thus oxidative damage was induced and activated the aging and adult disease. Many research was reported (7-9) the methods of strengthening the antioxidative defense system and weaking the free radical generation systen using the antioxidant substance, such as vitamin E, catechin, vitamin C and selenium.

On the other hand, the polyphenol compound, catechin, found in green tea has previously been reported to have pharmalogical effects (11-13), including antioxidation. Especially, catechin's polyphenol structure restricts cytotoxicity by hydrogen peroxide (H_2O_2) and is effective in eliminating singlet oxygen and free radicals in the early stage of lipid peroxide.

Free radicals, such as O_2^-, OH^- and H_2O_2 activate the initiation and promotion stages of cancer, as well as give damages on the cell membrane and DNA. It was reported (14,15) that green tea catechin suppress the free radical generation. Kim et al. (9) reported that catechin acted as a strong antioxidative substance through including gene expression so as to maintain the cell membranes' organelle in an optimum condition to facilitate enzyme activation against lipid peroxidation.

However, there was few studies on free radical generation mechanism and the change of antioxidative defense enzyme in lung tissue by microwave irradiation.

This study aims to show the changes in the MFO system and antioxidative defense system of lung tissue in microwave-exposed rats and examine the enforcing antioxidative effect of green tea catechin.

MATERIALS AND METHODS

Experimental animals and diets

Male Sprague-Dawley rats weighing about 100 g were purchased from KRITC (Daejon, Korea). The animals were individually housed in stainless steel cage in a room with controlled temperature (20~23°C), first adjusted for one week and then allotted to one normal group (which fed with normal diet) and microwave expose groups: microwave exposed groups were divided three groups: catechin free diet (MW-0C) group, 0.25% catechin (MW-0.25C) group and 0.5% catechin (MW-0.5C) group according to the levels of dietary catechin supplementation. The four groups fed experimental diet for 4 wks. The experimental animal number is 10 per group (Table 1). Crude catechin powder from green tea was prepared by the method of Matsuzaki and Hata (16). Crude catechin purity was 87.5% (Table 2). And the experimental design was approved by the committee of Catholic University of Daegu for care and use of laboratory animals.

Instrument and methods for irradiation of the microwaves on experimental animals

The experimental instrument included a high frequency generator which was designed to be available for a remote control to optionally adjust both output and exposure time, by remodeling a household electronic oven with 2.45 GHz continuous wave (CW) in microwave. The incident power density was measured using an America Hewlett Packard's EMC analyzer (8594EM). The measurement of power density was done, using the 35 cm distance of the Horn Antenna (EMCO 3115) as an exposure point, after adjusting the distance several times for maximum dose-rate to be about 15 minutes. The average whole-body, specific absorption rate (SAR) was measured using a calorimetric technique. The SAR was determined to be 9.2 W/kg for an incident power density of 40 mW (40 mW)/cm^2.

Experimental animals were sacrificed at the 6th day after

Table 1. Classification of experimental groups

Groups[1)]	Catechin % of diet	Microwave[2)]
Normal	0	-
MW-0C	0	+
MW-0.25C	0.25	+
MW-0.5C	0.5	+

[1)]Normal: No irradiation.
MW: Microwave irradiation, catechin free diet.
MW-0.25C: Microwave irradiation, catechin supplementation (0.25% catechin diet).
MW-0.5C: Microwave irradiation, catechin supplementation (0.5% catechin diet).
[2)]Irradiated 2.45 GHz microwave for 15 min.

Table 2. Composition of crude catechin powder from green tea

Catechin (μg)/100 μg powder	EGC	EC	EGCG	ECG	Total
	24.20±0.02	7.00±0.01	45.38±0.06	10.92±0.06	87.50

(-) EGC: Epigallo catechin, (-) EC: Epicatechin, (-) EGCg: Epigallo catechin gallate, (-) ECG: Epicatechin gallate.

microwave irradiation, because the previous research (9) has shown that oxidative damage was the most severe at the 6th day after microwave irradiation.

Pretreatment of enzyme specimen

After sacrificing the animals under the mild ether anesthetic, their lung was collected, washed with 0.9% NaCl, quick-frozen by liquid hydrogen, freeze-stored in a -80°C place and then used for this study. Lung microsome were prepared according to previous research (17).

Measuring mixed function oxidase system of lung tissue

The contents of cytochrome P_{450} in lung tissue was measured in compliance with the methods of Omura and Sato (18). The activity of NADPH-cytochrome P_{450} reductase in lung tissue was measured according to the method of Masters et al. (19).

Measuring antioxidative defense system of lung tissue

The activity of SOD in lung tissue was measured in compliance with the method of Marklund and Marklund (20). The activity of GSHpx in lung tissue was measured according to the method of Lawrence and Burk (21).

Measurement of lipofuscin content and carbonyl value

The content of lipofuscin in lung tissue was measured in compliance with the method of Fletcher et al. (22). The carbonyl value in lung tissue was measured in compliance with the method of Levin et al. (23).

Determination of lipid peroxide in lung tissue

The content of lipid peroxide in lung tissue was measured in according to the Satoh's method (24), which measured malondialdehyde produced by a reaction of thiobarbituric acid (TBA) and lipid peroxide.

Protein determanation

The protein in lung tissue was measured in compliance with the method of Lowry et al. (25) with bovine serum albumin as the standard solution.

Statistical analysis

Results were assessed by ANOVA and Tukey's Honestly Significant Difference test. Difference was considered significant at $p<0.05$.

RESULTS

Cytochrome P_{450} content and NADPH-cytochrome P_{450} reductase activity

The resulf on cytochrome P_{450} content is shown in Table 3. Cytochrome P_{450} content in MW-0C group was increased to 95%, compared with normal group. MW-0.25C and MW-0.5C groups were reduced to 16% and 31%, respectively, compared with MW-0C group. The activity of NADPH-cytochrome P_{450} reductase in MW-0C group was increased to 44%, compared with normal group. MW-0.25C and MW-0.5C groups were reduced to 12% and 17%, respectively, compared with MW-0C group. Accordingly, the enzyme activity of MFO system was significantly reduced by catechin supplementation.

Table 3. Effect of green tea catechin on cytochrome P_{450} content and NADPH cytochrome P_{450} reductase activity of lung in rats exposed to microwave

Group	Cytochrome P_{450} (nmol/mg protein/min)	NADPH cytochrome P_{450} reductase (nmol/mg protein/min)
Normal	$0.09\pm0.01^{1)c2)}$	2.14 ± 0.23^{c}
MW-0C	0.19 ± 0.01^{a}	3.09 ± 0.29^{a}
MW-0.25C	0.16 ± 0.01^{b}	2.72 ± 0.17^{b}
MW-0.5C	0.12 ± 0.01^{b}	2.56 ± 0.21^{b}

[1)]All values are the mean±SE (n=10).
[2)]Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions are the same as Table 1.

Antioxidative defense enzyme activity

The levels of SOD and GSHpx activities are shown in Table 4. The activity of SOD in MW-0C group was decreased to 21%, compared with normal group. MW-0.25C group and MW-0.5C group were significantly ($p<0.05$) increased, compared with MW-0C group. The activity of GSHpx in MW-0C group was significantly decreased, compared with normal group. MW-0.25C group and MW-0.5C group were recovered to normal level.

Levels of lipid peroxide and carbonyl value

The result of TBARS contents as an index oxidative damage was shown in Table 5. The TBARS concentration in MW-0C group was increased to 34%, compared to normal group. Catechin supplementation groups

Table 4. Effect of green tea catechin on superoxide dismutase (SOD) and glutathione peroxidase (GSHpx) activity of lung in rats exposed to microwave

Group	SOD (Unit/mg protein/min)	GSHpx (nmol NADPH/mg protein/min)
Normal	$5.70\pm0.48^{1)a2)}$	59.97 ± 3.72^{a}
MW-0C	4.50 ± 0.16^{c}	52.08 ± 1.15^{b}
MW-0.25C	5.24 ± 0.28^{b}	68.10 ± 5.43^{a}
MW-0.5C	5.25 ± 0.66^{b}	61.52 ± 6.43^{a}

[1)]All values are the mean±SE (n=10).
[2)]Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions are the same as Table 1.

Table 5. Effect of green tea catechin on thiobarbituric acid reactive substances (TBARS) contents and carbonyl value of lung in rats exposed to microwave

Group	TBARS (MDA nmol/mg protein)	Carbonyl value (ug / mg protein)
Normal	$0.39 \pm 0.02^{1)a2)}$	71.72 ± 1.31^{a}
MW-0C	0.52 ± 0.03^{b}	86.88 ± 1.85^{b}
MW-0.25C	0.38 ± 0.01^{a}	75.10 ± 1.22^{c}
MW-0.5C	0.32 ± 0.05^{a}	75.99 ± 1.27^{c}

[1)]All values are the mean ± SE (n=10).
[2)]Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions are same as Table 1.

were maintained the normal level. The level of carbonyl value in MW-0C group was increased to 21%, compared to normal group. MW-0.25C group and MW-0.5C group were reduced to 14% and 12%, respectively, compared with MW-0C group.

The contents of lipofuscin lung tissue

The result of lipofuscin contents was shown in Fig. 1. The lipofuscin contents in MW-0C group were increased to 23.4%, compared with normal group. The lipofuscin content in MW-0.5C group was significantly reduced, compared with MW-0C group.

DISCUSSION

The current study was conducted to observe the antioxidative effect of catechin in microwave-exposed rats. The study was carried out by having microwave-exposed rats and then observing any changes in both the MFO system and antioxidative defense system of lung tissue in microwave-exposed rats, as well as, its related oxidative damage.

MFO system generated the O_2 and H_2O_2 by detoxification process of carcinogen, exogeneous toxic material, thus induced the oxidative damage of tissue. In this study, the cytochrome P_{450} content in MW-0C group was increased to 95%, compared with normal group. MW-0.25C and MW-0.5C groups were significantly reduced, compared with MW-0C group. It seemed that cytochrome P_{450} contents was increased, due to exogeneous stress, such as microwave, and it was significantly reduced by catechin supplementation. These results are similar to those cited in the reports of Kim et al. (8), which showed that cytochrome P_{450} of liver tissue could be increased by microwave irradiation.

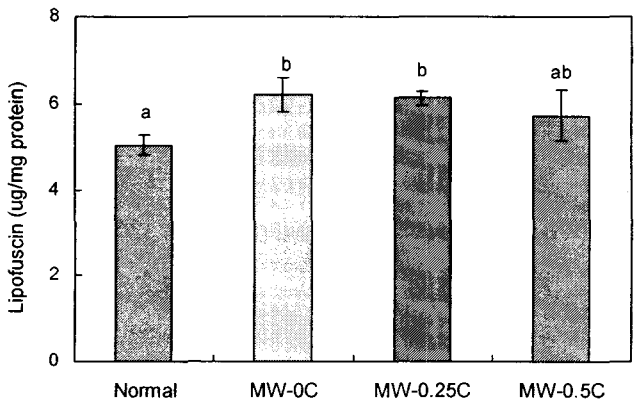

Fig. 1. Effect of green tea catechin on lipofuscin contents of lung in rats exposed to microwave.
All values are the mean ± SE (n=10).
Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test.
The experimental conditions are same as Table 1.

When living body was exposed to stress and an exogeneous toxic material, NADPH cytochrome P_{450} reductase activity was increased. In this study, the NADPH cytochrome P_{450} reductase activity has the same tendency in cytoochrome P_{450} content. These research seemed that when living body was exposed to exogeneous stress, enzyme related to detoxification process activity was activated and protected the living body.

Rhee et al. (26) and Park et al. (27) reported that the MFO system was activated in lung tissue of diabetic rats, but it was reduced by the antioxidant materials such as vitamin E and catechin.

SOD reduces superoxide radical to H_2O_2 when the generated H_2O_2 becomes unpoisoned by the action of GSHpx and catalase and, therefore, protects the living body from oxygen poisoned. When cell was exposed to oxidative stress, protein with protective capacity was activated and especially, SOD operates with first defense system against the tissue damage by radiation. In this study, The activity of SOD in MW-0C group was decreased to 21%, compared with normal group. MW-0.25C group and MW-0.5C group were significantly ($p<0.05$) increased, compared to MW-0C group. It seemed that the superoxide radical formation was induced by microwave irradiation and SOD activity was increased to eliminate the radical in catechin supplementation group.

The activity of GSHpx in MW-0C group was significantly decreased, compared with normal group. MW-0.25C group and MW-0.5C group were recovered to normal level. This would seem to indicate that the peroxidation of the unsaturated fatty acid in the existing membranes was activated by oxidative stress, in this case free radicals resulting from produced by microwave irradiation, thereby accelerating the cell tissue damage and decreasing the enzyme activities.

Especially, the pulmonary tissue, in particular, has high density of capillary and oxygen rate. Thus, the free radical is more likely to be generated and the possibility of damages from oxidization is high in pulmonary tissues.

The report of Kim et al. (9) stated that catechin reduced the antioxidative defense enzyme activity, as well

as it changed the gene expression of SOD and GSHpx of liver tissue in microwave-exposed rats. But catechin increased the antioxidative defense enzyme activity and gene expression by green tea catechin supplementation.

Lipid peroxide content as an index of lipid peroxidation in the MW-0C group was increased to 34%, compared with that of normal group. But catechin supplementation groups were maintained the normal level. The level of carbonyl value in MW-0C group was increased to 21%, compared with that of normal group. MW-0.25C group and MW-0.5C group were reduced to 14% and 12%, respectively, compared with MW-0C group.

The lipofuscin content in MW-0C group was increased to 23.4%, compared to normal group. The content in MW-0.5C group was significantly reduced, compared to MW-0C group. Accordingly, this research has the same tendency in antioxidative defense system.

In conclusion, MFO system was activated and the formation of oxidized protein, lipofuscin was increased and antioxidative defense system of weakened lung tissue in microwave-exposed rats, thus oxidative damage was increased. But it was rapidly recovered to normal level by green tea catechin supplementation.

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