The influence of chromium and iron on interleukin-1α and interleukin-6 concentration *in vitro* and *in vivo*

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Abstract

The aim of this study was to examine the effect of chromium and iron on interleukin-1α (IL-1α) and interleukin-6 (IL-6) concentration in vitro and in vivo.

The in vitro experiments were performed on the BALB/c 3T3 (mouse BALB/c embryo fibroblasts) cell line. The cells were incubated with 50 or 500 μM $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$ and 50 or 500 μM $FeCl_3$. Moreover, with 50 μM $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$ and 500 μM $FeCl_3$ or 50 μM $FeCl_3$ and 500 μm $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$. The IL-1α and IL-6 concentration was determined. In vivo experiments were performed on NRMI mice, which were intraperitoneally injected with 0.5 ml: NaCl (control), 1 mg Cr, 10 mg Cr, 150 mg Fe, 300 mg Fe, 1 mg Cr and 150 mg Fe, 1 mg Cr and 300 mg Fe,10 mg Cr and 150 mg Fe,10 mg Cr and 300 mg Fe per body weight as chromium chloride and iron chloride solution.

Iron and chromium used separately increase statistically significant IL-1α concentration after incubation with 50 and 500 μM of iron chloride or chromium chloride, whereas they decrease statistically significant IL-6 concentration. Simultaneously, incubation BALB/c 3T3 cells with 50 μM chromium chloride and 500 μM iron chloride decreases statistically IL-1α concentration when compared with cells incubated with iron chloride at concentration of 500 μM. Simultaneously, incubation with 50 μM iron chloride and 500 μM chromium chloride increases statistically significant IL-1α concentration. Interleukin-6 concentration decreases when compared with control cells.

Simultaneously, an injection with iron and chromium in all combinations has increased statistically significant serum IL-1α and IL-6 concentration in all groups when compared with control animals.

Key words: *chromium, iron, IL-1α, IL-6.*

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Introduction

Chromium and iron are trace elements necessary for growth and normal functioning of cells.

Iron is a central regulator of immune cell proliferation and functioning. The crucial role in immunology response play lymphocytes T and B, monocytes/macrophages and NK (natural killer) cells. There are few mechanisms regulating functions of iron in these cells. Lymphocytes and NK cells are dependent on transferrin/transferrin receptor (TfR) mediated iron uptake. Blockade of this pathway reduced the proliferation and differentiation of lymphocytes. It has been shown, that lymphocytes B are less sensitive to changes in iron homeostasis than lymphocytes T [1]. The various cells are dependent on transferrin/transferrin receptor mediated iron uptake. The blockade of this pathway leads to diminished proliferation, differentiation and cytokine production of these cells. Chromium is transported in the blood, predominantly, by transferrin and it competes with iron for binding at site B [2]. When saturation of transferrin with iron increases to over 50 percent, iron competes with chromium binding, affecting its transport [3]. On the basis on this findings we would like to check the influence of chromium and iron used separately and simultaneously on cells metabolism, especially to modu-

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late IL-1α and IL-6 production *in vitro* (on mouse embryo fibroblasts) and *in vivo* (on mice).

BALB/c 3T3 cell line was chosen for our investigation because it has been proposed as a cellular model in studying the morphological and biochemical changes induced by biometals [4]. The concentrations of chromium chloride and iron chloride for these studies were chosen on the basis of other reports [4, 5] and our earlier investigations. Our previous experiments have shown that both of them, at concentration of 50 μM, slightly stimulated cell proliferation, however, at concentration of 500 μM, significantly reduced the cell viability [6, 7].

The chromium and iron concentrations injected to mice were chosen on the basis of other authors' studies [8, 9].

Material and methods

Chemicals and materials

The Dulbecco's Modified Eagle Medium (DMEM), the heat-inactivated Fetal Bovine Serum (FBS), antibiotic/antimycotic (penicillin, streptomycin, amphotericin B), iron chloride ($FeCl_3 \times 6H_20$), chromium chloride ($[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$), IL-1α and IL-6 ELISA Kits were obtained from R&D Systems Europe (UK). Phosphate-buffered saline (PBS) and 0.25% trypsin were purchased from Biomed (Lublin, Poland) and tissue culture dishes were purchased from Nunc Brand Products (Denmark).

Cell culture and treatment

Mouse embryo fibroblasts (cell line BALB/c 3T3) were obtained from Dr D. Śladowski (Department of Transplantology & Central Tissue Bank, Centre of Biostructure, Medical University of Warsaw). The cells were cultured as adherent monolayers in plastic tissue culture dishes in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated FBS and penicillin (100 units/ml), streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml). Cells were maintained at 37°C in humidified incubator in atmosphere containing 5% of CO_2. The cells treated with 0.25% trypsin at 37°C for 5 minutes, were subcultured three times a week. Cells were used for cytokine assays during exponential phase of growth.

$FeCl_3 \times 6H_2O$ and $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$ was dissolved in PBS at the concentration of 1 mM. The final concentration was obtained by the dilution in culture medium (DMEM) supplemented with FBS and antibiotics.

In order to perform IL-1α and IL-6 assays, the cells were cultured on 96-well plates (2×10^5 cells/ml) in 100 μl DMEM, supplemented with 10% FBS and antibiotics. After 24 hours of incubation, the medium was exchanged for fresh DMEM (control), DMEM supplemented with 50 or 500 μM $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$, 50 or 500 μM $FeCl_3$, one supplemented with 50 μM $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$ and 500 μM $FeCl_3$ or 50 μM $FeCl_3$ and 500 μM $[Cr(H_2O)_4Cl_2]Cl \times 2H_2O$. After 24 hours of incubation IL-1α and IL-6 concentration was measured according to the original manufacture's instruction.

Interleukin-1α and interleukin-6 cytokine measurement

Cytokines (IL-1α and IL-6) concentrations 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.

Animals and treatment

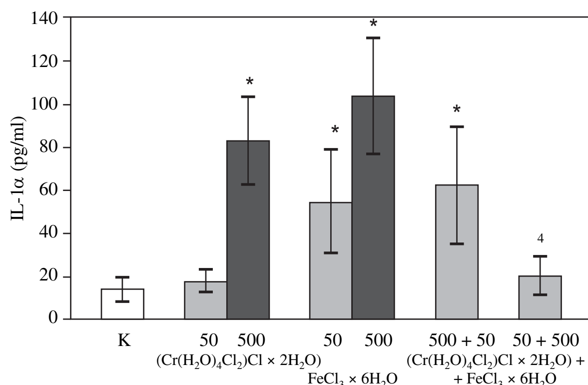
Investigations were performed on NRMI mice. The experimental protocol was approved by the Local Ethic Commitee for Animal Studies in Olsztyn (opinion number 28/2007). Mice were obtained from The Division of Pathophysiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn.

The animals were divided into 9 groups. Mice were intraperitoneally injected with 0.5 ml:
- group I – control (K): NaCl,
- group II (C1) 1 mg Cr per body weight as chromium chloride solution,
- group III (C10) 10 mg Cr per body weight as chromium chloride solution,
- group IV (Fe150) 150 mg Fe per body weight as iron chloride solution,
- group V (Fe300) 300 mg Fe per body weight as iron chloride solution,
- group VI (C1 + Fe150) 1 mg Cr and 150 mg Fe per body weight as chromium chloride and iron chloride solution,
- group VII (C1 + Fe300) 1 mg Cr and 300 mg Fe per body weight as chromium chloride and iron chloride solution,
- group VIII (C10 + Fe150) 10 mg Cr and 150 mg Fe per body weight as chromium chloride and iron chloride solution,
- group IX (C10 + Fe300) 10 mg Cr and 300 mg Fe per body weight as chromium chloride and iron chloride solution.

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

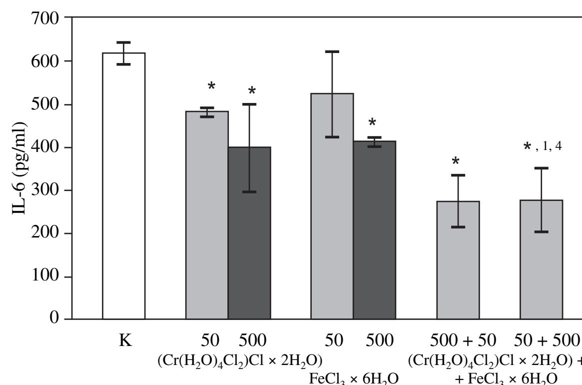
Interleukin-1α and interleukin-6 cytokine measurement

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 construct-



$p < 0.05$, significance of difference compared with control
[4]*$p < 0.05$, significance of difference compared with iron chloride at concentration of 500 μM*

Fig. 1. Interleukin-1α concentrations after incubation with iron chloride and/or chromium chloride



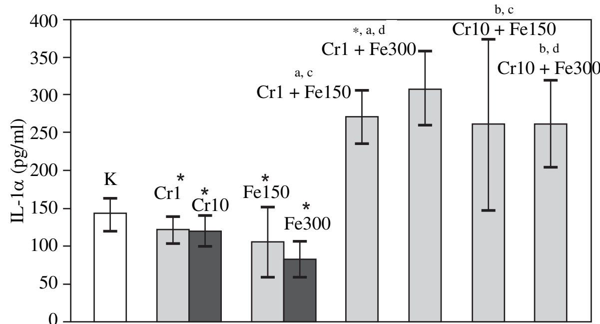
$p < 0.05$, significance of difference compared with control
[1]*$p < 0.05$, significance of difference compared with chromium chloride at concentration of 50 μM*
[4]*$p < 0.05$, significance of difference compared with iron chloride at concentration of 500 μM*

Fig. 2. Interleukin-6 concentrations after incubation with iron chloride and/or chromium chloride

ed 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.

Statistical analysis of data

The results were analysed with the use of Student's *t*-test with computer assistance (Statistica program). The accepted level of significance in all cases was $p < 0.05$. All results are presented as mean values ± SD.



$p < 0.05$, significance of difference compared with K group
[a]*$p < 0.05$, significance of difference compared with Cr1 group*
[b]*$p < 0.05$, significance of difference compared with Cr10 group*
[c]*$p < 0.05$, significance of difference compared with Fe150 group*
[d]*$p < 0.05$, significance of difference compared with Fe300 group*

Fig. 3. The serum IL-1α concentrations in mice (mean ± SD, *n* = 5)

Results

Figures 1 and 2 show the *in vitro* effects of iron and chromium on IL-1α and IL-6 concentrations. Iron and chromium used separately increase statistically significant IL-1α concentration after incubation with 50 and 500 μM of iron chloride or chromium chloride (Fig. 1), whereas they decrease statistically significant IL-6 concentration (Fig. 2).

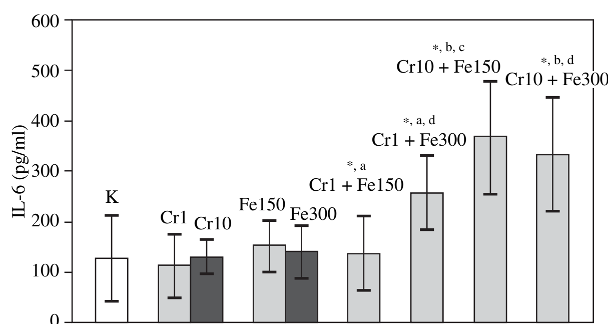
Simultaneously, incubation with 50 μM chromium chloride and 500 μM iron chloride decreases statistically IL-1α concentration when compared with cells incubated with iron chloride at concentration of 500 μM (Fig. 1) and IL-6 concentration when compared with control cells and cells incubated separately with chromium chloride and iron chloride at concentrations of 50 and 500 μm, respectively (Fig. 2).

Simultaneously, incubation with 50 μM iron chloride and 500 μM chromium chloride increases statistically significant IL-1α concentration (Fig. 1), whereas it decreases IL-6 concentration when compared with control cells (Fig. 2).

It can be seen from the Fig. 3 and 4, that a simultaneous injection with iron and chromium has increased statistically significant IL-1α and IL-6 concentration in groups: VI, VII, VIII and IX when compared with the control group and groups: II, III, IV, V.

Discussion

The relationship between microelements and cytokine production *in vitro* and *in vivo* has attracted of many investigators' attention. Availability of one nutrient may impair or enhance the action of another in the immune function.



$^{*}p < 0.05$, significance of difference compared with K group
$^{a}p < 0.05$, significance of difference compared with Cr1 group
$^{b}p < 0.05$, significance of difference compared with Cr10 group
$^{c}p < 0.05$, significance of difference compared with Fe150 group
$^{d}p < 0.05$, significance of difference compared with Fe300 group

Fig. 4. The serum interleukin-6 concentrations in mice (mean ± SD, *n* = 5)

Nutrient-nutrient interactions may negatively affect the immune function. The production of antibodies or cytokine can also be altered by the ability of vitamins and proteins [10].

This work is a first report on the influence of chromium and iron interaction on cytokine concentration. Our previous investigations have shown that chromium and iron used separately, increase statistically significant IL-1α concentration, whereas they decrease statistically significant IL-6 concentration *in vitro* [6, 7]. The present study shows that simultaneous incubation with chromium and iron increases statistically IL-1α at concentrations of 500 and 50 µm, respectively, and that it decreases IL-6 concentration when compared with control cells. However, simultaneous incubation of chromium chloride at concentration of 50 µm and iron chloride at concentration of 500 µm does not change IL-1α concentration when compared with control cells, but it decreases when compared with cells incubated with iron chloride at concentration of 500 µm. Moreover, chromium and iron used separately decrease statistically significant IL-1α concentration after the injection. The concentration of IL-6 does not differ from the control groups [11, 12]. Simultaneous, the injection of chromium and iron has increased IL-1α and IL-6 concentration in animal serum. Simultaneous treatment with chromium and iron suggests the synergistic interaction between these elements.

The ionic radii for Cr^{3+} and Fe^{3+} are close in size, 0.76 Å for chromium and 0.79 Å for high spin Fe^{3+} [13]. Moreover, chromium is transported in the blood predominantly by transferrin, and it competes with iron for binding capacity [2]. Chromium has been found to preferentially bind to the B site of transferrin. When saturation of transferrin with iron increases to over 50%, iron competes with chromium binding, affecting its transport [3]. *In vitro* experiments have shown that chromium III in the low-molecular-weight-binding protein was taken up by transferrin, and that chromium III inhibited iron uptake by apotransferrin. Daily feeding of chromium III decreases serum levels of iron and total iron binding capacity [2]. Moreover, men in a weight training program who were given chromium picolinate, showed a 24% decrease in transferrin saturation, when compared with men given chromic chloride or a placebo [14].

The relationship between iron and chromium metabolism needs to be further explored. It is not yet clear if chromium is decreases iron absorption or if it is also involved in the down regulation of iron absorption. Also it is not yet clear how this interaction affects the cells metabolism, especially immune cells.

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