

Curcumin blocks prostaglandin E₂ biosynthesis through direct inhibition of the microsomal prostaglandin E₂ synthase-1

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Abstract

Prostaglandin E₂ (PGE₂) plays a crucial role in the apparent link between tumor growth and chronic inflammation. Cyclooxygenase (COX)-2 and microsomal PGE₂ synthase-1, which are overexpressed in many cancers, are functionally coupled and thus produce massive PGE₂ in various tumors. Curcumin, a polyphenolic β-diketone from tumeric with anti-carcinogenic and anti-inflammatory activities, was shown to suppress PGE₂ formation and to block the expression of COX-2 and of microsomal PGE₂ synthase-1. Here, we identified microsomal PGE₂ synthase-1 as a molecular target of curcumin and we show that inhibition of microsomal PGE₂ synthase-1 activity is the predominant mechanism of curcumin to suppress PGE₂ biosynthesis. Curcumin reversibly inhibited the conversion of PGH₂ to PGE₂ by microsomal PGE₂ synthase-1 in microsomes of interleukin-1β-stimulated A549 lung carcinoma cells with an IC₅₀ of 0.2 to 0.3 μmol/L. Closely related polyphenols (e.g., resveratrol, coniferyl alcohol, eugenol, rosmarinic acid) failed in this respect, and isolated ovine COX-1 and human recombinant COX-2 were not inhibited by curcumin up to 30 μmol/L. In lipopolysaccharide-stimulated human whole blood, curcumin inhibited COX-2-derived PGE₂ formation from endogenous or from exogenous arachidonic acid, whereas the concomitant formation of COX-2-mediated 6-keto PGF_{1α} and COX-1-derived 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid was suppressed only at significant higher concentrations. Based on the key function of PGE₂ in inflammation and carcinogenesis, inhibition of microsomal PGE₂ synthase-1 by curcumin provides a molecular basis for its anticarcinogenic and

anti-inflammatory activities. [Mol Cancer Ther 2009;8(8):2348–55]

Introduction

Curcumin (diferuloylmethane; Fig. 1), an antioxidant polyphenol from *Curcuma longa* (tumeric), is a major ingredient of the curry spice tumeric and has been used for the therapy of inflammatory and infectious diseases in ayurvedic medicine. Results from preclinical and clinical studies indicate chemopreventive, antiproliferative, proapoptotic, antimetastatic, antiangiogenic, and anti-inflammatory effects of curcumin (for review, see refs. 1, 2). The pleiotropic activities of curcumin are supposed to be linked to its interference with the expression or activation of multiple key signaling molecules, including peroxisome proliferator-activated receptor γ, p53, nuclear factor-E2-related factor, nuclear factor κB (nuclear factor κB), activator protein-1, protein kinase C, protein kinase A, focal adhesion kinase, protein kinase B, tumor necrosis factor-α, interleukin 1β, chemokines, p300 histone acetyl transferase, cyclooxygenase (COX)-2, 5-lipoxygenase, and matrix metalloproteinase-9 (1, 2). Numerous molecular targets of curcumin have been identified thus far, including COX-1 (IC₅₀ = 25–50 μmol/L; refs. 3, 4), 5-lipoxygenase (IC₅₀ = 0.7 μmol/L; ref. 3), glycogen synthase kinase-3β (IC₅₀ = 0.07 μmol/L; ref. 5), DNA topoisomerase II (at 50 μmol/L; ref. 6), inhibitor of NFκB kinase (IC₅₀ = 20 μmol/L; ref. 7), protein kinase C (IC₅₀ = 15 μmol/L; ref. 8), and xanthine oxidase (IC₅₀ = 200–400 μmol/L). However, many of these interactions are characterized by low affinities as reflected by the respective high IC₅₀ values in functional assays, and the pharmacologic relevance of most of these target interactions is uncertain.

Prostaglandin E₂ (PGE₂) is a potent lipid mediator that is closely linked to inflammation and cancer. The biosynthesis of PGE₂ requires transformation of arachidonic acid by COX-1 or COX-2 [enzyme commission (EC) 1.14.99.1] to PGH₂, which is subsequently converted by PGE₂ synthases (EC 5.3.99.3) to PGE₂ (9). Whereas the cytosolic PGE₂ synthase is constitutively expressed and preferentially couples to COX-1, the microsomal PGE₂ synthase-1 is functionally linked to COX-2. COX-2 and microsomal PGE₂ synthase-1 are induced by proinflammatory stimuli, and both enzymes are overexpressed in various cancers (9, 10). Curcumin was shown to lower PGE₂ formation in cellular models (3, 11–14), in whole blood (15), and *in vivo* (16–19). Besides direct inhibition of COX-1 and -2 (IC₅₀ = 25–50 μmol/L for COX-1 and > 50 μmol/L for COX-2 (3, 4)), impaired activation of activator protein-1 and the NFκB signaling pathway, resulting in reduced expression of COX-2 (13, 20) and microsomal PGE₂ synthase-1 (11), might be responsible. However, the effects of curcumin on prostanoid biosynthesis are diverse, depending on the distinct assays used. For example, the

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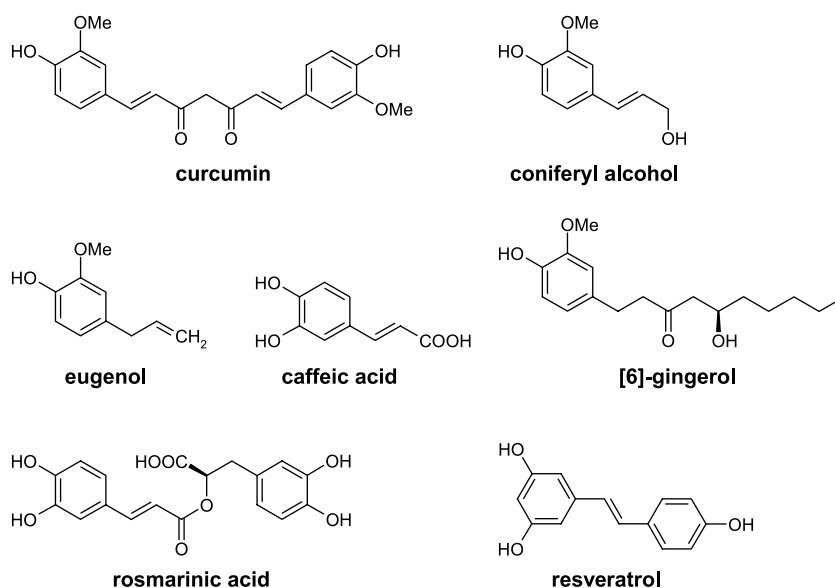


Figure 1. Structure of curcumin and related polyphenols.

conversion of arachidonic acid to PGD_2 and $\text{PGF}_2\alpha$ was blocked in epidermal microsomes (21), but curcumin increased the formation of $\text{PGF}_2\alpha$ and the stable PGL_2 degradation product 6-keto $\text{PGF}_{1\alpha}$ in interleukin- 1β -stimulated A549 cells (11). Here, we identified microsomal PGE_2 synthase-1 as functional and highly susceptible molecular target of curcumin. Our data show that suppression of PGE_2 biosynthesis in cell-based assays is primarily due to interference with microsomal PGE_2 synthase-1 rather than with COX enzymes, and this interaction occurs at low concentrations that may be achieved *in vivo*.

Materials and Methods

Reagents

Curcumin, purchased from Sigma-Aldrich was dissolved in DMSO and kept in the dark at -20°C , and freezing/thawing cycles were kept to a minimum. The thromboxane synthase inhibitor CV4151 (22) and the microsomal PGE_2 synthase-1 inhibitor 2-(2-chlorophenyl)-1*H*-phenanthro[9,10-*d*]-imidazole were generous gifts by Dr. S. Laufer (University of Tuebingen) and Dr. M. Schubert-Zsilavecz (University of Frankfurt), respectively. Materials used are DMEM/high glucose (4.5 g/L) medium, penicillin, streptomycin, and trypsin/EDTA solution (PAA); PGH_2 (Larodan); and 11β - PGE_2 , PGB_1 , 3-(3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-isopropyl-1*H*-indol-2-yl)-2,2-dimethylpropanoic acid (MK-886), human recombinant COX-2, and ovine COX-1 (Cayman Chemical). All other chemicals were obtained from Sigma-Aldrich, unless stated otherwise.

Cells

A549 cells were cultured in DMEM/high glucose (4.5 g/L) medium supplemented with heat-inactivated FCS (10%, v/v),

penicillin (100 U/mL), and streptomycin (100 $\mu\text{g}/\text{mL}$) at 37°C in a 5% CO_2 incubator. After 3 d, confluent cells were detached using $1\times$ trypsin/EDTA solution and reseeded at 2×10^6 cells in 20 mL medium in 175- cm^2 flasks.

For isolation of human platelets, venous blood was taken from healthy adult donors (Blood Center of the University Hospital Tuebingen) who did not take any medication for at least 7 d, and leukocyte concentrates were prepared by centrifugation ($4,000\times g$; 20 min; 20°C). Cells were immediately isolated by dextran sedimentation and centrifugation on Nycoprep cushions (PAA). Platelet-rich plasma was obtained from the supernatants, mixed with PBS (pH 5.9; 3:2 v/v), and centrifuged ($2,100\times g$; 15 min; room temperature), and the pelleted platelets were resuspended in PBS (pH 5.9)/0.9% NaCl (1:1 v/v). Platelets were finally resuspended in PBS (pH 7.4) and 1 mmol/L CaCl_2 .

Determination of PGE_2 and 6-keto $\text{PGF}_{1\alpha}$ Formation in Lipopolysaccharide-Stimulated Human Whole Blood

Peripheral blood from healthy adult volunteers (see above) was obtained by venipuncture and collected in syringes containing heparin (20 U/mL). For determination of PGE_2 and 6-keto $\text{PGF}_{1\alpha}$, aliquots of whole blood (0.8 mL) were mixed with the thromboxane synthase inhibitor CV4151 (1 $\mu\text{mol}/\text{L}$) and with aspirin (50 $\mu\text{mol}/\text{L}$). A total volume of 1 mL was adjusted with sample buffer [10 mmol/L potassium phosphate buffer (pH 7.4), 3 mmol/L KCl, 140 mmol/L NaCl, and 6 mmol/L D-glucose]. After preincubation with the indicated compounds for 5 min at room temperature, the samples were stimulated with lipopolysaccharide (10 $\mu\text{g}/\text{mL}$) for 5 h at 37°C . Prostanoid formation was stopped on ice, the samples were centrifuged ($2,300\times g$; 10 min; 4°C), and 6-keto $\text{PGF}_{1\alpha}$ was quantified in the supernatant using a 6-keto $\text{PGF}_{1\alpha}$ High Sensitivity EIA Kit (Assay

Designs), according to the manufacturer's protocol. PGE₂ was determined as described (23). In brief, the supernatant was acidified with citric acid (30 μ L; 2 mol/L), and after centrifugation (2,300 \times g; 10 min; 4°C), solid phase extraction and high-performance liquid chromatography analysis of PGE₂ were done to isolate PGE₂. The PGE₂ peak (3 mL), identified by coelution with the authentic standard, was collected, and acetonitrile was removed under a nitrogen stream. The pH was adjusted to 7.2 by addition of 10 \times PBS (pH 7.2; 230 μ L) before quantification of PGE₂ using a PGE₂ High Sensitivity EIA Kit (Assay Designs), according to the manufacturer's protocol.

Determination of Prostanoid Formation from Exogenous Arachidonic Acid in Human Whole Blood

Heparinized human whole blood, supplemented with penicillin (100 U/mL) and streptomycin (100 μ g/mL), was treated with 10 μ g/mL lipopolysaccharide for 16 h at 37°C and 5% CO₂. Then, CV4151 (1 μ mol/L) was added, and after preincubation with the indicated compounds for 10 min at 37°C, prostanoid formation was initiated by 100 μ mol/L arachidonic acid. PGE₂ and 6-keto PGF_{1 α} formation within 10 min was determined as described for lipopolysaccharide-stimulated whole blood. Calculated prostanoid levels were corrected by the amount of PGE₂ formed during prestimulation with lipopolysaccharide.

For determination of the COX product 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid, human whole blood (2 mL) was preincubated with the indicated compounds at 37°C for 10 min, and 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid formation was initiated by addition of 30 μ mol/L Ca²⁺-ionophore A23187 and 100 μ mol/L arachidonic acid. After 10 min at 37°C, the reaction was stopped on ice, and the samples were centrifuged (600 \times g; 10 min; 4°C). Aliquots of the resulting plasma (500 μ L) were then mixed with 2 mL of methanol, and 200 ng of PGB₁ was added as internal standard. The samples were placed at -20°C for 2 h and centrifuged again (600 \times g; 15 min; 4°C). The supernatants were collected and diluted with 2.5 mL PBS and 75 μ L 1 mol/L HCl. Formed 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid was extracted and analyzed by high-performance liquid chromatography, as described (24).

Activity Assays of Isolated COX-1 and -2

Inhibition of the activities of isolated COX-1 and -2 was done as described (23). Briefly, purified COX-1 (ovine; 50 units) or COX-2 (human recombinant; 20 units) were diluted in 1 mL reaction mixture containing 100 mmol/L Tris buffer (pH 8), 5 mmol/L glutathione, 5 μ mol/L hemoglobin, and 100 μ mol/L EDTA at 4°C, and preincubated with the test compounds for 5 min. Samples were prewarmed for 60 s at 37°C, and arachidonic acid (5 μ mol/L for COX-1, 2 μ mol/L for COX-2) was added to start the reaction. After 5 min at 37°C, 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid was extracted and then analyzed by high-performance liquid chromatography, as described (24).

Determination of COX-1 Product Formation in Washed Platelets

Freshly isolated platelets (10⁸/mL PBS containing 1 mmol/L CaCl₂) were preincubated with the indicated agents for 5 min at room temperature. After addition of

5 μ mol/L arachidonic acid and further incubation for 5 min at 37°C, 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid was extracted and then analyzed by high-performance liquid chromatography, as described (24).

Preparation of Crude Microsomal PGE₂ Synthase-1 in Microsomes of A549 Cells and Determination of PGE₂ Synthase Activity

Preparation of A549 cells and determination of microsomal PGE₂ synthase-1 activity was done as described previously (23). In brief, cells were incubated for 16 h at 37°C and 5% CO₂, the medium was replaced, interleukin-1 β (1 ng/mL) was added, and cells were incubated for another 48 h. Cells were harvested and frozen in liquid nitrogen, and ice-cold homogenization buffer [0.1 mol/L potassium phosphate buffer, (pH 7.4), 1 mmol/L phenylmethanesulphonyl fluoride, 60 μ g/mL soybean trypsin inhibitor, 1 μ g/mL leupeptin, 2.5 mmol/L glutathione, and 250 mmol/L sucrose] was added. Cells were sonicated on ice (3 \times 20 s), and the homogenate was subjected to differential centrifugation at 10,000 \times g for 10 min and 174,000 \times g for 1 h at 4°C. The pellet (microsomal fraction) was resuspended in 1 mL homogenization buffer, and the total protein concentration was determined by Coomassie protein assay. Microsomal membranes were diluted in potassium phosphate buffer (0.1 mol/L, pH 7.4) containing 2.5 mmol/L glutathione. Test compounds or vehicle were added, and after 15 min at 4°C, the reaction (100 μ L total volume) was initiated by addition of PGH₂ (20 μ mol/L final concentration). After 1 min at 4°C, the reaction was terminated using stop solution (100 μ L; 40 mmol/L FeCl₂, 80 mmol/L citric acid, and 10 μ mol/L of 11 β -PGE₂). PGE₂ was separated by solid phase extraction on reversed phase-C18 material and analyzed by reverse-phase high-performance liquid chromatography [30% acetonitrile/70% water + 0.007% TFA (v/v)] with UV detection at 195 nm. 11 β -PGE₂ was used as internal standard to quantify PGE₂ product formation by integration of the area under the peaks.

Statistics

Data are expressed as mean \pm SE. IC₅₀ values are approximations determined by graphical analysis (linear interpolation between the points between 50% activity). The program Graphpad InStat (Graphpad Software, Inc.) was used for statistical comparisons. Statistical evaluation of the data was done by one-way ANOVAs for independent or correlated samples followed by Tukey honestly significant differences (HSD) post hoc tests. A *P* of < 0.05 was considered significant.

Results

Curcumin Differentially Inhibits the Biosynthesis of PGE₂ and of 6-keto PGF_{1 α} in Human Whole Blood

We attempted to investigate nongenomic effects of curcumin on prostanoid biosynthesis using a modified whole blood assay. To minimize a potential interference with prostanoid generation at the level of gene expression, stimulation with lipopolysaccharide was restricted to 5 hours, instead of 24 hours (15). To avoid interference with other arachidonic acid metabolites in the ELISA, PGE₂ was

separated by reverse-phase high-performance liquid chromatography before its assessment by ELISA (23). Pretreatment of heparinized whole blood with curcumin resulted in a reduction of PGE₂ synthesis by ~40% at 3 μmol/L with an apparent IC₅₀ of 15 μmol/L (Fig. 2A). In analogy to the well-recognized microsomal PGE₂ synthase-1 inhibitors MK-886 and 2-(2-chlorophenyl)-1*H*-phenanthro[9,10-*d*]-imidazole (25), curcumin failed to completely suppress PGE₂ formation. The chosen concentrations of the microsomal PGE₂ synthase-1 reference inhibitors (30 and 2 μmol/L, respectively) markedly exceed their IC₅₀ values for inhibition of cell-free microsomal PGE₂ synthase-1 (2.1 and 0.09 μmol/L, respectively; refs. 25, 26) but are below the concentrations required to suppress the formation of other prostanoids (25). The COX inhibitors indomethacin and celecoxib used as controls efficiently blocked prostanoid formation, as expected (Fig. 2A). The concomitant generation of 6-keto PGF_{1α} was also reduced by curcumin under these experimental conditions, although less pronounced, and significant inhibition (40%) was evident only at 30 μmol/L (Fig. 2B). These results indicate that curcumin differentially interferes with the biosynthesis of PGE₂ and of 6-keto PGF_{1α}.

Because curcumin could block prostanoid formation by interference with lipopolysaccharide signaling or release of arachidonic acid (i.e., by phospholipase A₂ inhibition) as substrate for COX enzymes, receptor-coupled cell activation and substrate release was circumvented by supplementing exogenous arachidonic acid in the subsequent experiment. Human whole blood was first stimulated with lipopolysaccharide (16 hours) to induce expression of COX-2 and microsomal PGE₂ synthase-1. Then, the blood was preincubated with curcumin (10 min), and prostanoid formation was initiated by addition of exogenous arachidonic acid to provide ample substrate supply for COX-2. Under these experimental conditions, curcumin more efficiently suppressed PGE₂ synthesis with an IC₅₀ of ~1 μmol/L (Fig. 3A), and again, 6-keto PGF_{1α} synthesis in the same samples was suppressed only at 30 μmol/L (Fig. 3B). These data suggest that curcumin may directly interfere with the enzymatic conversion of PGH₂ to PGE₂.

Although COX-1 was found to be negligible for lipopolysaccharide-induced PGE₂ formation (27), we nevertheless assessed whether the activity of COX-1 was affected by curcumin. Heparinized whole blood (no lipopolysaccharide challenge) was preincubated with curcumin for 10 min and then the formation of 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid (as biomarker for COX activity) was elicited by Ca²⁺-ionophore and arachidonic acid. Curcumin moderately suppressed 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid formation with an IC₅₀ of 19 μmol/L (Fig. 3C).

Curcumin Inhibits Microsomal PGE₂ Synthase-1 Activity in Microsomes of A549 Lung Carcinoma Cells

Previously, curcumin was shown to moderately inhibit isolated ovine COX-1 (IC₅₀ = 25–50 μmol/L; refs. 3, 4) as well as COX-1-derived thromboxane A₂ formation in washed platelets (IC₅₀ = 40–70 μmol/L; ref. 28), whereas human recombinant COX-2 peroxidase activity was not signifi-

cantly affected up to 50 μmol/L (4). We could essentially confirm these results, showing that the isolated COX enzymes were not inhibited by curcumin at least up to 30 μmol/L (data not shown).

Suppression of PGE₂ synthesis might result from interference with enzymes distal of COX, namely, with PGE₂ synthases. Therefore, we investigated the effects of curcumin on microsomal PGE₂ synthase-1, which is functionally coupled to COX-2 (10). Microsomal preparations of interleukin-1β-treated A549 lung carcinoma cells, highly expressing microsomal PGE₂ synthase-1 (23), were preincubated with curcumin for 15 minutes before PGE₂ formation was initiated with 20 μmol/L PGH₂. Curcumin concentration dependently inhibited PGE₂ synthesis with an IC₅₀ of 0.3 μmol/L (Fig. 4A) being considerably superior over the reference compound MK-886 (IC₅₀ = 2.1 μmol/L; ref. 23). Decreasing the

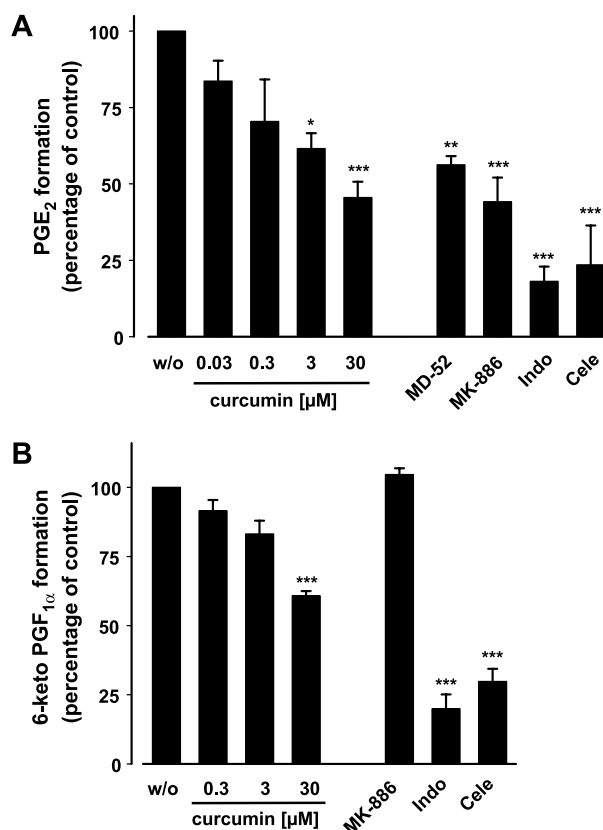


Figure 2. Effects of curcumin on prostanoid formation in lipopolysaccharide-stimulated human whole blood. Heparinized human whole blood, treated with 1 μmol/L CV4151 and 50 μmol/L aspirin, was preincubated with the test compounds or vehicle (DMSO) for 5 min at room temperature; 2-(2-chlorophenyl)-1*H*-phenanthro[9,10-*d*]-imidazole (2 μmol/L), MK-886 (30 μmol/L), indomethacin (50 μmol/L), and celecoxib (20 μmol/L) were used as controls. Indo, indomethacin; Cele, celecoxib. Then, 10 μg/ml lipopolysaccharide was added, and after 5 h at 37°C, PGE₂ was extracted from plasma by reversed phase-18 solid phase extraction, separated by reverse-phase high-performance liquid chromatography, and quantified by ELISA (A), as described, whereas 6-keto PGF_{1α} was directly determined in blood plasma by ELISA (B). Data are given as mean ± SE; n = 3 to 4. *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus vehicle (0.1% DMSO) control, ANOVA + Tukey HSD post hoc tests.

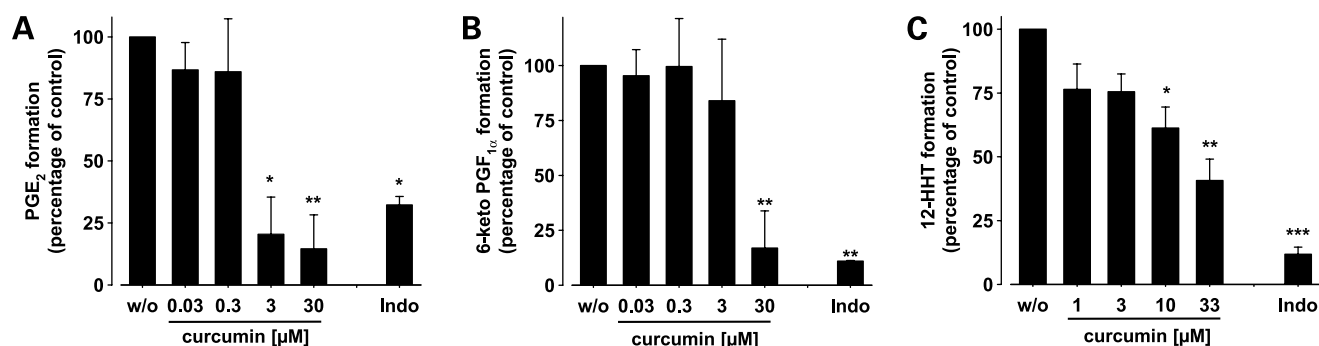


Figure 3. Effects of curcumin on arachidonic acid-induced prostanoid formation in human whole blood. Heparinized human whole blood was treated with 10 $\mu\text{g}/\text{mL}$ lipopolysaccharide for 16 h at 37°C, supplemented with thromboxane synthase inhibitor CV4151 (1 $\mu\text{mol}/\text{L}$), and preincubated with curcumin or vehicle (DMSO) for 10 min at 37°C. Then, 100 $\mu\text{mol}/\text{L}$ arachidonic acid was added, and PGE₂ (A) and 6-keto PGF_{1 α} (B) formed within 10 min were assessed as described. Indomethacin (50 $\mu\text{mol}/\text{L}$) was used as control. C, 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid formation. Heparinized whole blood was preincubated with curcumin or vehicle (DMSO) for 10 min, and arachidonic acid (100 $\mu\text{mol}/\text{L}$) and Ca²⁺-ionophore (30 $\mu\text{mol}/\text{L}$) were added. After 10 min at 37°C, 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid was extracted from blood plasma by reversed phase-18 solid phase extraction and analyzed by reverse-phase high-performance liquid chromatography, as described in Materials and Methods. Indomethacin (20 $\mu\text{mol}/\text{L}$) was used as control. Data are given as mean \pm SE; $n = 3$ to 5. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus vehicle (0.1% DMSO) control, ANOVA + Tukey HSD post hoc tests.

PGH₂ concentration to 1 $\mu\text{mol}/\text{L}$ even slightly increased the potency of curcumin (IC₅₀ = 0.17 $\mu\text{mol}/\text{L}$; Fig. 4B). To investigate whether curcumin reversibly inhibits microsomal PGE₂ synthase-1, microsomes preincubated with 1 $\mu\text{mol}/\text{L}$ curcumin were subjected to wash-out experiments. Ten-fold dilution of the sample to a final curcumin concentration of 0.1 $\mu\text{mol}/\text{L}$ recovered the enzymatic activity (Fig. 4C), implying a reversible mode of inhibition. Structurally related polyphenols (Fig. 1), such as coniferyl alcohol, eugenol, [6]-gingerol, caffeic acid, rosmarinic acid, and resveratrol, failed to significantly inhibit microsomal PGE₂ synthase-1 up to 10 $\mu\text{mol}/\text{L}$ (Fig. 4D), indicating that specific structural features are necessary for microsomal PGE₂ synthase-1 inhibition.

Discussion

Curcumin has received substantial attention as an effective antitumorogenic and anti-inflammatory compound, and many modes of action have been proposed that may rationalize its efficacy (for review, see refs. 1, 2). Curcumin modulates the expression or activation state of various transcription factors (e.g., NF κ B), protein kinases (e.g., protein kinase C), cytokines (e.g., tumor necrosis factor- α), enzymes (e.g., p300 histone acetyl transferase, COX-1), and many other regulators or effectors of cell proliferation, apoptosis, cell cycle regulation, angiogenesis, invasion, and inflammation (1). COX-1 (3), 5-lipoxygenase (3), glycogen synthase kinase-3 β (5), and inhibitor of NF κ B kinase (7) have been proposed as direct targets, but the functional link to the anticarcinogenic or anti-inflammatory effects is often unclear, and several of these interactions occur only at high curcumin concentrations, which are probably not pharmacologically relevant (see below). In this respect, human microsomal PGE₂ synthase-1 from A549 lung carcinoma cells represents a high-affinity target of curcumin with IC₅₀ values in the submicromolar range. Because closely related (poly)phenolic compounds failed to inhibit micro-

somal PGE₂ synthase-1, defined structural arrangements of curcumin are required for this interaction. Interestingly, curcumin represents a lipophilic acid similar to MK-886-derived microsomal PGE₂ synthase-1 inhibitors (29), suggesting that a common binding site at microsomal PGE₂ synthase-1 may exist (30). Moreover, the functional interference with microsomal PGE₂ synthase-1, reflected by inhibition of cellular PGE₂ formation, is also apparent in human whole blood (a clinically relevant pharmacologic test system), wherein many compounds fail due to unfavorable intracellular availability, degradation, and high plasma protein binding (31).

Our findings are in part consistent with previous studies showing effectiveness of curcumin on PGE₂ formation in human whole blood (15) and other cellular systems (IC₅₀ = 1–5 $\mu\text{mol}/\text{L}$; refs. 11, 13, 21), although the experimental settings of those studies did not allow to differentiate between nongenomic effects of curcumin on PGE₂ generation and effects at the level of gene expression (e.g., of COX-2, microsomal PGE₂ synthase-1). In particular, repression of COX-2 has been considered as major mechanism of curcumin underlying the reduced PGE₂ formation (1). However, substantially higher concentrations of curcumin are required to interfere with COX-2 expression (13, 21, 32) than suppressing microsomal PGE₂ synthase-1-derived PGE₂ biosynthesis. In addition, direct inhibition of isolated and cellular COX-1 and -2 activity (this study and refs. 3, 4, 28, 33) or modulation of arachidonic acid release through impaired activation of cytosolic phospholipase A₂ (3) are less pronounced. Consequently, other points of attack must exist, and interference of curcumin with microsomal PGE₂ synthase-1 may represent such a mechanism underlying the suppression of proinflammatory PGE₂ synthesis. On the other hand, effects on COX isoenzymes might contribute to the general suppression of cellular prostanoid biosynthesis observed at higher curcumin concentrations (≥ 10 $\mu\text{mol}/\text{L}$; refs. 21, 28, 34). Along these lines, curcumin at 30 $\mu\text{mol}/\text{L}$

significantly reduced the generation of PGE₂ and COX-2/prostacyclin synthase-derived 6-keto PGF₁α in our human whole blood assay.

The pharmacologic relevance of our findings is supported by data from clinical trials showing inhibition of PGE₂ *in vivo* (16–19). Thus, in a phase I trial, a daily dose of 3.6 g curcumin caused 62% and 57% reduction in inducible PGE₂ production in blood samples taken 1 hour after oral application (16). Interestingly, after daily oral uptake of 4 to 8 g curcumin, peak serum concentrations of 0.5 to 1.8 μmol/L were measured in a clinical study with 25 patients (35). Such plasma levels of curcumin are in the range of the effective concentrations needed to suppress microsomal PGE₂ synthase-1-derived PGE₂ formation in whole blood. Although lower daily doses of curcumin (36–180 mg) failed to achieve detectable plasma levels of curcumin (36), they might show pharmacologic relevance in the intestine, wherein tissue concentrations of up to 13 nmol/g in humans (3.6 g/d; ref. 37) and 1.8 μmol/g in rats (2% dietary curcumin) were achieved (36).

Recent advances in genetic and pharmacologic inhibition of microsomal PGE₂ synthase-1 indicate a crucial role of microsomal PGE₂ synthase-1 in the development and maintenance of inflammatory disorders, pain, fever, and cardiovascular diseases, and suggest microsomal PGE₂ synthase-1 inhibitors as alternative to nonsteroidal anti-inflammatory drugs showing comparable anti-inflammatory effectiveness while being essentially free of severe side effects (9, 31). Moreover, COX-2 and microsomal PGE₂ synthase-1 are overexpressed in various tumors (i.e., prostate, breast, lung, and colon; ref. 9), and preclinical studies indicate tumor-preventive effects of COX inhibition by nonsteroidal anti-inflammatory drugs and coxibs (38). Accordingly, genetic ablation of microsomal PGE₂ synthase-1 (9) or pharmacologic

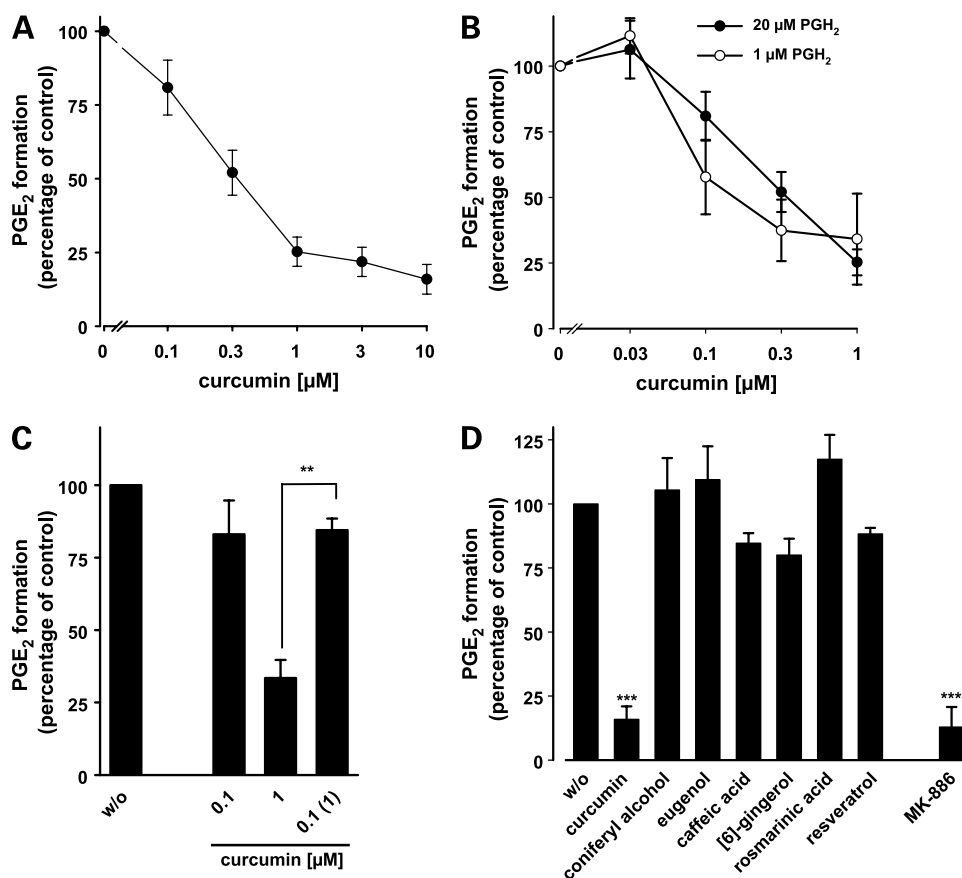


Figure 4. Effects of curcumin and related polyphenols on the activity of microsomal PGE₂ synthase-1. Microsomal preparations of interleukin-1β-stimulated A549 cells were preincubated with vehicle (DMSO) or the test compounds at the indicated concentrations for 15 min at 4°C, and the reaction was started with 20 μmol/L PGH₂. After 1 min at 4°C, the reaction was terminated using a stop solution containing FeCl₂ and 11β-PGE₂ (1 nmol) as internal standard. **A**, concentration-response curves for curcumin. **B**, the potency of curcumin for microsomal PGE₂ synthase-1 inhibition was compared at 1 and 20 μmol/L PGH₂ as substrate. The amount of PGE₂ was quantified for 1 μmol/L PGH₂ by use of a PGE₂ High Sensitivity EIA Kit. Data are given as mean ± SE; *n* = 3. **C**, reversibility of microsomal PGE₂ synthase-1 inhibition by curcumin. Microsomal preparations of interleukin-1β-stimulated A549 cells were preincubated with 1 μmol/L curcumin for 15 min at 4°C. An aliquot was diluted 10-fold to obtain an inhibitor concentration of 0.1 μmol/L. For comparison, microsomal preparations were preincubated for 15 min with 0.1 μmol/L curcumin or with vehicle (DMSO). Then, 20 μmol/L PGH₂ was added (no dilution), and PGE₂ formation was analyzed by reverse-phase high-performance liquid chromatography, as described. **D**, inhibition of microsomal PGE₂ synthase-1 by curcumin, related polyphenols, and MK-886 at 10 μmol/L, each. Data are given as mean ± SE; *n* = 3 to 4. **, *P* < 0.01; ***, *P* < 0.001, ANOVA + Tukey HSD post hoc tests.

inhibition of microsomal PGE₂ synthase-1 was shown to relieve fever and pain (25, 39) and to prevent intestinal tumorigenesis in APC^{min} mice (40). Of interest, induction of apoptosis of colorectal adenocarcinoma cell lines by curcumin was found to be correlated to inhibition of PGE₂ synthesis (41). Hence, inhibition of microsomal PGE₂ synthase-1 by curcumin might not only contribute to the efficacy of curcumin in the therapy of inflammation and cancer but might also be related to its high safety at daily dosages as high as 8 to 12 g (35, 42), for which neither gastrointestinal, renal, nor cardiovascular side effects (associated with COX inhibitors; ref. 43) were observed (7, 9).

Taken together, the extensive research over the last decades has rationalized the traditional use of curcumin for the treatment of various diseases (2). Although suppression of PGE₂ synthesis by curcumin was reported in numerous cellular studies as well as *in vivo* (3, 11–19), the molecular basis underlying this effect was still incompletely understood. Here, we provide strong evidence that curcumin preferentially suppresses PGE₂ synthesis by interference with microsomal PGE₂ synthase-1, and this action might essentially contribute to the anti-inflammatory and anticarcinogenic potential of curcumin.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. *Trends Pharmacol Sci* 2008;30:85–94.
- Hatcher H, Planalp R, Cho J, et al. Curcumin: from ancient medicine to current clinical trials. *Cell Mol Life Sci* 2008;65:1631–52.
- Hong J, Bose M, Ju J, et al. Modulation of arachidonic acid metabolism by curcumin and related β -diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. *Carcinogenesis* 2004;25:1671–9.
- Handler N, Jaeger W, Puschacher H, et al. Synthesis of novel curcumin analogues and their evaluation as selective cyclooxygenase-1 (COX-1) inhibitors. *Chem Pharm Bull (Tokyo)* 2007;55:64–71.
- Bustanji Y, Taha MO, Almasri IM, et al. Inhibition of glycogen synthase kinase by curcumin: investigation by simulated molecular docking and subsequent *in vitro/in vivo* evaluation. *J Enzyme Inhib Med Chem* 2008. In press.
- Martin-Cordero C, Lopez-Lazaro M, Galvez M, et al. Curcumin as a DNA topoisomerase II poison. *J Enzyme Inhib Med Chem* 2003;18:505–9.
- Kasinski AL, Du Y, Thomas SL, et al. Inhibition of I κ B kinase-nuclear factor- κ B signaling pathway by 3,5-bis(2-fluorobenzylidene)piperidin-4-one (EF24), a novel monoketone analog of curcumin. *Mol Pharmacol* 2008;74:654–61.
- Hasmeda M, Polya GM. Inhibition of cyclic AMP-dependent protein kinase by curcumin. *Phytochemistry* 1996;42:599–605.
- Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: a novel therapeutic target. *Pharmacol Rev* 2007;59:207–24.
- Murakami M, Nakatani Y, Tanioka T, et al. Prostaglandin E synthase. *Prostaglandins Other Lipid Mediat* 2002;68–69:383–99.
- Moon Y, Glasgow WC, Eling TE. Curcumin suppresses interleukin 1 β -mediated microsomal prostaglandin E synthase 1 by altering early growth response gene 1 and other signaling pathways. *J Pharmacol Exp Ther* 2005;315:788–95.
- Joe B, Lokesh BR. Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. *Lipids* 1997;32:1173–80.
- Zhang F, Altorki NK, Mestre JR, et al. Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial cells. *Carcinogenesis* 1999;20:445–51.
- Ireson C, Orr S, Jones DJ, et al. Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat *in vivo*, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E₂ production. *Cancer Res* 2001;61:1058–64.
- Plummer SM, Hill KA, Festing MF, et al. Clinical development of leukocyte cyclooxygenase 2 activity as a systemic biomarker for cancer chemopreventive agents. *Cancer Epidemiol Biomarkers Prev* 2001;10:1295–9.
- Sharma RA, Euden SA, Platton SL, et al. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004;10:6847–54.
- Rao CV, Simi B, Reddy BS. Inhibition by dietary curcumin of azoxy-methane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. *Carcinogenesis* 1993;14:2219–25.
- Rao CV, Rivenson A, Simi B, et al. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res* 1995;55:259–66.
- Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. *Eur J Cancer* 2005;41:1955–68.
- Surh YJ, Chun KS, Cha HH, et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF- κ B activation. *Mutat Res* 2001;480–481:243–68.
- Huang MT, Lysz T, Ferraro T, et al. Inhibitory effects of curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res* 1991;51:813–9.
- Kato K, Ohkawa S, Terao S, et al. Thromboxane synthetase inhibitors (TXSI). Design, synthesis, and evaluation of a novel series of ω -pyridylalkenoic acids. *J Med Chem* 1985;28:287–94.
- Koeberle A, Siemoneit U, Buehring U, et al. Licofelone suppresses prostaglandin E₂ formation by interference with the inducible microsomal prostaglandin E₂ synthase-1. *J Pharmacol Exp Ther* 2008;975–82.
- Albert D, Zundorf I, Dingermann T, et al. Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. *Biochem Pharmacol* 2002;64:1767–75.
- Cote B, Boulet L, Brideau C, et al. Substituted phenanthrene imidazoles as potent, selective, and orally active mPGES-1 inhibitors. *Bioorg Med Chem Lett* 2007;17:6816–20.
- Koeberle A, Siemoneit U, Buehring U, et al. Licofelone suppresses prostaglandin E₂ formation by interference with the inducible microsomal prostaglandin E₂ synthase-1. *J Pharmacol Exp Ther* 2008;326:975–82.
- Brideau C, Kargman S, Liu S, et al. A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. *Inflamm Res* 1996;45:68–74.
- Shah BH, Nawaz Z, Pertani SA, et al. Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor- and arachidonic acid-mediated platelet aggregation through inhibition of thromboxane formation and Ca²⁺ signaling. *Biochem Pharmacol* 1999;58:1167–72.
- Riendeau D, Aspiotis R, Ethier D, et al. Inhibitors of the inducible microsomal prostaglandin E₂ synthase (mPGES-1) derived from MK-886. *Bioorg Med Chem Lett* 2005;15:3352–5.
- San Juan AA, Cho SJ. 3D-QSAR study of microsomal prostaglandin E₂ synthase (mPGES-1) inhibitors. *J Mol Model* 2007;13:601–10.
- Friesen RW, Mancini JA. Microsomal prostaglandin E₂ synthase-1 (mPGES-1): a novel anti-inflammatory therapeutic target. *J Med Chem* 2008;51:4059–67.
- Gafner S, Lee SK, Cuendet M, et al. Biologic evaluation of curcumin and structural derivatives in cancer chemoprevention model systems. *Phytochemistry* 2004;65:2849–59.
- Ammon HP, Safayhi H, Mack T, et al. Mechanism of anti-inflammatory actions of curcumin and boswellic acids. *J Ethnopharmacol* 1993;38:113–9.
- Srivastava KC, Bordia A, Verma SK. Curcumin, a major component

of food spice turmeric (*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. Prostaglandins Leukot Essent Fatty Acids 1995;52:223–7.

35. Cheng AL, Hsu CH, Lin JK, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res 2001;21:2895–900.
36. Sharma RA, Ireson CR, Verschoyle RD, et al. Effects of dietary curcumin on glutathione *S*-transferase and malondialdehyde-DNA adducts in rat liver and colon mucosa: relationship with drug levels. Clin Cancer Res 2001;7:1452–8.
37. Garcea G, Berry DP, Jones DJ, et al. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. Cancer Epidemiol Biomarkers Prev 2005;14:120–5.
38. Gasparini G, Longo R, Sarmiento R, et al. Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? Lancet Oncol 2003;4:605–15.
39. Xu D, Rowland SE, Clark P, et al. MF63 {2-(6-chloro-1H-phenanthro[9,10-d]imidazol-2-yl)isophthalonitrile}, a selective microsomal prostaglandin E synthase 1 inhibitor, relieves pyresis and pain in preclinical models of inflammation. J Pharmacol Exp Ther 2008;326:754–63.
40. Collett GP, Robson CN, Mathers JC, et al. Curcumin modifies Apc(min) apoptosis resistance and inhibits 2-amino 1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) induced tumour formation in Apc(min) mice. Carcinogenesis 2001;22:821–5.
41. Lev-Ari S, Maimon Y, Strier L, et al. Down-regulation of prostaglandin E2 by curcumin is correlated with inhibition of cell growth and induction of apoptosis in human colon carcinoma cell lines. J Soc Integr Oncol 2006;4:21–6.
42. Lao CD, Ruffin MTt, Normolle D, et al. Dose escalation of a curcuminoid formulation. BMC Complement Altern Med 2006;6:10.
43. Rainsford KD. Anti-inflammatory drugs in the 21st century. Subcell Biochem 2007;42:3–27.

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