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Iron and age-related macular degeneration

Żelazo i zwyrodnienie plamki związane z wiekiem

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Summary: Iron can be involved in the pathogenesis of age-related macular degeneration (AMD) through the oxidative stress. In siderosis, exogenous iron can cause retinal degeneration which can be also associated with elevated retinal iron levels resulting in hereditary defects in iron homeostasis. Iron is transported into the retina by the endocytosis of iron complexed with transferrin and stored in complex with ferritin. The retinal pigmented epithelium and the neuroretinal vasculature serve as blood-retina barriers and disruption of homeostasis at these barriers may result in iron overload. There is firm experimental evidence that retinas of AMD patients contain more iron than retinas of the healthy subjects, but the question whether it is the reason or a consequence of AMD remains open. Excessive iron can cause damage to protein, lipids and DNA through the generation of free radicals in the Fenton reaction. Therefore, iron may play a role in the pathogenesis of AMD as a source of free radical damage but this hypothesis has not been verified experimentally and further studies are needed to establish the relationship between disturbance in iron homeostasis and AMD.

Key words: age related macular degeneration, AMD, iron ions, oxidative stress, transferring, ferritin, RPE cells, celuroplazmin, DNA damage.

Słowa kluczowe: zwyrodnienie plamki związane z wiekiem, AMD, jony żelaza, stres oksydacyjny, transferryna, ferrytyna, komórki RPE, celuroplazmina, uszkodzenia DNA.

Introduction

Age-related macular degeneration (AMD) is a disease of the central retina, the macula, characterized by progressive degeneration of the retina, retinal pigmented epithelium (RPE) and choroid. On the degeneration of the macula, central vision is impaired, or even lost, and peripheral vision dominates, or remains. In addition to central vision impairment, AMD patients suffer also from impairment of distance visual acuity, near visual acuity, color discrimination, contrast sensitivity and other sense functions. They have problems with reading, recognizing other people's faces, playing or even watching sports (1). It is considered as the main cause of vision loss and blindness in individuals aged over 65 (2). Mechanisms underlying occurrence and progression of this disease are largely unknown.

Oxidative stress and damage caused by its product, mainly reactive oxygen species (ROS), may be implicated in the pathogenesis of AMD, but this concept remains unproven (3). High polyunsaturated fatty acid content in photoreceptor outer segments combined with oxygen-rich environment may provide reactive oxygen species, but the source of oxidative stress playing a role in the pathogenesis of AMD is still unknown. There are experimental data and hypotheses on several factors, which can contribute to this disease, with iron ions among them.

Iron homeostasis

Iron is an element which is essential for cellular homeostasis. The lack of it may lead to serious disturbances in the cell's functioning, which, in consequence, may result in a disease phenotype of an organism. On the other hand, iron ions can contribute, through the Fenton reaction, to the production of reactive oxygen species (ROS), including free radicals, which can be toxic for the cell. Iron ions are carried in the bloodstream attached to transferrin (Tf), an 80 kDa transporter protein, upon binding to its receptor (4). Iron homeostasis is managed by the regulation of the expression of iron-regulatory proteins (IRPs), which can bind iron-responsive elements (IREs) on the mRNA of regulated proteins (5). Most non-heme iron in the circulation is bound to transferrin, which can bind two molecules of ferric (3+) iron with a high affinity (4). Adults normally have approximately 3 mg of circulating non-heme iron, with transferrin binding sites only approximately 30% saturated.

Most of the metabolically active iron in the cell is processed in the mitochondria, which contain their own mitochondrial ferritin (MtF), distinct from its cytoplasmatic counterpart (6). MtF has been shown to possess ferroxidase activity and its function is unclear. The results of some studies suggest that MtF may protect mitochondria from iron-induced oxidative damage, since its elevated level was observed in the mitochondria of iron-overloaded sideroblasts in sideroblastic anemia (7).

Iron that is not utilized or stored by the cell is extruded by ferroportin, a transport protein (8). Iron transported by ferroportin is in ferrous state and must be oxidized to be accepted by transferrin. This process is assisted by several proteins, including ceruloplasmin, a copper binding protein, containing about 95% of plasma.

As mentioned above, the interaction between IRPs and IREs is essential for the iron homeostasis. This interaction allows the

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cell to regulate iron uptake, sequestration, and export according to their status. IRPs detect intracellular iron status and, in the case of deficiency, bind to IREs on the mRNA of the regulated protein. In particular, the binding of IRPs to the IRE of ferritin, disturb the process of translation, resulting in a decreased ferritin levels in iron deficiency. Iron is absorbed in the intestine, but very little iron is excreted, leading to an increase in tissue iron levels with age.

Iron ions in the retina

Retina is separated from the bloodstream by the blood-retina barrier. Transferrin cannot diffuse trough the blood-brain barrier and the same applies to the barrier between blood and retina, although transferrin can be found in the retina. The expression of transferrin mRNA was detected in RPE cells, which could suggest that RPE is the main site of the transferrin synthesis. Transferrin with iron can be endocytosed into cells following binding to the cell surface transferrin receptor (9). Transferrin receptors were detected in RPE.

Iron complexed with transferrin may be taken up by transferrin receptors on the inner segments of photoreceptors. It was shown that rat's photoreceptor inner segments were immunopositive for transferrin receptor (10). Transferrin is also present in the aqueous and vitreous humor, which suggests that they may constitute a route for iron delivery to ocular cells (11,12). Some experimental data suggests that part of the transferrin can be synthesized in the eye (13). Iron can be transported across the blood-retina barrier by the transcytosis of Tf-bound iron and endocytosis of Tf-bound iron followed by the removal of iron from Tf within endosomes (14). In the same research it was suggested that there was a mechanism regulating iron uptake by the retina. This mechanism decreases the uptake when the retina has sufficient amount of it.

It has been reproted that iron ions in the retina may be also transported by divalent metal transporter-1 (DMT1), moving one atom of ferrous iron and a proton in the same direction. DMT1 was localized in rod bipolar cell bodies, photoreceptor inner bodies, rod bipolar cell axon termini and horizontal cell bodies (15). Another protein which can be involved in iron transport in the retina is Dexras1, a 30 kDa protein belonging to the Ras subfamily. It can be induced by the activation of some receptors to signal iron uptake in the brain (16). Iron in the cell is primarily stored in cytoplasmic ferritin, one molecule of which can hold about 4500 iron molecules (17). Ferritin has heavy and light subunits and it is its central core that is responsible for iron binding. Although ferritin is a cytoplasmatic protein, it can be found in the nucleus of corneal epithelial cells, where it likely sequesters iron to prevent UV-induced DNA damage (18).

Another protein which can be involved in iron homeostasis, ceruloplasminhas ferroxidase activity and oxidizes iron from Fe^{2+} to Fe^{3+}. This activity represents antioxidant properties of ceruloplasmin, since this is Fe^{2+} which catalyzes free radicals production via the Fenton reaction. Moreover, ceruloplasmin facilitates iron export by the same reaction, since only ferrous iron can be exported across the plasma membrane, but only ferric iron can be taken up by transferrin (19).

Therefore, iron supplied with the diet and iron coming from the environment can both be present in the retina. Moreover, it was observed that retinal iron levels are higher in maculas from post mortem donors aged over 65 than in those younger than 65 (20). This is consistent with the effect of iron accumulation with age. Obviously, this iron accumulation is potentially toxic.

Iron in AMD

Results of some research suggest that iron ions may contribute to the pathogenesis of AMD. Probably the most direct evidence for the involvement of iron ions in the etiology of AMD arises from the results of *post-mortem* research comparing the iron content in the macula of AMD patients and sex- and age-matched individuals without visual disturbances (21). Moreover, it was shown in the same study that the retinas from AMD patients had more transferrin than retinas persons without AMD. AMD patients showed not only iron ions themselves, but also higher concentrations of transferrin than in an age-matched control group (22). However, the fact that the retinas of AMD patients had more iron and transferrin than those of healthy subjects does not indicate unambiguously that iron is the cause of AMD, for it can be a byproduct of AMD pathology. Transferrin was reported to be upregulated at the mRNA and protein levels in patients with AMD compared to age-matched healthy controls (23).

Through its involvement in the Fenton reaction, iron is implicated in the oxidative stress, which, in turn, can be involved in the pathogenesis of AMD. Therefore, a link between iron and AMD seems to be straigthforward. Moreover, it seems that antioxidants and iron chelators can be beneficial in preventing and curing AMD. But in fact, the source of oxidants, which may play a role in the etiology of AMD, is unknown. The thesis that iron can be this source is controversial, albeit – in our opinion – rational.

It is remarkable that iron content in the retina increases with age, as shown in eyes of individuals below the age of 35 compared with subjects older than 65 (22). An early onset of macular drusen-like opacities was reported in a patient with retinal iron overload resulting from the hereditary disease aceruloplasminemia. Mice with the iron overload in RPE resulting from disturbances in the iron exporter celuroplazmin developed a retinal degeneration with some features of AMD, including sub-RPE deposits and subretinal neovascularization (24)).

An intraocular iron overload was shown to initiate oxidative damage to the retina induced by superoxide radicals in photoreceptor inner segments (25). Therefore, if we assume that oxidative damage to the retina can be a prerequisite to AMD, iron ions can initiate a cascade of events leading to the development of the disease.

If iron indeed plays a role in the pathogenesis of AMD, iron chelators could be effective in protecting against the pathological effects of iron. Moreover, if we assume that the harmful effect of iron is carried out through oxidative stress, similar protective effects should be manifested following antioxidant supplementation. In fact, the results obtained in the Age-Related Eye Disease Study have shown that substances recognized as antioxidants: zinc, vitamin C, vitamin E and -carotene may slow down the progression of AMD (26).

Concluding remarks

In the light of a very likely role of iron in the pathogenesis of AMD it seems imperative to establish the relationship betwe-

en dietary iron and retinal iron. Until then, patients with retinal disease, including AMD, should avoid taking iron as dietary supplement and eating red meat, unless they are instructed to do so due to disturbances in iron homeostasis, such as iron deficiency anemia. It also seems important to consider the role of iron ions in the degeneration of the retina in general, for it can potentiate the effect of aging in AMD.

Since studies on antioxidants in AMD brought promising results, they could inspire their expansion by using iron chelators to modulate the occurrence and/ or progression of AMD. It is justified by the reports suggesting that iron chelation may play a role in the treatment of a number of neurological degenerative diseases such as Alzheimer's disease and Parkinson's disease, Huntington's disease and others (27,28). However, from an ophthalmologic point of view, considering iron chelation as a therapeutic or preventive strategy against AMD must be done with great caution, since in some cases this process may induce retinal toxicity (29).

The research on the role of iron in AMD should also involve a search for a correlation between markers of AMD and polymorphism of the genes coding for proteins involved in iron transport and storage in the retina: ferritin, ceruloplasmin, ferroprotin and others. The results of such studies may be useful for constructing a microarray for the assessment of the risk of AMD occurrence and progression linked with disturbances of iron homeostasis. More detailed study should be also directed to a role of cerulopasmin in AMD, since it can play a pronounced role in the iron homeostasis and exert an antioxidant effect.

In summary we can state that there is no doubts that disturbed homeostasis of iron is associated with AMD, but the question whether it is the reason or a result of AMD remains open.

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