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Mechanisms of cancer prevention by tea polyphenols based on inhibition of TNF-α expression

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Abstract. Among various biochemical and biological activities of tea polyphenols, we believe inhibition of the expression and release of tumor necrosis factor-α (TNF-α) is crucial, since our study with TNF-α-deficient mice has revealed that TNF-α is an essential factor in tumor promotion. We found that EGCG dose-dependently inhibited AP-1 and NF-κB activation in BALB/3T3 cells treated with okadaic acid, resulting in inhibition of TNF-α gene expression. Furthermore, treatment with 0.1% green tea extract in drinking water reduced TNF-α gene expression as well as TNF-α protein level in the lung of TNF-α transgenic mice; and IL-1β and IL-10 gene expression in the lung was also inhibited by treatment with green tea extract, indicating that green tea inhibits both TNF-α and the cytokines induced by TNF-α in organs. We recently found synergistic effects of EGCG and cancer preventive agents such as tamoxifen and sulindac, on cancer preventive activity. Taken together, the results show that green tea is efficacious as a non-toxic cancer preventive for humans.

Keywords: EGCG, sulindac, tamoxifen, tumor promotion

1. Introduction

Green tea is rapidly being acknowledged as one of the most practical cancer preventives, based on results of various rodent carcinogenesis experiments on the inhibitory effects of (−)-epigallocatechin gallate (EGCG) and green tea extract, along with results of a prospective cohort study of humans [1–3]. In 1987, we first reported the anti-tumor promoting activity of EGCG, the main constituent of green tea, in a two-stage carcinogenesis experiment on mouse skin [4]. Topical applications of EGCG before treatment with various tumor promoters – teleocidin, one of the 12-*O*-tetradecanoylphorbol-13-acetate – (TPA)-types, or okadaic acid – inhibited tumor promotion on mouse skin initiated with 7,12-dimethylbenz(a)anthracene [1]. Based on these results, we next looked at how EGCG inhibits the process of tumor promotion. Our study of tumor promotion with okadaic acid had provided strong indications that tumor necrosis factor-α (TNF-α) is an endogenous tumor promoter and the essential

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cytokine for tumor promotion [5]. Recently we were able to prove our hypothesis using TNF-α-deficient (TNF-$\alpha^{-/-}$) mice, which were established by Lloyd J. Old's group in 1997 [6,7]: Repeated applications of okadaic acid did not induce any tumors in TNF-$\alpha^{-/-}$ mice by 19 weeks of tumor promotion, whereas 100% of TNF-$\alpha^{+/+}$ mice developed tumors by week 17. These results clearly demonstrated that TNF-α is the key cytokine for tumor promotion in mouse skin. We have also found that EGCG as well as cancer preventive agents inhibited TNF-α release from BALB/3T3 cells treated with okadaic acid [8]. Based on all of our results, we think that, among various biochemical and biological activities of tea polyphenols, inhibition of TNF-α production is crucial. So, we examined inhibitory mechanisms of EGCG on TNF-α production.

Next, inhibitory effects of EGCG on cytokine production in vivo were studied using TNF-α transgenic mice. The TNF-α transgenic mice overexpressed TNF-α only in the lung and developed interstitial pneumonitis resembling idiopathic pulmonary fibrosis in humans [9]. Treatment with green tea extract in drinking water reduced TNF-α mRNA expression and its protein level in the lung of these mice, along with reduction of IL-1β and IL-10 gene expression. This is the first evidence that green tea inhibits TNF-α production as well as other cytokine production in mouse organs.

More recently, we found synergistic effects of EGCG and (−)-epicatechin (EC) on cancer preventive activity [10]. We extended our investigation of synergistic effects by examining EGCG with other cancer preventive agents, such as tamoxifen and sulindac. That is, cotreatment with EGCG and tamoxifen or EGCG and sulindac enhanced cancer preventive activity. Tamoxifen is the first cancer preventive drug for breast cancer, approved by FDA in the United States in 1998 [11]; and sulindac is a cancer preventive agent for colon cancer and a nonsteroidal anti-inflammatory agent [12]. This paper presents our recent findings with tea polyphenols and green tea extract looking mainly at inhibition of TNF-α gene expression in the cells and in TNF-α transgenic mice. The synergistic effects of EGCG and sulindac are also discussed.

2. Materials and methods

2.1. Inhibition of AP-1 and NF-κB activation by EGCG

BALB/3T3 cells were pretreated with EGCG for one hr, and treated with 200 nM okadaic acid for another 8 hr. Binding activity of AP-1 and NF-κB in nuclear extract to each consensus sequence of DNA was examined by electrophoretic mobility gel-shift assay (EMSA) [13].

2.2. Reduction of TNF-α in the lung of TNF-α transgenic mice by consumption of green tea extract

Transgenic mice carry a chimeric gene consisting of the promoter region of the human surfactant protein-C (SP-C) gene and mouse TNF-α gene. Transgenic mice and transgenic negative littermates were given 0.1% green tea extract in drinking water from embryo to 4 month-old. Expression of TNF-α, IL-1β, and IL-10 genes was examined by reverse transcription (RT)-PCR in the presence of ^{32}P-dCTP [9]. TNF-α protein level in the lung was determined by enzyme-linked immunosorbent assay (ELISA) kit for mouse TNF-α (Genzyme Corporation, USA) [9].

2.3. Induction of apoptosis of PC-9 cells by EGCG and sulindac

PC-9 cells were incubated in sulindac with or without 75 μM EGCG for 2 days. DNA fragmentation was quantitatively measured using ELISA kit (Boehringer Mannheim, Mannheim, Germany) [10].

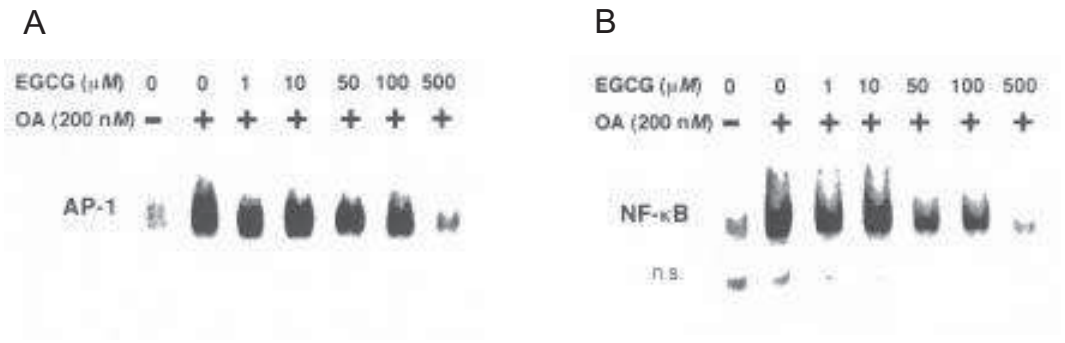

Fig. 1. Inhibition with EGCG of AP-1 and NF-κB activation induced by okadaic acid. Binding activity in nuclear extracts to AP-1 (A) and NF-κB (B) binding sites was examined by EMSA.

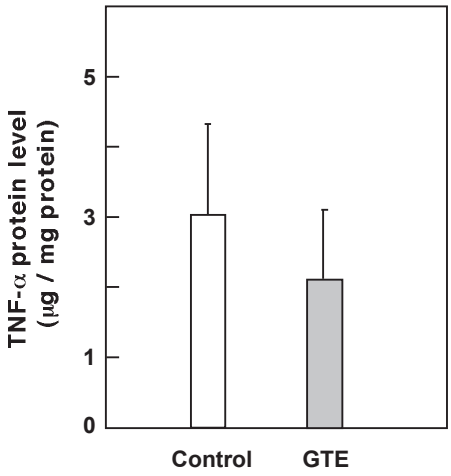

Fig. 2. Reduction of TNF-α protein level in the lung of TNF-α transgenic mice. Transgenic mice were treated with 0.1% green tea extract in drinking water from embryo to 4 month-old. GTE: green tea extract.

3. Results

3.1. Inhibition of TNF-α gene expression by EGCG

Tea polyphenols, such as EGCG, (−)-epicatechin gallate (ECG) and (−)-epigallocatechin (EGC), inhibit TNF-α release from BALB/3T3 cells and KATO III cells treated with okadaic acid [8,13]. TNF-α gene expression was enhanced by treatment with okadaic acid in both BALB/3T3 cells and KATO III cells, and EGCG dose-dependently inhibited this TNF-α gene expression, just as it inhibited TNF-α release. Since TNF-α gene expression is regulated by several transcription factors, such as AP-1 and NF-κB, we examined inhibition of AP-1 and NF-κB activation with EGCG using EMSA: 200 nM okadaic acid significantly enhanced AP-1 and NF-κB binding to each consensus sequence of DNA in BALB/3T3 cells, and pretreatment with 500 μM EGCG clearly inhibited these binding activities, as shown in Fig. 1(A) and Fig. 1(B) [13]. These results demonstrated that EGCG inhibits TNF-α production by inhibiting AP-1 and NF-κB activation.

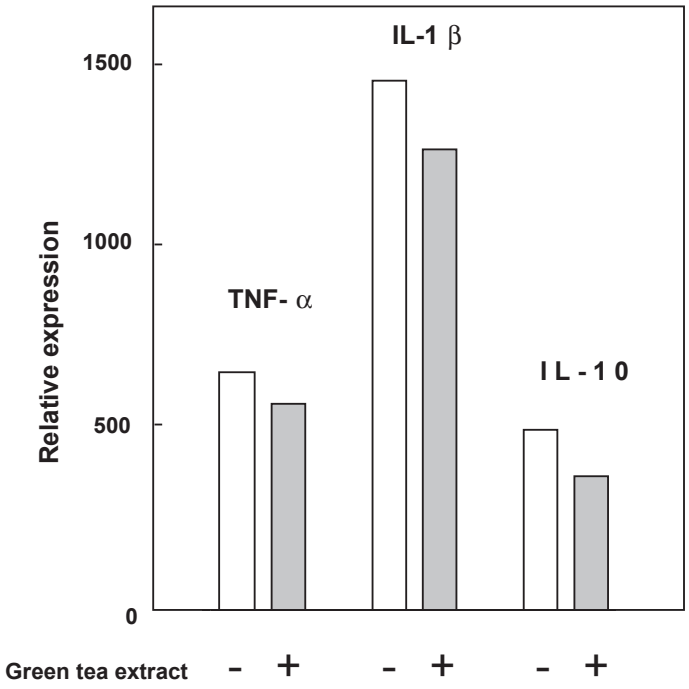

Fig. 3. Effects of EGCG on expression of TNF-α, IL-1β, and IL-10 genes in the lung of TNF-α transgenic mice. Transgenic mice were treated with 0.1% green tea extract in drinking water from embryo to 4 month old.

3.2. Reduction of TNF-α in the lung of TNF-α transgenic mice by consumption of green tea extract

TNF-α transgenic mice had enhanced TNF-α gene expression just after birth and continuously over-expressed it in the lung. At 4 month-old, TNF-α protein level in the lung of TNF-α transgenic mice was significantly reduced, from 3.0 to 2.1 μg/mg protein, by treatment with 0.1% green tea extract in drinking water (Fig. 2). This reduction level was well correlated with reduction of TNF-α gene expression (Fig. 3). Treatment with green tea extract also reduced expression of IL-1β and IL-10 genes about 15–20% in the lung (Fig. 3). These results indicated that green tea inhibited TNF-α gene expression as well as expression of other cytokine genes induced by TNF-α.

3.3. Enhanced effects of EGCG and tamoxifen or EGCG and sulindac on cancer preventive activity

Based on our discovery that synergistic induction of apoptosis by EC and other tea polyphenols, EGCG, ECG and EGC, we examined synergistic effects of tea polyphenols with other cancer preventive agents, such as tamoxifen and sulindac. We found that cotreatment with EGCG and tamoxifen inhibited growth of MCF-7 cells and induction of apoptosis of PC-9 cells even more strongly than either did alone (data not shown) [10]. These results suggest that green tea enhances tamoxifen's preventive activity against breast cancer in humans.

We next looked at the synergistic effects of EGCG and sulindac or its metabolites, sulindac sulfide and sulindac sulfone, on induction of apoptosis of PC-9 cells. Sulindac at a concentration of 10 μM induced almost no apoptosis of PC-9 cells, while 10 μM sulindac with 75 μM EGCG induced apoptosis more than 20 times as strongly as sulindac alone (Fig. 4). Sulindac sulfide, an inhibitor of cyclooxyganase (COX)-1

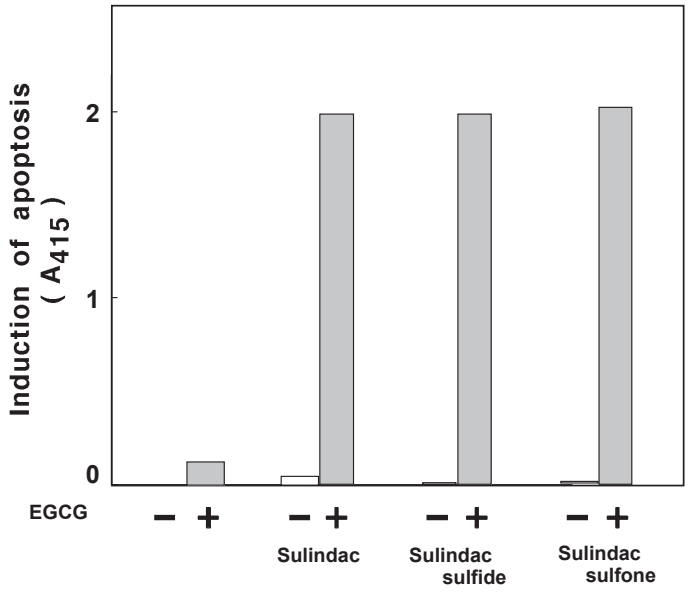

Fig. 4. Synergistic induction of apoptosis of PC-9 cells by cotreatment with EGCG and sulindac or its metabolites.

and COX-2, and sulindac sulfone, an inactive metabolite, both synergistically induced apoptosis of the cells in the presence of 75 μM EGCG, whereas neither was effective alone (Fig. 4). We therefore believe that the synergistic effects on apoptosis by cotreatment with EGCG and sulindac are not directly related to inhibition of COX. Clearly, cotreatment with EGCG and sulindac will result in increased cancer preventive activity in humans.

4. Discussion

This paper reports that green tea extract in drinking water inhibits TNF-α gene expression and reduces TNF-α protein level in the lung of TNF-α transgenic mice. Since treatment with green tea extract did not inhibit expression of SP-C gene, we think that green tea inhibits expression of TNF-α gene and other cytokine genes subsequently induced by TNF-α through cytokine network. This important evidence strongly suggests that administration of green tea extract somehow inhibits TNF-α gene expression, resulting in reduction of an endogenous tumor promoter in the lung. We previously reported that ^{3}H-EGCG orally administered was distributed into the lung as well [14], so it is reasonable to suppose that tea polyphenols reach the lung and inhibit TNF-α gene expression mediated through inhibition of AP-1 and NF-κB activation in the lung. It is now known that various cancer preventive agents, such as green tea polyphenols, sodium salicylate and curcumin, inhibit AP-1 and NF-κB activation [15–17]: Our results here indicate that inhibition of AP-1 and NF-κB activation is a further step toward inhibition of TNF-α gene expression.

The synergistic effects of EGCG and sulindac were significant for cancer preventive activity in cells, and we recently demonstrated their synergistic effects on inhibition of tumor formation in C57BL/6J Min mice (manuscript in preparation). All our results show that green tea is a promising candidate for use in combination with cancer preventive agents such as sulindac and tamoxifen, for the purpose of reducing their adverse effects [10].

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