ABSTRACT

Iron is a metal element with crucial roles in human organism. Both iron deficiency and iron overload are important pathologies. Hepcidin, a peptide synthetized in the liver, is a key iron regulatory hormone. Increased amount of iron and inflammation stimulate its expression while iron deficiency and activated erythropoiesis cause hepcidin downregulation. The regulation of hepcidin expression on the molecular level and its hierarchy and interactions are not completely known. The main regulatory pathway is BMP/ SMAD which reacts to the iron amount in the organism. Several molecules, including hemojuvelin and HFE, are involved in this pathway and their mutations are linked to inappropriately low hepcidin production, iron overload and hereditary hemochromatosis. Erythroid regulation with suppressive action on hepcidin expression is known only partially as well as its connection to the BMP/ SMAD pathway. Recently, two new negative regulators of hepcidin expression have been described. Membrane enzyme present in hepatocytes – matriptase-2 (MT-2, TMPRSS6) and soluble factor secreted by erythroblasts – erythroferrone (ERFE).

The aim of our work was to investigate how MT-2 is involved in the erythroid regulatory pathway, and whether it can represent the molecule where various regulatory pathways interact. We determined gene expression as the amount of mRNA by real-time PCR and as protein level by immunoblots. Firstly, we used male mice and female rats which were given erythropoietin (EPO), iron or were kept on low iron diet. We proved that neither iron nor EPO influenced MT-2 expression on the mRNA level but that MT-2 reacted to both on protein level. EPO and iron deficiency induced by low iron diet increased the amount of MT-2 protein while iron administration decreased it. The changes were more pronounced in rats. In next experiments we therefore administered the combination of iron and EPO and studied their influence on MT-2 and ERFE in rats. We confirmed previous data in mice that the previous administration of high iron doses blocks the EPO mediated downregulation of hepcidin expression despite the increased ERFE expression in the spleen. As iron administration prevented the increase of MT-2 protein amount observed after EPO, we suppose that MT-2 plays role in the limitation of hepcidin downregulation.

Our results show that both increased ERFE and decreased amount of phosphorylated SMADs are not able to sufficiently decrease hepcidin expression if iron was previously administered. Hepcidin expression remains high (i.e. normal) even in the presence of high amount of ERFE in the plasma and activated BMP/ SMAD pathway. Iron administration

restraint the increase of MT-2 protein amount which could be the molecular cause of the fact that hepcidin expression is not increased despite enhanced erythroid stimulation. It could indicate that MT-2 could have a role in long term erythroid hepcidin regulation and could be one of the points where erythroid and store regulatory systems interact. Moreover, the mechanisms of hepcidin regulation and iron metabolism are probably strongly influenced by time course and can differ in acute and long term changes in erythropoiesis.

Key words: iron, hepcidin, erythropoietin, matriptase-2, erythroferrone, inflammation