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Streszczenie w języku angielskim

Identification of the novel route for hemoglobin clearance in the liver.

Hemoglobin (Hb) is a protein found in red blood cells that transports oxygen and carbon dioxide throughout the body. When released from erythrocytes into the circulation or surrounding tissues, Hb can exhibit cytotoxic, pro-inflammatory and pro-oxidative properties. Some amounts of Hb are released during the physiological lysis of erythrocytes, and excessive hemolysis accompanies many congenital and acquired diseases. Physiologically, up to 10% of erythrocytes can be lysed in the circulation, especially in the spleen where the removal of senescent erythrocytes takes place. Canonically, free Hb is bound by the plasma acute-phase protein- haptoglobin. Hb- haptoglobin complexes are bound by the CD163 receptor present on spleen and liver macrophages, where Hb is degraded. However, hemolytic conditions can lead to saturation of these mechanisms including macrophages and haptoglobin, suggesting alternative mechanisms for Hb clearance. To date, no other mechanisms of removing free Hb have been described.

The aim of this study was to identify and investigate a novel pathway of free Hb uptake in the liver, that is independent of haptoglobin and the CD163 receptor. The study was performed in a mouse model and in primary cell cultures obtained from mouse liver. In the experiments, we used Hb isolated from mouse blood to mimic physiological conditions. To track Hb in the body, the protein was fluorescently labeled with Alexa Fluor or an iodine isotope.

Studies using intravenous administration of clodronic acid liposomes, which depletes tissue macrophages, and fluorescently labeled Hb have shown that Hb accumulates primarily in the liver, regardless of the presence of macrophages. Using flow cytometry and confocal microscopy, liver sinusoidal endothelial cells (LSECs) were observed to be the major liver cells population responsible for the uptake of free Hb at both low and high concentrations. Interestingly, it was shown that LSECs do not have the CD163 receptor on their surface, and that free Hb was captured more effectively than the Hb-haptoglobin complex. Staining of primary LSECs with the early endosome marker (EEA1) and fluorescently labeled Hb showed

the presence of Hb in macropinosome-sized intracellular vesicles. Co-localization of Hb and dextran, a marker of the liquid phase, which is taken up in macropinosomes, was also observed. In addition, the macropinocytosis inhibitor (EIPA) was shown to inhibit Hb uptake by LSECs, in contrast to the endocytosis inhibitor (CPZ). The obtained results indicate that under physiological conditions LSECs are the major liver cell population responsible for the uptake of free Hb, which they sequester by macropinocytosis.