SHORT COMMUNICATION

The influence of selenium supplementation on the immunity of corticoid-dependent asthmatics

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Abstract

Selenium (Se) is a trace element that is essential for immune functions, and protects the immune system from oxidative damage.

Aim: **The aim of the pilot clinical study was to assess the influence of selenium supplementation (SeS) on the selected immune parameters analyzed from peripheral blood of corticoid-dependent asthmatics (CDAs).**

Material and methods: **Seventeen CDAs aged from 30 to 74 years (7 females, 10 males) with suboptimal levels of Se in plasma were enrolled into the study. The follow up of SeS lasted 96 weeks. It is daily dose was 200 µg (2x2 tbl daily, 1 tbl contained 50 µg of Se). Before (-4 weeks) and after the 12th, 48th, 72nd and 96th weeks of SeS, the following parameters were observed: Epitopes EG1, EG2 expressed on intracellular eosinophil (Eo) cationic protein and eosinophil peroxidase, the numbers of CD3, CD4, CD8, CD19 and CD3 HLADR positive T lymhocytes (Ly), lymphocyte blastogenesis test (LTT) with mitogens concanavalin A, (Conc A) phytohemaglutin (PHA), the levels of C3, C4 complement components, activation of complement by classic and alternative pathways (CP50, AP50), the levels of immunoglobulins (Ig) G, A, M and total IgE, circulating immune complexes (CIC).**

Results: **Epitopes EG1and EG2 in cytoplasma of Eo decreased significantly after 12 weeks of SeS, ($p<0.01$) and 96 weeks of follow up. In parameters of T cell mediated immunity the relative number of CD3 HLADR+ T Ly increased after 24, 48 and 96 weeks of SeS ($p<0.0008$, $p<0.009$, $p<0.07$). Proliferative activity of T Ly to mitogenes PHA and ConcA in LTT decreased significantly after 12, 48, 72 and 96 weeks of SeS ($p<0.0005$, $p<0.009$, $p<0.04$, $p<0.02$, respectively). In humoral parameters activation of CP50 decreased after 24, 72 and 96 weeks of SeS to the reference range ($p<0.001$, $p<0.03$, $p<0.02$) and AP50 after 96 weeks, respectively ($p<0.02$). The levels of IgG elevated after 24 weeks ($p<0.02$), IgA after 24, 48 weeks ($p<0.0007$, $p<0.02$, respectively). The level of total IgE significantly decreased after 96 weeks of SeS ($p<0.003$).**

Conclusion: **Our pilot clinical study with the CDAs demonstrates the significant changes particularly in functional parameters of both cellular and humoral types of immunity. These results support the immunomodulating effects of SeS.** ***(Tab. 5, Ref. 15.)***

Key words: supplementation of selenium, corticoid-dependent asthmatics, humoral and cellular immunity.

Asthma bronchiale (AB) represents a chronic disease, the pathogenesis of which is dominated by inflammation. This has not been completely elucidated, however the multifactorial nature of the disease is assumed. In this respect, free oxygen radicals produced from cellular membranes of accumulated eosinophils (Eo), polymorphonuclears (PMN), alveolar macrophages and mastocytes participate also in the process of inflammation (Strieter et al, 1993; Horváthová et al, 1998).

The enzyme selenium glutathione peroxidase (Se Gpx) represents one of the major scavengers of free oxygen radicals. Several authors have published their results of decreased levels of selenium (Se) in plasma, correlating with the decreased activity of Se Gpx (Hasselmark et al, 1990; Kadrabová et al, 1996;

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Pearson et al, 1991). Many studies suggest that an adequate intake of Se is required to ensure the optimal immune function and to prevent malignancy. Various components of the immune system fail to function correctly should the dietary Se be deficient. The currently published data focuse on the significance of Se especially in anti-infectious and anti-tumour fields of immunity (McKenzie et al, 1998).

The aim of the study

The aim of the pilot clinical study was to assess the influence of selenium supplementation (SeS) on the selected immune cellular and humoral parameters analyzed from peripheral blood of corticoid-dependent asthmatics (CDAs).

Patients and methods

Seventeen CDAs aged from 30 to 74 years (7 females, 10 males) with suboptimal levels of Se in plasma were enrolled into the study. Asthmatics fulfilled the criteria of asthma recommended by the American Thoracic Society (American Thoracic Society, 1987). The follow up of SeS lasted 96 weeks. The daily dose was 200 µg (2x2 tbl daily, 1 tbl contained 50 µg of Se). All patients were treated by inhaled corticosteroids (CS) (beclomethasone propionate and 4 of them also by systemic CS (methylprednisolon).

The clinical trial was performed by form of a simple opened clinical pilot study. We assessed the parameters of cellular immunity from peripheral blood before (-4th week) and after SeS (12th, 24th and 96th week) as follows: count of leukocytes (Le) and differential count, absolute number of lymphocytes (Ly) and eosinophils (Eo).

Before (-4 weeks) and after 12, 48, 72 and 96 weeks of SeS the following parameters were observed: relative numbers of CD3, CD4, CD8, CD19 positive Ly, CD3 HLADR positive Ly, CD4/CD8 (immunoregulatory index). CD markers were assessed by the method of flow cytometry analysis (Coulter Epics XL) using standard monoclonal antibodies (Becton Dickinson).

Before (-4 weeks) and after 12, 72 and 96 weeks of SeS, epitopes EG1, EG2 in Eo were determined using FOG method (Hed a Halldém, 1993). Epitopes EG1 and EG2 were assessed by monoclonal antibodies directed against intracellular eosinophil cationic protein (ECP) and eosinophil peroxidase (EPX), respectively.

During of the -4th week and in intervals of the 12th, 48th, 72nd and 96th weeks lymphocyte blastogenesis test (LTT) with mitogens concanavalin A, (Conc A) phytohemaglutin (PHA) were assessed (istotope method with marked 3H-thymidine).

During the -4th, 24th, 48th, 72nd and 96th weeks of SeS we observed parameters of humoral immunity as follows: the levels of C3, C4 complement components (turbidimetric method), alpha macroglobulin (turbidimetric method), activation of complement by classic and alternative pathway (CP50, AP50), assessed by method of the lysis of sheep erythrocytes (haemolytic assay), the levels of circulating immune complexes (CIC) by method PEG IKEM, the levels of immunoglobulins (Ig) G, A, M (turbidimetric method) and total IgE (ELISA method).

Statistical analysis

The data were evaluated using pair Wilcoxon test, analyzed and expressed as the standard error. p-Values less than 0.05 were regarded as significant.

Results

The parameters of cellular immunity as to the numbers of Le, Ly, Eo did not change significantly (Tab. 1).

Mean fluorescence intensity (MFI) of EG1 and EG2 (epitope expression of EG1 on ECP molecule and epitope EG2 on molecules ECP and EPX, respectively) significantly decreased after the 12th and 96th weeks of SeS, respectively ($p<0.01$ and $p<0.007$, $p<0.04$, respectively) (Tab. 2).

Tab. 1. The influence of SeS on the leukocytes and differential count (n=17).

	Week			
	-4	**12**	**24**	**96**
Leukocytes (x10^9)	6,41	5,41	6,17	7,49
±SEM	0,6	0,4	0,4	0,5
p		NS	NS	NS
Neutrophils (%)	56,35	58,69	57,53	58,2
±SEM	2,1	2,6	2,1	2,4
p		NS	NS	NS
Lymphocytes (%)	36,47	36,50	37,12	33,44
±SEM	1,9	2,7	1,9	2,3
p		NS	NS	NS
Eosinophils (x 10^9/l)	0,342	0,318	0,334	0,370
±SEM	0,05	0,05	0,05	0,07
p		NS	NS	NS

Notes: n — number of patients, SEM — standard error mean, p — statistical significance

Tab. 2. The influence of SeS on the mean fluorescence intensity expression of epitopes EG1 a EG2 in eosinophils (%) (n=17).

	Week			
	-4	**12**	**24**	**96**
MFI EG1	2,04	1,55	2,09	1,72
±SEM	0,2	0,07	0,09	0,08
p		0,01	NS	NS
MFI EG2	2,16	1,61	2,48	1,68
±SEM	0,22	0,05	0,16	0,06
p		0,007	NS	0,04

Notes: n — number of patients, SEM — standard error mean, p — statistical significance, MFI — mean fluorescence intensity, EG1 — epitope on eosinophilic cationic protein (ECP), EG2 — epitope on ECP and eosinophil peroxidase

Tab. 3. The influence of SeS on the parameters of cellular immunity (n=17).

	Week					
	-4	12	24	48	72	96
CD3+ Ly (%)	71,53	71,81	73,50	68,75	67,69	69,44
±SEM	1,56	1,60	1,40	2,1	1,5	1,50
p		NS	NS	NS	NS	0,02
CD4+ Ly (%)	43,76	44,06	45,12	43,19	44,18	44,44
±SEM	1,80	2,50	2,00	2,6	2,7	2,4
p		NS	NS	NS	NS	NS
CD8+ Ly (%)	28,06	30,19	28,82	25,56	25,59	26,19
±SEM	2,20	2,60	2,00	2,4	1,80	1,7
p		NS	NS	0,02	0,03	NS
CD19+ Ly (%)	12,24	12,19	12,18	13,06	13,71	12,50
±SEM	1,10	1,10	1,20	1,50	1,10	1,10
p		NS	NS	NS	NS	NS
IRI (CD4+/CD8+)	1,76	1,94	1,78	2,04	1,88	1,88
±SEM	0,20	0,30	0,20	0,30	0,30	0,30
p		NS	NS	NS	NS	NS
CD3 HLADR+	7,82	9,06	12,35	9,62	7,94	10,69
±SEM	0,70	0,70	0,90	0,80	0,97	0,80
p			0,0008	0,009		0,0007

Notes: n — number of patients, Ly — lymfocytes, IRI — immunoregulatory index, HLA — human leukocytes antigens, SEM — standard error mean, p — statistical significance

Tab. 4. The influence of SeS on the LTT with mitogens phytohemaglutinin (PHA), concanavalin A (Conc A) (dpm^3) and immunoregulatory index (IRI) (n=17).

	Week				
	-4	12	48	72	96
LTT-PHA	109,0	43,0	81,0	56,5	90,8
±SEM	13,43	5,47	6,95	5,48	9,41
p		0,003	0,02	0,02	NS
LTT-ConcA	70,9	38,1	53,4	38,6	56,9
±SEM	7,6	4,9	5,7	6,1	6,8
p		0,0005	0,0009	0,004	0,02
IRI	1,57	1,28	1,68	1,72	1,68
±SEM	0,12	0,16	0,13	0,13	0,14
p		NS	NS	NS	NS

Notes: n — number of patients, SEM — standard error mean, p — statistical significance

In parameters of specific cellular immunity the numbers of CD3, CD4, CD8 and CD19 positive T Ly did not change (Tab. 3). The numbers of CD3 HLADR positive T Ly increased after the 24th, 48th and 96th weeks ($p<0.0008$, $p<0.009$, $p<0.0007$) (Tab. 3).

The proliferative activity of Ly in LTT after mitogen stimulation with PHA decreased significantly after the 12th, 48th and 72nd weeks ($p<0.003$, $p<0.02$, $p<0.02$) (Tab. 4).

Tab. 5. The influence of SeS on the parameters of natural and specific humoral immunity (n=17).

	Week				
	-4	12	48	72	96
C3 (g/l)	0,94	1,03	0,88	0,71	1,04
±SEM	0,06	0,08	0,06	0,06	0,1
p		NS	NS	0,01	NS
C4 (g/l)	0,43	0,36	0,41	0,28	0,32
±SEM	0,04	0,02	0,04	0,02	0,03
p		NS	NS	0,001	0,049
CP50 (units/ml)	2455,53	1662,71	2129,35	1811,65	1722,50
±SEM	219,7	151,3	159,7	186,7	155,4
p		0,001	NS	0,03	0,02
AP50 (units/ml)	208,5	156,5	150,2	139,8	125,8
±SEM	58,1	8,0	14,8	9,5	8,5
p		NS	NS	NS	0,02
CIC (units)	34,12	34,41	50,29	39,76	32,69
±SEM	3,29	2,19	4,66	2,54	3,88
p		NS	0,005	NS	NS
A2M (g/l)	2,04	2,41	2,45	2,57	2,16
±SEM	0,17	0,20	0,10	0,30	0,20
p		NS	NS	NS	NS
IgG (g/l)	13,8	17,0	15,6	14,8	15,0
±SEM	0,8	1,1	1,5	1,1	1,3
p		0,02	NS	NS	NS
IgA (g/l)	2,32	3,63	2,95	2,79	2,38
±SEM	0,40	0,40	0,40	0,30	0,40
p		0,0007	0,02	NS	NS
IgM (g/l)	1,79	2,14	2,19	2,06	1,02
±SEM	0,20	0,30	0,30	0,20	0,20
p		0,04	NS	NS	0,003
IgE (g/l)	185,1	271,7	325,6	266,4	107,5
±SEM	73,7	102,2	137,1	85,9	33,3
p		NS	NS	NS	0,003

Notes: n — number of patients, CIC — circulating immune complex, Ig — immunoglobulin, A2M — alpha$_2$-macroglobulin, SEM — standard error mean, p — statistical significance, CP 50 — classic pathway, AP 50 — alternative pathway, C3, C4 — complement components

A significant decrease of proliferative activity was noticed also after stimulation with Conc A in all observed intervals, including week 96 ($p<0.0005$, $p<0.009$, $p<0.004$, $p<0.02$). The immunoregulatory index did not change significantly (Tab. 4).

The parameters of natural humoral immunity showed a drop in CP50, and AP50 was seen after the 12th, 72nd and 96th weeks and after the 96th week of SeS respectively ($p<0.001$, $p<0.03$, $p<0.02$, and $p<0.02$, respectively) (Tab. 5).

A decrease in C4 and C3 complement components was seen after the 72nd, 96th and 72nd weeks, respectively ($p<0.001$, $p<0.049$ and $p<0.01$, respectively). These changes were within the reference ranges (Tab. 5).

The level of IgG increased significantly after the 24th week of SeS ($p<0.02$). The level of IgA increased also after the 24th and 48th weeks ($p<0.0007$, $p<0.02$). The level of IgM increased after the 24th week ($p<0.04$) and decreased after the 96th week ($p<0.003$), but the changes were within the reference range.

The level of total IgE changed significantly after the 96th week of SeS ($p<0.003$) (Tab. 5).

Discussion

The presented pilot clinical study investigates the effects of SeS during a period of 96 weeks in a group of CDAs on selected parameters of cellular and humoral immunity assessed from peripheral blood.

The majority of data about the influence of Se on the immune system are provided from experiments on animals or in vitro tests. In general, it is known that the deficiency of Se leads to the attenuation of effector immune mechanisms. However, it is SeS that enhances them.

Eo with T Ly and mastocytes play the key role in the immunopathogenesis of AB by production of pro-inflammatory cytokines as well as cytotoxic enzymes (Bousquet et al, 1990; Weller, 1991). In the study we did not observe any changes in the number of Eo within peripheral blood but we noticed a decrease in expression of epitopes EG1 and EG2. Epitope EG1 is localized on the intracellular molecule ECP, while epitope EG2 on intracellular ECP and EPX, and is characteristic for activated Eo. This phenomenon (decrease in epitopes expression) we consider to be favourable and associated with anti-inflammatoty effects. In this respect, also Roquet et al (1996) reported a positive correlation between the degree of bronchial hyperreactivity and the plasma levels of ECP and expression of EG2 (Roquet et al, 1996).

Generally, Se deficiency depresses the effectiveness of immune cells. SeS appears to boost cellular immunity by 3 mechanisms. First, it upregulates the expression of the T-cell high-affinity IL-2 receptor and provides a vehicle for enhanced T-cells responses. Since the T-cells is a key component in providing B-cell help for antibody synthesis, this may explain the stimulatory effects of Se on antibody production. Second, it prevents oxidative-stress-induced damage to immune cells. Third, it alters platelet aggregation by decreasing the ratio of thromboxane to leukotriene production (McKenzie et al, 1998).

In our investigation in parameters of specific cellular immunity we found an increase in the relative number of CD3 HLADR positive T Ly. This subpopulation of T Ly participates in the immune humoral response. In this respect we could speculate about the enhancing effects of SeS on effector immune mechanisms.

Interesting results were observed in LTT. Ly of CDAs proportionally decreased their proliferative activity to both mitogenes, PHA and Conc A, without significant changes in immunoregulatory index. The proliferative activity of both subpopulations, CD4 and CD8 reduced proportionally. These results are in contrast with reports of authors from Finland. They did not confirm any changes in LTT in subjects with lower levels of Se, and after SeS (Arvilommi et al, 1983).

The effects of Se on T cell-mediated immunity is more complex. In animal experiments, increased levels of Se enhanced the delayed type of hypersensitivity in skin tests (Martin et al, 1976). Aleksondrovicz et al (1977) reported the effects of SeS on the depression of alotransplantate rejection in an experimental animal model.

As to the parameters of humoral immunity, we observed changes in complement system and in the levels of imunoglobulins. Although these changes were in reference ranges it is likely that SeS has immunomodulatoring, and immunonormalizing effects.

An interesting laboratory finding was observed, namely that the total IgE level decreased after the 96th week of SeS. In this respect we could speculate about antiallergic effects of long-term SeS, resulting in the reduction of IgE syntesis. Flatt et al (1990) reported the relation between the decrease in the Se status and the high prevalence of AB in New Zealand. There is a suspicion that the low Se status represents one of the risk factors of the manifestation of AB. In order to confirm this thesis, a prospective, long-term placebo controlled studies will have to be performed in the future.

During the long-term pilot study with SeS we demonstrated changes especially in functional parameters of both cellular and humoral types of immunity supporting the immunomodulatory effects of Se. No immunotoxic effects were observed during SeS.

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