The influence of iron 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 iron on 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 in mice peripheral blood.

The experiments were performed on NRMI mice, which were intraperitoneally injected with 0.5 ml: NaCl (control), 150 mg Fe, or 300 mg Fe per body weight, in the form of iron chloride solution.

The results showed that iron decreased statistically significantly IL-1α concentration in mouse serum after injection of both iron doses. No differences between control and experimental groups were observed in other studied parameters.

Key words: *iron, cellular immunity, humoral immunity, mice.*

(Centr Eur J Immunol 2009; 34 (2): 57-60)

Introduction

Iron is an essential element for the survival of almost all living organisms. It is required for many metabolic processes such as: oxygen transport, drug metabolism, steroid synthesis, cellular respiration, electron transport, DNA synthesis, cell proliferation and differentiation and gene regulation [1]. Iron is the most important part of hemoglobin, ferritin, myoglobin and many of enzymes. Moreover, it is present in iron transport protein, such as transferrin [2].

However, excess free iron promotes the formation of reactive oxygen species (ROS), which attack cellular lipids, proteins and nucleic acids. Moreover, excess iron is toxic, and tissue iron concentration must be strictly regulated [3, 4].

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 III (Fe150): 150 mg Fe per body weight as $FeCl_3 \times 6H_2O$ solution (Sigma),
- group III (Fe300): 300 mg Fe per body weight $FeCl_3 \times 6H_2O$ solution.

24 hours later the animals were sacrificed.

Blood samples were taken form the jugular vein of anesthetized mice into plastic tubes with heparin as an anticoagulant.

IL-1α and IL-6 cytokine measurement

Serum cytokines IL-1α and IL-6 were measured by sandwich-linked immunosorbent assay using 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 the assays were as follows: 2.5 pg/ml for IL-1α and 1.6 pg/ml for IL-6.

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Proliferative response of lymphocytes

The proliferative response of the lymphocytes was determined by MTT method after 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% Foetal Calf Serum (FCS, Sigma). The isolated lymphocytes (100 µl) were resuspended in RPMI medium supplemented with 10% FCS, 2mM L-glutamine, 0.02 mM 2-mercaptoethanol, 1% Hepes buffer and ConA at concentration 5 µg/ml and distributed in 96-well plates. After 72 hours of incubation 50 µl MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, Sigma) was added. The plates were incubated for 4 hours at room temperature. After incubation plates were centrifuged at 1400 g at 15°C for 15 minutes, supernatants were removed and 100 µl DMSO was added to each well and incubated for 15 minutes at room temperature. The absorbance was measured using 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 based on 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 1 mg/ml. Plates were incubated for 30 minutes at 37°C. After incubation supernatant was removed and cells were washed in 70% ethanol three times and next dried at room temperature. Next, cells were incubated with 2M KOH and DMSO (domethylsulfoxide, Sigma). The absorbance was measured using 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 level were determined based on method adapted by Siwicki and Anderson [6, 7].

Statistical method

Statistical differences were analysed using Student's *t*-test. $P<0.05$ were considered as statistically significant. All results are presented as mean values ± SEM.

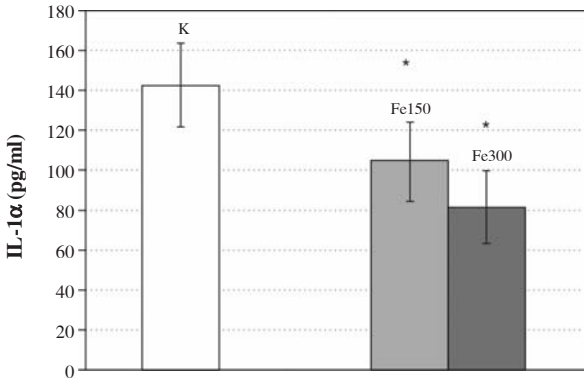
Results

The results of influence of iron chloride on IL-1α are presented on Fig. 1. It can be seen that iron decreased statistically significantly IL-1α concentration in mice injected with a dose 150 and 300 mg Fe per body weight, in the form of iron chloride solution. Figure 2 shows the influence of iron on IL-6 concentration in tested groups sera. The concentration of IL-6 did not differ from the control in both groups.

We observed no differences between experimental and control groups in proliferative response of lymphocytes and metabolic activity of phagocyting cells (Figs. 3-4). Similar results were observed in lysozyme and gamma-globulins levels (Figs. 5-6).

Discussion

Iron is an essential growth factor for proliferation and differentiation of all living cells. Because of this function



*Statistically significant differences compared with the control ($p<0.05$)

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

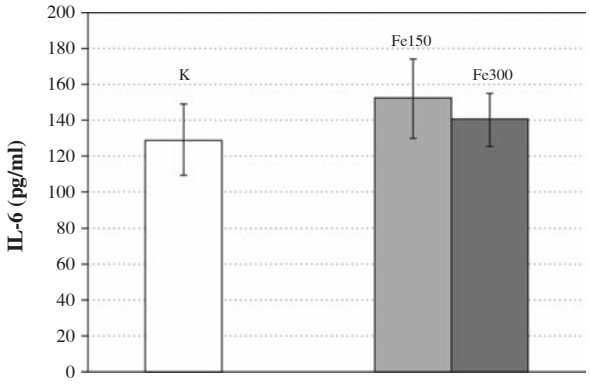


Fig. 2. The serum interleukin IL-6 concentrations in mice (mean ± SD, n=5)

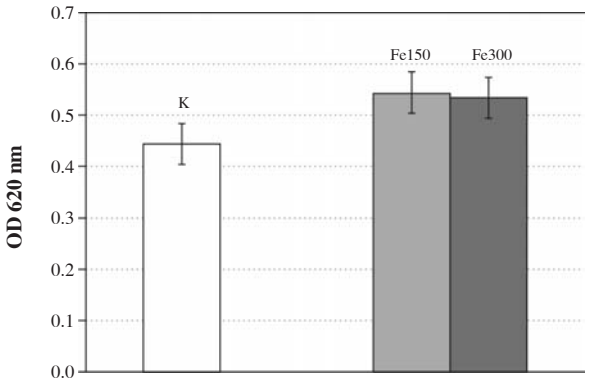


Fig. 3. The effect of injection of 150 and 300 mg Fe per body weight as iron chloride solution on proliferative response of lymphocytes in mice (mean ± SD, n = 5)

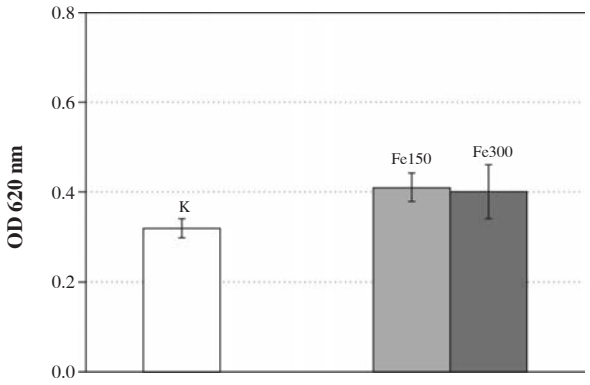


Fig. 4. The effect of injection of 150 and 300 mg Fe per body weight as iron chloride solution on metabolic activity of blood phagocyting cells in mice (mean ± SD, n = 5)

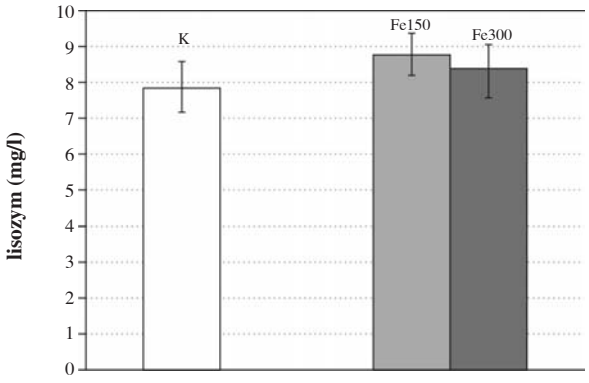


Fig. 5. The effect of injection of 150 and 300 mg Fe per body weight as iron chloride solution on lysozyme activity in mice (mean ± SD, n = 5)

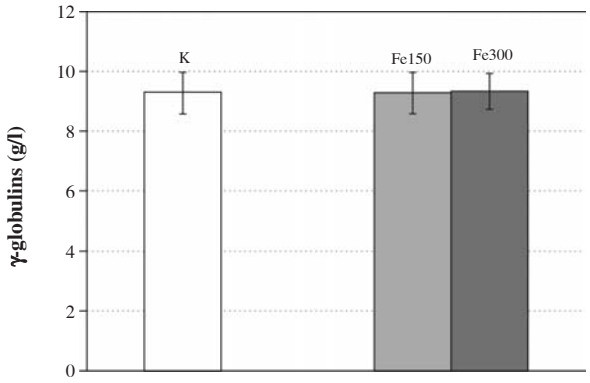


Fig. 6. The effect of injection of 150 and 300 mg Fe per body weight as iron chloride solution on gamma-globulins concentration in mice (mean ± SD, n = 5)

iron plays an important role in immunity regulation. The crucial role in immunology response plays lymphocytes T and B, monocytes/macrophages and NK (natural killer) cells. There are some 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 proliferation and differentiation of lymphocytes. It has been shown, that lymphocytes B are less sensitive to changes in iron homeostasis than lymphocytes T. Moreover, in malignant B lymphocytes a non-transferrin iron uptake mechanism has been described. The major role in this pathway plays divalent metal transporter DMT-I. Apart from these mechanisms all lymphocyte subtypes express receptors for H-ferritin, which is involved in the iron turnover by lymphocytes, but also by macrophages. The proliferation of lymphocytes is regulated also by iron- binding protein – Lactoferrin [8]. The present study shows that proliferative response of mice blood lymphocytes were not affected after iron injection.

The investigations performed by Zhdanova et al. on nonpregnant women with latent deficiency anemia have shown, that phagocytic index of leukocytes (mostly neutrophils and monocytes) increased as compared to the control [9]. However, the investigations performed by Barkova et al. have shown that, phagocytic index (the percentage of phagocyting cells) of monocytes obtained from breast-feeding women with iron deficiency anemia (IDA) and latent iron deficiency (LID) decreases when compared with control [10]. That confirms Bergman et al. investigations, which have shown, that percentage of phagocyting neutrophils from IDA patients was lover as compared with the control group. The

percentage of monocytes engaged in phagocytosis was similar in both groups and was not affected by addition of iron [11]. That correspond with our investigations, which have shown, that iron have any effect on metabolic activity of phagocyting cells.

The relationship between microelements and cytokine production has attracted the attention of several investigators. It has been shown, that exposure of human astrocytoma cells to IL-1α increases ferritin synthesis. Investigations performed by Bergman et al. have shown that peripheral blood mononuclear cells (PBMC) incubated with 50 and 100 μg% iron secreted significantly lover amounts of IL-1α than control cells. Moreover, iron at concentrations 50, 100 and 200 μg% had no effect on IL-6 release by these cells [12]. The investigations performed by Bergman et al. are in agreement with our investigations, which have shown the decrease of IL-1α production and no changes in IL-6 release after iron injection in mice.

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