Specific Proteins in Relation with Iron Overload in Experimental Study

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Iron is an essential mineral for organisms and due to environment iron deficiency and pollution it is very important to understand and monitor the iron homeostasis. Thus, the concentration of iron is very important in hematological and biochemical parameters because iron is component of hemoglobin, erythrocytes, and myoglobin -being part of oxygen-binding proteins. The aim of our experiment was to try a natural protection with fresh, organic plants administration – of iron overload in rabbits. Thereby, we administrated ferrous gluconate (hydro-solution, 15g Fe^{2+}/body weight) as intraperitoneal injection for 43 days to German Lop Eared breed rabbits, and in the same time we tried to protect the animals with a diet consisting in black cumin seeds; clover, chives, leek, parsley, coriander, rucola, radish, fenugreek - leaves; cucumber and carrots. At the end of experiment we analyzed the protein electrophoresis (albumin, α_1-globulin, α_2-globulin, β_1-globulin, β_2-globulin and γ-globulin), total iron binding capacity, and transferrin concentration. The analytical results demonstrated that plant administration was beneficial for iron homeostasis and the tested blood parameters did not record significant variations between control and experimental group, which proves that diet is very important in health and homeostatic mechanisms in living organisms.

Keywords: iron, proteins, rabbits, homeostasis

Iron is an essential mineral for living organisms, being the fourth most common element in the crust of Earth, being component of outer and inner core of Earth. Iron is a transition metal, and because of this chemical property it can exist in different oxidation states, Fe^{+2} and Fe^{+3} being the most common. Naturally iron is found in soil and water, being mandatory for living organisms, but in certain concentrations. Generally in biology, iron is involved in red blood cells (RBC or erythrocytes) production, being in direct relation with hematocrit and hemoglobin concentration; is important for cellular respiration, more exactly in oxygen carrying proteins in vertebrates – such as hemoglobin and myoglobin; is component of various enzymes, especially redox enzymes; and iron's final target together with oxygen, is mitochondria where iron modulates the oxidative phosphorylation [1-3]. Iron and oxygen homeostasis are correlated with cell homeostasis and some hypoxia-inducible factors (transcription factors) and specific genes [4,5].

Unbalance of iron metabolism are due to depletion or overload, resulting in perturbation of iron homeostasis which contribute to several pathological condition that can affect the kidney, liver, brain, gonads, cardiovascular or neurological function [6-8]. In iron deficiency the erythrocytes production decrease, the hematocrit and hemoglobin concentration leading to anemia, but for correct diagnosis- the cell blood count, iron and ferritin from serum have to be also evaluated [9]. The erythropoiesis is the process that consumes important quantities of iron, being regulated by hepcidin - a gene transcription which stimulates the hepatocytes to release the iron and stimulates the iron absorption. In turn, the hepcidin in also mediated by erythroferrone which is synthesized in the marrow by erythroblasts. A high iron diet for a medium or long term can modify the hepcidin activity, which can negatively affect others minerals homeostasis, such as cooper and zinc. Hepcidin is a liver-expressed peptide hormone (form from 25 amino acids), being a key regulator in iron homeostasis. In some medical conditions, like inflammatory processes, hepcidin level goes very high, the cells are signaling, iron serum falls down due to decreases of iron absorption and due to inhibition of iron transport by binding to ferroportin from gut erythrocytes, plasma membrane of reticuloendothelial cells and liver cells, and finally the anemia occurrence because the iron is not available for erythropoiesis [10,11].

Also iron excess is not well tolerated by living organisms. Thus, there are natural plants that can help in iron overload using two different strategies: reduction based iron update (due to low iron availability from basic matrix; and chelation based iron uptake [12,13]. Since ancient, there were used plants, microorganisms and algae to treat deficiencies and overloads of various chemical compounds, even minerals [14-17]. Thus, because iron can change its oxidation states from Fe^{2+} to Fe^{3+} is usually part of the Fenton reaction, which forms different reactive oxygen species (ROS) - very aggressive chemical form for cells [18,19]. Experimental studies demonstrated that various anatomical parts of plants can be used to decrease the mineral concentration from mammalians. This type of experimental research use small animals (mice, rats, rabbits) most of the time, with similar metabolism with humans, which can be genetically improved to produce the best animal breed for experiment [20-24].

Experimental part

Our research topic was to study whether in an area where the soil and groundwater are rich in iron - assuming that animals and people living in that area accumulate there minerals in concentration above normal levels-when dieting based on antioxidant and detoxification properties of plants the effects of iron overloaded cannot be attenuated, thus protecting the living organism. Thus, our experiment was carried out on two groups of rabbits German Lop Eared breed, each group being formed from five animals: one control group (C) and one experimental

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group (E). The mean weight of rabbits was 703g, and the experiment was conducted during summer time for consecutive 43 days. Experimental rabbits were subjected to a period of accommodation time at the experiment conditions, providing adequate ventilation and following the conditions imposed by the legislation in force [25,26]. After accommodation time, we have chosen to administrate iron as a chemical form that was easy to be absorbed being water soluble, having good bioavailability in living organisms, and being commercially available. To the experimental group we administrated intraperitoneal injection with ferrous-gluconate hydrated (Fe^{2+}) from Fluka company, in concentration of 15mg Fe^{2+}/body weight, two times during our experiment. During the experiment, to protect the organism from iron overload, we set a specific diet based on organic, fresh vegetables with very good antioxidant activity, consisting in seeds of black cumin; leaves of clover, chives, coriander, fenugreek, leek, parsley, rucola, radish; and also cucumber and carrots. At the end of experiment we analyzed the protein electrophoresis (albumin, α_1-globuline, α_2-globuline, β_1-globuline, β_2-globuline and γ-globulin), total iron binding capacity, and transferrin concentration in an authorized clinical laboratory. Analytical tests were performed in a clinical laboratory equipped with modern laboratory equipment, with software that allows the calculation and expression of the results in an easily centralized and statistically evaluated form [27,28].

Results and discussions

Protein electrophoresis evaluated the concentration of albumin (ALB), α_1-globuline (A1G), α_2-globuline (A2G), β_1-globuline (B1G), β_2-globuline (B2G) and γ-globulin (GG). The sequence of the serum protein fractions is giving by the position they occupy after migration into the electric field, namely albumin -the largest peak - will be the closest to the positive electrode, followed by alpha1- and alpha2-globulins, then beta1- and beta2-globulins, and the last will be gamma-globulin. The concentrations of protein fractions were expressed as g/dL and the values in the graphic presentation were given in mean ± standard deviation (fig. 1).

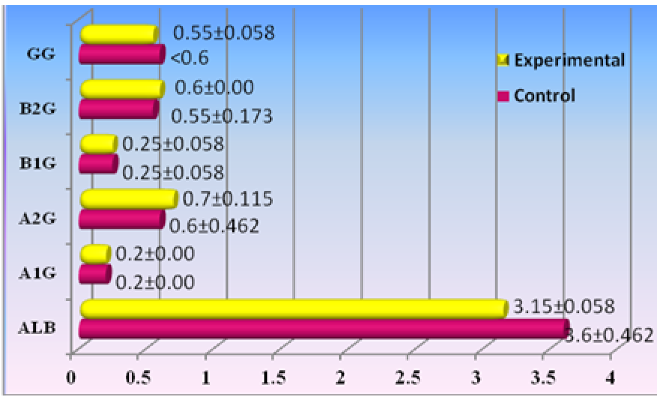

Fig. 1. Protein fraction electrophoresis from rabbits serum [g/dL] (albumin - ALB, α_1-globulin - A1G, α_2-globulin - A2G, β_1-globulin - B1G, β_2-globulin - B2G, γ-globulin - GG)

Protein electrophoresis is giving important information because iron is usually chemically bonded to proteins, and protein fractions offer information about possible chemical injury, inflammation, trauma, necrosis, and malignancy. The albumin (ALB) has normally the highest concentration in blood serum, 3.6g/dL for control group and a small decrease of 3.15g/dL for experimental group. A decrease value for albumin can be associated with a low synthesis of protein in liver or a high catabolism of proteins, but the decrease is not in pathological range [29]. Alpha-globulin fractions represented Alpha 1-antitrypsin, thyroid-binding globulin and transcortin and in our experiment the concentration of Alpha2-globulin increased compared to control values.Alpha-globulins can increased in inflammations, and also the concentration can be analytical modified due to the activity of ceruplasmin, alpha2-macroglobulin and haptoglobin. Beta-globulins represent beta1-globulin - form mainly from transferrin, and beta2-globulin form mainly from beta-lipoprotein, IgA, IgM, possible IgG, but also other proteins. Gamma-globulin is the mostly clinical interesting fraction because the migration part of gamma-globulin electrophoresis is the place of immunoglobulins. Between beta- and gamma-globulin migrates the C-reactive protein - another protein fraction related with inflammatory process.

In our experiment we also evaluated the serum total iron binding capacity and the concentrations are presented in figure 2.

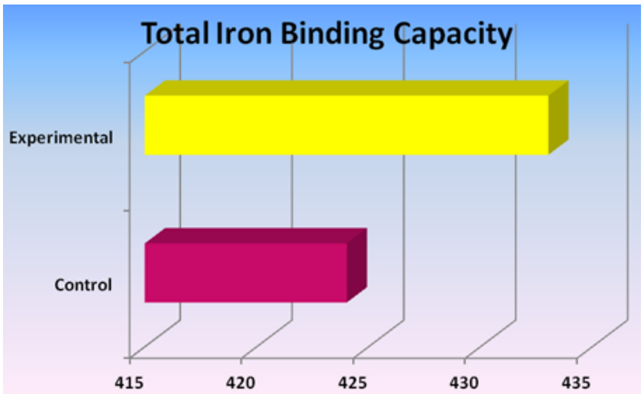

Fig. 2. Total iron binding capacity from rabbit's serum [μg/dL]

Serum total iron binding capacity (TIBC) evaluates the iron homeostasis from the blood, giving information about anemia-when serum iron is low or high values for serum iron. In our experiment the TIBC increased insignificant for experimental group compared to control group, but this is expected due to the administration of ferrous-gluconate. Cases of high TIBC, mostly associated with high serum iron concentration and transferrin, may explain a possible hemolytic anemia (when red blood cells are destroyed and the iron is released into the blood), iron or lean intoxication, often blood transfusions or liver injuries.

Serum transferrin presented values under 0.09 g/L for both control and experimental groups. The values of TIBC, serum iron and transferrin are very important in iron toxicity because if TIBC has a higher value compared to the concentration of serum iron that proves that in the organism there is no free iron that can cause toxicity [30]. The toxicity of iron is treated by administration of deferoxamine. Also, TIBC or concentration of transferrin can predict the iron deficiency or iron overload in organism [31]. Transferrin being the transporter of iron in organism, it could be used to modulate low or high serum iron in case of anemia or iron toxicity. Transferrin has two main functions in organism: assure the iron mobilization to the bone marrow for erythropoiesis, and bind the iron and prevents the reactive oxygen species (ROS) formation. Due to this function, a low concentration of transferrin in organism (hypotransferrinemia) will determine anemia and oxidative stress due to ROS. So, transferrin supplementation can correct anemia by assuring an optimal iron quantity for erythropoiesis and also binding the free iron in case of oxidative stress [32].

Conclusions

Serum protein electrophoresis, total iron binding capacity and transferrin are parameters which can give information about the iron homeostasis in organism.

In regards to our experimental study, a short term iron overload associated with a detoxifying vegetable diet, did not unbalance the iron homeostasis.

Serum proteins fractions and transferrin are proteins that can modulate the iron circulation and concentration in organism to assure optimum erythropoiesis and to prevent blood free iron that lead to reactive oxygen species formation, which affect the cell signaling and homeostasis.

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