Physiol. Res. 47: 439–443, 1998

Expression of Adhesion Molecules and Effect of Disodium Cromoglycate Treatment in Asthmatics

E. JAHNOVÁ, M. HORVÁTHOVÁ, F. GAZDÍK

Institute of Preventive and Clinical Medicine, Department of Clinical Immunology, Bratislava, Slovak Republic

Received June 6, 1998
Accepted July 14, 1998

Summary

Allergic processes are complex disorders in which inflammatory and immunological mechanisms are involved. Many studies indicate that the adhesion molecules are upregulated in allergic inflammation, and play a critical role in the pathogenesis of allergic inflammation. Modulation of the expression of adhesion molecules may provide a potential new target for therapeutic intervention in allergic diseases. In the present study the changes expression of adhesion molecules CD11a, CD18 (LFA-1), CD54 (ICAM-1) and L-selectin (CD62L) and VLA-4 (CD49d) were analysed by flow cytometry. Serum concentrations of soluble ICAM-1, VCAM-1 and soluble low affinity receptor for IgE concentrations sCD23 were measured by ELISA in atopic patients with mild asthma before and after treatment by disodium cromoglycate (DSCG). The most significant finding was a significant decrease of ICAM-1 expression on monocytes and CD49d on monocytes and lymphocytes as well as an increase of L-selectin expression on monocytes after treatment with DSCG, without any associated effect on CD11a and CD18. The levels of soluble ICAM-1 and VCAM-1 were not changed, only the levels of soluble CD23 that plays a regulatory role in ongoing IgE production, were decreased in asthmatic patients after the treatment with DSCG. These results suggest that DSCG diminishes cell activation.

Key words

Allergic rhinitis – Adhesion molecules – Asthma – Disodium cromoglycate

Introduction

Cell adhesion molecules are glycoproteins, identified within the last decade, expressed on all surfaces that allow cell-to-cell contact. They have a variety of functions. Adhesion molecules act through cell adhesion, promote leukocyte-vascular endothelium and leukocyte-extracellular tissue matrix adhesion, are leukocyte migration from vascular compartments into extravascular tissues, stimulate antigen-specific recognition by T lymphocytes, are costimulatory signals for T cell activation, stimulate effector mechanisms of activated lymphocytes and enhance cell proliferation and regulation of cell growth (Patarroyo and Makgoba 1989, Springer 1990). Many studies indicate that these adhesion molecules are upregulated in allergic inflammation, and play a critical role in the pathogenesis of allergic inflammation (Leung *et al.* 1991, Kyan-Aung *et al.* 1991, Gundel *et al.* 1991, Wegner *et al.* 1990). The modulation of adhesion molecule expression may provide a potential new target for therapeutic intervention in allergic diseases. Cell adhesion molecules include a wide variety of member receptors that belong to the selectin, integrin, and immunoglobulin superfamilies (Springer 1990).

Fax +420 2 293 894
E-mail physres@sun1.biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

In the present study, we investigated the expression of intercellular adhesion molecule-1 (ICAM-1, CD54), L-selectin (CD62L), β2-integrin LFA-1 (CD11a/CD18) and the expression of α4 subunit of β1-integrins (CD49d) in patients with allergic rhinitis and mild asthma before and after treatment by disodium cromoglycate (DSCG) with the aim of assessing how and which adhesion molecules are participating in the mechanisms of DSCG.

Material and Methods

Patients

Forty-two patients (average age 31 years) with the history of seasonal pollen rhinoconjunctivitis and mild asthma were treated with DSCG in a regular dosage scheme for 6 weeks (administered by inhalation of the powder by a spinhaler, 1 capsule (20 mg) 3–4 times daily for 6 weeks). Rhinitis patients were defined as having rhinitis symptoms (nasal blockage, sneezing, and nasal discharge). All of them exhibited positive responses to the skin prick test with a standard set of allergens and positive nonspecific inhalation challenge test (assessment of PD 15). At the time of the study the patients were not in the state of acute respiratory distress. No patient had any signs of upper or lower respiratory affliction for at least 3 weeks before the study and were not receiving any concomitant antiinflammatory therapy. Informed consent was obtained from all patients. The control group (n = 45, mean age 36 years) consisted of volunteers who had no history of allergic or nasal disease.

Flow cytometry analysis

Differentiation antigens CD11a, CD18, CD54, CD49d, CD62-L and control mouse IgG_1,IgG_{2a} were quantified with monoclonal antibodies conjugated to fluorescein (FITC) or phycoerytrin (PE) from Becton Dickinson. The leukocyte subsets were assessed by setting gates due to forward and side scatter characteristics. Phenotypical analysis of the cells was performed using one-colour flow cytometry. Ten microlitres of fluorescein isothiocyanate (FITC)-conjugated anti CD11a, anti CD18, anti CD62L and 10 μl of phycoerythrin-conjugated anti CD54, anti CD49d were added to 50 μl of whole blood and incubated for 15 min at room temperature. Thereafter, 250 μl lysing solution OptiLyse C were added and incubated for 10 min. After lysis of red blood cells, 250 μl phosphate-buffered saline were added followed by brief vortex mixing. After 20 min, the cells were analysed by flow cytometry using an Epics XL Coulter. Cells were gated using forward *versus* side scatter to exclude dead cells and debris. Nonspecific immunofluorescence was determined using isotype-controls.

Soluble CD23 and sICAM-1 and sVCAM-1 assay

Serum sICAM-1, sVCAM-1 and sCD23 levels were measured by Sandwich enzyme-linked immunoassay (Bender MedSystems). The limit of detection for sCD23 defined as the analytic concentration resulting in absorption significantly higher than the absorption of the dilution medium (mean plus three standard deviations) was determined to be 1.3 U/ml. The limit of detection for sICAM-1 was determined to be 3.3 ng/ml. A coloured product was formed in proportion to the amount of sICAM-1, sVCAM-1 and sCD23 present in the sample. The absorbance was measured at 450 nm.

Statistical analysis

Statistical analysis was performed by Wilcoxon test. Differences associated with probability values of $p<0.05$ were considered to be statistically significant.

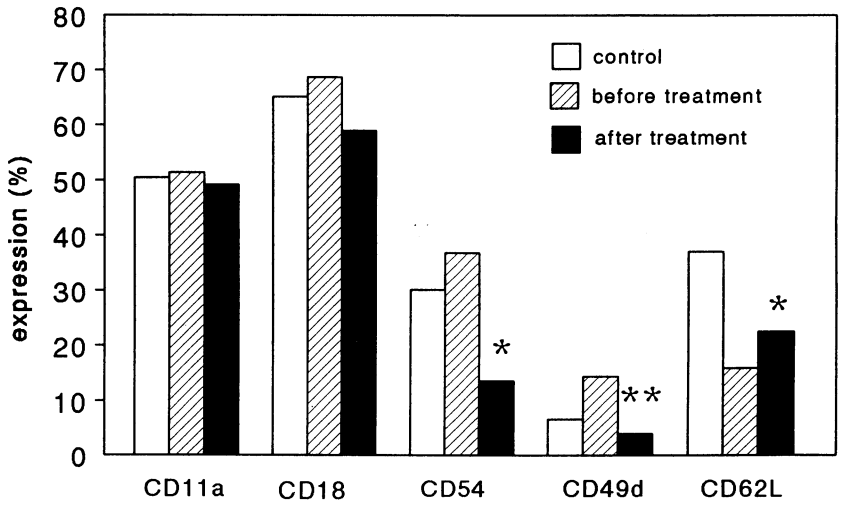

Fig. 1. *Effect of DSCG on the CD11a, CD18, CD54, CD62L and CD49d expression on lymphocytes or monocytes in patients with allergic rhinitis and mild asthma (n = 42) before and after treatment by DSCG and in the control group (n = 45). Data are expressed as lymphocyte or monocyte percentage mean values. * $P<0.05$, ** $P<0.005$ as compared with the group before therapy.*

Results

Patients

The basic spirometric parameters (FVC and FEV1) were not changed after therapy. The total amount of acetylcholine, which led to a 15 % decrease of FEV1 (PD 15), was calculated in each patients (490 mg vs 1894 mg). The administration of DSCG was very effective in suppressing bronchial hyperreactivity. In our study, bronchial hyperreactivity disappeared or decreased in 71.4 % of patients with this affliction.

Effect of disodium cromoglycate (DSCG) on the expression of adhesion molecules in peripheral blood mononuclear cells

After treating patients suffering from allergic rhinitis and mild asthma with DSCG, we observed a decreased adhesion molecules expression of CD54 36.93±9.40 vs 13.65±4.55 on monocytes ($p<0.05$) and CD49d 14.38±6.71 vs 4.00±2.43 on lymphocytes ($p<0.005$), 20.74±11.6 vs 6.3±3.24 on monocytes ($p<0.01$) and increased CD62L 15.94±7.78 vs 22.49±11.51 on monocytes ($p<0.05$). The expression of CD11a and CD18 were not significantly affected (Fig. 1).

Effects of DSCG on sICAM-1, sVCAM and sCD23

The levels of serum sICAM-1 and sVCAM-1 were not changed after treatment with DSCG (Fig. 2), but IgE soluble binding receptor sCD23 was significantly decreased after the treatment with DSCG (280.71±138.94 vs 172.41±138.62, $p<0.002$).

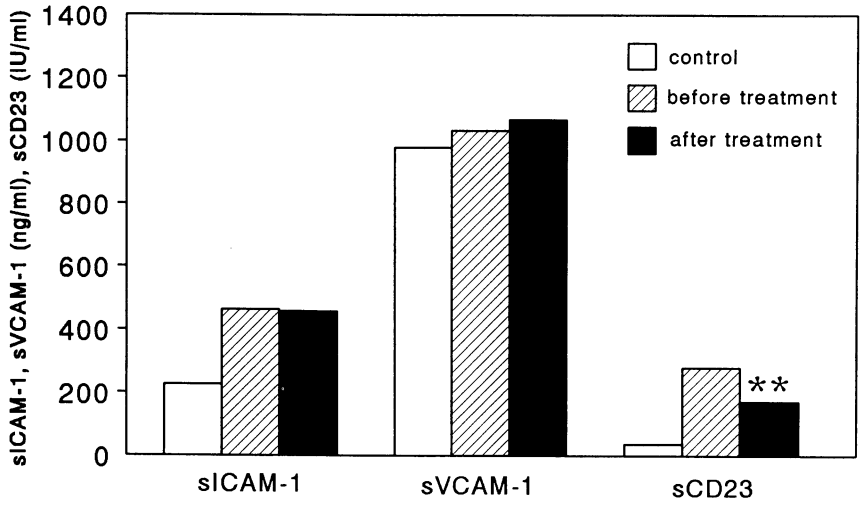

Fig. 2. *Effect of DSCG on serum levels of sICAM-1 (ng/ml), sVCAM-1 (ng/ml) and sCD23 (U/ml) in patients with allergic rhinitis and mild asthma (n = 42) and control group (n = 45) before and after treatment by DSCG. ** $P<0.002$ as compared with the group before therapy.*

Discussion

In this study, the change of expression of adhesion molecules CD11a, CD18 (LFA-1), CD54 (ICAM-1) and L-selectin (CD62L) and VLA-4 (CD49d) were analysed by flow cytometry. The serum concentrations of soluble ICAM-1, VCAM-1 and soluble low affinity receptor for IgE concentrations sCD23 were measured by ELISA in atopic patients before and after the treatment by disodium cromoglycate (DSCG).

After the treatment with DSCG, a decrease of CD54 on monocytes and CD49d on monocytes and lymphocytes as well as an increase of L-selectin on monocytes was observed, but was without any effect on CD11a and CD18.

Several reports have studied the effect of various antiinflammatory drugs on adhesion molecules expression. Cyclosporin A has been shown to inhibit ICAM-1 synthesis by endothelial cells in healing psoriatic plaques (Petezelbauer 1991) and glucocorticoids inhibit IL-1-induced expression of selectin E (Crostein 1992). Deflazacord, an effective oxazoline derivate of prednisone, was found to block late-phase reaction events, including ICAM-1 expression on the endothelium. Deflazacord also downregulated ICAM-1 expression during the early phase. The latter effect is the first description of steroid activity during the early phase of an allergic reaction (Ciprandi 1993). Another study compared the effects of terfenadine and placebo in patients with allergic rhinitis during the pollen season. Terfenadine was also found to be effective in reducing infiltration by proinflammatory cells, particularly eosinophils, and in the reduction of ICAM-1 expression by epithelial cells. The new generation of antihistamines such as cetirizine and terfenadine might exert subsidiary antiinflammatory effects *via* their effect on ICAM-1 expression (Butcher 1991, Springer 1994).

It has been established that interaction of leukocytes with the endothelium is a key step in the development of inflammation. Migration of immune cells from the blood stream to inflammatory foci follows a pattern that includes rolling of leukocytes on the endothelium and their firm adhesion and extravasation (Bevilacqua 1993, Bevilacqua and Nelson 1993). The first step is reversible rolling mediated by the selectins. The second step (firm adhesion) involves leukocyte activation in which L-selectin is downregulated and β_2 integrins CD11a-c/CD18 are upregulated. We found that after treatment with DSCG, lymphocytes and monocytes are less activated that before the treatment. L-selectin is constitutively expressed by resting leukocytes and, upon cell activation, this molecule is proteolytically removed from the cell membrane (Kishimoto *et al.* 1989, Kahn *et al.* 1994, Dougherty *et al.* 1988). The activated lymphocytes demonstrate enhanced ICAM-1 expression (Dustin *et al.* 1986). Previous reports have shown that DSCG inhibits the adhesion molecules expression and may reduce the activation of neutrophils, eosinophils and monocytes *in vitro* (Hoag and McFadden 1991). The expression of CD54 (ICAM-1) and β_1 integrin VLA-4 (CD49d) was decreased in patients with DSCG but that of CD62L (L-selectin) was increased after the treatment, while no effects on CD11a and CD18 (LFA-1) were found. In biopsies of bronchial mucosa, the expression of ICAM-1, VCAM-1 and ELAM-1 was decreased after the administration of DSCG in asthmatic patients (Hoshino and Nakamura 1995). It was also concluded that sodium cromoglycate does have an effect on the infiltration of bronchial mucosa by inflammatory cells and also on the expression of adhesion molecules (Hoshino and Nakamura 1997).

The levels of soluble ICAM-1 and sVCAM-1 were not decreased after the DSCG treatment. Circulating forms of cell adhesion molecules can be regarded as markers for the presence of inflammation (Gundel *et al.* 1992). The circulating levels of ICAM-1 and E-selectin, but not VCAM-1 were significantly elevated in peripheral blood of patients with acute asthma compared to those of stable asthmatic patients or normal volunteers (Schroth 1996). The functional activity of sICAM-1 has also been investigated. Soluble ICAM-1 has been shown to inhibit the natural killer and lymphokine-activated lymphocyte activities (Calderon and Lockey 1992). This effect is presumably mediated by competitive inhibition of adherence. The levels of the soluble CD23, that plays a regulatory role in IgE production, were decreased in asthmatic patients after treatment with DSCG.

It can be hypothesized that the reduced expression of inflammatory cellular adhesion molecules will reduce the susceptibility to common multifactorial diseases that also have an inflammatory component. Furthermore, this hypothesis raises the possibility that drugs which block the expression of inflammatory cellular adhesion molecules might be of therapeutic benefit in selected diseases (Bevilacqua 1993).

We may thus conclude that the effect of DSCG in the activation of lymphocyte and monocyte cells is mediated by modulating the expression of cell adhesion molecules. The results suggest that DSCG diminishes the effect on cells. We suppose that this phenomenon could be important in the explanation of antinflammatory effect of DSCG.

Acknowledgements

I would like to thank dr. I. Kozlovská, and T. Faybíková for clinical management of patients during the study.

References

BEVILACQUA M.P.: Endothelial-leukocyte adhesion molecules. *Annu. Rev. Immunol.* **11:** 767–804, 1993.

BEVILACQUA M.P., NELSON R.M.: Selectins. *J. Clin. Invest.* **91:** 379–387, 1993.

BUTCHER E.C.: Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* **67:** 1033–1036 1991.

CALDERON E., LOCKE R.F.: A possible role for adhesion molecules in asthma. *J. Allergy Clin. Immunol.* **90:** 852–865, 1992.

CIPRANDI G., BUSCAGLIA S., PESCE G.P., IUDICE A., BAGNASCO M., CANONICA G.W.: Deflazacort protects against late-phase but not early-phase reactions induced by the allergen-specific conjuctival provocation test. *Allergy* **48:** 421–430, 1993.

CROSTEIN B.N., KINNEL S.C., LEVIN R.I., MARTINIUK F., WEISSMAN G.: A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of ELAM-1 and ICAM-1. *Proc. Natl. Acad. Sci. USA* **89:** 45–56, 1992.

DOUGHERTY G.J., MURDOCH S., HOGG N.: The function of human intercellular adhesion molecule-1 (ICAM-1) in the generation of an immune response. *Eur. J. Immunol.* **18:** 35–39, 1988.

DUSTIN M.L., ROTHLEIN R., BHAN A.K., DINARELLO C.A., SPRINGER T.A.: Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of an intercellular adhesion molecule (ICAM-1). *J. Immunol.* **137:** 245–254, 1986.

GUNDEL R.H., WEGNER C.D., TORCELLINI C.A.: Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *J. Clin. Invest.* **88:** 1407–1411, 1991.

GUNDEL R.H., WEGNER C.D., TORCELLINI C.A., LETTS L.G.: The role of intercellular adhesion molecule-1 in chronic airway inflammation. *Clin. Exp. Allergy* **22:** 569–575, 1992.

HOAG J.E., McFADDEN E.R.: Long-term effect of cromolyn sodium on nonspecific bronchial hyperresponsiveness: a review. *Ann. Allergy* **66:** 53–63, 1991.

HOSHINO M., NAKAMURA Y.: The effect of disodium cromoglycate (DSCG) on infiltration of inflammatory cells into bronchial mucosa and expression of adhesion molecules in asthmatics. *Arerugi* **44:** 593–601, 1995.

HOSHINO M., NAKAMURA Y.: The effect of inhaled disodium cromoglycate on cellular infiltration into the bronchial mucosa and expression of adhesion molecules in asthmatics. *Eur. Resp. J.* **10:** 885–865. 1997.

KAHN J., INGRAHAM R.H., SHIRLEY F., MIGAKI G.I., KISHIMOTO T.K.: Membrane proximal cleavage of L-selectin: identification of the cleavage site and a 6-kD transmembrane peptide fragment of L-selectin. *J. Cell. Biol.* **25:** 461–470, 1994.

KISHIMOTO T.K., JUTILA M.A., BERG E.L., BUTCHER E.C.: Neurophil MAC-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science* **245:** 1238–1241, 1989.

KYAN-AUNG U., HASKARD D.O., POSTON R.N., THORNILL M.H., LEE T.H.: Endothelial leukocyte adhesion molecule-1 and intracellular adhesion molecule-1 mediate the adhesion of eosinophils to endothelial cells in vitro and are expressed by endothelium in allergic cutaneous inflammation in vivo. *J. Immunol.* **146:** 521–528, 1991.

LEUNG D.Y.M., POBER J.S., CORTON R.S.: Expression of endothelial leukocyte conjunctiva provocation test. *Allergy* **48:** 421–30, 1993.

PATARROYO M., MAKGOBA M.W.: Leukocyte adhesion to cells in immune and inflammatory response. *Lancet* **2:** 1139–1142, 1989.

PETEZELBAUER P., STINGL G., WOLFF K., VOLC-PLATZER B.: Cyclosporin A suppress ICAM-1 expression by papillary endothelium in healing psoriatic plaques. *J. Invest. Dermatol.* **96:** 362–369, 1991.

SCHROTH M.K.: Adhesion molecules in asthma and allergy. *Mol. Biol. Allergy Immunol.* **16:** 643–657, 1996.

SPRINGER T.A.: Adhesion receptor of the immune system. *Nature* **346:** 425–434, 1990.

SPRINGER T.A.: Traffic signals for lymphocyte recirculation and leucocyte emigration: the multistep paradigm. *Cell* **76:** 301–314. 1994.

WEGNER C.D., GUNDEL R.H., PEILLY P.: Intercellular adhesion molecules-1 (ICAM-1) in the pathogenesis of asthma. *Science* **247:** 456–459,1990..

Reprint requests

Dr. E. Jahnová, Institute of Preventive and Clinical Medicine, Department of Clinical Immunology, Limbová 14, SK-833 01 Bratislava, Slovak Republic.