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Procyanidin dimer B2 [epicatechin-(4β-8)-epicatechin] suppresses the expression of cyclooxygenase-2 in endotoxin-treated monocytic cells ☆

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Abstract

The anti-inflammatory activity of the predominant procyanidin dimer in cocoa, dimer B2, was investigated in this study. Pretreatment of the procyanidin dimer B2 reduced COX-2 expression induced by the endotoxin lipopolysaccharide (LPS) in differentiated human monocytic cells (THP-1) in culture. To further elucidate the underlying mechanism of COX-2 inhibition by procyanidin, we examined their effects on the activation of extracellular signal-regulated protein kinase (ERK), Jun-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK), which are upstream enzymes known to regulate COX-2 expression in many cell types. Pretreatment with procyanidin dimer B2 decreased the activation of ERK, JNK, and p38 MAPK. In addition, procyanidin dimer B2 suppressed the NF-κB activation through stabilization of IκB proteins, suggesting that these signal-transducing enzymes could be potential targets for procyanidin dimer B2. By affecting the expression rather than the activity of COX-2, these in vitro data reported herein give further evidence on the anti-inflammatory protection by procyanidins.

Keywords: Cocoa; Procyanidin; Cyclooxygenase-2 (COX-2); Mitogen-activated protein kinase (MAPK); Nuclear factor κB (NF-κB)

Cyclooxygenase (COX), the key enzyme that catalyzes the rate-limiting step in prostaglandin (PG) biosynthesis, exists in at least two isoforms, designated as COX-1 and COX-2. Although these enzymes share considerable sequence homology, these isoforms differ considerably in the tissue distribution, regulation, and function. Cyclooxygenase-1 is a housekeeping enzyme, being constitutively expressed in almost all mammalian tissues. In contrast, COX-2 is barely detectable under normal physiological conditions; however, like other early-response gene products, COX-2 can be induced rapidly and transiently by pro-inflammatory mediators and mitogenic stimuli including cytokines, endotoxins, growth factors, oncogenes, and phorbol esters. As such, COX-2 has received considerable attention for its potential role in the inflammation and disease development.

Several lines of compelling evidence from genetic and clinical studies indicate that improper upregulation of COX-2 is implicated in the progression of neurodegenerative diseases, atherosclerosis, and cancer. COX-2 expression has been shown to be increased in the frontal cortex of individuals diagnosed with Alzheimer's disease, and synthetic β-amyloid peptides induced COX-2 expression in SH-SY5Y neuroblastoma cells in vitro [1]. Furthermore, COX-2 induction occurs through a JNK/c-Jun-dependent mechanism after administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), suggesting

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that COX-2 may be playing a role in the neuropathology of Parkinson's disease. [2] Selective COX-2 inhibition has been shown to reduce early lesion formation in LDL receptor-deficient ($LDLR^{-/-}$) mice and that macrophage COX-2 expression contributes to atherogenesis in $LDLR^{-/-}$ mice [3]. The elevated expression of COX-2 is also reported in multiple malignancies, including those of esophagus, stomach, breast, pancreas, lung, colon, skin, urinary bladder, and prostate [4]. Thus, COX- 2 is recognized as a potential therapeutic target for the prevention and treatment of a number of diseases.

Epidemiologic studies have linked flavonoid-rich foods with a reduced risk of cancer and cardiovascular disease [5]. Some cocoas and chocolate can be flavonoid-rich, composed of primarily the monomeric flavanols (−)-epicatechin and (+)-catechin, and oligomeric procyanidins formed from these monomeric units. While the mechanisms underlying the suggested health benefits of flavonoid-rich foods remain to be fully elucidated, in vitro and in vivo studies have demonstrated that the flavanols and procyanidins from cocoa have a number of potential biological effects including the ability to reduce oxidative damage [6], promote endothelium-dependent relaxation [7], and decrease platelet aggregation [8]. Specifically related to inflammation, cocoa flavanols and procyanidins have been shown to modulate the production of inflammatory cytokines [9], as well as inhibit lipoxygenase activity in vitro [10]. These data together suggest that the flavanols found in cocoa may have important biological activities.

Although the potent in vitro effects of the procyanidins from cocoa have generated considerable interest in these compounds, it has been suggested that their in vivo effects may be minimal because of gastric degradation [11]. While the flavanol monomers in cocoa ((−)epicatechin and (+) catechin) are readily absorbed and metabolized in humans [12], less is known about the bioavailability and metabolism of procyanidins. Human feeding trials done with cocoa demonstrate that the procyandins can survive the acidic milieu of the stomach and as such, are not initially broken down and likely enter into the small intestine intact. In support of this, the predominant procyanidin dimer in cocoa, dimer B2 [epicatechin-(4β-8)-epicatechin] (Fig. 1), has been detected in human plasma as early as 30 min after the consumption of a flavanol-rich food such as cocoa [13]. Thus, while the metabolic fate of dimer B2 has yet to be elucidated, it is clear from this work that it can be absorbed, and as such, suggests that dimer B2 may contribute to some of the benefits observed following the consumption of flavanol/procyanidin-rich cocoa and chocolate.

For this reason, we investigated whether dimer B2 could suppress COX-2 expression induced by the endotoxin lipopolysaccharide (LPS) in a differentiated human monocytic leukemia cell line, THP-1. As the expression of COX-2 has been shown to be linked to mitogen-activated protein kinase (MAPK) signaling cascades, we also examined the cellular signaling mediators and events involved in COX-2 expression, particularly transcription factors NF-κB and upstream regulatory kinases, such as extracellular signal-regulated kinase (ERK), Jun-N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK), as potential molecular targets of dimer B2.

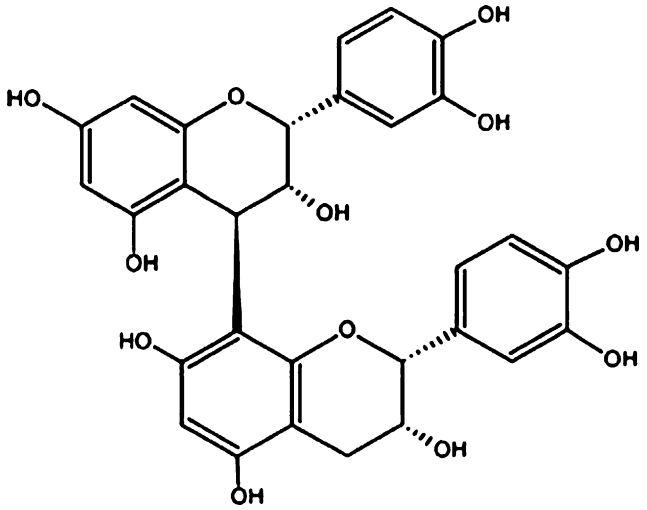

Fig. 1. Chemical structures of procyanidin dimer B2, epicatechin-(4β-8)-epicatechin (cocoa).

Materials and methods

Materials. Procyanidin dimer B2 was supplied by Mars Incorporated (purity 98.34%). Phorbol 12-myristate 13-acetate (PMA), lipopolysaccharides (LPS, from *Escherichia coli* serotype 0111:B4), arachidonic acid, and NS398 were purchased from Sigma (St. Louis, MO). RPMI 1640, Grace's supplemented medium, L-glutamine, Hepes, 2-mercaptoethanol, fetal bovine serum, and penicillin/streptomycin were purchased from Gibco-BRL (Grand Island, NY). Anti-COX-2, anti-IκBα, and anti-NF-κB p65 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-ERK, anti-JNK, anti-p38 MAPK, and their phosphor antibodies were purchased from Cell signaling technology (Beverly, MA). Alexa Fluor 488 goat anti-mouse IgG was purchased from Molecular probes (Eugene, OR). SuperSignal West Pico chemiluminescent substrate was purchased from Pierce (Rockford, IL). All other chemicals used were in the purest form available commercially.

Cell culture. Human monocytic THP-1 cells (American Type Culture Collection, Manassas, VA) in RPMI 1640 medium, with 4.5 g/L glucose, 10 mM Hepes, 1 mM sodium pyruvate, and 50 μM 2-ME supplemented with 10% FBS, were cultured under a humidified 5% CO_2 atmosphere at 37 °C. For differentiation, THP-1 cells were plated at 1×10^6 cells/ml in the medium containing 100 nM PMA and allowed to adhere for 48 h, after which they were fed with PMA-free medium and cultured for 24 h prior to use. LPS was used at a concentration of 1 μg/ml in the medium. Insect cell line sf9 was cultured in monolayer at 28 °C in Grace's supplemented medium with 10% heat-inactivated fetal bovine serum.

Determination of COX-2 enzyme activity. The effect of procyanidin dimer B2 and selective COX-2 inhibitor NS398 on the activity of COX-2 was measured using baculovirus-expressed recombinant human COX-2 enzyme as previously described [14].

Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) to assess COX-2 mRNA expression. Total RNA was extracted from macrophages with TRIzol reagent. Real-time quantitative RT-PCR was performed using the Opticon 2 (MJ Research Inc., Waltham, MA). Sequence-specific PCR primers for COX-2 [Accession No. NM_000963; forward primer: 5′-GGGCAAAGACTGCGAAGA AG-3′; reverse primer: 5′-CCCATGTGACGAAATGACTG-3′] and