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Original Article

Effect of green tea catechin on arachidonic acid cascade in chronic cadmium-poisoned rats

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The purpose of this study was to investigate the effect of green tea catechin on the cyclooxygenase and lipoxygenase pathways in chronic cadmium-poisoned rats. Sprague–Dawley male rats weighing 100 ± 10 g were randomly assigned to one normal and three cadmium-poisoned groups. The cadmium groups were classified as catechin-free diet group (Cd-0C), 0.25% catechin diet group (Cd-0.25C) and 0.5% catechin diet group (Cd-0.5C), in accordance with the level of catechin supplement. The phospholipase A_2 activity was remarkably increased 117% in the Cd-0C group and 60% in the Cd-0.25C group compared with the normal group, and the level in the Cd-0.5C group was the same as the normal group. Activity of platelet cyclooxygenase increased 284% in the Cd-0C group, 147% in the Cd-0.25C group and 193% in the Cd-0.5C group. The synthesis of platelet thromboxane A_2 (TXA_2) increased 157% in the Cd-0C group and 105% in the Cd-0.25C group, compared with the normal group. The Cd-0.5C group showed the same level as the normal group. Prostacyclin (PGI_2) formation in the aorta decreased 24% in the Cd-0C group and 18% in the Cd-0.25C group. The ratio of PGI_2/TXA_2, the thrombocyte synthesis index, decreased 70% in the Cd-0C group and 59% in the Cd-0.25C group. The activity of 5′-lipoxygenase in the polymorphonuclear leukocyte was increased 40% in the Cd-0C group as compared with the normal group. Catechin-supplemented Cd-0.25C and Cd-0.5C groups showed the level of the normal group. In this study, the observed content of leukotriene B_4, which induces the inflammatory process, increased 54% in the Cd-0C group, and in catechin-supplemented groups, showed the same level as in the normal group. The serum peroxide value increased 60% in the Cd-0C group compared with the normal group; but in the Cd-0.5C group, it showed the level of the normal group. These results indicate that chronic cadmium poisoning in rats accelerates arachidonic acid metabolism. Inhibition of arachidonic acid metabolism due to catechin supplementation, however, decreases platelet aggregation and inflammatory action. In conclusion, it would appear that green tea catechin supplementation in chronic cadmium-poisoned rats inhibits the arachidonic acid cascade by regulating the activity of phospholipase A_2.

Key words: cadmium, cyclooxygenase, green tea catechin, lipoxygenase, prostaglandin.

Introduction

In proportion to increasing industrial development, environmental contamination by heavy metals (such as lead, cadmium and mercury) has produced severe problems with its pollution-mediated diseases. Cadmium can be absorbed orally through contaminated food and can be carried into the body subconsciously by smoking or respiration with contaminated air. Therefore, about 20–30 mg of cadmium can be accumulated through 40–60 years of our lifetime.[1] Hence, absorbed cadmium, even though in small doses, shows a high residual accumulation in the body because of its long biological half-life of 13–37 years.[2] Much research into cadmium intoxication has been completed.[3–6] Well-documented human disorders resulting from cadmium intoxication are subclassified as acute intoxication and chronic intoxication. Acute intoxication is characterised by illnesses of the liver, stomach and central nervous system, loss of kidney function, metabolic disorders involving calcium and itai-itai disease. Chronic intoxication causes the bone-mediated disease osteoporosis.[7,8] Moreover, it has been reported that cadmium kinetically affects the heart and/or vascular circulatory system, and causes cardiovascular diseases such as hypertension and arteriosclerosis.[9]

Caprino and Togna[10] performed animal experiments focusing on cadmium intoxication that caused cardiovascular disease. Observing the effect of prostaglandin in cadmium-injected rabbits, they reported that the production of prostacyclin (PGI_2) was decreased while thromboxane A_2 (TXA_2) was increased. The report indicated that arterial hypertension caused by cadmium intoxication is due to an unbalanced PGI_2/TXA_2 ratio. A previous report[11] has referred to changes in cyclooxygenase (COX), PGI_2 and

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TXA_2, which are induced by the activation of phospholipase A_2 (PLA_2), the rate-limiting enzyme of the arachidonic acid (AA) cascade. It is well known that most symptoms of acute inflammation are due to the acceleration of vessel permeability caused by chemical mediators such as histamine, serotonin, bradykinin and leukotriene.[12] According to these results, the regulation of the AA cascades, the 5′-lipoxygenase (5′-LPx) pathway and the COX pathway (as well as modulation of PLA_2 activation), are needed to improve vascular disorders. This control involves the formation of leukotriene B_4 (LTB_4) and PGI_2/TXA_2, respectively, and has anti-inflammatory and antithrombotic effects.

The following conditions can decrease the accumulation of internal cadmium, thereby providing cadmium detoxification: increasing the Ca^{2+} concentration (1.5%); going on a high-protein diet; increasing calcium and vitamin concentrations; and taking natural minerals such as seaweed (marine algae), chitoic acid and arginate. Thus, various nutrients and functional minerals can contribute to cadmium detoxification.

Green tea catechin, among other natural substances, is known for its various pharmacological actions, such as decreasing blood cholesterol,[13] antioxidation[14] and the possibility of inhibiting platelet coagulation.[15] The green tea beverage promotes detoxification activity by inhibiting the body's absorption of heavy metals and promoting their excretion.[16] The catechin in green tea is a polyphenol-type chemical that is highly available in the green tea. This substance binds with the metal ions to form an insoluble complex with ionic salts to remove the heavy metals.

It is very difficult to find any *in vivo* studies on pure catechin extracts and their detoxification effects or mechanisms regarding bone metabolic disorders caused by cadmium. Also, cadmium detoxification using physiologically active natural materials, such as green tea catechin, as well as the clinical symptoms of circulation disorders and hypertension due to cadmium intoxication have been insufficiently researched. This research seeks a pathological mechanism for cardiovascular disease due to chronic cadmium intoxication and a detoxification solution. Rats chronically intoxicated with cadmium were observed for improvement of blood circulation after supplementation with green tea catechin.

Materials and methods

Experimental animals and diets

Male Sprague–Dawley rats weighing 100 ± 10 g were purchased from KRITC (Daejon, Korea). The animals were housed individually in stainless steel cages in a room with controlled temperature (20–23°C) and lighting (alternating 12 h periods of light and dark), and fed a palletised, commercial non-purified diet for 6 days after arrival. They were divided randomly into one normal group and three cadmium groups. The cadmium groups were classified into Cd-0C (catechin free), Cd-0.25C (2.5 g catechin/kg diet), and Cd-0.5C (5 g catechin/kg diet), according to the level of catechin supplemented

Crude catechin powder was prepared by the method of Matsuzaki and Hata.[14] Catechin content in the crude powder and dietary composition of the basal diet are shown in Tables 1 and 2. The cadmium supply was made by adding $2CdCl_2.H_2O$ to distilled water to make the cadmium concentration 500 p.p.m. The rats were allowed to take water and diet freely and were fed in this manner for 20 weeks. The experimental design was approved by the committee for the care and use of laboratory animals of the Catholic University of Daegu.

Measurement of phospholipase A_2 activity in platelets

Blood mixed with acid citrate dextrose was centrifuged at 400 *g* for 15 min. The supernatant was collected and respun at 1400 *g* for 15 min. Platelets obtained from the supernatant were supplemented with 2.5 mL of platelet-poor plasma. Two hours after incubation at 37°C with 120 μL of [^3H]-AA (4.68×10^8 Bq), according to the method of Yang *et al.*,[17] the platelet suspension was diluted with 15 mL of platelet-poor plasma and centrifuged at 1400 *g*. The resulting [^3H]-AA-labelled platelets were blended in 5 mL Tris-tyroid

Table 1. Catechin content of crude catechin preparations made from green tea

	Catechin in dry powder (% on a dry weight basis)
Epigallocatechin	4.56 ± 0.02
Epicatechin	4.52 ± 0.01
Epigallocatechin gallate	38.56 ± 0.06
Epicatechin gallate	20.76 ± 0.06
Total	68.40

Table 2. Composition of basal diet

Ingredients	Amount (g/kg diet)
Corn starch†	668
Casein‡	180
D-L-methionine§	2
Corn oil¶	50
Salt mixture††	40
Vitamin mixture‡‡	10
Cellulose§§	50

Total energy content of diet is 3850 kcal/kg. †Pung Jin Chemical Company, Seoul, Korea; ‡lactic casein, 30 mesh; New Zealand Dairy Board, Wellington, NZ; §Sigma Chemical Company, St Louis, MO, USA; ¶Dong Bang Oil Company, Seoul, Korea; ††AIN-76 salt mixture (g/kg): dibasic calcium phosphate, 500; sodium chloride, 74; potassium citrateminohydrate, 220; potassium sulphate, 52; magnesium oxide, 24; manganious carbonate (45–48% Mn), 3.5; ferric citrate (16–17% Fe), 6; zinc carbonate (70% ZnO), 1.6; cuppric carbonate (53–55% Cu), 0.3; potasium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulphate, 0.55; finely powdered sucrose, 1000; ‡‡AIN-76 vitamin mixture (mg/kg): thiamine.HCl, 600; riboflavin, 600; pyridoxine.HCl, 700; nicotinic acid, 3000; D-calcium pantothenate, 1600; folic acid, 200; D-biotin, 20; cyanocobalamin (vitamin B_{12}), 1; retinyl palmitate or acetate (vitamin A) as stabilised powder to provide 400 000 IU vitamin A activity or 120 000 retinol equivalent; D-L-α-tocopheryl acetate, 5000 IU; cholecalciferol (100 000 IU, may be in powder form), 2.5; menaquinone (vitamin K, menadione), 5; finely powdered sucrose, 1000; §§Sodium carboxyl methyl cellulose, Sigma Chemical Company.

buffer containing ethylenediaminetetraacetic acid (EDTA), centrifuged at 1400 *g* for 15 min and resuspended in 1–2 mL Tris-tyroid buffer excluding EDTA. The platelet suspension (1×10^6 cell/mL) was incubated in 50 mL 200 mol/L propyl gallate and used for measurement of phospholipase A_2 activity. The sample was incubated at 37°C for 5 min in 50 µL thrombin (1 U/mL). It was then centrifuged at 400 *g* in 5 µL 1.5 mol/L formaldehyde. The pellet was mixed with 0.5 mL distilled water, 0.6 mL $CHCl_3$ and 1.2 mL methanol, followed by addition of a further 1.2 mL $CHCl_3$. After the lower layer was removed and evaporated under a stream of nitrogen gas, 300 µL $CHCl_3$ was added to the residue. Two-thirds of the resulting suspension (200 µL) was analysed by thin-layer chromatography (TLC) using ethylacetate/isooctane/acetic acid/water (90:50:20:10 v/v) as a developing solvent. Arachidonic acid separated on the TLC plate was measured using a liquid scintillation counter.

Measurement of cyclooxygenase activity in platelets

Platelets were separated using a method previously described.[17] A total of 1.5×10^6 cells in washed platelet suspension (WPS) (3×10^8 cells/mL) were taken and arachidonic acid was added to make a final concentration of 10 µmol/L. This was incubated at 37°C for 6 min before 50 µL indomethacin (final concentration = 120 µmol/L) was added to stop the reaction. The amount of thromboxane B_2 (TXB_2) formed by COX was measured using the RIA Kit (TRK 780; Amersham, Cleveland, OH, USA).

Measurement of thromboxane A_2 and 6-keto prostaglandin $F_{1\alpha}$

Assays for TXB_2 and 6-keto prostaglandin $F_{1\alpha}$ were carried out instead of those for TXA_2 and PGI_2. TXA_2 can easily change into a semistable form (i.e., TXB_2). Accordingly, using an RIA specific for TXB_2 with an assay kit from Amersham (TRK 780; Amersham), TXB_2 was determined as an indicator of TXA_2. WPS (1.5×10^9 cells/L) was subjected to a reaction with either collagen (final concentration 50 µg/mL) or thrombin (final concentration 0.5 U/mL). The reaction was st. WPS (1.5×10^9 cells/L) was subjected to a reaction with either collagen (final concentration 50 µg/mL) or thrombin (final concentration 0.5 U/mL). The reaction was stopped by adding indomethacin (final concentration 20 µmol/L). After centrifugation at 1400 *g* for 15 min, the supernatant was used for the TXB_2 assay. Because PGI_2 has a half-life that is too short for measurement, 6-keto prostaglandin $F_{1\alpha}$ (a physiologically stable metabolite of PGI_2) was determined instead. Slices of aorta (approximately 2 cm thick) were incubated in 2 mL of 50 mmol/L Tris buffer (pH 7.4) at 37°C for 30 min to stimulate the production of 6-keto prostaglandin $F_{1\alpha}$. Formic acid was then added to the medium at a concentration of 4.8 mol/L. After extraction of fat with n-hexane, the aorta slices were air dried and weighed. The content of 6-keto prostaglandin $F_{1\alpha}$ was measured by the use of a commercially available RIA kit (TRK 790; Amersham). The radioactivity was measured with a Packard liquid scintillation counter (Tricarb 1600TR; Packard Instruments, Meriden, CT, USA). The amount of 6-keto prostaglandin $F_{1\alpha}$ production was expressed as pg/mg of dry aorta.

Measurement of leukotriene B_4 formation in polymorphonuclear leucocytes

Leukotriene B_4 synthesis in the enzyme fraction. Synthesis of leukotriene B_4 was determined according to the method of Koshihara *et al.*,[18] using prostaglandin B_1 (PGB_1) as an internal standard. The sample was adsorbed to a Sep-pack C_{18} cartridge (Waters, Massachusetts, USA) cartridge and dissolved with anhydrous methanol. It was then analysed by high-performance liquid chromatography for 5′-LPx (Waters) using the $CH_3CN : MeOH : H_2O : AcOH$ (33.6:5.4:61.1:1.0 v/v, pH 5.6) solvent system and absorbance measured at 280 nm.

Leukotriene B_4 synthesis in cells. The method of Min *et al.* was used to generate a pellet of polymorphonuclear leucocytes (PMNL).[19] The pellet was then resuspended at 1×10^7 cells/mL in Eagle's minimal essential medium (including L-glutamine) containing 30 mmol/L HEPES, and ionophore A_{23187} solution (Sigma, St Louis, IL, USA) was added to a final concentration of 5 µmoL. The arachidonic acid and PMNL were separated out before HPLC analysis for 5′-LPx.

Determination of serum lipid peroxide

The lipid peroxide (malondialdehyde) content of the serum was fluorometrically measured by using the thiobarbituric acid (TBA) method of Yagi,[20] with modifications.

Statistical analysis

All results were assessed by variance analysis (ANOVA) to investigate the standard difference among groups. If significance was found by variance analysis, the level of significance among the groups was analysed by Tukey's honestly significant difference test.

Results

Phospholipase A_2 activity in platelets

The results of measuring PLA_2 activity, defined as the first rate-limiting enzyme in the AA cascade, are shown in Fig. 1. The PLA_2 activity in the Cd-0C group (33.82 ± 0.60 Bq/1.5 x 10^9 platelets) and the Cd-0.25C group (24.79 ± 0.44 Bq/1.5 x 10^9 platelets) was increased by 117 and 60%, respectively, as compared with that in the normal group (15.45 ± 0.52 Bq/1.5 x 10^9 platelets). Conversely, the PLA_2 activity level in the Cd-0.5C group (16.07 ± 0.32 Bq/1.5 x 10^9 platelets) was not significantly different from that in the normal group, indicating that catechin-dosed groups are significantly inferior to the Cd-0C group in platelet PLA_2 activity.

Platelet PLA_2 activity was increased with chronic cadmium intoxication, but when dietary catechin was administered, the activity decreased. This tendency was inversely proportional to platelet PLA_2 activity according to the amount of green tea catechin administered.

Cyclooygenase activity in platelets

Cyclooxygenase activity is illustrated graphically in Fig. 2. The COX activity level was increased by 284% in the Cd-0C group (5.983 ± 0.22 Bq/1.5 x 10^9 platelets) compared with normal group (1.563 ± 0.10 Bq/1.5 x 10^9 platelets). In contrast, COX activity in the Cd-0.25C group (3.832 ± 0.12 Bq/ 1.5 x 10^9 platelets) and Cd-0.5C group (4.555 ± 0.14 Bq/ 1.5 x 10^9 platelets) were increased by 193% and 147%, respectively. This indicates that catechin-dosed groups were comparably lower than the catechin-free group in activating cyclooxygenase.

Thromboxane A_2 and prostacyclin synthesis

The production of TXA_2, PGI_2 and the PGI_2/TXA_2 ratio in platelets and the aorta of rats supplemented with different levels of green tea catechin are shown in Table 3. The formation of TXA_2 in platelets increased by 157% in the Cd-0C group (555 ± 32.2 pg/1.5 x 10^9 platelets) and 105% in the Cd-0.25C group (442 ± 30.9 pg/1.5 x 10^9 platelets), compared with that in the normal group (216 ± 22.5 pg/ 1.5 x 10^9 platelets). However, the Cd-0.5C group (283 ± 18.0 pg/1.5 x 10^9 platelets) and control groups had a similar level of TXA_2 formation.

The formation of PGI_2 in the aorta decreased by 23 and 18% in the Cd-0C (942 ± 52.2 pg/mg fat-free aorta) and Cd-0.25C groups (1013 ± 49.5 pg/mg fat-free aorta), respectively, compared with that in the normal group (1231 ± 90.3 pg/mg fat-free aorta). However, PGI_2 formation in the Cd-0.5C group (1114 ± 47.0 pg/mg fat-free aorta) was not significantly different from that in the normal group. The ratio of PGI_2/TXA_2 decreased by 70% in the Cd-0C group (1.74 ± 0.34) and 59% in the Cd-0.25C group (2.41 ± 0.26), compared with that in the normal group (5.85 ± 0.84), while the ratio in the Cd-0.5C group (3.96 ± 0.61) was similar to that in the normal group.

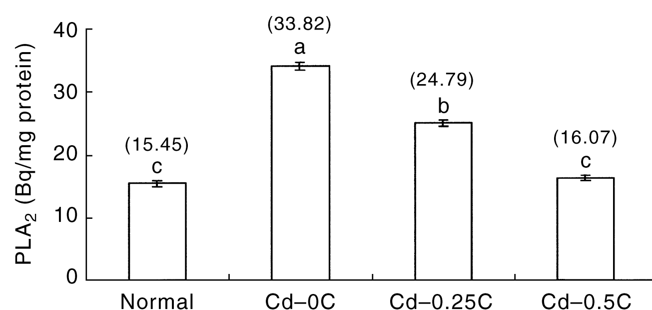

Figure 1. Effects of green tea catechin on platelet phospholipase A_2 activities in rats administered for 20 weeks. All values are mean ± SE, $n = 10$. Values with different superscripts are significantly different at $P < 0.05$.

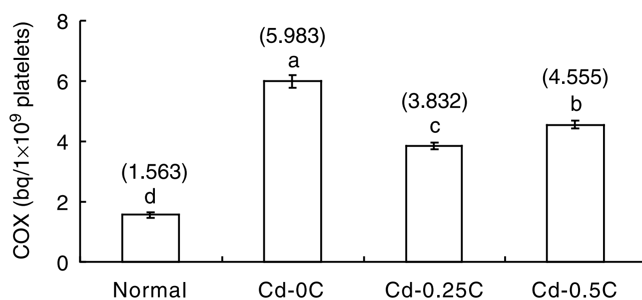

Figure 2. Effect of green tea catechin on platelet cyclooxygenase in rats administered cadmium for 20 weeks. All values are mean ± SE, $n = 10$.

Activity of 5′-lipoxygenase and leukotriene B_4 content in polymorphonuclear leucocytes

The effects of green tea catechin on 5′-LPx and LTB_4 are presented in Table 4. As the major enzyme of the leukotriene synthesis pathway (one of two AA cascades) is lipoxygenase, the activity of 5′-LPx was measured in polymorphonuclear leucocytes. Only the Cd-0C group (97.1 ± 9.82 ng/ 1×10^7 cells) showed an increase (40%; $P < 0.05$) compared with the normal group (69.2 ± 7.83 ng/1×10^7 cells). The catechin-administered groups (Cd-0.25C, 70.5 ± 4.63 ng/ 1×10^7 cells; and Cd-0.5C, 69.5 ± 8.95 ng/1×10^7 cells)

Table 4. Effects of green tea catechin on polymorphonuclear leucocyte 5′-lipoxygenase and leukotriene B_4 in rats administered cadmium over 20 weeks

Group	5′-LPx (ng/1 × 10^7 cells)	LTB_4 (ng/1 × 10^7 cell)
Normal	69.2 ± 7.83^b	9.65 ± 1.69^b
Cd-0C	97.1 ± 9.82^a	14.8 ± 0.39^a
Cd-0.25C	70.0 ± 4.63^b	11.1 ± 1.31^b
Cd-0.5C	69.5 ± 8.95^b	11.6 ± 0.46^b

All values are mean ± SEM ($n = 10$). $^{a–d}$Values with different superscripts are significantly different at $P < 0.05$. Cd-0C, catechin-free diet; Cd-0.25C, 0.25% catechin diet; Cd-0.5C, 0.5% catechin diet; 5′-LPx, 5′-lipoxygenase; LTB_4, leukotriene B_4.

Table 3. Effects of green tea catechin on platelet TXA_2, PGI_2 and PGI_2/TXA_2 ratio in rats administered cadmium over 20 weeks

Group	TXA_2 (pg/1.5 × 10^9 platelets)	PGI_2 (pg/mg fat-free aorta)	PGI_2/TXA_2 ratio
Normal	216 ± 22.5^d	1231 ± 90.3^a	5.85 ± 0.84^a
Cd-0C	555 ± 32.2^a	942 ± 52.2^b	1.74 ± 0.34^b
Cd-0.25C	442 ± 30.9^b	$1013 \pm 49.5^{b,c}$	2.41 ± 0.26^b
Cd-0.5C	283 ± 18.0^c	$1114 \pm 47.0^{a,c}$	3.96 ± 0.61^a

All values are mean ± SEM ($n = 10$). $^{a–d}$Values with different superscripts are significantly different at $P < 0.05$. Cd-0C, catechin-free diet; Cd-0.25C, 0.25% catechin diet; Cd-0.5C, 0.5% catechin diet; PGI_2, prostacyclin; TXA_2, thromboxane A_2.

were the same level as the normal group. As mentioned, the activity of 5′-LPx is in inverse proportion to the administration level of catechin, as indicated by COX measurements. The amount of LTB_4 formed was therefore proportional to the production of 5′-LPx and related to the inflammation reaction. The Cd-0C group (14.8 ± 0.39 ng/1×10^7 cells) showed a 54% increase in LTB_4 ($P < 0.01$) compared with the normal group (9.65 ± 1.69 ng/1×10^7 cells). But the Cd-0.25C group (11.1 ± 1.31 ng/1×10^7 cells) and Cd-0.5C group (11.6 ± 0.46 ng/1×10^7 cells) showed a decrease in catechin levels similar to that of the normal group. Thus, the production of LTB_4 was significantly decreased by administering catechin, as was the activity of 5′-LPx.

Serum thiobarbituric acid-reactive substance levels

The results of measuring thiobarbituric acid-reactive substances (TBARS), recognised as an index of peroxidational damage in biological membranes, are shown in Fig. 3. The total TBARS value increased by 61 and 34% in the Cd-0C and Cd-0.25C groups, respectively, compared with the normal group; however, the Cd-0.5C group was not significantly different from the normal group. The low-density lipoprotein (LDL)-TBARS increased by 133% in the Cd-0C group and 113% in the Cd-0.25C group, compared with that in the normal group. Again, the Cd-0.5C group was not significantly different from the normal group. The ratio of PGI_2/TXA_2 decreased by 70% in the Cd-0C group and 59% in the Cd-0.25C group, compared with the normal group, while the ratio in the Cd-0.5C group was similar to that in the normal group.

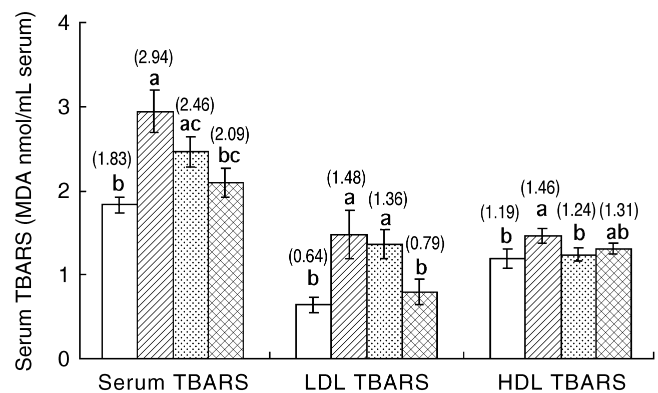

Figure 3. Effect of green tea catechin on serum thiobarbituric acid reactive substance (TBARS) levels in rats administered cadmium for 20 weeks. All values are mean ± SE, *n* = 10.

Discussion

The aim of this study was to investigate the effects of green tea catechin on pathological phenomena (e.g., thrombus, inflammation) caused by cadmium poisoning in rats. We observed the antithrombotic and anti-inflammatory effects in chronically cadmium-poisoned rats by focusing on the AA cascade. PLA_2 hydrolyses the *sn*-2 fatty acid ester bonds of glycero-3-phospholipid to produce free fatty acids,[21] accelerating the AA cascade. This metabolic procedure makes free radical groups, and increasing the PLA_2 activity stimulates free radical production. Free oxygen radicals (or lipid peroxides) activate PLA_2.[22] In this study, the PLA_2 activity is remarkably increased by 117% in the Cd-0C group and 60% in the Cd-0.25C group, compared with the normal group, but has the same level in the Cd-0.5C group as in the normal group. These results agree with those of Yang *et al.*[17] that showed that increased PLA_2 activity in diabetic rats was inhibited by administration of catechin. This is due to the inhibitive production of lipid peroxides, according to the antioxidant function of catechin. In the catechin-administered group, the decreased activity of PLA_2 agrees with the report of Moon *et al.*[23] that the antioxidant hydroxybrazilin inhibited thrombotic PLA_2 activity, and indicates catechin's role as an antioxidant.

Activity of platelet COX, a major enzyme of prostaglandin synthesis in two AA metabolism pathways, increased 284% in the Cd-0C group, 147% in the Cd-0.25C group and 193% in the Cd-0.5C group. This study showed a similar result to the diabetic rat research carried out by Takahashi *et al.*[24] Panganamala and Cornwell reported that antioxidants such as vitamin E regulate the acceleration of AA metabolism and reduce the activity of lipoxygenase and cyclooxygenase.[25] This involves an inhibition of free radical synthesis along with a simultaneous role as a chain-breaking antioxidant.

The synthesis of platelet TXA_2, which stimulates aggregation of platelets and blood vessel contraction, increased 157% in the Cd-0C group and 105% in the Cd-0.25C group, compared with the normal group. The Cd-0.5C group did show a difference; however, it recovered a level close to the normal group. This result agrees with the report of Salonen *et al.*[26] where an overdose of vitamin C (a known antioxidant, like catechin) provides a protective function to the synthesis of TXA_2. PGI_2 biosynthesis at the wall of the blood vessel inhibits platelet aggregation and stimulates dilation of blood vessels. In this study, PGI_2 levels in the aorta decreased 24% in the Cd-0C group and 18% in the Cd-0.25C group. The ratio of PGI_2/TXA_2 (thrombocyte synthesis index) decreased 70% in the Cd-0C group and 59% in the Cd-0.25C group. This is in agreement with the report of Caprino and Togna,[10] where the PGI_2/TXA_2 ratio is reduced when cadmium is administered to rats and rabbits.

The activity of 5′-LPx, the main enzyme in one of the two AA cascades, in polymornuclear leucocytes was increased 40% in the Cd-0C group compared with the normal group. The catechin-administered groups, Cd-0.25C and Cd-0.5C, showed the same levels as the normal group. LTB_4, produced by 5′LPx, acts as an inflammatory chemical mediator by inhibiting material that would otherwise have an anti-inflammatory function. In this study, the observed amount of LTB_4 increased 54% in the Cd-0C group compared with the normal group. Min *et al.*[19] have reported that the protopanasadiol saponin of ginseng reduced LTB_4 (i.e., it inhibited 5′-LPx) and affected the synthesis of LTB_4 from arachidonic acid. The serum

peroxide value, an index of internal peroxide, increased 60% in the non-catechin-administered Cd-0C group, as compared with the normal group, but in the Cd-0.5C group administered 0.5% catechin, it was reduced to the level of the normal group. In addition, LDL-TBARS increased 133% and 113% in the Cd-0C and Cd-0.25C groups, respectively. In the Cd-0.5C group, however, it decreased to the level of the normal group. High-density lipoprotein-TBARS increased in the Cd-0C group (i.e., no catechin administration), compared with the normal group, and it was reduced to the level of the normal group with catechin supplementation. LDL-TBARS of the catechin-administered groups was lower than the Cd-0C group, which means that the epicatechin and epigallocatechin of green tea strongly inhibit LDL oxidation, considered to be a significant step in the aetiological theory of arteriosclerosis. The high concentrations of total serum lipid peroxides and distinctively high LDL-TBARS concentrations observed in the Cd-0C group rather than the catechin-administered groups in this study add weight to the theories above. Integrating all the experiments, PLA_2, COX and 5′-LPx activity are increased significantly in the platelets of chronically cadmium-poisoned rats. However, the activity of these enzymes is substantially decreased in cadmium-poisoned rats administered with the antioxidant catechin. It is thought that the addition of cadmium causes increased TXA_2 synthesis and decreased PGI_2 synthesis, causing a decrease in the thrombogenesis index (PGI_2/TXA_2 ratio). Catechin was able to inhibit the activity of PLA_2 and COX, thereby improving the PGI_2/TXA_2 ratio, which indicates that catechin has an antithrombotic function. Catechin also inhibits the activity of 5′-LPx involved in normal LTB_4 synthesis, which shows that catechin has an anti-inflammatory function.

Conclusion

In rats, the activities of PLA_2, COX and 5′-LPx and the synthesis of TXA_2 were significantly increased due to cadmium poisoning. A decrease in PGI_2 synthesis caused a reduction in the PGI_2/TXA_2 ratio (considered to be an index of thrombus synthesis) and in the amount of LTB_4 (inflammatory-related matter). However, administering high doses of catechin, which has remarkable antioxidative capabilities, inhibited the activity of PLA_2, COX and 5′-LPx, improved the PGI_2/TXA_2 ratio and normalised LTB_4 synthesis. Therefore, catechin has an antithrombotic and anti-inflammatory action.

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