Curcumin blocks prostaglandin E$_2$ biosynthesis through direct inhibition of the microsomal prostaglandin E$_2$ synthase-1

Andreas Koeberle,$^1$ Hinnak Northoff,$^2$ and Oliver Werz$^1$

$^1$Department of Pharmaceutical Analytics, Pharmaceutical Institute, University of Tuebingen, and $^2$Institute for Clinical and Experimental Transfusion Medicine, University Medical Center Tuebingen, Tuebingen, Germany

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 linked 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$_2$α and COX-1-derived 12(S)-hydroxy-5-cis-8,10-trans-heptadecaenoic 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 (diferylulymethane; 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, 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 prostanooid biosynthesis are diverse, depending on the distinct assays used. For example, the
conversion of arachidonic acid to PGD₂ and PGF₂α was blocked in epidermal microsomes (21), but curcumin increased the formation of PGF₂α and the stable PGI₂ degradation product 6-keto PGF₁₂ in interleukin-1β-stimulated A549 cells (11). Here, we identified microsomal PGE₂ synthase-1 as functional and highly susceptible molecular target of curcumin. Our data show that suppression of PGE₂ biosynthesis in cell-based assays is primarily due to interference with microsomal PGE₂ 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 PGE₂ synthase-1 inhibitor 2-(2-chlorophenyl)-1H-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); PGH₂ (Larodan); and 11β-PGE₂, PGB₁, 3-(3-tert-butythio)-1-(4-chlorobenzyl)-5-isopropyl-IH-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% CO₂ incubator. After 3 d, confluent cells were detached using 1× trypsin/EDTA solution and reseeded at 2 × 10⁶ cells in 20 mL medium in 175-cm² 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 CaCl₂.

Determination of PGE₂ and 6-keto PGF₁₂ 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 PGE₂ and 6-keto PGF₁₂, 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 KC, 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 PGF₁₂ was quantified in the supernatant using a 6-keto PGF₁₂ 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 μL; 2 mol/L), and after centrifugation (2,300 × 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 acetoneitrile 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 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 μg/mL), was treated with 10 μg/mL lipopolysaccharide for 16 h at 37°C and 5% CO2. 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. PGE2 and 6-keto PGFα 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 preincubulation 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 Ca2+-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 PGFβ, 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 CaCl2) 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 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-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 phenylmethylsulfonyl 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 PGH2 (20 μmol/L final concentration). After 1 min at 4°C, the reaction was terminated using stop solution (100 μL; 40 mmol/L FeCl3, 80 mmol/L citric acid, and 10 μmol/L of 11β-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. 11β-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 ± 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 PGFα 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 hirpanarized whole blood with curcumin resulted in a reduction of PGE2 synthesis by \( \sim 40\% \) at 3 \( \mu \)mol/L with an apparent IC50 of 15 \( \mu \)mol/L (Fig. 2A). In analogy to the well-recognized microsomal PGF2 synthase-1 inhibitors MK-886 and 2-(2-chlorophenyl)-1H-phenanthro[9,10-d]-imidazole (25), curcumin failed to completely suppress PGE2 formation. The chosen concentrations of the microsomal PGF2 synthase-1 reference inhibitors (30 and 2 \( \mu \)mol/L, respectively) markedly exceed their IC50 values for inhibition of cell-free microsomal PGF2 synthase-1 (2.1 and 0.09 \( \mu \)mol/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 prostanoïd formation, as expected (Fig. 2A). The concomitant generation of 6-keto PGF1\( \alpha \) was also reduced by curcumin under these experimental conditions, although less pronounced, and significant inhibition (40%) was evident only at 30 \( \mu \)mol/L (Fig. 2B). These results indicate that curcumin differentially interferes with the biosynthesis of PGE2 and of 6-keto PGF1\( \alpha \).

Because curcumin could block prostanoïd 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 for 10 min, and prostanoïd 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 \( \sim 1 \) \( \mu \)mol/L (Fig. 3A), and again, 6-keto PGF1\( \alpha \) synthesis in the same samples was suppressed only at 30 \( \mu \)mol/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 Ca\( ^{2+} \)-ionophore and arachidonic acid. Curcumin moderately suppressed 12(S)-hydroxy-5-cis,8,10-trans-heptadecatrienoic acid formation with an IC50 of 19 \( \mu \)mol/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 \( \mu \)mol/L; refs. 3, 4) as well as COX-1-derived thromboxane A2 formation in washed platelets (IC50 = 40–70 \( \mu \)mol/L; ref. 28), whereas human recombinant COX-2 peroxidase activity was not significantly affected up to 50 \( \mu \)mol/L (4). We could essentially confirm these results, showing that the isolated COX enzymes were not inhibited by curcumin at least up to 30 \( \mu \)mol/L (data not shown).

Suppression of PGE2 synthesis might result from interference with enzymes distal of COX, namely, with PGH2 synthases. Therefore, we investigated the effects of curcumin on microsomal PGF2 synthase-1, which is functionally coupled to COX-2 (10). Microsomal preparations of interleukin-1\( \beta \)-treated A549 lung carcinoma cells, highly expressing microsomal PGF2 synthase-1 (23), were preincubated with curcumin for 15 minutes before PGE2 formation was initiated with 20 \( \mu \)mol/L PGH2. Curcumin concentration dependently inhibited PGE2 synthesis with an IC50 of 0.3 \( \mu \)mol/L (Fig. 4A) being considerably superior over the reference compound MK-886 (IC50 = 2.1 \( \mu \)mol/L; ref. 23). Decreasing the
PGE$_2$ 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 exist (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 prostanooid biosynthesis observed at higher curcumin concentrations (≥10 μmol/L; refs. 21, 28, 34). Along these lines, curcumin at 30 μmol/L

![Figure 3](Mol Cancer Ther 2009;8(8). August 2009)
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 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.

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 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.

**Acknowledgments**

We thank Gertrud Kleefeld for the expert technical assistance.

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Molecular Cancer Therapeutics

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Andreas Koeberle, Hinnak Northoff and Oliver Werz

Mol Cancer Ther 2009;8:2348-2355. Published OnlineFirst August 11, 2009.

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