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Inhibitory Effects of Epigallocatechin-3-Gallate on Microsomal Cyclooxygenase-1 Activity in Platelets

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Abstract

In this study, we investigated the effect of (–)-epigallocatechin-3-gallate (EGCG), a major component of green tea catechins from green tea leaves, on activities of cyclooxygenase (COX)-1 and thromboxane synthase (TXAS), thromboxane A_2 (TXA_2) production associated microsomal enzymes. EGCG inhibited COX-1 activity to 96.9%, and TXAS activity to 20% in platelet microsomal fraction having cytochrome c reductase (an endoplasmic reticulum marker enzyme) activity and expressing COX-1 (70 kDa) and TXAS (58 kDa) proteins. The inhibitory ratio of COX-1 to TXAS by EGCG was 4.8. These results mean that EGCG has a stronger selectivity in COX-1 inhibition than TXAS inhibition. In special, a nonsteroid anti-inflammatory drug aspirin, a COX-1 inhibitor, inhibited COX-1 activity by 11.3% at the same concentration (50 μM) as EGCG that inhibited COX-1 activity to 96.9% as compared with that of control. This suggests that EGCG has a stronger effect than that of aspirin on inhibition of COX-1 activity. Accordingly, we demonstrate that EGCG might be used as a crucial tool for a strong negative regulator of COX-1/TXA_2 signaling pathway to inhibit thrombotic disease-associated platelet aggregation.

Key Words: (–)-Epigallocatechin-3-gallate (EGCG), Aspirin, Microsomal fraction, Cyclooxygenase-1, Thromboxane synthase

INTRODUCTION

An important role in the mechanism that agonists induce platelet aggregation is played by an aggregation-inducing molecule thromboxane A_2 (TXA_2) formation (Malmsten *et al.*, 1975; Lewis and Watts, 1982; Li *et al.*, 2010), which can cause circulatory disorders such as thrombosis, atherosclerosis, and myocardial infarction (Schwartz *et al.*, 1990). TXA_2 is generated from arachidonic acid (AA) released when membrane phospholipids are broken down by diverse agonists such as collagen, thrombin, and ADP, and powerfully induces platelet activation and vasoconstriction as an autacodial action (Hamberg *et al.*, 1975; Samuelsson *et al.*, 1978; Gresele *et al.*, 1991). TXA_2 production associated enzymes are cyclooxygenase (COX)-1 and thromboxane synthase (TXAS), which are located at microsomes (Carey *et al.*, 1982). COX-1 produces prostaglandin (PG)G_2 from substrate AA, TXAS produces TXA_2 from PGH_2 that oxidized from PGG_2 by endoperoxidase. Therefore, inhibition of COX-1 or TXAS is very useful to evaluate an antiplatelet effect of any substance or compound. For instance, COX-1 inhibitor aspirin and TXAS inhibitor ozagrel are being used as anti-platelet agents (Patrono, 2001).

A major catechin analogue (-)-epigallocatechin-3-gallate (EGCG, Fig. 2A) from green tea has a galloyl group at the 3' position of catechin, and is known to have an anti-platelet activity by inhibiting p38 mitogen-activated protein kinase, and extracellular signal-regulated kinase-1/2 (Lill *et al.*, 2003) and by reducing thrombin-induced $[Ca^{2+}]_i$ increase *via* inhibition of Syk and Lyn activities (Deana *et al.*, 2003). EGCG has also been reported to inhibit collagen-induced phospholipase C-γ_2 activity and TXA_2 production (Jin *et al.*, 2008). With regard to the regulatory effects of EGCG on COX-1 or TXAS activity, it is reported that EGCG failed to inhibit COX-1 and TXAS activity (Jin *et al.*, 2008). In addition, it is known that EGCG did not affect COX-1 expression in human prostate carcinoma cells (Hussain *et al.*, 2005). In this study, however, we clarified and characterized the inhibitory effects of EGCG on COX-1 and TXAS activities by using microsomal enzymes both having cy-

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tochrome c reductase activity and expressing COX-1 (70 kDa) and TXAS (58 kDa) proteins. This provides a novel information of EGCG-mediated antiplatelet activity.

MATERIALS AND METHODS

Materials

Collagen was obtained from the Chrono-Log Corporation (Havertown, PA, USA). (–)-Epigallocatechin-3-gallate (EGCG), aspirin, and other reagents were obtained Sigma Chemical Corporation (St. Louis, MO, USA). COX-fluorescent activity assay kit and thromboxane A_2 synthase substrate prostaglandin H_2 were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Cytochrome c reductase (NADPH) assay kit and other reagents were obtained Sigma Chemical Corporation (St. Louis, MO, USA). All other chemicals and reagents used in the present study were of the analytical grade.

Preparation of rat washed platelets

We prepared rat washed platelets to obtain microsomal fraction, a source of COX-1 and TXAS. Blood was collected from Sprague-Dawley (SPD) rats (6-7 weeks, male), and anticoagulated with acid-citrate-dextrose (ACD) solution (0.8% citric acid, 2.2% sodium citrate, 2.45% glucose). Platelet-rich plasma was centrifuged at 125 × *g* for 10 min to remove the red blood cells, and the platelets were washed twice with washing buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 5.5 mM glucose, and 1 mM EDTA, pH 7.4). The washed platelets were then resuspended in suspension buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 0.49 mM $MgCl_2$, 5.5 mM glucose, 0.25% gelatin, pH 7.4) to a final concentration of 5×10^8/ml. All of the above procedures were carried out at 25°C to avoid platelet aggregation on cooling. The ethics committee for animal experiments of Inje University (Gimhae, Gyungnam, Korea) approved these animal experiments.

Isolation of microsomal fraction

Washed platelets (10^8 platelets/ml) containing suspension buffer (pH 7.4) with 1% protease inhibitor were sonicated at sensitivity 100% for 20 sec, 1 cycle, and 10 times on ice with a sonicator (Bandelin, HD2070, Germany) to obtain platelet lysates. Homogenates was centrifuged at 1,500 × *g* for 15 min, then the supernatant was centrifuged at 10,000 × *g* for 15 min, which pellets were termed to F1-cellular fraction (Mancuso *et al.*, 2003). Then, the remnants were ultracentrifuged at 105,000 × *g* for 1 hr at 4°C to obtain microsomal fraction (F2-cellular fraction) containing endoplasmic reticulum (ER) membrane (Carey *et al.*, 1982). The supernatant was termed to F3-cellular fraction. All of the separated fractions (homogenates, F1-, F2-, and F3-cellular fraction) were identified by cytochrome c reductase (a marker enzyme of ER membrane) (Lagarde *et al.*, 1981) and used as enzyme sources in Western blot as described below.

Cytochrome c reductase activity assay

Cytochrome c reductase is a flavoprotein localized in the ER. Cytochrome c reductase activity of the fractions (homogenates, F1-, F2-, and F3-cellular fraction) was assayed by using cytochrome c reductase (NADPH) assay kit (Sigma Chemical Corp, St. Louis, MO, USA). The reaction was initiated by addition of NADPH and the reduction of cytochrome c is monitored by the increase of absorbance at 550 nm for 7 min with kinetic program.

Western blot analysis of cyclooxygenase-1 (COX-1) and TXA_2 synthase (TXAS)

Protein concentrations in the separated fractions were measured by using Bradford method. Proteins of each fraction (30 μg) were separated on 8% SDS-PAGE. The separated proteins were electrophoretically transferred to polyvinylidine difluoride (PVDF) membrane (GE Healthcare, New Jersey, USA). Immunoblot was prepared by using primary antibody (COX-1, 1:200, TXAS, 1:1000) and horseradish peroxidase conjugate secondary antibody at a dilution of 1:10,000 from Santa Cruz Biotechnology Inc., CA (USA). After secondary antibody (anti-mouse IgG-horseradish peroxidase conjugate (HRP) or anti-goat IgG-HRP) treatment, detection of antibody-bound protein in the membrane was performed with enhanced chemiluminesence (ECL) solution (GE Healthcare, Buckinghamshire, UK).

Cyclooxygenase-1 (COX-1) activity assay

For the measurement of COX-1 activity, the F2-cellular fraction (microsomal fraction, 20 μg proteins) of platelets was pre-incubated with or without various concentrations of EGCG or aspirin at 37°C for 30 min. COX-1 activity of the treated F2-cellular fraction was assayed with COX-1 fluorescent assay kit (Cayman Chemical Co, Ann Arbor, MI, USA).

Thromboxane A_2 synthase (TXAS) activity

For the measurement of TXAS activity of the F2-cellular fraction (microsomal fraction, 20 μg proteins) was preincubated with ozagrel (11 nM, IC_{50}), a positive control as a TXAS inhibitor, and with or without various concentrations of EGCG at 37°C for 5 min. The reaction is initiated by the addition of PGH_2. Incubation is allowed to proceed for 1 min at 37°C. The reactions are terminated by the addition of 1 M citric acid. After neutralization with 1 N NaOH, the amount of TXB_2, a stable metabolite of TXA_2, was determined by using TXB_2 EIA kit according to the procedure described by manufacturer.

Statistical analysis

The experimental results are expressed as the means ± S.E.M. accompanied by the numbers of observations. Data were assessed by analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared by the Newman-Keuls method. *p* less than 0.05 was considered statistically significant.

RESULTS

Determination of enzyme source of COX-1 and TXAS

In our previous reports (Ok *et al.*, 2012), we reported that the amount of TXA_2 (determined as TXB_2) in intact platelets was 4.0 ± 0.1 ng/10^8 platelets, and this was markedly increased to 356.1 ± 46.9 ng/10^8 platelets when platelets were stimulated with collagen (10 μg/ml) (Ok *et al.*, 2012). EGCG (50 μM), however, powerfully reduced TXA_2 production to 89.5% (Ok *et al.*, 2012). These results show that EGCG may inhibit the activity of COX-1 or TXAS to suppress the production of TXA_2 in collagen-induced platelet aggregation. Therefore, to deter-

mine whether the suppression of TXA_2 by EGCG was involved in inhibition of COX-1 or TXAS, we needed enzyme sources expressing both COX-1 and TXAS proteins. Therefore, we separated F1-, F2-, and F3- cellular fractions from platelet lysates, and measured the activity of cytochrome c reductase, an endoplasmic reticulum (microsomes) marker enzyme. As shown in Fig. 1A, we found that the F2-cellular fraction has the highest specific activity of cytochrome c reductase. Next, we determined which fraction from platelet lysates expresses both COX-1 and TXAS proteins. As the result, remarkably high expressions of COX-1 (70 kDa) and TXAS (58 kDa) were observed in F2-cellular fraction (Fig. 1B). Because these are accordance with that F2-cellular fraction has the highest activity of cytochrome c reductase, it is thought that F2-cellular fraction is microsomal fraction. This microsomal fraction was used to determine the activity of COX-1 and TXAS.

Effects of EGCG on COX-1 and TXAS activities

As shown in Fig. 2B, COX-1 activity in the absence of EGCG (control) was 1.59 ± 0.11 nmol/min/protein-mg. However, EGCG inhibited dose-dependently COX-1 activity, and EGCG at 50 μM inhibited COX-1 activity to 96.9% as compared with that (1.59 ± 0.11 nmol/min/protein-mg) of control (Table 1). TXAS activity in EGCG-untreated control was 0.18 ± 0.01 nmol/min/protein-mg. However, EGCG (50 μM) inhibited TXAS activity to 0.14 ± 0.01 nmol/min/protein-mg. In addition, 11 nM of ozagrel used as a positive control inhibited strongly TXAS activity to 0.02 ± 0.00 nmol/mim/protein-mg (Fig. 3). The inhibitory degree by 50 μM of EGCG in TXAS activity was 20% (Table 1). Comparing the inhibitory effects of EGCG (50 μM) in COX-1 and TXAS activities, the inhibitory degree (96.9%) of COX-1 by EGCG is about 4.8 fold high as compared with that (20%) of TXAS (Table 1). We next investigated the effect of aspirin, COX-1 inhibitor, on inhibition of COX-1 activity.

Effects of aspirin on COX-1 activity

As shown in Fig. 4, various concentrations of aspirin (50 to 800 μM) inhibited mildly COX-1 activity (Fig. 4). Fifty μM of aspirin that corresponds to EGCG inhibiting COX-1 activity to 96.9% inhibited COX-1 activity to 11.3% (Table 2). The inhibitory degree ratio of EGCG (50 μM) to aspirin (50 μM) is calculated as 8.6, which means that EGCG (50 μM) has stronger inhibitory effect of 8.6 fold than aspirin (50 μM) on COX-1 activity. In addition, 100 μM of aspirin suppressed control activity (1.59 ± 0.11 nmol/protein-mg/min) to 1.36 ± 0.10 nmol/protein-mg/min (inhibition of 14.5%) (Fig. 4, Table 2). We analyzed the combination effect (Berenbaum, 1989; Gaddum, 1940) of EGCG (50 μM) with aspirin (100 μM) on COX-1 activity inhibition. Basal COX-1 activity (1.59 ± 0.11 mmol/mg-protein/min) was inhibited to 14.5 % by aspirin (100 μM) (Table 2). In the presence of both EGCG (50 μM) and aspirin (100 μM), COX-1 activity was inhibited 98.7% as compared with that of basal activity (Table 2), which inhibitory degree (98.7%) is lower than the sum (111.4%) of the inhibitory degree (96.9%) by EGCG (50 μM), and the inhibitory degree (14.5%) by aspirin (100 μM). This means that the combination of EGCG (50 μM) and aspirin (100 μM) has no combination effect on COX-1 activity, but may have antagonistic effect each other. The inhibitory

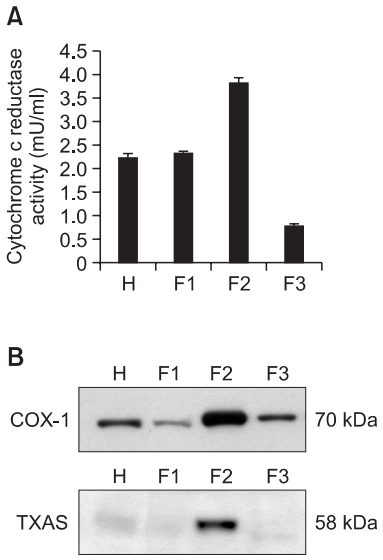

Fig. 1. Determination of enzyme source of cyclooxygenase (COX)-1 and thromboxane A_2 synthase (TXAS). (A) Cytochrome c reductase activities in homogenates, F1-, F2-, and F3-cellular fraction. NADPH-cytochrome c reductase, the marker enzyme for dense tubular system in platelets (100 μg of total protein) was assayed as using NADPH cytochrome c reductase kit (Sigma). (B) Western blot analysis of COX-1 and TXAS in homogenates, F1-, F2-, and F3-cellular fraction. 30 μg proteins in homogenates, F1-, F2-, and F3-cellular fraction from platelets were separated by 8% SDS-PAGE, transferred to PVDF membranes, and detected by ECL. H: homogenates; F1: F1-cellular fraction; F2: F2-cellular fraction (microsomal fraction); F3: F3-cellular fraction.

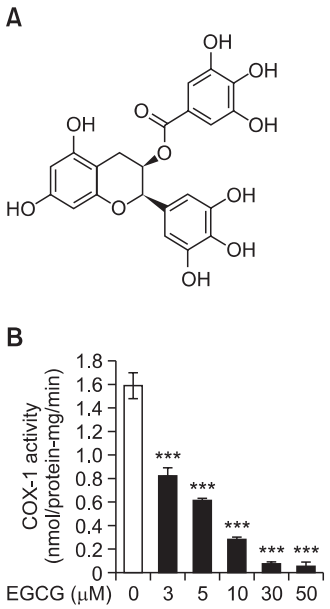

Fig. 2. Effects of EGCG on cyclooxygenase (COX)-1 activity. (A) Chemical structure of EGCG from Green tea leaves. (B) Effect of EGCG on COX-1 activity in F2-cellular fraction (microsomal fraction). The platelet F2-cellular fraction (20 μg of protein) was preincubated for 30 min at 37°C with or without various concentration of EGCG. The enzyme activity was measured with COX fluorescent assay kit (Cayman). The data are given as the means ± S.E.M. (n=4). ***p<0.001 compared with preincubated F2-cellular fraction (microsomal fraction) without EGCG.

Table 1. Inhibitory degree of EGCG in COX-1- and TXAS- activities

	COX-1 (nmol/protein-mg/min)	Inhibition (%)	TXAS (nmol/protein-mg/min)	Inhibition (%)	COX-1 / TXAS
Control	1.59 ± 0.11	0	0.18 ± 0.01	0	0
EGCG (50 μM)	0.05 ± 0.04	96.9	0.14 ± 0.01	20	4.8

Data were from Fig. 2B, and Fig. 3. Inhibition (%) = (Control–EGCG (50 μM)) / Control× 100. COX-1 / TXAS: inhibitory ratio of COX-1 to TXAS by EGCG.

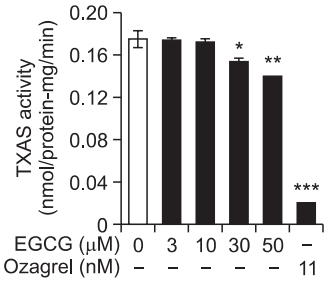

Fig. 3. Effect of EGCG on thromboxane synthase activity The platelet F2-cellular fraction (microsomal fraction, 20 μg proteins) was preincubated for 5 min at 37°C with or without various concentration of EGCG. The reaction was initiated by the addition of 5 μM PGH_2 and terminated by the addition by 1 M citric acid. The activity was reflected by the production of PGH_2 mediated TXB_2, which was determined by TXB_2 EIA kit (GE Healthcare). The data are given as the means ± S.E.M. (n=4). **p*<0.05, ***p*<0.01, ****p*<0.001 compared with preincubated F2-cellular fraction without EGCG.

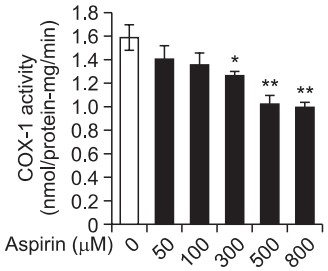

Fig. 4. Effects of aspirin on COX-1 activity. The platelet F2-cellular fraction (microsomal fraction, 20 μg proteins) was preincubated with or without various concentration of aspirin for 30 min at 37°C. The enzyme activity was measured with COX fluorescent assay kit (Cayman). The data are given as the means ± S.E.M. (n=4). **p*<0.05, ***p*<0.01 compared with preincubated F2-cellular fraction without aspirin.

degree of COX-1 by aspirin was largely beyond the scope of those concentrations (50 to 800 μM) (Fig. 4), IC_{50} value of aspirin in COX-1 inhibition could not be calculated.

Effects of EGCG on COX-1 protein expression

Western blot analyses were carried out to investigate if the inhibition of COX-1 by EGCG or aspirin is resulted from suppression of COX-1 protein (70 kDa) expression. As shown in Fig. 5, the degree of COX-1 protein expression by various concentration (10 to 50 μM) of EGCG, and aspirin (50, 100 μM) were not altered as compared with that of control, without of EGCG or aspirin. These results suggest that the inhibition of COX-1 activity by EGCG or aspirin is independent on reduction in amount of COX-1 protein, COX-1 mRNA.

DISCUSSION

TXA_2 is generated from arachidonic acid (AA) released when membrane phospholipids are broken down by diverse agonists such as collagen, thrombin, and ADP (Hamberg *et al.*, 1975; Samuelsson *et al.*, 1978; Gresele *et al.*, 1991). In previous our report (Ok *et al.*, 2012), it was obvious that EGCG elevates cAMP without affecting cGMP to reduce intracellular Ca^{2+}, required for the release of TXA_2 precursor AA from platelet membrane phosphoslipids. EGCG is also known to inhibit collagen-induced phospholipase C-γ_2 activity to reduce intracellular Ca^{2+} mobilization and TXA_2 production (Jin *et al.*, 2008). TXA_2 is a strong agonist on resting platelets as a positive feedback promoter on activated platelets (Ruggeri, 2002; Jennings, 2009), and is also a vasoconstrictor and a bronchoconstrictor (FitzGerald, 1991). Thus, a compound that can inhibit TXA_2 production-associated enzymes (COX-1 or TXAS), TXA_2 production or TXA_2 action has a potential application as an anti-thrombotic agent (Clutton *et al.*, 2001). Phytochemicals such as ginseng saponin (Lee *et al.*, 2012), chlorogenic acid (Cho *et al.*, 2012), caffedymine (Park, 2007) and sanguinarine (Jeng *et al.*, 2007) inhibited TXA_2 production in agonists-stimulated platelets, which were involved in inhibition of COX-1 activity. These previous reports are accord with fact that the inhibition of agonists-induced platelet aggregation is supported by inhibition of COX-1 rather than TXAS (Lewis and Watts, 1982; Jang *et al.*, 2002). This is supported from result that EGCG inhibits potently COX-1 activity to 96.9%, but mildly inhibited TXAS activity to 20% as compared with those of control (Fig. 2B, 3, Table 1). Because these results suggest that inhibition of TXA_2 by EGCG (Ok *et al.*, 2012) is responsible for the suppression of microsomal COX-1 rather than TXAS, it is shown that EGCG may be used as a COX-1 inhibitor. In addition, it is known that EGCG elevated prostaglandin D_2 (PGD_2), an inhibitor of platelet aggregation, even though EGCG did not inhibit COX-1 and TXAS activities in AA-induced platelet aggregation (Jin *et al.*, 2008). Because intracellular PGD_2 is known to elevate cAMP level to inhibit platelet aggregation (Armstrong, 1996), EGCG that only elevates Ca^{2+}-antagonistic cAMP level (Ok *et al.*, 2012) may involve also in inhibition of Ca^{2+}-mobilization (Jin *et al.*, 2008; Ok *et al.*, 2012), then Ca^{2+}-dependent phospholipase C-γ_2 ($PLC\gamma_2$) is inhibited by EGCG, which is resulted in inhibition of AA release from membrane phosphatidylinositol 1,4,5-tri-

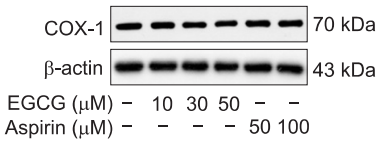

Fig. 5. Effects of EGCG on COX-1 protein expression. 30 μg proteins in F2-cellular fraction were separated by 8% SDS-PAGE, transferred to PVDF membranes, and detected by ECL.

Table 2. Effects of EGCG and aspirin on COX-1 activity

	COX-1 activity (nmol/protein-mg/min)	Inhibitory degree (%)
Basal	1.59 ± 0.11	0
EGCG (50 μM)	0.05 ± 0.004**	96.9
Aspirin (50 μM)	1.41 ± 0.11	11.3
Aspirin (100 μM)	1.36 ± 0.01	14.5
EGCG (50 μM) + Aspirin (100 μM)	0.02 ± 0.01	98.7

The platelet F2-cellular fraction (microsomal fraction, 20 μg proteins) was preincubated for 30 min at 37°C with or without EGCG (50 μM) or aspirin (50, 100 μM). The enzyme activity was measured with COX fluorescent assay kit (Cayman). The data are given as the means ± S.E.M. (n=4). ***p*<0.01 compared with preincubated F2-cellular fraction without EGCG or aspirin. Inhibitory degree (%) = (Basal – factors) / Basal × 100 (factors; EGCG, aspirin, EGCG + aspirin)

sphosphate (Jin *et al.*, 2008). Therefore, EGCG might have multiple action modes, such as cAMP elevation, $PLC\gamma_2$ inhibition, Ca^{2+}-mobilization inhibition and VASP-Ser^{157} phosphorylation, to inhibit platelet aggregation (Jin *et al.*, 2008; Ok *et al.*, 2012). IC_{50} value of a substance is contributed to understand the inhibitory sensitivity against an enzyme activity. Jeng *et al.* (2007) reported the IC_{50} value of sanguinarine in COX-1 inhibition, phytochemical, is 28 μM. In this study, IC_{50} value of EGCG in inhibition of COX-1 activity was calculated as 3.37 μM (Fig. 2), which value is lower than sanguinarine. It was obvious that the inhibition of COX-1 activity by EGCG is independent on reduction of COX-1 protein expression (Fig. 5), indicating reduction of COX-1 mRNA. It is known that aspirin is involved in inhibition of COX-1 activity *via* acetylation of COX-1 protein (Roth *et al.*, 1975; Dewitt *et al.*, 1990). At present, it is unknown how EGCG inhibits COX-1 activity, and if EGCG acetylates COX-1 protein in the same as aspirin. With regard to this, further more study is necessary in the future. It is known that increased cellular cAMP selectively inhibits COX-1 activity without altering COX-1 protein expression in bovine aortic endothelial cells (Samokovlisky *et al.*, 1999). In special, platelet COX-1 activity is known to inhibit by cAMP (Schafer *et al.*, 1980). Considering these reports (Schafer *et al.*, 1980; Samokovlisky *et al.*, 1999), and the effects of EGCG that increases markedly cAMP *via* adenylate cyclase activation and subsequently phosphorylates VASP-Ser^{157} through A-kinase activation to inhibit Ca^{2+}-mobilization and TXA_2 production on collagen-induced platelet aggregation (Ok *et al.*, 2012), it is thought that EGCG might involve in inhibition of COX-1 activity (Fig. 2B) by stimulating adenylate cyclase/cAMP/A-kinase/VASP-Ser^{157} phosphorylation pathway. Aspirin for the prevention of cardiovascular disease is known to increase the risk of gastrointestinal bleeding, cerebral haemorrhage (Sanmuganathan *et al.*, 2001; Pignone and Williams, 2010). EGCG from traditional green tea, one of the most popular beverages, may be used to treat platelet-mediated thrombotic disease by inhibiting potently TXA_2 production (Ok *et al.*, 2012). It is known that EGCG inhibits prostate- and colon- carcinogenesis by suppressing COX-2 protein expression (Hussain *et al.*, 2005; Peng *et al.*, 2006). PGE_2 that is produced from AA by COX-2/PGE_2 synthase results in inflammation (Trebino *et al.*, 2003). If so, it is thought that EGCG may have also anti-inflammatory effect by inhibiting COX-2 activity in inflammatory leukocytes, in the same way as EGCG has anti-prostate and –colon carcinogenic effects by inhibiting COX-2 activity (Hussain *et al.*, 2005; Peng *et al.*, 2006). Because both COX-1/TXA_2 pathway-induced platelet aggregation and COX-2/PGE_2 pathway-induced inflammation are the cause of atherosclerosis, it is thought that EGCG could contribute to the treatment of cardiovascular disease.

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