Iron, Zinc and Copper in Selected Tissues of Rabbits under Increasing Exposure to Iron

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Abstract

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The aim of this study was to demonstrate the disturbance of zinc/copper balance in tissues as the possible accelerating factor of liver damage after short- and long-term exposure to high doses of iron in case of its accidental overdosing or experimental haemochromatosis. Thirty chinchilla rabbits were administered Jectofer /iron (III) - sorbitol iron complex/

Short-term administration was given to groups A and B, respectively. Long term administration was given to groups C, D and E, respectively. The contents of metals in the fur, muscles, skin and liver were determined by atomic absorption spectrometry (AAS).

After short-term administration the highest concentrations of iron in comparison to the control group were observed in liver (about 42-46%) at each collection ($p < 0.05$), copper concentration in liver slightly decreased (≈23%), but increased in other tissues (by a maximum of 55%). Accumulation of zinc in fur was stable, but a decrease in other tissues like liver, muscles and skin was observed (4 - 35%).

After long-term administrations changes in zinc concentration were found (higher in the fur of groups C and D and in the liver in group E; lower in the skin of groups: C and E). On the other hand, the long-term administration of Jectofer caused a decrease in copper concentration in fur (23-70%) but caused a 3-to-5 fold increase in the skin of experimental rabbits.

Short-term as well as long-term administration of iron affect changes of copper/zinc equilibrium concentrations in different tissues and in livers of rabbits.

Liver, skin, muscles, fur, selected metals

The maintenance of homeostasis in a living organism ensures appropriate, physiological contents of both organic and inorganic compounds including ions of specific metals (iron, zinc and copper). An excess of iron present in an organism may affect numerous functions of the whole organism (e.g. liver dysfunction). Haemo-chromatosis is the most significant disorder (Barisani et al. 1996; Adams et al. 1997). Primary (idiopathic) haemo-chromatosis is a metabolic, genetically conditioned disease. Secondary haemo-chromatosis is mainly caused by increased absorption of iron from the alimentary tract (Little 1996). In this case the liver is the most affected organ due to absorption of iron; this may consequently lead to progressive hepatocellular damage resulting in hepatic cirrhosis with a frequent fatal outcome (Little 1996).

According to many authors, toxicity of iron ions lies in their participation in reactions that generate free oxygen radicals (Higashiyama et al. 1990), and thus lead to increased peroxidation of cellular lipids (Qian et al. 1996).

Copper is another metal that is accumulated in the liver. Ions of copper absorbed in the alimentary tract are transported with albumin to the liver, where they take part in the synthesis of coeruloplasmin. Copper plays an important role in releasing iron from the liver and in synthesis of haem (Brewer et al. 1993).

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Zinc plays an important protecting role in hepatocellular damage caused by e.g. D-galactosamine. Supplementation of zinc ions decreases mortality in animals due to cessation of lipid peroxidation, increasing protein biosynthesis and general improvement in the functioning of the organ (Milanino et al. 1992). All these metal ions participate in redox reactions, and thus contribute to regulation of homeostasis**.**

To our knowledge, no data are available on changes in zinc and copper after iron administration in rabbits. The aim of the present work therefore was to evaluate the quantitative distribution of zinc, copper and iron in selected tissues (fur, skin, muscles and liver) of rabbits after repeated administrations of an iron preparation.

Materials and Methods

The experiment was conducted on 30 chinchilla rabbits of 3.2 ± 0.1 kg body mass each. They were randomly assigned to six groups (five animals per group) A, B, C, D, E and K (controls). During the experiment, each animal was placed in a separate cage. The room with cages was air-conditioned. Temperature was maintained at a constant level (about 22-24 °C) and humidity about 70% with a constant day/night cycle (08.00 - 08.00 h). The animals had free access to water and food. A standard Labofeed was used. The animals were treated according to detailed recommendations of the Polish National Academy of Sciences concerning experiments on animals.

The pharmacological preparation of iron - Jectofer /iron (III) - sorbitol iron complex/ produced by Astra D. Bosnolijek (Yugoslavia) was administered i. m. as follows: group A received 1 dose of Jectofer (6 mg·kg^{-1} body mass each) twice a day at 3-h intervals for one day. Group B was given 1 dose of Jectofer (6 mg·kg^{-1} body mass each) four times a day at 3-h intervals for one day. Animals in Group C received a single daily dose of Jectofer (6 mg·kg^{-1} body mass) during 3 subsequent days, Group D received the same dose once a day for 10 days, and Group E received this dose (6 mg·kg^{-1} body mass) once a day for 90 days.

The animals of group K (control) obtained i.m. injections of isotonic saline solution. All animals survived the procedures. No serious pathological symptoms were observed, except for the animals of group E that were apathetic after 90 days of Jectofer administration.

The animals were anaesthetized and euthanized with pentobarbital sodium 24 hours after being given the last dose of the iron preparation.

Examined samples (10g each) were taken from muscles of the thigh, the right part of the liver, the skin of the back, and the fur taken from the abdominal area.

The contents of iron, zinc and copper were then determined in mineralised samples of tissues by atomic absorption spectrometry (AAS) using the Perkin – Elmer atomic absorption spectrophotometer (Pinta 1997).

The concentrations of Fe, Cu, and Zn were determined by the Perkin-Elmer apparatus with an air-acetylene flame and a background deuther correction. Detectability of the particular metals was as follows: 10 μg Fe/cm^3, 1 μg Zn/cm3, 1 μg Cu/cm^3.

As for the results obtained during determination of iron in the solution by means of the AAS method in the range of pattern concentrations 0.0-4.0 μg/cm^3, the value of SD (standard deviation) was 0.015 and the value of γ (variability coefficient) was 6.6%; for zinc solutions in concentrations 0.0 – 1.0 μg/cm^3 the value of SD = 0.001, γ = 5.14%; and for copper solutions 0.0 – 1.0 μg/cm^3 SD = 0.003 and γ = 4.05%. The collected results were presented in the tables as average ± SD. The results were analyzed by analysis of variance with a reliability level of 95%, and by the Student's *t*-test. The value $p < 0.05$ was considered to be statistically significant.

Results

The results (Table 1) show that two and four administrations of the iron preparation to rabbits given at short intervals (group A and B) for one day did not cause significant changes in the contents of these elements in fur and muscles as compared with the control group K (see also Tables 1 and 2).

Table 2 shows the results collected from the groups of rabbits given Jectofer for 3, 10 and 90 days, respectively.

Iron, copper and zinc in tissues of rabbits following a short-term Jectofer administration

Administration of Jectofer for 1 day (group A) did not cause substantial changes in the iron concentration in the fur, muscles and skin of animals as compared to the control group. However, in group B, the mean concentration of iron in the muscle tissue was 63% higher compared to the controls. Increased concentration of iron in the liver was observed in groups A and B. However, the mean iron concentration in the skin of group B rabbits was a 75%

Table 1.The mean contents of iron, zinc and copper (μg/g of dry mass) in the fur, muscles, skin and liver of rabbits administered in two or four doses during a very short period (groups A, B and control group K)

	Group	Fur	Muscle	Skin	Liver
Iron	K (control)	60.0 ± 4.0	18.6 ± 1.2	6.8 ± 0.5	13.6 ± 0.9
	A	57.8 ± 3.8	18.7 ± 1.2	7.7 ± 0.5	19.4 ±1.3*
	B	61.0 ± 4.0	30.3 ± 2.0*	11.9 ± 0.8*	19.9 ±1.3*
zinc	K (control)	40.4 ± 2.1	12.3 ± 0.6	12.1 ± 0.6	34.5 ± 1.8
	A	39.7 ± 2.0	11.6 ± 0.6	10.4 ± 0.5*	30.1 ±1.0*
	B	39.5 ± 2.0	9.6 ± 0.5*	8.0 ± 0.4*	33.2 ±1.7
Copper	K (control)	11.3 ± 0.5	4.1 ± 0.2	3.1 ± 0.1	7.6 ± 0.3
	A	11.5 ± 0.5	4.6 ± 0.2	4.1 ± 0.2*	5.9 ± 0.2*
	B	14.7 ± 0.6*	5.8 ± 0.2*	4.8 ± 0.2*	5.9 ± 0.2*

* significantly different from the control *$p < 0.05$

Table 2. The average contents of iron, zinc and copper (μg/g of dry mass) in the fur, muscles, skin and liver of rabbits administered as one dose of iron per day for 3, 10 and 90 days (groups C, D and E, respectively) vs. the control group K

	Group	Fur	Muscle	Skin	Liver
Iron	K (control)	60.0 ± 4.0	18.6 ± 1.2	6.8 ± 0.5	13.6 ± 0.9
	C	66.6 ± 4.4	23.1 ± 1.5*	7.2 ± 0.5	14.1 ± 0.9
	D	78.9 ± 5.2*	26.4 ± 1.7*	11.8 ± 0.8*	39.6 ± 2.6*
	E	87.4 ± 5.8*	34.0 ± 2.2*	24.0 ± 1.6*	190.0 ± 12.5*
Zinc	K (control)	40.4 ± 2.1	12.3 ± 0.6	12.1 ± 0.6	34.5 ± 1.8
	C	185.1 ± 9.5*	13.6 ± 0.7	8.7 ± 0.4*	40.3 ± 2.1*
	D	259.3± 13.3*	12.5 ± 0.6	11.2 ± 0.6	33.4 ± 1.7
	E	57.9 ± 3.0*	11.7 ± 0.6	8.1 ± 0.4*	481.6 ± 24.7*
Copper	K (control)	11.3 ± 0.5	4.1 ± 0.2	3.1 ± 0.1	7.6 ± 0.3
	C	7.0 ± 0.3*	3.4 ± 0.1*	11.3 ± 0.5*	8.2 ± 0.3
	D	8.8 ± 0.4*	5.6 ± 0.2*	16.7 ± 0.7*	4.8 ± 0.2*
	E	3.3 ± 0.1*	3.9 ± 0.2	11.7 ± 0.5*	4.7 ± 0.2*

* significantly different from the control $p < 0.05$

increase compared to the controls. The iron content of the liver increased 43% in group A and 46% in group B, respectively.

Zinc concentrations in the fur of rabbits (groups A and B) were similar to those obtained in the controls. In the muscle, skin and liver tissues, decreased zinc concentration was observed in comparison to the control group. The muscles of rabbits of group B contained 22% less zinc than the controls. Zinc concentration in the skin (group B) was 43% lower than levels in the control group. The mean amount of copper in the fur from group A was similar to the control group.

Copper amount observed in the skin of animals from group A was 32% of the control levels but the same variable in the liver decreased 22% in comparison to the control group.

In the tissues examined from group B animals (fur, muscles, skin), the average amount of copper increased 30%, 42% and 55%, respectively. On the other hand, in the liver a decrease of copper level (22% compared to the controls) was observed.

Iron, copper and zinc in tissues of rabbits after 3, 10 and 90 days of Jectofer administration

After 3, 10 and 90 days of administration of single daily doses of Jectofer to rabbits (group C, D and E), the average amount of iron in the fur of animals in group C was 66.6 μg/g dry

mass. The same variable in group D was 78.9 μg/g dry mass and in group E – 87.4 μg/g dry mass. In comparison to the control group, an increase of iron concentration in the fur in groups D and E was observed; the values were 31% and 46%, respectively.

The amount of iron in the muscles increased, too, and was 24% in group C compared to the controls. The same variable in group D and E was 42% and 83%, respectively. In the skin, the average iron amount was 73% higher in group D than the controls. In group E, the mean concentration of iron in the skin tissue of the examined rabbits had increased three times as compared to the control.

After a 3-day period of Jectofer administration, the average amount of iron was close to the control, but after 10 days it increased to 39.6 μg/g dry mass (Table 2), nearly twice compared to the control. A fourteen times higher concentration of iron in the liver tissue in the group of rabbits given Jectofer for 90 days was observed.

The average amount of zinc in the fur of animals after 3 days of Jectofer administration was 185.1 μg/g dry mass, after 10 days - 259.3 μg/g dry mass, and after 90 days - 57.9 μg/g dry mass (Table 2).

In the fur of animals from group C and D, the average amount of zinc increased five times after 3 days and six times after 10 days, but after 90 days (group E) the same variable increased only 43% compared to the control. After 3, 10 and 90 days, the average concentration of zinc in muscles equalized to the value of the control group. In the skin, zinc concentration decreased to 35% after 3 days of the experiment; after 10 days it approached the control; after 90 days it decreased again and was similar to the value observed on the third day. The most conspicuous changes in zinc concentration were observed in the liver tissue.

The average amount of zinc after 90 days was 14 times higher than the control.

The amount of copper in the fur decreased during the experiment and after 90 days was only 29% of the control. The same variable in muscles was 17% lower after 3 days in comparison to the control group; after 10 days it increased (37%); and after 90 days it decreased for about 5% in comparison to the control group.

Substantial decrease of copper concentration in the liver (37%-38%) was observed especially after 10 and 90 days of iron administration. Copper concentration in the skin increased 3 times after 3 and 90 days, but maximum level was observed on the 10th day of the experiment, exceeding the controls 5 times.

Discussion

The amount of administered iron was established on the basis of estimations available in medical publications concerning the bioaccessibility of iron (Dutra-de-Oliveira et al. 1995; Hulten et al. 1995).

The time schedule of the experiment was arranged in order to measure the dynamics of quantitative changes of the examined metals.

Observation of changes in the amounts of metal ions in the examined tissues of groups A and B (apart from fur) compared to the controls showed an almost immediate response. Iron was accumulated along with a simultaneous non-significant decrease of zinc concentration and an increase of the amount of copper in muscles, with a simultaneous decrease of its amount in the liver.

The quantity of metals except for the content of copper in group B practically did not change. It is probably due to the short time of the experiment; this assumption is supported by changes in the amounts of the examined metals noted in the fur of animals from groups C, D and E.

As the experiment proceeded, iron and zinc were being accumulated in the fur, but copper was not. Metabolism of copper is probably faster than that of iron and zinc.

The increase of the content of iron in the muscles of groups C, D and E does not influence the change in the content of zinc in this tissue. It seems that changes in the content of copper in the muscle tissue are not connected with regular changes in the content of iron in this tissue.

The increase of the content of iron in the skin is accompanied by an increase of copper concentration and not explicit decrease of the content of zinc.

The increase of the content of iron in the liver observed in groups A, B, C and E was accompanied, except for group C, by a decrease of the content of copper.

In conclusion, our results show that a long-term administration of the iron preparation Jectofer causes its accumulation in all the examined tissues, with the most pronounced effect on the liver. Such accumulation of iron may lead to serious disturbance of the balance of ions, such as copper and zinc in the liver.

Železo, zinek a měd ve vybraných tkáních králíků při zvýšené expozici železu

Cílem práce bylo prokázat narušení rovnováhy zinku a mědi v tkáních, jako možný faktor urychlující poškození jater po krátkodobé a dlouhodobé expozici vysokým dávkám železa při jeho náhodném předávkování či experimentální hemochromatóze. Králíkům plemene činčila (n = 30) byl aplikován Jectofer (Fe^{3+}) – komplex sorbitolu a železa. Krátkodobá aplikace (2 dávky Jectoferu v dávce 6 $mg \cdot kg^{-1}$ živé hmotnosti dvakrát a třikrát denně v 3 h intervalech) byla provedena u skupiny A a B. Dlouhodobá aplikace (6 $mg \cdot kg^{-1}$ ž.h. jednou denně a to 3., 10. a 90. den) byla provedena u skupin C, D a E. Obsah kovů v srsti, svalech, kůži a játrech byly zjišťovány pomocí atomové absorpční spektrometrie (AAS).

Po krátkodobé aplikaci byla ve srovnání s kontrolní skupinou ze všech odběrů ($p < 0,05$) zjištěna nejvyšší koncentrace železa v játrech (42-46%), koncentrace mědi se mírně snížila v játrech (23%), ale v ostatních tkáních se zvýšila (s maximem 55%). Akumulace zinku v srsti byla stabilní, ale v ostatních tkáních jako jsou játra, svaly a kůže se jeho obsah snížil (4-35%).

Po dlouhodobé aplikaci v 3., 10. a 90. den byly zjištěny změny v koncentraci zinku (vyšší obsah v srsti skupin C, D a v játrech skupiny E; nižší obsah v kůži skupin C a E). Naopak dlouhodobá aplikace Jectoferu způsobila pokles koncentrace mědi v srsti (23-70%), ale 3 až 5 násobný nárůst v kůži experimentálních králíků. Krátkodobá stejně jako dlouhodobá aplikace železa narušuje rovnováhu mědi a zinku v sledovaných tkáních a játrech králíků.

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