

SHORT COMMUNICATION

Effects of selenium supplementation on expression of adhesion molecules in corticoid-dependent asthmatics

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*Institute of Preventive and Clinical Medicine, Department of Clinical Immunology, Bratislava, Slovakia. jahnova@upkm.sk***Abstract**

Selenium (Se) deficiency attenuates the host immune response, thereby increasing the risk of bacterial and viral infections. We have examined the effects of selenium supplementation (SeS) in corticoid-dependent asthmatics (CDAs) with lowered circulatory Se status. Twenty CDAs (10 males and 10 females, average age 54.5 yrs) were enrolled into the study. The average duration of the disease was 10 yrs. The asthmatics were receiving 200 µg of Se per day for a period of 6 months, in addition to regular treatment with inhaled corticosteroids and β-agonists. The expression of adhesion molecules (CD11a, CD11b, CD18, CD49d, CD54, CD62L) on peripheral blood mononuclear cells (PBMCs) of asthmatics and the expression of E- and P-selectins, ICAM-1, VCAM-1 on cultured human umbilical vein endothelial cells (HUVEC) after stimulation with PBMCs from CDAs before and after 3 and 6 months of SeS were assessed by standard monoclonal antibodies and analyzed by flow cytometry. The concentrations of soluble adhesion molecules P-selectin, E-selectin, ICAM-1 and VCAM-1 were determined by ELISA method.

The expression of adhesion molecules on PBMCs: After 3- and 6-months of SeS, a decreased expression of molecules CD11a, CD11b and CD62L was observed ($p < 0.02$, $p < 0.005$, $p < 0.003$). No changes were seen in the expression of CD18, CD49d except for the increased expression of CD54 ($p < 0.005$). Modulation of adhesion molecules expression on HUVEC: We observed a significant increase in VCAM-1, P- and E-selectins expressions in the group of asthmatics without SeS in comparison with the control group ($p < 0.05$, $p < 0.01$, $p < 0.05$). During SeS a significant decrease in molecules VCAM-1, E-selectin (after 3 months) ($p < 0.05$, $p < 0.05$) and P-selectins and ICAM-1 (after 6 months) ($p < 0.05$, $p < 0.01$) were observed. Soluble adhesion molecules: After 3 month of SeS we noticed a significant decrease in VCAM-1 and P-selectin expressions ($p < 0.05$, $p < 0.05$) and after 6 months the level of VCAM-1 decreased ($p < 0.01$). The effect of Se on the adhesion molecules expression in endothelial cells in vitro experiments: Se blocks the expression of adhesion molecules stimulated by IFN-γ in a dose-dependent way after addition of Se into a culture of endothelial cells. Concentration of 10 µg/ml inhibits the increase in expression of ICAM-1 ($p < 0.05$) but not that of VCAM-1, E- or P-selectins. The inhibition of expression in Se concentration of 10 µg/ml is over 80 % ($p < 0.01$).

Our data demonstrate that Se is able to affect the adhesion molecules expressions that are crucial in the inflammatory process. (*Fig. 5, Ref. 22.*)

Key words: corticoid-dependent asthmatics, selenium supplementation, adhesion molecules, endothelial cells.

Adhesion molecules represent glycoproteins expressed on cellular surface mediating interactions between both the cells and the extracellular matrix. They have a crucial role in the migration of cells from blood vessels to the site of inflammation (Etzioni, 1994). Selectins, integrins and molecules of immunoglobulin superfamily mediate the cellular interactions, influence the

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adhesion and migration of cells into the site of inflammation. These molecules represent an important step in response to the infectious stimulus or tissue damage (Faull, 1995). Cytokines are low-molecular-weight proteins that regulate the expression of adhesion molecules (Flatt et al, 1995). Endothelial expression of adhesion molecules is a dynamic process influenced by specific mediators and the duration of the provoking agent. Some adhesion molecules act as signal molecules, which affect various cellular activities, for example degranulation and secretion of leukotrien C4 and the production of superoxid in eosinophils (Eo) (Hirai et al, 1996; Kadrabová et al, 1996; Shiota et al, 1996) and etc.

Selenium (Se) is important for the optimal function of the immune system. Deficiencies in Se attenuate especially the cellular immune response by oxidative stress, and increase the risk of bacterial and viral infections (Henricks et al, 1996). The function of glutathione peroxidase enzyme depends on Se and protects the cells from oxidative damage. Asthma bronchiale (AB) represents a disease with an increased production of free oxygen radicals produced from accumulated Eo, T-lymphocytes, polymorfonuclears and mastocytes (Beasley et al, 1991; Calderon et al, 1992; Lockey, 1992; Horváthová et al, 1999). Impaired antioxidative defence system (decreased activity of scavenger free oxygen radicals) may participate in the maintenance of chronic inflammation. The deficiency of Se in asthmatics could be independent from the activity of glutathione peroxidase caused by a decreased intake of Se in food or from an increased consumption of the enzyme in site of chronic inflammation (Xia et al, 1998).

The aims of the study

The aim of the study was to assess the influence of selenium supplementation (SeS) in corticoid-dependent asthmatics (CDAs) on adhesion molecules expression in peripheral blood on mononuclear cells (PBMCs), endothelial cells and plasma levels of soluble adhesion molecules, respectively. The next aim of the study was to determine the *in vitro* effect of Se on adhesion molecules expression on endothelial cells, by previous stimulation by IFN-gamma.

Material and methods

Twenty CDAs (10 males and 10 females) at average age of 54.5 yrs, treated by inhaled and systemic corticosteroids for more than 10 yrs were enrolled into the study. In all asthmatics suboptimal Se status was determined. The SeS was done by the administration of 200 µg Se daily during a six-month follow up. All included subjects were nonsmokers and had not been treated by Se preparation less than 6 months before the inclusion into the study. The blood was collected before the supplementation and after 3 and 6 months of SeS.

The expression of adhesion molecules on PBMCs and endothelial cells before and after SeS was assessed by flow cytometry analysis (Coulter EPICS — XL). The assessment of Se

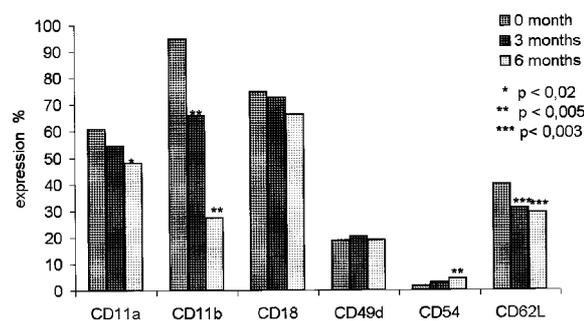


Fig. 1. Expression of adhesion molecules on the mononuclears in the peripheral blood (PBMCs). The expression was measured after 3 and 6 months of selenium supplementation. Values are expressed as the mean \pm SD of groups in the study period. The statistical significance is expressed by values $p < 0.05$.

effects on the expression of adhesion molecules on endothelial cells after the previous stimulation by IFN-gamma was done by the same method. Monoclonal antibodies conjugated with fluorescein (FITC) or phycoerthrin (PE) (produced by Becton Dickinson and Immunotech companies) directed against antigens CD11a, CD11b, CD18, CD49d, CD54, CD 62L and controls IgG1 and IgG2 were used. The soluble adhesion molecules ICAM-1, VCAM-1, P-selectin, E-selectin were assessed by ELISA method (tests produced by company Bender Ned Systems) with detected limits of 0.5 µg/ml for E and P-selectins, 0.9 µg/ml for VCAM and 3.3 µg/ml for ICAM-1, respectively.

Human umbilical vein endothelial cells (HUVEC) were isolated and cultured on the gelatine-coated Petri dishes, with RPMI 1640 (BioWhittaker, Inc.) and addition of 5 % bovine fetal serum (BioWhittaker, Inc.), antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml gentamicin), 1 % L-glutamine and growth factor for endothelial cells (30 µg/ml, Sigma Chemical Co.).

Results

The expression of adhesion molecules on PBMCs: After 3- and 6-months of SeS decreased expression of molecules CD11a, CD11b and CD62L were observed ($p < 0.02$, $p < 0.005$, $p < 0.003$). No changes have been seen in the expression of CD18, CD49d except for the increased expression of CD54 ($p < 0.005$) (Fig. 1).

Modulation of adhesion molecules expression on HUVEC: Vascular endothelium represents a crucial role in immune reactions. Adhesion molecules localized on the cell surface (for example ICAM-1, VCAM-1, P- and E-selectins) are activated in process of leukocytes migration in many inflammatory diseases including AB. The presented study is further focused on the assessment as to whether SeS in CDAs modulates the expression of adhesion molecules in cultured endothelium. HUVEC were stimulated by PBMC, isolated from peripheral blood of healthy subjects and CDAs before and after SeS. We observed a significant increase in VCAM-1, P- and E-selectins expression in gro-

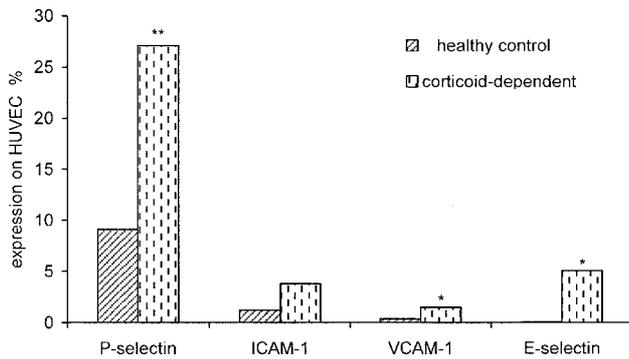


Fig. 2. Modulation of adhesion molecules expression on HUVEC after PBMCs stimulation. PBMCs were isolated from corticoid-dependent asthmatics. HUVEC were exposed to PBMCs from normal controls (n=21) or asthmatic individuals (n=17) in standard incubation periods (see Material and Methods). The cells were stained for P-selectin, ICAM-1, VCAM-1, ELAM-1 or isotype control and analyzed by flow cytometry. * $p < 0.05$ comparison to healthy donors.

up of asthmatics without SeS in comparison with the control group ($p < 0.05$, $p < 0.01$, $p < 0.05$) (Fig. 2).

During SeS, a significant decrease in molecules VCAM-1, E-selectin (after 3 months) ($p < 0.05$, $p < 0.05$) and P-selectin and ICAM-1 (after 6 months) ($p < 0.05$, $p < 0.01$) were observed (Fig. 3).

Effect of SeS on soluble adhesion molecules: After 3 months of SeS we noticed a significant decrease in VCAM-1 and P-selectin expressions ($p < 0.05$, $p < 0.05$), and after 6 months the level of VCAM-1 decreased ($p < 0.01$) (Fig. 4).

The effect of Se on the adhesion molecules expression in endothelial cells in vitro experiments: Se blocks the expression of adhesion molecules stimulated by IFN-gamma in a dose-dependent way after addition of Se into a culture of endothelial cells. The concentration of 10 $\mu\text{g/ml}$ inhibits the increase in expression of ICAM-1 ($p < 0.05$) but not that of VCAM-1, E- or P-selectins. The inhibition of expression in the Se concentration of 10 $\mu\text{g/ml}$ is higher than 80 % ($p < 0.01$) (Fig. 5).

Discussion

AB represents a chronic inflammatory disease with infiltration of mucosa by Eo, mastocytes, T lymphocytes and other cells. Mediators of inflammation are released from activated cells and change the physiology of the airways (Caldéron and Lockey, 1992; D'alesio et al, 1998). The migration and activation of cells is also accompanied by an increase in expression of adhesion molecules localized on leukocytes, epithelial and endothelial cells (Beasley et al, 1991). In asthmatics a decrease in Se status and an increase in production of free reactive oxygen radicals were reported (Imhof and Dunon, 1997; Kobayashi et al, 1994). Se incorporated within the enzyme glutathione peroxidase represents one of most important antioxidants in humans (Hasselmark et al, 1990; Kobayashi et al, 1994; Pichetti et al, 1998; Pitzalis, 1993).

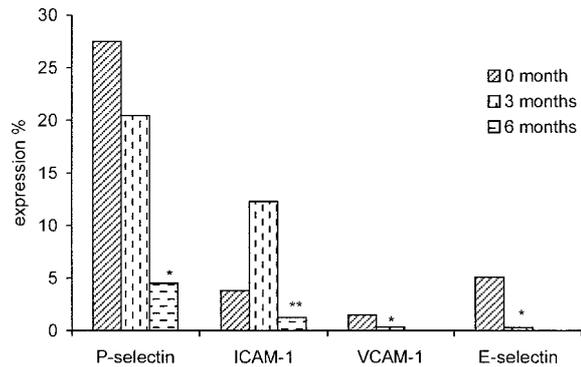


Fig. 3. Effect of selenium supplementation in asthmatics on the adhesion molecules expression on HUVEC. Cells were treated in standard incubation periods. The expression of adhesion molecules P-selectin, ICAM-1, VCAM-1 and E-selectin were measured by flow cytometry before selenium supplementation (month 0) and after 3 and 6 months thereof. The zero level represents HUVEC control medium (alone). Statistical significance: * $p < 0.05$, ** $p < 0.01$.

The group of CDA with suboptimal Se plasma levels showed a significant increase in molecules expression CD11a, CD11b and CD49d on PBMC in comparison with healthy volunteers. An increase in expression of adhesion molecules usually accompanies the activation of cells. This phenomenon is seen in diseases with immunopathogenetic origin such as autoimmune or allergic diseases (Brezinček et al, 1999). Our results showed a significant decrease in CD11a, CD11b and CD62L expression on PBMCs after SeS. The levels of soluble adhesion molecules such as P-selectin and VCAM-1 in plasma of asthmatics were also decreased. It is known that the total status of nutrition limits the integrity and function of endothelial cells by the development of defined pathologic conditions.

Adhesion molecules play the key role in the pathogenesis of AB (Beasley, 1991; Pichetti et al, 1998; Xia et al, 1998). Several authors reported the results of increased concentrations of soluble ICAM-1, VCAM-1 and E-selectin in plasma or in bronchoalveolar lavage in acute phase of AB (Moutet et al, 1998; Winn et al, 1998). Our in vitro tests showed a higher expression of P-, E-selectins and VCAM-1 on endothelial cells after incubation with asthmatic PBMCs in comparison with controls. Expression of ICAM-1, VCAM-1, E- and P-selectins on endothelial cells is regulated by cytokines with proinflammatory activity such as: IL-1, TNF-alpha, IFN-gamma or IL-5 (Etzioni, 1996; Hori et al, 1997; Moutet et al, 1998; Pitzalia, 1993; Winn et al, 1998). After SeS we observed a decrease in adhesion molecules expression on endothelial cells. The results presented study widen our knowledge about the role of SeS in CDAs. The significance of Se was demonstrated also in vitro experiments. An addition of Se decreases the expression of adhesion molecules on endothelial cells that were stimulated by IFN-gamma. We demonstrated that Se inhibited the expression of the above mentioned adhesion molecules in a dose-dependent way. To assess the nature of mechanism by which Se inhibits the adhesion molecules expression, it is necessary to perform experiments focused on molecular levels.

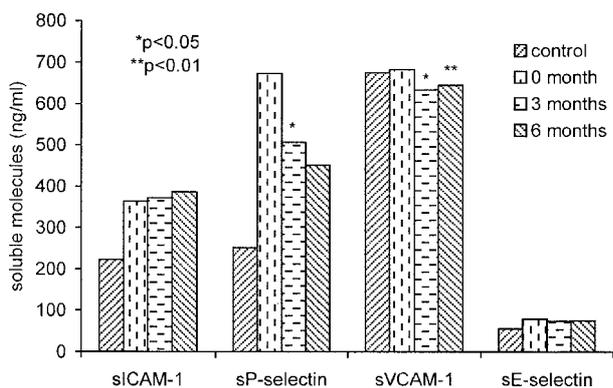


Fig. 4. Effect of selenium supplementation on the levels of soluble molecules. The sICAM-1, sP-selectin, sVCAM-1 and sE-selectin were determined in patients with corticoid-dependent asthma bronchiale (n=20) before and after 3 and 6 months of supplementation. The values represent mean SD and are compared with the group before therapy * p<0.05, ** p<0.01.

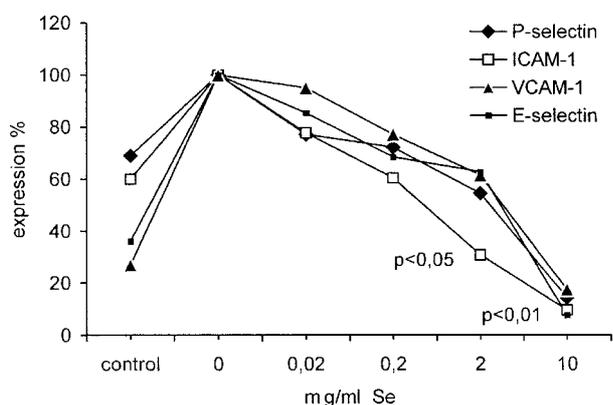


Fig. 5. Inhibition of adhesion molecules expression by selenium in IFN-gamma-stimulated endothelial cells. This experiment was performed on HUVEC isolated from different umbilical cord veins in each experiment. Cells were treated without IFN-gamma (control) or with IFN-gamma (100 U/ml). Selenium was added simultaneously with IFN-gamma. The quantification was performed by flow cytometry. The values represent means ±SD of 5 separate experiments performed in duplicate. p<0.05 a p<0.01 compared to adhesion molecules expression measured in the presence of IFN-gamma alone.

In the presented study we found a significant increase in the expression of adhesion molecules on PBMCs and endothelial cells of CDAs as well as increased levels of soluble adhesion molecules in comparison with healthy subjects. The increased expression of adhesion molecules is also accompanied by an increase in cellular activation of the immune system. After SeS we demonstrated a decrease in the expression of the following adhesion molecules on PBMCs: CD11a, CD11b, CD62L and also on endothelial cells: P- and E-selectins, ICAM-1, VCAM-1. After SeS soluble adhesion molecules responded by a decrease in levels of P-selectin and VCAM-1.

In vitro tests, the addition of Se inhibits the expression of adhesion molecules on endothelial cells stimulated by IFN-gamma in a dose-dependent way.

Based on the presented results, we suppose that SeS decreases the cellular activation of the immune system. In this respect Se has immunomodulatory and antiinflammatory effects.

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Received October 18, 2001.

Accepted December 7, 2001.