The influence of chromium on cell-mediated and humoral-mediated immunity in mice

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Abstract

The aim of this study was to examine the effect of chromium on interleukine 1α and 6 (IL-1α and IL-6 production. Moreover, the proliferative response of lymphocytes, the metabolic activity of phagocyting cells, the lysozyme activity and γ-globulins level were examined.

The experiments were performed on NRMI mice, which were intraperitoneally injected with 0,5 ml: NaCl (control), 1 or 10 mg Cr per body weight, which was administered in the form of chromium chloride solution.

The present study has shown that chromium decreases statistically significantly IL-1α concentration after injection of chromium at a dose of 1 and 10 mg Cr per body weight. The concentration of IL-6 does not differ from the control group in both experimental groups. We have observed no differences in the proliferative response of lymphocytes and in the metabolic activity of phagocyting cells. Similar results have been observed in lysozyme and γ-globulins levels.

Key words: *chromium, cellular and humoral immunity, mice.*

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Introduction

Chromium is an element commonly occurring in nature. Chromium exists primarily in two valence states: trivalent (Cr III) and hexavalent (Cr VI). It has been shown that Cr VI is cytotoxic, genotoxic and carcinogenic, while Cr III appears to be relatively non toxic [1]. It has been proved that trivalent chromium is essential for human life. Chromium III is essential for proper insulin functioning (insulin-receptor activation) and is required for normal protein, fat and carbohydrate metabolism [2].

However, the excess of mentioned element in cells can generate reactive oxygen species. Moreover, inside the cells chromium may interact with microfilaments, mitochondria, lysosomes and nucleus [1]. Cr(III) compounds can bind directly to DNA *in vitro*, forming Cr-DNA adducts and DNA-DNA crosslinks [3]. Moreover, Cr III has been shown to be able to increase the catalytic activity and decrease the fidelity of DNA polymerase [4]. The effects of chromium supplemented diet have been examined in numerous animal and human studies, but the results of the investigations are not equivocal.

Material and methods

Animals and treatment

The investigations were performed on NRMI mice. The experimental protocol was approved by the Local Ethic Commitee for Animal Studies in Olsztyn (opinion number 28/2007). Mice were obtained from The Division of Pathophysiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn.

The animals were divided into 3 groups. Mice were intraperitoneally injected with 0.5 ml:

- Group I control (K): NaCl,
- Group II (Cr1): 1 mg Cr per body weight as $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$ solution (Sigma),
- Group III (Cr10): 10 mg Cr per body weight as $[Cr(H_2O)4Cl_2]Cl \times 2H_2O$ solution.

Twenty-four hours later blood samples were taken form the jugular vein of anesthetized mice into plastic tubes with heparin as an anticoagulant.

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Interleukin-1α and interleukin-6 cytokine measurement

Serum cytokines IL-1α and IL-6 were measured by the sandwich-linked immunosorbent assay with the use of commercially available kits (R&D Systems) according to the manufacture's instruction. A standard curve was constructed by plotting the absorbance of each standard vs. the corresponding standard concentration, and then the cytokine levels of unknown samples were calculated. The sensitivities of assays were as follows: 2.5 pg/ml for IL-1α and 1.6 pg/ml for IL-6.

Proliferative response of lymphocytes

The proliferative response of the lymphocytes was determined by MTT method after the Concanavalin A (ConA, Sigma) stimulation. Leucocytes were isolated from blood by centrifugation for 30 minutes at 2000 g and 4°C on the Gradisol L gradient. Next, the cells were washed three times in PBS and resuspended at stock concentration 2×10^6 cells/ml in RPMI cell culture medium (Sigma) supplemented with 10% Fetal Calf Serum (FCS, Sigma). The isolated lymphocytes (100 µl) were resuspended in RPMI medium supplemented with 10% FCS, 2 mM L-glutamine, 0,02 mM 2-mercaptoethanol, 1% Hepes buffer and ConA at concentration of 5 µg/ml and distributed in 96-well plates. After 72 hours of incubation 50 µl of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, Sigma) was added. The plates were incubated for 4 h at room temperature. After incubation plates were centrifuged at 1400 g at 15°C for 15 min, supernatants were removed and 100 µl DMSO was added to each well and incubated for 15 min at room temperature. The absorbance was measured with the use of the microplate reader at 620 nm wavelength. Experiments were independently performed at least five times.

RBA (Respiratory Burst Acivity) test

The metabolic activity of phagocyting cells (granulocytes and monocytes) was determined on the basis of Respiratory Burst Acivity test - the method described by Chung and Secombes and adapted by Siwicki [5]. Blood samples were centrifuged on the Gradisol G gradient. Next, isolated cells were resuspended in RPMI 1640 cell culture medium (Sigma) at 10^6 cells/ml. Then, 100 µl resuspended cells were distributed in 96-well U-shaped plates and mixed with 100 µl of 0,2% nitro blue tetrazolium (NBT, Sigma) and phorbol myristate acetate (PMA, Sigma) at concentration of 1 mg/ml. Plates were incubated for 30 minutes at 37°C. After the incubation the supernatant was removed and cells were washed in 70% ethanol three times and then dried at room temperature. Next, cells were incubated with 2M KOH and DMSO (domethylsulfoxide, Sigma). The absorbance was measured with the use of the microplate reader at 620 nm wavelength. Experiments were independently performed at least five times.

Lysozyme activity and γ-globulins level

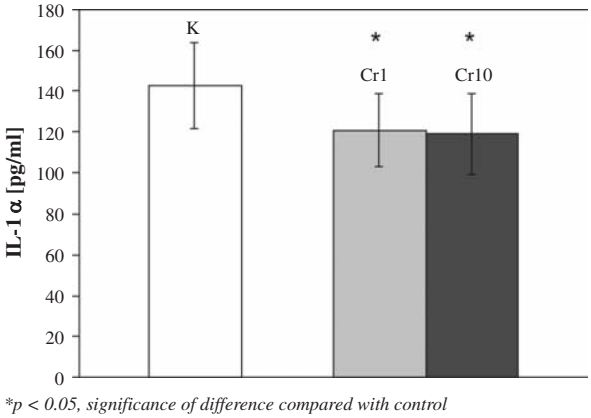
The lysozyme activity and γ-globulins levels were determined on the basis on method adapted by Siwicki and Anderson [6, 7].

Statistical method

Statistical differences were analysed by Student's t-test. $P < 0,05$ were considered to be statistically significant. All results are presented as mean values ± SD.

Results

Figure 1 shows the IL-1α levels induced by chromium chloride. It can be seen that chromium decreases statistically significantly IL-1α concentration after the injection of chromium at concentration of 1 and 10 mg Cr per body weight in the form of chromium chloride solution. Figure 2 shows the influ-



**p < 0.05, significance of difference compared with control*

Fig. 1. The serum interleukin IL-1α concentrations in mice (mean ± SD, $n = 5$)

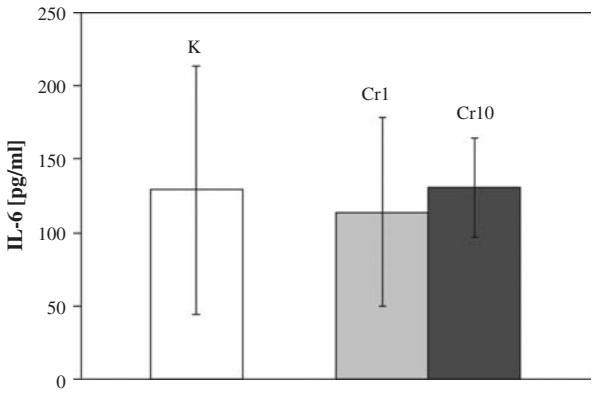


Fig. 2. The serum interleukin IL-6 concentrations in mice (mean ± SD, $n = 5$)

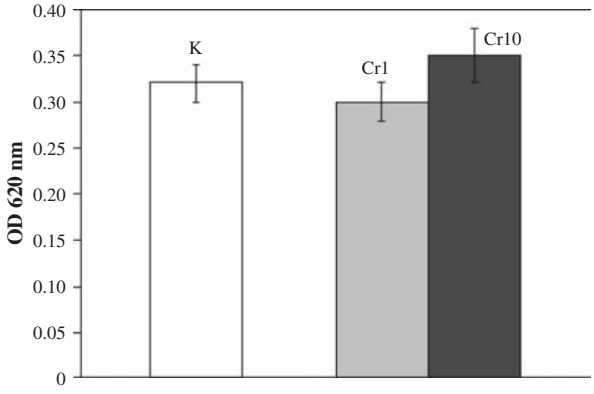


Fig. 3. The effect of injection of 1 and 10 mg Cr per body weight as chromium chloride solution on metabolic activity of blood phagocyting cells in mice (mean ± SD, *n* = 5)

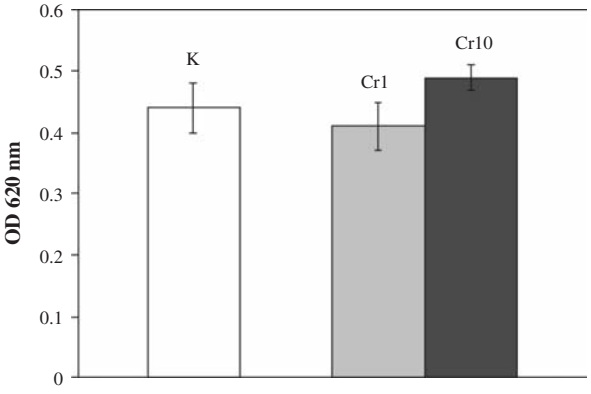


Fig. 4. The effect of injection of 1 and 10 mg Cr per body weight as chromium chloride solution on proliferative response of lymphocytes in mice (mean ± SD, *n* = 5)

ence of chromium on IL-6 concentration in tested groups. The concentration of IL-6 does not differ from the control group in both groups.

We have observed no differences in proliferative response of lymphocytes and metabolic activity of phagocyting cells (Figures 3-4). Similar results have been observed in lysozyme activity and γ-globulins levels (Figures 5-6).

Discussion

The role of dietary nutrients in modulating immunity attracted considerable attention in animal research. Microelements are of significant importance of altering the immune response by immunostimulatory or immunosuppressive mechanisms. It has been shown that chromium is an essential nutrient necessary for the normal metabolism of glucose, cholesterol and fat. However, high doses of this microelement and long term exposure of it can give rise to various, cytotoxic and genotoxic reactions that affect the immune system of the body. The influence of chromium on cytokines, immunoglobulins and proliferation of both, T and B cells have been investigated.

The relationship between microelements and cytokine production has attracted the attention of several investigators. The investigations performed by Bhagat *et al*. have shown that IFN-γ mRNA expression on day 1 post immunization of NDV in animals which received 500 ppb of chromium was four times higher than control animals. Later on day 3 post immunization, IFN-γ mRNA expression increased and reached approximately 27 times significantly higher in treatment group compared to control [8]. The investigations provided by Burton *et al*. have shown, that chromium in dose 0,5 mg Cr per diet caused increase of IL-2, IFN and TNF-α production by cow lymphocytes [9]. Myers *et al.* investigations have shown that chromium picolinate treated swines have very high IL-6 levels when compared with control group [10]. Our investigations have shown,

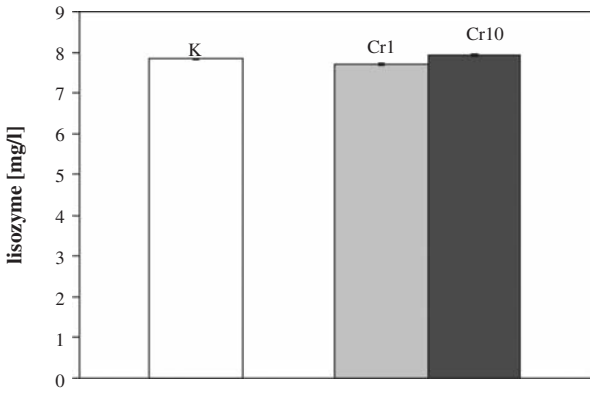


Fig. 5. The effect of injection of 1 and 10 mg Cr per body weight as chromium chloride solution on lysozyme activity in mice (mean ± SD, *n* = 5).

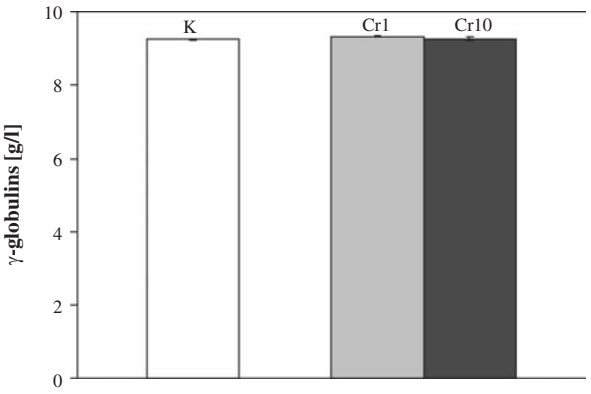


Fig. 6. The effect of injection of 1 and 10 mg Cr per body weight as chromium chloride solution on γ-globulin concentration in mice (mean ± SD, *n* = 5)

that chromium chloride caused the statistically significant increase of IL-α concentration and no changes in IL-6 concentration.

Investigations provided by Burton *et al.* have shown the decrease of leucocytes blastogenesis [11]. That is confirmed by Kegley *et al*. investigations. Moreover, investigations performed by these authors have shown that chromium chloride and chromium picolinate in dose 0.4 mg Cr per body weight in milk caused the decrease of IgG and IgM serum concentration in calves [12]. However, the investigations provided by van den Ligt *et al*. have shown no effect of chromium on IgG and IgM concentration in gilt's milk [13]. The investigations performed by Chang *et al*. have shown that chromium caused decrease of IgM concentration, but no differences in IgG and IgA was observed. The investigations performed by these authors have also shown no effect of chromium on lymphocytes blastogenesis [14, 15]. However, other authors reported, that the proliferation of both T and B cells was inhibited by chromium after intraperitoneal injection in mice [16]. These investigations are in contrast with our investigations which have shown that chromium have no effect on the metabolic activity of leukocytes and on the proliferative response of T lymphocytes.

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