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Iron metabolism

Non-transferrin-bound iron (NTBI): an ignored lethal toxin.

Abstract

Iron overload disorders are common untreated a cause of severe systemic symptoms and organ damage to the liver, heart, brain, spleen, pancreas, gonads and generally in parenchymal tissue. After exceeding the iron binding capacity of the body, toxic free iron (non-transferrin bound iron = NTBI) can enter the circulation and from there into the cells. NTBI is only treatable by chelation therapy in emergency cases (e.g. after transfusions). In the long term, chelation therapies are risky, sometimes even life-threatening, in cases of NTBI overload. NTBI is formed in the body after the uptake of iron and very specifically from a transferrin saturation of 50% in women and towards 55% in men. From 60% to 70%, the total NTBI load increases exponentially. The lack of knowledge about NTBI still costs the lives of numerous people today. This is because it is neither directly measurable nor stainable in biopsies.

Non-transferrin bound iron (NTBI)

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exceeding the iron binding capacity of the body, toxic free iron (non-transferrin bound iron = NTBI) can enter the circulation and from there into the cells. NTBI is only treatable by chelation therapy in emergency cases (e.g. after transfusions). In the long term, chelation therapies for NTBI are risky,

sometimes even life-threatening. NTBI is formed in the body after iron uptake, most notably at transferrin saturation above 50% in women and ~55% in men. In the range between 60% and 70% saturation, the total NTBI load increases exponentially. The lack of knowledge about NTBI continues to cost thousands of lives today, even in first world countries. The reason is that the properties of NTBI iron are still not sufficiently known, it is not directly measurable, even not stainable in biopsies. Yet it is as real as radioactivity, which also kills without being smellable, tastable or visually detectable. However, the full knowledge of the catastrophic effects and nature of NTBI-type iron must finally become part of the basic knowledge of internal medicine. For a long time little was known about it, but in the last twenty years the understanding of the pathophysiology of iron overload disease and also of the role of free iron in it has improved considerably. The laboratory diagnosis is simple: a chronically or very frequently elevated value of transferrin saturation (TfSat) is sufficient to indirectly detect NTBI. The reason: if the TfSat value is (in absence of a severe inflammatory condition) above 50%, the probability of NTBI formation is high, above 60% it is very high and above 70% it can be considered certain. Even at saturation values of only 60 %, peak values between 90 % and 100 % must be assumed.^{2,5,6,7,10,12,13}

Iron regulation

Iron, in physiological concentrations, is essential for numerous cellular processes and for oxygen transport by erythrocytes. However, any iron excess can lead to tissue damage. There is no mechanism in the body to actively excrete iron. Therefore, serum iron concentration can only be kept within physiological limits by regulating its uptake from the intestine and release into the blood by enterocytes and macrophages. Dietary iron enters the duodenum as heme-mainly in meat-or in the free, trivalent form (Fe^{3+}), mainly in leafy vegetables and cereals. After reduction to the divalent form (Fe^{2+}), the non-heme iron is taken up by the enterocyte via divalent metal transporter-1 (DMT1). The iron is stored in the cell in the form of ferritin

or released into the blood via the iron-exporting protein ferroportin. In the blood, iron ions, after oxidation to Fe^{3+} , are coupled to the glycoprotein transferrin, which safely transports iron to tissues. Transferrin receptor 1 (TfR1) is responsible for the uptake of transferrin iron into the cell via endocytosis. The main consumer is the bone marrow, where iron is incorporated into the hemoglobin of young erythrocytes. However, most of the iron in serum does not come from food, but from macrophages of the reticuloendothelial system (RES). These release iron from hemoglobin from absorbed old or damaged red blood cells and release it back into the blood via ferroportin. In the cell membrane of the hepatocyte is a sensor for the transferrin-iron complex, which consists of the HFE protein and the transferrin receptors TfR1 and TfR2.² The serum concentration of the transferrin-iron complex, together with the serum ferritin concentration, reflects the iron status of the body. An increase in it is recorded by the hepatocyte and leads to increased production of the peptide hormone hepcidin. The latter regulates the release of iron into the blood by binding to ferroportin and then breaking it down. This slows a further increase in iron concentration in the blood and body and creates a new equilibrium.

Pathophysiology of iron overload

Disorders in the regulation of iron metabolism can have both hereditary and acquired causes. Congenital mutations in the HFE genes can lead to hemochromatosis (HH) a relatively common systemic disease in northern Europe which can be treated easily if diagnosed early enough. In HH the culprit is an overload in ferritin. Another cause of markedly reduced hepcidin concentrations is the combination of ineffective and increased erythropoiesis. This is characterized by a strong proliferation of “young” erythrocytes in the bone marrow to compensate for an increased apoptosis rate of these cells. This combination is observed in patients with β -thalassemia major or intermedia and in some forms of MDS. There is evidence that during this ineffective erythropoiesis, substances are released that suppress the synthesis of

hepcidin by the hepatocyte. In addition, a homozygous mutation in HFE gene H63D can result in so-called H63D- (or Oslo-) syndrome, usually caused by a relative transferrin deficiency. Iron overload can also be triggered by multiple blood transfusions, such as in patients with β -thalassemia major, sickle cell anemia, or a form of MDS. Each unit of red blood cells transfused contains approximately 250 mg of iron.

Toxic NTBI

A high concentration of iron in the circulation is likely to have consequences only if the binding capacity of transferrin is exceeded, such as by a genetically determined relative deficiency of transferrin (H63D syndrome). As a result, iron is loosely bound to albumin, acetate or citrate, among others, as a substitute, detaches from these quite rapidly and then circulates in the bloodstream as a toxin, NTBI, a chemically heterogeneous group of iron complexes. The reactive and chelation therapy-accessible fraction of this is termed "labile plasma iron" (LPI). In recent years, NTBI has received too little attention in iron toxicity research. Iron ions in the form of NTBI, unlike the relatively harmless transferrin or ferritin iron, are not bound in a "safe" manner. NTBI complexes are therefore considered toxic and a pathophysiological substrate of oxidative organ damage in iron overload diseases. Thus, even patients with very low ferritin levels can be poisoned by iron. The mechanism is remotely reminiscent of what happens in Wilson's disease. Intracellular uptake of NTBI is a major contributor to this damage and is mediated by various receptors and transporters, many of which are organ-specific. The best studied examples are the transporter Zip14, the receptor TfR2, and calcium channels. Zip14 appears to be expressed primarily in liver, pancreas, and myocardium, the major organs that are particularly susceptible to uptake of toxic NTBI iron, as are the brain and gonads. In the cell, iron ions can catalyze the so-called Fenton reaction, a redox reaction in which reactive oxygen radicals are formed. These oxygen radicals (severely) damage various intracellular macromolecules, including membrane lipids,

DNA, and proteins. In animal models, this iron-induced damage has been extensively studied and clearly demonstrated. It is now also considered unequivocal that NTBI causes organ damage in humans. It has just not yet arrived as general knowledge in practices and clinics. In patients with β -thalassemia major, as one example of many, a correlation has been found between the amount of NTBI and the degree of damage to the heart. In patients with β -thalassemia intermedia, there is a correlation between NTBI and markers of oxidative stress, and in patients with hereditary hemochromatosis, LPI correlated with the serum level of transaminases. The toxicity of NTBI may be compounded by the fact that it is thought to promote its own formation and cellular uptake by paradoxically reducing hepcidin concentrations. Whenever hepcidin is showing low concentrations, iron release continues into the blood via ferroportin. Thus, there appears to be a vicious cycle in which the negative feedback mechanisms have been replaced by positive feedback. It is also striking that significantly more iron-mediated organ damage is observed in patients with β -thalassemia than in patients with sickle cell anemia. The subclinical, chronic inflammation in sickle cell anemia may lead to increased production of the acute phase protein hepcidin, which contributes to lower NTBI concentrations. In addition to oxygen radical production, NTBI can cause damage in other ways. For example, iron-rich serum is known to provide a good breeding ground for various microorganisms, increasing the risk of sepsis.^{1,2,3,4,5,13,14,15}

There is no longer any scientific doubt that NTBI iron can be responsible for organ damage of any severity. Knowledge of the hazardous nature of NTBI helps in the prevention of organ damage in the context of screening and as a biomarker to initiate the indication for an iron-controlled diet before irreversible organ damage occurs. The development of specific NTBI receptor antagonists would be desirable but is not expected in the foreseeable future.¹⁻¹⁵

Conclusion

With the discovery of hepcidin and various cellular iron transporters, the understanding of the pathophysiology of iron overload diseases has improved considerably over the past decades. In addition, it is becoming increasingly clear that NTBI plays an outstanding role in the development of tissue damage. However, knowledge of this is not yet as widespread as it urgently needs to be. In addition, a number of questions remain unanswered. For example, not all details about the exact processes involved in the intracellular uptake of NTBI are yet known. Nevertheless, clinicians increasingly need to address NTBI if they are to avert the risk of severe organ damage. Physicians who still do not take persistently or intermittently elevated transferrin saturation levels seriously are acting negligently and are jeopardizing the health of their patients due to functional and structural organ damage.

Conflicts of interest

None declared.

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