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Curcumin blocks prostaglandin E_2 biosynthesis through direct inhibition of the microsomal prostaglandin E_2 synthase-1

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Abstract

Prostaglandin E_2 (PGE_2) plays a crucial role in the apparent link between tumor growth and chronic inflammation. Cyclooxygenase (COX)-2 and microsomal PGE_2 synthase-1, which are overexpressed in many cancers, are functionally coupled and thus produce massive PGE_2 in various tumors. Curcumin, a polyphenolic β-diketone from tumeric with anti-carcinogenic and anti-inflammatory activities, was shown to suppress PGE_2 formation and to block the expression of COX-2 and of microsomal PGE_2 synthase-1. Here, we identified microsomal PGE_2 synthase-1 as a molecular target of curcumin and we show that inhibition of microsomal PGE_2 synthase-1 activity is the predominant mechanism of curcumin to suppress PGE_2 biosynthesis. Curcumin reversibly inhibited the conversion of PGH_2 to PGE_2 by microsomal PGE_2 synthase-1 in microsomes of interleukin-1β–stimulated A549 lung carcinoma cells with an IC_{50} 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 PGE_2 formation from endogenous or from exogenous arachidonic acid, whereas the concomitant formation of COX-2–mediated 6-keto $PGF_1\alpha$ 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 PGE_2 in inflammation and carcinogenesis, inhibition of microsomal PGE_2 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 (IC_{50} = 25–50 μmol/L; refs. 3, 4), 5-lipoxygenase (IC_{50} = 0.7 μmol/L; ref. 3), glycogen synthase kinase-3β (IC_{50} = 0.07 μmol/L; ref. 5), DNA topoisomerase II (at 50 μmol/L; ref. 6), inhibitor of NFκB kinase (IC_{50} = 20 μmol/L; ref. 7), protein kinase C (IC_{50} = 15 μmol/L; ref. 8), and xanthine oxidase (IC_{50} = 200–400 μmol/L). However, many of these interactions are characterized by low affinities as reflected by the respective high IC_{50} values in functional assays, and the pharmacologic relevance of most of these target interactions is uncertain.

Prostaglandin E_2 (PGE_2) is a potent lipid mediator that is closely linked to inflammation and cancer. The biosynthesis of PGE_2 requires transformation of arachidonic acid by COX-1 or COX-2 [enzyme commission (EC) 1.14.99.1] to PGH_2, which is subsequently converted by PGE_2 synthases (EC 5.3.99.3) to PGE_2 (9). Whereas the cytosolic PGE_2 synthase is constitutively expressed and preferentially couples to COX-1, the microsomal PGE_2 synthase-1 is functionally linked to COX-2. COX-2 and microsomal PGE_2 synthase-1 are induced by proinflammatory stimuli, and both enzymes are overexpressed in various cancers (9, 10). Curcumin was shown to lower PGE_2 formation in cellular models (3, 11–14), in whole blood (15), and *in vivo* (16–19). Besides direct inhibition of COX-1 and -2 (IC_{50} = 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 PGE_2 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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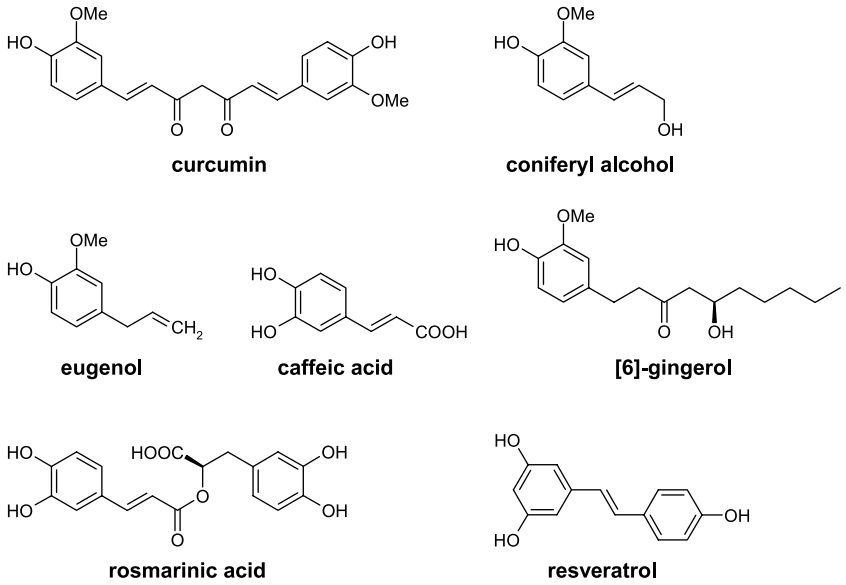

Figure 1. Structure of curcumin and related polyphenols.

conversion of arachidonic acid to PGD_2 and $PGF_2\alpha$ was blocked in epidermal microsomes (21), but curcumin increased the formation of $PGF_2\alpha$ and the stable PGI_2 degradation product 6-keto $PGF_1\alpha$ 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 μg/mL) at 37°C in a 5% CO_2 incubator. After 3 d, confluent cells were detached using 1× 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 × *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 × *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 $PGF_1\alpha$ 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 $PGF_1\alpha$, aliquots of whole blood (0.8 mL) were mixed with the thromboxane synthase inhibitor CV4151 (1 μmol/L) and with aspirin (50 μmol/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 μg/mL) for 5 h at 37°C. Prostanoid formation was stopped on ice, the samples were centrifuged (2,300 × *g*; 10 min; 4°C), and 6-keto $PGF_1\alpha$ was quantified in the supernatant using a 6-keto $PGF_1\alpha$ High Sensitivity EIA Kit (Assay

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Designs), according to the manufacturer's protocol. PGE_2 was determined as described (23). In brief, the supernatant was acidified with citric acid (30 μL; 2 mol/L), and after centrifugation (2,300 × *g*; 10 min; 4°C), solid phase extraction and high-performance liquid chromatography analysis of PGE_2 were done to isolate PGE_2. The PGE_2 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× PBS (pH 7.2; 230 μL) before quantification of PGE_2 using a PGE_2 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 μg/mL), was treated with 10 μg/mL lipopolysaccharide for 16 h at 37°C and 5% CO_2. Then, CV4151 (1 μmol/L) was added, and after preincubation with the indicated compounds for 10 min at 37°C, prostanoid formation was initiated by 100 μmol/L arachidonic acid. PGE_2 and 6-keto $PGF_1\alpha$ formation within 10 min was determined as described for lipopolysaccharide-stimulated whole blood. Calculated prostanoid levels were corrected by the amount of PGE_2 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 μmol/L Ca^{2+}-ionophore A23187 and 100 μmol/L arachidonic acid. After 10 min at 37°C, the reaction was stopped on ice, and the samples were centrifuged (600 × g; 10 min; 4°C). Aliquots of the resulting plasma (500 μL) were then mixed with 2 mL of methanol, and 200 ng of PGB_1 was added as internal standard. The samples were placed at -20°C for 2 h and centrifuged again (600 × g; 15 min; 4°C). The supernatants were collected and diluted with 2.5 mL PBS and 75 μL 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 μmol/L hemoglobin, and 100 μmol/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 μmol/L for COX-1, 2 μmol/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 (10^8/mL PBS containing 1 mmol/L $CaCl_2$) were preincubated with the indicated agents for 5 min at room temperature. After addition of 5 μmol/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 PGE_2 Synthase-1 in Microsomes of A549 Cells and Determination of PGE_2 Synthase Activity

Preparation of A549 cells and determination of microsomal PGE_2 synthase-1 activity was done as described previously (23). In brief, cells were incubated for 16 h at 37°C and 5% CO_2, the medium was replaced, interleukin-1β (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 μg/mL soybean trypsin inhibitor, 1 μg/mL leupeptin, 2.5 mmol/L glutathione, and 250 mmol/L sucrose] was added. Cells were sonicated on ice (3 × 20 s), and the homogenate was subjected to differential centrifugation at 10,000 × g for 10 min and 174,000 × 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 μL total volume) was initiated by addition of PGH_2 (20 μmol/L final concentration). After 1 min at 4°C, the reaction was terminated using stop solution (100 μL; 40 mmol/L $FeCl_2$, 80 mmol/L citric acid, and 10 μmol/L of 11β-PGE_2). PGE_2 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. 11β-PGE_2 was used as internal standard to quantify PGE_2 product formation by integration of the area under the peaks.

Statistics

Data are expressed as mean ± SE. IC_{50} 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 PGE_2 and of 6-keto $PGF_1\alpha$ 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, PGE_2 was

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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 PGE_2 synthesis by ~40% at 3 μmol/L with an apparent IC_{50} of 15 μmol/L (Fig. 2A). In analogy to the well-recognized microsomal PGE_2 synthase-1 inhibitors MK-886 and 2-(2-chlorophenyl)-1*H*-phenanthro[9,10-d]-imidazole (25), curcumin failed to completely suppress PGE_2 formation. The chosen concentrations of the microsomal PGE_2 synthase-1 reference inhibitors (30 and 2 μmol/L, respectively) markedly exceed their IC_{50} values for inhibition of cell-free microsomal PGE_2 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 $PGF_1\alpha$ 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 PGE_2 and of 6-keto $PGF_1\alpha$.

Because curcumin could block prostanoid formation by interference with lipopolysaccharide signaling or release of arachidonic acid (i.e., by phospholipase A_2 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 PGE_2 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 PGE_2 synthesis with an IC_{50} of ~1 μmol/L (Fig. 3A), and again, 6-keto $PGF_1\alpha$ 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 PGH_2 to PGE_2.

Although COX-1 was found to be negligible for lipopolysaccharide-induced PGE_2 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 Ca^{2+}-ionophore and arachidonic acid. Curcumin moderately suppressed 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid formation with an IC_{50} of 19 μmol/L (Fig. 3C).

Curcumin Inhibits Microsomal PGE_2 Synthase-1 Activity in Microsomes of A549 Lung Carcinoma Cells

Previously, curcumin was shown to moderately inhibit isolated ovine COX-1 (IC_{50} = 25–50 μmol/L; refs. 3, 4) as well as COX-1–derived thromboxane A_2 formation in washed platelets (IC_{50} = 40–70 μmol/L; ref. 28), whereas human recombinant COX-2 peroxidase activity was not significantly 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 PGE_2 synthesis might result from interference with enzymes distal of COX, namely, with PGE_2 synthases. Therefore, we investigated the effects of curcumin on microsomal PGE_2 synthase-1, which is functionally coupled to COX-2 (10). Microsomal preparations of interleukin-1β–treated A549 lung carcinoma cells, highly expressing microsomal PGE_2 synthase-1 (23), were preincubated with curcumin for 15 minutes before PGE_2 formation was initiated with 20 μmol/L PGH_2. Curcumin concentration dependently inhibited PGE_2 synthesis with an IC_{50} of 0.3 μmol/L (Fig. 4A) being considerably superior over the reference compound MK-886 (IC_{50} = 2.1 μmol/L; ref. 23). Decreasing the

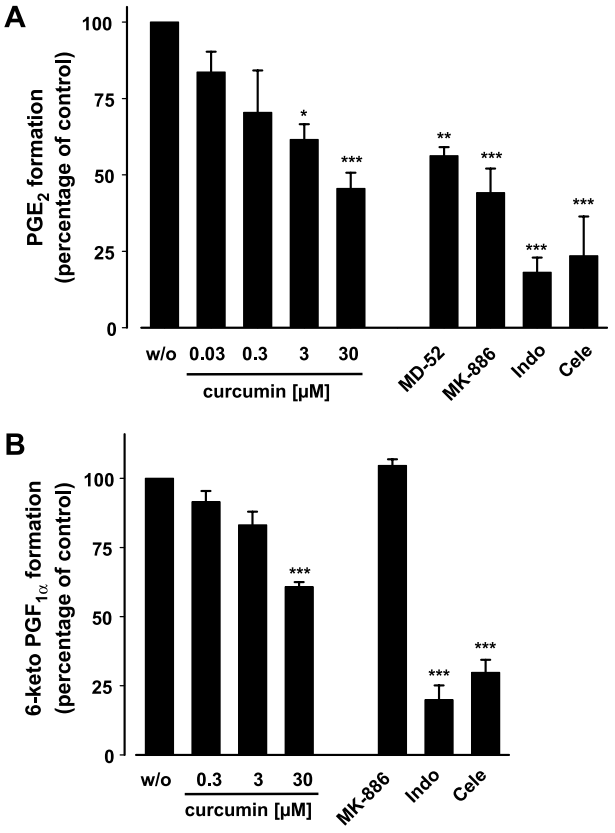

Figure 2. Effects of curcumin on prostanoid formation in lipopolysaccharide-stimulated human whole blood. Heparinized human whole blood, treated with 1 μmol/L CV4151 and 50 μmol/L aspirin, was preincubated with the test compounds or vehicle (DMSO) for 5 min at room temperature; 2-(2-chlorophenyl)-1*H*-phenanthro[9,10-d]-imidazole (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, PGE_2 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 $PGF_1\alpha$ 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.

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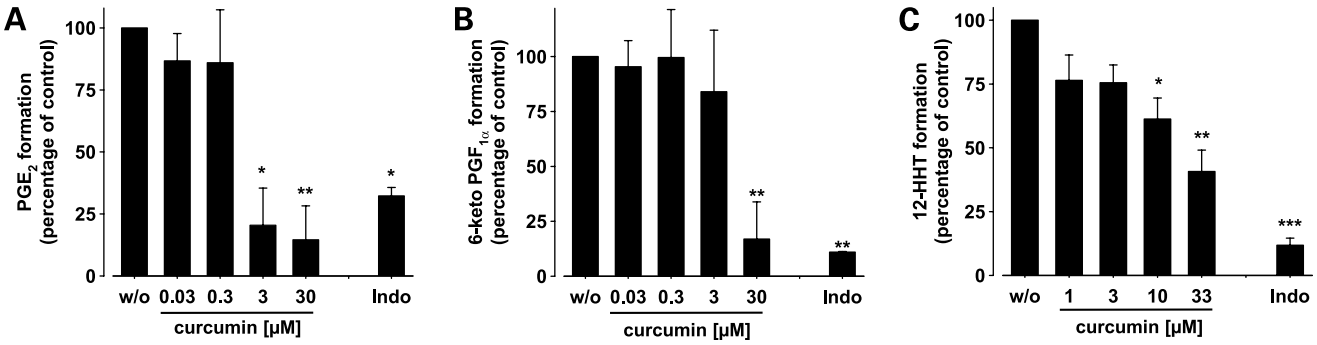

Figure 3. Effects of curcumin on arachidonic acid–induced prostanoid formation in human whole blood. Heparinized human whole blood was treated with 10 μg/mL lipopolysaccharide for 16 h at 37°C, supplemented with thromboxane synthase inhibitor CV4151 (1 μmol/L), and preincubated with curcumin or vehicle (DMSO) for 10 min at 37°C. Then, 100 μmol/L arachidonic acid was added, and PGE_2 (**A**) and 6-keto $PGF_{1\alpha}$ (**B**) formed within 10 min were assessed as described. Indomethacin (50 μmol/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 μmol/L) and Ca^{2+}-ionophore (30 μmol/L) were added. After 10 min at 37°C, 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid was extracted form 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 μmol/L) was used as control. Data are given as mean ± 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.

PGH_2 concentration to 1 μmol/L even slightly increased the potency of curcumin (IC_{50} = 0.17 μmol/L; Fig. 4B). To investigate whether curcumin reversibly inhibits microsomal PGE_2 synthase-1, microsomes preincubated with 1 μmol/L curcumin were subjected to wash-out experiments. Ten-fold dilution of the sample to a final curcumin concentration of 0.1 μmol/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 PGE_2 synthase-1 up to 10 μmol/L (Fig. 4D), indicating that specific structural features are necessary for microsomal PGE_2 synthase-1 inhibition.

Discussion

Curcumin has received substantial attention as an effective antitumorigenic 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., NFκB), 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-3β (5), and inhibitor of NFκB 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 PGE_2 synthase-1 from A549 lung carcinoma cells represents a high-affinity target of curcumin with IC_{50} values in the submicromolar range. Because closely related (poly)phenolic compounds failed to inhibit microsomal PGE_2 synthase-1, defined structural arrangements of curcumin are required for this interaction. Interestingly, curcumin represents a lipophilic acid similar to MK-886–derived microsomal PGE_2 synthase-1 inhibitors (29), suggesting that a common binding site at microsomal PGE_2 synthase-1 may exists (30). Moreover, the functional interference with microsomal PGE_2 synthase-1, reflected by inhibition of cellular PGE_2 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 PGE_2 formation in human whole blood (15) and other cellular systems (IC_{50} = 1–5 μmol/L; refs. 11, 13, 21), although the experimental settings of those studies did not allow to differentiate between nongenomic effects of curcumin on PGE_2 generation and effects at the level of gene expression (e.g., of COX-2, microsomal PGE_2 synthase-1). In particular, repression of COX-2 has been considered as major mechanism of curcumin underlying the reduced PGE_2 formation (1). However, substantially higher concentrations of curcumin are required to interfere with COX-2 expression (13, 21, 32) than suppressing microsomal PGE_2 synthase-1–derived PGE_2 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 A_2 (3) are less pronounced. Consequently, other points of attack must exist, and interference of curcumin with microsomal PGE_2 synthase-1 may represent such a mechanism underlying the suppression of proinflammatory PGE_2 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 μmol/L; refs. 21, 28, 34). Along these lines, curcumin at 30 μmol/L

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significantly reduced the generation of PGE_2 and COX-2/prostacyclin synthase–derived 6-keto $PGF_1\alpha$ in our human whole blood assay.

The pharmacologic relevance of our findings is supported by data from clinical trials showing inhibition of PGE_2 *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 PGE_2 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 PGE_2 synthase-1–derived PGE_2 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 PGE_2 synthase-1 indicate a crucial role of microsomal PGE_2 synthase-1 in the development and maintenance of inflammatory disorders, pain, fever, and cardiovascular diseases, and suggest microsomal PGE_2 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 PGE_2 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 PGE_2 synthase-1 (9) or pharmacologic

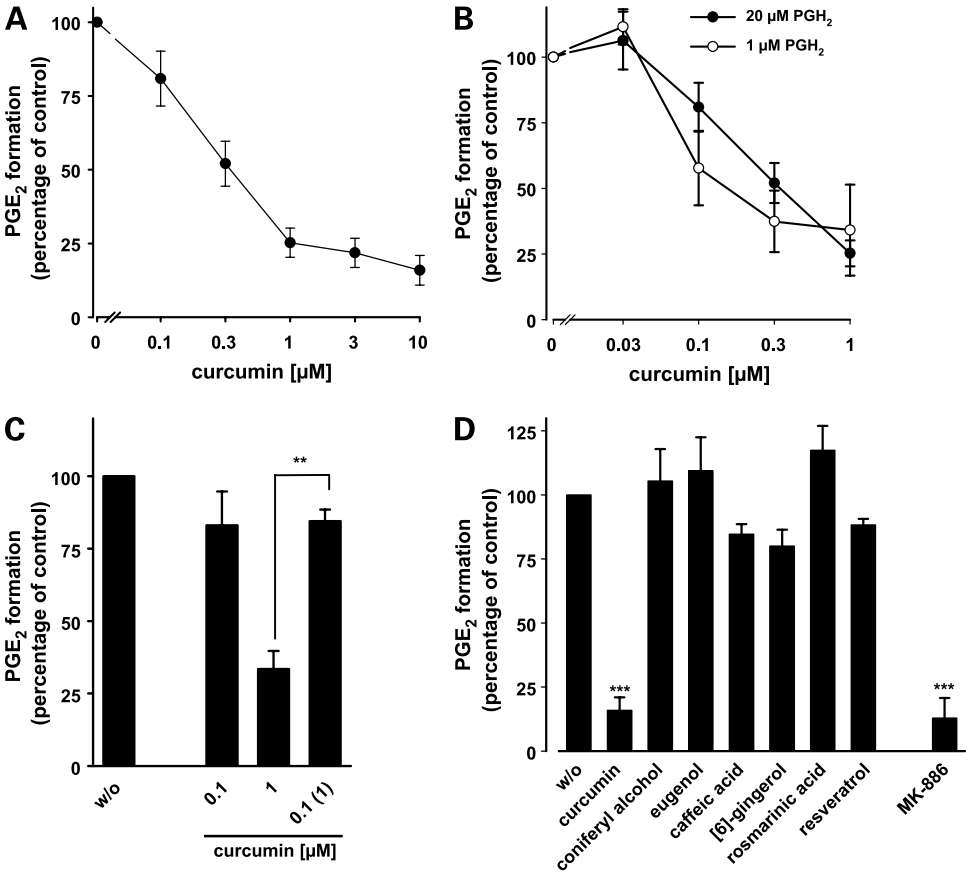

Figure 4. Effects of curcumin and related polyphenols on the activity of microsomal PGE_2 synthase-1. Microsomal preparations of interleukin-1β–stimulated A549 cells were preincubated with vehicle (DMSO) or the test compounds at the indicated concentrations for 15 min at 4°C, and the reaction was started with 20 μmol/L PGH_2. After 1 min at 4°C, the reaction was terminated using a stop solution containing $FeCl_2$ and 11β-PGE_2 (1 nmol) as internal standard. **A,** concentration-response curves for curcumin. **B,** the potency of curcumin for microsomal PGE_2 synthase-1 inhibition was compared at 1 and 20 μmol/L PGH_2 as substrate. The amount of PGE_2 was quantified for 1 μmol/L PGH_2 by use of a PGE_2 High Sensitivity EIA Kit. Data are given as mean ± SE; $n = 3$. **C,** reversibility of microsomal PGE_2 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 PGH_2 was added (no dilution), and PGE_2 formation was analyzed by reverse-phase high-performance liquid chromatography, as described. **D,** inhibition of microsomal PGE_2 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 PGE_2 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 PGE_2 synthesis (41). Hence, inhibition of microsomal PGE_2 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 PGE_2 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 PGE_2 synthesis by interference with microsomal PGE_2 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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