ELSEVIER

Biochemical Pharmacology 62 (2001) 1175–1183

Biochemical Pharmacology

Effects of purified green and black tea polyphenols on cyclooxygenase- and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues

Jungil Hong[a], Theresa J. Smith[b], Chi-Tang Ho[c], David A. August[d], Chung S. Yang[a,*]

[a]*Laboratory for Cancer Research, College of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ, 08854-8020, USA*

[b]*Basic Pharmaceutical Science, College of Pharmacy, University of South Carolina, Columbia, SC, 29208, USA*

[c]*Department of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ, 08901, USA*

[d]*Department of Surgery, University of Medicine & Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ, 08901, USA*

Received 19 December 2000; accepted 23 March 2001

Abstract

The effects of green and black tea polyphenols on cyclooxygenase (COX)- and lipoxygenase (LOX)-dependent arachidonic acid metabolism in normal human colon mucosa and colon cancers were investigated. At a concentration of 30 μg/mL, (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), and (-)-epicatechin-3-gallate (ECG) from green tea and theaflavins from black tea inhibited LOX-dependent activity by 30–75%. The formation of 5-, 12-, and 15-LOX metabolites was inhibited to a similar extent. Tea polyphenols also inhibited COX-dependent arachidonic acid metabolism in microsomes from normal colon mucosa, with ECG showing the strongest inhibition. The formation of thromboxane (TBX) and 12-hydroxyheptadecatrienoic acid (HHT) was decreased to a greater extent than other metabolites. The inhibitory effects of tea polyphenols on COX activity, however, were less pronounced in tumor microsomes than in normal colon mucosal microsomes. Theaflavins strongly inhibited the formation of TBX and HHT, but increased the production of prostaglandin E_2 (PGE_2) in tumor microsomes. The enhancing effect of theaflavins on PGE_2 production was related to the COX-2 level in the microsomes. Although theaflavin inhibited ovine COX-2, its activity in the formation of PGE_2 was stimulated by theaflavin when ovine COX-2 was mixed with microsomes, suggesting that theaflavin affects the interaction of COX-2 with other microsomal factors (e.g. PGE synthase). The present results indicate that tea polyphenols can affect arachidonic acid metabolism in human colon mucosa and colon tumors, and this action may alter the risk for colon cancer in humans.

Keywords: Tea; Polyphenols; Cyclooxygenase; Lipoxygenase; Colon; Arachidonic acid

1. Introduction

Tea (*Camellia sinensis*) is one of the most commonly consumed beverages in the world. Green tea is the dried leaves of the tea plant, containing various polyphenols such as flavanols, flavonols, flavandiols, and phenolic acids as well as alkaloids such as caffeine. Catechins, the major constituents of green tea polyphenols, including EGCG, EGC, ECG, and EC, account for about 30% of the dry weight of the water-extractable materials. EGCG is the most abundant catechin, followed by EGC [1]. In the manufacturing of black tea, a large portion of the catechins are converted to TFs and thearubigens, which are responsible for the taste and the dark brown color of black tea. The remaining catechins account for 3–10% of solids in brewed black tea [2].

The biological effects of tea and tea constituents have been studied by many investigators, and this subject has been reviewed [3]. The inhibitory action of tea and tea components against chemically induced carcinogenesis has been demonstrated in animal models, including cancer of the skin, lung, esophagus, stomach, liver, small intestine, pancreas, colon, bladder, prostate, and mammary glands

* Corresponding author. Tel.: +1–732-445–5360; fax: +1–732-445–0687.

E-mail address: csyang@rci.rutgers.edu (C.S. Yang).

Abbreviations: COX, cyclooxygenase; LOX, lipoxygenase; EGCG, (-)-epigallocatechin-3-gallate; EGC, (-)-epigallocatechin; ECG, (-)-epicatechin-3-gallate; EC, (-)-epicatechin; TF, theaflavin; TF3-G, theaflavin 3-gallate; TF3′-G, theaflavin 3′-gallate; TFdiG, theaflavin 3,3′-digallate; PGE_2, prostaglandin E_2; HETE, hydroxyeicosatetraenoic acid; HHT, 12-hydroxyheptadecatrienoic acid; and TBX, thromboxane.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00767-5

[1,3–7]. Although epidemiological studies on the relationship between tea consumption and human cancers have not been conclusive, many studies suggest a protective role of tea consumption against the development of various types of cancers [8–10]. Many mechanisms have been suggested for the anticarcinogenic effect of tea polyphenols, including antioxidative activities, inhibition of enzymes related to tumor promotion such as ornithine decarboxylase, protein kinase C, cyclooxygenase, and lipoxygenase, inhibition of activator protein-1 (AP-1), and inhibition of angiogenesis [1,11–15].

Colorectal cancer is one of the most frequent types of cancer in Western countries and is the second leading cause of cancer death in the United States [16]. Significant reduction in the incidence and mortality rates of colorectal cancer among regular users of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs), which are inhibitors of COX, has been observed [17]. Interestingly, overexpression of COX-2, an inducible isoform of COX, has been observed in various types of cancers such as those of the colon, lung, breast, and esophagus [18–21]. PGE_2, a metabolite derived from COX-catalyzed arachidonic acid metabolism, was reported to be involved in cell hyperproliferation, mitogenesis, invasiveness, and angiogenesis as well as in the inhibition of apoptosis [22–25]. Accordingly, the inhibition of COX is of particular interest in the search for chemopreventive agents. LOX catalyzes the oxidation of arachidonic acid to HETEs. Three LOXs (5-LOX, 12-LOX, and 15-LOX) have been reported to be present in human tissues. LOXs and their HETE products have also been reported to be important regulators in the proliferation and apoptosis of cancer cell lines [26–28]. It was observed that the expression of 15-LOX was increased significantly in human colorectal cancer cells compared with the adjacent normal epithelial cells [29]. These results suggest that the regulation of arachidonic acid metabolism is important in the prevention of many types of cancer, especially cancers of the digestive tract.

The colonic epithelium is directly exposed to dietary compounds en route to their excretion. Accordingly, the risk of neoplastic development in the colon is dependent upon the diet. Inhibition of arachidonic acid metabolism could be an effective way of decreasing the risk for colorectal cancer. Recently, we observed that the PGE_2 level in human rectal mucosa biopsy tissues was decreased significantly at 4 and 8 hr after the ingestion of green tea [30]. It is possible that tea polyphenols may inhibit COX- and LOX-dependent arachidonic acid metabolism in human colonic tissues. Decreased skin COX and LOX activities after feeding green or black tea to SENCAR mice have been reported by Katiyar *et al.* [12,13]; however, it is not clear whether the decreased COX and LOX activities were due to lower levels of COX and LOX enzymes in the tea-treated mice or to the inhibition of these enzyme activities by residual tea polyphenols in the skin. The effects of tea polyphenols on human colon tissues are not clear. In this study, the effects of tea polyphenols on COX- and LOX-dependent activities on human colon mucosa and colon tumors were investigated. Our results indicate that, whereas tea polyphenols are inhibitors of COX and LOX activities in general, TFs enhanced the formation of PGE_2 in tumor microsomes.

2. Materials and methods

2.1. Chemicals

[1-^{14}C]Arachidonic acid (54.6 mCi/mmol) was purchased from NEN Life Science. NS-398, indomethacin, arachidonic acid metabolite standards, ovine COX-2, and human COX-2 monoclonal antibody were purchased from the Cayman Chemical Co. The HHT standard was purchased from Biomol Research Laboratory. Ovine COX-1 and human recombinant COX-2 were purchased from the Oxford Biomedical Research Co. All other chemicals were purchased from the Sigma Chemical Co.

2.2. Tea polyphenols

The purified green tea polyphenols EGCG, EGC, and ECG, and the TF mixture (TFs) from black tea were gifts from the Thomas J. Lipton Co. TFdiG was provided by the Misui Norin Co. Ltd. TFs consisted of TF, TF3-G, TF3′-G, and TFdiG. The TF, TF3-G, and TF3′-G were purified from a mixture of TFs by reverse-phase HPLC using an ODS column (MCM-pak C_{18}, 4.6 mm $\times$ 150 mm, ESA Inc.). HPLC was performed under isocratic conditions using 65% solvent A (1.75% acetonitrile, 0.15% tetrahydrofuran, 0.5% acetic acid, and 97.6% water; pH 3.4) and 35% solvent B (58% acetonitrile, 12.5% tetrahydrofuran, 0.5% acetic acid, and 29% water; pH 3.4) at a flow rate of 1 mL/min. The purities of TF3-G, TF3′-G, and other tea polyphenols were determined to be $>$ 98% by HPLC. All of the tea polyphenols were dissolved in 100 mM Tris–HCl buffer solution (pH 7.4) at a concentration of 0.5 mg/mL and stored at -80^{o}.

2.3. Human colon tissues

Tissue samples were surgical resections obtained from the Tissue Retrieval Service of the Cancer Institute of New Jersey. The tumor colon tissues included: a case of poorly differentiated carcinoma (from an 82-year-old female patient), a case of moderately differentiated adenoma (from a 47-year-old female patient), and a case of carcinoma with mucin (from a 55-year-old female patient). Normal colon tissues were from a 53-year-old female, a 65-year-old male, and a 72-year-old male patient. At the time of surgery, 2–10 g of colon tissue was obtained, fresh-frozen in liquid nitrogen within 1 hr, and stored at -80^{o} before use.

2.4. Preparations of microsomes and cytosols

Normal colonic mucosa was isolated by stripping off the muscle layer just before homogenization. For preparation of tumor microsomes, the tumor tissues were dissected and homogenized. The normal colon mucosa and colon tumor samples were homogenized with a polytron (Brinkmann homogenizer) in 4 vol. of homogenizing buffer (100 mM Tris–HCl, pH 7.4) on ice. The homogenates were centrifuged at 9000 *g* for 20 min at 4°. The supernatants were then centrifuged at 105,000 *g* for 90 min at 4°. The cytosolic fraction was collected and kept at -80^{o} for further analysis. The pellets were washed with 3–4 vol. of washing buffer (1.15% KCl, 10 mM EDTA, pH 7.4), and the microsomes were recovered by centrifugation at 105,000 *g* for 60 min at 4°. The microsomal pellets were resuspended in 0.25 M sucrose solution and stored at −80°. The protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce).

2.5. COX and LOX assays

COX activity in microsomes was assayed at 37^{o} for 30 min in a reaction mixture (200 μL) consisting of 0.1 mg microsomal protein, 20 μM (0.22 μCi) arachidonic acid, 1 mM reduced glutathione, 1 mM epinephrine, and 10 mM EDTA in a 100 mM Tris–HCl buffer, pH 7.4. Cytosolic LOX activity was measured at 37^{o} for 30 min in a reaction mixture (200 μL) containing 0.6 mg cytosolic protein, 20 μM arachidonic acid, and 2 mM $CaCl_2$ in a 100 mM Tris–HCl buffer, pH 7.4. Tea polyphenols were dissolved in 100 mM Tris–HCl buffer, NS-398 in DMSO, and indomethacin in ethanol. The inhibitor solutions or vehicles accounted for less than 1% of the volume of the reaction mixture. At this concentration, DMSO and ethanol had no effect on the enzyme activity. Ovine COX-1 and COX-2 activities were assayed under similar conditions, except that 3 units of the enzyme and an incubation time of 10 min were used. For determination of the kinetics of the inhibition, arachidonic acid concentrations of 3–40 μM were used. Both reactions of COX and LOX were initiated by the addition of 20 μL of sonicated substrate in buffer solution after a 5-min preincubation. The reactions were terminated by the addition of 15 μL of 0.5 N HCl, and the reaction tubes were immediately placed on ice for 5 min. Unmetabolized arachidonic acid and its metabolites were extracted from aqueous solution by vortexing with 0.6 mL of ethyl acetate for 1 min. After centrifuging at 3000 rpm (Sorvall RT6000B, Dupont) for 10 min at 4°, the organic layer was transferred to a clean vial, and the remaining aqueous layer was extracted again with 0.5 mL ethyl acetate. The pooled organic extracts were dried in a N_2 purging-evaporator. The dried extracts were stored at -20^{o} before HPLC analysis.

2.6. HPLC analysis

The dried metabolites in the vials were redissolved in acetonitrile. After centrifugation, 40 μL of the acetonitrile solutions was mixed with 60 μL of nanopure water, and the mixture was injected onto a reverse-phase HPLC system equipped with a Waters automated gradient controller, two Waters 6000A pumps, Waters 440 UV detectors, a Waters 712B WISP autoinjector, a symmetry C18 column (4.6 × 150 mm, Waters), and a Radiomatic Flo-One/Beta radioflow detector (Radiomatic Instruments and Chemical Co.). It was eluted with a linear gradient from 7% solvent A (100% acetonitrile containing 0.01% acetic acid) and 93% solvent B (75% water and 25% acetonitrile containing 0.01% acetic acid) to 14% solvent A and 86% solvent B over a 27-min time period. The gradient was then changed to 40% solvent A and 60% solvent B over a 2-min period, followed by a linear gradient to 47% solvent A and 53% solvent B over 25 min, and then a 10-min period of 100% solvent A at a flow rate of 1 mL/min. The radioactive fractions were detected using the radioflow detector, while the non-radioactive co-eluting standards were detected at wavelengths of 200 nm for COX-dependent metabolites and at 230 nm for LOX-dependent metabolites.

2.7. Data analysis

The mechanism of inhibition and apparent kinetic parameter were determined by Dixon plots. Statistical significance was evaluated using Student's *t*-test.

3. Results

3.1. Identification of arachidonic acid metabolites

After incubation of 0.1 mg of colon microsomal protein with 20 μM arachidonic acid for 30 min, approximately 3–6% of arachidonic acid was converted to COX metabolites, and several products were identified. With human colon microsomes, the metabolites were PGE_2, 6-keto $PGF_1\alpha$, TBX, HHT, and HETE products (Fig. 1A). The identities of the arachidonic acid metabolites were established by co-elution with authentic standards. The retention times of 6-keto $PGF_1\alpha$, TBX B_2, $PGF_2\alpha$, PGE_2, HHT, 15-HETE, 11-HETE, 8,12-HETE, 5-HETE, and arachidonic acid were 7.5, 14.0, 18.0, 21.0, 40.5, 51.0, 53.0, 55.0, 57.0, and 61.0 min, respectively. Since TBX A_2 and prostacyclin (PGI_2) are labile, they were detected as TBX B_2 and 6-keto $PGF_1\alpha$, respectively. The peaks found at 33–35 min are believed to be PGH_2 and PGG_2; however, the amounts of these metabolites in all of the samples were small. The product profiles varied somewhat among microsomes. PGE_2 was the most abundant product in all of the normal and two tumor tissues, whereas HHT was the most abundant in the microsomes from a sample of poorly dif-

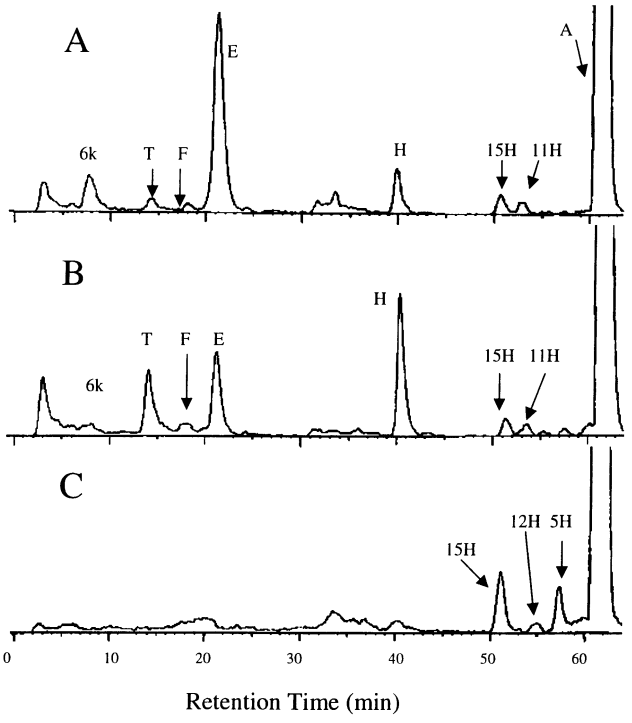

Fig. 1. Typical chromatograms of microsomal [1-^{14}C]arachidonic acid metabolites by microsomes from normal colon mucosa (A) and tumor tissue (B) and normal cytosol (C) in the HPLC system. Key: 6k, 6-keto $PGF_1\alpha$; T, TBX; F, $PGF_2\alpha$; E, PGE_2; H, HHT; 15H, 15-HETE; 11H, 11-HETE; 12H, 12-HETE; 5H, 5-HETE; and A, arachidonic acid.

ferentiated carcinoma (Fig. 1B). The activities of tumor microsomes were slightly higher than those of normal microsomes. In the incubation with cytosols, formation of 15-HETE, 12-HETE, and 5-HETE was observed. 15-HETE was the main product, accounting for over 60% of the total metabolites (Fig. 1C). There was no significant difference in catalytic activity or product profiles between the cytosolic preparations from three normal and three tumor samples (data not shown).

3.2. Effects of tea polyphenols on LOX-dependent arachidonic acid metabolism

The formation of 15-, 12-, and 5-HETEs in human colon cytosol was inhibited almost completely by 20 μM nordihydroguaiaretic acid, a known LOX inhibitor, suggesting that the reaction products are derived from LOX-dependent arachidonic acid metabolism (data not shown). All tea polyphenols (30 μg/mL) inhibited LOX activity, and the inhibitory effect was uniform on all products. Among the tea polyphenols, on a weighted basis ECG exhibited the strongest inhibitory effect, followed by EGCG, EGC, TFdiG and TFs (Table 1). In a separate experiment with 50 μM EGCG, EGC, ECG, TFs, or TFdiG, the percent inhibition was 41.2, 18.9, 63.9, 40.2, and 51.0, respectively. For all tea polyphenols, the percent inhibition and the inhibitory pattern on LOX-dependent arachidonic acid metabolism

Table 1
Inhibitory effect of tea polyphenols on colonic LOX- and COX-dependent arachidonic acid metabolism

	% Inhibition		
	LOX	COX	
	Normal mucosal cytosol	Normal mucosal microsomes	Tumor microsomes
EGCG	68.2 ± 3.1	51.3 ± 3.3	40.5 ± 0.8*
EGC	44.1 ± 3.3	38.9 ± 3.4	30.1 ± 2.0
ECG	74.4 ± 0.8	62.4 ± 2.4	64.4 ± 2.4
TFs	29.8 ± 1.1	36.6 ± 4.6	1.41 ± 1.1*
TFdiG	32.0 ± 2.1	51.2 ± 1.8	26.5 ± 7.0*

Products were analyzed from the reactions with 0.6 mg cytosolic protein, 20 μM (0.22 μCi) arachidonic acid, and 2 mM $CaCl_2$ for LOX or with 0.1 mg microsomal protein, 20 μM (0.22 μCi) arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA for COX with or without 30 μg/ml of tea polyphenols in 100 mM Tris-HCl buffer, pH 7.4. The reactions were carried out at 37° for 30 min. Percent inhibition was calculated based on the sum of the total metabolites. Data are the means ± SEM from three (LOX)-, and five or four (COX)-independent experiments.

* Significantly different ($P < 0.02$) from the corresponding values in the normal samples.

were not significantly different when either normal or tumor cytosol was used as the enzyme source (data not shown).

3.3. Effects of tea polyphenols on COX-dependent arachidonic acid metabolism

To determine the effect of tea polyphenols on COX-dependent arachidonic acid metabolism, microsomes from normal colon mucosa and tumors were utilized. The arachidonic acid metabolism in these microsomes was inhibited almost completely by 5 μM indomethacin, suggesting that the formation of metabolites is COX-dependent (data not shown). On a weighted basis, ECG exhibited the strongest inhibitory effect and inhibited the activities in normal and tumor microsomes to the same extent (Table 1). EGCG and EGC (30 μg/mL) inhibited the production of COX-dependent metabolites in normal colon by 51 and 39%, respectively. TFs and TFdiG (30 μg/mL) inhibited COX-dependent arachidonic acid metabolism in normal colon mucosa by 37 and 51%, respectively (Table 1). The inhibitory effects, however, were much lower in the incubations with the microsomes from colon carcinomas, showing 1.4 and 26.5% inhibition by 30 μg/mL of TFs and TFdiG, respectively (Table 1). In a separate experiment, the inhibition of arachidonic acid metabolism in normal microsomes by 50 μM EGCG, EGC, ECG, TFs, or TFdiG was 42.0, 27.4, 59.1, 32.2, and 59.3%, respectively (data not shown). In terms of specific metabolites, EGCG and ECG inhibited the formation of TBX and HHT to a greater extent than other COX-dependent metabolites in both normal colon and tumor microsomes (Table 2). In normal colon mucosa, TFs and TFdiG also inhibited the formation of TBX and HHT more severely than other metabolites (Table 2).

Table 2
Effects of tea polyphenols on COX-dependent arachidonic acid metabolites in normal colon mucosa and tumor microsomes

	% Inhibition					
	6-k $PGF_{1\alpha}$	TBX	PGE_2	HHT	HETEs	Total
(a) Normal colon mucosa						
EGCG	52.4 ± 8.30	69.5 ± 1.76*	47.7 ± 4.09	63.9 ± 1.78*	47.3 ± 5.27	51.3 ± 3.32
EGC	41.2 ± 10.8	39.6 ± 5.42	40.8 ± 4.43	34.2 ± 1.08	32.3 ± 3.76	38.9 ± 3.38
ECG	48.9 ± 5.67	76.3 ± 3.44*	63.5 ± 3.10	70.7 ± 2.94*	61.0 ± 2.71	62.4 ± 2.42
TFs	26.4 ± 6.54	69.3 ± 3.99*	22.4 ± 4.64	66.5 ± 2.55*	25.6 ± 5.48	36.6 ± 4.61
TFdiG	52.9 ± 7.99	71.6 ± 5.00*	47.0 ± 3.53	70.2 ± 4.16*	33.6 ± 6.24	51.2 ± 1.83
(b) Colon tumor microsomes						
EGCG		52.0 ± 3.21*	27.9 ± 2.29*	51.6 ± 3.78*	45.0 ± 3.39	40.5 ± 0.77
EGC		33.0 ± 5.45	29.7 ± 1.59	34.2 ± 3.67	32.2 ± 7.80	30.1 ± 2.01
ECG		78.3 ± 3.03*	60.0 ± 3.30	81.1 ± 1.40*	58.3 ± 3.68	64.4 ± 2.56

Products were analyzed from the reactions with 0.1 mg microsomal protein, 20 μM (0.22 μCi) arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA with or without 30 μg/mL of tea polyphenols in 100 mM Tris-HCl buffer, pH 7.4. The reactions were carried out at 37° for 30 min. Values are the means ± SEM of five experiments (section a) or four experiments (section b).

* Significantly different ($P < 0.05$) from inhibition of the total metabolites.

3.4. Mechanisms of inhibition by ECG against COX-1

To elucidate the mechanisms of inhibition by tea polyphenols, isolated ovine COX-1 was used. PGE_2 was the major product formed by this preparation of isolated COX-1 due to the presence of PGE_2 isomerase, and ECG showed more potent inhibition against PGE_2 than against total metabolite formation (Fig. 2, inset). The presence of ECG caused the formation of several unidentified new metabolites with retention times of 25–35 min in the reaction with isolated ovine COX-1, but the formation of these metabolites was negligible in the reactions with microsomes (data not shown). ECG inhibited ovine COX-1 in a noncompetitive manner with a K_i value of 16.9 ± 1.3 μM, as determined by Dixon plotting (Fig. 2). EGCG also showed a noncompetitive inhibitory pattern on ovine COX-1 with an estimated K_i of 19.7 μM, and the product modifying effect was similar to that observed with ECG (data not shown). In the experiments with isolated COX-1, the percent inhibition by tea polyphenols was not changed significantly with different periods of preincubation (from 0 to 10 min) and reaction times (from 1 to 30 min) (data not shown).

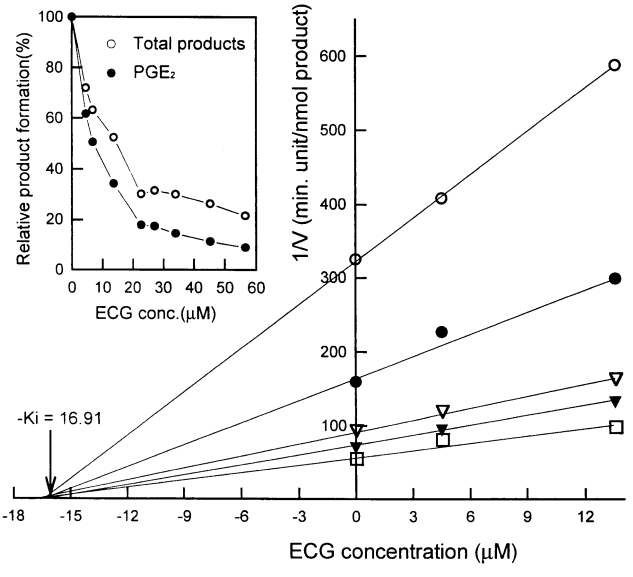

Fig. 2. Inhibitory mechanism of ECG on ovine COX-1. The reaction was carried out at 37° for 10 min. The incubation mixture was comprised of 3 units of ovine COX-1, ECG as indicated, arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA in 100 mM Tris–HCl buffer, pH 7.4. The data are means of at least two experiments. Arachidonic acid concentrations: (○) 3 μM; (●) 6 μM; (▽) 10 μM; (▼) 20 μM; and (□) 40 μM.

3.5. Effects of black tea polyphenols on PGE_2 formation in colon tumor microsomes

In tumor microsomes, PGE_2, TBX, and HHT were the main metabolites (Fig. 1B). TFs significantly inhibited the production of HHT and TBX, but increased the formation of PGE_2 in tumor microsomes. The enhancement of PGE_2 formation by TFs and TFdiG was observed with colon tumor samples, but not in normal colon mucosa (Fig. 3). The same conclusion was reached in experiments with two other normal and two other tumor samples (data not shown). With colon tumor microsomes, the formation of HHT and TBX decreased in a concentration-dependent manner, but PGE_2 production was increased markedly by TFs, even at 2 μg/mL (Fig. 4). Purified TF, TF3-G, TF3′-G, and TFdiG also inhibited TBX and HHT formation in colon tumor microsomes, and enhanced PGE_2 production (Fig. 5). Among these compounds, TF was the most effective in enhancing PGE_2 formation.

3.6. Effect of TFs on COX-2-dependent arachidonic acid metabolism

To determine whether the observed effects were related to COX-2, the COX-2 protein level of the microsomes and

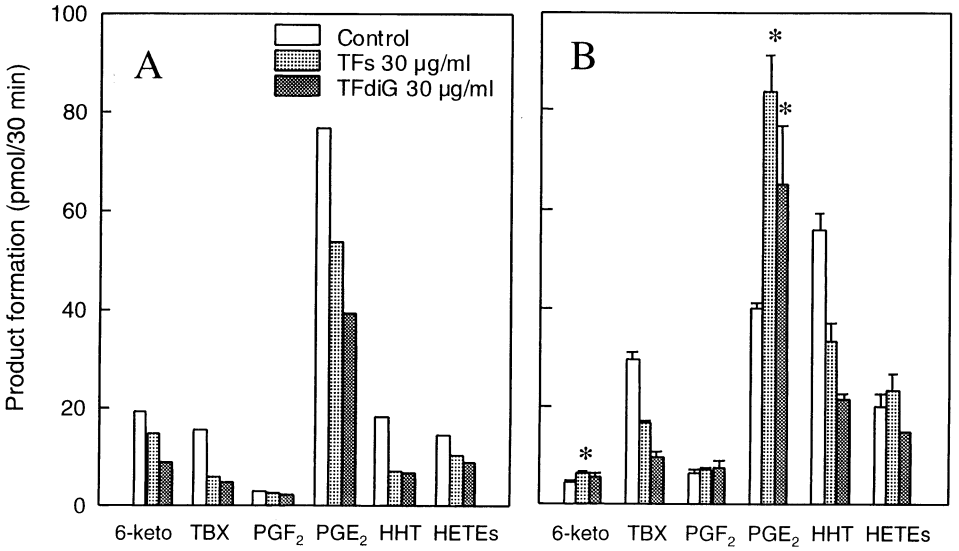

Fig. 3. Effect of black tea polyphenols on COX-dependent arachidonic acid metabolites in microsomes from normal colon mucosa (A) and a poorly differentiated colon carcinoma (B). Products were analyzed from the reactions with 0.1 mg microsomal protein, 20 μM (0.22 μCi) arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA with or without 30 μg/mL of black tea polyphenols in 100 mM Tris–HCl buffer, pH 7.4. The reactions were carried out at 37° for 30 min. The data in panel A are the means of duplicate experiments. The results in panel B are means ± SEM from three independent experiments. Key: (*) significantly higher ($P < 0.05$) than control.

the effect of NS-398, a specific COX-2 inhibitor, were determined. Normal colon mucosa had a low level of COX-2 and was inhibited slightly by NS-398, whereas tumor microsomes had COX-2 levels that were 10–40 times higher than the normal tissue and their COX-dependent activities were more susceptible to inhibition by NS-398 (data not shown). The increased formation of PGE_2 by TFs and TFdiG in tumor microsomes appears to be related to the COX-2 expression level in tumor tissues.

To determine whether TFs enhance COX-2-dependent

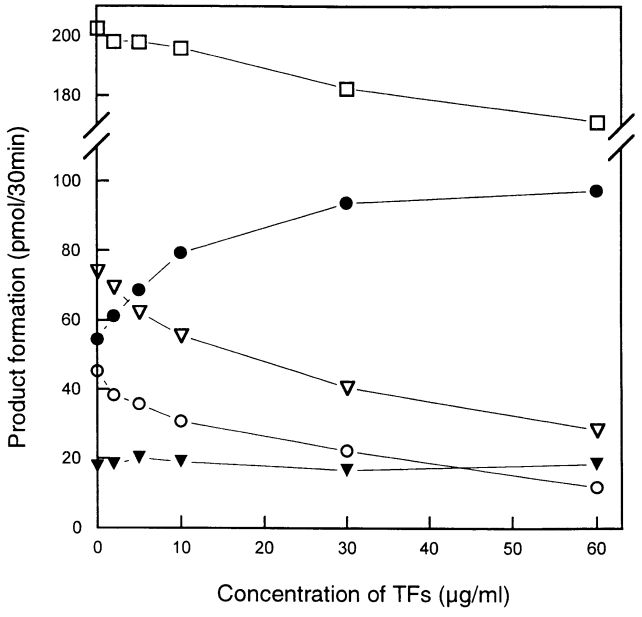

Fig. 4. Concentration-dependent effect of TFs on changes of COX-dependent metabolites in a poorly differentiated colon carcinoma. The incubation mixture contained 0.1 mg microsomal protein, 20 μM (0.22 μCi) arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA with various concentrations of TFs in 100 mM Tris–HCl buffer, pH 7.4. The reactions were carried out at 37° for 30 min. The reaction products were analyzed by HPLC. The results are means of duplicate determinations. Key: (□) total products; (●) PGE_2; (▽), HHT; (○) TBX; and (▼) HETEs.

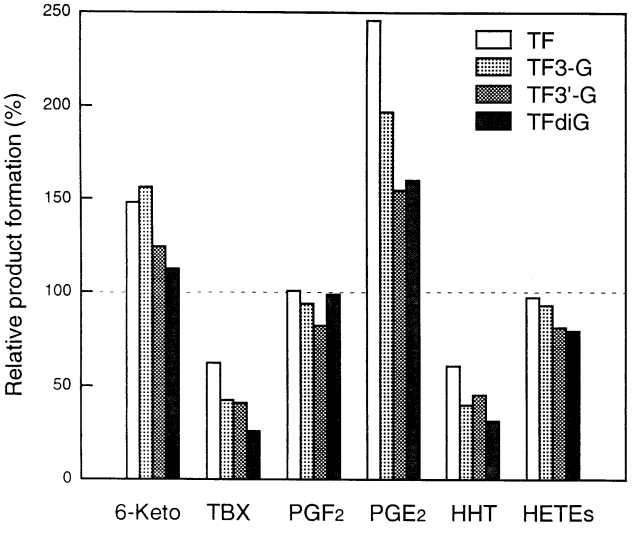

Fig. 5. Effect of purified black tea polyphenols on COX-dependent arachidonic acid metabolites of tumor colon microsomes. The incubation mixture contained 0.1 mg microsomal protein (from a poorly differentiated colon carcinoma), 20 μM (0.22 μCi) arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA with 30 μg/mL of black tea polyphenols in 100 mM Tris–HCl buffer, pH 7.4. The relative product formation (%) was calculated based on metabolite formation (100%) in a reaction without tea polyphenols, as the control. The results are means of duplicate determinations.

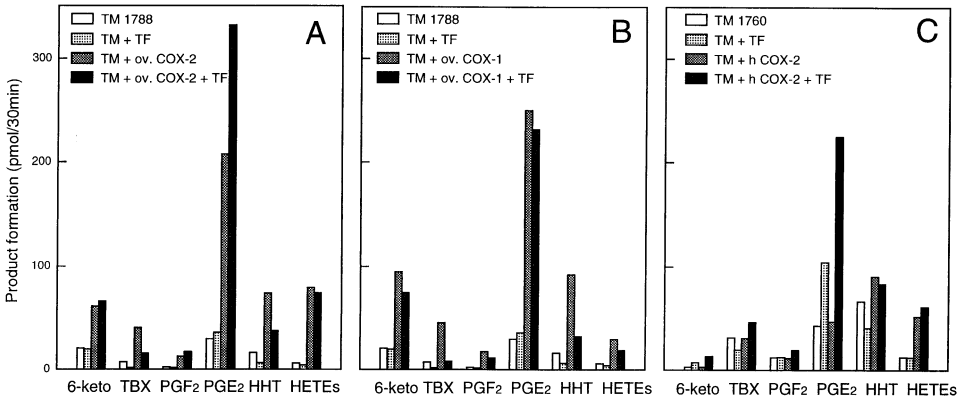

Fig. 6. Effect of TF (30 μg/mL) on arachidonic acid metabolism by tumor microsomes [from a carcinoma with mucin (TM 1788) or a poorly differentiated colon carcinoma (TM 1760)] including ovine COX-2 (A), ovine COX-1 (B), or human recombinant COX-2 (C). Products were analyzed from the reactions with 0.1 mg microsomal protein with or without 1.5 units of ovine COX-2 or -1 (in the case of panels A and B, respectively), or 1 unit of human recombinant COX-2 (in the case of panel C), 20 μM (0.22 μCi) arachidonic acid, 1 mM glutathione, 1 mM epinephrine, and 10 mM EDTA in 100 mM Tris–HCl buffer, pH 7.4. The reactions were carried out at 37° for 30 min. The data in panels A and B are means of duplicate experiments. The results in panel C are single determinations.

arachidonic acid metabolism, their effects on ovine COX-2 were investigated. All purified TFs inhibited ovine COX-2 activity in a concentration-dependent manner with TFdiG being the most potent (data not shown). When ovine COX-2 was added to tumor microsomes, it increased the formation of all metabolites measured; TF further enhanced the formation of PGE_2, but decreased the formation of TBX and HHT (Fig. 6A). Similar effects due to the addition of COX-2 and TF were also observed in normal microsomes (data not shown). Addition of COX-1 to tumor microsomes also enhanced the formation of all metabolites, but TF did not enhance PGE_2 formation (Fig. 6B). The addition of human recombinant COX-2 to tumor microsomes did not cause significant changes in the metabolite profile, except for an increase in HETEs; however, PGE_2 formation was increased dramatically in the presence of TF (Fig. 6C). It appears that TF does not activate COX-2 directly, but increases PGE_2 formation by affecting COX-2 through its interaction with other microsomal factors.

4. Discussion

Arachidonic acid is metabolized by three important enzyme systems: cyclooxygenase, lipoxygenases, and cytochromes P450 [31]. In our study, COX activity was found exclusively in the microsomes, whereas LOX activity was found predominantly in the cytosol (Fig. 1). This finding regarding the cellular distribution of COX and LOX is consistent with the findings in previous reports [12,32]. COX-1 and COX-2 are localized in both the endoplamic reticulum membrane and nuclear membrane [33]. Accordingly, the microsomal preparation can be a representative for cellular COXs. In the present assay system, without added NADPH, microsomal P450 is not expected to play an appreciable role in the oxidation of arachidonic acid. 5-, 12-, and 15-LOX and their products have been reported to be closely related to carcinogenic events [26–28]. They are believed to be involved in tumor development by affecting cell proliferation, inflammation, apoptosis, and angiogenesis [reviewed in Ref. 34]. Increased levels of LOX, and its mRNA have been observed in tumor tissues [29,35]. Our results indicate that tea polyphenols can inhibit the formation of LOX metabolites, thus suggesting their anticarcinogenic potentials.

All of the green tea polyphenols in this study inhibited COX-dependent arachidonic acid metabolism in both tumor and normal microsomes. EGCG and ECG displayed noncompetitive inhibition against ovine COX-1. Notably, the formation of TBX and HHT was inhibited more extensively than that of other metabolites. TBX and HHT are the subsequent metabolites formed from PGH_2 by TBX synthase, which is also located in the microsomal fraction [36]. The more extensive inhibition of the formation of these two metabolites than of other metabolites suggests the inhibition of TBX synthase in addition to COX-1 by tea polyphenols (Table 2).

It is known that COX-1 is constitutively expressed, whereas COX-2 is overexpressed in many types of tumors [18–21]. Overexpression of COX-2 was also observed in all the tumor microsomal samples in our study. Apparently COX-2 is generally less susceptible to inhibition by tea polyphenols, and this may be explained by the milder inhibition observed in colon tumor than in normal microsomes (Table 1). Comparison of the inhibitory effect of EGCG vs EGC and TFdiG vs TF suggests that the gallate group plays an important role in the inhibitory action against COX activities. The precise inhibitory effect and the structure relationship of tea polyphenols on both COX-1 and COX-2 need to be investigated further.

Most interestingly, we found that TFs increased the formation of PGE_2 in tumor microsomes, but not in normal

microsomes, whereas TBX and HHT formation was inhibited at a similar level in both tumor and normal microsomes. Previously, Katiyar and Mukhtar [13] reported that black tea polyphenols inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced formation of COX metabolites including PGE_2 in mouse skin. The inhibition of PGE_2 formation appears to be due to compounds other than the TFs in their black tea polyphenol preparation.

COX-1 and COX-2 metabolize arachidonic acid to PGG_2, which is subsequently reduced to PGH_2. PGH_2 is converted to PGE_2, TBX, and PGI_2 by PGE synthase, TBX synthase, and prostacyclin synthase, respectively. PGH_2 is also converted to HHT by TBX synthase or nonenzymetically [37]. Recently, it was reported that PGH_2 from COX-1 is preferentially metabolized to TBX and HHT, but the same metabolite from COX-2 is mainly metabolized to PGE_2 and PGI_2 [38]. Our results are consistent with this concept in that the enhanced PGE_2 formation by TFs appears to be related to the COX-2 content in tumor microsomes. Although the activity of isolated ovine COX-2 was inhibited by TFs, when ovine COX-2 was added to normal colon or tumor microsomes, its activity in catalyzing the formation of PGE_2 was stimulated by TF; the formation of TBX and HHT was inhibited by TF (Fig. 6). The results suggest that TF stimulates PGE_2 formation in tumor tissues by enhancing the interaction of COX-2 with other microsomal factors. These observations may provide an important clue for a mechanism of the PGE_2 formation through COX-2. We observed that the stimulatory effect by TF on PGE_2 formation was abolished in the absence of glutathione. Since human microsomal PGE synthase is known to be a glutathione-dependent enzyme [39], it is likely that this enzyme is involved in the stimulation of PGE_2 formation by TF, i.e. TF affects the interaction of COX-2 with PGE synthase in catalyzing the formation of PGE_2.

The possible importance of the present observation in understanding the effect of tea consumption on colon cancer remains to be investigated. Colonic cells are in contact with rather high concentrations of tea polyphenols because a large portion of the ingested compounds are left unabsorbed in the upper gastrointestinal tract. Even the absorbed EGCG (and probably ECG) is known to be excreted through the bile into the colon. The inhibition of COX and LOX activities by catechins in green tea is expected to reduce the risk for colon cancer. The catechins in black tea are expected to have a similar effect, but the black tea TFs may have an opposite effect due to their enhancement of PGE_2 formation in colon cells containing COX-2.

Acknowledgments

This study was supported by NIH Grant CA56673. We would like to thank Dr. Jannelle Landau for careful reading of the manuscript.

References

[1] Yang CS, Wang Z-Y. Tea and cancer: a review. J Natl Cancer Inst 2000;85:1038–49.
[2] Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 1997;37:693–704.
[3] Yang CS, Landau JM. Effect of tea consumption on nutrition and health. J Nutr 2000;130:2409–12.
[4] Yang CS, Chen L, Lee M-J, Landau JM. Effects of tea on carcinogenesis in animal models and humans. In: American Institute for Cancer Research, editor. Dietary phytochemicals in cancer prevention and treatment. New York: Plenum Press, 1996. p. 51–61.
[5] Yang CS, Kim S, Yang G-Y, Lee M-J, Liao J, Chung JY, Ho C-T. Inhibition of carcinogenesis by tea: bioavailability of tea polyphenols and mechanisms of actions. Proc Soc Exp Biol Med 1999;220:213–7.
[6] Conney AH, Lu Y-P, Lou Y-R, Xie J-G, Huang M-T. Inhibitory effect of green and black tea on tumor growth. Proc Soc Exp Biol Med 1999;220:229–33.
[7] Yamane T, Takahashi T, Kuwata K, Oya K, Inagake M, Kaito Y, Suganuma M, Fujiki H. Inhibition of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine-induced carcinogenesis by (-)-epigallocatechin gallate in the rat glandular stomach. Cancer Res 1995;55:2081–4.
[8] Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. Eur J Cancer Prev 1996;5:425–38.
[9] Kohlmeier L, Weterings KGC, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. Nutr Cancer 1997;27:1–13.
[10] Buschman JL. Green tea and cancer in humans: a review of the literature. Nutr Cancer 1998;31:151–9.
[11] Huang MT, Ho C-T, Wang ZY, Ferraro T, Finnegan-Olive T, Lou YR, Mitchell JM, Laskin JD, Newmark H, Yang CS, Conney AH. Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. Carcinogenesis 1992;13:947–54.
[12] Katiyar SK, Agarwal R, Wood GS, Mukhtar H. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz[*a*]anthracene-initiated SENCAR mouse skin by a polyphenolic fraction isolated from green tea. Cancer Res 1992;52:6890–7.
[13] Katiyar SK, Mukhtar H. Inhibition of phorbol ester tumor promoter 12-O-tetradecanoylphorbol-13-acetate-caused inflammatory responses in SENCAR mouse skin by black tea polyphenols. Carcinogenesis 1997;18:1911–6.
[14] Dong Z, Ma W-Y, Huang C, Yang CS. Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-)-epigallocatechin gallate and theaflavins. Cancer Res 1997;57:4414–9.
[15] Cao Y, Cao R. Angiogenesis inhibited by drinking tea. Nature 1999; 398:381.
[16] Boring CC, Squires TS, Tong T, Montgomery S. Cancer statistics. CA Cancer J Clin 1994;44:7–26.
[17] Levy GN. Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer. FASEB J 1997;11:234–47.
[18] Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–8.
[19] Fujita T, Matsui M, Takaku K, Uetake H, Ichikawa W, Taketo MM, Sugihara K. Size-, and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. Cancer Res 1998;58: 4823–6.
[20] Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res 1998;58:4997–5001.

[21] Zimmermann KC, Sarbia M, Weber A-A, Borchard F, Gabbert HE, Schror K. Cyclooxygenase-2 expression in human esophageal carcinoma. Cancer Res 1999;59:198–204.
[22] Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493–501.
[23] Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 1997;94:3336–40.
[24] Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E_2 in human colon cancer cells. Cancer Res 1998;58:362–6.
[25] Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998;93:705–16.
[26] Ghosh J, Myers CE, Arachidonic acid stimulates prostate cancer cell growth: critical role of 5-lipoxygenase. Biochem Biophys Res Commun 1997;235:418–23.
[27] Tang DG, Chen YQ, Honn KV. Arachidonate lipoxygenases as essential regulators of cell survival and apoptosis. Proc Natl Acad Sci USA 1996;93:5241–6.
[28] Kamitani H, Geller M, Eling T. Expression of 15-lipoxygenase by human colorectal carcinoma Caco-2 cells during apoptosis and cell differentiation. J Biol Chem 1998;273:21569–77.
[29] Ikawa H, Kamitani H, Calvo BF, Foley JF, Eling TE. Expression of 15-lipoxygenase-1 in human colorectal cancer. Cancer Res 1999;59:360–6.
[30] August DA, Landau J, Caputo D, Hong J, Lee M-J, Yang CS. Ingestion of green tea rapidly decreases prostaglandin E_2 levels in rectal mucosa in humans. Cancer Epidemiol Biomarkers Prev 1999;8:709–13.
[31] Subbaramaiah K, Zakim D, Weksler BB, Dannenberg AJ. Inhibition of cyclooxygenase: a novel approach to cancer prevention. Proc Soc Exp Biol Med 1997;216:201–10.
[32] Huang M-T, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH. Inhibitory effects of curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res 1991;51:813–9.
[33] Spencer AG, Woods JW, Arakawa T, Singer II, Smith WL. Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. J Biol Chem 1998;273:9886–93.
[34] Steele VE, Holmes CA, Hawk ET, Kopelovich L, Lubet RA, Crowell JA, Sigman CC, Kelloff GJ. Lipoxygenase inhibitors as potential cancer chemopreventives. Cancer Epidemiol Biomarkers Prev 1999;8:467–83.
[35] Gao X, Grignon DJ, Chbihi T, Zacharek A, Chen YQ, Sakr W, Porter AT, Crissman JD, Pontes JE, Powell IJ, Honn KV. Elevated 12-lipoxygenase mRNA expression correlates with advanced stage, and poor differentiation of human prostate cancer. Urology 1995;46:227–37.
[36] Arita H, Nakano T, Hanasaki K. Thromboxane A_2: its generation, and role in platelet activation. Prog Lipid Res 1989;28:273–301.
[37] John H, Cammann K, Schlegel W. Development and review of radioimmunoassay of 12-S-hydroxyheptadecatrienoic acid. Prostaglandins Other Lipid Mediators 1998;56:53–76.
[38] Brock TG, McNish RW, Peters-Golden M. Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E_2. J Biol Chem 1999;274:11660–6.
[39] Jakobsson P-J, Thoren S, Morgenstern R, Samuelson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci USA 1999;96:7220–5.