INHIBITORY EFFECT OF CATIONIZED CATALASE ON RELEASES OF INFLAMMATORY MEDIATORS IN LPS-INDUCED RAT ALVEOLAR MACROPHAGES
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Lipopolysaccharide (LPS) activates alveolar macrophages to release inflammatory mediators, leading to lung inflammation. Reactive oxygen species, which are intracellularly produced by LPS, enhance the release of the inflammatory mediators. Catalase, an antioxidant enzyme, has a potential to inhibit the release of the inflammatory mediators. However, the accessibility of catalase to intracellular target sites is very low due to its high molecular weight and negative charge. To enhance its intracellular accessibility, cationized catalase (HMD-catalase) was synthesized by coupling catalase with hexamethylenediamine. In this study, the cellular uptake characteristics of HMD-catalase were examined in primary cultured rat alveolar macrophages. Then, the effect of HMD-catalase on release of TNF-α and CINC-1 (a member of IL-8 family), which are the major inflammatory mediators, and intracellular reactive oxygen species produced by the stimulation of LPS was studied. The number of modified amino groups was 24.5 per a catalase molecule. HMD-catalase more effectively inhibited TNF-α and CINC-1 release than catalase. The cellular uptake study showed that the cellular association of [111In]-labeled HMD-catalase was much higher than that of [111In]-labeled catalase. Confocal microscopic study demonstrated that HMD-catalase effectively eliminated intracellular reactive oxygen species produced by LPS in rat alveolar macrophages. These results indicate that internalized HMD-catalase eliminated hydrogen peroxide in LPS-stimulated rat alveolar macrophages, leading to the inhibition of TNF-α and CINC-1 release; therefore, HMD-catalase would be a potent candidate for the treatment of lung inflammation.

HEPATIC CLEARANCE OF AMYLOD β PEPTIDE (1-40) IS REGULATED BY INSULIN
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The liver is the major organ that eliminates amyloid β-peptide (Aβ) from the circulation, and we have revealed that low-density lipoprotein receptor-related protein 1 (LRP-1) is a molecule responsible for the hepatic clearance. Since epidemiologic investigations suggest the high incidence of Alzheimer’s disease in diabetes mellitus, the purpose of this study was to clarify the effect of insulin on the hepatic clearance of human Aβ(1-40) in rat. Insulin infusion into the rat portal vein increased LRP-1 expression in plasma membrane fraction of liver, but did not affect the expression in whole cell lysate. LRP-1 protein levels in plasma membrane were also greater in the fed conditions compared with the fasted conditions. These results indicate that insulin promotes the translocation of LRP-1 at the plasma membrane of hepatocytes. Insulin treatment induced hepatic uptake of α2-macroglobulin, an LRP-1 ligand, in concentration dependent manner. Insulin also increased the hepatic uptake of Aβ(1-40), which reached 1.6-fold greater uptake than non-treated control after 10 min treatment as well as that of α2-macroglobulin. Increase of the hepatic uptake of Aβ(1-40) by insulin was concentration dependent (EC₅₀ = 230 pM), and was completely suppressed by RAP (2 μM), an LRP inhibitor. These results suggest that insulin induces translocation of LRP-1 to the plasma membrane of hepatocytes, leading to increase of Aβ hepatic clearance from the circulation. It is possible that reduction of insulin signaling in diabetics leads reduction of Aβ hepatic clearance, which may affect the Aβ levels in the plasma and the brain.