Thesis Title The Role of Oxidative Stress on the Pathophysiology of Beta-thalassemic Red Cell Membrane.

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ABSTRACT

Current studies indicate that the pathophysiology of thalassemic red blood cells is associated with binding of free globin chains to the cell membrane leading to oxidative stress which perturbs membrane organization and finally to rapid clearance of such cells by the reticuloendothelial system. However, problems in the study of oxidative damage in thalassemic red blood cells are complicated by individual variations. Having an *in vitro* model that mimics thalassemic red blood cells will be useful in determining the detailed effects of oxidative stress on membrane components.

In this study, normal red blood cells were oxidized with either phenazine methosulfate, phenylhydrazine or t-butylhydroperoxide. Following oxidation, electrophoretic pattern of membrane proteins were analyzed by SDS-PAGE, the thiol contents of individual membrane proteins were quantitated by radiolabeling with [3H] N-ethylmaleimide and procoagulant activity on the outer surface of red blood cells were measured by prothrombin converting activity assay. In oxidized red blood cells, there were evidences of protein damage as shown by increase in membrane-bound globin, protein degradation and formation of high molecular weight protein complex

which was partially reduced by β-mercaptoethanol. Sulfhydryl contents of spectrin and protein 4.1 were decreased in dose-dependent manner. All of these oxidizing agents could induce in a dose-dependent manner the presence of procoagulant activity on the outer surface of red blood cells. These alterations were also observed in thalassemic red blood cell membranes. The degree of changes was highest in splenectomized β-thalassemia/Hb E, followed by nonsplenectomized β-thalassemia/Hb E and α-thalassemia (Hb H disease). In addition, the reduction in the level of lipid antioxidant, vitamin E, also supported the important role of oxidative stress in the pathophysiology of thalassemic red blood cells. Antioxidants, butylated hydroxytoluene (25-200 μM) and d-α-tocopherol (5-40 μM), had partially protective effect on the presence of procoagulant activity on oxidized red cell outer surface but had no effect on the membrane protein damage.

To determine the effect of vitamin E status on red blood cell procoagulant activity, one group of β-thalassemia/Hb E patients was supplemented with vitamin E (325 mg daily) for three months and another group received placebo. The results indicated that vitamin E supplementation was not only able to raise the patients' plasma vitamin E status but also reduced their red cell procoagulant activity to near normal level.

It can be concluded from this study that thalassemic red blood cells were altered by oxidative stress. The degree of red cell membrane damage was highest in splenectomized β-thalassemia/Hb E followed by nonsplenectomized β-thalassemia/Hb E and Hb H disease respectively. Supplementation of vitamin E restored abnormal high procoagulant activity back towards the level of normal control. It is thus suggested that vitamin E may have therapeutic benefit in β-thalassemia/Hb E patients.