Influence of Iron Administration on Kidney Serum Parameters

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Some minerals are essential nutrients for organisms being necessary in different quantities, but any homeostasis disturbance (deficiency or excess) can lead to various diseases. Iron in the body plays a critical role being directly involved in the respiration process – more precisely O_2 and CO_2 transportation, and also in beneficial oxidation-reduction processes. But sometimes the iron can also lead to the formation of substances with toxicogenic potential, more precisely to free radicals formed as a result of oxidative stress. In the liver, there is a specialized protein – hepcidin – which is the key regulator of circulating iron in the organism. In several cases of overdose, this protein blocks the iron transportation, binds it to the export channels of ferroportin and finally sends the excess iron to the kidney for urinal excretion. Our experiment tried to evaluate if in case of iron intraperitoneal administration in excess to rabbits, for a short period of time, it influences the serum creatinine, uric acid, and blood urea nitrogen if the rabbits were fed with a special organic diet. The results of the experiment presented a lower concentration of creatinine (with 24.46%), uric acid (with more than 41.48%), and blood urea nitrogen - BUN (6.45%) for experimental rabbits compared to the control group, proving that a well-documented diet influences the accumulation and excretion of excess iron.

Keywords: iron, creatinine, uric acid, blood urea nitrogen

Living organisms live in balance with the environment and ingested substances must be in accordance with the needs of the organism. Any insufficiency or excess has repercussions on the good functioning of the metabolic pathways which, in the long term, can lead to the onset of various disorders [1-3]. Iron is a very important nutrient for animal and human organism and usually a balanced diet bring sufficient iron to meet the organism requirements. The needs for iron could be higher in case of some physiological special situations like childhood, pregnancy, menstruation, hemorrhages, when the absorption of iron could be increased up to 20%. Also, normally the epithelia cells need iron because due to the desquamation these cells lose daily about 1-2mg iron. From dietary intake only about 10% from ingested iron is absorbed (about 10-20mg) every day and the iron in excess is excreted via renal function. If the kidney function is altered the homeostasis of iron could be disturb, and the iron concentration will increase and could lead to toxicity effects [4].

Iron is essential mineral for life being involving in oxygen transportation, electron transfer in mitochondria and not only, in intermediary and xenobiotic metabolism, in nucleic acid processing, DNA replication and repair, in cell signaling and in oxidative stress process [5]. In organism iron is usually bond to proteins – as organic cofactors and in iron-sulphur clusters – as inorganic cofactors. Iron has the ability to donate electrons and change its chemical form between Fe^{2+} and Fe^{3+}, participating to reduce oxygen being part of the factors that can generate reactive oxygen species [6].

The main source of inorganic and organic iron is the diet, and only in deficiency the supplementation is assured by iron intake. Iron absorption is made mainly in the duodenum and the upper part of jejunum. Transportation of iron is possible only if iron is bounded on some transporters like divalent metal transporter 1 (DMT1) or transferrin – which is the iron transporter in the bloodstream.

One of the most important proteins related to iron is the hemoglobin (a metalloprotein) which is responsible for oxygen transportation – oxyhemoglobin, and carbon dioxide transportation – carbaminohemoglobin [7]. The modulation of iron is possible due to involving of iron regulatory proteins IRP1 and IRP2. When high doses of iron come from diet, the absorption blocks the additional uptake, the concentration of iron increases in liver which releases the hepatic peptide – hepcidin. The hepcidin has the role to diminish the iron ferroportin release from intestinal mucosa which sends the absorbed iron into the circulation. The erythrocytes lead the greatest quantity in the spleen and another quantity is

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deposited in the renal parenchyma [8]. Ferritin is an iron-protein complex which stores initially the iron, but part of ferritin is uploaded by phagolysosomes in order to form hemosiderin granules.

The tests for iron that are performed into the clinical laboratory are serum iron, serum iron binding capacity, serum ferritin, complete blood count (CBC), bone marrow biopsy, liver biopsy. But usually the serum iron and total iron binding capacity are the most relevant and easy to do, which could be used to calculate the percent of transferrin saturation. Also, for iron excess renal function is also evaluated with laboratory tests for serum creatinine, blood urea nitrogen (BUN), uric acid (UA), glomerular filtration rate (GFR) [9, 10].

Iron homeostasis can be unbalanced either by iron deficiency or by iron overload. Iron deficiency is the most common iron nutritional deficiency that affects around 2 billion of people around the world [11]. The iron deficiency is a severe medical condition because the iron is part of the cytoplasm of red blood cells (erythrocytes) – which is rich in hemoglobin. The hemoglobin is an iron-protein that can bind oxygen, with the main function to transport oxygen from the lungs to all tissues. On the other hand, iron overload can be a cause of specific mutations in genes responsible with the iron transportation and regulation (hemochromatosis); of blood transfusions; or iron dietary supplementation. The biochemical explanation of this process is the reduction of hepcidin expression which cannot limit the iron absorption as the load is increasing [12].

Our experiment objective was to evaluate the kidney function after a short time of iron overload in rabbits.

Experimental part

The source of iron for the human and animal organism is primarily the food or feed, but in certain geographical areas, soil and water are also important sources of iron due to the soil rich in this mineral. Having in view these aspects, to prevent the occurrence of too much ingestion of iron there is the possibility of introducing into the diet of plants that could reduce or potentiate the absorption of iron and the overloading with iron. Starting to his idea our experiment was conducted on two groups of experimental rabbits – control group (C) and experimental group (E), every group having 5 rabbits weighing on average 703g. The rabbits were German Lop Eared breed, the experiment was conducted in the summer time for 43 days, and at the first period of time we give time to the animals to get comfortable, providing all the conditions imposed by the rules and legislation in force [13-17]. We administrated iron to the rabbits from the experimental group as ferrous gluconate hydrated (as Fe^{2+}, from Fluka company), administrated as intraperioteneal injections, twice during the experiment, in concentration of 15 mg Fe^{2+} / body weight. To assure the same stress for intraperitoneal administration also for the control group, we administrated physiological serum to the control rabbits. The ferrous gluconate was chosen having in view a good chemical form for absorption, bioavailability and commercially available. For the experiment we also thought to provide a special rabbits diet for the experiment to assure a minimum protection for iron overload, the diet consisting of the administration of organic vegetables as clover, coriander, parsley, radish, rucola, leek, chives, and fenugreek leaves; black cumin seeds; and fresh carrots and cucumber. At the end of experiment we collect blood serum from all the rabbits and we analyzed the serum concentration of creatinine, blood urea nitrogen (BUN), and uric acid (UA) in an authorized clinical laboratory.

Results and discussions

In order to evaluate the renal function of the rabbits as following the iron administration, we evaluated the concentration of creatinine and uric acid (table 1), and also blood urea nitrogen (table 2) from serum at the final of the experiment, presented in the chart as comparative data.

As we can observe from the graphical results presentation, the uric acid and creatinine concentration in blood serum decrease in the experimental rabbits compared to the control one. This fact shows that the administrated iron did not – in any way – impair the renal function, and by administering a protective diet the renal function was optimized.

We decided to analyze the serum creatinine and uric acid concentration because these are important indicators of kidney function. The creatine phosphate from muscle is converted in creatinine, but the quantity of creatine depends on the muscular mass. In men, about 1.5% of creatine is breakdown in creatinine. The creatinine is considered the most stable nitrogenous component of blood, which is directly correlated with muscular metabolism and is not influenced by food or feed intake, by physical or psychical effort, by circadian rhythm or other biological components. Serum creatinine test is very useful because it is the most specific indicator of renal function and a signal for kidney failure, but more important is to evaluate the BUN/creatinine rate for chronic renal diseases.

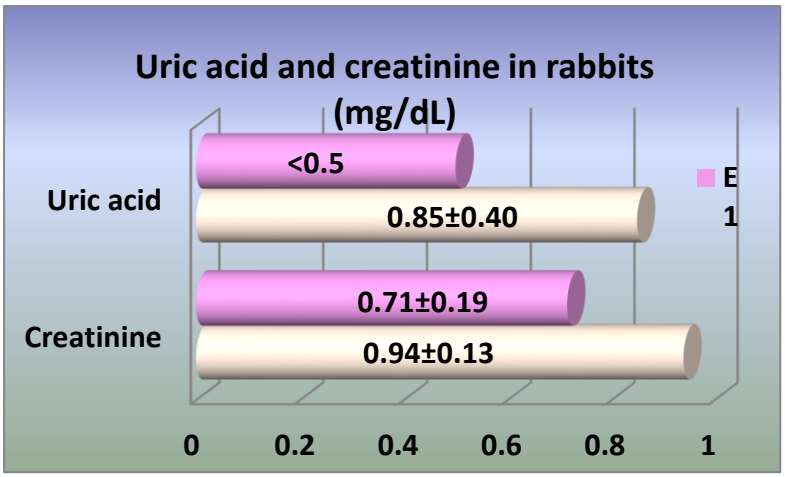

Fig. 1. Serum creatinine and uric acid after iron administration in rabbits

Different studies present the pathogenesis of excess iron on kidney function. Iron it could be accumulated in kidney due to a high ingestion of iron for a longer period of time, or due to the chronic kidney diseases. The catalytic iron can induce reactive oxygen species and oxidative stress that damage the renal cells. But, more of this, due to this oxidative stress the lipid peroxide is formatting and is accumulated, and this leads to activation of a specific iron-dependent regulated cell death that act like ferroptosis which is implicated in the renal ischemia reperfusion injury pathogenesis [18,19].

Urea is a biochemical compound synthetized in the liver from ammonium and represents the end product of the protein metabolism namely Krebs-Henseleit Cycle – that took place in mitochondria from hepatocytes and cytosol. From the liver the urea is transported to the kidney in order to be excreted through urine. The concentration of blood urea nitrogen (BUN) from serum depends on the protein intake, the liver metabolic function, and also of excretory kidney function [20, 21].

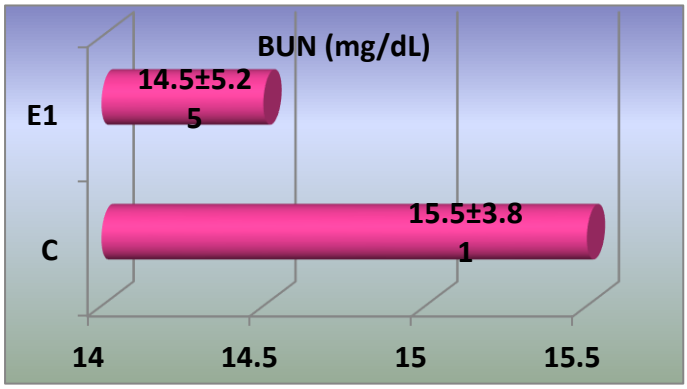

Fig. 2. Serum blood urea nitrogen of rabbits of control and experimental group

As the liver is the main detoxifying organ, so the kidney is responsible for the excretion of waste or excess substances. When iron is in elevated concentration in organism, this becomes toxic and the hepcidin – a specific protein, starts mediate it in the liver and iron is excreted through kidney and urine [10,22].

In our experiment the BUN concentration decrease slowly in the experimental group compared to the control group. This fact can be explained either by a moderate iron administration for a short period of time, or by the fact that the diet based on fresh organic vegetables provided very good protection of the liver – for metabolism, and of the kidney – for a very good excretion o iron excess.

Different studies present the effect of the iron overload on the kidney function. In thalassemia patients, the iron overload due to transfusions negatively affects the renal function and ferritin, blood urea nitrogen, creatinine, uric acid, albumin were significantly higher in patients compared to the control. Ferritin is a protein located inside the cell that plays a decisive role in the iron storage, being useful in the evaluation of deficiency or overload of iron, but it is an insufficient and unreliable test if it is not associated with other blood tests [23].

There are many studies which demonstrated that excess of metals or other chemicals could impair the organs function, mainly the liver and the kidney. Some of the heavy metals are essential for living organisms, but in large quantities, they are accumulated in tissues and cells, being able to change the oxidative status leading to oxidative stress allowing the formation of toxic products for the cell. These products could inactivate some enzymes, could damage the proteins and nucleic acids or could allow lipid peroxidation with the formation of free radicals which are very reactive and toxic [24-29].

Recent researches have reported that for a better evaluation of the damages produced by iron overload it is necessary to test the transferrin saturation – calculated as 100 x serum iron / total iron binding capacity. If the transferrin saturation

is higher than 45% it is recommended to do further tests for the specific hemochromatosis gene, whether is or not is hyperferritinemia [30].

The evaluation of the effects of iron excess intake has to be done taking in consideration the chemical form of administered iron, the time period of experiment, the iron concentration administered, the diet, the environment, and of course the blood tests and the histopathological examination together with other analytical tests with allowed evaluating the concentration of different minerals that could be influenced by one metal in excess. Anywhere, these studies are very important due to the presence of mineral all over in all environments, and also due to the pollution in air, soil and water that can negatively affect the living organisms.

Conclusions

Iron is an essential element for living organisms, found all over in environment. Due to the possibility of participating in the processes of oxide reduction, iron can change its oxidation state and can lead to the formation of free radicals, leading to oxidative stress, with negative consequences on the different physiological and biochemical processes.

Intraperitoneal administration of iron in excess to rabbits can impair mainly the liver and kidney function. In the liver, some specialized proteins block the intake and accumulation of iron and the excess is transferred to the kidney to excrete it through the urine. Creatinine, uric acid, and blood urea nitrogen are good parameters for renal function evaluation. In our experiment, the kidney function has not been altered, because the rabbits had a detoxification diet that protected the body from excessive iron loading and prevent impair renal function.

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