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HISTOPATHOLOGICAL STUDY

Iron-rich complexes in human spleen in hereditary spherocytosis

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Abstract: *Objective:* Tissue iron plays an important role in the development of certain diseases. Although it is one of biogenic elements, its excess induces the reactive oxygen species (ROS) formation. The aim of the present work is to examine the protection against free or loosely bound iron from the view of morphology and chemical composition of iron-rich complexes in human spleen tissues with hereditary spherocytosis (HS) by scanning and transmission electron microscope with energy-dispersive microanalysis (EDX).
Results: The examination of human spleen tissues by scanning and transmission electron microscope showed covering of iron-rich particles. EDX revealed many iron-rich complexes of multi-element composition in HS samples with sulphur and phosphorus as the major elements. Detection was negative in the reference samples.
Conclusion: The covering of iron-rich particles can be explained by elimination and isolation of ferritin/iron complexes from surrounding environment to prevent the ROS formation. Sulphur, phosphorus and their compounds are probably the most significant elements that influence the ROS formation *(Fig. 5, Ref. 16)*. Full Text in PDF *www.elis.sk.*
Key words: iron, spleen, EDX microanalysis, electron microscopy, reactive oxygen species.

Tissue iron plays an important role in the etiology of several diseases. Iron in brain plays a decisive role in the process of aging and neurodegenerative diseases (1). It is one of the biogenic elements and its excess induces oxidative stress in tissues. Our knowledge about the iron presence comes mainly from measurements by nuclear magnetic resonance (NMR, 2). A disadvantage of this method, however, is that it provides only qualitative information about iron in the analyzed tissue. Compared to NMR methods, histochemical methods have a higher specificity and a higher specificity level. However, they do not provide the information about physicochemical properties of iron and its compounds in the examined samples. Another weak point of the histochemical methods is the inability to present iron compounds if their dimensions or content are below the discrimination power of the light microscope.

Iron is present in the human body predominantly in the form of ferritin (3). This protein forms spherical formations of the size about 12 nm. Under physiological conditions, in the spleen old or damaged red blood cells (RBC) are destroyed (4) and subsequently engulfed by its lysosomes of macrophages (5). Hereditary spherocytosis is a spherocytic malformation of the red blood cells. The shape abnormalities cause a defect in spectrin-a RBC membrane protein (6). The change in shape of RBC affects their sequestration in the spleen. They are mistaken for old or damaged RBC and are phagocyted and destroyed. Iron accumulation in cords can result in their fibrosis (7). Iron is a prominent inductor of ROS. Their overproduction is believed to play a role in the pathogenesis of many diseases in general (8).

The aim of the present work was to examine the protection against free or loosely bound iron from the view of morphology and chemical composition of iron-rich particles in human spleen tissues with hereditary spherocytosis (HS) by the scanning and transmission electron microscope with energy-dispersive microanalysis (EDX).

Methods

Samples

Five samples of human tissues with clinicopathological diagnosis of hereditary spherocytosis were studied by scanning and transmission electron microscope with EDX. As the reference samples, the samples of human spleen after a crash accident were used.

Scanning electron microscope (SEM) and EDX microanalysis

The samples of human tissues were embedded in paraffin blocks, cut by microtome to 5 μm thin sections and mounted on glass support. Unstained and uncovered samples were analyzed by the scanning electron microscope on the instrument JXA 840 A (JEOL, Japan) with the accelerating voltage of 20 kV. Simultaneous EDX analysis was performed with KEVEX 3205-1200 (Kevex, Valencia, Ca). The time period of spectrum collection was 200 s with the energy range 0.160 to 9 keV.

Transmission electron microscope (TEM)

The samples for the transmission electron microscopy study were fixed in 3% solution of glutar(di)aldehyde (SERVA, Hei-

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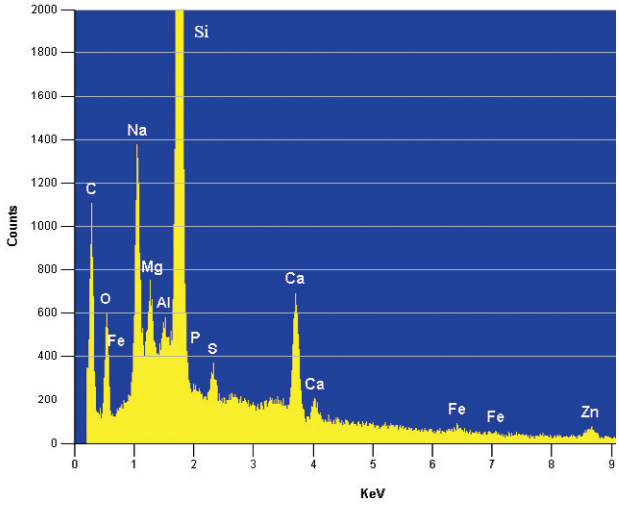

Fig. 1. Human spleen, reference sample, EDX microanalysis. Spectrum showed the element composition with various concentration of C, O, Na, Mg, Al, Si, S, Ca, Fe and Zn. The presence of silicon and sodium in the sample is caused by a glass support. Carbon-coated, energy range 0.160–9 keV, collection time 200 s.

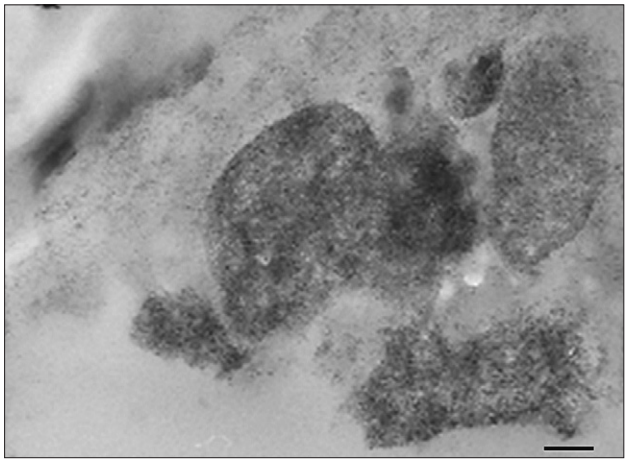

Fig. 2. Human spleen, reference sample. TEM micrograph reveal a cluster of round shape particles of ferritin. EDX of this cluster revealed O and Fe. Scale bar = 100 nm.

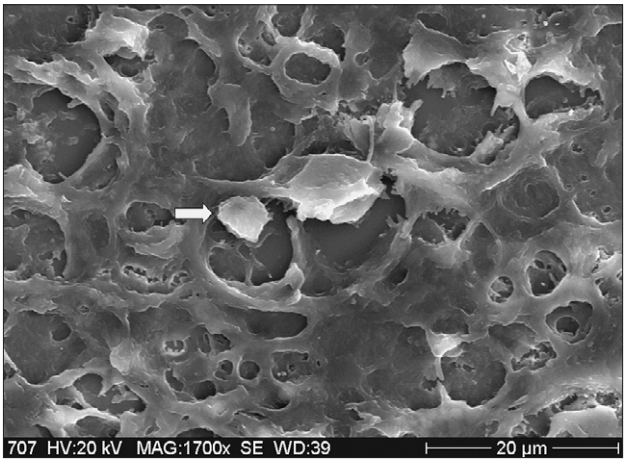

Fig. 3. Human spleen, hereditary spherocytosis. Histological section. The sharp-edged iron-rich complex with an irregular shape (arrow). Scanning electron microscopy. Scale bar: 20 µm.

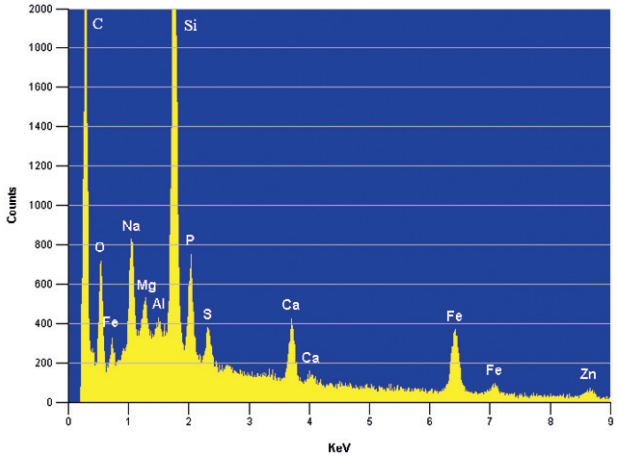

Fig. 4. Human spleen, hereditary spherocytosis, EDX microanalysis. Spectrum showed a composition of iron-rich complex in human spleen consisting of C, O, Na, Mg, Al, Si, P, S, Ca and Zn. The presence of silicon and sodium in the sample is caused by a glass support. Carbon-coated, energy range 0.160–9 keV, collection time 200 s.

delberg, Germany) for two hours and buffered by phosphate (pH 7.2–7.4). After dehydrating the tissue by alcohol, samples were embedded into Durcupan ACM (Fluka AG, Busch, Switzerland) as recommended by the manufacturer and cut by ultramicrotome (C. Reichert, Wien, Austria). The thickness of samples was 200 nm. Non-contrasted ultrathin sections were mounted on the nickel grids and studied by the transmission electron microscope CM 100 (Philips, Eindhoven, Netherlands) with an acceleration voltage of 120 kV. To find the iron-rich complexes, chemical analysis by EDX was applied.

Results

Reference samples contained no iron-rich complexes. EDX microanalysis revealed a multi-element composition. Carbon, oxygen, sodium, magnesium, aluminium, silicon, sulphur, calcium and zinc were identified (Fig. 1).

Transmission electron micrographs of reference samples revealed ferritin particles, sometimes of their clusters (Fig. 2). EDX of ferritin clusters revealed O and Fe.

Sharp-edged complexes with a high concentration of iron (sometimes in the shape of a block) with dimensions of about 5–7 µm were observed by scanning electron microscopy in samples with HS (Fig. 3).

EDX microanalysis revealed a multi-element composition of these complexes. In addition to iron, carbon, oxygen, sodium, magnesium, aluminium, silicon, phosphorus, sulphur, calcium and zinc was identified (Fig. 4).

The transmission electron micrographs of HS samples revealed bumpy, solid particles of platy, well-defined crystal faces (Fig. 5). EDX of this crystal revealed besides O and Fe also Si, Ti, Cu, Cr,

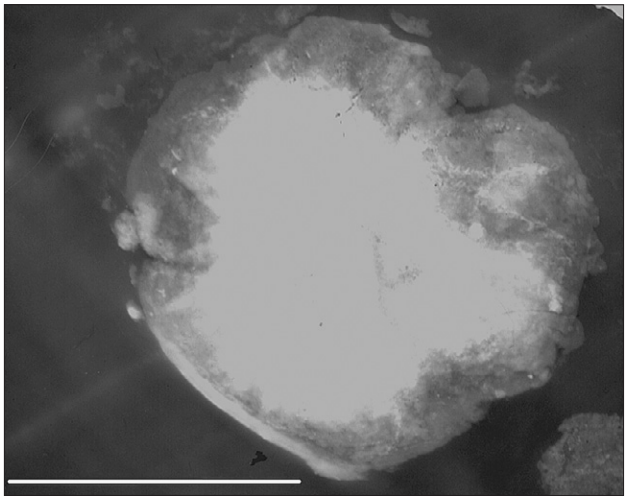

Fig. 5. Human spleen, hereditary spherocytosis. TEM micrograph revealed a bumpy, irregular particle. Sometimes, rounded shape particles were seen. The shape of smaller particles was more regular. EDX of this particle revealed besides O and Fe also Si, Ti, Cu, Cr, Pb. Scale bar = 3 µm.

Pb. Sometimes there were irregular, rounded shape particles seen. The shape of the smaller particles was more regular.

Discussion

Under physiological conditions, ferritin forms spherical formations of the size about 12 nm. The core of this protein consists of iron and oxygen. Ferritin is the iron storage protein found in cells of human spleen. Its physiological function causes the presence of iron in spleen. Zinc is one of the essential dietary factors and physiological element. A decline of zinc level can contribute to metabolic diseases, high blood pressure or diminished immune response (Tubek, 2007, Maggini et al, 2007). Vayenas et al. (1998) examined changes in the concentration of zinc, manganese, and copper in the liver, spleen, and brain of rats after an iron overload by atomic absorption spectrophotometry. They found an increased amount of iron, zinc and manganese in the liver and spleen. They explained the increased amount of these elements by an increased uptake from the cell.

Hereditary spherocytosis affects sequestration of RBC and their accumulation in the spleen. Numerous macrophages can be found and the sinus lining cells are hypertrophied. In certain circumstances, a lot of ferritin is seen both intra and extracytoplasmically (7). Free or loosely bound iron is a substantial inductor of ROS via the Fenton reaction (12). The ferric iron Fe (III) is permanently reduced to ferrous iron Fe (II) and vice versa to form ROS. They are able to damage lipids, DNA and proteins (13). There exists an effect against ROS formation by elimination and isolation of ferritin/iron complexes from surrounding environment by their covering. This protection effect can be seen from the size and EDX microanalysis of iron-rich complexes. SEM revealed around 10 µm iron-rich complexes, but the size of iron-containing particles detected by TEM is around 3 µm. The rest of complexes are organic compounds that prevent ROS formation. Compounds containing the thiol group exhibit a high radical-trapping activity (14). Iron overload leads to oxidative stress and subsequently to the regulation of antioxidant defensive mechanisms involving thiol metabolism that may play a major role in the resistance to iron-induced oxidative damage (15). By comparison of the results from EDX microanalysis of reference and HS samples, the presence of phosphorus in the HS sample can be seen. Phosphorus and its compounds significantly affect the morphology and redox activity of iron what can markedly influence the ROS formation. In addition, trace amounts of other elements significantly affect the structure, chemical composition and steichiometry of iron oxides (16).

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