Suppression of pro-inflammatory and proliferative pathways by diferuloylmethane (curcumin) and its analogues dibenzoylmethane, dibenzoylpropane, and dibenzylideneacetone: Role of Michael acceptors and Michael donors

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A R T I C L E   I N F O

A B S T R A C T

Curcumin, a diferuloylmethane, has been shown to exhibit anti-inflammatory and anti-proliferative activities. Whereas curcumin has both a Michael acceptor and a Michael donor units, its analogues dibenzoylmethane (DBM, a component of licorice) and dibenzoylpropane (DBP) have a Michael donor but not a Michael acceptor unit, and the analogue dibenzylideneacetone (DBA) has a Michael acceptor unit. In the current report, we investigated the potency of DBM, DBP, and DBA in relation to curcumin for their ability to suppress TNF-induced NF-κB activation, NF-κB-regulated gene products, and cell proliferation. We found that all four agents were active in suppressing NF-κB activation; curcumin was most active and DBM was least active. When examined for its ability to inhibit the direct DNA binding activity of p65, a subunit of NF-κB, only DBP inhibited the binding. For inhibition of TNF-induced IKK activation, DBA was most active. For suppression of TNF-induced expression of NF-κB-regulated gene products such as COX-2 (inflammation marker), cyclin D1 (proliferation marker), and VEGF (angiogenesis marker), DBA and curcumin were more active than DBM. Similarly for suppression of proliferation of leukemia (KBM-5), T cell leukemia (Jurkat), prostate (DU145), and breast (MDA-MB-231) cancer cells, curcumin and DBA were most active and DBP was least active. Overall, our results indicate that although curcumin and its analogues exhibit activities to suppress inflammatory pathways and cellular proliferation, a lack of Michael acceptor units in DBM and DBP can reduce their activities.

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1. Introduction

Dibenzoylmethane (DBM), a β-diketone, a constituent of licorice (Glycyrrhiza glabra), is used in sunscreens as an anti-melanoma agent [1]. It exhibits anti-cancer effects in vitro and in vivo and has been shown to suppress the proliferation of breast, colon [2], and prostate cancer cells [3]. DBM also induces apoptosis in human oral squamous cell carcinoma (HSC-2) and leukemia cells [4]. Its ability to inhibit the formation of benzo(a)pyrene (BP)-induced DNA adducts in lungs [5], the development of intestinal adenomas in the ApcMin/+ mouse model [6], DMBA-induced mammary tumors [7–10], and lymphomas/leukemias [10] has been demonstrated. DBM can also prevent polycyclic aromatic hydrocarbon (PAH)-induced tumorigenesis through binding to aryl hydrocarbon receptor (AhR) function in rodents [11], suppress androgen receptor expression in

advanced prostate cancer [12], and contribute to the detoxification of food-derived procarcinogens such as BP [13]. In addition, DBM is also known to inhibit estradiol (E2)-induced mammary cell proliferation through the E2-estrogen receptor (ER)- estrogen response elements (ERE)-dependent pathway [14] and to also inhibit BP- and 1,6-dinitropyrene-DNA adduct formation in human mammary epithelial cells [7,15]. DBM has also been found to increase the expression of vascular endothelial growth factor (VEGF) through induction of hypoxia inducible factor-1α [16], block the phosphorylation of cytosolic phospholipase A2, decrease the expression of cyclooxygenases, inhibit the catalytic activity of 5-lipoxygenase [17] and inhibit the binding of ER to ERE in the promoter region of c-myc, hTERT, and bcl-2 genes [14]. Despite this myriad of reports on the bioactivity of DBM, the mechanism by which DBM mediates these effects is yet to be established.

Several of the above bioactivities of DBM are similar to those of curcumin, a diferuloylmethane (Fig. 1). Both of these molecules have a β-diketone group flanked by two aryl groups, and both are metal-chelating agents. Compared with curcumin, however, DBM
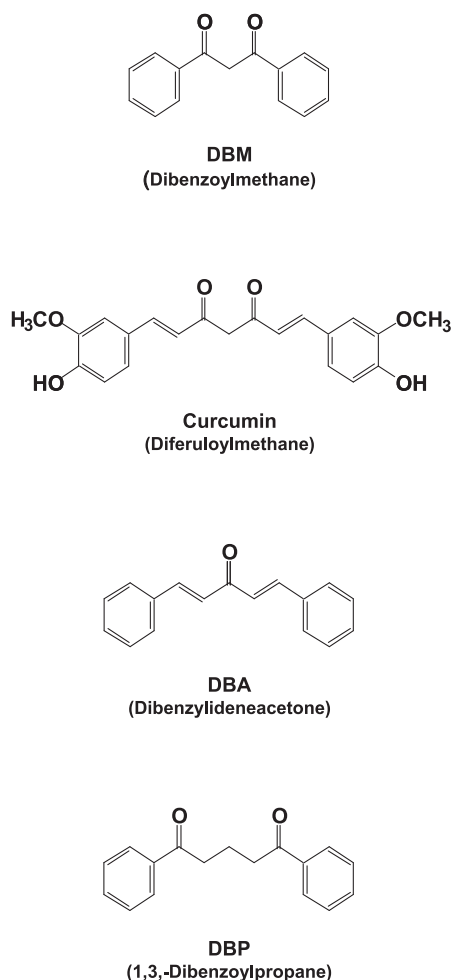
Fig. 1. The chemical structures of curcumin, dibenzoylmethane (DBM), dibenzylideneacetone (DBA) and 1,3-dibenzoylpropane (DBP).

is smaller in molecular size and lacks hydroxyl and methoxy groups. Whereas curcumin has Michael acceptor and Michael donor sites, DBM has a Michael donor, but no Michael acceptor, site. The closely related dibenzoylpropane (DBP) also is not a Michael acceptor, whereas dibenzylideneacetone (DBA) has one keto group and is a Michael acceptor. DBA has been shown to be more active than curcumin in inhibiting ubiquitin isopeptidase [18]. Curcumin is known to exhibit strong anti-oxidant and free radical-scavenging activities, whereas in these respects, DBM is inactive [10,19]. It is yet to be established whether the anti-oxidant activity of a compound has any correlation with its anti-inflammatory, anti-proliferative, or chemopreventive activity. In the present report, we investigated the potency of DBM, DBP, and DBA, in relation to curcumin, for their ability to suppress TNF-induced NF-κB activation, NF-κB-regulated gene products, and cell proliferation.

2. Materials and methods

2.1. Chemicals

Curcumin with purity greater than 95% was kindly supplied by Sabinsa Corporation (East Windsor, NJ); DBM, DBA, and DBP were obtained from Sigma–Aldrich (St. Louis, MO). Stock solutions (50 mM each) of curcumin, DBM, DBA, and DBP (Fig. 1) were prepared in DMSO and used in the studies. Bacteria-derived human recombinant TNF, purified to homogeneity with a specific activity

of 5×10^7 units/mg, was kindly provided by Genentech (South San Francisco, CA). Penicillin, streptomycin, RPMI 1640, Iscove's modified Dulbecco's medium, and Dulbecco's modified Eagle medium (DMEM) fetal bovine serum were obtained from Invitrogen (Grand Island, NY). Anti-β-actin was obtained from Sigma–Aldrich. Antibodies against cyclin D1 and cyclooxygenase (COX)-2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody against vascular endothelial growth factor (VEGF) was purchased from Thermo Fisher Scientific (Waltham, MA). Anti-IKK (IκBα kinase)-α and anti-IKK-β antibodies were kindly provided by Imgenex (San Diego, CA).

2.2. Cell lines

The human cell lines A293 (embryonic kidney carcinoma), Jurkat (acute T cell leukemia), DU145 (prostate carcinoma), and MDA-MB-231 (breast adenocarcinoma) were obtained from American Type Culture Collection (Manassas, VA). Human myeloid leukemia KBM-5 cells were kindly supplied by Dr. Nicholas Donato (University of Michigan Comprehensive Cancer Center, Ann Arbor, MI). Cells were cultured as follows: KBM-5 cells in Iscove's modified Dulbecco's medium with 15% fetal bovine serum; Jurkat and DU145 cells in RPMI 1640; A293 and MDA-MB-231 cells in DMEM supplemented with 10% fetal bovine serum. Culture media were supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin.

2.3. Electrophoretic mobility shift assay

To determine NF-κB activation, we used electrophoretic mobility shift assays (EMSAs), as described previously [20]. In brief, nuclear extracts prepared from TNF-treated cells were incubated with ^{32}P-end-labeled 45-mer double-stranded NF-κB oligonucleotide (15 μg of protein with 16 fmol of DNA) from the HIV long terminal repeat 5′-TTGTTACAAGGGACTTTCCGCTGGG-GACTTTCCAGGGAGGCGTGG-3′ (underline indicates NF-κB binding sites) for 30 min at 37 °C. The DNA–protein complex that formed was separated from the free oligonucleotide on 6.6% native polyacrylamide gels. The dried gels were visualized, and the radioactive bands were quantified with a Storm 220 Phosphor-Imager using ImageQuant software (GE Healthcare, Piscataway, NJ).

2.4. Western blot analysis

To determine the levels of protein expression in the cytoplasm and nucleus, we fractionated extracts using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as described previously [21]. The proteins were then electrotransferred to nitrocellulose membranes and blotted with each antibody, and the expression of proteins was detected with an enhanced chemiluminescence reagent (GE Healthcare). We quantified the bands with National Institutes of Health imaging software (Bethesda, MD).

2.5. IKK assay

To determine the effects of DBM on TNF-induced IKK activation, we used the IKK assay, as described previously [21]. In brief, to determine the total amounts of IKK-α and IKK-β in each sample, we resolved 30 μg of whole-cell protein by 7.5% SDS-PAGE, electrotransferred it to a nitrocellulose membrane, and blotted it with anti-IKK-α or anti-IKK-β antibodies.

2.6. NF-κB-dependent reporter gene expression assay

We performed an NF-κB-dependent reporter gene expression assay, as previously described [21]. The effects of curcumin and its

analogues on transforming growth factor (TGF)-β-activated kinase 1 (TAK1)-induced NF-κB-dependent reporter gene transcription were analyzed by the secretory alkaline phosphatase (SEAP) assay, as described previously [21].

3. Results

We determined the effects of DBM and its analogues on the NF-κB activation pathway induced by TNF. We also evaluated the effect of DBM on NF-κB-regulated gene expression and NF-κB-mediated cellular proliferation. Because TNF is one of the most potent proinflammatory cytokines and the most potent activator of NF-κB and because the TNF-induced NF-κB activation pathway has been well characterized, we investigated the effects of various curcumin analogues on TNF-induced NF-κB activation.

### 3.1. Curcumin analogues differentially inhibit NF-κB activation

Cells were exposed to different concentrations of curcumin and its analogues, and these cells were then examined for TNF-induced NF-κB activation. We found that none of the presently studied agents by themselves activated NF-κB (Fig. 2A–D). However, all agents inhibited TNF-induced NF-κB activation, although at dissimilar concentrations. Whereas curcumin completely inhibited TNF-induced NF-κB activation at a concentration of approximately 30 μM, DBM, DBA, and DBP required concentrations of 200, 40, and 100 μM, respectively, for complete inhibition.

Next, we determined the minimum time of exposure required to inhibit TNF-mediated NF-κB activation. The cells were exposed to curcumin and its analogues for various time-periods and then treated with TNF for 30 min. The results showed that an exposure
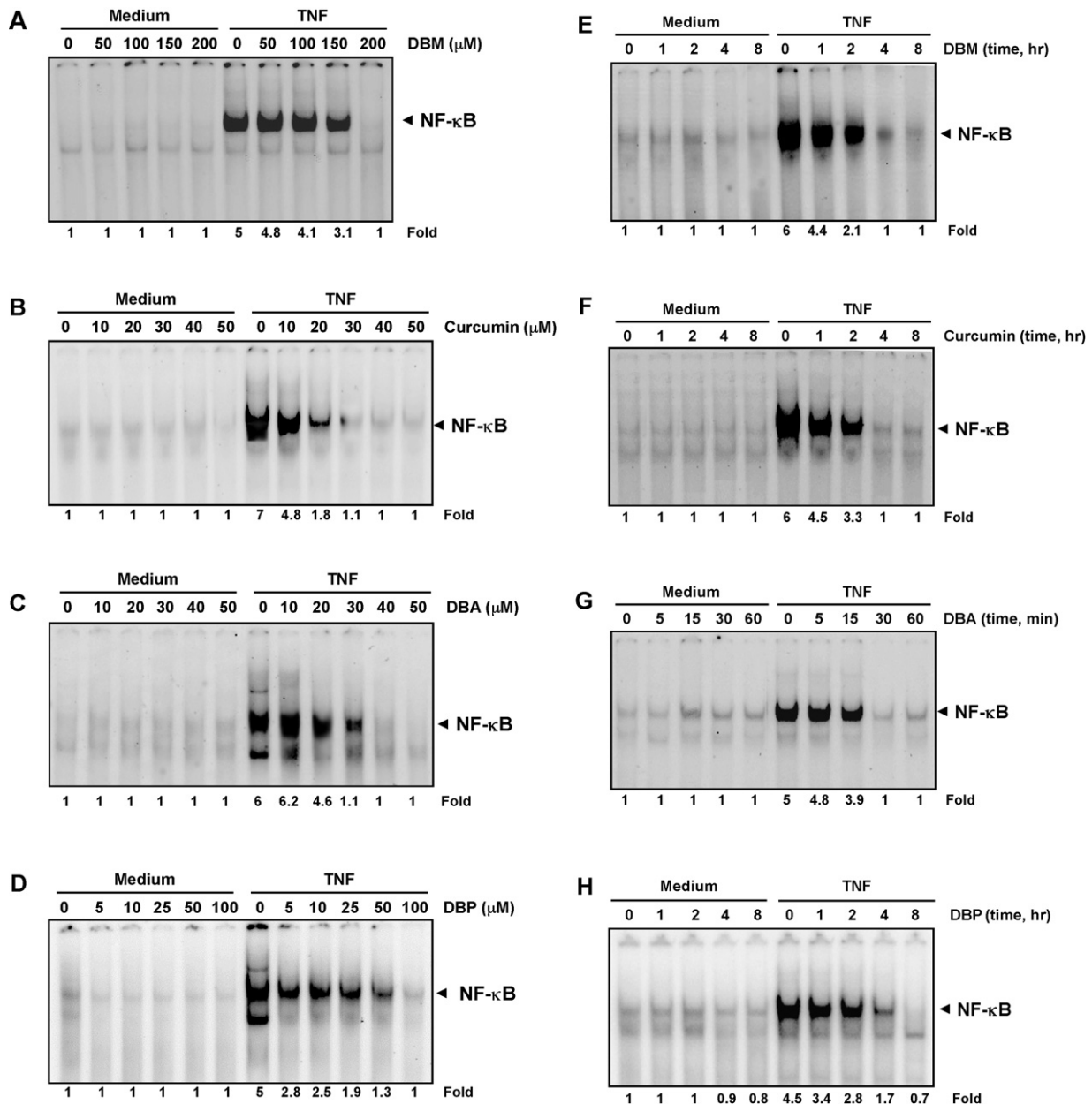
Fig. 2. Curcumin and its structural analogues block NF-κB activation induced by TNF-α. KBM-5 cells were preincubated with the indicated concentrations of DBM (A), curcumin (B), DBA (C), and DBP (D) for 4 h and then treated with 0.1 nM TNF-α for 30 min. The nuclear extracts were prepared and assayed for NF-κB activation by EMSA. Cell viability (C.V.) was determined by the trypan blue exclusion assay. (E–H) Time-dependent effect of curcumin, DBA, DBM, and DBP on TNF-α-induced NF-κB activation. KBM-5 cells were preincubated with DBM (panel E; 200 μM), curcumin (panel F; 50 μM), DBA (panel G; 50 μM) and DBP (panel H; 100 μM) for the indicated times and then treated with 0.1 nM TNF-α for 30 min. The nuclear extracts were prepared and assayed for NF-κB activation by EMSA.
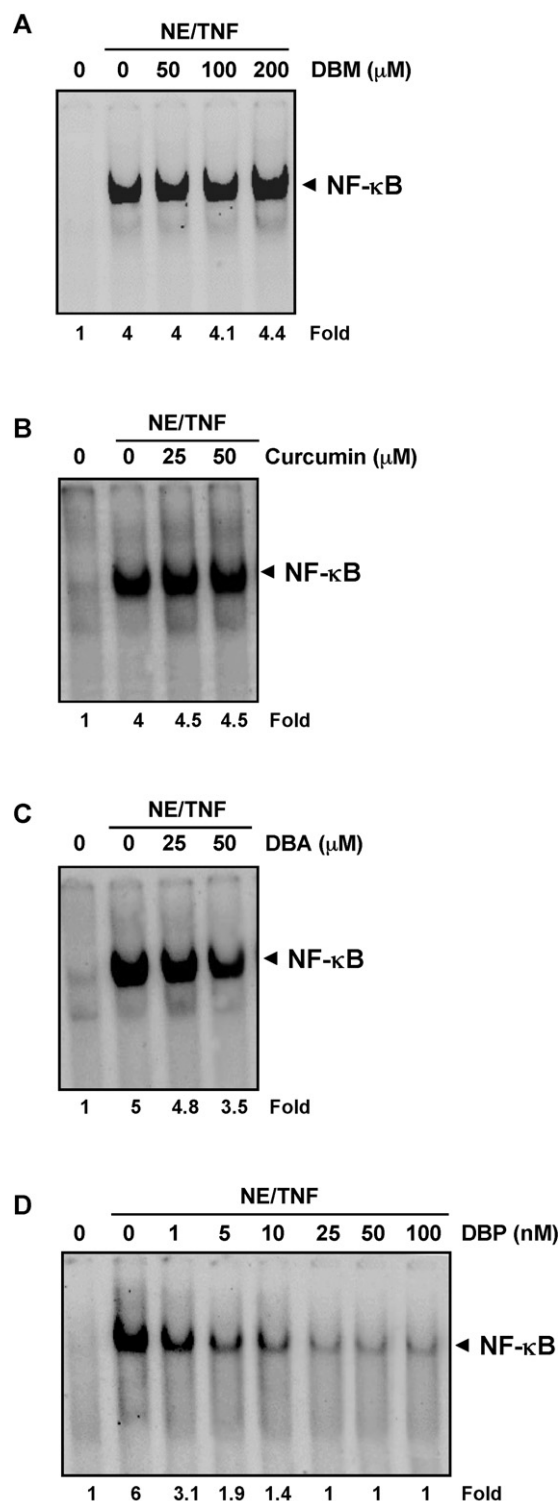
Fig. 3. The direct effect of curcumin and its analogues on NF-κB complex. Nuclear extracts were prepared from untreated cells or cells treated with 0.1 nM TNF-α and then incubated for 30 min with the indicated concentrations of DBM (A), curcumin (B), DBA (C), and DBP (D). They were then assayed for NF-κB activation by EMSA.

of cells to DBM or curcumin for 4 h was sufficient to inhibit TNF-induced NF-κB activation completely (Fig. 2E and F). DBP, however, required 8 h to fully suppress NF-κB activation (Fig. 2H). Interestingly, the kinetics of suppression of NF-κB activation by DBA, was found to be very rapid as complete suppression was observed within 30 min (Fig. 2G).

3.2. DBP, but not other curcumin analogues, inhibits directly the DNA binding of NF-κB (p65)

Activated NF-κB consists of p50 and p65 subunits, among which the p65 subunit has transcriptional activity. We next investigated whether curcumin and its analogues could directly bind to p65 and thereby inhibit NF-κB (p65) binding to DNA. We incubated TNF-activated nuclear extracts with different concentrations of curcumin analogues and then examined these for DNA binding. We found that DBM, curcumin, and DBA had no effect on DNA binding of NF-κB, but observed that DBP inhibited the DNA binding of p65 (Fig. 3).

3.3. Glutathione can reverse the suppression of NF-κB activation induced by curcumin but not the suppression induced by its analogues

Although Michael adduct formation involves a covalent bond formation between a Michael acceptor system and a Michael donor unit, this reaction under certain conditions is reversible. For example, compounds with Michael acceptor ability are known to react with sulfhydryl (–SH) groups present in proteins, and this covalent bond formation may sometimes be reversed by glutathione. We therefore next examined the ability of glutathione to reverse the inhibition of NF-κB activation. For this, cells were treated with curcumin or its analogues in the presence or absence of glutathione and then examined for TNF-induced NF-κB activation. As shown in Fig. 4, the inhibition of NF-κB activation by curcumin was reversible by GSH but the inhibition by DBM, DBA or DBP was not reversible.

3.4. Curcumin and its analogues can directly inhibit TNF-induced IKK activation

Because IKK is required for TNF-induced NF-κB activation, we investigated next whether curcumin analogues can directly inhibit the activity of IKK. In order to probe this aspect, IKK was activated by TNF, whole-cell extracts were prepared, and the IKK was immunoprecipitated and then incubated with various concentrations of curcumin analogues (Fig. 5). We found that curcumin and its analogues inhibited the IKK activity; however, with DBA (Fig. 5C), DBP (Fig. 5D), and curcumin (Fig. 5B), the inhibition occurred at a concentration of 5 μM, whereas in the case of DBM (Fig. 5A), a concentration of 50 μM was required for optimal inhibition of IKK activity.

3.5. Curcumin and its analogues can inhibit NF-κB–dependent reporter gene expression induced by TAK1

The activation of IKK is induced by TAK1 [22]. Because DNA binding is not always related to NF-κB-dependent gene transcription, we determined the effects of curcumin analogues on NF-κB reporter activity induced by TAK1. Cells were transiently transfected with the NF-κB-regulated SEAP reporter construct and TAK1 construct and then incubated with curcumin analogues. We found that all three analogues of curcumin suppressed the NF-κB reporter activity (Fig. 6).

3.6. Curcumin and its analogues can inhibit TNF-induced expression of inflammatory, proliferative, and angiogenic gene products

NF-κB activation has been shown to regulate the expression of COX-2, cyclin D1, and VEGF [23–25]. Whether curcumin analogues can modulate their expression was next examined, and the results indicated that DBM, curcumin, DBA, and DBP could down regulate the expression of COX-2, but at different doses (Fig. 6B–E). We also observed that DBM and DBP had only a marginal effect on the
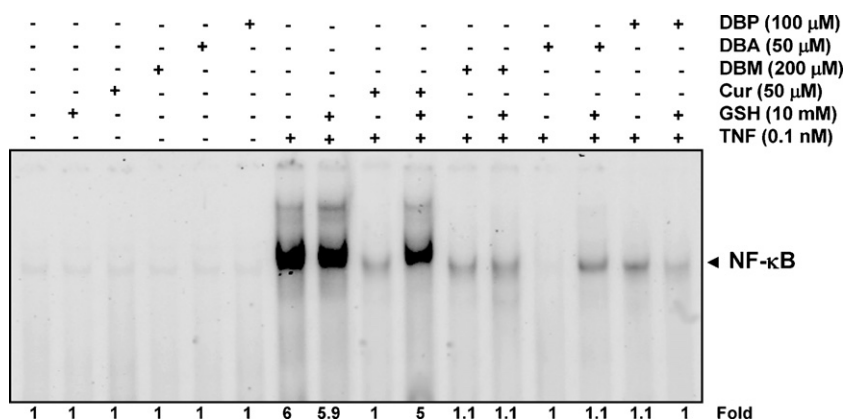
Fig. 4. Glutathione abrogates curcumin-mediated inhibition of NF-κB but has no effect on DBA-, DBM-, or DBP-mediated inhibition of NF-κB. KBM-5 cells were incubated with 10 mM GSH for 2 h and treated with 100 μM DBP, 50 μM DBA, 200 μM DBM and 50 μM curcumin for 4 h. Then the cells were activated with 0.1 nM TNF-α for 30 min and subjected to EMSA assay for NF-κB activation. Cell viability (C.V.) was determined by the trypan blue exclusion assay.
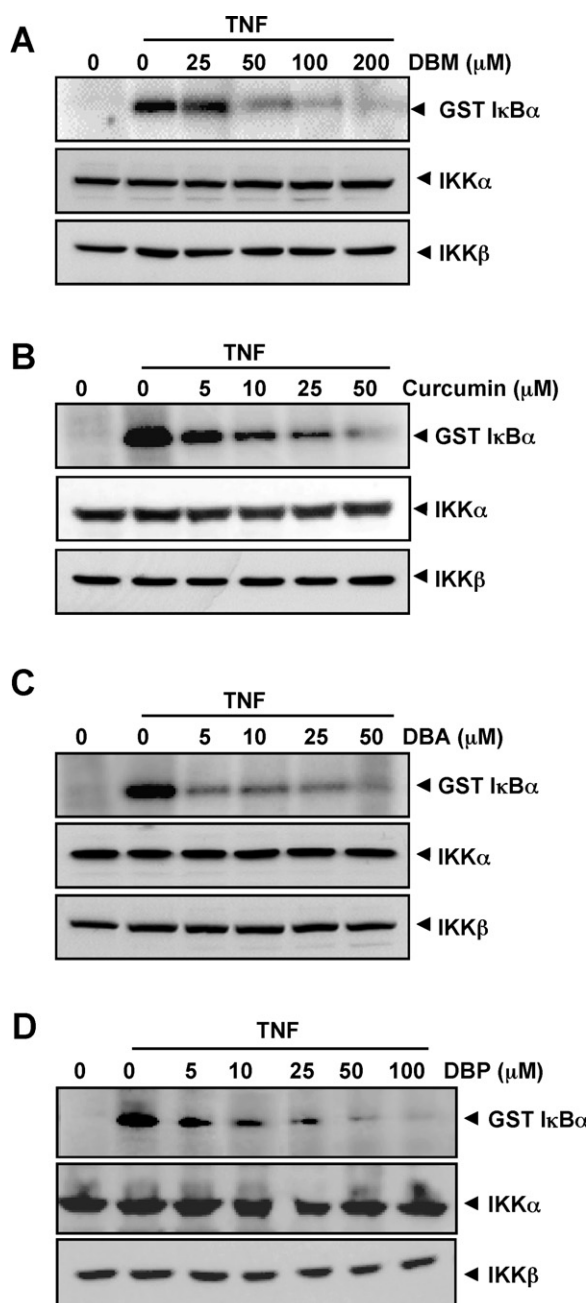
expression of cyclin D1, whereas curcumin and DBA down regulated cyclin D1 expression (Fig. 6B–E).

VEGF is the most potent angiogenic factor, and recent studies have found that its expression is regulated by NF-κB [25]. The downregulation of TNF-induced expression of VEGF was more pronounced with curcumin, DBA or DBP than with DBM (Fig. 6B–E).

### 3.7. Curcumin and its analogues can inhibit the proliferation of tumor cells

Because NF-κB and NF-κB-regulated cyclin D1 play a major role in cell proliferation, we investigated the effect of curcumin and its analogues on four different human cancer cell lines, KBM-5, Jurkat, MDA-MB-231 and DU145. Curcumin, DBA and DBM inhibited cell proliferation in a dose-dependent manner, but curcumin and DBA were more active than DBM in suppressing the growth of all four different tumor cell lines (Fig. 7A). DBP, that lacks Michael acceptor, was found to be least effective for suppression of tumor cell proliferation.

When examined at a given concentration (25 and 50 μM), the results showed that the abilities of curcumin and DBA on the suppression of tumor cell proliferation were similar, but DBM and DBP were much less effective (Fig. 7B). These results suggest that the suppression of NF-κB and of cell proliferation by the curcumin and its analogues, could be due to different groups in the molecule.

4. Discussion

Although a considerable body of information exists about the mechanism of action of curcumin, little is known about the bioactivity profile of the curcumin analogues DBM, DBA, and DBP. The present study reports for the first time that these analogues can modulate TNF-induced NF-κB activation, IKK activity, NF-κB-regulated gene products, and cellular proliferation. A fairly good structural similarity exists among curcumin, DBM, DBA, and DBP, as can be gleaned from Fig. 1, and these four compounds may be looked upon as α,ω-bisaryl alkanone derivatives having differential Michael acceptor and donor properties. Based on structural considerations, the four compounds now studied may be classified

Fig. 5. Curcumin and its analogues inhibit IKK activity directly. Whole-cell extracts were prepared from KBM-5 cells treated with 1 nmol/L TNF-α and immunoprecipitated with anti-IKK-β antibody. The immunocomplex kinase assay was performed in the absence or presence of the indicated concentrations of DBM (A), curcumin (B), DBA (C), and DBP (D). To examine the effect of curcumin and its analogues on the level of expression of IKK proteins, whole-cell extracts were fractionated on SDS-PAGE and examined by Western blot analysis using anti-IKK-α and anti-IKK-β antibodies.
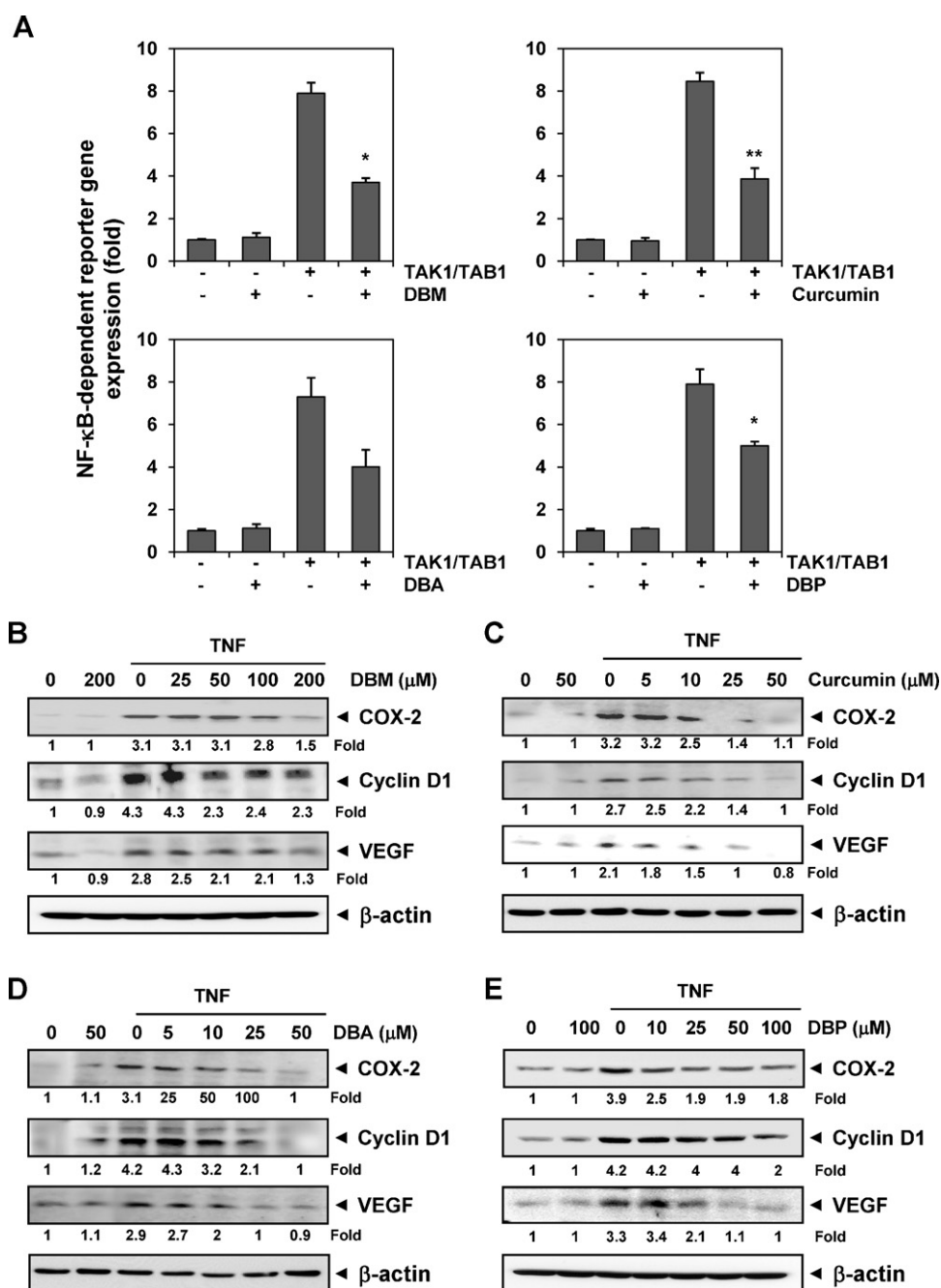
Fig. 6. (A) Curcumin, DBA, DBM and DBP inhibit TAK-1-induced NF-κB-reporter gene (SEAP) expression. A293 cells were transiently transfected with a NF-κB-containing plasmid linked to the SEAP gene or co-transfected with TAK1/TAB1 gene plasmid along with NF-κB-SEAP-containing plasmid, and then treated with DBM (200 μM), curcumin (50 μM), DBA (50 μM), or DBP (100 μM) for 4 h. Cell supernatants were collected and assayed for SEAP activity. Results are expressed as fold activity over the activity of the vector control. Columns, mean of three measurements; bars, SD. *P < 0.05; *P < 0.01 versus TAK1/TAB1 transfection. (B-E) Effect of curcumin, DBA, DBM, and DBP on NF-κB-dependent gene expression. KBM-5 cells were incubated with the indicated concentrations of each compound for 4 h and then treated with 1 nmol/L TNF-α for 20 h. Whole-cell extracts were prepared and subjected to western blot analysis using antibodies against COX-2, cyclin D1, and VEGF. The same blots were stripped and reprobed with β-actin antibody to verify equal protein loading.

depending on their Michael acceptor and donor properties. The reactivity of Michael acceptor–donor systems is shown in Fig. 8. The central methylene group, –CO–CH$_2$–CO–, of DBM is a good Michael donor due to its 1,3-diketone moiety, but the compound is not a Michael acceptor. DBP is a weak Michael donor, since it is a 1,5-diketone, as well as an unexceptional alkyl aryl ketone, and has no Michael acceptor property. DBA has no Michael donor group; however, it is a good Michael acceptor due to its two cross-conjugated to –CH=CH–CO– enone units. In contrast to these, curcumin is deemed to be a weak Michael donor since the

1,3-diketone unit in it is bis-α,β-conjugated, thereby decreasing the reactivity of its central methylene unit. It also is comparatively less active as a Michael acceptor since the electron releasing substituents in its phenyl ring on both sides are in conjugation with the enone unit.

We now report, for the first time, that DBM, a minor component of licorice and some sunscreens, can suppress NF-κB activation, although at a concentration six to seven times higher than that required for curcumin. Unlike DBM, curcumin contains two hydroxy groups, two methoxy groups, a pair of Michael acceptor
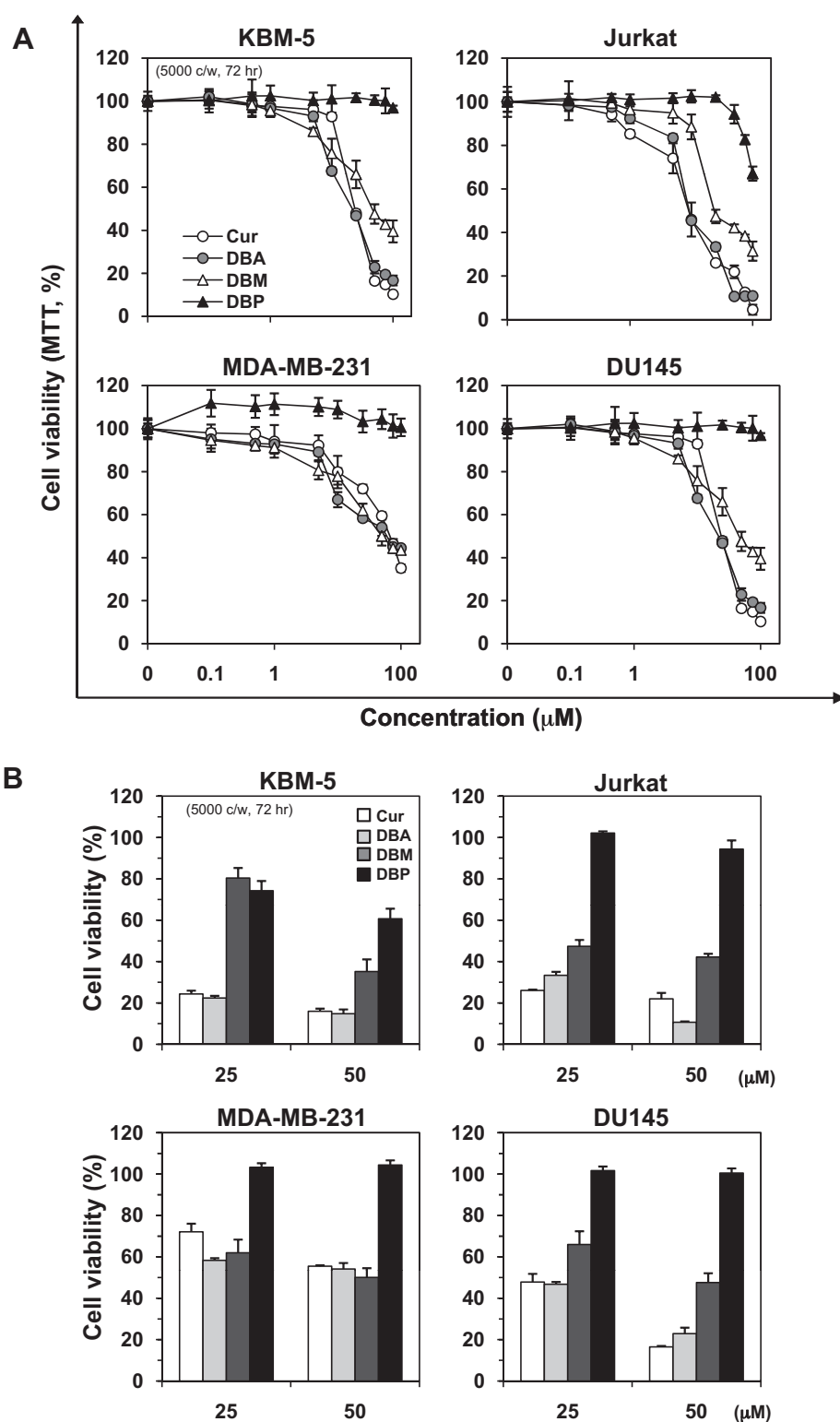
Fig. 7. Effect of curcumin, DBA, DBM, and DBP on cell proliferation. (A) Five thousand KBM-5, Jurkat, MDA-MB-231 and DU145 cells per well were seeded in triplicate onto 96-well plates and treated with each compound at 1, 5, 10, 25, 50, 100, or 200 μM for 72 h. Cell viability was then measured by the MTT method and presented as percent cell viability. (B) The percent change in cell viability at 25 and 50 μM for each compound is shown. The results are expressed as the mean ± standard error of 3 independent experiments.

moieties, and an active methylene Michael donor unit. Because curcumin and the three analogues here tested suppressed TNF-induced NF-κB activation, it appears that none of the above mentioned groups are by themselves essential for the suppression of NF-κB activation. It emerges that a minimal structural motif of two aromatic rings linked by a short alkanone chain of three to five

carbons would be sufficient to suppress TNF-induced NF-κB activation. This surmise is based on the observation that DBM was also active, although the least active, among the four compounds in suppressing TNF-induced NF-κB activation. The results further showed that DBA was almost as active as curcumin, thereby indicating that enone type Michael acceptor units present in
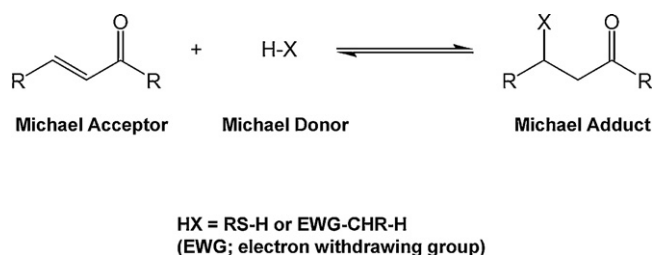
HX = RS-H or EWG-CHR-H
(EWG; electron withdrawing group)

Fig. 8. Michael acceptor donor chemistry. EWG; electron withdrawing group.

curcumin and DBA could augment the ability to suppress the NF-κB activity. Thus, it seems safe to presume that the presence of a α,ω-bisaryl alkanone would be a minimal structural parameter in the suppression of TNF-induced NF-κB activation and this would be facilitated further by the presence of an enone moiety. We also found that the kinetics of suppression of NF-κB activation by DBA was more rapid. Despite its lack of Michael donor ability, DBA is the most effective Michael acceptor among DBM, DBP, DBA and curcumin. This result also points to the facilitating effect of an enone type Michael acceptor unit in the suppression of the NF-κB activity.

Further, we now observe that the inhibition of NF-κB activation by curcumin was reversible by GSH, but not that by DBM, DBA or DBP. This implies that if at all a Michael pathway is involved; it is reversible in the case of curcumin. This may be explained by the fact that the substituents on the aryl moiety of curcumin are electron releasing, and hence the Michael activity of the enone system of curcumin will be different from that of DBA.

Our results showed that suppression of NF-κB activation by DBA was very rapid as compared to other analogues (Fig. 2G). Why DBA is fast-acting as compared to other analogues is not clear. Lack of Michael donor ability of DBA may contribute to its fast-acting nature. In addition, structural arguments suggest that, among DBM, DBP, DBA and curcumin; DBA would be a most effective Michael acceptor. These factors account for the difference.

Our investigation of the ability of curcumin, DBM, DBA, and DBP to bind directly to the NF-κB (p65) subunit, thus leading to the inhibition of DNA binding of NF-κB (p65), seemed to suggest that the modus operandi of DBP in inhibiting TNF-induced NF-κB activation differs from that of curcumin, DBM, and DBA. Curcumin, DBM and DBA did not affect the DNA binding of NF-κB p65, whereas DBP inhibited it. This suggests that DBP mediates its effects through a mechanism different from those of curcumin, DBM, and DBA. It therefore appears that the Michael reactivity, either as acceptor or donor, may be insignificant as a determinant for the inhibition of DNA binding of NF-κB (p65).

We found that DBP directly inhibited the DNA binding at nM range (Fig. 3D). But, DBP needed higher concentrations (100 μM) for the TNF-induced NF-κB activation by EMSA and it needed a long pre-incubation time (Fig. 2D and H). Direct inhibition of DNA binding of NF-κB by DBP at low concentration, indicates a probable reactivity of its ketone groups with cysteine residues of NF-κB in a thio-hemiketal formation reaction. Among DBM, DBP, DBA and curcumin, this type of nucleophilic carbonyl addition reactivity would be highest for DBP, since the others have carbonyl groups that are either tautomerisable to enolic form and/or are α,β-unsaturated. Also, the position of the carbonyl groups in DBP is different from that of DBA and curcumin. However, the ability of DBP to suppress cellular NF-κB was weak indicating perhaps poor cellular uptake.

The effects of curcumin, DBM, DBA, and DBP on IKK activity showed that all four can inhibit IKK activity; with DBM, with its C3 alkanone linker unit, being the least effective among the four. This result appeared to indicate that a minimal structural motif of two aromatic rings linked by a short alkanone chain of five carbons

would be sufficient to express inhibition of IKK activity. The results of our experiments examining the effects of curcumin and these three analogues on NF-κB reporter activity induced by TAK1 also could be explained on the basis of the hypothesis that a structural scaffold of a short alkanone chain of three to five carbons linking two aryl rings would be sufficient to affect the NF-κB reporter activity induced by TAK1. The results of the experiments designed to address the ability of curcumin, DBM, DBA and DBP on the expression of COX-2, cyclin D1, and VEGF indicated that these could downregulate the expression of COX-2 but with varying efficacy. Furthermore, we also observed that in comparison with curcumin and DBA, DBM (with its shorter alkanone chain), and DBP showed a much smaller effect on the expression of cyclin D1 expression. Although potent inhibitors of NF-κB, both DBP and DBM were weak inhibitor of cyclin D1 expression, thus suggesting the role of other transcription factors. DBM, however, has stronger cytotoxic effects than DBA, which highlights the role of 1,3-diketones and their position. A similar conclusion could be arrived at based on the results of the inhibition studies on the cancer cells. In conclusion, we now propose that a α,ω-bisaryl alkanone unit would be a minimal structural requirement for the suppression of inflammation and cell proliferation and this would be facilitated further by the presence of a Michael acceptor enone moiety.

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