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Curcuma longa inhibits TNF-α induced expression of adhesion molecules on human umbilical vein endothelial cells ☆

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Abstract

Identification of non-steroidal anti-inflammatory small molecules is very important for the development of anti-inflammatory drugs. We demonstrate here that out of three compounds, viz diferuloylmethane, *p*-coumaroylferuloylmethane and di-*p*-coumaroylmethane, present in the ethyl acetate extract of *Curcuma longa*, diferuloylmethane is most potent in inhibiting TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin on human umbilical vein endothelial cells. The inhibition by diferuloylmethane is time dependent and is reversible. By using RT-PCR, we demonstrate that it inhibits the induction of steady state transcript levels of ICAM-1, VCAM-1 and E-selectin, and therefore it may interfere with the transcription of their genes. As diferuloylmethane significantly blocks the cytokine induced transcript levels for the leukocyte adhesion molecules, it may be interfering at an early stage of signalling event induced by TNF-α. © 1999 International Society for Immunopharmacology. Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Curcuma longa*; Cell adhesion molecules; Endothelial cells; TNF-α; ICAM-1; VCAM-1; E-selectin

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Abbreviations: IL-1, interleukin-1; ELISA, enzyme linked immunosorbent assay; FCS, foetal calf serum; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; TNF-α, tumor necrosis factor-α; FITC, fluorescein isothiocynate; FACS, fluorescence-activated cell sorter; PCR, polymerase chain reaction; PBS, phosphate buffer saline; TLC, thin layer chromatography.

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1. Introduction

Extracts prepared from *Curcuma longa* have been used for centuries for the treatment of various inflammatory and other diseases [1]. *C. longa* has been shown to have anti-inflammatory activity as tested in carrageenan induced paw edema and Freund's adjuvant induced arthritis model [2,3]. The induction of various cell adhesion proteins such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on the endothelial cells is directly involved in inflammation (reviewed by Springer [4]). These cell adhesion proteins are not normally present on the endothelial cell surface but are induced by various pro-inflammatory cytokines such as IL-1 and TNF-α [5,6].

As the upregulation of these proteins on endothelial cells has been shown to be associated with various inflammatory diseases (reviewed by Gorski [7]), various strategies have been tried to down-regulate the expression of these molecules [8]. Also it has been shown that ICAM-1 deficient mice are protected against ischemic renal injury [9]. Various small molecules, such as glucocorticoids, aspirin, pentoxifylline, etc. have also been shown to block the expression of cell adhesion molecules and have been found to be effective in controlling various inflammatory diseases [10–12].

Since *C. longa* has anti-inflammatory activity and expression of adhesion molecules plays an important role in inflammation, we sought to identify and characterise the active principles in the ethyl acetate extract of ground-dried rhizome of *C. longa*. We report here that diferuloylmethane is the most active component present in the ethyl acetate extract of *C. longa* for inhibiting TNF-α induced ICAM-1, VCAM-1 and E-selectin expression on the human umbilical vein endothelial cells (HUVECs). We also report that the inhibition by diferuloylmethane is time dependent and is reversible, and it probably interferes with the induction of transcription by TNF-α.

2. Experimental procedures

2.1. Materials

The human umbilical vein endothelial cell line CRL 1730 was obtained from American Type Culture Collection (Rockville, MD). TNF-α, anti-ICAM-1 (BBA3), anti-VCAM-1 (BBA6) and anti-E-selectin (BBA1) antibodies were purchased from R and D Systems, California. M199, l-glutamine, penicillin, streptomycin, amphotericin, endothelial cell growth factor, trypsin, Pucks saline, HEPES, *o*-phenylenediamine dihydrochloride, anti-mouse IgG-HRP, anti-mouse IgG-FITC were purchased from Sigma Chemical Co., USA. ICAM-1, VCAM-1 and E-selectin primer sets were synthesized by Genset Corp., Japan and β-actin primer set was purchased from Stratagene, USA. Fetal calf serum was purchased from Biological industries, Israel. TLC plates were purchased from E. Merk, Germany.

2.2. Cells and cell culture

Primary endothelial cells were isolated from human umbilical cord as described before [13].

Endothelial cells were cultured in M 199 medium supplemented with 20% heat inactivated fetal calf serum, 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin and endothelial cell growth factor (50 μg/ml) supplemented with heparin (5 U/ml) in gelatin coated tissue culture flasks. For subculturing, the cells were dislodged using 0.125% trypsin–0.01 M EDTA solution in Pucks saline and HEPES buffer. Each donor yielded about 10 million cells after three passages. The cells thus obtained were used as primary cells in our experiments. The viability of endothelial cells in culture was checked by trypan blue exclusion test and the purity was determined by E-selectin staining. Endothelial cell line CRL 1730 was used for flow cytometry and some preliminary ELISAs.

2.2.1. Preparation of the extract and fractionation by thin layer chromatography

Dried powdered rhizomes of *C. longa*, 10 g, were macerated in 100 ml of 50% ethanol overnight at room temperature with shaking. The percolate was collected and vacuum dried. The dried material was then partitioned with ethyl acetate and water (1:1) four times. The ethyl acetate fraction was then dried under vacuum and resuspended in 1.0 ml DMSO. The ethyl acetate portion was fractionated using chloroform and methanol (47:1) using thin layer chromatography. Three major components (F1, F2 and F3) were purified from this fraction on silica gel and eluted with chloroform and methanol.

2.3. Modified ELISA for measurement of ICAM-1 and E-selectin

The cell surface expression of ICAM-1 and E-selectin on endothelial monolayers was quantified using cell-ELISA by modification of previously published methods [14]. HUVECs were grown to confluency in 96 well, flat bottom, gelatin-coated plates. The cells were incubated without or with the extract of *C. longa* or diferuloylmethane (solubilized in DMSO, final concentration 0.4%) for desired time periods, followed by induction with TNF-α (10 ηg/ml) for 16 h for ICAM-1 and for 4 h for E-selectin expression. Following incubation, the cells were washed with phosphate buffer saline pH 7.4 (PBS) and fixed with 1.0% glutaraldehyde for 30 min at 4°C. Non-fat dry milk (3.0% in PBS) was added to the monolayers to reduce non-specific binding. Cells were incubated with anti-ICAM-1, anti-E-selectin monoclonal antibody or isotype matched control antibody (0.25 μg/ml, diluted in blocking buffer) overnight at 4°C, washed with PBS followed by incubation with peroxidase-conjugated goat anti-mouse secondary antibody (1 μg/ml, diluted in PBS). Following this, the cells were washed with PBS and exposed to the peroxidase substrate (*o*-phenylenediamine dihydrochloride 40 mg/100 ml in citrate phosphate buffer, pH 4.5). Absorbance was determined at 492 nm by an automated microplate reader (Anthos Labtech HT2, Austria). The absorbance values of isotype matched control antibody were taken as blank and subtracted from the experimental values.

2.4. Flow cytometry

The expression of ICAM-1 on endothelial cells was evaluated by flow cytometry as previously described [15]. Briefly, the endothelial cells were incubated without or with ethyl

acetate extract of *C. longa* for 1 h followed by induction with TNF-α (10 ηg/ml) for 16 h. After induction the cells were washed with PBS twice and then dislodged using mild trypsinization (0.01% trypsin and 4 mM EDTA). After washing twice with PBS the cells were incubated with anti-ICAM-1 or relevant control antibody (1.0 μg/10^6 cells) for 30 min at 4°C. To remove the unbound antibody the cells were washed twice with PBS and then incubated with FITC-conjugated goat anti-mouse IgG (1:100 diluted) for 30 min at 4°C. The cells were fixed with 0.1% paraformaldehyde and were analysed by using a flow cytometer (Becton and Dickinson, USA). For each analysis, 20,000 events were collected and histograms were generated.

2.5. Total RNA isolation and reverse transcription polymerase chain reaction

RNA was isolated according to a modified guanidium thiocyanate procedure [16]. The expression of the transcripts for ICAM-1 (upper primer: 5′-CAGTGACCATCTACA GCTTTCCGG-3′; and lower primer: 5′-GCTGCTACCACAGTGATGATGACAA-3′), VCAM-1 (upper primer: 5′-ACCCTCCCAAGGCACACACAG; and lower primer: 5′-GTAAGTCTATCTCCAGCCTGTC-3′) and E-selectin (upper primer: 5′-GATGTGG GCATGTGGAATGATG; and lower primer: 5′-AGGTACACTGAAGGCTCTGG-3′) was evaluated by RT-PCR. The primers were synthesised according to the published cDNA sequences to yield products of length 555, 533 and 479 bp, respectively [17–19]. As a control, β-actin mRNA was also amplified by RT-PCR (upper primer: 5′-TGACGGGG TCACCCACACTGTGCCCATCTA-3′; lower primer: 5′-CTAGAAGCATTT GCGGGGACGATGGAGGG-3′) and a product of 661 bp was obtained [20]. The RT-PCR was performed as a single step reaction in a reaction mixture containing 0.2 mM dNTPs, 1 mM $MgSO_4$, 1 μM upstream and downstream primer each, 0.1 unit/μl AMV reverse transcriptase and 0.1 unit/μl T*fl* polymerase following the manufacturer's protocol (Access RT-PCR system, Promega, Madison). Briefly, 100 ng of the total RNA was used for reverse transcription using AMV reverse transcriptase at 48°C for 45 min followed by amplification using T*fl* polymerase for 25 cycles. The conditions for PCR were as follows: denaturation at 92°C for 1 min, primer annealing at 52°C for 90 s, extension at 72°C for 2 min and a final extension at 72°C for 10 min. Of the PCR products, 8 μl were analysed in 1% agarose gel and visualised by ethidium bromide staining. The densitometric measurements of the bands were done using Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (KDS-EDAS, Kodak, Rochester, NY, USA) and the ratio of the intensities of individual cell adhesion molecule (CAMs) bands (viz ICAM-1, VCAM-1 and E-selectin) to their corresponding β-actin bands were calculated. The percentage inhibition in the presence of diferuloylmethane was calculated using the following formula

$$\frac{(\text{CAMs}/\beta\text{-actin of TNF-}\alpha\text{ induced cells} - \text{CAMs}/\beta\text{-actin of uninduced cells}) - (\text{CAMs}/\beta\text{-actin of TNF-}\alpha\text{ and diferuloylmethane treated cells})}{(\text{CAMs}/\beta\text{-actin of TNF-}\alpha\text{ induced cells} - \text{CAMs}/\beta\text{-actin of uninduced cells})} \times 100$$

3. Results

3.1. Ethyl acetate extract of C. longa inhibits TNF-α induced ICAM-1 expression on endothelial cells in a dose dependent manner

To examine the effect of ethyl acetate extract of *C. longa*, HUVECs were incubated without or with the extract at various dilutions for 1 h prior to induction with TNF-α (10 ηg/ml) for 16 h. The time of incubation and concentration of the extracts used in these experiments had no effect on the viability as determined by trypan blue staining and morphology of the endothelial cells (data not shown). As detected by ELISA, ICAM-1 was expressed at low levels on unstimulated endothelial cells and was induced about 5-fold by TNF-α stimulation (Fig. 1A). The extract had no effect on the basal level of ICAM-1 expression, whereas it led to a reduction in the TNF-α induced ICAM-1 expression in a dose dependent manner. This was further confirmed by measuring the expression of ICAM-1 by flow cytometry (Fig. 1B).

3.1.1. Fractionation of ethyl acetate fraction of C. longa

To identify the active component(s) responsible for inhibiting the TNF-α induced ICAM-1 expression, we further fractionated the ethyl acetate fraction of *C. longa* by using TLC as mentioned in the experimental procedures (Fig 2A). Three fractions F1, F2 and F3 were isolated and analysed for their effect on TNF-α induced ICAM-1 expression. It was observed that fraction F1 is the most potent component for inhibiting the TNF-α induced ICAM-1 expression. The other fractions, F2 and F3, are only partially able to inhibit the ICAM-1 expression (Table 1).

3.2. Fraction 1 of ethyl acetate extract is diferuloylmethane

The fractionation of alcoholic extracts of *C. longa* yields three major components, viz diferuloylmethane, *p*-coumaroylferuloylmethane and di-*p*-coumaroylmethane [21]. To characterise F1, we compared the fastest moving component of the ethyl acetate fraction, F1, with the commercially available pure diferuloylmethane by thin layer chromatography (TLC). The mobility of F1 was found to be identical with the mobility of diferuloylmethane (Fig. 2B, compare lanes a and b). In contrast the mobility of F2 and F3 was not identical to that of diferuloylmethane (compare lane a with c and d). Next, we tested the effect of pure diferuloylmethane in inhibiting TNF-α induced ICAM-1 expression. Diferuloylmethane inhibits TNF-α induced ICAM-1 expression in a dose dependent manner from a concentration ranging from 10 to 40 μM (Fig. 3). The maximal inhibition was observed at a concentration of 40 μM.

3.3. Diferuloylmethane also inhibits TNF-α induced VCAM-1 and E-selectin expression

In addition to ICAM-1, TNF-α also induces VCAM-1 and E-selectin expression in inflammation. To examine the effect of diferuloylmethane on TNF-α induced expression of E-selectin and VCAM-1, the endothelial cells were incubated with varying concentrations of diferuloylmethane for 1 h prior to induction with TNF-α (10 ηg/ml) for 16 h for the expression of VCAM-1 and for 4 h for E-selectin. As measured by ELISA, the unstimulated

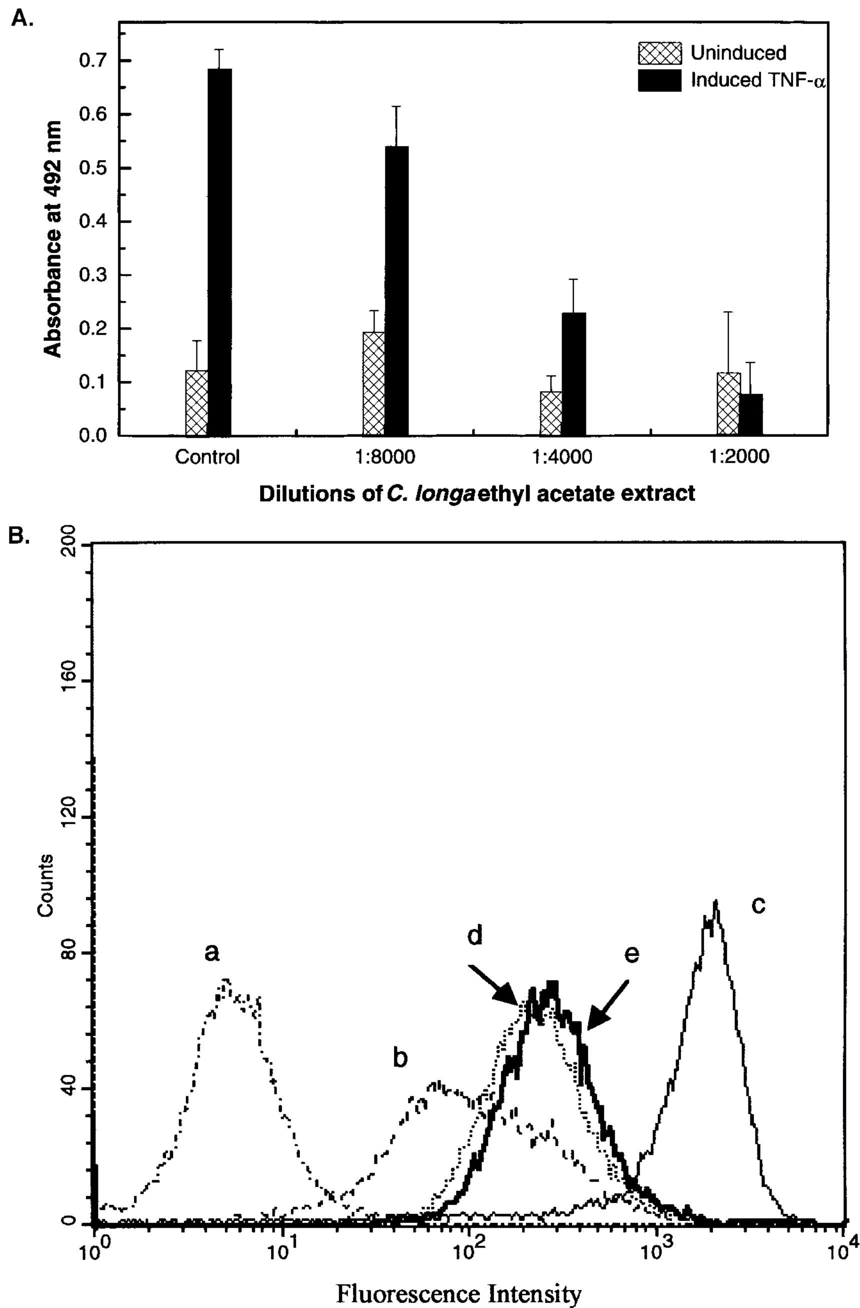

Fig. 1. Dose dependent inhibition of TNF-α induced ICAM-1 expression by *C. longa* ethyl acetate extract: (A) Endothelial cells grown to confluency in 96 well plates were incubated without or with indicated concentrations of extract for 1 h prior to induction without (hatched bars) or with TNF-α (closed bars) for 16 h. Following this, ICAM-1 level on the cells was measured by ELISA as described in Section 2. The data are representative of three independent experiments. Values shown are mean ± SD of quadruplicate wells. (B) Cells were treated as in (A) and the expression of ICAM-1 was measured by flow cytometry as described in Section 2. The data are expressed in histograms representing cell number (y-axis) vs fluorescence intensity (x-axis) plotted on a log scale. Autofluorescence (a), unstimulated (b), stimulated by TNF-α (c), *C. longa* extract alone (d), *C. longa* extract and TNF-α (e).

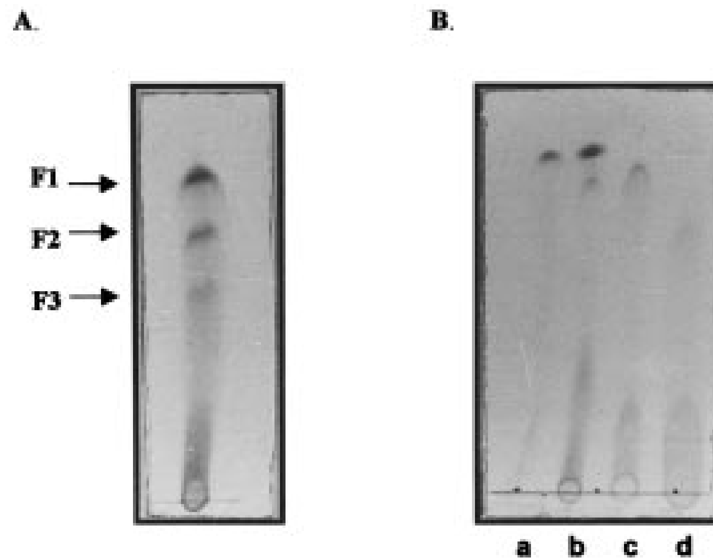

Fig. 2. Fractionation of ethyl acetate extract by TLC: (A) Ethyl acetate extract of *C. longa* was fractionated on silica gel using thin layer chromatography using chloroform:methanol (47:1) and three major fractions were obtained F1, F2 and F3. (B) Fractions F1, F2, and F3 isolated from the ethyl acetate extract and diferuloylmethane were run on silica coated TLC plate and their mobility compared: (a) diferuloylmethane; (b) fraction 1; (c) fraction

Table 1
Comparison of inhibitory effects of F1, F2 and F3: endothelial cells were incubated for equal amounts of fractions F1, F2 and F3 for 1 h prior to induction with TNF-α for 16 h. Following this the expression of ICAM-1 was measured by ELISA as described in Section 2

Fraction no.	Percentage inhibition of ICAM-1 expression
F1	63
F2	12.7
F3	36.15

cells expressed undetectable amounts of E-selectin (Fig. 4). Upon induction with TNF-α, a significant increase in the expression of E-selectin was observed. Pretreatment with diferuloylmethane blocked the TNF-α induced E-selectin expression (Fig 4). Similarly, TNF-α induced VCAM-1 expression was also inhibited by diferuloylmethane in a dose dependent manner (data not shown). Taken together, these results suggest that diferuloylmethane is effective in blocking the induced level of expression of ICAM-1, VCAM-1 and E-selectin.

3.4. Diferuloylmethane inhibits TNF-α induced ICAM-1 expression in a time dependent manner

To determine the time dependency of inhibition, endothelial cells were incubated with

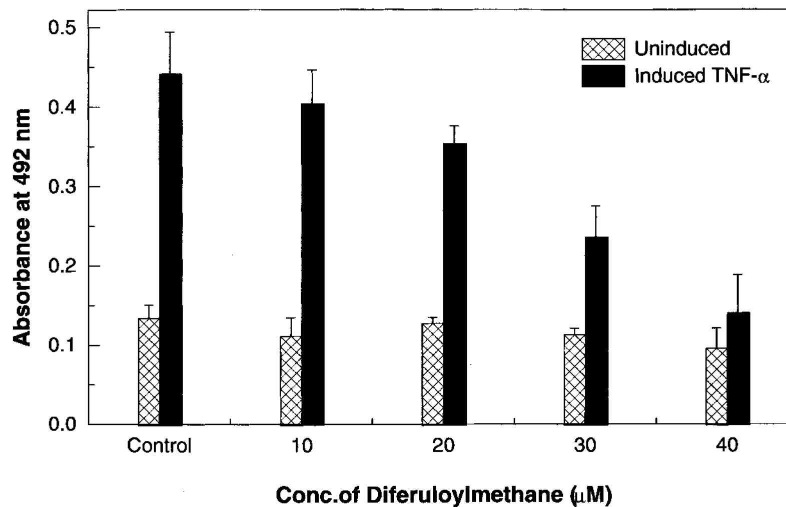

Fig. 3. Dose dependent inhibition of TNF-α induced ICAM-1 expression by diferuloylmethane. Endothelial cells grown to confluency in 96 well plates were incubated without or with indicated concentrations of diferuloylmethane for 1 h prior to induction without (hatched bars) or with TNF-α (closed bars) for 16 h. Following this, ICAM-1 level on the cells was measured by ELISA as described in Section 2. The data are representative of three independent experiments. Values shown are mean ± SD of quadruplicate wells.

diferuloylmethane (40 μM) at the indicated times before addition of TNF-α, simultaneously with TNF-α or at the indicated times after addition of TNF-α (Fig. 5). Diferuloylmethane

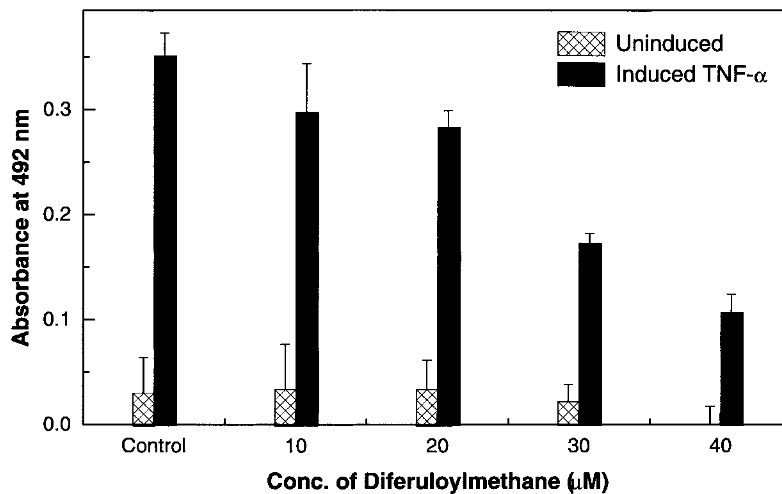

Fig. 4. Dose dependent inhibition of TNF-α induced E-selectin expression by diferuloylmethane. Endothelial cells grown to confluency in 96 well plates were incubated without or with indicated concentrations of diferuloylmethane for 1 h prior to induction without (hatched bars) or with TNF-α (closed bars) for 4 h. Following this, E-selectin level on cells was measured by ELISA as described in Section 2. Values shown are mean ± SD of quadruplicate wells.

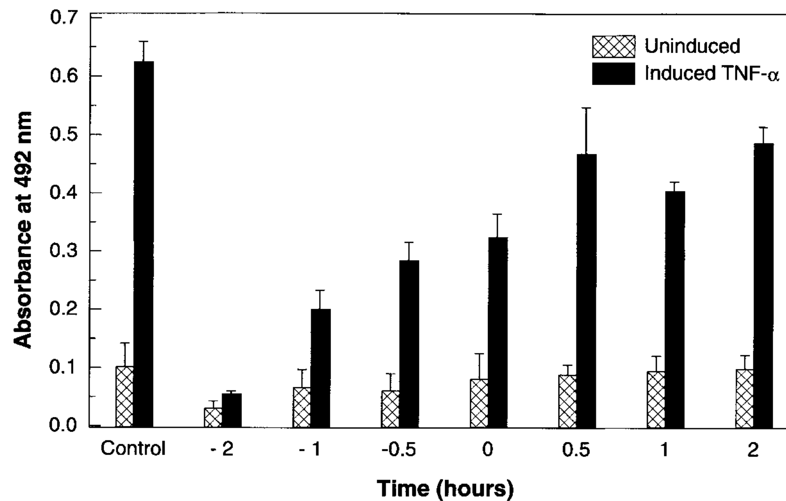

Fig. 5. Time dependent Inhibition of TNF-α induced ICAM-1 expression. Endothelial cells grown to confluency were incubated without or with 40 μM diferuloylmethane at indicated time, followed by induction without (hatched bars) or with (closed bars) TNF-α (10 ηg/ml) for 16 h. Following this, ICAM-1 level on the cells was measured by ELISA as described in Section 2. The data are representative of two independent experiments. Values shown are mean ± SD of quadruplicate wells.

inhibited ICAM-1 cell surface expression when added 1 h prior to induction with TNF-α (Fig. 5). Treatment for 2 h with diferuloylmethane was found to inhibit further the induced level of ICAM-1 expression. However, it also inhibited the basal level of ICAM-1 expression. When diferuloylmethane was added simultaneously or 30 min to 2 h after induction, the inhibition of ICAM-1 expression was not significant (Fig. 5). These results indicate that diferuloylmethane interferes with early signalling events in response to TNF-α.

3.5. Inhibition by diferuloylmethane is reversible

To determine if the effect of diferuloylmethane is reversible, the cells were pre-incubated with diferuloylmethane (40 μM) for 1–4 h, washed, and allowed to recover for 1 h before adding TNF-α for 16 h (Fig. 6). Effect of diferuloylmethane was found to be reversible over a period of time and even after 4 h no permanent change was observed as the cells were fully capable of responding to TNF-α. In control experiments, however, where curcumin was not removed from the media, inhibition of ICAM-1 was observed (Fig. 6).

3.6. Transcript levels of ICAM-1, VCAM-1 and E-selectin are decreased significantly by diferuloylmethane

As the activation of ICAM-1, VCAM-1 and E-selectin gene expression occurs at the level of transcription, we examined whether diferuloylmethane blocks the induction of the steady-state levels of transcripts by RT-PCR. Endothelial cells were incubated without or with 40 μM diferuloylmethane for 1 h prior to induction with TNF-α. As revealed by RT-PCR analysis

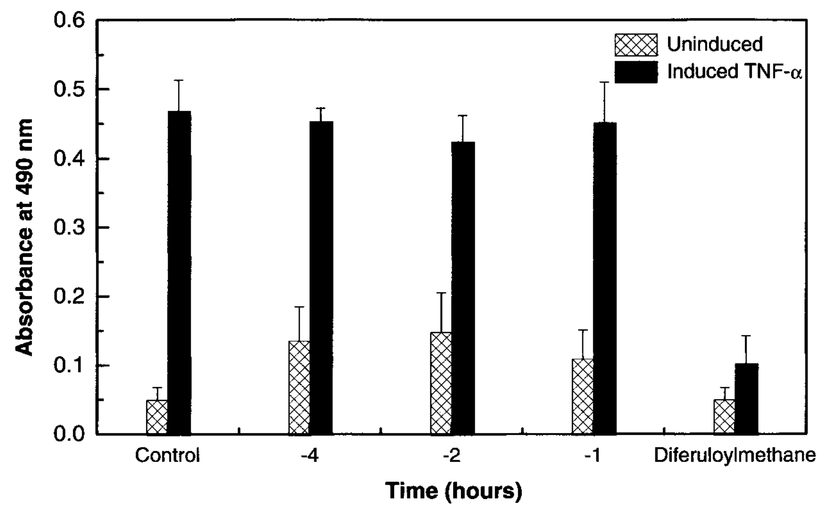

Fig. 6. Inhibition by diferuloylmethane is reversible. Endothelial cells were preincubated with 40 μM diferuloylmethane for various time periods (1–4 h), washed or not washed and incubated in complete medium for 1 h. ICAM-1 expression was measured as described in Section 2. The data are representative of two independent experiments. Values shown are mean ± SD of quadruplicate wells.

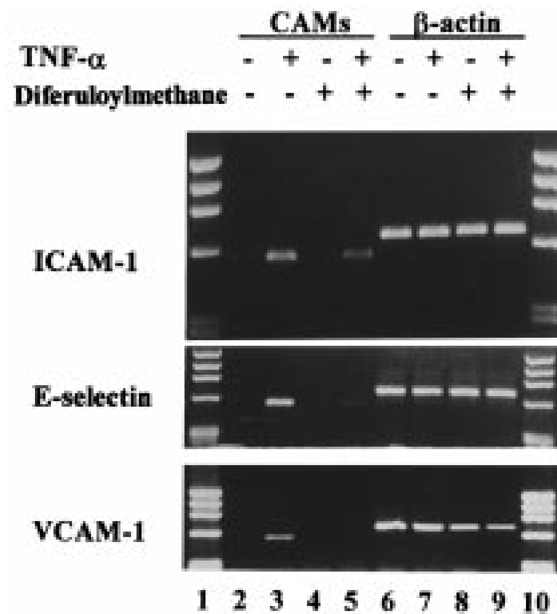

Fig. 7. Analysis of ICAM-1, VCAM-1 and E-selectin transcript levels in diferuloylmethane treated cells by RT-PCR. Endothelial cells were incubated without or with 40 μM of diferuloylmethane for 1 h prior to induction with TNF-α (10 ηg/ml) for 4 h. After this the total RNA of the cells was isolated and analysed by RT-PCR as described in Section 2. Lanes 1 and 10, marker ϕX174 Hae III digest; lane 2, unstimulated cells; lane 4, diferuloylmethane alone; lane 3, stimulated with TNF-α; lane 5, stimulated with TNF-α after diferuloylmethane pre-treatment for 1 h. Corresponding samples were analysed for β-actin mRNA as controls (lanes 6–9).

there was an increase in ICAM-1 mRNA following stimulation with TNF-α (Fig. 7, lane 3) as compared to low levels in control cells or cells treated with diferuloylmethane alone (Fig. 7, lanes 2 and 4). E-selectin and VCAM-1 transcript levels were undetectable in unstimulated cells (lane 2), while their expression was upregulated in TNF-α induced cells (lanes 3). However, treatment with diferuloylmethane for 1 h prior to addition of TNF-α reduced the transcript levels of ICAM-1 by 78%, VCAM-1 by 90% and E-selectin by 100% (lane 5). In contrast, the levels of β-actin mRNA expressed under these conditions remained the same (lanes 6–9). These results indicate that diferuloylmethane may affect the transcription of ICAM-1, VCAM-1 and E-selectin genes.

4. Discussion

Although *C. longa* is found to have potent antioxidant and anti-inflammatory properties, very little is known in regard to its effect on the induction of cell adhesion molecules by TNF-α. In our study, ethyl acetate fraction of *C. longa* blocked TNF-α-induced expression of leukocyte adhesion molecules, ICAM-1, VCAM-1 and E-selectin. Further fractionation of the ethyl acetate fraction by TLC produced three main fractions (Fig. 2). Earlier fractionation of the alcoholic extract has also produced similar three main components, viz diferuloylmethane, *p*-coumaroylferuloylmethane and di-*p*-coumaroylmethane [22,23]. A comparison of their inhibitory activities indicated that diferuloylmethane is most active in inhibiting TNF-α-induced expression of leukocyte adhesion molecules, ICAM-1, VCAM-1 and E-selectin (Table 1, Figs. 3 and 4). These results are in good agreement with earlier studies where it has been shown that curcumin (diferuloylmethane) is the active principle of many activities found in *C. longa* [23–25]. The inhibition by diferuloylmethane at 40 μM is specific as the expression of MHC class I protein (data not shown) or the expression of actin mRNA (Fig. 7) in presence of 40 μM of diferuloylmethane is unaffected. We also observed that the inhibition by diferuloylmethane is time dependent as it should be added prior to or simultaneously with TNF-α to be effective (Fig. 5).

As diferuloylmethane significantly blocked the cytokine induced transcript levels for the leukocyte adhesion molecules (Fig. 7) it may be interfering at an early stage of signalling event induced by TNF-α or LPS. This suggests that diferuloylmethane may block the expression of the cell adhesion molecules by interfering with the transcription of their respective genes. It may inhibit either the initiation of transcription or the stability of the mRNAs encoding these molecules. In fact, it has been shown that activation of transcription factor NF-κB is required for the TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin genes [26]. Induction of NF-κB activation by TNF-α requires the phosphorylation and degradation of IκB-α [27]. Recently, a cyclic AMP-independent protein kinase A associated to IκB, and an IκB kinase complex (IKK), have been found to be involved in the activation of NF-κB [28,29]. Interestingly, curcumin is found to be a selective inhibitor of phosphorylase kinase [24]. It is, therefore, possible that curcumin may affect the early stages of signalling events by blocking the activation of NF-κB by inhibiting important protein kinases.

Under normal conditions the functional properties of the vasculature are properly maintained. However, under inflammatory conditions due to the production of various

cytokines and other mediators, the functional integrity of the vasculature is altered. The adhesive property of the vasculature is primarily altered due to the upregulation of expression of cell adhesion molecules. Thus various approaches, such as monoclonal antibodies specific to cell adhesion molecules, small molecules, peptides derived from adhesion molecules to prevent receptor-ligand interactions, and antisense oligonucleotides have been employed to inhibit the cell adhesion molecules. For example, a number of small molecules, such as glucocorticoids, aspirin, acetylsalicylate, serine proteinase and proteasome inhibitors have been shown to inhibit TNF-α induced cell adhesion molecule expression [10–12,30,31]. Here we demonstrated that the active principle of *C. longa*, diferuloylmethane, effectively blocked the expression of leukocyte adhesion molecules. It is also a natural compound and thus offers a better therapeutic target for controlling various pathological conditions associated with upregulation of endothelial leukocyte adhesion molecules.

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References

[1] Leung AY, Foster S. In: Encyclopedia of common natural ingredients used in food drugs and cosmetics. New York: John Wiley and Sons Inc, 1996. p. 499–501.
[2] Chandra D, Gupta SS. Anti-inflammatory and anti-arthritic activity of volatile oil of *Curcuma longa*. Ind J Med Res 1972;60:138–42.
[3] Yegnanarayan R, Saraf AP, Balwani JH. Comparison of anti-inflammatory activity of various extracts of *Curcuma longa*. Indian J Med Res 1976;64:601–8.
[4] Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994;76:301–14.
[5] Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. FASEB 1992;6:2591–9.
[6] Mantovani A, Bussolino F, Introna M. Cytokine regulation of endothelial cell function: from molecular level to bedside. Immunol Today 1997;18:231–40.
[7] Gorski A. The role of cell adhesion molecules in immunopathology. Immunol Today 1994;15:251–5.
[8] Paul LC, Issekutz TB. In: Adh. Mol. in Health and Disease. New York: John Wiley and Sons Inc, 1997. p. 55–86.
[9] Kelly KJ, Williams WW, Colvin RB, Mechan SM, Springer TA, Carlos J, Ramos G, Bonventre JV. Intercellular adhesion molecule-1-defecient mice are protected against ischemic renal injury. J Clin Invest 1996;97:1056–63.
[10] Neuner P, Klosner G, Pourmojib M, Knobler R, Schwarz T. Pentoxifylline in vivo and in vitro down-regulates the expression of the intercellular adhesion molecule-1 in monocytes. Immunology 1997;90:435–9.
[11] Weber C, Erl W, Pietsch A, Weber PC. Aspirin inhibits nuclear factor-κB mobilization and monocyte adhesion in stimulated human endothelial cells. Circulation 1995;91:1914–7.
[12] Brostjan C, Anrather J, Csizmadia V, Natrajan G, Winkler H. Glucocrticoids inhibit E-selectin expression by targeting NF-κB and not ATF/c-jun. J Immunol 1997;158:3836–44.

[13] Schleimer RP, Rutledge BK. Cultured human vascular endothelial cells acquire adhesiveness for leukocytes following stimulation with interleukin-1, endotoxin and tumor promoting blood cells. J Immunol 1986;136:649–54.
[14] Foreman KE, Vaproyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM, Eddy SM, Ward PA. C5a-induced expression of P-selectin in endothelial cells. J Clin Invest 1994;95:1147–55.
[15] Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Luscinskas FW, Gimbrone MA, McIntyre BW, Bochner BC. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. J Immunol 1992;148:1086–92.
[16] Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.
[17] Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 1988;52:925–33.
[18] Collins T, Williams A, Johnston GI, Kim J, Eddy R, Shows T, Gimbrone MA, Bevilacqua MP. Structure and chromosomal location of the gene for endothelial leukocyte adhesion molecule 1. J Biol Chem 1991;266:2466–73.
[19] Cybulsky MI, Fries JWU, Williams AJ, Sultan P, Eddy R, Byers M, Shows T, Gimbrone MA, Collins T. Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM-1 gene. Proc Natl Acad Sci USA 1991;88:7859–63.
[20] Iijima SA, Hamada H, Reddy P, Kakunaga T. Molecular structure of the human cytoplasmic β-actin gene: interspecies homology of sequences in the introns. Proc Natl Acad Sci USA 1985;82:6133–7.
[21] Kiso Y, Suzuki Y, Wantanabe N, Oshima Y, Hikino H. Antihepatotoxic principles of *Curcuma longa* rhizomes. Planta Med 1983;49:185–7.
[22] Srinivasan KR. A chromatographic study of the curcuminoids in *Curcuma longa*. J Pharm Pharmacol 1953;5:448–57.
[23] Ammon HPT, Wahl MA. Pharmacology of *Curcuma longa*. Planta Med 1991;57:1–7.
[24] Reddy S, Aggarwal BB. Curcumin is a non-competitive and selective inhibitor of phosphorylase kinase. FEBS Lett 1994;341:19–22.
[25] Haung MT, Smart RC, Wong CQ, Conney AH. Inhibitory effect of curcumin, chlrogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-*o*-tetradeanoylphorbol-13-acetate. Cancer Res 1988;48:5941–6.
[26] Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine inducible enhancers. FASEB J 1995;9:899–909.
[27] Baldwin AS. The NF-κB and I-κB proteins: new discoveries and insights. Ann Rev Immunol 1996;14:649–83.
[28] Zhong H, Su Yang H, Bromage HE, Tempst P, Ghosh S. The transcriptional activity of NF-κB is regulated by the Iκ-B-associated PKAc subunit through cyclic AMP-independent mechanism. Cell 1997;89:413–24.
[29] Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. Cell 1997;91:243–52.
[30] Read MA, Neish AS, Luscinskans FW, Palombella VJ, Maniatis T, Collins T. The proteasome pathway is required for cytokine induced endothelial leukocyte adhesion molecule expression. Immunity 1995;2:493–506.
[31] Kumar A, Dhawan S, Hardegen NJ, Aggarwal BB. Curcumin (diferuloylmethane) inhibition of the tumor necrosis factor (TNF) mediated adhesion of monocytes to endothelial cells by suppression of cell surface expression of adhesion molecules and of nuclear factor-κB activation. Biochem Pharmacol 1998;55:775–83.