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Inhibitory Effect of Curcumin, a Food Spice from Turmeric, on Platelet-Activating Factor- and Arachidonic Acid-Mediated Platelet Aggregation through Inhibition of Thromboxane Formation and Ca^{2+} Signaling

Bukhtiar H. Shah,† Zafar Nawaz, Shamim A. Pertani, Asad Roomi, Hammad Mahmood, Sheikh A. Saeed and Anwar H. Gilani*

Department of Physiology and Pharmacology, The Aga Khan University, Karachi, Pakistan

ABSTRACT. Curcumin, a dietary spice from turmeric, is known to be anti-inflammatory, anticarcinogenic, and antithrombotic. Here, we studied the mechanism of the antiplatelet action of curcumin. We show that curcumin inhibited platelet aggregation mediated by the platelet agonists epinephrine (200 μM), ADP (4 μM), platelet-activating factor (PAF; 800 nM), collagen (20 μg/mL), and arachidonic acid (AA: 0.75 mM). Curcumin preferentially inhibited PAF- and AA-induced aggregation (IC_{50}; 25–20 μM), whereas much higher concentrations of curcumin were required to inhibit aggregation induced by other platelet agonists. Pretreatment of platelets with curcumin resulted in inhibition of platelet aggregation induced by calcium ionophore A-23187 (IC_{50}; 100 μM), but curcumin up to 250 μM had no inhibitory effect on aggregation induced by the protein kinase C (PKC) activator phorbol myrsitate acetate (1 μM). Curcumin (100 μM) inhibited the A-23187-induced mobilization of intracellular Ca^{2+} as determined by using fura-2 acetoxymethyl ester. Curcumin also inhibited the formation of thromboxane A_2 (TXA_2) by platelets (IC_{50}; 70 μM). These results suggest that the curcumin-mediated preferential inhibition of PAF- and AA-induced platelet aggregation involves inhibitory effects on TXA_2 synthesis and Ca^{2+} signaling, but without the involvement of PKC. BIOCHEM PHARMACOL **58**;7:1167–1172, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. human platelets; curcumin; platelet aggregation; calcium influx; cyclooxygenase; thromboxane A_2

Platelet aggregation plays an important role in thrombosis and haemostasis. The first step in the response of platelets to vascular injury is irreversible attachment to the altered surfaces followed by platelet aggregation. This mainly takes place by the action of endogenous agonists such as AA,‡ PAF, ADP, collagen, and thrombin and through adhesiveness of platelets to the site of injury [1]. Most platelet agonists activate platelets through binding to seven transmembrane receptors coupled with G-proteins. Second messengers such as (Ca^{2+} and IP_3), PKC, and protein tyrosine kinases increase platelet aggregation [2, 3], whereas cAMP inhibits it [4].

Recent studies have shown that dietary factors can modify the processes of inflammation and carcinogenesis [5, 6]. We and others have shown that vegetables, fruits, and plants contain an abundance of compounds with anticancer and anti-inflammatory properties, antioxidants, protein kinase inhibitors, and calcium channel blockers [7–9]. Curcumin (diferuloylmethane), a major component of the food flavor turmeric, has been isolated from the rhizomes of *Curcuma longa*. The powder of this rhizome is a vital ingredient in the Asian diet and has been widely used for centuries in traditional medicine for the treatment of a variety of inflammatory conditions and other diseases [10]. The anti-inflammatory and cancer chemoprevention mechanisms of curcumin involve inhibitory effects on multiple signaling pathways which include COX, PKC, and oncogene expression [7, 11], protein tyrosine kinase, AA metabolism [5, 12], and lysosomal enzyme secretions [13]. This study was conducted to examine the effect of curcumin in human platelet aggregation. We show that curcumin selectively inhibits PAF- and AA-mediated aggre-

*Present address: Endocrinology and Reproduction Research Branch, The National Institute of Health, Building 49, Room 6A-36, 49 Convent Drive MSC 4510, Bethesda, MD 20892.

† Corresponding author: Bukhtiar H. Shah, Ph.D., Department of Physiology and Pharmacology, The Aga Khan University Medical College, Karachi-74800, Pakistan. Tel. (92) 21 4930051; FAX (92) 21 4934294; E-mail: bukhtiar.shah@aku.edu

‡ *Abbreviations:* AA, arachidonic acid; fura-2 AM, fura-2 acetoxymethyl ester; cAMP, 3′,5′-cyclic adenosine monophosphate; COX; cyclooxygenase; G_i protein, inhibitory GTP-binding protein; IP_3, inositol 1,4,5-triphosphate; PAF, platelet-activating factor; PKC, protein kinase C; PMA, phorbol myristate acetate; PRP, platelet-rich plasma; and TXA_2, thromboxane A_2.

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gation and that such effects are exerted predominantly through inhibition of COX activity and partly via blocking of Ca^{2+} signaling.

MATERIALS AND METHODS

Chemicals

ADP, AA, PAF, collagen, epinephrine, A-23187, and curcumin were purchased from the Sigma Chemical Co. [1-^{14}C]-Arachidonic acid (specific activity 54 mCi/mmol) was from Amersham. Fura 2-AM was from Calbiochem-Novabiochem. All other chemicals were of the highest purity grade available.

Preparation of Human Platelets

Blood was taken by venipuncture from normal human volunteers (N = 35) reported to be free of medication for at least one week. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at 260 *g* for 15 min at 20° to obtain PRP with platelet counts between 2.5 and 3 × 10^8/mL of plasma. Experiments were performed within 2 hr of PRP preparation.

Measurement of Platelet Aggregation

Aggregation was monitored using a Dual-channel Lumi-aggrometer (Model 400; Chronolog Corporation) using 0.45-mL aliquots of PRP [14, 15]. The final volume was made up to 0.5 mL with the test drug dissolved either in normal saline or appropriate vehicle (curcumin stock solution in DMSO) known to be devoid of any effect on aggregation. Aggregation was induced with ADP (4.2 μM), AA (0.75 mM), epinephrine (200 μM), PAF (800 nM), or collagen (20 μg/mL). The anti-aggregatory effects of curcumin were studied by pretreatment of human platelets with curcumin for 1 min prior to addition of the agonists at varying concentrations. The resulting aggregation was recorded at 5-min challenge by the change in light transmission as a function of time. Once the antiplatelet activity of the curcumin against Ca^{2+} ionophore and other agonists was established, dose–response curves were constructed to calculate the IC_{50} values of the drug.

Measurement of Ca^{2+} Influx

The agonist-induced influx of Ca^{2+} was measured using fura-2 AM as described previously [16]. Briefly, platelets (1 × 10^8/mL) were suspended in Ca^{2+}-free standard medium (145 mM NaC, 5 mM KC, 1 mM MgC_2, 10 mM HEPES, 10 mM glucose, pH 7.4). Fura-2 AM dissolved in DMSO was added to the platelet suspension at 37° for 45 min. The cell suspension was centrifuged at 350 *g* for 15 min and the cell pellet resuspended in fresh standard medium. Fura-2 fluorescence was monitored at 340 and 505 (excitation and emission) in cells treated with the agonist in the absence and presence of calcium (1 mM).

Arachidonic Acid Metabolism by Platelets

The formation of TXA_2 in platelets was estimated as described previously [6]. The PRP was centrifuged at 1200 *g* for 20 min and the sedimented platelets were washed twice with an ice-cold phosphate buffer (50 mM, pH 7.4) containing sodium chloride (0.15 M) and EDTA (0.2 mM). After centrifugation, washed platelets were resuspended in the same buffer without EDTA and homogenized at 4° using a polytron homogenizer for 15 sec. The homogenate was centrifuged at 1200 *g* for 20 min and 300 μL of the supernatant (containing 0.4 mg of protein) was incubated with 10 μg unlabeled AA and 0.1 μCi [1-^{14} C]-AA in the presence and absence of curcumin. After 15-min gentle shaking in air at 37°, the reaction was stopped by adding 0.4 mL of citric acid (0.4 M) and ethyl acetate (7.0 mL). After mixing and centrifugation at 600 *g* for 5 min at 4°, the organic layer was separated and evaporated to dryness under nitrogen. Residues were dissolved in 40 μL ethanol and 20 μL was applied to silica gel G TLC plates. The AA, TXB_2 (a stable degradation product of TXA_2), and 12-hydroxy-5*Z*,8*Z*,10*E*,14*Z*, ecostate traenoic acid standards were spotted separately. The plates were developed in ethylacetate/isooctane/water/acetic acid (11:5:10:2, v/v, upper phase) to a distance of 17 cm. Radioactive zones were located and quantified by use of a Berthold TLC linear analyzer and chromatography data system (Model LKB 511).

RESULTS

The platelet agonists PAF, ADP, epinephrine, AA, and collagen induced platelet aggregation in a dose-dependent manner in human PRP. Pretreatment of platelets with curcumin for 1 min inhibited the platelet aggregation mediated by ADP (4.2 μM), epinephrine (200 μM), collagen (20 μg/mL), PAF (800 nM), and AA (0.75 mM) in a dose-dependent manner (Fig. 1). Curcumin showed different half-maximal inhibitory concentrations (IC_{50}) against platelet aggregation induced by various agonists (Table 1). The order of effectiveness of curcumin in blocking the platelet aggregation induced by these agonists was PAF = AA > epinephrine > collagen = ADP. The curcumin was distinctly potent against PAF and AA with IC_{50} values of 25 and 30 μM, respectively.

To investigate the mechanism(s) involved in this effect, we measured platelet activity after treatment with calcium ionophore A-23187 and PMA (a PKC activator) and also determined the levels of COX and lipoxygenase products. Curcumin decreased the aggregatory effect of A-23187 in a concentration-dependent manner, with an IC_{50} of 100 μM (Fig. 2). We also measured changes in the cytosolic Ca^{2+} in platelets stimulated with Ca^{2+} ionophore A-23187. Results show that pretreatment of platelets with curcumin reduced the A-23187–induced rise in intracellular calcium $[Ca^{2+}]_i$ (Fig. 3).

Activation of PKC is known to stimulate human platelet

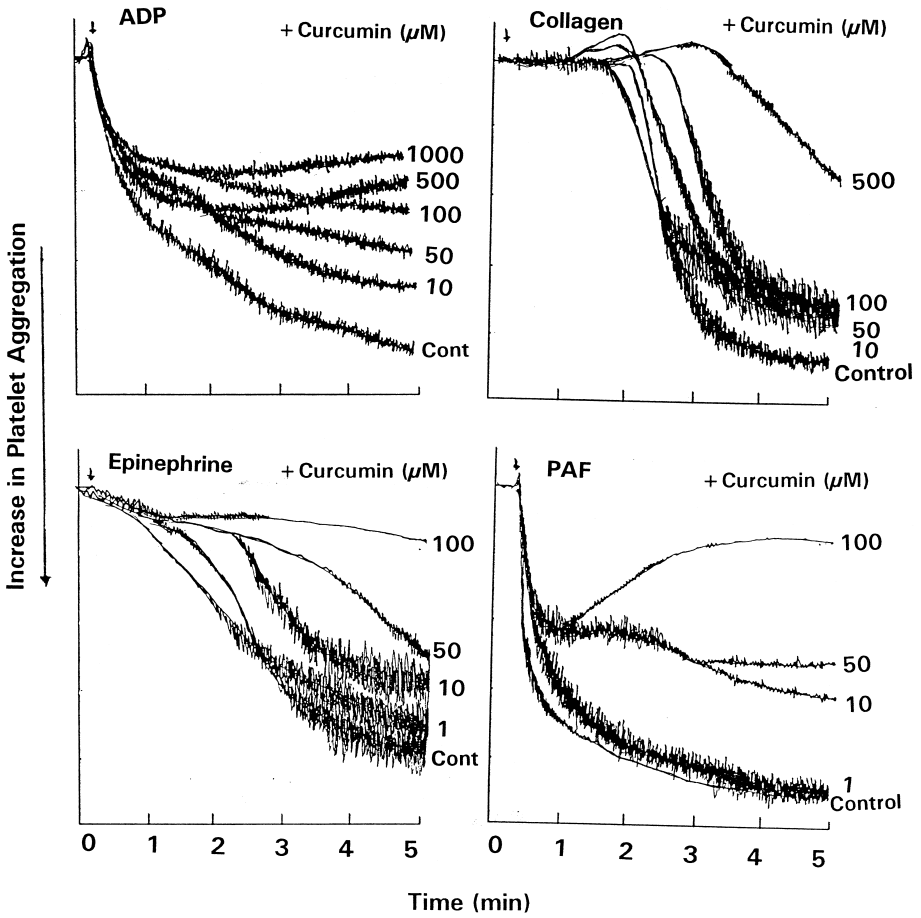

FIG. 1. Curcumin inhibits platelet aggregation induced by various platelet agonists in a concentration-dependent manner. Tracings of a representative experiment are shown here. Control represents the full aggregating dose of PAF (800 nM), ADP (4.2 μM), epinephrine (200 μM), and collagen (20 μg/mL). The platelets pretreated with different concentrations of curcumin for 1 min were challenged with the agonist and the effect recorded for 5 min. The IC_{50} values calculated from these experiments (N = 5–7) are given in Table 1.

aggregation [15, 16]. Phorbol esters such as PMA are frequently used in monitoring the effects of PKC in aggregation [8, 17]. Pretreatment of PRP with curcumin up to 1 mM did not completely inhibit the aggregation induced by PMA (0.1–1 μM). There was only 25% inhibition of aggregation at quite high concentrations (500–1000 μM) of curcumin (data not shown). Since platelet activation following treatment with AA and possibly other agonists (PAF and A-23187) occurs through activation of the COX pathway leading to the production and release of TXA_2, we measured the effects of curcumin on TXA_2 production in agonist-stimulated platelets. The results in Fig. 4 show that curcumin inhibits TXA_2 production in platelets with an IC_{50} of 70 μM.

TABLE 1. Inhibitory effects of curcumin on human platelet aggregation induced by various platelet agonists

Agonists	Concentration	IC_{50} of curcumin (μM)
PAF	(800 nM)	25 ± 5
AA	(0.75 mM)	30 ± 4
Epinephrine	(200 μM)	50 ± 6
A-23187	(6 μM)	100 ± 12
Collagen	(20 μg/mL)	450 ± 24
ADP	(4.2 μM)	650 ± 40
PMA	(1 μM)	NE

IC_{50} values of curcumin are means ± SEM of 5–7 experiments for each agonist treatment. NE indicates no effect. Curcumin up to 1 mM could not inhibit platelet aggregation induced by PMA.

DISCUSSION

Curcumin, a spice food pigment quite commonly used in natural medicine, exerts its potent antiplatelet activity through inhibition of COX activity and the blockade of calcium signaling. We have shown that the sensitivity of platelet agonists to the inhibitory effect of curcumin varied, PAF- and AA-mediated aggregation being the most sensitive. Both these agonists are directly or indirectly involved in the stimulation of TXA_2 production. TXA_2 interacts with its receptors on platelets in an autocrine fashion to activate G_q protein [18]. PAF is known to activate the G_q-phospholipase C signaling pathway [19, 20]. Stimula-

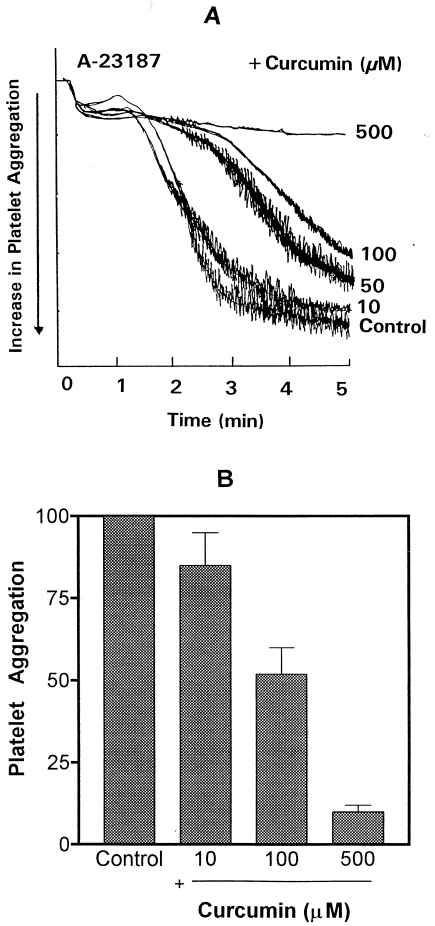

FIG. 2. Effect of curcumin on platelet aggregation induced by Ca^{2+} ionophore A-23187. (A) A representative experiment showing the inhibitory effect of curcumin against A-23187-induced aggregation. Platelets pretreated with curcumin with varying concentrations as shown in the figure were subjected to treatment with A-23187 (6 μM), which is indicated as control. (B) The curcumin-mediated inhibition of aggregation is quantitated (mean ± SEM, N = 5). A-23187 (6 μM)-induced platelet aggregation is taken as 100% and indicated as control.

tion of G_q protein leads to generation of second messenger IP_3 and diacylglycerol, which in turn induce the release of Ca^{2+} from the dense tubular system and the activation of PKC, respectively [1, 3, 20, 21]. Multiple isoforms of both IP_3 receptor and PKC are present in platelets [22–24]. Defects at any of the signaling cascade, receptor or G_q protein activation, IP_3 formation, or Ca^{2+} release can impair the platelet response to agonists. Recent studies have shown that transgenic mice deficient in G_q protein lack platelet aggregation capabilities [25].

We have demonstrated that curcumin inhibited platelet aggregation induced by calcium ionophore A-23187 and other agonists known to increase cytosolic Ca^{2+}. A rise in cytosolic Ca^{2+} levels accompanies platelet activation through stimulation of enzymes which are not fully functional at the low Ca^{2+} concentration present in the resulting platelets [3]. In platelets multiple signaling mechanisms cause an increase in cytosolic Ca^{2+}. These include stimulation of phospholipase C, activation of inhibitory G-protein (G_i), opening of receptor-operated calcium channels, and mobilization of intracellular calcium [1–4]. The common point in the action of the platelet agonists (ADP, epinephrine, collagen, A-23187, PAF) used in the present studies is the increase in the levels of cytosolic Ca^{2+}, either due to its release from internal stores or through Ca^{2+} influx [3, 26]. Since curcumin inhibits the aggregation induced by these agents, it is likely that it interferes with the Ca^{2+} signaling influx in activated platelets. However, the agonists that are linked with the activation of the COX pathway were more sensitive to the inhibitory effect of curcumin. It seems that curcumin exerts its inhibitory effects at points distal to any specific receptor of G-protein, most likely at COX- and Ca^{2+}-activated signaling cascades. Therefore, curcumin may exhibit preferential inhibitory effects on aggregation mediated by agonists such as (PAF and AA) which activate either one or both of the signaling targets [1, 6, 27].

Cells possess the capabilities of maintaining *milieu intérieur* through regulation and desensitization of signaling molecules which may include receptors, G-proteins, or second messengers [28]. PKC plays an important role in signal transduction pathways including platelets, as it directly regulates adenylyl cyclase activity through phosphorylation and inactivation of the inhibitory G-protein, G_i [29]. PKC acts in synergy with Ca^{2+} mobilization for the activation of platelets [22]. Curcumin (up to 500 μM) did not block the aggregation induced by PMA. This excludes the possible role of PKC in curcumin-mediated inhibition of aggregation. These findings are in contrast to previous studies suggesting the inhibitory activity of curcumin on PKC in NIH 3T3 cells [7, 11], but are in accordance with

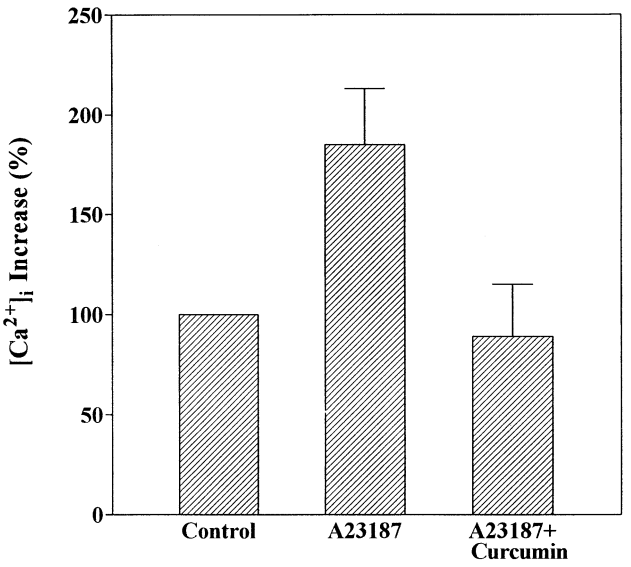

FIG. 3. Effect of curcumin (100 μM) on the A-23187 (6 μM)-induced rise in intracellular calcium $[Ca^{2+}]_i$. Platelets were loaded with fura-2 AM and assays done as described in Methods. Control represents unstimulated platelets and is taken as 100 per cent. Data are means ± SEM (N = 4).

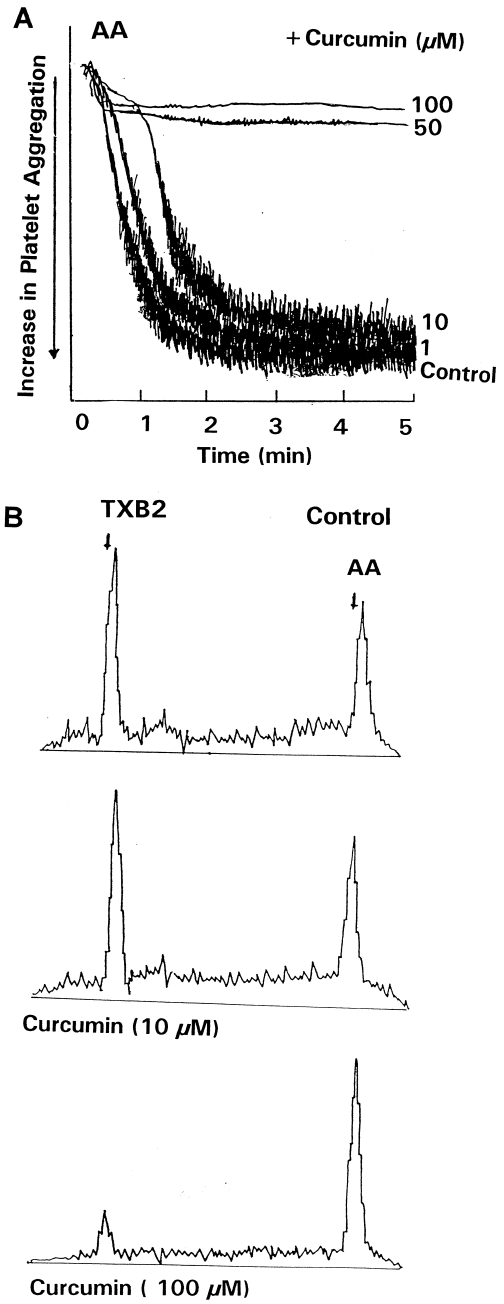

FIG. 4. Tracings of a representative experiment showing the inhibitory effect of curcumin on AA-induced platelet aggregation. (B) A representative radiochromatogram of the product of COX acitivity (TXB_2) formed upon incubation of homogenate of human platelets with 1-[^{14}C]-AA in the absence and presence of varying concentrations of curcumin. TXB_2, thromboxane B_2.

the findings of Huang *et al.* [12], who did not observe any inhibition of rat brain PKC by curcumin. Since PKC has multiple isoforms—the α, β, and ϵ isoforms of PKC are predominantly expressed in platelets [22]—it is possible that curcumin exerts differential effects on PKC in a tissue-specific manner. Further studies are needed in this direction.

Curcumin also inhibited platelet aggregation induced by epinephrine with an almost 2-fold higher IC_{50} (50 μM) compared to AA and PAF (25–30 μM). Epinephrine, through interaction with α_2-adrenoceptors, activates G_i protein, and inhibits adenylyl cyclase activity, thus causing a decrease in intracellular cAMP levels in platelets. Agents which decrease cAMP levels stimulate platelet aggregation [2, 4]. In fact, the effects of epinephrine in platelets may be mediated through multiple pathways: a decrease in intracellular cAMP levels, stimulation of phospholipase Cβ and thus IP_3 production by G-protein βγ subunits, an increase in Ca^{2+} influx, and activation of other proteins such as Syk and related adhesion focal tyrosine kinase (RAFTK) [30–32]. Besides epinephrine, other platelet agonists, such as ADP, collagen, and A-23187, which were inhibited by curcumin (IC_{50}; 650, 450, and 100 μM, respectively) also induce RAFTK phosphorylation. It may be interesting to examine the effect of curcumin on Syk and RAFTK.

The effect of curcumin on ADP- and collagen-mediated aggregation was not well pronounced. ADP causes a transient increase in IP_3-mediated Ca^{2+} levels which may eventually lead to a store-depleted influx of Ca^{2+} through calcium channels [3, 26]. PAF, which is known to activate phospholipase C (PLC), can enhance aggregation through an increase in Ca^{2+} influx via receptor-operated Ca^{2+} channels with such an effect at low concentrations of PAF (picomolar) being independent of PLC activation [19]. Our studies provide evidence that curcumin possibly inhibits Ca^{2+} influx in platelets, as demonstrated by inhibition of platelet aggregation induced by calcium ionophore A-23187. Since both ADP- and collagen-mediated aggregation have distinct signaling pathways which do not involve activation of COX activity, higher concentrations of curcumin (IC_{50}, 450–600 μM) were required to block their effect. In conclusion, these results imply that curcumin not only inhibits COX activity as shown previously [33], but also impairs Ca^{2+} ionophore-mediated platelet aggregation.

Turmeric has been traditionally used in the Asian diet for centuries. According to a WHO/FAO report (1974), the dietary intake of turmeric in Indian populations ranges between 2–2.5 g per person (approx. weight 60 kg) per day. Keeping in view the amount of curcumin (3–5%) present in dry rhizome [34], an adult individual might be consuming 60–100 mg of curcumin per day. Besides its antiplatelet action, curcumin is also reported to possess anticarcinogenic and anti-inflammatory effects, especially in stomach and colon cancers [5, 7, 10]. The use of curcumin in diet for prophylaxis of these ailments may be beneficial. Further work in this direction using *in vivo* models is needed.

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References

1. Siess W, Molecular mechanisms of platelet activation. *Physiol Rev* **69:** 58–178, 1989.
2. Brass LF, Hoxie JA and Manning DR, Signalling through G

proteins and G protein-coupled receptors during platelet activation. *Thromb Haemost* **70:** 217–223, 1993.
3. Heemskerk JWM and Sage O, Calcium signalling in platelets and other cells. *Platelets* **5:** 295–316, 1994.
4. Siess W, Grunberg B and Luber K, Functional relationship between cyclic AMP-dependent protein phosphorylation and platelet inhibition. *Adv Exp Med Biol* **344:** 229–235, 1993.
5. Rao CV, Simi B and Reddy BS. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. *Carcinogenesis* **14:** 2219–2225, 1993.
6. Saeed SA, Gilani AH, Majoo RU and Shah BH, Anti-thrombotic and anti-inflammatory activities of protopine. *Pharmacol Res* **36:** 1–8, 1997.
7. Lin J-K, Chen Y-C, Huang Y-T and Lin-Shiau S-Y, Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin. *J Cell Biochem Suppl* **28/29:** 39–48, 1997.
8. Shah BH, Safdar B, Virani SS, Nazaw Z, Saeed SA and Gilani AH, The antiplatelet aggregatory activity of *Acacia nilotica* is due to blockade of calcium influx through membrane calcium channels. *Gen Pharmacol* **29:** 251–255, 1997.
9. Shah BH, Nawaz Z, Virani SS, Ali IQ, Saeed SA and Gilani AH, The inhibitory effect of cinchonine on human platelet aggregation due to blockade of calcium influx. *Biochem Pharmacol* **56:** 956–960, 1998.
10. Srivastava KC and Mustafa T, Pharmacological effects of spices: Eicosanoid modulating activities and their significance in human health. *Biomed Rev* **2:** 15–29, 1993.
11. Liu J-Y, Lin S-J and Lin J-K, Inhibitory effects of curcumin on protein kinase C activity induced by 12-*O*-tetradecanoyl-phorbol-13-acetate in NIH 3T3 cells. *Carcinogenesis* **14:** 857–861, 1993.
12. Huang M-T, Lysz T, Ferraro T, Abidi TF, Laskin JD and Conney AH, Inhibitory effects of curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res* **51:** 813–819, 1991.
13. Joe B and Lokesh BR, Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. *Lipids* **32:** 1173–1180, 1997.
14. Shah BH and Saeed SA, Phosphatidylinositol 3-kinase inhibitor, wortmannin, inhibits 5-hydroxytryptamine-mediated potentiation of platelet aggregation induced by epinephrine. *Res Comm Mol Pathol Pharmacol* **89:** 157–164, 1995.
15. Shah BH, Shamim G, Khan S and Saeed SA, Protein kinase C inhibitor, chelerythrine, potentiates the adrenaline-mediated aggregation of human platelets through calcium influx. *Biochem Mol Biol Int* **38:** 1135–1141, 1996.
16. Font J, Azula FJ, Marino A, Nieva N, Trueba M and Macrulla JM, Intracellular Ca^{2+} mobilization and not calcium influx promotes phorbol ester-stimulated thromboxane A_2 synthesis in human platelets. *Prostaglandins* **43:** 383–395, 1992.
17. Turini ME, Gaudette DC, Holub BJ and Kirkland JB, Correlation between platelet aggregation and dephosphorylation of a 68 kDa protein revealed through the use of putative PKC inhibitors. *Thromb Haemost* **70:** 648–653, 1993.
18. Offermans S, Laugwitz Kl, Spicher K and Schultz G, G proteins of the G12 family are activated via thromboxane A_2 and thrombin receptors in human platelets. *Proc Natl Acad Sci USA* **91:** 504–508, 1994.
19. James-Kracke MR, Sexe RB and Shukla SD, Picomolar platelet activating factor mobilizes Ca^{2+} to change platelet shape without activating phospholipase C or protein kinase C; simultaneous measurements of intracellular free Ca^{2+} concentration and aggregation. *J Pharmacol Exp Ther* **271:** 824–831, 1994.
20. Shukla SD, Franklin CC and Carter MG, Activation of phospholipase C in platelets by platelet activating factor and thrombin causes hydrolysis of a common pool of phosphatidylinositol 4,5-bisphosphate. *Biochim Biophys Acta* **929:** 134–141, 1987.
21. Clapham DE, Calcium signalling. *Cell* **80:** 259–268, 1995.
22. Crabos M, Fabbro D, Stabel S and Erne P, Effect of tumour-promoting phorbol ester, thrombin platelets and regulation by calcium. *Biochem J* **288:** 891–896, 1992.
23. Newton AC, Protein kinase C: Structure, function and regulation. *J Biol Chem* **270:** 28495–28498, 1995.
24. Quinton TM and Dean WL, Multiple inositol 1,4,5-triphosphate receptor isoforms are present in platelets. *Biochem Biophys Res Commun* **224:** 740–746, 1996.
25. Offermanns S, Toombs CF, Hu Y-H and Simon MI, Defective platelet activation in $G\alpha_q$-deficient mice. *Nature* **389:** 183–186, 1997.
26. Hourani SMO and Hall DA, Receptors for ADP on human blood platelets. *Trends Pharmacol Sci* **15:** 103–108, 1994.
27. Chao W and Olsom MS, Platelet-activating factor: Receptors and signal transduction. *Biochem J* **292:** 617–629, 1993.
28. Shah BH, McEwan DJ and Milligan G, Gonadotrophin-releasing hormone receptor agonist-mediated down-regulation of Gqα/G11 α (pertussis toxin-insensitive) G proteins in αT3–1 gonadotroph cells reflects increased G protein turnover but not alterations in mRNA levels. *Proc Natl Acad Sci USA* **92:** 1886–1890, 1995.
29. Kawabe J, Iwami, G, Ebina T, Ohno S, Katada T, Ueda Y, Homcy CJ and Ishikawa Y, Differential activation of adenylyl cyclase by protein kinase C isoenzymes. *J Biol Chem* **269:** 16554–16558, 1994.
30. Banno Y, Asano T and Nozawa Y, Stimulation by G protein βγ subunits of phospholipase Cβ isoforms in human platelets. *Thromb Haemost* **79:** 1008–1013, 1998.
31. Wang X, Yanagi S, Yang C, Inatome R and Yamamura H, Tyrosine phosphorylation and SYK activation are involved in thrombin-induced aggregation of epinephrine-potentiated platelets. *J Biochem* **121:** 325–330, 1997.
32. Raja S, Avraham S and Avraham H, Tyrosine phosphorylation of the novel protein-tyrosine kinase RAFTK during an early phase of platelet activation by an integrin glycoprotein IIb-IIIa-independent mechanism. *J Biol Chem* **272:** 10941–10947, 1997.
33. Srivastava KC, Bordia A and Verma SK. Curcumin, a major component of food spice turmeric (*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. *Prostaglandins Leukot Essent Fatty Acids* **52:** 223–227, 1995.
34. Govindrajan VS, Turmeric-chemistry, technology and quality. *Crit Rev Food Sci Nutr* **12:** 199–301, 1980.