Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 1431–1437
Bioorganic & Medicinal Chemistry

1,4-Dihydroxyxanthone modulates the adhesive property of endothelial cells by inhibiting intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin

Babita Madan,[a] Ashok K. Prasad,[b] Virinder S. Parmar[b] and Balaram Ghosh[a,*]

[a]*Molecular Immunology and Immunogenetics Laboratory, Institute of Genomics and Integrative Biology, University of Delhi Campus (North), Mall Road, Delhi-110007, India*
[b]*Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110007, India*

Received 1 November 2003; revised 6 November 2003; accepted 27 December 2003

Abstract—Cell adhesion molecules, particularly intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1) and E-selectin, play important roles in the recruitment of leukocytes to the site of inflammation. Blocking the expression of these molecules or preventing their interaction with the receptors has been shown to be important in controlling various inflammatory diseases. These cell adhesion molecules are induced on endothelial cells by various proinflammatory cytokines like IL-1β and TNF-α and also by bacterial LPS. We demonstrate here that 1,4-Dihydroxyxanthone (1,4 DHX) inhibits the expression of cell adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin, on endothelial cells in a concentration and time dependent manner. The inhibition by 1,4 DHX is reversible. On further analysis, our results also show that 1,4 DHX inhibits the adhesion of peripheral neutrophils to the endothelial cell monolayers. 1,4 DHX, therefore, could be used as a novel target for controlling various pathological conditions associated with upregulation of endothelial leukocyte adhesion molecules.

1. Introduction

Under normal physiological conditions, the leukocytes keep freely circulating in the blood vessels. The endothelium lining the blood vessels helps to maintain the vascular integrity by preventing the migration of leukocytes from the blood vessels to the underlying tissues. However, in various vascular and inflammatory diseases, the adhesive property of the vasculature is altered primarily due to the increased expression of cell adhesion molecules on the surface of endothelial cells, thus allowing indiscriminate infiltration of the leukocytes across the blood vessels leading to inflammation.[1,2] The cell adhesion molecules that are upregulated include intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin.[3–5] The expression of these molecules on the endothelium is induced by various pro-inflammatory cytokines like IL-1β and TNF-α and also by bacterial LPS.[6]

Inhibition of cell adhesion molecules has shown to be a useful therapeutic approach to regulate inflammatory response.[7] Various strategies including monoclonal antibodies (mAbs) specific to cell adhesion molecules and small molecules from natural and synthetic sources such as curcumin, diclofenac have been used successfully to downregulate the induced expression of cell adhesion molecules, hence preventing inflammation.[8,9] However, due to the problems like endotoxin contamination, secondary antibody formation, cellular activation, and other complications like sensitisation leading to serum sickness and anaphylaxis the practical use of mAbs is limited.[10]

Cell adhesion molecules viz. ICAM-1, VCAM-1 and E-selectin share common regulatory features in that the expression of all these molecules is increased in response to pro-inflammatory cytokines like TNF-α and IL-1β.[11]

Keywords: 1,4-Dihydroxyxanthone; Endothelial cells; Neutrophils; Adhesion molecules; Immunomodulators; NF-κB.
Abbreviations: 1,4-DHX, 1,4-Dihydroxyxanthone; HUVECs, Human umbilical vein endothelial cells; ICAM-1, Intercellular adhesion molecule-1; IL-1, Interleukin-1; LPS, Lipopolysaccharide; TNF-α, Tumor necrosis factor-α; VCAM-1, Vascular cell adhesion molecule-1.
* Corresponding author. Tel.: +91-11-2766-7602; fax: +91-11-2766-7471; e-mail: bghosh@igib.res.in

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2003.12.027

Xanthones are obtained from natural sources like plants and can also be synthesised in the laboratory. Anti-inflammatory and anti-oxidant activities of various xanthones have been reported using diverse biological systems. Xanthones with one to four hydroxyl substituents on the xanthone nucleus, such as norathyriol (1,3,6,7-tetrahydroxyxanthone) have been reported to have potent inhibitory effects on superoxide formation by rat neutrophils stimulated with fMLP, to prevent histamine release from stimulated rat peritoneal mast cells and also have profound inhibitory effects on hind paw oedema in mice induced by inflammatory mediators[12–14]. Another study demonstrated that 1,7-dihydroxy-2,3-dimethoxyxanthone isolated from the plant *Polygala cyparissias* antagonises the contractions induced by chemical inflammatory mediators like histamines, bradykinin in guinea pig trachea in vitro.[15] In addition, xanthones are also reported to have activities against various pathogens including fungi, bacteria, virus and malarial parasites.[16–19] Anti-diabetic and anti-carcinogenic activities of xanthones are also reported.[20,21] Based on these observations, we hypothesised that xanthones may be able to modulate cellular trafficking process by controlling the expression of cell adhesion molecules. In our recent study, the structure–activity relationship of various hydroxy-, acetoxy- and methoxy-xanthones was compared for their ability to modulate TNF-α induced ICAM-1 expression and nicotinamide adenine dinucleotide phosphate (NADPH)-catalysed liver microsomal lipid peroxidation.[22]

In this study, we further analysed the activity of 1,4-DHX for its ability to modulate the expression of cell adhesion molecules. We found that 1,4-DHX inhibited

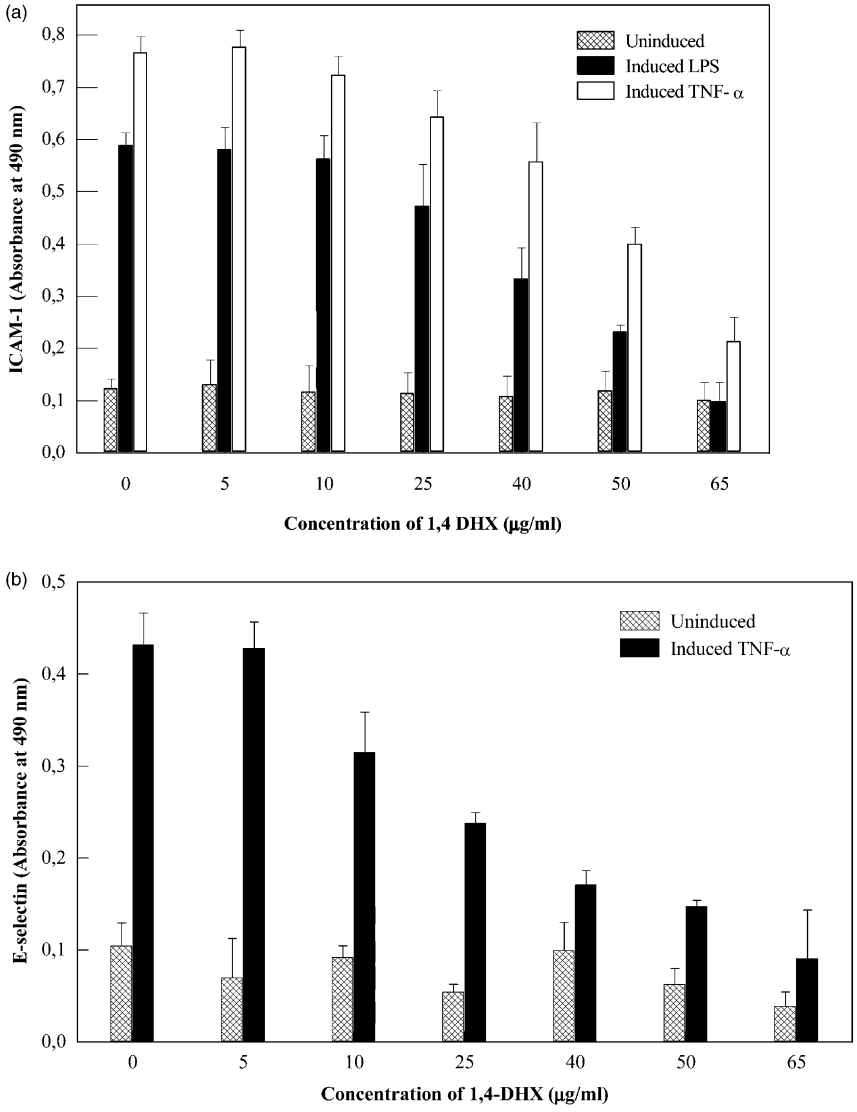

Figure 1. (A) Concentration dependent inhibition of TNF-α or LPS induced ICAM-1 expression by 1,4-DHX: Endothelial cells were grown to confluence in 96-well plates and incubated with or without indicated concentrations of 1,4-DHX for 1 h prior to induction with TNF-α (10 ng/mL) (open bars), LPS (1μg/mL) (closed bars) for 16 h or without induction (hatched bars). The ICAM-1 level on the cells was measured by ELISA as described in Material and Methods. The data presented are representative of three independent experiments. The values obtained using IgG Ab as a control have been subtracted from the corresponding values. Values shown are mean + SD of quadruplicate wells. (B) Concentration dependent inhibition of TNF-a induced E-selectin expression by 1,4-DHX: Endothelial cells were treated as in A, followed by induction without (hatched bars) or with TNF-α (10 ng/mL) (closed bars) for 4 h. E-selectin level on the cells was measured by ELISA as described in Material and Methods. The data presented are representative of 4 independent experiments. Values shown are mean + SD of quadruplicate wells.

the induced expression of cell adhesion molecules. It blocks the adhesion of neutrophils to endothelial monolayers by preventing TNF-α or LPS induced upregulation of cell adhesion molecule expression on endothelial cells.

2. Results

2.1. 1,4-DHX inhibits the TNF-α/LPS induced expression of ICAM-1, VCAM-1 and E-selectin on endothelial cells

The endothelial cells plated to confluence in 96-well plates were incubated with varying concentrations of 1,4-DHX. Its effect on the viability (determined by trypan blue exclusion test) and the morphology of the endothelial cells (observed under microscope) were tested. The time of incubation (up to 24 h) and the concentration of 1,4-DHX used (up to 65 μg/mL) in the presence or absence of LPS/TNF-α in the subsequent experiments had no effect on the viability or morphology of the endothelial cells (data not shown).

As cell adhesion molecules play an important role during inflammation, we analysed the effect of 1,4-DHX on expression of these molecules. The effect of 1,4-DHX on TNF-α induced ICAM-1 and E-selectin expression was analyzed using cell-ELISA as detailed in Material and Methods. Our results using cell-ELISA demonstrate that ICAM-1 and E-selectin were expressed at low levels on unstimulated endothelial cells and there was over three to five-fold increase in their expression upon stimulation with TNF-α (Fig. 1A and B). Pre-treatment of endothelial cells with 1,4-DHX had no effect on the constitutively expressed levels of ICAM-1 or E-selectin. The inhibitory activity of 1,4-DHX on ICAM-1 expression was first evident at a concentration of 25 μg/mL with maximal inhibition by 86.0% at a concentration of 65 μg/mL (Fig. 1A). The inhibition of TNF-α induced E-selectin expression was observed at a concentration of 10 μg/mL with maximal inhibition by 80.0% at a concentration of 65 μg/mL (Fig. 1B).

The inhibitory activity of 1,4-DHX on ICAM-1 and E-selectin expression was further confirmed by flow cytometry (Fig. 2). In addition, the effect of 1,4-DHX on VCAM-1 expression was also analysed (Fig. 2). The unstimulated cells expressed low levels of ICAM-1 and undetectable levels of VCAM-1 and E-selectin. Upon stimulation with TNF-α a substantial increase in the expression of all these three molecules was observed (Fig. 2). Pre-treatment of endothelial cells with 1,4-DHX (65 μg/mL) inhibited TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin significantly (Fig. 2). Thus, 1,4-DHX inhibits the induced expression of cell adhesion molecules as measured using cell-ELISA and flow cytometry.

As LPS is also an effective inducer of ICAM-1 expression, we studied whether 1,4-DHX is equally effective in downregulating LPS induced ICAM-1 expression. Our results demonstrate that 1,4-DHX also inhibited the

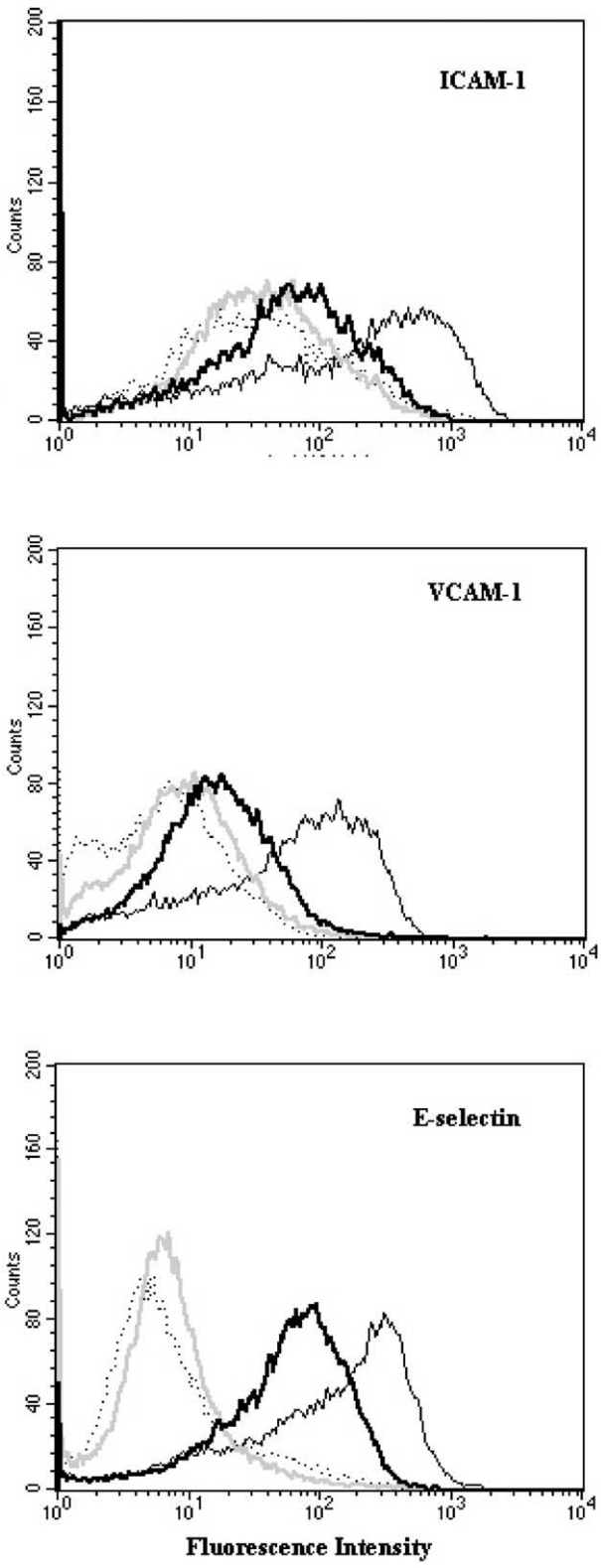

Figure 2. Flow cytometric analysis of inhibition of TNF-α induced ICAM-1, VCAM-1 and E-selectin expression by 1,4-DHX: The endothelial cells were treated with 65 μg/mL 1,4-DHX for 1 h followed by stimulation with TNF-α (10 ng/mL) for 16 h for VCAM-1 & ICAM-1 and for 4 h for E-selectin. Expression of these molecules was measured by flow cytometry as described in Material and Methods. The data are expressed in histograms representing cell number (y-axis) v/s fluorescence intensity (x-axis) plotted on a log scale. Unstimulated (dotted line), stimulated by TNF-α (thin line), 1,4-DHX and TNF-α (heavy line), 1,4-DHX alone (light line).

LPS induced expression of ICAM-1 on endothelial cells in a concentration dependent manner with almost complete inhibition upto the basal level at a concentration of 65 μg/mL (Fig. 1A).

2.2. The inhibition of ICAM-1 expression by 1,4-DHX is reversible and time dependent

To study if the inhibition of ICAM-1 expression by 1,4-DHX is reversible, the endothelial cells were pre-incubated with 50 μg/mL of 1,4-DHX for varying time periods up to 5 h, washed and allowed to recover for 1 h. The cells were then induced with TNF-α (10 ng/mL) for 16 h. The effect of 1,4-DHX was found to be reversible over a period of time and the cells were fully capable of responding to TNF-α (Fig. 3A). This indicates that 1,4-DHX causes no permanent change in endothelial cells.

To determine the kinetics of inhibition, endothelial cells were incubated with 65 μg/mL of 1,4-DHX for 1–4 h prior to, simultaneously or 1–2 h after induction with TNF-α for 16 h. 1,4-DHX inhibited ICAM-1 expression when added prior to or simultaneously with induction with TNF-α. When added after induction, the inhibition of ICAM-1 expression was not significant (Fig. 3B). These results, therefore, indicate that 1,4-DHX may be interfering with the early signalling events in response to TNF-α.

2.3. Neutrophil adhesion to endothelial cells is inhibited by 1,4-DHX

The adhesion of neutrophils to endothelial cells requires an increased expression of cell adhesion molecules, namely ICAM-1 and E-selectin. VCAM-1 has also

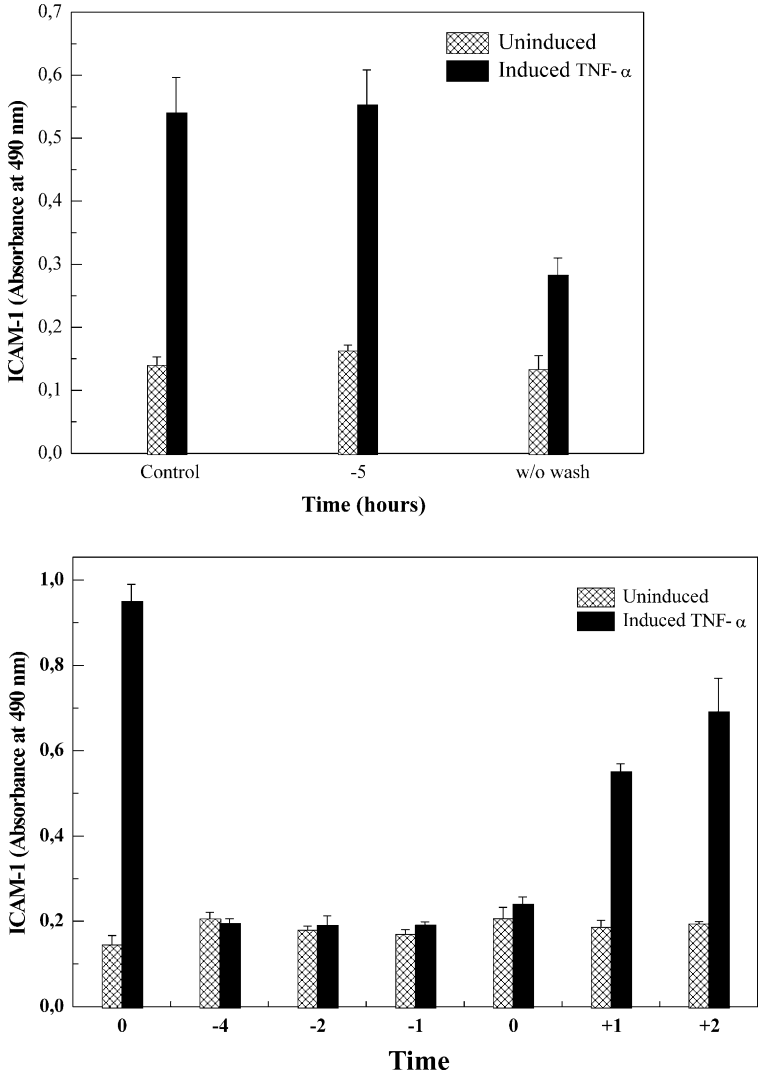

Figure 3. Inhibition by 1,4-DHX is time dependent and is reversible: A. Endothelial cells grown to confluence in 96 well plates were incubated with or without 50 μg/mL 1,4-DHX for indicated time periods. The cells were washed twice with medium and incubated in complete medium for 1 h. This was followed by induction without (hatched bars) or with (closed bars) TNF-α (10 ng/mL) for 16 h. The ICAM-1 level on the cells was measured by cell ELISA as described in Material and Methods. The data are representative of three independent experiments. Values shown are mean + SD of quadruplicate wells. B. Endothelial cells grown to confluence were incubated without or with 65 μg/mL 1,4-DHX at indicated time, followed by induction without (hatched bars) or with (closed bars) TNF-α (10 ng/mL) for 16 hrs. Following this ICAM-1 level on the cells was measured by ELISA as described in Experimental Procedures. The data are representative of 2 independent experiments. Values shown mean + SD of quadruplicate wells.

found to be involved in the adhesion of neutrophils to endothelial cells.[23] The functional significance of inhibition of cell adhesion molecules by 1,4-DHX was evaluated by testing the adhesion of neutrophils to endothelial cells in the presence of 1,4-DHX. Our results using adherence assay demonstrate that the adhesion of neutrophils to the unstimulated endothelial cells was low as compared to the adhesion to TNF-α stimulated endothelial cells (Fig. 4). Treatment with TNF-α increased the adhesion of neutrophils by five- to six-folds. Pre-treatment of endothelial cells with 1,4-DHX inhibited the adhesion of neutrophils to TNF-α stimulated endothelial cells in a concentration dependent manner with almost 70.0% inhibition at a concentration of 50 μg/mL (Fig. 4). 1,4-DHX did not affect the adhesion of neutrophils to unstimulated endothelial monolayers. This suggests that 1,4-DHX is effective in blocking adhesion of neutrophils to the endothelial cells by inhibiting the TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin.

3. Discussion

In the present study we demonstrate for the first time that 1,4-DHX can be used for controlling expression of cell adhesion molecules and thus may be useful in the regulation of cellular trafficking. We analyzed its ability to inhibit TNF-α induced ICAM-1, VCAM-1 and E-selectin expression on endothelial cells. Further studies with 1,4-DHX demonstrate that it is equally effective in inhibiting LPS induced expression of ICAM-1. The inhibition by 1,4-DHX was found to be reversible implying it does not cause any permanent change in the endothelial cells.

Adhesion of leukocytes to the endothelial cells is the first step in the process of cellular migration where the expression of cell adhesion molecules on endothelial cells is a prerequisite. The receptors to ICAM-1, VCAM-1 and E-selectin are present on the circulating leukocytes, which then get arrested and migrate across the endothelium into the underlying tissues. As a functional consequence of inhibition of cell adhesion molecules, as expected, 1,4-DHX was found to inhibit the adhesion of neutrophils to endothelial cells induced with TNF-α.

To analyse the mechanism of inhibition of cell adhesion molecules by 1,4-DHX, we studied the time kinetics of ICAM-1 inhibition and found that 1,4-DHX should be added prior to or simultaneously with TNF-α to be effective. These results suggest that it may be interfering at an early stage of signalling event induced by TNF-α or LPS.

Studies by many groups including our study shows that the presence of hydroxyl group on the xanthone nucleus is useful for imparting anti-inflammatory or anti-oxidant activity. For instance, 1,4- and 2,6-dihydroxyxanthone, 3- and 1-hydroxyxanthone inhibit NADPH-catalysed liver microsomal lipid peroxidation,[22] norathyriol (1,3,6,7-tetrahydroxyxanthone), 1,3- and 3,5-dihydroxyxanthone prevent histamine release from stimulated rat peritoneal mast cells .[14] Norathyriol, 1,3- and 1,6-dihydroxyxanthone, 1,3,7-trihydroxyxanthone, 1,3,5,6-, 2,3,6,7-, and 3,4,5,6-tetrahydroxy xanthones have potent inhibitory effects on superoxide formation by rat neutrophils stimulated with fMLP.[12] 1,6 dihydroxyxanthone and Norathyriol also have profound inhibitory effects on hind paw oedema induced by various inflammatory mediators in mice.[13,14] Our study demonstrates the ability of 1,4-DHX to inhibit the expression of cell adhesion molecules. As the activation of cell adhesion molecules is involved in various pathways associated with inflammation, the results reported here may explain the mechanism underlying the activities of xanthones observed by other investigators.

The generation of reactive oxygen species (ROS) by inflammatory cytokines like TNF-α is a critical signal-

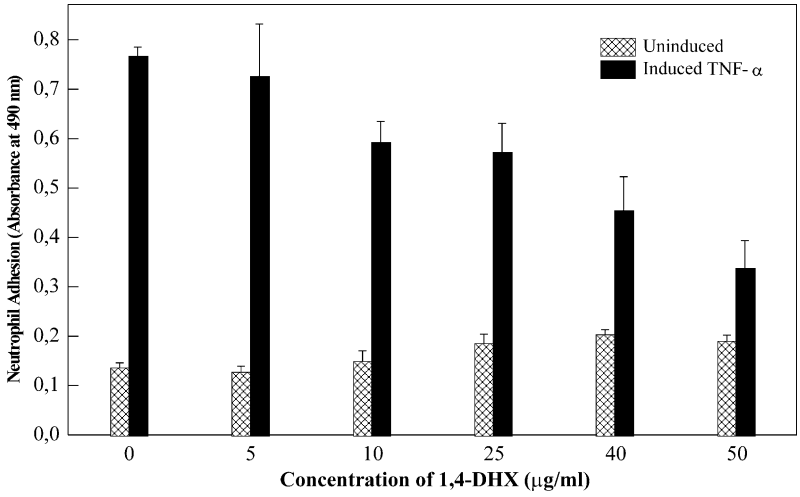

Figure 4. Inhibition of neutrophil adhesion to endothelial cells: Endothelial cells were grown to confluence in 96-well plates and incubated with or without indicated concentrations of 1,4-DHX for 1 h followed by induction without (open bars) or with (closed bars) TNF-α (10 ng/mL) for 6 h. The cells were then incubated with human peripheral neutrophils for 1 h. The amount of neutrophils adhering to the endothelial cell monolayers were measured by a colorimetric assay as described in Material and Methods. The data are representative of three independent experiments. Values shown mean + SD of quadruplicate wells.

ling mechanism mediating cell adhesion molecule expression in endothelial cells. Reactive oxygen species (ROS) generation serves as an important signal in the activation of transcription factors like NF-κB leading to the activation of cell adhesion molecules. Antioxidants like pyrrolidine dithiocarbamate (PDTC) and *N*-acetyl cysteine (NAC) are shown to exert their inhibitory effects on cell adhesion molecule expression by inhibiting the activation these transcription factors.[24] 1,4 DHX is a potent inhibitor of NADPH-catalysed microsomal lipid peroxidation thus has antioxidant activity.[22] Therefore, 1,4-DHX may interfere with the signalling pathways leading to activation cell adhesion molecules by preventing the generation of reactive oxygen species. The effect of 1,4 DHX on these transcription factors needs to be tested in future.

The ability of 1,4-DHX to effectively block the induced expression of cell adhesion molecules demonstrates its potential that may be tested in various inflammatory conditions where down-regulation of cell adhesion molecules is required. In our study, 1,4-DHX at a concentration of 280 μM (65 μg/mL) is found to be a potent inhibitor of cell adhesion molecule expression. It is comparable to the concentrations (10 μM to 1mM) required by other cell adhesion molecule inhibitors. For example, diclofenac inhibits at a concentration of 750 μM.[9] Pyrrolidine dithiocarbamate and *N*-acetyl cysteine are most effective at concentrations of 1 mM and 100 μM, respectively.[25] 1,4-DHX, therefore, works at a comparatively lower range of concentration and can be used as a lead molecule towards the development of anti-inflammatory drugs. It also offers a novel target for controlling various pathological conditions associated with upregulation of endothelial leukocyte adhesion molecules.

4. Experimental

4.1. Materials

Anti-E-selectin (BBA1) antibody was purchased from R&D Systems, California. Anti-ICAM-1, anti-VCAM-1 antibodies and TNF-α, were purchased from Pharmingen, USA. 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 and anti-mouse IgG-HRP were purchased from Sigma Chemical Co., USA. Fetal calf serum was purchased from Biological Industries, Israel. Anti-mouse-IgG-FITC was purchased from Becton & Dickinson, USA. 1,4-Dihydroxyxanthone was synthesized using published protocols.[26,27] It was identified on the basis of comparison of its spectral data and melting point with that reported in the literature. Its structure is shown in Figure 5.

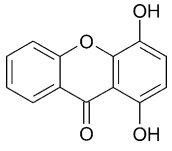

Figure 5. Structure of 1,4 Dihydroxyxanthone.

4.2. Cells and cell culture

For isolating the primary endothelial cells from the umbilical cord we used mild trypsinisation as described before.[8] Cells were maintained in gelatin coated tissue culture flasks 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, endothelial cell growth factor (50 μg/mL) and heparin (5 U/mL). The cells were subcultured by dislodging with 0.125% trypsin-0.01 M EDTA solution in Pucks saline and HEPES buffer. For the present analysis cells were used between passages three to four and the viability of cells was determined by trypan blue exclusion test. E-selectin expression was employed to determine the purity of endothelial cells.

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

ICAM-1 and E-selectin expression on surface of endothelial cells was quantified using cell-ELISA as described previously.[8] Endothelial cells were plated to confluence in gelatin coated 96-well plates. The cells were then incubated with or without 1,4-DHX at desired concentrations for the required period followed by treatment with TNF-α (10 ng/mL) or LPS (1 μg/mL) for 16 h for ICAM-1 expression and for 4 h for E-selectin expression. The cells were fixed with 1.0% glutaraldehyde and non-specific binding of antibody was blocked by using non-fat dry milk (3.0% in PBS). The cells were incubated overnight at 4 °C with ICAM-1 mAb or E-selectin mAb or control IgG Ab (0.25 μg/mL, diluted in blocking buffer). Following this the cells were washed with PBS and incubated with peroxidase-conjugated goat anti-mouse secondary Ab (1:1000 diluted in PBS). The cells were again washed with PBS and exposed to the peroxidase substrate (*o*-phenylenediamine dihydrochloride 40 mg/100 mL in citrate phosphate buffer, pH 4.5). 2 N sulphuric acid was added to stop the reaction and absorbance at 490 nm was measured using an automated microplate reader (Spectramax 190, Molecular Devices, USA).

4.4. Flow cytometry

Flow cytometric assays were performed for measuring the expression of ICAM-1, VCAM-1 and E-selectin on endothelial cells as described before.[8] The endothelial cells were incubated with or without 1,4-DHX for 1 h followed by induction with TNF-α (10 ng/mL) for 16 h for ICAM-1 and VCAM-1, and for 4 h for E-selectin expression. Following this the cells were washed with PBS and then dislodged. The cells were then incubated with anti-ICAM-1, anti-VCAM-1, anti-E-selectin or control IgG antibody (1.0 μg/10^6 cells, 30 min, 4 °C). After incubation the cells were washed twice with PBS for removing the unbound antibody and then incubated

with goat anti-mouse IgG-FITC antibody for 30 min at 4C. Paraformaldehyde (1.0%) was used to fix the cells. The cells were analysed using a flow cytometer (FACS-Vantage, Becton & Dickinson, USA). For each analysis, 20,000 events were collected and histograms generated. Histograms are presented as mean channel fluroscence (4 decades log scale) v/s relative cell number.

4.5. Neutrophil isolation

Neutrophils were isolated from peripheral blood of healthy individuals as described before.[28] The peripheral blood was collected in heparin solution (20 U/mL) and erythrocytes were removed by sedimentation against 6% dextran solution. Plasma rich in white blood cells was layered over ficoll-hypaque solution followed by centrifugation (300 *g* for 20 min, 20 °C). The top saline layer and the ficoll-hypaque layer were aspirated leaving the neutrophil/RBC pellet.

The residual red blood cells were removed by hypotonic lysis. Isolated cells were washed with PBS and resuspended in PBS containing 5 mM glucose, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ at a final concentration of 6×10^5 cells/mL. This procedure usually resulted in approximately 95% neutrophils and the cell viability was more than 95% as detected by trypan blue exclusion test.

4.6. Cell adherence assay

Adhesion of neutrophils to endothelial monolayers was assayed as described previously.[28] The endothelial cells were plated in 96-well culture plates at a density of 2×10^4 cells/well and allowed to adhere for 24 h. The cells were incubated with or without 1,4-DHX for 1 h followed by induction with TNF-α (10 ng/mL) for 6 h. Following incubation, the endothelial monolayers were washed twice with PBS and neutrophils (6×10^4/well) were added. After incubation for 1 h at 37 °C, the non-adherent neutrophils were removed by washing with PBS. The adherent neutrophils were assayed by adding a substrate solution (100 μL/well) consisting of *o*-phenylenediamine dihydrochloride (40 mg/100 mL in citrate phosphate buffer, pH 4.5) containing 0.1% cetitrimethyl ammonium bromide as peroxidase solubilising agent. The interference by few contaminating eosinophils was abolished by adding a selective eosinophil peroxidase inhibitor, 3-amino-1,2,4 triazole (1 mM) to the substrate solution. The reaction was stopped after 2 min of incubation by using 2N H_2SO_4 (50 μL/well). The absorbance was determined at 490 nm using an automated microplate reader (Spectramax 190, Molecular Devices, USA).

Acknowledgements

This work was supported by Council of Scientific and Industrial Research, India, and Department of Biotechnology, India. We are grateful to Dr. G. W. Rembhotkar for helping us with the flow cytometric analysis. We thank Dr. K. Muralidhar, University of Delhi for his advice and constructive criticisms.

References and notes

1. Bochner, B. S.; Luscinskas, F. W.; Gimbrone, M. A.; Newman, W.; Sterbinsky, S. A.; Anthony, C. P. D.; Klunk, D.; Schliemer, R. P. *J. Exp. Med.* **1991**, *173*, 1553.
2. Gorski, A. *Immunol. Today* **1994**, *15*, 251.
3. Osborn, L. *Cell* **1990**, *62*, 3.
4. Butcher, E. C. *Cell* **1991**, *67*, 1033.
5. Springer, T. A. *Cell* **1994**, *76*, 301.
6. Mantovani, A.; Bussolino, F.; Introna, M. *Immunol. Today* **1997**, *18*, 231.
7. Yusuf-Makagiansar, H.; Anderson, M. E.; Yakovleva, T. V.; Murray, J. S.; Siahaan, T. J. *Med. Res. Rev.* **2002**, *22*, 146.
8. Gupta, B.; Ghosh, B. *Int. J. Immunopharmacol.* **1999**, *21*, 745.
9. Sakai, A. *Life Sci.* **1996**, *58*, 2377.
10. Weiser,M.R.; Gibbs, S.A.L.; Hechtman, H.B. In *Adhesion Molecules in Health and Disease*; Paul, L.C.; Issekutz, T.B.; Ed Marcel Dekker Inc: New York: 1997; p 55.
11. Collins, T.; Read, M. A.; Neish, A. S.; Whitley, M. Z.; Thanos, D.; Maniatis, T. *FASEB J.* **1995**, *9*, 899.
12. Hsu, M. F.; Raung, S. L.; Tsao, L. T.; Lin, C. N.; Wang, J. P. *Free Radic. Biol. Med.* **1997**, *23*, 1035.
13. Wang, J. P.; Raung, S. L.; Lin, C. N.; Teng, C. M. *Eur. J. Pharmacol.* **1994**, *251*, 35.
14. Lin, C. N.; Chung, M. I.; Liou, S. J.; Lee, T. H.; Wang, J. P. *J. Pharm. Pharmacol.* **1996**, *48*, 532.
15. El Sayah, M.; Cechinel-Filho, V.; Pinheiro, T. R.; Yunes, R. A.; Calixto, J. B. *Inflamm. Res.* **1999**, *48*, 218.
16. Ito, C.; Itoigawa, M.; Furukawa, H.; Rao, K. S.; Enjo, F.; Bu, P.; Takayasu, J.; Tokuda, H.; Nishino, H. *Cancer Lett.* **1998**, *132*, 113.
17. Hein, S. M.; Gloer, J. B.; Koster, B.; Malloch, D. *J. Nat. Prod.* **1998**, *61*, 1566.
18. Iinuma, M.; Tosa, H.; Tanaka, T.; Asai, F.; Kobayashi, Y.; Shimano, R.; Miyauchi, K. *J. Pharm. Pharmacol.* **1996**, *48*, 861.
19. Ignatushchenko, M. V.; Winter, R. V.; Riscoe, M. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 77.
20. Saxena, A. M.; Bajpai, M. B.; Mukherjee, S. K. *Indian J. Exp. Biol.* **1991**, *29*, 674.
21. Liu, H. S.; Lin, C. N.; Won, S. J. *Anticancer Res.* **1997**, *17*, 1107.
22. Madan, B.; Singh, I.; Kumar, A.; Prasad, A. K.; Raj, H. G.; Parmar, V. S.; Ghosh, B. *Bioorg. Med. Chem.* **2002**, *10*, 3431.
23. Essani, N. A.; Bajt, M. L.; Farhood, A.; Vonderfecht, S. L.; Jaesckhe, H. *J. Immunol.* **1997**, *158*, 5941.
24. Rahman, A.; Kefer, J.; Bando, M.; Niles, W. D.; Malik, A. B. *Am. J. Physiol.* **1998**, *275*, L533.
25. Weber, C.; Erl, W.; Pietsch, A.; Strobel, M.; Ziegler-Heitbrock, H. W. L.; Weber, P. C. *Arterioscler. Thromb.* **1994**, *14*, 1665.
26. Patel, G. N.; Trivedi, K. N. *Indian J. Chem.* **1988**, *27B*, 458.
27. Pankajamani, K. S.; Sheshadri, T. R. *J. Scient. Ind. Res.* **1954**, *13B*, 396.
28. Madan, B.; Batra, S.; Ghosh, B. *Mol. Pharmacol.* **2000**, *58*, 526.