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Effects of Green Tea Catechin on Platelet Phospholipase A_2 Activity and the Liver Antioxidative Defense System in Streptozotocin-induced Diabetic Rats

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Abstract

The purpose of this study was to investigate the effects of dietary green tea catechin and vitamin E on the phospholipase A_2 activity and the antioxidative defense system in streptozotocin (STZ)-induced diabetic rats. Sprague-Dawley male rats weighing 100±10 gm were randomly assigned to one normal and five STZ-induced diabetic groups. The diabetic groups were assigned either a catechin-free diet (DM group), 0.5% catechin diet (DM-0.5C group), 1% catechin diet (DM-1C group), vitamin E-free diet (DM-0E group), and 400 mg vitamin E per kg diet (DM-400E group) according to the levels of dietary catechin or vitamin E supplementation. The vitamin E levels of the normal, DM, DM-0.5C, and DM-1C groups were 40 mg per kg diet. Diabetes was experimentally induced by an intravenous injection of streptozotocin after 4 weeks of feeding the five experimental diets. The animals were sacrificed on the 6th day of the diabetic state. The body weight gains were lower in all five diabetic groups after the STZ injection. The platelet phospholipase A_2 (PLA_2) activity in the diabetic groups was higher than that in the normal group. However, the enzyme activity in the DM-0.5C, DM-1C, and DM-400E groups was lower than that in the DM and DM-0E groups. The cytochrome P_{450} and cytochrome b_5 content and NADPH-cytochrome P_{450} reductase activity were about 50~110% higher in the DM and DM-0E groups than in the normal group, yet significantly reduced by either catechin or vitamin E supplementation. The superoxide dismutase (SOD) content in the liver did not differ significantly in any of the groups. However, the glutathione peroxidase (GSHpx) activity was generally lower in the diabetic groups, compared with the normal group, whereas that of the DM-0.5C, DM-1C, and DM-400E groups was significantly higher compared with that of the DM and DM-0E groups. The levels of thiobarbituric acid reactive substances (TBARS) in the liver tissue were 148% and 201% higher in the DM and DM-0E groups, respectively, compared with the normal group, however, these levels were reduced by either catechin or vitamin E supplementation (DM-0.5, DM-1C and DM-400E). Accordingly, the present results indicate that STZ-induced diabetic rats exhibited an imbalance between free radical generation and scavenger systems in the liver which led to the acceleration of lipid peroxidation. However, these abnormalities were reduced and the antioxidative defense system was restored by either dietary catechin or vitamin E supplementation. In conclusion, the effects of dietary catechin or vitamin E in streptozotocin-induced diabetic rats would appear to inhibit lipid peroxidation as an antioxidant by regulating the activity of PLA_2.

Key words: diabetes mellitus, lipid peroxidation, phospholipase A_2, antioxidative defense system, catechin

INTRODUCTION

With the recent changes in living style and westernization of eating habits in Korea, there has been an increase in the number of patients suffering from various adult diseases. In particular, the pathogenesis rate of diabetes has seen a consistent increase (1). Consequently, there has been a sharp rise in the number of deaths resulting from diabetic complications.

Diabetic metabolism causes vascular disorders over the entire body such as microangiopathy, including retinopathy and nephropathy, arteriosclerotic vascular disorders and neuropathy, hypertension, hyperlipemia, obesity, cerebrovascular disorders, and cardiac disorders (2-6).

One of the reasons these vascular disorders occur so frequently is related to the oxidative damage of tissues by in vivo lipid peroxide (7). The oxidative damage of tissues results from the peroxidation of phospholipid on the cell membrane by a noxious oxygen radical such as O_2^- produced *in vivo* (8,9).

The free radical production system includes the mixed function oxidase system (MFO system), which is an enzyme system that plays an central role in metabolizing foreign substances. The MFO system serves to detoxicate and metabolize xenobiotics such as environmental pollutants or harmful drugs which are absorbed into the body. However, during the process of detoxicating these toxic substances, free radicals such as O_2^- and H_2O_2, which have a stronger toxicity than the original compounds, can be produced, thereby causing the peroxidation of tissue (10).

It has been also reported that if phospholipase A_2 (PLA_2), known as a rate-limiting enzyme of the arachidonic acid cascade system, is activated by several factors, free arachidonic

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acid, a metabolite of PLA_2, can be excessively produced resulting in the production of free radicals during the process of metabolism (11).

The human body uses the free radical scavenger system as a physiological defense system to protect tissues from peroxidative damage by free radicals and lipid peroxide. This defense system is divided into a non-enzyme defense system (vitamin E, β-carotene, etc.) and an enzyme defense system (superoxide dismutase (SOD), glutathione peroxidase (GSHpx), catalase, etc.) (12,13). It is said that in a normal physiological condition these defense systems protect the body from oxidative damage, however, in a pathological condition like diabetes, the free radical scavenger system can become weak whereas the free radical production system is promoted resulting in an imbalance between the systems (14). Therefore, in a pathological condition like diabetes, it is important to identify a way of normalizing this inbalance. Yang et al. (15) reported that lipid peroxidation is more accelerated in diabetic rats than in a normal group, yet if a substantial amount of dietary vitamin E is given, the antioxidative defense system is strengthened and peroxidative tissue damage is reduced. Thus, the role of the antioxidative system was highlighted for the first time.

In contrast, the excellence of the antioxidative function of catechin contained in green tea, a polyphenol-based compound, has also been recognized by several reports (14). Therefore, this study attempted to identify the antioxidative level and mechanism of catechin by comparatively observing the activities of the free radical production system and scavenger system relative to the feeding level of vitamin E and catechin after the dietary addition of these antioxidants for which the function is only partially known.

MATERIALS AND METHODS

Experimental animals and diet

After male Sprague-Dawley rats weighing about 100 g were acclimatized to the laboratory for one week, they were allotted to a normal group and five experimental diabetic groups using a completely randomised block design (Table 1). The six groups (n=10) were maintained on their respective experimental diets for four weeks *ad libitum*. The diabetic groups were classified as DM (catechin-free), DM-0.5C (catechin, 0.5%), DM-1.0C (catechin, 1%), DM-0E (vitamin E-free), and DM-400E (400 mg vitamin E/kg diet), respectively, according to the level of catechin or vitamin E supplementation. Crude catechin powder was prepared by the method of Matsuzaki and Hata (16), and used as such. Catechin contents in the crude catechin are shown in Table 2.

Inducing diabetes, measurements of body weight, and the food efficiency ratio

Diabetes were induced by intravenous injection 55 mg/kg body weight of STZ in sodium citrate buffer (pH 4.3) after 4 weeks of feeding three experimental diets. The rats were sacrificed on the 6th day of the diabetic states. The animals whose whole blood glucose concentration exceeded 300 mg/dL after six days were selected for the further experiments. The body weight was measured regularly at the same time every other day throughout the experimental period. The efficiency of the diet was calculated by dividing the body weight by the dietary intake during the experimental period.

Table 1. Classification of experimental groups

Groups	Vitamin E[1]	Catechin[2]	Streptozotocin[3]
	mg/kg diet	% (g/kg diet)	55 mg/kg B.W.
Nomal	40	-	-
DM	40	-	+
DM-0.5C	40	0.5% (5 g)	+
DM-1C	40	1% (10 g)	+
DM-0E	0	-	+
DM-400E	400	-	+

[1]Vitamin E : dL-α-tocopherylacetate
[2]Catechin : Crude catechin was extracted from green tea
[3]Intravenous injection of streptozotocin (55 mg/kg of body weight) in a 0.1 M sodium citrate buffer (pH 4.3) via a tail vein.

Table 2. Catechin contents in crude catechin preparation from green tea

	Catechin in dry powder (% on a dry weight basis)
Epigallo catechin	24.2
Epicatechin	7.0
Epigallo catechin gallate	45.3
Epicatechin gallate	10.9
Total	87.5

Collecting samples and sample preparation

Streptozotocin (STZ)-induced diabetic rats were fasted for 12h and sacrificed on the sixth day. The livers were excised, washed in 0.9% of NaCl, frozen rapidly in liquid nitrogen, stored at -80°C, then prepared for the experiment. The microsomes were isolated from the liver tissues by using a previously reported method (17).

Activity of platelet phospholipase A_2

Separation of platelets and preparation of [^{3}H]-arachidonic acid-labelled platelets

The number of platelets was measured and set at 3×10^8 cells/μL by separating the platelets, using the same method as reported previously (15), from the blood collected after the end of the experiment. The [^{3}H]-AA labelled platelets were prepared using the same method as reported previously (15) by adding [^{3}H]-arachidonic acid (AA) of 12.5 μCi to 2.5 mL of the platelet suspension.

Measurement of activity of platelet phospholipase A_2 (PLA_2)

The sample was incubated at 37°C for 5 min in 50 μL of

thrombin (1 unit/mL). Thereafter, the sample was centrifuged at 400×g in 5 μL of 1.5 M formaldehyde. The pellet was mixed with 0.5 mL of distilled water, 0.6 mL of $CHCl_3$, and 1.2 mL of MeOH, followed by the addition of 1.2 mL of $CHCl_3$. After the lower layer was evaporated under a stream of nitrogen gas, 300 μL of $CHCl_3$ was added to the residue. Two-thirds of the resulting suspension (200 μL) was analyzed by thin-layer chromatography using ethyl acetate/isooctane/acetic acid/water (90 : 50 : 20 : 10) as the developing solvent. The arachidonic acid separated on a TLC plate was measured using a liquid scintillation counter (LSC, Packard Co.).

Measurement of mixed function oxidase system in liver tissues

The microsome preparation of the liver tissues was performed using the same method as reported previously (17). The content of cytochrome P_{450} was measured with reduced carbon monooxide complexes of 450 nm and 490 nm by the method of Omura and Sato (18) using a spectrophotometer. The NADPH-cytochrome P_{450} reductase activity was measured by observing the decrease in the absorbance of dichlorophenolindophenol (DCIP) for one minute at 600 nm according to the method developed by Master et al. (19).

Measurement of antioxidative enzyme activity in liver tissues

The activity of superoxide dismutase (SOD) in the liver tissues was measured by the method of Marklund and Marklund (20), based on the color development of pyrogallol autoxidation in an alkaline state, whereas the glutathione peroxidase (GSHpx) activity was measured using the method of Lawerence and Burk (21).

Lipid peroxide (TBARS) content in liver tissues

The content of lipid peroxide in the liver tissues was measured using the Satoh method (22) which measures the TBARS reaction to thiobarbituric acid (TBA).

Statistical analysis

The data were analyzed by ANOVA, and when a significance was identified, the differences between the groups were evaluated using Tukey's HDS test.

RESULTS AND DISCUSSION

Body weight, food intake, and food efficiency ratio

The results of the body weight gain, food intake, and food efficiency ratio are shown in Table 3. During the period of the experiment, no significant differences were found between the normal and the experimental groups in terms of changes in body weight, food intake, and food efficiency before the injection of streptozotocin (STZ).

Activity of platelet phospholipase A_2

The result of measuring the PLA_2 activity, known as a rate-limiting enzyme of the arachidonic acid (AA) cascade (Fig. 1), showed that the DM and DM-0E groups with deficient vitamin E had increases of 42% and 91%, respectively, compared to the normal group. However, the catechin-administered DM-0.5C and DM-1C groups, and the high vitamin E-administered DM-400E group showed only slight increases of 17%, 23%, and 19%, respectively.

Measurement of mixed function oxidase system in liver tissues

The process of metabolizing environmental substances which enter the body from outside is mostly performed by the cytochrome P_{450}-dependent mixed function oxidase (MFO) system. The result of observing cytochrome P_{450}, which plays the most important role in this MFO system, is shown in

Table 3. Food intake, weight gain, liver weight, and food efficiency ratio (FER) of experimental rats

Groups	Body weight gains (g)	Food intake (g)	FER	Liver weight (g/100 g body wt)
	During 4 weeks before STZ injection[1)]			
Normal	168.57±7.0[2)NS3)]	576.91±19.6[NS]	0.28±0.01[NS]	
DM	164.50±7.8	564.00±9.2	0.37±0.11	
DM-0.5C	158.71±6.1	543.76±16.6	0.32±0.04	
DM-1C	155.25±5.7	522.02±8.4	0.40±0.18	
DM-0E	157.00±0.4	624.33±4.0	0.31±0.07	
DM-400E	163.38±1.4	602.51±18.4	0.32±0.08	
	During 6 days after STZ injection[1)]			
Normal	24.64±13.0[a4)]	104.01±6.5[a]	0.23±0.11[a]	2.92±0.5[NS]
DM	-67.11±19.1[b]	129.04±10.2[b]	-0.52±0.15[b]	3.36±0.4
DM-0.5C	-50.63±22.3[b]	122.79±7.8[b]	-0.45±0.17[b]	3.11±0.3
DM-1C	-67.89±23.6[b]	127.19±10.1[b]	-0.58±0.21[b]	3.19±0.4
DM-0E	-63.67±15.4[b]	130.78±7.9[b]	-0.53±0.09[b]	3.10±0.4
DM-400E	-62.08±25.0[b]	123.96±8.7[b]	-0.50±0.30[b]	3.18±0.6

[1)]Experimental and control groups were injected with or without 55 mg STZ/Kg B.W. via tail vein.
[2)]All values are mean±SE (n=10).
[3)]NS: Not significant
[4)]Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test.

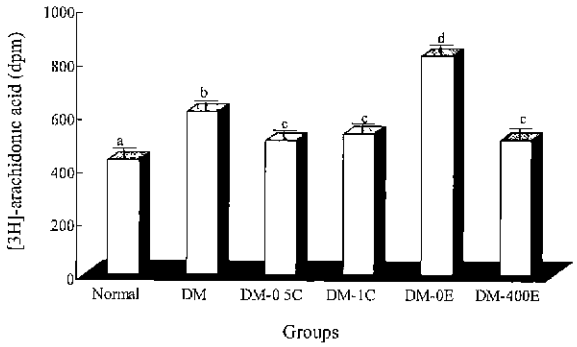

Fig. 1. Effect of green tea catechin and vitamin E on phospholipase A_2 activity in streptozotocin-induced diabetic rats. All values are mean±SE. (n=10). Bars with different letters are significantly different at $p<0.05$ by Tukey's-HSD test.

Table 4.

The concentration of cytochrome P_{450} in the DM group increased by 113% when compared with the normal group. In contrast, the cytochrome P_{450} concentration in the DM-0.5C and DM-1C groups increased by 31% and 38%, respectively, compared with the normal group, and decreased by 38% and 35%, respectively, compared with the DM group. The concentration of cytochrome P_{450} in the DM-400E and DM-0E groups increased significantly by 56% and 113%, respectively, when compared with that in the normal group.

The content of cytochrome b_5 in the DM and DM-0E groups increased 60% and 87%, respectively, compared with that in the normal group yet no significant diffrence was found in the DM-0.5C, DM-1C, and DM-400E groups.

The activity of NADPH-cytochrome P_{450} reductase in the DM-0E, DM, and DM- 0.5C groups significantly increased by 63%, 49%, and 26%, respectively, yet no significant difference was found in the DM-1C and DM-400E groups when compared with the normal group.

Measurement of antioxidative enzyme activity in liver tissues

The activity (Table 5) of SOD, an antioxidative defense system that protects the body from oxidative damage by reducing

Table 4. Effect of green tea catechin and vitamin E on the mixed function oxidase system in streptozotocin-induced diabetic rats

Groups	Cytochrome P_{450} (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	NADPH-Cytochrome P_{450} reductase (nmol/mg protein/min)
Normal	0.16±0.01[1)a]	0.15±0.02[a2)]	22.38±1.12[a]
DM	0.34±0.03[b]	0.24±0.01[b]	33.23±0.90[b]
DM-0.5C	0.21±0.01[c]	0.17±0.01[a]	28.14±0.47[c]
DM-1C	0.22±0.02[c]	0.16±0.01[a]	27.82±1.30[c]
DM-0E	0.36±0.01[b]	0.28±0.02[b]	36.55±1.43[b]
DM-400E	0.25±0.02[c]	0.16±0.01[a]	25.10±1.25[ac]

[1)]All values are mean±SE (n=10).
[2)]Values within a column with different superscripts are significantly different at $p<0.05$.

Table 5. Effect of green tea catechin and vitamin E on superoxide dismutase and glutathion peroxidase activities in liver tissue of streptozotocin-induced diabetic rats.

Groups	SOD (unit/mg protein/min)	GSHpx (nmol NADPH/mg protein/min)
Normal	3.59±0.16[1)NS]	230.41±12.41[a2)]
DM	3.27±0.18	196.89± 9.50[c]
DM-0.5C	3.42±0.29	207.77± 8.73[ac]
DM-1C	3.34±0.29	236.61± 8.60[a]
DM-0E	3.53±0.28	168.49± 7.58[b]
DM-400E	3.30±0.13	264.75± 8.62[d]

[1)]All values are mean±SE (n=10).
[2)]Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test.

superoxide radicals (O_2^-) to H_2O_2, showed a significant difference in the various experimental groups.

The activity of GSHpx decreased 15%, 10%, and 27%, respectively, in the DM, DM-0.5C, and DM-0E groups, yet maintained the same level in the DM-1C group as that in the normal group. In contrast, the DM-400E group showed a significant increase of 15% compared to the normal group.

Levels of lipid peroxide (TBARS) in liver tissues

The result of measuring the content of lipid peroxide in the liver tissues (Fig. 2) showed that the DM group with no added dietary catechin exhibited a liver tissue TBARS content that was 148% higher than that of the normal group. The DM-0.5C, DM-1C, and DM-400E groups with catechin added to their diets exhibited content values that were 42%, 24%, and 73% higher, respectively, than the content in the normal group yet 43%, 50% and 30% lower, respectively, than the content in the DM group.

In contrast, the DM-0E group that was deficient in vitamin E showed a greater increase of 201%, compared to the normal group. Accordingly, it would appear that lipid peroxidation was substantially accelerated in the liver tissues of diabetic rats.

The purpose of this study was to observe the effect of

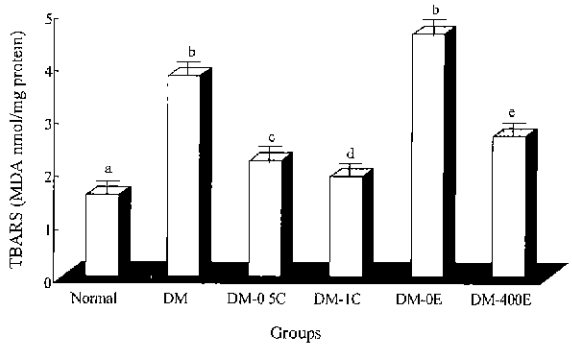

Fig. 2. Effect of green tea catechin and vitamin E on TBARS values of liver tissue in streptozotocin-induced diabetic rats. All values are mean±SE (n=10). Bars with different letters are significantly different at $p<0.05$ by Tukey's-HSD test.

catechin contained in green tea, on the free radical production system and free radical scavenger system in the liver tissues of STZ-induced diabetic rats. Accordingly, this study also compared and examined the function of vitamin E, an antioxidant. Based on the weight increase observed in this study, even though the dietary intake increased after the STZ injection, the weight and food efficiency ratio were remarkably reduced. This result is similar to that reported in other diabetic studies (23). However, there was no significant difference between the experimental groups.

PLA_2 produces free fatty acids (24) by hydrolyzing the fatty acid ester conjugation in the sn-2 position of glycero-3-phospholipid, and then accentuates the arachidonic acid cascade system. With production of free radicals in this metabolic process, the increase in the PLA_2 activity then accelerates the production of free radicals. In this experiment, the DM group showed an increase of 42%, compared with the normal group. This increase was significantly reduced by administering catechin and vitamin E. The finding that PLA_2 increased in the diabetic group with insufficient vitamin E and yet its activity was reduced in the vitamin E or catechin-administered group agrees with Moon's report (25) that an antioxidant-like hydroxybrazillin serves to inhibit the PLA_2 activity of platelets. This indicates that catechin and vitamin E function as antioxidants. In this study, the change of PLA_2 showed a similar trend to the change in the concentration of lipid peroxide in the liver tissues. This would appear to result from the fact that PLA_2 is activated by free oxygen radicals or lipid peroxide.

In conjunction with cytochrome b_5, cytochrome P_{450}, which exists in the endoplasmic reticulum (ER) of organs such as the liver, lungs, kidneys, brain, skin, and placenta, is an enzyme which plays a central role in detoxicating foreign substances. In this study, the activity of the MFO system as a free radical production system, to which cytochrome P_{450}, cytochrome b_5, and NADPH-cytochrome P_{450} reductase belong, increased significantly yet also tended to decrease according to the concentration of catechin or vitamin E.

SOD reduces a superoxide radical to H_2O_2 which is then detoxicated by the action of GSHpx and catalase to protect the body from oxygen poison. In a previous study (14), an increase in SOD was observed on the fourth day after diabetic induction, yet this study showed no significant difference between the normal group and the diabetic group. This may have been due to the difference in the period of the experiment which in this study was terminated on the sixth day after inducing diabetes. GSHpx catalyzes the reactions to produce oxidative glutathione (GSSG) and H_2O from H_2O_2 and reductive glutathione (GSH) *in vivo*, and produce alcohol (ROH) and H_2O from other peroxides (ROOH) *in vivo*. In this experiment, the DM and DM-0E groups showed a more significant decrease in the activity of GSHpx than the normal group. However, the activity in both groups was restored to the level of the normal group after administering catechin. The DM-400E group to which a large amount of vitamin E was administered showed a 14% increase compared to the normal group and a 34% increase compared to the DM group. The reduction of GSHpx activity in the STZ-induced diabetic group would seem to imply that in diabetic rats, as the level of unsaturated fatty acid content in the biomembrane increases, the biomembrane becomes more sensitive to oxidative stress thereby resulting in accelerated lipid peroxidation and peroxidative damage of the organelles, plus the activity of the enzymes decreases. The value of lipid peroxide in the liver tissues was 2.5 times higher in the DM group with no dietary catechin than in the normal group, whereas the DM-0E group that was vitamin E deficient showed a remarkable 3-fold increase. These increases were lowered by vitamin E or catechin supplementation. The reduction of the peroxide value in the liver tissues by the dietary addition of catechin or vitamin E, implies that the *in vivo* peroxidative reaction is inhibited by the chain-breaking antioxidative activities of catechin and vitamin E along with their ability to capture free radicals (4-15).

From the above results, it would appear that the remarkable increase in the lipid peroxide value in the STZ-induced diabetic group comes from an increase in the activity of free radical producing enzymes such as cytochrome P_{450}, cytochrome b_5, and NADPH-cytochrome P_{450} reductase, which belong to the MFO system, an increase in the production of free radicals due to a decrease in the activity of antioxidative enzymes, and an increase in the activity of phospholipase A_2 in the biomembrane lipid (26).

The results of comparing catechin and vitamin E showed that the proper administration of these two antioxidants reduced the free radical production system to the level of the normal group and strengthened the antioxidative defense system, thereby causing a decrease in the accumulation of lipid peroxide in the liver tissues. Plus, catechin appeared to have a slightly better antioxidative effect than vitamin E.

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