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Green tea epigallocatechin-3-gallate inhibits microsomal prostaglandin E_2 synthase-1

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ABSTRACT

Prostaglandin (PG)E_2 is a critical lipid mediator connecting chronic inflammation to cancer. The anti-carcinogenic epigallocatechin-3-gallate (EGCG) from green tea (*Camellia sinensis*) suppresses cellular PGE_2 biosynthesis, but the underlying molecular mechanisms are unclear. Here, we investigated the interference of EGCG with enzymes involved in PGE_2 biosynthesis, namely cytosolic phospholipase (cPL)A_2, cyclooxygenase (COX)-1 and -2, and microsomal prostaglandin E_2 synthase-1 (mPGES-1). EGCG failed to significantly inhibit isolated COX-2 and $cPLA_2$ up to 30 μM and moderately blocked isolated COX-1 (IC_{50} > 30 μM). However, EGCG efficiently inhibited the transformation of PGH_2 to PGE_2 catalyzed by mPGES-1 (IC_{50} = 1.8 μM). In lipopolysaccharide-stimulated human whole blood, EGCG significantly inhibited PGE_2 generation, whereas the concomitant synthesis of other prostanoids (i.e., 12(*S*)-hydroxy-5-cis-8,10-trans-heptadecatrienoic acid and 6-keto $PGF_{1\alpha}$) was not suppressed. Conclusively, mPGES-1 is a molecular target of EGCG, and inhibition of mPGES-1 is seemingly the predominant mechanism underlying suppression of cellular PGE_2 biosynthesis by EGCG.

Introduction

Epigallocatechin-3-gallate (EGCG, Fig. 1) from green tea (*Camellia sinensis*) has shown chemopreventive and anti-inflammatory activity in animal and epidemiologic human studies [1]. Diverse biochemical mechanisms of EGCG have been described including interference with cyclin-dependent kinases 2 and 4 ($IC_{50} \approx 20$ μM) [2], NFκB signaling (IC_{50} = 15 μM) [3], mitogen-activated protein kinase pathways (IC_{50} = 10–20 μM) [4], proteasome activity (IC_{50} = 0.1–10 μM) [5], and inhibition of cellular generation of eicosanoids [6,7], particularly of prostaglandin (PG)E_2. PGE_2, a key mediator in inflammation, fever, and pain, is synthesized from arachidonic acid by the concerted action of cyclooxygenases (COX) and PGE_2 synthases (PGES). The molecular targets underlying the suppression of PGE_2 formation by EGCG are incompletely understood. Thus, PGE_2 formation is inhibited in different cellular systems at low micromolar concentrations of EGCG (IC_{50} = 5–25 μM), whereas an interference with cyclooxygenase (COX)-2 mRNA or protein expression is markedly less pronounced (IC_{50} = 50–250 μM) [8–12] and therefore fails to explain the potent effects of EGCG on cellular PGE_2 biosynthesis.

Different terminal isoforms of PGES exist, and co-expression studies have indicated preferential functional couplings between COX and PGES isoenzymes [13]. Thus, the microsomal PGE_2 synthase-1 (mPGES-1) receives its substrate (i.e., PGH_2) from COX-2, and both COX-2 and mPGES-1 are co-expressed in response to pro-inflammatory stimuli (e.g., interleukin (IL)-1β) resulting in increased PGE_2 formation during inflammation [14]. On the other hand, cPGES and mPGES-2, which are constitutively expressed and essentially coupled to COX-1, are seeming dispensable for massive PGE_2 formation under pro-inflammatory conditions [15–17]. Here we performed a detailed investigation of the effects of EGCG on enzymes involved in PGE_2 biosynthesis. Our data exclude $cPLA_2$ and COX enzymes as primary targets of EGCG but instead led to the identification of EGCG as potent inhibitor of mPGES-1. Because PGE_2 plays a critical role in inflammation and also in inflammation-triggered cancer [18,19], we suggest that suppression of PGE_2 generation through interference with mPGES-1 may contribute to the anti-carcinogenic effects of EGCG.

Materials and methods

Reagents. (−)-EGCG and (−)-EGC, isolated from green tea (*Camellia sinensis*), were from Enzo Life Sciences AG (Lörrach, Germany) and LKT Laboratories (St. Paul, MN, USA), respectively. Both were dissolved in dimethyl sulphoxide (DMSO) and kept in the dark at

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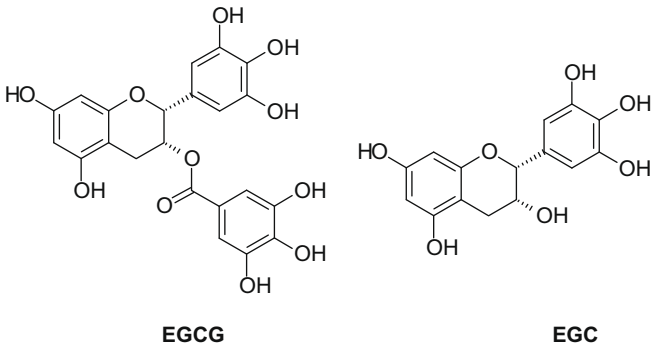

Fig. 1. Chemical structure of EGCG and EGC.

−20 °C, and freezing/thawing cycles were kept to a minimum. The thromboxane synthase inhibitor CV4151 and the mPGES-1 inhibitor 2-(2-chlorophenyl)-1*H*-phenanthro[9,10-d]-imidazole (MD-52) were generous gifts by Dr. S. Laufer (University Tuebingen, Germany) and Dr. M. Schubert-Zsilavecz (University Frankfurt, Germany), respectively. Materials used: DMEM/high glucose (4.5 g/l) medium, penicillin, streptomycin, trypsin/EDTA solution, PAA (Coelbe, Germany); PGH_2, Larodan (Malmö, Sweden); 11β-PGE_2, PGB_1, MK-886, human recombinant COX-2, ovine COX-1, Cayman Chemical (Ann Arbor, MI). All other chemicals were obtained from Sigma–Aldrich (Deisenhofen, Germany) unless stated otherwise.

Cells. A549 cells were cultured in DMEM/high glucose (4.5 g/l) medium supplemented with heat-inactivated fetal calf serum (10%, v/v), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37 °C in a 5% CO_2 incubator.

Determination of cell-free activity of human recombinant cPLA$_2$. The $cPLA_2$ coding sequence was cloned from pVL1393 plasmid (kindly provided by Dr. Wonhwa Cho, University of Illinois at Chicago) into pFastBac™ HT A containing a 6× his-tag coding sequence. The recombinant plasmid was transformed into DH10Bac™ Escherichia coli. Sf9 cells were transfected with recombinant bacmid DNA using Cellfectin® Reagent and the generated baculovirus was amplified. Overexpression of his-tagged $cPLA_2$ in baculovirus-infected Sf9 cells and isolation using Ni–NTA agarose beads was performed as described [20]. Multilamelar vesicles (MLVs) were prepared by drying 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine (PAPC) and 1-palmitoyl-2-oleoyl-*sn*-glycerol (POG) in a ratio of 2:1 (n/n, in chloroform) under nitrogen. MLVs were downsized to large unilamelar vesicles (LUVs) by extrusion (100 nm pore diameter). Final total concentration of lipids was 250 μM in 200 μl. Test compounds and 1 mM Ca^{2+} was added to the vesicles and the reaction was started by addition of 500 ng his-tagged $cPLA_2$ (in 10 μl buffer). After 1 h at 37 °C, 1.6 ml MeOH was added, and arachidonic acid was extracted by RP-18 solid phase extraction. Following derivatization with *p*-anisidium chloride, the resulting derivate was analyzed by RP-HPLC at 249 nm as described [21].

Activity assays of isolated COX-1 and -2. Inhibition of the activities of isolated ovine COX-1 and human recombinant COX-2 was performed as described [22]. Briefly, purified COX-1 (ovine, 50 U) or COX-2 (human recombinant, 20 U) were diluted in 1 ml reaction mixture containing 100 mM Tris buffer, pH 8, 5 mM glutathione, 5 μM hemoglobin, and 100 μM EDTA at 4 °C and pre-incubated with the test compounds for 5 min. Samples were pre-warmed for 60 s at 37 °C, and arachidonic acid (5 μM for COX-1, 2 μM for COX-2) was added. After 5 min at 37 °C, the COX-derived 12-HHT was extracted and then analyzed by HPLC.

Preparation of crude mPGES-1 in microsomes of A549 cells and determination of PGE$_2$ synthase activity. Preparation of A549 cells and determination of mPGES-1 activity was performed as described [22]. In brief, cells were incubated with interleukin-1β (1 ng/ml) for 48 h, cells were harvested, frozen in liquid nitrogen, and ice-cold homogenization buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethanesulphonyl fluoride, 60 μg/ml soybean trypsin inhibitor, 1 μg/ml leupeptin, 2.5 mM glutathione, and 250 mM sucrose) was added. Cells were sonicated on ice (3 × 20 s), and the homogenate was subjected to differential

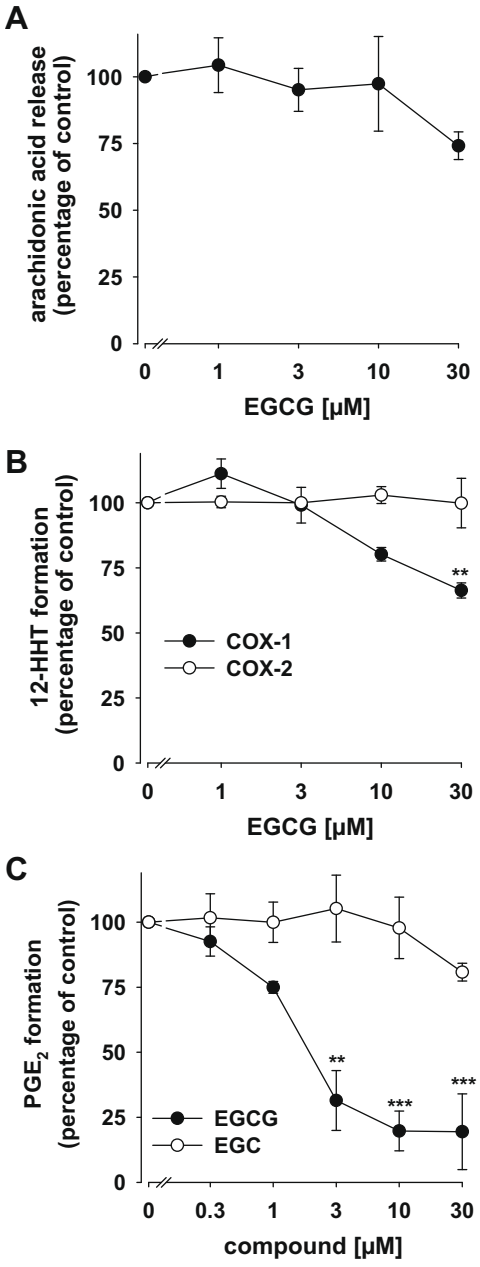

Fig. 2. Effects of EGCG on key enzymes involved in prostanoid formation in cell-free assays. (A) Purified $cPLA_2$ (500 ng) was added to liposomes, which were pre-treated with EGCG or vehicle (DMSO). After another 60 min at 37 °C, released arachidonic acid was analyzed by RP-HPLC as described. (B) Purified ovine COX-1 (50 U) or human recombinant COX-2 (20 U) were added to a COX reaction mix, and after pre-incubation with EGCG or vehicle (DMSO) for 5 min, the reaction was started with 5 μM (COX-1) or 2 μM (COX-2) arachidonic acid. After 5 min at 37 °C, the formation of 12-HHT was determined by RP-HPLC as described. (C) Microsomal preparations of interleukin-1β-stimulated A549 cells were pre-incubated with vehicle (DMSO) or test compounds for 15 min at 4 °C, and the reaction was started by addition of 20 μM PGH_2. After 1 min at 4 °C, the reaction was terminated, and formed PGE_2 was assessed as described. Data are given as means + SE, $n = 3$–4, $^{**}p < 0.01$, or $^{***}p < 0.001$ vs. vehicle (0.1% DMSO) control, ANOVA + Tukey HSD *post hoc* tests.