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The effect of turmeric extracts on inflammatory mediator production

R.C. Lantz[a,c,*], G.J. Chen[a,c], A.M. Solyom[b,c], S.D. Jolad[b], B.N. Timmermann[b,c]

[a]*Department of Cell Biology and Anatomy, University of Arizona, Tucson, AZ, USA*
[b]*Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ, USA*
[c]*Arizona Center for Phytomedicine Research, P.O. Box 210207, University of Arizona, Tucson, AZ, USA*

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Abstract

Major compounds of several commonly used botanicals, including turmeric, have been purported to have anti-inflammatory actions. In order to test the anti-inflammatory activity of compounds isolated from rhizomes of *Curcuma longa* L. (Zingiberaceae), we have established an *in vitro* test system. HL-60 cells were differentiated and exposed to lipopolysaccharide (LPS) from *Escherichia coli* (1 μg/ml) in the presence or absence of botanical compounds for 24 h. Supernatants were collected and analyzed for the production of tumor necrosis factor alpha (TNF-α) and prostaglandin E_2 (PGE_2) using standard ELISA assays. Water-soluble extracts were not cytotoxic and did not exhibit biological activity. Organic extracts of turmeric were cytotoxic only at concentrations above 50 μg/ml. Crude organic extracts of turmeric were capable of inhibiting LPS-induced TNF-α (IC_{50} value = 15.2 μg/ml) and PGE_2 (IC_{50} value = 0.92 μg/ml) production. Purified curcumin was more active than either demethoxy- or bisdemethoxycurcumin. Fractions and subfractions of turmeric extracts collected via preparative HPLC had differing biological activity, ranging from no activity to IC_{50} values of <1 μg/ml. For some fractions, subfractionation resulted in a loss of activity, indicating interaction of the compounds within the fraction to produce an anti-inflammatory effect. A combination of several of the fractions that contain the turmeric oils was more effective than the curcuminoids at inhibiting PGE_2. While curcumin inhibited COX-2 expression, turmeric oils had no effect on levels of COX-2 mRNA.

Keywords: *Curcuma longa* L. (Zingiberaceae); Turmeric; Anti-inflammatory; Cytokines; Prostaglandins; TNF-α; PGE_2

Introduction

The use of dietary supplements containing botanical products is expanding rapidly in the United States. In the mass market alone, over $650 million is spent yearly on botanical supplements. The public is using these products for a wide range of health-related problems, including chronic inflammatory diseases such as chronic obstructive pulmonary disease, asthma and rheumatoid arthritis. Firm scientific information about these products and their active ingredients is not, however, currently available. A number of these botanical supplements have been used for centuries in Ayurvedic medicine, and some have been purported to have anti-inflammatory actions. Turmeric, the powdered rhizome of the herb *Curcuma longa* L. (Zingiberaceae), is used extensively in curries and mustards as a coloring and flavoring agent. In Ayurvedic medicine, turmeric has traditionally been used as a treatment for inflammation, skin wounds and tumors (Ammon and Wahl, 1991).

*Corresponding author. Tel.: +1 520 626 6716; fax: +1 520 626 2097.
E-mail address: lantz@email.arizona.edu (R.C. Lantz).

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Extracts have been reported to have effect as antimicrobial, anti-inflammatory, anti-oxidant and anticancer agents. In preclinical animal studies, turmeric has shown anti-inflammatory, cancer-chemopreventive and antineoplastic properties (Kelloff et al., 1996). Powdered turmeric, or its extract, is found in numerous commercially available botanical supplements. The best characterized of the compounds found in turmeric is curcumin, which appears to be able to act at multiple sites to reduce inflammation (see Aggarwal et al., 2003, for a current review).

Inflammation is associated with a large range of mediators which initiate inflammatory response, recruit and activate other cells to the site of inflammation and subsequently resolve the inflammation (Gallin and Snyderman, 1999). Cytokines are regulatory polypeptides that are produced by virtually all cells (Thompson, 1998). In general, cytokines are not constitutively produced. However, in the presence of appropriate stimuli, for example lipopolysaccharide (LPS) from gram negative bacteria, increased gene expression and production of the cytokines will occur, leading to the initiation of an inflammatory response. Two major cytokines involved in the initiation of inflammation are TNF-α and IL-1. These proteins have multiple sites of action. Responses can include induction of other cytokines, activation of arachidonic acid metabolism, priming of polymorphonuclear leukocytes (PMN), and up-regulation of adhesion molecules. Regulation of gene expression for these cytokines is in part controlled by activation of transcription factors such as NF-κB and AP-1.

In addition to cytokines, metabolites of arachidonic acid also participate in the inflammatory process. Products produced by the metabolism include both cyclooxygenase products (prostaglandins, thromboxanes) and lipooxygenase products (leukotrienes). Products such as LTB_4 and PGE_2, which are representative of these two pathways, can initiate PMN recruitment and changes in vascular tone and blood flow. Increased production of prostaglandins during an inflammatory response is achieved by induction of cyclooxygenase 2 (COX-2). COX-2 expression is mediated by NF-κB activation (Plummer et al., 1999).

The current treatment of inflammation includes aspirin, nonsteriodal anti-inflammatory medications and dexamethasone. The sites of action of these compounds range from inhibition of enzymes responsible for production of arachidonic acid metabolites to inhibition of cytokine expression.

Evaluation of the active ingredients in turmeric has focused primarily on curcumin, a polyphenolic responsible for the yellow color of turmeric. *In vitro* studies have demonstrated that curcumin will inhibit the production of inflammatory mediators, such as TNF-α and IL-1 (Chan, 1995; Chan et al., 1998; Abe et al., 1999). In addition, curcumin has been reported to also inhibit superoxide and PGE_2 production and to inhibit expression of inducible nitric oxide synthase (iNOS) and COX-2 (Ruby et al., 1995; Joe and Lokesh, 1997; Chan et al., 1998; Hong et al., 2002a). For curcumin, data indicate that a major site of action is inhibition of transcription factor activation (Chan et al., 1998; Plummer et al., 1999; Jobin et al., 1999; Zhang et al., 1999). Several well-characterized transcription factors are known to participate in the production of cytokines and arachidonic acid-metabolizing enzymes during inflammation. These include NF-κB and AP-1. LPS induction of inflammatory mediators will activate these transcription factors, leading to increased gene expression and protein for TNF-α, IL-1, IL-12, iNOS and COX-2 (inducible cyclooxygenase), among others. Inhibition of these transcription factors would explain the wide range of inhibitory effects ascribed to curcumin. Further studies have indicated that curcumin inhibits activation of these transcription factors by inhibiting kinases necessary for their activation. Before activation, the NF-κB complex resides in the cytoplasm. Sequestration of the complex to the cytoplasm and inactivation are accomplished by association of NF-κB with an inhibitory subunit, IκB. In order to activate NF-κB and allow it to migrate to the nucleus, IκB must be phosphorylated. Plummer et al. (1999) and Jobin et al. (1999) have shown that curcumin can inhibit the kinase that is responsible for the phosphorylation of IκB, thus inhibiting activation of NF-κB. Additionally, Chen and Tan (1998) have shown that curcumin can inhibit kinase activity in the c-Jun N-terminal kinase pathway. This pathway is also responsible for activation of NF-κB and AP-1 transcription factors.

While the activity and sites of action of curcumin have been studied, the potential anti-inflammatory activity of other compounds in turmeric has not been systematically examined. Other potential anti-inflammatory compounds may be present in *C. longa* extracts. For example, sesquiterpenoids from *Curcuma xanthorrhiza* and *Curcuma zedoaria* have been shown to inhibit COX-2 and iNOS activity at concentrations similar to those found for curcumin inhibition (Lee et al., 2002).

To test the potential anti-inflammatory activity of turmeric, we have prepared an organic extract. Furthermore, we have prepared and tested fractions and subfractions from this extract to discover active compounds. Anti-inflammatory activity was measured using a human promyelocytic leukemia cell line, the HL-60 cell, differentiated by PMA and stimulated by LPS, *in vitro*. The production of TNF-α and PGE_2 were inhibited by curcuminoids, as reported previously. In addition, other fractions tested demonstrated inhibition of TNF-α and PGE_2 at concentrations similar to those seen for curcumin.

Methods and materials

Supplies

Turmeric (rhizome of *C. longa* L.) was obtained from Botanicals International as a powder. Purified curcumin, demethoxycurcumin and bisdemethoxycurcumin were provided by S.D. Jolad, a co-author. The HL-60 cell line (ATCC, CCL-240) was purchased from ATCC. PMA (Sigma, P 1585), LPS (Sigma, L2630), Curcumin (Sigma, C7727), MTT (Sigma M5655) and XTT (Sigma X4626) were all purchased from Sigma Chemical Co. PMS (Fluka. Cat No. 68600) was purchased from Fluka Biochemika. IMDM medium was purchased from Gibco BRL. Human TNF-α and PGE_2 immunoassay kits were purchased from R&D systems (Minneapolis, MN, USA, Cat Nos. DTA 50 and DEO 100).

HPLC fractionation

Following dichloromethane-methanol (1:1 v/v) extraction, components of the turmeric sample were separated using a gradient method with a flow rate of 21.2 ml/min at ambient temperature. The mobile phase consisted of (A) Milli-Q water (Millipore, Billerica, MA, USA) and (B) acetonitrile (Burdick and Jackson). The following gradient elution was used: 0 min, 40% B; 10 min, 60% B; 32 min, 100% B; 38 min, 100% B; 40 min, 40% B.

The 1100 series purification system consisted of two preparative HPLC pumps, a multi-wavelength detector, a solvent delivery system, and a 220 microplate sampler (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was accomplished using a Luna C18 (2) column (5 μm, 250 × 21.2 mm) with a C18 guard column (60.0 mm x 21.2 mm) from Phenomenex (Torrance, CA). The eluent was monitored at 425 nm (signal A) and 250 nm (signal B). ChemStation for LC 3D (Rev. A.08.04, Agilent Technologies) with CC-Mode (Rev. A.03.02, Nederland B.V.) was used to control the separation and fractionation.

Cell culture

HL-60 cells were cultured in media (IMDM with 4 mM L-glutamine, 1.5 g/l sodium bicarbonate, 20% of FBS at 37 °C, 5% CO_2). Cells growing actively were distributed into 48 well plates (1 × 106/ml, 0.5 ml/well) and cultured with 10 nM PMA for 24 h at 37 °C, 5% CO_2 to differentiate the cells. Cells were washed with culture media and different concentrations of extract, fractions or subfractions and LPS (1 μg/ml) were added. Cells were cultured for another 24 h. Supernatants were picked up and stored at −80 °C until assayed for human TNF-α and PGE_2.

Immunoassay for TNF-α and PGE

Immunoassay kits were purchased from R&D Systems. OD was measured on plate reader (Molecular Devices, Spectra max plus) at 450 nm for TNF-α and 405 nm for PGE_2. The reference was 570 nm. Data were analyzed using Molecular Devices plate reader software.

COX-2 expression assay

Levels of mRNA for COX-2 were determined using a Quantikine mRNA assay kit available from R&D Systems. Cells were stimulated with LPS in the absence or presence of botanical extracts. At 24 h, cells were lysed and total RNA was isolated using Qiagen kits. RNA samples were processed and added to the reagents as described in the manufacturers' instructions. Values were compared against a standard curve. Data were recorded as percent of LPS alone (LPS alone = 100%). Preliminary tests showed that COX-2 expression reached a plateau at 4 h after LPS stimulation. However, since these same levels of expression were maintained for 24 h, data were collected at 24 h for correlation with PGE_2 production measurements.

Cytotoxicity assay

HL-60 cells were cultured as described above. Cells (1×10^5 cells/ml) were distributed to 96-well plates, 0.1 ml/well, and cultured with PMA (10 nM) for 24 h. Cells were washed with culture media and different concentrations of extract, fractions or subfractions and LPS (1 μg/ml) were added. Cells were cultured for another 24 h. For the MTT assay, 20 μl of MTT (5 mg/ml) were added to each well and plates were cultured for another 4 h. Supernatants were aspirated and 100 μl of isopropanol-HCl (0.04% HCl) were added to each well. The plates were protected from the light at room temperature overnight. The OD was measured at 570 nm (660 nm as reference wavelength). For XTT assay, 25 μl of XTT (1 mg/ml with PMS) were added to each well and the plates were cultured for another 4 h in the dark. OD was measured at 450 nm (650 nm as reference wavelength).

Data collection

All data concerning plant sources, extraction procedures, chemical analysis and bioassay results were stored in a relational database (NAPIS) (White Point Systems, Friday Harbor, WA) for easy retrieval and searches.

Results

The turmeric sample (*C. longa*) extracted with dichloromethane-methanol (1:1, v/v) was able to inhibit LPS-induced production of TNF-α and PGE_2 (Fig. 1). The IC_{50} value for inhibition was 15.2 μg/ml for TNF-α and 0.92 μg/ml for PGE_2. Water-soluble extracts showed no biological activity. Cytotoxicity was seen only at levels above 50 μg/ml.

Preparative HPLC separation of the above crude extract was performed and yielded ten fractions. The 425/250 nm chromatograms are shown in Fig. 2, with the ten separate fractions indicated. The three major peaks in fraction 5 are the three curcuminoid compounds, curcumin (RT: 18.4 min), demethoxycurcumin (RT: 17.5 min) and bisdemethoxycurcumin (RT: 16.7 min). This fraction accounted for 32.2% of the mass of the ten fractions. Each of these three compounds was separated, purified and tested separately for their ability to inhibit LPS-induced inflammatory mediator production. Fig. 1 shows that all three compounds are effective in inhibiting LPS-induced PGE_2 production. Of the three, the most active was curcumin, followed by bisdemethoxy- and demethoxycurcumin. These compounds were also effective at inhibiting TNF-α production, but at significantly higher concentrations (Table 1).

We investigated whether differences in TNF-α and PGE_2 IC_{50} values in our experiments were due to loss of curcumin from the media, as has been reported (Wang et al., 1997). The curcumin standard was placed in IMDM media and incubated for 24 h under our culture

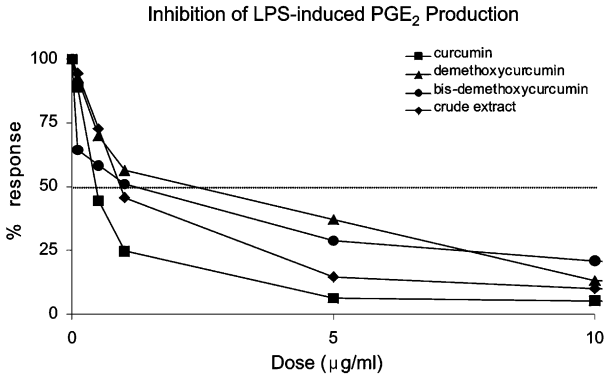

Fig. 1. Dose–response curves for inhibition of LPS-induced PGE_2 production. HL-60 cells were cultured for 24 h in the presence of 1 μg/ml of LPS and various concentrations of turmeric compounds. Supernatants were analyzed for production of PGE_2 by standard ELISA. All values were normalized to LPS alone values = 100%. IC_{50} value concentrations are where the curves cross the 50% line.

Table 1. IC_{50} values of curcuminoids from fraction 5 of turmeric extract

	Curcumin	Demethoxy-curcumin	Bisdemethoxy-curcumin
TNF-α	28.8	24.7	24.6
PGE_2	0.45	2.6	1.2

Values are concentration of each fraction required to lead to 50% inhibition of LPS-induced mediator production. Values are in μg/ml.

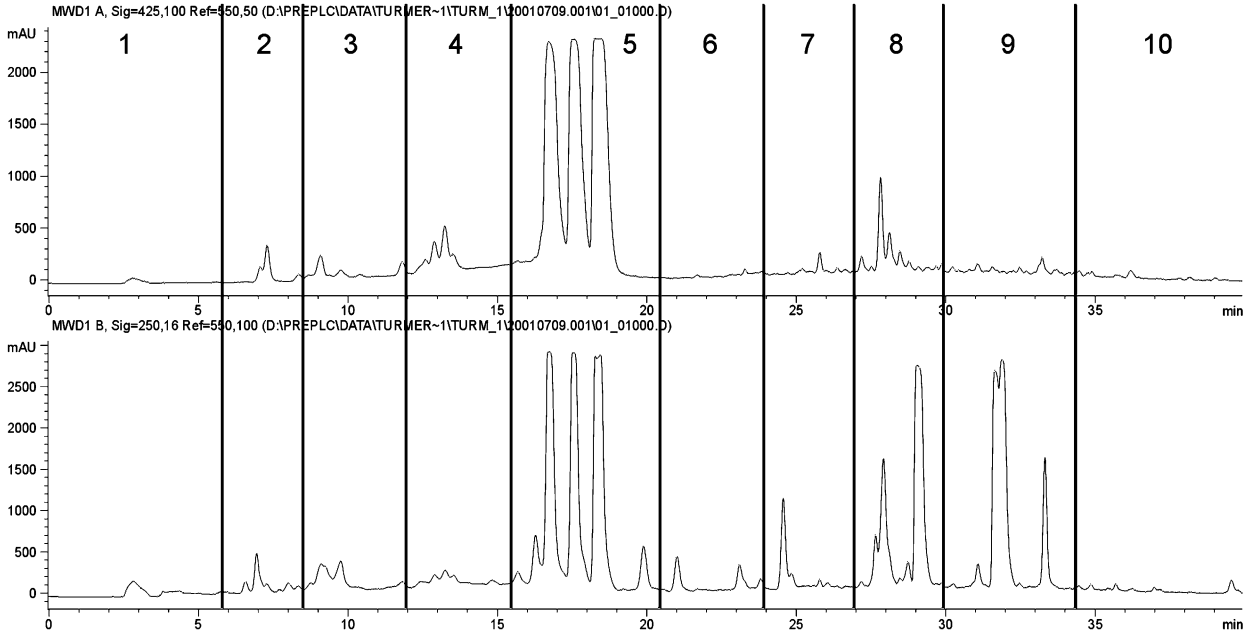

Fig. 2. Chromatogram demonstrating the peaks present in the crude extract of turmeric. Spectra at both 425 and 250 nm are shown. The extract was subdivided into ten fractions as indicated on the chromatogram. The curcuminoids are found in fraction 5.

Table 2. IC_{50} values of 10 primary fractions from turmeric extract

	1	2	3	4	5	6	7	8	9	10
TNF-α	29.2	6.5	5.8	11.7	18.9	39.4	—	26.7	25.6	—
PGE_2	2.2	4.7	3.5	1.0	0.9	3.7	7.5	1.7	6.3	6.3

Values are concentration of each fraction required to lead to 50% inhibition of LPS-induced mediator production. Dashes indicate no inhibitory activity for that fraction. Values are in μg/ml.

Table 3. Mass of fractions isolated from crude turmeric extract (T 1-1-F0).

T 1-1-F 0	Total amount (mg)	%
2.0611 g/20 ml (5 ml loop, 17 injections)		
TP1/08_1	148.5	10.2
TP1/08_2	60.6	4.2
TP1/08_3	69.4	4.8
TP1/08_4	141.8	9.8
TP1/08_5	467.3	32.2
TP1/08_6	48.2	3.3
TP1/08_7	57.2	3.9
TP1/08_8	171.0	11.8
TP1/08_9	202.6	14.0
TP1/08_10	83.6	5.8
Total	1450.2	
Recovery %	70.4	

Table 4. IC_{50} values of 8 subfractions from fraction 1 of turmeric extract

	A	B	C	D	E	F	G	H
TNF-α	—	—	—	—	—	9.9	—	—
PGE_2	—	—	40.3	—	31.4	2.8	47.8	—

Values are concentration of each fraction required to lead to 50% inhibition of LPS-induced mediator production. Dashes indicate no inhibitory activity for that fraction. Values are in μg/ml.

Table 5. IC_{50} values of 5 subfractions from fraction 2 of turmeric extract

	A	B	C	D	E
TNF-α	—	—	30.1	—	—
PGE_2	44.0	9.3	14.0	25.5	21.7

Values are concentration of each fraction required to lead to 50% inhibition of LPS-induced mediator production. Dashes indicate no inhibitory activity for that fraction. Values are in μg/ml.

Table 6. IC_{50} values of 8 subfractions from fraction 8 of turmeric extract

	A	B	C	D	E	F	G	H
TNF-α	—	—	35.1	—	—	—	—	—
PGE_2	—	36.5	9.9	—	8.7	16.4	7.4	—

Values are concentration of each fraction required to lead to 50% inhibition of LPS-induced mediator production. Dashes indicate no inhibitory activity for that fraction. Values are in μg/ml.

conditions. Aliquots were collected and sampled for curcumin. Concentrations recovered after 24 h were 90–95% of the original concentration. Therefore, curcumin is not being lost during the incubation period.

As expected, the curcuminoids were effective in inhibition of inflammatory mediator production. However, other fractions isolated from the original extract were also found to have significant activity. IC_{50} value data for each of the ten fractions are shown in Table 2. Several of the fractions (2–4) showed lower IC_{50} values for inhibition of TNF-α production than the curcuminoids (fraction 5). In addition, the IC_{50} values for inhibition of PGE_2 were within the same order of magnitude, regardless of the fraction tested. The percentage of the total mass in each fraction is shown in Table 3.

In order to further isolate the active compounds, subfractions were made from fractions 1, 2 and 8. Although no large apparent peak is seen at 425/250 nm in fraction 1, this fraction does make up over 10% of the mass. Eight subfractions (A–H) from fraction 1 were isolated and tested for activity (Table 4). The majority of the activity appears to reside in subfraction F, with an IC_{50} value for TNF-α of 9.9 μg/ml and for PGE_2 of 2.8 μg/ml. All the other subfractions showed either no or very limited inhibitory activity.

Fractions 2 and 8 were subfractioned into five (A–E) and eight (A–H) subfractions, respectively (Tables 5 and 6). In both cases, none of the subfractions showed greater activity than the parent fraction, indicating that compounds in this fraction may interact to inhibit mediator production.

In order to further characterize the effect of interaction of compounds, we analyzed the compounds in fractions 6–10 combined in mass ratios found in the crude extracts. These fractions should contain the turmeric oils. The fraction consists primarily of two major peaks seen in the UV range. Other minor peaks are also evident. Combination of these five fractions, which together make up 38.8% of the mass, resulted in

IC_{50} values that were lower than or equivalent to those seen for the curcuminoids. The IC_{50} value for TNF-α was 7.23 and the IC_{50} value for PGE_2 was 0.47 μg/ml. This same mixture of compounds was also isolated as a byproduct of the isolation of the curcuminoids. The IC_{50} value for LPS-induced TNF-α production for this mixture was 17.32. This mixture was extremely effective at inhibiting LPS-induced PGE_2 production, with an IC_{50} value for PGE_2 equal to 0.084 μg/ml. For comparison, we have found an IC_{50} value of 0.052 μg/ml for indomethacin in our assay system, indicating that the effectiveness of fractions 6–10 at inhibition of LPS-induced PGE_2 production is approaching the effectiveness of commercially available, nonsteriodal, anti-inflammatory compounds.

It is possible that a portion of the inhibition caused by fractions 6–10 is due to binding or sequestration of LPS. This would lower the effective stimulation concentration and would appear as an inhibition of inflammatory mediator production. This possibility was tested by using zymosan stimulation in place of LPS. Inhibition of opsonized zymosan-induced PGE_2 production occurred at concentrations equivalent to those seen with LPS stimulation. Therefore, LPS was not being sequestered by compounds found in fractions 6–10.

Previous reports have shown that curcumin can inhibit expression of inflammatory mediators by inhibiting activation of transcription factors responsible for inflammatory gene expression. Inhibition of PGE_2 by curcumin is therefore believed to be due to inhibition of the expression of COX-2. Only limited data exist, however, on the site(s) of inhibition of PGE_2 production by turmeric oils. HL-60 cells were exposed to LPS in the presence of curcumin or turmeric oils (fractions 6–10). Cells were lysed and mRNA was collected and analyzed for the presence of COX-2 message. Values were expressed as a percentage of LPS alone (100%). Data are shown in Fig. 3. As expected, curcumin at concentrations as low as 0.5 μg/ml resulted in a greater than 50% decrease in COX-2 expression. Even at concentrations up to 10 μg/ml, however, turmeric oils did not cause any significant decrease in COX-2 expression.

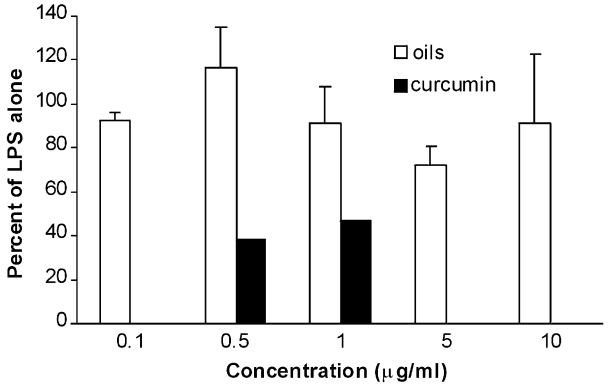

Fig. 3. Inhibition of COX-2 expression by curcumin and turmeric oils. HL-60 cells were stimulated with LPS in the presence of curcumin or turmeric oils (fractions 6–10). Curcumin at 0.5 and 1.0 μg/ml resulted in a greater than 50% inhibition of COX-2 expression. However, turmeric oils did not show any significant inhibition of COX-2 expression. Values are expressed as a percentage of COX-2 expression following LPS stimulation alone.

Discussion

The powdered rhizome of the herb *Curcuma longa* L. (Zingiberaceae) is used extensively in Ayurvedic medicine, traditionally as a treatment for inflammation, skin wounds and tumors (Ammon and Wahl, 1991). The best-characterized of the compounds found in turmeric is curcumin, which appears to be able to act at multiple sites to reduce inflammation (see Aggarwal et al. (2003), for a current review). Curcumin has been shown to alter the production of numerous cytokines and inflammatory mediators. The wide range of action of curcumin is most likely due to its interaction and inhibition of transcription factor activation, specifically NFκB and AP-1 pathways.

Reports in the literature show that curcumin led to 50% decreases in the production of TNF-α at concentrations ranging from 2 to 10 μg/ml (Chan, 1995; Abe et al., 1999). IC_{50} values for inhibition of PGE_2 and/or COX-2 expression are in the same range (Zhang et al., 1999; Goel et al., 2001). Our data for inhibition of PGE_2 are similar to those reported elsewhere. Inhibition of TNF-α only occurred, however, at concentrations an order of magnitude higher than that necessary to inhibit PGE_2. Production of TNF-α and PGE_2 depend on induction of gene expression by activation of transcription factors (Jobin et al., 1999). Previous research has demonstrated that curcumin inhibits these pathways at steps prior to the activation of the transcription factors. This should result in similar IC_{50} values for mediators that are induced through similar pathways.

It is possible that the timing of application of the curcumin may differentially affect the expression of the two mediators. Curcumin is most effective at inhibiting NFκB activation if added an hour prior to inflammatory stimulation (Singh and Aggarwal, 1995). If activation of TNF-α gene expression occurs more rapidly than up regulation of COX-2, differential levels of inhibition may occur following simultaneous addition of stimulus and inhibitor, as performed in our experiments. Direct inhibition of COX-2 enzyme activity by anti-inflammatory compounds may also contribute to the lower IC_{50} value for PGE_2 seen in our experiments (Zhang et al., 1999).

The curcuminoids exist as three primary compounds seen in the crude rhizome extract (Fig. 2). We have isolated each of these three compounds (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and analyzed them separately to evaluate their anti-inflammatory potential. All three of the compounds showed an ability to inhibit PGE_2 production in the range of 0.5–2.5 μg/ml IC_{50} values, with curcumin being the most effective. Ramsewak et al. (2000) and Kim et al. (2001) have both compared the activity of all three curcuminoids. In assays to test the direct inhibition of COX-2 enzyme activity, Ramsewak et al. (2000) found that bisdemethoxycurcumin was the most effective. Curcumin resulted only in a slight inhibition of the enzyme activity. Ramsewak analyzed inhibition of COX-2 at concentrations of 125 mg/ml, values that are significantly higher than the IC_{50} values we have shown for PGE_2 production and COX-2 mRNA expression. It is therefore unlikely that the curcuminoids exert their major action through direct inhibition of COX-2 activity, and may do so through inhibition of COX-2 expression. IC50 values for antioxidant activity for each of the curcuminoids were all similar, but an order of magnitude higher than those reported here (Kim et al., 2001).

In addition to the curcuminoids, other fractions isolated from the crude rhizome extract showed significant activity and ability to inhibit LPS-induced PGE_2 production. Specifically the combination of fractions 6–10, which contains the turmeric oils, showed an IC_{50} value of 84 ng/ml, a value approaching that seen for indomethacin. Hong et al. (2002a, b) and Lee et al. (2002) have reported that sesquiterpenoids (*β*-tumerone, ar-tumerone and xanthorrhizol) isolated from *Curcuma zedoaria* and *Curcuma xanthorrhiza* can inhibit PGE_2 production. IC_{50} values were in the range of 2–5 μg/ml. These IC_{50} values are similar to those we have seen for the individual fractions 6–10 (Table 2). Lee et al. (2002b) were able to show inhibition of COX-2 expression by xanthorrhizol at concentrations above 5–10 μg/ml; the IC_{50} values were not reported. This inhibition is in contradiction to our findings. Exposure to the 6–10 fractions did not lead to any significant reduction in COX-2 mRNA levels up to 10 μg/ml, even though PGE_2 IC_{50} values were 84 ng/ml. These data suggest that fractions 6–10 (turmeric oils) inhibit PGE_2 production either by direct inhibition of COX-2 enzyme activity or by some other mechanism downstream from induction of COX-2 expression.

Fractions 6–10, in isolation, demonstrated inhibitory activity in the 2–6 μg/ml range, but together, chemicals contained in these fractions had potent inhibitory activity for inhibition of PGE_2 production. This implies a synergy between two or more of the chemicals in these fractions. Synergy could be produced in several different ways. For example, combinations of the fractions could result in better bioavailability of the active compound(s) inside the cell. In addition, several of the compounds could act at different sites in the transduction involved in LPS-induced expression of COX-2. Or finally, some of the compounds could inhibit COX-2 expression, while others inhibit the enzyme activity directly. These are only a few of the potential mechanisms that could lead to the synergy.

In conclusion, compounds isolated from *Curcuma longa* rhizomes are effective inhibitors of inflammatory mediator production *in vitro*. These compounds include the curcuminoids and the turmeric oils. The most effective compounds were mixtures of several of the fractions containing the oils. Different mechanisms of action appear to apply with curcumin inhibiting COX-2 expression. The oils act at some point distal to COX-2 expression. While these compounds are effective under the current experimental conditions, their efficacy must be tested in an *in vivo* system. Lack of bioavailability and/or metabolism of the compounds may make them ineffective during *in vivo* testing, especially when the compounds are administered orally. For example, while iNOS induction in LPS-stimulated macrophages is inhibited almost completely by 10 μM curcumin, the reduced forms of tetra-, hexa-and octahydrocurcumin, curcumin metabolites found in mice, were not effective (Pan et al., 2000). It is therefore important that these results be verified in an *in vivo* model.

Acknowledgments

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