THE EXPERIMENTAL IRON DISORDERS OF THE LIVER AND SMALL INTESTINE

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ABSTRACT
Two feeding studies with rats and cockerels were conducted to evaluate the role of iron deficiency and overload in the morphofunctional changes in the liver and intestine. Administration of an iron – deficient diet to Wistar-Kyoto rats resulted within 20 days in significantly reduced serum iron concentrations, a hypochromic anemia, and increased intestinal villus height but no pronounced effects on cell proliferation was observed. The iron concentration in the liver of iron – deficient rats was found to increase. At the same time, the most common laboratory tests for the detection of liver injury – serum aminotransferases (ASAT and ALAT) were altered. We considered that moderate iron deficiency results in complex systemic disorders, including hypochromic anemia, duodenum mucosal hyperplasia and hepatocellular injury. Our observations emphasize also the importance of vacuolar compartmentalization in controlling iron fluxes in enterocyte and suggest that TRPV1- dependent iron uptake by subapical vesicles may play an important role in metal ion homeostasis of intestinal epithelium.

KEY WORDS: iron deficiency, iron overload, TRPV1 expression.

INTRODUCTION
Iron is essential to life, because of its ability to donate and accept electrons. The most important group of iron-binding proteins contains the heme molecule, all of which contain iron at their centers. For example, animals use iron in the hemoglobin of erythrocytes. Iron is absorbed from food by enterocytes in the duodenum. It circulates in the bloodstream bound to transferrin and is delivered to sites of utilization and storage. In mammals the limited availability of soluble dietary iron is reflected in the lack of a true excretory system (iron is lost from the body through bleeding and sloughing of skin and mucosal cells) and the use of high affinity binding proteins for iron transport and storage. Iron deficiency and iron overload are among the most prevalent nutritional disorders worldwide. Severe iron deficiency results in complex systemic disorders including metabolism of energy and minerals. The role of iron deficiency as cause of liver and small intestine disfunction has never been studied in detail (8, 14). It should also be pointed out, that the data regarding villus height and crypt cell mitosis is such circumstances are still controversial (1, 10, 11**).** Other aspects of iron homeostasis, such as regulation of intracellular iron distribution, also remais largerly unexplored. Experimental approaches to analyze these mechanisms have been considerably advanced by the discovery of mammalian homologs of the Drosophila transient receptor potential cation channel, subfamily V, member 1 (TRPV1).TRPV1 is now known to be expressed not only in the peripheral sensory neurons, but also in the spinal cord, brain and wide-range of non-neural cells (e.g. enterocytes, urothelium, fibroblasts and hepatocytes) (2).

Therefore, the objective of present experimental study conducted on laboratory animals was to evaluate the duodenal morphometry, the extent of hepatocellular injury and iron status indices of rats with iron – deficiency anemia under controlled environmental conditions. We wished to examine also the role of TRPV1 in the intracellular iron compartmentalization within the enterocyte.

MATERIAL AND METHODS

The effect of iron deficiency on liver and small intestine morphology have been studied in the first block of experiments. The experiment was carried out with 8 male rats Wistar-Kyoto, which were given a semi-synthetic ID-diet with suboptimal iron (3 mg iron per kg diet) over a total of 20 days. The study also included healthy control group (n=8) rats fed with the same diet, but enriched by iron - 270 mg iron/kg diet (7).Blood erythrocytes and hemoglobin were detected on ILAB-300 analyzer (Instrumentation Laboratory), and blood serum ASAT, ALAT - on Colter Hmx (Beckman) ones. Fe determinations in liver dry ashed material (heated to 480ºC for 48h in silica crucibles), as well as in deproteinized blood serum were measured by flame atomic absorption spectroscopy (Perkin-Elmer, Analyst 700).

One-to-thirty-day old Lohmann Brown cockerels have been used to investigate the intracellular distribution of TRPV1 and compartmentalization of iron in intestinal epithelial cells. One-day-old birds were received from Latvian poultry manufacture 'BALTICOVO'. Chickens were housed in cage units with free access to food and water. Animals were divided into two groups of seven heads in each. Birds of the first group were fed on a wheat-barely full-fed basal diet containing all the necessary nutrients (control). Chickens from the second group were provided with the same basal diet plus 1000 mg Fe/kg. At the end of the experiment, the 30-day-old nonfasted cockerels were killed by decapitation in accordance with the Recommendations for thr Euthanasia of Experimental Animals of the Europe Convention. The abdomen was immediately opened, and the small intestine (duodenum) was dissected out. Each segment was washed out with an ice-cold saline solution (0, 9% NaCl, Merck, in nano-pure water), to remove food remnants. The experiments were approved by the local animal ethics committee.

Routine hematoxylin – eosin sections were prepared from formalin – fixed, paraffin – embedded tissues. Immunohistochemical studies were performed by indirect staining methods using antibodies against CD68 (macrophages), CD 235 (glycophorin A), and TRPV1. Perl's stain is used to detect stainable tissue hemosiderin. The mitotic index representing the percentage of cells in mitosis in the crypt was calculated. The length of villi in the duodenum was also measured. The datasets were statistically compared using the Student's unpaired test.

RESULTS AND DISCUSSION

In the liver of control rats Perl's stain did not show the stainable tissue hemosiderin. Otherwise in experimental group Perl's stain showed the presence of hemosiderin in Kupffer cells and in some hepatocytes (Fig.1). Immunohistochemical analysis of liver specimens from iron – deficient rats revealed that CD235-positive erythrocytes are frequently aggregated around Kupffer cells. Some of these cells show erythrophagocytosis.These events was also accompanied with an accumulation of iron in the liver. Liver iron concentrations in the iron – deficient rats were 128% of control values (183.43 +/- 4.93 vs 236.8 +/- 6.31 micrograms/g dry tissue weight; $p < 0.02$). Rats fed an iron – deficient diet were anemic. These rats had significantly lower mean serum iron concentrations (1.15 +/- 0.35 vs 41.25 +/- 19.72 micrograms/ml serum; p <

0.01), and mean hemoglobin concentrations (10.10 +/- 0.54 vs 17.71 +/- 0.92 g/dL; $p < 0.001$). Statistically insignificant decrease in the erythrocyte count was also observed (6.11 +/- 0.55 vs 7.93 +/- 0.66 million/mm^3).

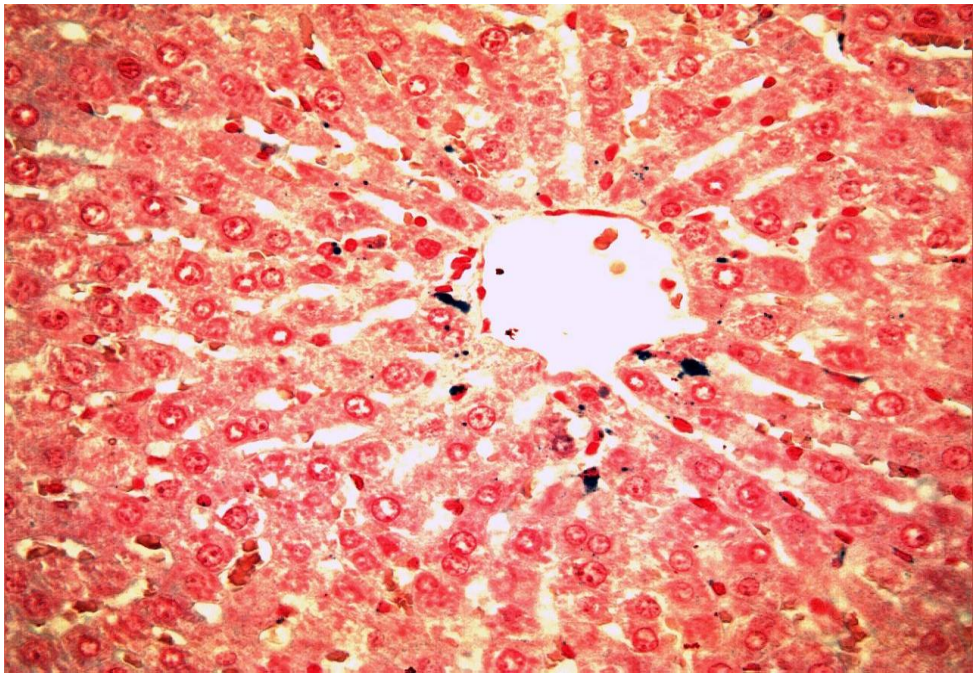

Figure 1. **Positive Perls' staining is localized to some Kupffer cells and hepatocytes**

A slight, but statistically insignificant increase of ASAT (289.53 +/- 74.07 vs 278.28 +/- 56.22) and ALAT (57.50 +/- 13.07 vs 52.00 +/- 6.08) was noted after 20 days of iron restriction. ASAT and ALAT were 114% and 111% of control values.

The cell proliferation parameters (mitotic index) did not differ between groups (5.1 +/- 0.1 vs 5.3 +/- 0.2 in the experimental group). In the experimental group, the mean length of the villi increased up to 623 +/- 41.2 μm in comparison to control group (437 +/- 36.7 μm).

Chickens fed a high iron diet had blue Perls'-positive material within a subapical vesicular compartment of enterocytes, which may represent recycling endosomes. At the same time, enterocytes showed a preferred apical distribution of TRPV1, as demonstrated by immunohistochemistry (Fig.2).

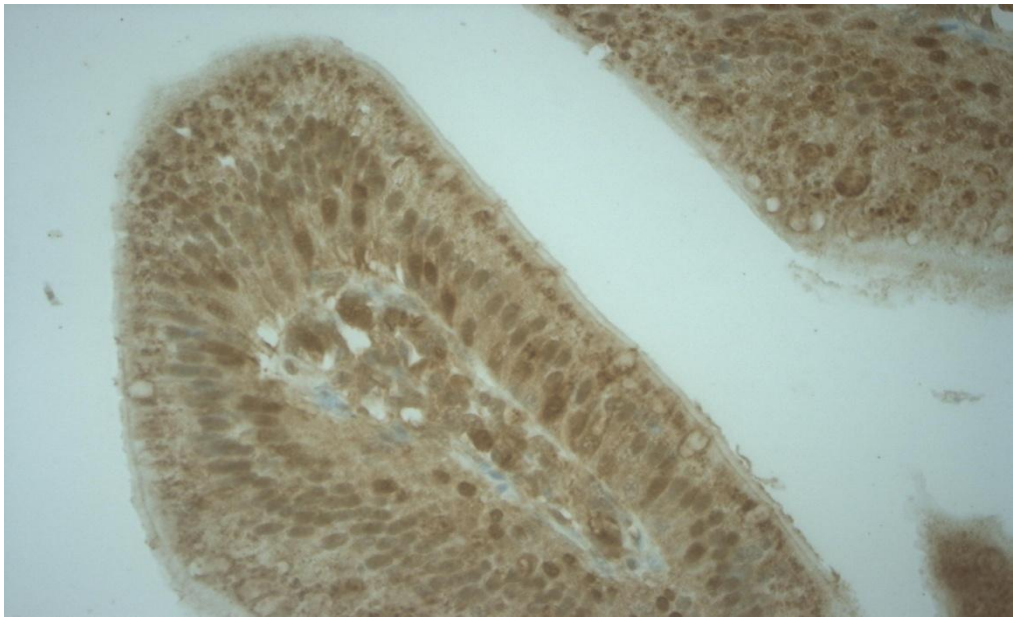

Figure 2. **Positive apical vesicle TRPV1 immunohistochemical staining (arrows)**

In enterocytes, the polarizesd distribution of membrane enzymes, channels and transport proteins provide the basis for the vectorial transport functions of the epithelium. Intestinal iron absorption involves proteins located in the brush border membrane, cytoplasm, and basolateral membrane of duodenal enterocytes. The apical divalent metal transporter-1 transports ferrous iron from the lumen into the cells, while the basolateral transporter ferroportin extrudes iron from the enterocytes into the circulation. Once inside the enterocyte, there are two fates for iron: it may leave the cell or it can be bound to ferritin. We hypothesized, that the third homeostatic mechanism may include the uptake of iron into the apical vesicles. This conclusion is consistent with the some of our early investigations directed at clarifying the role of subapical vesicles in the regulation of zinc homeostasis (6). It appears reasonable to attribute the observed expression of TRPV1 on the apical vesicles to this phenomenon. Cellular iron homeostasis must be balanced to supply enough iron for metabolism and to avoid excessive, toxic levels. To perform intracellular compartmentalization, iron can cross a vesicle membrane with the aid of TRPV1. There would be a high probability that TRPV1 is not specific to calcium, and can transport other metal ions. Moreover, at higher iron concentrations other mechanisms (perhaps associated with the vesicular transport of iron) may come into play (4).

As iron metabolism principally takes place in liver, intestines and blood, we studied the interference of iron deficient anemia in the morphofunctional changes of these organs and tissues. Intriguingly, iron deficiency results in accumulation of iron in the liver. We do not yet understand how this occurs, but we envision two possibilities. First of all, the induction of transferrin receptors might be one of the mechanisms of iron deposition in hepatocytes (9, 12). In the second place it may be that phagocytosis of damaged erytrocytes is closely related to the storage of iron derived from hemoglobin. Most of the iron in the organism is recycled when old erythrocytes are taken out of circulation and destroyed with their iron scavenged by macrophages, and returned to the storage pool for re-use. It is well established that peroxidative damage results in significant reduction in erythrocyte lifespan in iron deficiency (3). Present study showed

that iron deposition in the liver occurs primarily in Kupffer cells and then "spills over" into hepatocytes to a lesser degree.

Both inadequate and excessive iron cause significant mitochondrial malfunction (13). In our previous studies we have also demonstrated that mitochondria are target organelles for iron (5). Therefore, alterations of the most reliable markers of hepatocellulas injury (ALAT and ASAT) seemed to have been due more to iron induced damage of hepatocyte mitochondria and subsequent cell death in hepatocytes.

After 20 days of iron deficiency a duodenum mucosal hyperplasia was observed. The increase of villus height in iron-deficient rats is most probably due to the decreased rate of cell death among mature enterocytes. We are of the opinion that these changes reflect an attempt to increase iron absorption to counteract iron deficiency. Serum iron concentration decreased approximately 35 times in iron – deficient rats compared to controls. It can be concluded that serum iron value is a sensitive indicator of possible iron deficiency anemia. A short – term moderate iron deficiency with ranging hemoglobin from 17.71 to 10.1, was accompanied by a decrease of erythrocyte count. The results obtained in this study indicate the occurrence of hypochromic anemia in experimental animals.

We consider that our results offer a valuable tool for investigating nutritional disorders and for screening pharmaceutical compositions for improving iron absorbtion. Our observations also provide a basis for elucidation of the physiological role of TRPVI in iron homeostasis.

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