Effect of Curcumin and Capsaicin on Arachidonic Acid Metabolism and Lysosomal Enzyme Secretion by Rat Peritoneal Macrophages

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ABSTRACT: The inflammatory mediators secreted by macrophages play an important role in autoimmune diseases. Spice components, such as curcumin from turmeric and capsaicin from red pepper, are shown to exhibit antiinflammatory properties. The influence of these spice components on arachidonic acid metabolism and secretion of lysosomal enzymes by macrophages was investigated. Rat peritoneal macrophages preincubated with 10 μM curcumin or capsaicin for 1 h inhibited the incorporation of arachidonic acid into membrane lipids by 82 and 76%: prostaglandin E_2 by 45 and 48%; leukotriene B_4 by 61 and 46%, and leukotriene C_4 by 34 and 48%, respectively, but did not affect the release of arachidonic acid from macrophages stimulated by phorbol myristate acetate. However, the secretion of 6-keto PG $F_{1\alpha}$ was enhanced by 40 and 29% from macrophages preincubated with 10 μM curcumin or capsaicin, respectively, as compared to those produced by control cells. Curcumin and capsaicin also inhibited the secretion of collagenase, elastase, and hyaluronidase to the maximum extent of 57, 61, 66%, and 46, 69, 67%, respectively. These results demonstrated that curcumin and capsaicin can control the release of inflammatory mediators such as eicosanoids and hydrolytic enzymes secreted by macrophages and thereby may exhibit antiinflammatory properties.

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Inflammation is a necessary response of the host to counteract the threat of infectious agents and other foreign bodies (1). The inflammatory response is coordinated by the number of immune cells such as macrophages, B and T lymphocytes, basophils, eosinophils, and mast cells. A large number of mediators produced by these cells play a key role in inflammatory response. These include arachidonic acid metabolites like prostaglandins (PG) and leukotrienes (LT), reactive oxygen species like superoxide anions and hydroxyl radicals, hydrolytic enzymes like proteases and glycosidases, cytokines, complement-derived peptides, and kinins (2–4). These mediators act in concert to eliminate the source of infection. However if these mediators are produced in an uncontrolled manner, they can lead to the destruction of connective tissue matrix as has been observed in autoimmune diseases like rheumatoid arthritis (5). Enzymes such as collagenase, elastase, and hyaluronidase play a key role in the destruction of cartilage in the joints in arthritic conditions (6). The inflammatory responses can also be exacerbated by cytokines, reactive oxygen species, and arachidonic acid metabolites (4,7). Macrophages present at the site of inflammation are an important source of these various mediators (5). The tissue macrophages at the site of inflammation are supplemented by blood-derived monocytes and lymphocytes which infiltrate the vasculature in large numbers in response to complement activation products, connective tissue fragments, and other endogenously derived stimuli (4,8). Binding of the stimuli to cells leads to transmembrane signaling which ultimately leads to the secretion of lysosomal enzymes (9). To restrict the uncontrolled response of accumulated cells, treatments such as lymphocytopheresis, nonsteroidal antiinflammatory drugs, gold compounds, D-penicillamine, hydroxychloroquinine, corticosteroids, and immunosuppressants are often used (6). These treatments reduce the secretory functions of macrophages and other cells, resulting in the lowering of inflammatory responses.

Although usage of antiinflammatory drugs is in vogue, the continued administration of these drugs over a long period of time can have adverse side effects (5,6,10). Therefore, there is a need to explore alternative strategies to lower the formation of inflammatory mediators with the help of nontoxic natural products. Recently we have demonstrated that phenolic compounds, such as curcumin from the spice turmeric and capsaicin from red pepper, exhibit antiinflammatory properties on carrageenan-induced paw inflammation as well as in adjuvant-induced arthritis in rats (11,12). These effects are comparble to some of the known antiinflammatory drugs such as aspirin, indomethacin, piroxicam, and phenylbutazone (12,13). However, the mechanism by which curcumin and capsaicin exhibit antiinflammatory properties is not very clearly understood yet.

Activation of mononuclear cells plays a very important role in inflammation (5). Since our earlier studies and that of other investigators clearly established the efficacy of cur-

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Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hanks' balanced salt solution; LT, leukotriene; PG, prostaglandin; PMA, phorbol myristate acetate.

cumin and capsaicin to reduce the inflammation in arthritis and other model systems (11,12,14), it is of interest to study the effect of these phenolic compounds on macrophage activation with reference to secretion of inflammatory mediators. We report in this investigation that curcumin and capsaicin inhibit the incorporation of arachidonic acid into macrophage lipids, which in turn decreases the formation of PGE_2, LTB_4, and LTC_4. Curcumin and capsaicin also transiently reduced the secretion of lysosomal enzymes such as collagenase, elastase, and hyaluronidase in activated macrophages. These studies indicated that the antiinflammatory properties of curcumin and capsaicin may be mediated by their inhibitory effects on the secretion of inflammatory mediators of mononuclear cells.

MATERIALS AND METHODS

Dulbecco's modified Eagle's medium (DMEM), phorbol myristate acetate (PMA), calcium ionophore A23187, Zymosan A, fatty acid-free bovine serum albumin (BSA), indomethacin, *N*-acetyl glucosamine, collagenase (Type VII from *Clostridium histolyticum*), elastase (from bovine pancreas), hyaluronidase (from bovine testes), hyaluronic acid, Dextran T-70, PGE_2, 6-keto prostaglandin $F_{1\alpha}$ and LTB_4 and LTC_4 were obtained from Sigma Chemical Co. (St. Louis, MO). 5,6,8,9,11,12,14,15[^{3}H]Arachidonic acid (specific activity 211.2 Ci/mmol), 6-keto [5,8,9,11,12,14,15(*N*)]-[^{3}H]-$PGF_{1\alpha}$ (specific activity 185 Ci/mmol), and [5,6,8,11,12,14,15(*N*)-[^{3}H]-PGE_2 (specific activity 183 Ci/mmol) were purchased from Amersham International plc (Amersham, United Kingdom). Antisera for 6-keto $PGF_{1\alpha}$ and PGE_2 were purchased from Advanced Magnetics Inc. (Cambridge, MA). DNP-peptide III and Suc-Ala-pNA were bought from Peptide Research Institute (Osaka, Japan). Natural capsaicin was from Aldrich Chemical Co. (Milwaukee, WI). Curcumin (99% pure) was purchased from Flavours and Essences (Mysore, India). Linalool (97% pure) and cuminaldehyde (90% pure) were purchased from Fluka (Buchs, Switzerland). Gelatin was obtained from Difco Laboratories (Detroit, MI). All other chemicals and solvents used were of analytical grade.

Animals. Adult male Wistar rats [OUTB-Wistar, IND-cft (2c), CFTRI animal house facility, Mysore, India] weighing approximately 250 g were used for all the studies.

Isolation of peritoneal macrophages. Macrophages were isolated from the rat peritoneal exudates in Hanks' balanced salt solution (HBSS) as described previously (15).

Uptake of [^{3}H]-arachidonic acid by macrophages. Macrophage monolayers (2.5×10^6 cells) in 1.5 mL of DMEM were incubated with [^{3}H]-arachidonic acid (0.01 μCi complexed to 1 mg BSA/mL) for 15 h at 37°C in a humidified incubator (16). The culture supernatant was aspirated, and the adhering cells were washed three times with DMEM containing 0.1% BSA. The adhering cells were scraped in 1 mL of saline containing 0.01% Triton-X 100, and the lipids were extracted by the method of Bligh and Dyer (17). The lipid extracts were taken in scintillation vials, evaporated to dryness with nitrogen, and dissolved in 5 mL of toluene-based scintillant. The incorporation of [^{3}H]-arachidonic acid was quantitated using a Beckman liquid scintillation counter (Model LS-100; Fullerton, CA).

Release of [^{3}H] arachidonic acid. Macrophage lipids were labeled with [^{3}H]-arachidonic acid as described above and washed three times with DMEM containing 0.1% BSA. The cells were incubated for 2 h in 1.5 mL of DMEM containing 2.5 mg/mL BSA, 3 μM indomethacin, and 0.1 μM PMA (16). Control cells were incubated under similar conditions in the absence of PMA. The radiolabeled arachidonic acid released into the medium was extracted by the Bligh and Dyer procedure (17). The [^{3}H]-arachidonic acid released was quantitated in a Beckman scintillation counter (16).

Release of PG. Macrophage monolayers (2.5×10^6 cells/1.5 mL DMEM) were incubated with 5 μg/mL of Ca^{2+} ionophore A23187 for 90 min. The PGE_2 and 6-keto $PGF_{1\alpha}$ released were extracted from the medium (in 2 mL of ethyl acetate × 3) after acidifying the supernatant with 100 μL of 3% formic acid to pH 3 (18). The combined ethyl acetate extracts were evaporated under a stream of nitrogen and suspended in 100 μL of phosphate-buffered saline containing 1% gelatin. Appropriate dilutions of this solution were used for quantifying the PG by radioimmunoassay (18).

Analysis of LT. Macrophages (2.5×10^6 cells/1.5 mL of DMEM) were activated with 5 μg/mL of Ca^{2+} ionophore A23187 for 90 min. The LT released into the medium were extracted with 2 vol of methanol. The extract was acidified with 3% formic acid to pH 3.0 and prepurified on Sep-Pak C-18 column (Waters, Millipore Corp., Milford, MA) (19). The LT extract was concentrated and loaded on a Supelcosil C-18 high-performance liquid chromatographic column (Supelco, Bellefonte, PA) of pore size of 5 μ. The LT were eluted with acetonitrile/methanol/acetic acid/water (65:10:1:24, by vol) adjusted to pH 5.6 with ammonia. LTC_4 and LTB_4, which eluted at the retention times of 4.7 and 16.9 min, were monitored at 280 nm. The LT were identified and quantitated by comparison with chromatography of authentic standards.

Effect of spice principles on the incorporation and release of arachidonic acid. The macrophages were preincubated with increasing concentration of spice principles (in 10 μL of ethanol) for 60 min prior to the addition of [^{3}H]-arachidonic acid for incorporation studies or prior to the addition of activators in the release reaction. The cells that were incubated with spice principles were thoroughly washed with HBSS containing 2% BSA and used for further studies. Uptake of spice principles by macrophages was monitored by high-performance liquid chromatography analysis as described earlier (15).

Hydrolytic enzymes. Macrophage monolayers (2.5×10^6 cells) were incubated with or without spice principles in 1 mL of HBSS containing 500 μg of Zymosan A for 18–72 h. The hydrolytic enzymes collagenase, elastase and hyaluronidase, released in the culture supernatants by activated macrophages, were measured.

Collagenase activity. Collagenase activity was measured colorimetrically at 365 nm using the synthetic substrate DNP peptide III (DNP-pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg) (20). The amount of DNP peptide hydrolyzed was quantitated using a molar extinction coefficient of 1.49. Collagenase activity is expressed as µM/mg protein (20).

Elastase activity. The activity of elastase was assayed at 405 nm as described by Kawabata *et al.* (21) using the synthetic substrate Suc-Ala-Pro-Ala-pNA in 0.1 M Tris-HCl buffer (pH 5.0). One unit of elastase activity was defined as the quantity of enzyme that liberates 1 µmol of *p*-nitrosanilide in 60 min.

Hyaluronidase activity. Hyaluronidase activity in the culture supernatant was determined by the amount of *N*-acetyl glucosamine released from hyaluronic acid (22,23).

Protein estimation. Total protein was estimated by the method of Sedmak and Grossberg (24) using BSA as reference standard. Macrophage protein was quantified after digesting the cells (2.5×10^6 cells) in 1 mL of 1 N NaOH overnight.

Statistical analysis. The data were statistically analyzed by Student's *t*-test (25).

RESULTS

Effect of spice principles on: (i) Incorporation of arachidonic acid. Macrophages preincubated with curcumin or capsaicin for 1 h incorporated a lesser amount of [^{3}H]-arachidonic acid in membrane lipids as compared to control cells which were incubated with vehicle (10 µL of ethanol) alone. This effect was dependent on the concentration of spice principles used in the incubation medium. Thus, 1, 2.5, 5, 7.5, and 10 µM curcumin inhibited [^{3}H]-arachidonic acid incorporation by 9, 27, 42, 54, and 82%, respectively, at 1 h of incubation (Fig. 1). However, this inhibitory effect of curcumin on fatty acid incorporation was reduced if macrophages were incubated with [^{3}H]-arachidonic acid for a longer period of time. Thus curcumin at 1, 2.5, 5, 7.5, and 10 µM reduced the incorporation of [^{3}H]-arachidonic acid by only 0, 3, 11, 28, and 78% by the end of 2 h. By 6 h of incubation, curcumin at 1 to 5 µM did not inhibit the incorporation of [^{3}H]-arachidonic acid by macrophages. Similarly, the inhibitory effects of curcumin at 10 µM on [^{3}H]-arachidonic acid incorporation were decreased with time, and by 10 h of incubation, curcumin even at high concentrations failed to influence the ability of macrophages to incorporate arachidonic acid (Fig. 1). Capsaicin at 1, 2.5, 5, 7.5, and 10 µM reduced the incorporation of [^{3}H]-arachidonic acid into macrophage lipids by 21, 46, 52, 66, and 76% at 1 h, 3, 11, 18, 36, and 45% at 2 h, 0, 0, 0, 20, and 35% at 6 h, 0, 0, 0, 10, and 27% at 8 h, and there was no reduction at 10 h of incubation. These studies indicated that the inhibitory effect of curcumin and capsaicin on the incorporation of arachidonic acid by macrophages is short-lived and is fully reversible with time. Therefore, the incorporation of arachidonic acid into macrophage lipids was inhibited by spice principles only in the initial period of incubation (<10 h at 10 µM). The macrophages incubated with spice principles were fully viable at all the time points as judged by trypan blue exclusion. Macrophages preincubated with other spice principles such as cuminaldehyde (from cumin) and linalool (from coriander) even at 500 µM had no effect on the incorporation of [^{3}H]-arachidonic acid at all the times tested (Fig. 1). These studies indicate the specificity and the efficacy of individual spice principles in reducing the incorporation of arachidonic acid into macrophage lipids.

The inhibitory pattern of arachidonic acid incorporation into macrophage lipids was associated with intact curcumin or capsaicin inside the cells (Table 1). However, when these spice principles were modified by the cells, the inhibition was reduced. More than 95% of intact curcumin and 87% of capsaicin internalized by the cells are modified by the end of 9 h of incubation (Table 1). However, we were unable to identify the metabolites of curcumin or capsaicin by spectroscopic methods, thin-layer chromatography, or high-performance liquid chromatography. Cuminaldehyde and linalool, though taken up to a maximum extent of 92%, failed to influence the incorporation of arachidonic acid. The cells were fully viable as judged by trypan blue exclusion method at all concentrations of spices used for preincubation.

(ii) Release of arachidonic acid. The macrophages labeled with [^{3}H]-arachidonic acid were preincubated with various spice principles for 1 h. After washing the cells, they were activated with PMA, and arachidonic acid release was quantitated. There was no significant difference in the amount of arachidonic acid released by macrophages preincubated with 1–10 µM curcumin, 1–10 µM capsaicin, 500 µM cuminaldehyde, or 500 µM linalool as compared to that observed with control cells (Table 2). This indicated that spice principles do not affect the phospholipase activity in macrophages. Spice principles alone did not cause the release of arachidonic acid from macrophages. Known inhibitors of phospholipase A_2, like bromophenacyl bromide, inhibited the release of arachidonic acid in a dose-dependent manner (Table 2).

TABLE 1
Internalization of Spice Principles by Macrophages[a]

Time of incubation (h)	Curcumin uptake (µM)	Capsaicin (µM)	Cuminaldehyde (µM)	Linalool (µM)
0.25	0.96	3.29	202	44
0.50	2.75	4.82	300	97
0.75	6.13	7.80	365	239
1.00	9.30	0.68	456	460
2.00	8.50	8.24	426	418
3.00	8.13	8.23	326	307
5.00	3.38	4.10	204	174
7.00	1.75	1.63	89	80
9.00	n.d.	1.24	0.45	22
24.00	n.d.	0.63	0.53	n.d.

[a]Macrophages (2.5×10^6 cells) were incubated in 1 mL of Hanks' balanced salt solution containing curcumin (10 µM), capsaicin (10 µM), cuminaldehyde (500 µM), or linalool (500 µM) in 10 µL ethanol. The spice principles internalized were quantitated by high-performance liquid chromatography (Ref. 15). Values are mean of three independent experiments; n.d., not detected.

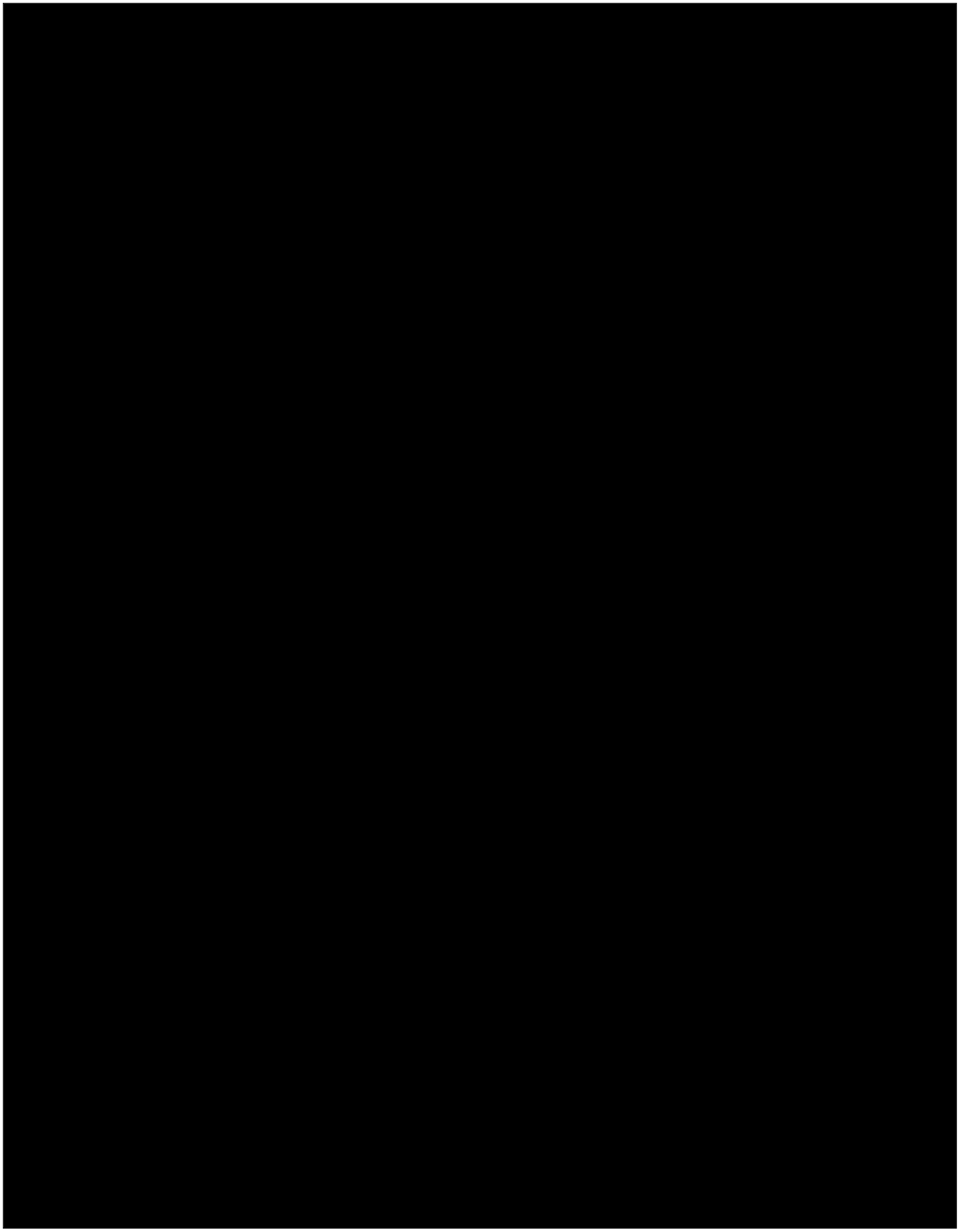

FIG. 1. Effect of spice principles on the incorporation of [^{3}H]-arachidonic acid into macrophage lipids *in vitro*. The incorporation of [^{3}H]-arachidonic acid into macrophage lipids was determined after incubation with (A) curcumin or (B) capsaicin at the following concentrations: A: 0; B: 1 μM; C: 2.5 μM; D: 5 μM; E: 7.5 μM, and F: 10 μM or (C) cuminaldehyde or (D) linalool at 500 μM concentrations. Arachidonic acid incorporation was measured at the time intervals indicated. Values with same superscripts are not significantly different from each other at $P < 0.05$. Abbreviation: NS, not significantly different from the control value.

TABLE 2
Effect of Spice Principles on the Release of [^{3}H]-Arachidonic Acid from Macrophages

Compound added (μM)	[^{3}H]-Arachidonic acid released (cpm/mg protein/h)
Nil	16338 ± 235
Curcumin	
1.0	16637 ± 400
5.0	15987 ± 259
10.0	16365 ± 103
Capsaicin	
1.0	15907 ± 395
5.0	16100 ± 288
10.0	16564 ± 269
Cuminaldehyde (500)	16054 ± 203
Linalool (500)	16299 ± 270
Bromophenacyl bromide	
1.0	7414 ± 410
5.0	4392 ± 186
10.0	2024 ± 398

[a]Macrophages (2.5×10^6 cells/mL Dulbecco's modified Eagle's medium) were preincubated with [^{3}H]-arachidonic acid–bovine serum albumin complex followed by spice principles for 60 min. The release of [^{3}H]-arachidonic acid was measured in the supernatant after activating the cells with phorbol ester as described in the Materials and Methods section. Values are means ± SD, n = six experiments.

(iii) Effect on PG formation. Since arachidonic acid uptake was influenced by curcumin and capsaicin, their effects on arachidonic acid metabolites (PG and LT) were determined. Macrophages preincubated with curcumin or capsaicin secreted lower levels of PGE_2 as compared to that produced by control cells when activated with calcium ionophore (Table 3). Thus, macrophages preincubated with 1 and 10 μM curcumin produced 22% ($P < 0.05$) and 45% ($P < 0.05$) lower amounts of PGE_2, respectively, compared to control cells. Similarly, preincubation of macrophages with 1 and 10 μM capsaicin produced 40% ($P < 0.05$) and 48% ($P < 0.05$) lower amounts of PGE_2, respectively, compared to that produced by control cells.

TABLE 3
Effect of Spice Principles on the Release of Prostaglandins by Macrophages *in vitro*[a]

Spice principle added (μM)	PGE_2 (μg/mg protein)	6-Keto-$PGF_{1\alpha}$ (μg/mg protein)
Nil	49.16^a ± 5.95	58.08^a ± 3.05
Curcumin		
0.1	47.50^a ± 4.93	55.17^a ± 13.82
1.0	38.33^b ± 1.66	$70.b8^b$ ± 6.54
10.0	27.00^c ± 3.32	81.25^c ± 5.84
Capsaicin		
0.1	44.17^a ± 2.76	52.38^a ± 3.05
1.0	29.33^c ± 6.63	67.04^b ± 9.74
10.0	25.34^c ± 3.23	75.10^b ± 13.82
Cuminaldehyde		
500	50.14^a ± 2.93	56.77^a ± 2.19
Linalool		
500	52.49^a ± 4.15	49.56^a ± 3.96

[a]Values with different superscripts in the same column are significantly different from the control values (without spice principles) ($P < 0.05$). Values are means ± SD of three experiments. PGE_2, prostaglandin E_2; $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$.

However, 6-keto $PGF_{1\alpha}$ release was consistently increased in macrophages incubated with curcumin or capsaicin. Curcumin treatment at 1 and 10 μM enhanced 6-keto $PGF_{1\alpha}$ by 22% ($P < 0.05$) and 40% ($P < 0.05$), respectively, while capsaicin at 1 and 10 μM enhanced 6-keto $PGF_{1\alpha}$ by 15% ($P < 0.1$) and 29% ($P < 0.05$), respectively, compared to control cells (Table 3). Macrophages treated with cuminaldehyde and linalool, even at 500 μM levels, had no influence on secretion of PGE_2 and 6-keto $PGF_{1\alpha}$.

(iv) Effect on LT formation. LT are potent inflammatory lipoxygenase products. Macrophages preincubated with increasing concentrations of curcumin or capsaicin (1–10 μM) secreted lower amounts of LTB_4 and LTC_4 (Fig. 2). The inhibitory effects on LTB_4 and LTC_4 secretion were observed even at low concentrations of curcumin or capsaicin. A maximum inhibition of 61 and 46% LTB_4 formation was observed ($P < 0.05$) when macrophages incubated with 10 μM curcumin or capsaicin, respectively. Similarly, LTC_4 was decreased by 34 and 48% ($P < 0.05$) when macrophages were incubated with 10 μM curcumin or capsaicin, respectively (Fig. 2). Cuminaldehyde and linalool even at 500 μM did not affect LT formation in macrophages (data not shown).

(v) Effect on lysosomal enzymes. Hydrolytic enzymes play an important role in the tissue destruction observed in autoimmune diseases. Macrophages, when treated with curcumin (10 μM) or capsaicin (10 μM), secreted lower levels of lysosomal enzymes like collagenase, elastase, and hyaluronidase (Fig. 3). With curcumin, the amount of collagenase secreted by macrophages activated with Zymosan was reduced by

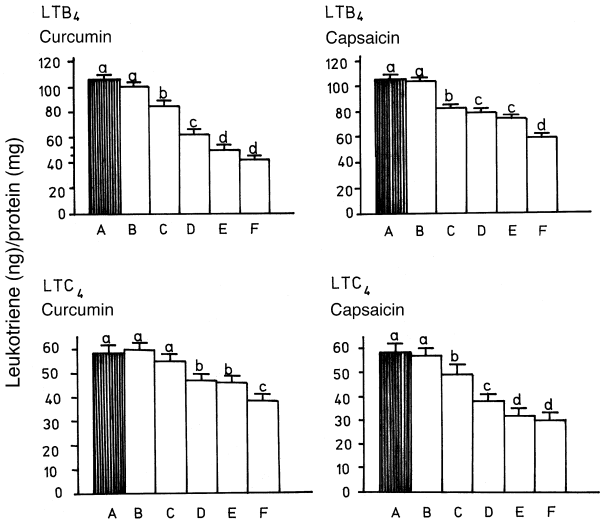

FIG. 2. Effect of spice principles on the release of leukotrienes by macrophages *in vitro*. Leukotrienes B_4 (LTB_4) and C_4 (LTC_4) released by macrophages preincubated with curcumin or capsaicin were quantitated by high-performance liquid chromatography as described in the Materials and Methods section. The concentrations of curcumin or capsaicin added to macrophage monolayers in Dulbecco's modified Eagle's medium (DMEM) were as follows: A: 0, B: 1 μM; C: 2.5 μM; D: 5 μM; E: 7.5 μM; and F: 10 μM. Values are means ± SD of six experiments. Values with the same superscripts are not significantly different from each other at $P < 0.05$.

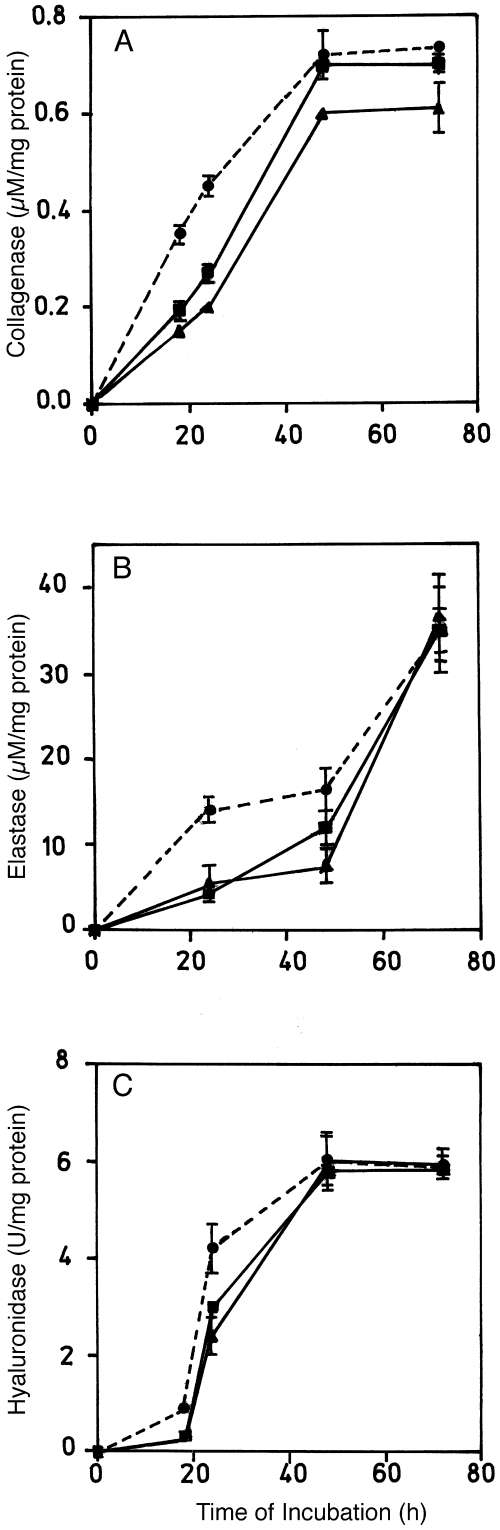

FIG. 3. Effect of spice principles on the secretion of hydrolytic enzymes by macrophages *in vitro*. Macrophages (2.5×10^6 cells/mL DMEM) were incubated with 10 μM curcumin (----) or 10 μM capsaicin (——) along with Zymosan A for different time intervals. The release of hydrolytic enzymes (A) collagenase, (B) elastase, and (C) hyaluronidase was assayed as described in the Materials and Methods section and compared with that of control cells (●---●) incubated under similar conditions in the absence of spice principles. Values are means ± SD of six experiments. See Figure 2 for abbreviation.

57% ($P < 0.001$), 56% ($P < 0.001$), 17% ($P < 0.001$), and 17% ($P < 0.001$) at the end of 18, 24, 48 and 72 h, respectively. Similarly, elastase secretion was reduced in curcumin-treated macrophages by 61% ($P < 0.001$) and 55% ($P < 0.001$), respectively, at the end of 24 and 48 h (Fig. 3). Hyaluronidase secretion was decreased in macrophages incubated with curcumin by 66% ($P < 0.001$) and 43% ($P < 0.001$) at 18 and 24 h, respectively (Fig. 3). The inhibitory effect of curcumin on elastase and hyaluronidase secretion by macrophages was, however, completely overcome when macrophages were incubated for a longer period of time (>72 h). Similarly, capsaicin-inhibited collagenase secretion from macrophages by 46% ($P < 0.001$) and 40% ($P < 0.001$) at the end of 18 and 24 h, respectively; elastase secretion by 69% ($P < 0.001$) and 25% ($P < 0.05$) at 24 and 48 h, respectively; and hyauronidase secretion by 67 ($P < 0.001$) and 30% ($P < 0.05$) at the end of 18 and 24 h of incubation, respectively (Fig. 3). Here again, the inhibitory effects on lysosomal enzyme secretion were not observed when the cells were incubated for longer periods of time with capsaicin (>72 h) (Fig. 3). Spice principles themselves did not affect the activities of collagenase, elastase, and hyaluronidase (data not shown).

DISCUSSION

These studies indicate that macrophage functions are altered by curcumin and capsaicin. Both these phenolic compounds, in a dose-dependent manner, inhibit the incorporation of arachidonic acid into macrophage lipids. However, when macrophages were subsequently activated with PMA after the incorporation of arachidonic acid, no significant differences in the percentage of arachidonic acid released were observed in curcumin- and capsaicin-treated macrophages as compared to that released by control cells. This indicated that curcumin and capsaicin did not affect the release of arachidonic acid from macrophage lipids. The incorporation of arachidonic acid into tissue lipids requires activation of fatty acids by fatty acyl CoA synthetase and transfer by fatty acyl transferase (26). The specific effects of curcumin and capsaicin on these two enzymes have yet to be ascertained. Curcumin is reported to inhibit Δ^6 desaturase in *Mortierella alpina* and rat liver microsomes (27). This lowers the conversion of linoleic to arachidonic acid. As a consequence of these effects on Δ^6 desaturase and fatty acid uptake, curcumin lowers the overall arachidonic acid levels in the tissues. The lower levels of arachidonic acid subsequently reflected in reduced production of PGE_2, LTB_4, and LTC_4, which are major arachidonic acid metabolites formed from cyclooxygenase and lipoxygenase pathways. These results are in agreement with those found in neutrophils (28) and skin fibroblasts (29) where curcumin decreased eicosanoid formation. However, the formation of 6-keto $PGF_{1\alpha}$ was consistently enhanced after the treatment of macrophages with curcumin and capsaicin. This indicated a differential influence of these phenolic compounds on PG and prostacyclin synthetases. It is also possi-

ble that arachidonic acid may be channeled into prostacyclin synthesis by these compounds. Antioxidants such as ascorbate have been shown to enhance the formation of 6-oxo $PGF_{1\alpha}$ in ram seminal vesicles (30). Capsaicin also produces activation of a number of biochemical systems increasing cellular cGMP, diacylglycerol, and inositol triphosphate (31). It is interesting to note that capsaicin and its structural analogs are reported to have vasodilatory effects (32). Prostacyclin is a vasodilatory compound which is enhanced in macrophages by curcumin and capsaicin treatment. Analogs of capsaicin have analgesic and antiinflammatory properties (31). Capsaicin has been used to treat a number of painful clinical conditions such as cluster headache, postmastectomy pain, diabetic neuropathy, and rheumatoid arthritis (33,34). Similarly, antiinflammatory properties of curcumin have also been reported. Srivastava *et al.* (35) have demonstrated that curcumin inhibits platelet aggregation and eicosanoid formation in human blood platelets. Thus, curcumin and capsaicin can alter arachidonic acid metabolism and the generation of other inflammatory mediators.

The mononuclear cells are known to generate enzymes which are potent mediators of inflammation that can destroy tissues (5). These cells may contribute to the excessive levels of collagenase and PG in synovitis (9,36). This, in turn, may contribute to the destruction of connective tissues and the erosion of bone, cartilage, and soft connective tissues. Therefore, controlling the secretion of lysosomal enzymes, such as collagenase, elastase and hyaluronidase, from mononuclear cells may reduce inflammatory responses. Curcumin and capsaicin inhibit the secretion of these various enzymes from macrophages in the early stages of activation. Nirmala and Puvanakrishnan (37) reported that curcumin can lower the elevated levels of lysosomal hydrolases such as β-glucuronidase, β-*N*-acetyl glucoseaminidase, cathepsin B, cathepsin D, and acid phosphatase in myocardial infarction. Similarly, Srivastava and Srimal (34) have shown that curcumin stablizes the lysosomal membranes and inhibits the liver acid phosphatase and cathepsin D activities. The release of collagenase by human leucocytes is enhanced by PGE_2 and cAMP (38). Curcumin and capsaicin inhibited the synthesis of PGE_2. As a consequence of this, the release of collagenase and other lysosomal enzymes may have been decreased in the cells treated with curcumin and capsaicin. Our earlier studies have shown that the formation of reactive oxygen species in macrophages is also inhibited by curcumin and capsaicin (15). Nitric oxide is known to activate metalloprotease enzymes in articular cartilage (39). Reducing nitric oxide by spice principles lowers the activation of proteases and thereby exerts antiinflammatory properties. Therefore, several of the mediators of inflammation secreted by macrophages could be reduced by curcumin and capsaicin.

The effects of curcumin and capsaicin on macrophages, however, are reversible. The inhibitory effects of these compounds on arachidonic acid metabolism are evident only when intact molecules are present in the cells. Macrophages are capable of metabolizing these compounds since the parent molecules were not found in the cells with increasing time of incubation. Our attempts to identify the metabolites of curcumin and capsaicin by conventional methods were not successful. Similarly, Vijayalakshmi and Chandrasekhara (40) earlier demonstrated the rapid turnover of curcumin in rats but were unable to detect the metabolites. Recently Oi *et al.* (41) have shown a capsaicin-degrading enzyme in rat liver microsomes. Here, again, the end product was not identified. However, the effects of curcumin and capsaicin on lysosomal enzymes were observed even after intact spice principles disappeared from macrophages.

The studies with spice components have already established the nontoxic, antimutagenic, antioxidant, antimicrobial, and antiinflammatory effects of curcumin and capsaicin (33,42–44). No adverse side effects have been found with the consumption of curcumin even at a very high dose of 1% in the diet (45). The spices are unlikely to accumulate in the body and cause any adverse side effects due to their rapid turnover. Mega doses of curcumin (1800 to 2500 mg/day) have been successfully used on human arthritic subjects with encouraging results in the alleviation of inflammation (42). Therefore, phenolic compounds from spices, like curcumin and capsaicin, may provide an adjunctive means to reduce the production of inflammatory mediators from mononuclear cells.

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