Abstract

Excessive hemoglobin (Hb) enhances production of free radicals *via* Haber Weiss-Fenton reactions. Haptoglobin (Hp) is a cellular protective protein which protect cells from heme iron toxicity. Hp polymorphism has been proposed as a risk factor for developing diabetic mellitus and atherosclerotic vascular disease. Patients carrying Hp 2-2 phenotype are at risk for developing coronary artery disease. We investigated the pathophysiologic mechanisms of heme iron derived from Hb, glycated Hb (Hb(gly)) and Hp-Hb complex. HepG2 cells were treated with various forms of heme iron. labile iron pool (LIP) was measured using calcein-AM technique. Reactive oxygen species (ROS) was investigated using 2,7-dichlorofluorescein and the amount of redox active chelatable iron associated with Hb and Hp-Hb complex was assessed by fluorometric assay with dihydrorhodamine. HDL-associated lipid peroxide was determined using spectrophotometric method. Free Hb and Hb(gly) significantly increased amounts of LIP, ROS, redox active chelatable iron and lipid peroxide. Cells treated with Hb(gly) showed higher levels compared to Hb treated cells. As a part of Hp-Hb complex, Hp stabilized heme iron in heme pocket of Hb and prevented cells from oxidative damage. It was found that Hp 1-1 was superior in reduction of LIP, ROS, redox active chelatable iron and lipid peroxide levels as compared to Hp 2-2. Interestingly, curcumin (25-100 ng/ml) resulted in dramatically decreased heme iron-mediated oxidation. In comparison, curcumin showed a greater decrease in Hp 2-2 treated cells than Hp1-1. These result provide evidence that heme iron, especially, Hb(gly) associated Hp 2-2-phenotype is more redox active further to increase oxidative stress. However, the use of curcumin has the potential to reduce this oxidative damage

Keywords: haptoglobin, hemoglobin, curcumin, oxidative damage, labile iron pool, reactive oxygen species, redox active chelatable iron, lipid peroxide

Introduction to the research problem and its significance

Under increased red cell hemolysis in β-thalassemia a patient, excessive hemoglobin (Hb) is released into plasma and becomes highly toxic substance This hemoprotein can enhance production of free radicals *via* Haber-Weiss-Fenton reactions**.** Under physiological condition, free Hb is captured by haptoglobin (Hp) to form a Hb-Hp complex and the resulting complex is directed to CD163-expressing macrophages and further internalized in Kuffer cells in the liver. This mechanism prevents cells from toxic free Hb. There are many evidences illustrate that iron overloaded-β-thalassemic patients are relate with diabetes (1-3). Frequency of diabetic mellitus (DM) in patients with clinical hemochromatosis ranges in 20-50% (4-7). The main pathophysiologic mechanism that leads to diabetes in hereditary hemochromatosis is thought to involve β-cell dysfunction due to iron toxicity resulting in relative decrease of insulin secretion (8). Iron overload is thought to have direct toxic effects on β-cells in the pancreas. Importantly, patients with β-thalassemia and DM are more prone to develop cardiomyopathy which this serious complication is a major cause of mortality (9).

Hyperglycemia plays an important role in the pathogenesis of long-term complications and further affects vital organs such as heart, nervous system, kidneys and blood vessels. Protein glycation is thought to be a major cause of diabetic pathologies which it can form advanced glycation end-products (AGEs). Glucose can undergo autoxidation in the present of transition metals, especially iron and copper. Reactive oxygen species (ROS) is generated at multiple steps during this process and more generated in thalassemic patients with iron

overload. Recent interest has focused on strategies to prevent, reverse or retard this glycoxidation in order to improve thalassemic-diabetic cardiovascular complications.

Hp is a cellular protective protein which functions in the protection of cells from heme iron toxicity. However, it might be a genetic modifier of *Hfe*-associated hemochromatosis. Hp(2-2) type was over-expressed not only in iron overloaded patients but also in type 2 diabetes mellitus (type 2 DM) or noninsulin dependent diabetes mellitus (NIDDM). It may be exaggerated in diabetic stage given the glycosylation of hemoglobin molecule. More recently, Hp polymorphism has been proposed as a risk factor for developing atherosclerotic vascular disease. Patients carrying Hp(2-2) phenotype are at risk for developing premature coronary artery disease whereas Hp(1-1) phenotype has no risk of myocardial infarction. As mentioned above, myocardial complication is a major cause of death in the β-thalassemic patients. Many researchers have proposed that it is a consequence of nonheme iron catalyzed oxidative damage and involved in lipoprotein functions. In addition, heme is known to exhibit peroxidase-like activity that can oxidize many biomolecules. However, the mechanisms responsible for cardiovascular complication in severe hemolytic-β-thalassemia and diabetes which involved in heme iron, Hb and Hp phenotype have not been completely defined.

Furthermore, inhibition of heme iron-induced toxicity would provide a therapeutic strategy to prevent the cardiovascular diseases in diabetic thalassemic patients. Therefore, the inspection of compounds which could ameliorate above complications is necessary and should be encouraged. In this study, we will investigate the pathophysiologic mechanisms of

heme iron derived from non-glycated Hb, glycated Hb, Hp-bound Hb (both Hp(1-1) and Hp(2-2) phetotypes) *ex vivo* and further study the protective effects of curcumin, best-studied natural compound, in these events.