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Journal of Ethnopharmacology 75 (2001) 25–32

Journal of ETHNO-PHARMACOLOGY

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Curcuma longa activates NF-κB and promotes adhesion of neutrophils to human umbilical vein endothelial cells [1]

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Received 14 November 2000; received in revised form 14 November 2000; accepted 21 November 2000

Abstract

Upregulation of expression of cell adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin, is important for immune surveillance. Extravasation and migration of body's effector cells to the site of immune activation is controlled by the expression of cell adhesion molecules on endothelial cells. We demonstrate here that an aqueous extract prepared from *Curcuma longa* (ClAqE), a dietary component, promotes the adhesion of peripheral neutrophils to human umbilical vein endothelial cells. To delineate the mechanism of increased adhesion, we investigated the possibility that ClAqE induces the expression of ICAM-1 and E-selectin on endothelial cells. ClAqE increases the steady state transcript levels of ICAM-1, VCAM-1, and E-selectin as determined by RT-PCR. We also show that ClAqE activates nuclear transcription factor NF-κB, a major transcription factor involved in the transcription of genes encoding ICAM-1, VCAM-1 and E-selectin. These results have implications for the usage of aqueous preparation of *C. longa* for upregulation of cell adhesion molecule expression and/or NF-κB.

Keywords: *Curcuma longa*; Endothelial cells; Neutrophils; Adhesion molecules; Immunomodulators

1. Introduction

The regulated expression of cell adhesion molecules is an essential part of immune surveillance. Cell adhesion molecules are expressed on both immune and non-immune cells. Adhesion molecules such as ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), and E-selectin mediate the extravasation of leukocytes from blood vessels to the underlying tissues at the sites of injury or infection. The function of these adhesion molecules and their ligands is to regulate cell migration, cellular differentiation and development, and tissue repair. These molecules also play an important role in signal transduction and cell activation (Butcher, 1991; Yurochko, 1997).

The expression of cell adhesion molecules on various cell types is induced by proinflammatory cytokines, including interferon-γ, TNF-α, and interleukin-1, as well as by bacterial products (e.g. lipopolysaccharide) and phorbol esters (Mantovani et al., 1997). TNF-α, IL-1β, and LPS upregulate ICAM-1, VCAM-1, and E-selectin expression on endothelial cells at the transcriptional level through the activation of NF-κB. The promoters of these molecules contain recognition sequences for nuclear transcription factor NF-κB which is essentially required for the induced expression of these molecules (Collins et al., 1995; Baldwin, 1996).

Failure to express cell adhesion molecules leads to various pathological disorders since it will impair the individual's ability to mount an inflammatory response. One such disorder is leukocyte adhesion deficiency-1

Abbreviations: ICAM-1, Intercellular adhesion molecule-1; VCAM-1, Vascular cell adhesion molecule-1; TNF-α, Tumor necrosis factor-α; LPS, Lipopolysaccharide; NF-κB, Nuclear factor κB; EMSA, Electrophoretic mobility shift assay; IL-1, Interleukin-1; Mac-1, CD11b/CD18; PAGE, Polyacrylamide gel electrophoresis; HUVECs, Human umbilical vein endothelial cells; RT-PCR, Reverse transcription-polymerase chain reaction; mAb, Monoclonal antibody; PBS, Phosphate buffer saline.

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[1] Council of Scientific and Industrial Research and Department of Biotechnology, India supported this work.

[2] Babita Madan is a recipient of CSIR fellowships.

0378-8741/01/$ - see front matter
PII: S0378-8741(00)00386-X

(LAD-1), an autosomal recessive disorder, characterized by recurrent bacterial and fungal infections and impaired wound healing. In these patients, most of the adhesion-dependent functions of leukocytes including adherence to endothelium, neutrophil aggregation and chemotaxis, phagocytosis, and cytotoxicity mediated by neutrophils, NK cells and T lymphocytes are abnormal. The molecular basis of the defect is deficient expression of β2 integrins, which include leukocyte-function-associated antigen-1 (LFA-1), Mac-1, and p150, 95. Another pathological disorder, leukocyte adhesion deficiency-2 (LAD-2), results from an absence of sialyl-Lewis X, the carbohydrate ligand on neutrophils that is required for the binding to E-selectin and P-selectin on cytokine-activated endothelium. LAD-2 patients have mutations in genes encoding enzymes involved in fucose metabolism (Abbas, 1994). Also there is lack of expression of ICAM-1 on endothelial cells in the skin of patients with lepromatous leprosy, resulting in a failure to develop immune granulomata in response to *Mycobacteruim leprae* (Moncada et al., 1993).

Identification of compounds that can upregulate the expression of adhesion molecules on the surface of endothelial cells could be useful in the treatment of abnormalities resulting from the absence or reduced expression levels of cell adhesion molecules. *C. longa*, popularly known as turmeric, has been reported to posses a wide array of biological activities that include anti-inflammatory, antibacterial, antiviral, as well as antioxidant property (Ammon and Wahl, 1991). Alcoholic extracts of *C. longa* have been studied in detail. The major component isolated from an alcoholic extract of *C. longa* is curcumin, which has been studied well for its biological properties and mode of action (Huang et al., 1991; Kobayashi et al., 1997). Aqueous extracts have been reported to contain components that include an antioxidant peptide turmerin, an antioxidant protein and ar-turmerone that inhibits lymphocyte proliferation and has natural killer activity (Ferreira et al., 1992; Srinivas et al., 1992; Selvam et al., 1995). A series of polysaccharides Ukanon A, B, C and D that have reticulo-endothelial-system potentiating activities are also reported to be present in aqueous extracts of *C. longa* (Gonda et al., 1992). Although antioxidant, antimutagenic, and reticulo-endothelial-potentiating activities of *C. longa* have been reported, very little is known about the molecular basis of these activities.

In this study we show that an aqueous extract of *C. longa* promotes the adhesion of neutrophils to endothelial cells by upregulating the expression of cell adhesion molecules on endothelial cells. We also show that this particular aqueous extract of *C. longa* promotes the transcription of the genes encoding these adhesion molecules by enhancing the intracellular level of factor NF-κB.

2. Methodology

2.1. Materials

Anti-ICAM-1 (BBA3) and *anti*-E-selectin (BBA1) antibodies were purchased from R&D Systems (Minneapolis, MN). M199, l-glutamine, penicillin, streptomycin, amphotericin, endothelial cell growth factor, trypsin, Pucks saline, HEPES, *o*-phenylenediamine dihydrochloride, ficoll-hypaque, cetitrimethyl ammonium bromide, **3**-amino-1,2,4 triazole, *anti*-mouse IgG-HRP were purchased from Sigma Chemical Co., USA. NF-κB oligonucleotide was purchased from Promega (Madison, WI). The ICAM-1, VCAM-1, E-selectin and β-actin primer sets employed were synthesized by Genset Corp. (Tokyo, Japan). Fetal calf serum was purchased from Biological industries (Kibbutz Beit Haemek, Israel). Limulus amebocyte assay kit was purchased from Charles River Endosafe (Charleston, USA).

2.2. Preparation of the extract

Dried powdered rhizomes of *C. longa* (10 g), were macerated in 100 ml of distilled water overnight at 25°C under shaking and the percolate was collected. Maceration and percolate collection was continued for 3 days. The percolate was pooled and vacuum dried. The yield of this process was 0.73% (w/w). The dried material was resuspended in water before use and is designated ClAqE.

2.3. Cells and cell culture

Endothelial cells were isolated from human umbilical cords by mild trypsinisation as described before (Gupta and Ghosh, 1999). The endothelial cells were cultured in M199 medium supplemented with 20% 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) 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. The cells were used in passages three and four. The viability of cells was determined by trypan blue staining and their identity was established by E-selectin expression.

2.4. Neutrophil isolation

Neutrophils were isolated from peripheral blood as described previously (Clark and Nauseef, 1996). Blood from healthy individuals was collected in heparin solution (20 U/ml, final concentration). The red blood cells were removed by sedimentation in 6% dextran; subsequently, the white-blood-cell rich plasma layer was

collected, and layered on a ficoll-hypaque solution in order to be centrifuged for 20 min at 300 × *g* and 20°C. Following centrifugation, the top saline layer and the ficoll-hypaque layer were removed and the neutrophil/RBC pellet was collected. Residual RBC's were removed by hypotonic lysis. The isolated cells were washed with PBS and resuspended to a concentration of 6×10^5 cells/ml in PBS containing 5 mM glucose, 1 mM $CaCl_2$ and $MgCl_2$. This procedure usually results in approximately 95% pure neutrophils with a cell viability of more than 95% as detected by trypan blue exclusion.

2.5. Cell-adherence assay

The adherence assay was performed as described before (Dobrina et al., 1991). To assay the adhesion of neutrophils to endothelial monolayers, the endothelial cells were plated to confluence in 96-well culture plates and allowed to adhere for 24 h. The cells were incubated with or without ClAqE and/or LPS (1 μg/ml) for 6 h. Upon incubation, the cells were twice washed with PBS and then incubated with neutrophils (6×10^4 per well) for 1 h at 37°C. To remove the non-adherent neutrophils, the endothelial monolayers were washed thrice with PBS. Adherent neutrophils were assayed colorimetrically by adding substrate solution (100 μl/well) consisting of peroxidase substrate (*o*-phenylenediamine dihydrochloride 40 mg per 100 ml in citrate phosphate buffer, pH 4.5) containing 0.1% cetitrimethyl ammonium bromide as peroxidase-solubilising agent. To reduce the interference by contaminating eosinophils, 3-amino-1,2,4 triazole (1 mM), a selective eosinophil peroxidase inhibitor, was added to the substrate solution. Color development reaction was stopped by adding 2N H_2SO_4 (50 μl/well). The absorbance was determined at 490 nm using an automated microplate reader (Anthos Labtech HT2, Austria).

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

For estimating the expression of ICAM-1 and E-selectin on the surface of endothelial cells, cell-ELISA was used as described previously (Gupta and Ghosh, 1999). HUVECs grown to confluence in 96 well plates were incubated with or without ClAqE or with LPS (1 μg/ml) for 16 h for ICAM-1 or for 4 h for E-selectin expression. After incubation the cells were washed with PBS and fixed with glutaraldehyde. Following fixation, the cells were blocked with 3.0% non-fat dry milk, and then incubated with *anti*-ICAM-1 or *anti*-E-selectin monoclonal antibody overnight at 4°C. Following this, the cells were washed with PBS and incubated with peroxidase-conjugated goat *anti*-mouse secondary antibody. The cells were subsequently washed with PBS and exposed to peroxidase substrate (*o*-phenylenediamine dihydrochloride). Absorbance was measured at 490 nm.

2.7. Total RNA isolation and reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from endothelial cells according to a modified guanidium thiocyanate procedure (Chomczynski and Sacchi, 1987). Expression of the transcripts for ICAM-1 (upper primer: 5′-CAGTGACCATCTACAGCTTTCCGG-3′; and lower primer 5′-GCTGCTACCACAGTGATGATGACAA-3′), VCAM-1 (upper primer: 5′- ACCCTCCCAAGGCACACACAG-3′; and lower primer 5′-GTAAGTCTATCTCCAGCCTGTC-3′), and E-selectin (upper primer: 5′-GATGTGGGCATGTGGAATGATG-3′; and lower primer 5′-AGGTACACTGAAGGCTCTGG-3′) was evaluated by RT-PCR to yield products of length 555,533 and 479 bp, respectively as detailed before (Gupta and Ghosh, 1999). β-actin was amplified simultaneously, using identical conditions (upper primer 5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′; and lower primer 5′-CTAGAAGCATTTGCGGGGACGATGGAGGG-3′) as a control resulting in an amplified product of 661 bp. RT-PCR was performed as a single step reaction following manufacturer's protocol (Access RT-PCR system, Promega, Madison). PCR products were analyzed by 1% agarose gel electrophoresis.

2.8. Preparation of nuclear extracts

Nuclear extracts were prepared by slightly modified previously published methods (Dignam et al., 1983). Primary endothelial cells were incubated without or with ClAqE (700 ng/ml) or with LPS (1 μg/ml) for 30 min. The cells were washed with PBS, dislodged with a cell scraper, and collected by centrifugation at 300 × *g*. The cell pellet was resuspended in cell lysis buffer (10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM PMSF, 1 mM DTT, 0.5% Noniodet P40, 0.1 mM EGTA and 0.1mM EDTA) and allowed to swell on ice for 5 min, followed by centrifugation at 3300 × *g* for 15 min. The nuclear pellet thus obtained was resuspended in nuclear extraction buffer (20 mM HEPES pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 420 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM PMSF, and 1 mM DTT), and incubated on ice for 30 min. The extracted nuclei were pelleted at 25 000 × *g* for 15 min at 4°C, the supernatant being collected as nuclear extract and stored at -70°C. Protein concentrations were estimated by using standard Bradford's method.

2.9. Electrophoretic mobility shift assay

The electrophoretic mobility shift assay was performed with modifications of a previously published procedure (Marrugo et al., 1996). Briefly, nuclear extracts (6–8 μg) were incubated with 40–80 fmoles of ^{32}P-end labeled, double-stranded NF-κB oligonucleotide (5′- AGTTGAGGGGACTTTCCCAGG-3′) in binding buffer (12 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM TrisCl pH 7.5, 10% glycerol, 1 mM EDTA, 1 mM DTT and 1.0 μg poly dI–dC) for 30 min at room temperature. DNA–protein complexes were analyzed by electrophoresis on a 4% native polyacrylamide gel using Tris–glycine buffer pH 8.5, and, were visualized by autoradiography.

3. Results

3.1. ClAqE is non-toxic to endothelial cells and its activity is not due to endotoxin contamination

To determine any possible toxic effect of ClAqE on endothelial cells, the cells grown to confluence in 96 well plates were incubated with varying concentrations of ClAqE for 24 h. Viability of the cells was determined by trypan-blue exclusion and their morphology was observed under microscope. It was observed that the time of incubation (upto 24 h) and the extract concentration used (700 ng/ml) in subsequent experiments did neither affect the viability nor morphology of the endothelial cells (data not shown). As analysed using thio-barbituric acid method, the presence of 2-keto 3-deoxy octonate (KDO), a major constituent of LPS was not detected in the extract. The extract was further tested for the presence of endotoxin by limulus amebocyte assay, using manufacturer's protocol. Using this sensitive assay it was found that ClAqE contained trace amounts of LPS (20–30 pg LPS per 1000 ng ClAqE). However, these amounts of LPS were unable to activate expression of cell adhesion molecules or promote adhesion of neutrophils to endothelial cells (data not shown). Previous studies have also shown that 100–1000 ng of LPS is required for inducing expression of cell adhesion molecules on endothelial cells (Swerlick et al., 1992).

3.2. ClAqE promotes the adhesion of neutrophils to endothelial cells

To study the effect of ClAqE on the adhesion of neutrophils to endothelial cells, the endothelial cells were treated with ClAqE at concentrations varying from 70 to 700 ng/ml whereafter the adhesion of neutrophils to endothelial monolayers was evaluated. As studied by cell-adherence, the adhesion of neutrophils to the unstimulated endothelial cells was low Fig. 1. However, when endothelial cells were incubated with ClAqE, a concentration-dependent increase in the adhesion of neutrophils to endothelial cells occurred, with maximal adhesion at a concentration of 700 ng/ml. The level of adhesion was comparable to the adhesion induced by LPS. Moreover, there was no further increase in adhesion induced by ClAqE upon further treatment of endothelial cells with LPS (data not shown).

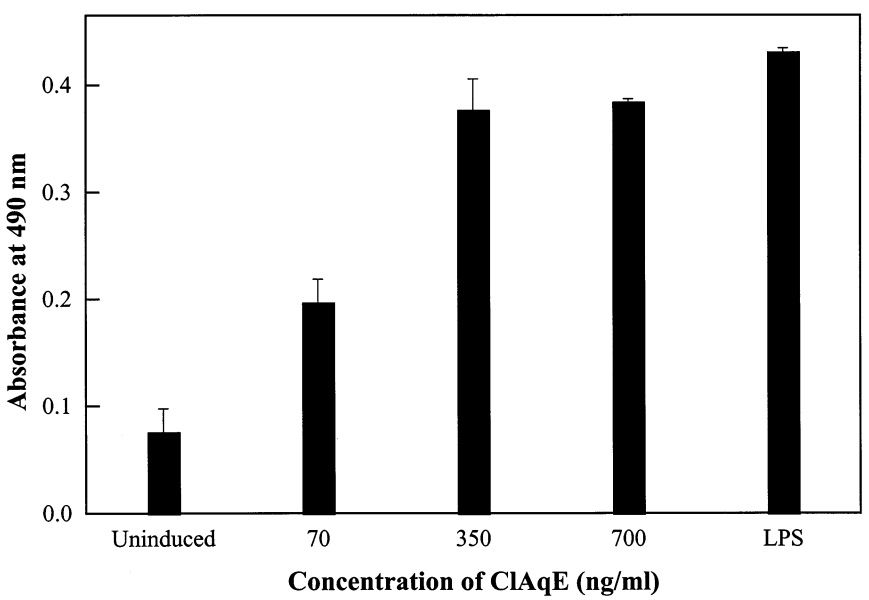

Fig. 1. Increase of neutrophil adhesion to endothelial cells. Endothelial cells grown to confluence in 96 well plates were incubated with or without indicated concentrations of ClAqE or with LPS (1 μg/ml) for 6 h. The cells were then incubated with human peripheral neutrophils for 1 h. The amounts of neutrophils adhering to the endothelial cell monolayers were measured by a colorimetric assay as described in Section 2. Data are representative of three independent experiments. Values shown are mean ± SD of duplicate wells.

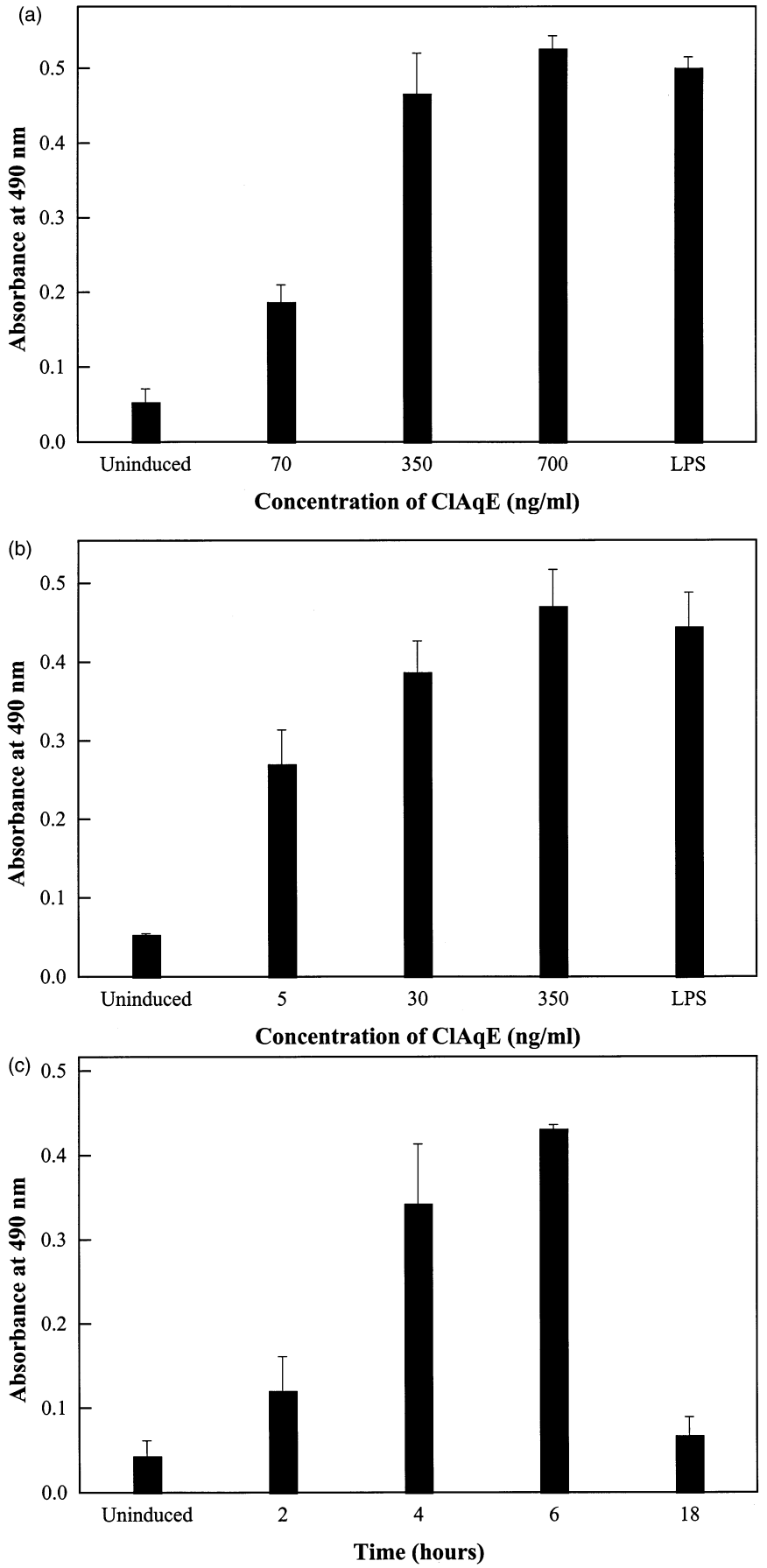
(a)
Absorbance at 490 nm
0.0
0.1
0.2
0.3
0.4
0.5
Uninduced
70
350
700
LPS
Concentration of ClAqE (ng/ml)
(b)
Absorbance at 490 nm
0.0
0.1
0.2
0.3
0.4
0.5
Uninduced
5
30
350
LPS
Concentration of ClAqE (ng/ml)
(c)
Absorbance at 490 nm
0.0
0.1
0.2
0.3
0.4
0.5
Uninduced
2
4
6
18
Time (hours)

Fig. 2.

3.3. ClAqE upregulates ICAM-1 and E-selectin expression on endothelial cells in a concentration-dependent manner

Cell-adhesion molecules such as ICAM-1 and E-selectin are required for the adhesion of neutrophils to endothelial cells. Therefore, we studied the effect of ClAqE on the expression of these molecules. As detected by ELISA and shown in Fig. 2A–B, ICAM-1 and E-selectin were expressed at low levels on unstimulated endothelial cells. When endothelial cells were incubated with ClAqE, a concentration-dependent upregulation of ICAM-1 and E-selectin expression was observed. Interestingly, the concentration of the extract that caused maximal induction of E-selectin expression was much less (350 ng/ml) as compared to the concentration required for maximal ICAM-1 expression (700 ng/ml). Maximal levels of ICAM-1 and E-selectin expression induced by ClAqE were comparable with those for LPS-induced expression. This suggests that ClAqE promotes neutrophil adhesion to endothelial cells by inducing the expression of ICAM-1 and E-selectin on endothelial cells. Kinetics of E-selectin expression by ClAqE was also evaluated. Six hours incubation with ClAqE was found to be optimal for maximal E-selectin expression as shown in Fig. 2C.

3.4. Transcript levels of ICAM-1, E-selectin and VCAM-1 are significantly increased by ClAqE

In order to understand the mechanism of induction of cell adhesion molecules by ClAqE, we analyzed the steady-state levels of transcripts of ICAM-1 and E-selectin in the presence of ClAqE. The endothelial cells were incubated without or with varying concentrations of ClAqE and/or induced with LPS for 4 h. As revealed by RT-PCR analysis and shown in Fig. 3, there were low levels of ICAM-1 mRNA and undetectable levels of E-selectin mRNA in unstimulated cells (lane 1). Treatment with ClAqE (70–700 ng/ml) significantly increased the transcript levels of ICAM-1 and E-selectin (lanes 3–5, respectively) with no further increase upon induction with LPS (lanes 6–8). The level of expression of ICAM-1 and E-selectin in cells treated with ClAqE was similar to the level in LPS induced cells (lane 2). The levels of β-actin mRNA expressed under these conditions remained the same. These results indicate that ClAqE upregulates the expression of ICAM-1 and E-selectin by activating transcription of their genes.

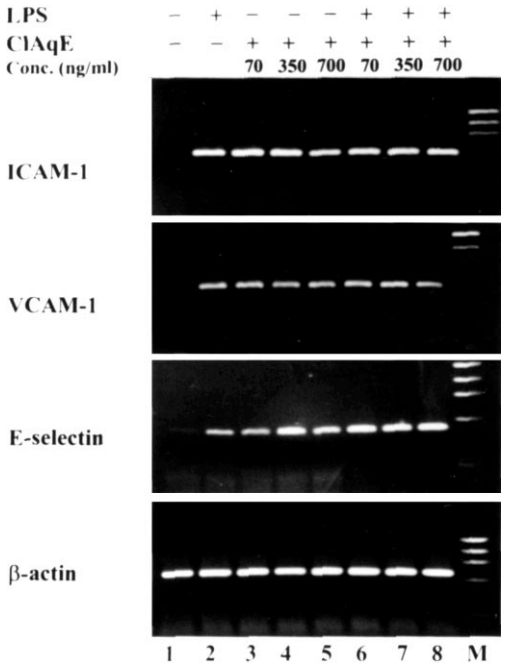

Fig. 3. Analysis of ICAM-1, VCAM-1, and E-selectin transcript levels in cells treated with ClAqE: Endothelial cells were incubated with or without varying concentrations of ClAqE and/or induced with LPS (1 μg/ml) for 4 h. Total RNA of the cells was isolated and analyzed by RT-PCR as described in the Section 2. Lane M, marker ϕX174 *Hae*III digest; Lane 1, unstimulated cells; Lane 2, stimulated with LPS; Lanes 3–5 ClAqE; Lanes 6–8, stimulated with LPS after pre-treatment with ClAqE. Corresponding samples were analyzed for β-actin mRNA as control (lanes 1–8).

VCAM-1 is also an important cell adhesion molecule required for the adhesion of eosinophils, lymphocytes, and monocytes to the endothelial cells. For studying the effect of ClAqE on expression of VCAM-1, we analyzed its steady-state transcript levels in endothelial cells in the presence of ClAqE. The endothelial cells were incubated without or with varying concentrations of ClAqE and/or induced with LPS for 4 h. As revealed by RT-PCR analysis and shown in Fig. 3, there were undetectable levels of VCAM-1 mRNA in unstimulated cells (lane 1). Treatment with ClAqE (70–700 ng/ml) significantly increased the transcript levels of VCAM-1 (lanes 3–5, respectively) with no further increase upon induction with LPS (lanes 6–8). The level of expression of VCAM-1 in cells treated with ClAqE was similar to the level in LPS induced cells (lane 2). This indicates that in addition to ICAM-1 and E-selectin ClAqE also induces the expression of VCAM-1 on endothelial cells.

Fig. 2. Concentration-dependent increase of ICAM-1 and E-selectin expression by ClAqE: (A) Endothelial cells grown to confluence in 96 well plates were incubated with or without indicated concentrations of ClAqE or with LPS (1 μg/ml) for 16 h. ICAM-1 level on the cells was measured by ELISA as described in Section 2. (B) Endothelial cells were treated as in 'A' without or with ClAqE or with LPS (1 μg/ml) for 4 h. Level of E-selectin on the cells was measured by ELISA as described in Section 2. (C) Endothelial cells grown to confluence in 96 well plates were incubated with ClAqE (350 ng/ml) for indicated time. E-selectin level on the cells was measured by ELISA as described in Section 2. The data are representative of four independent experiments. Values shown are mean ± SD of quadruplicate wells.

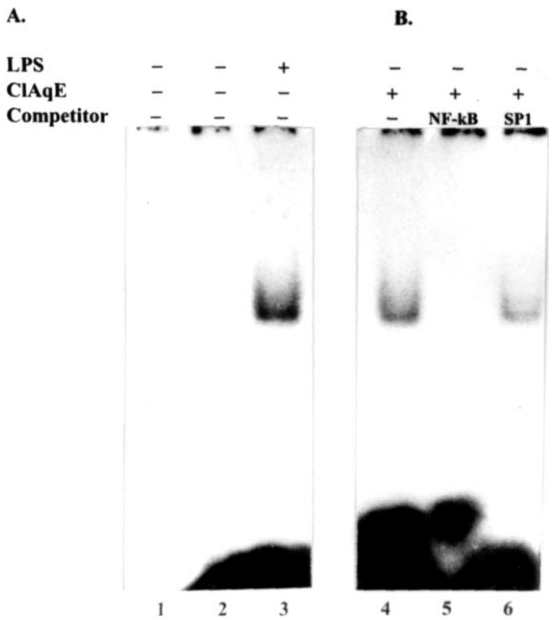

Fig. 4. Activation of NF-κB in endothelial cells treated with ClAqE: Endothelial cells were incubated with or without ClAqE or with LPS (1 μg/ml) for 30 min. Nuclear extracts were prepared, incubated with ^{32}P-labelled NF-κB oligonucleotide and analyzed on a 4% polyacrylamide gel electrophoresis as described in the Section 2.

3.5. ClAqE activates NF-κB

It has been shown that for the induction of ICAM-1, VCAM-1, and E-selectin expression, activation of transcription factor NF-κB is required (Collins et al., 1995). To examine whether activation of NF-κB is involved in ClAqE-mediated induction of cell-adhesion molecules, we investigated the status of NF-κB by EMSA in cells treated with ClAqE. As shown in Fig. 4, there were low levels of NF-κB in unstimulated cells (lane 2). The treatment of cells with ClAqE caused a substantial increase in the level of NF-κB (lane 4), thus causing retardation in the mobility of the labeled oligonucleotide. This level was comparable to the LPS-induced levels of NF-κB (lane 3). The specificity of the NF-κB DNA complex induced by ClAqE was confirmed in control experiments. Incubation with an excess of unlabelled NF-κB inhibited the formation of the complex, whereas competition with an excess of an irrelevant oligonucleotide, SP1, did not inhibit the complex (compare lanes 5–6). These results indicate that ClAqE upregulates the expression of cell-adhesion molecules through the activation of NF-κB.

4. Discussion and conclusions

We demonstrate here for the first time that an aqueous preparation of a naturally and widely occurring plant *C. longa*, can be used for promoting the adhesion of peripheral neutrophils to human umbilical vein endothelial cells. ClAqE promotes the adhesion of neutrophils by inducing the expression of cell adhesion molecules ICAM-1 and E-selectin on endothelial cells. Our RT-PCR results demonstrate that ClAqE increases the steady state transcript levels of these adhesion molecules suggesting that it may be activating at an early stage of signaling event. We also demonstrate by gel-retardation assays that ClAqE activates the transcription factor NF-κB. As NF-κB is essentially required for the inducible expression of ICAM-1, VCAM-1, and E-selectin, this suggests that ClAqE induces transcription of these genes by activating NF-κB. The induction of NF-κB activation requires the phosphorylation and degradation of IκB-α (Baldwin, 1996). This process is dependent on the activation of both protein kinase C and protein tyrosine kinase (Meichvle et al., 1990). It is possible that ClAqE may activate any of these protein kinases. The activation of cell-adhesion molecules and/or NF-κB may explain some of the previously reported antioxidant, *anti*-mutagenic, and reticulo-endothelial potentiating activities of aqueous preparations of *C. longa*. Identifying the active component(s) of ClAqE will be interesting. The active principle present in ClAqE is found to be resistant to heat treatment, mild hydrolysis by acetic acid and digestion by proteinase K (unpublished observation).

The cell adhesion molecules are important in the regulation of cellular immune responses as they control the extravasation and migration of body's effector cells to the site of immune activation (Butcher, 1991). An aqueous preparation of *C. longa* may be useful in conditions where upregulation of cell adhesion molecules and/or activation of NF-κB is required. Topical application of ClAqE may be useful for inducing the expression of ICAM-1 on endothelial cells where there is a lack of its expression, for example, for developing an immune granuloma in response to *M. leprae* in patients with lepromatous leprosy. An aqueous preparation of *C. longa* may also be used for inducing low levels of NF-κB for inducing tolerance to septic shock. It has been shown that sublethal hemorrhage causes activation of NF-κB at low levels, this prevents its further upregulation upon subsequent endotoxic shock and hence induces tolerance in rats (Kramerr et al., 1999). For the treatment of localized infections and in wound healing also ClAqE may be useful as it can promote the infiltration of neutrophils at that site. The aqueous extract of *C. longa* may thus find new therapeutic applications in the future.

Acknowledgements

We thank Dr K. Muralidhar, University of Delhi for his advice and constructive criticisms.

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