CHAPTER I

INTRODUCTION

Thalassemias are inherited autosomal recessive disease that involves the decreased and defective production of hemoglobin in red blood cells. In thalassemia, the genetic defect result in reduced rate of hemoglobin synthesis. Thalassemia occurs when one or more of genes fail to synthesis of the globin chains that make up hemoglobin can cause the formation of abnormal hemoglobin molecules. Thalassemia red blood cells are subjected to oxidative stress due to the imbalance of hemoglobin chains to the cytoplasmic side of the plasma membrane leading to destruction of red blood cells and shortened life span, thus causing anemia [15, 42, 43].

Thalassemia has been an important public health problem in many countries. In Thailand, thalassemia occurs with high incidence and presents individual, social and economic burdens. Approximately 1% of Thai people suffer from thalassemia and 30 – 40% of Thai people are carriers of abnormal globin genes that can be transmitted to their offsprings [13, 39].

In recent years, many children continue to be born with thalassemia disease. The basic sign and symptoms of thalassemia disease include yellow skin, growth failure, anemia, enlarged spleen, and increased susceptibility for infections. Ingeneral thalassemias are caused by an imbalance of globin chains synthesis, ineffective erythropoiesis, increased apoptosis of the blood genitor cells and increased oxidative stress leading to shortened life span and eryptosis or programmed cell death of thalassemic red blood cells. The mainstay treatment of thalassemia patiens have been regular blood transfusions, the judicious use of splenectomy, and the removal of iron with chelating agents. However this treatment is expensive, iron overload that can damage the heart, liver and endocrine system and there is increasing concern about the safety of blood products [9, 10, 13, 48].

In study of modulation of eryptosis in thalassemia red blood cells might be exploited for the improving of the treatment program of patients suffering from thalassemia. Eryptosis is apoptosis or programmed cell death of erythrocytes and shares similarities with apoptosis of nucleated cells [7, 11]. However, a functional impact of CD95 on the induction of eryptosis has not been analysed in depth although this death receptor is expressed in the red blood cells and although some forming of functional death receptor complexes could be found (Fas-receptor: Fas-associated death domain (FAS:FADD) complex, reactive oxygen species (ROS) -mediated, [36]. On the other hand, stimulation of caspases-8 could not be observed by another group upon stimulation of the CD95 pathway by an agonistic antibody [7], and it is open, if stimulation of CD95 leads to eryptosis in red blood cells. This study wants to find out, if CD95 has a functional role for the induction of eryptosis in red blood cells and might influence the survival of thalassemic red blood cells. This is the first goal of the study.

Since several studies have suggested that insulin may protect cell from apoptosis by decreased oxidative stress in different cell lines and red blood cells react on insulin with increased glycolysis [26, 50]. Human erythrocytes express a receptor for insulin respond to insulin by increasing phosphorylation of tyrosine residues in several proteins. These effects occur through activation of phosphofructokinas (PFK), the key regulator glycolytic enzyme of the glycolytic pathway [26]. Indeed, insulin stimulates glycolysis in red blood cells [26]. Enhanced glycolysis leads to an increased turnover rate of NAD/ NADH and to an increased level of ATP. Thus, we postulate that insulin reduces eryptosis by enhanced production of ATP which is an inhibitor of the proapoptotic protein kinase C (PKC). Furthermore, NADH generated by glycolysis can serve as an essential cofactor to reduce methemoglobin and thus reducing oxidative strees [38]. On the other hand, activation of glycolysis by insulin will reduce the amount of glucose metabolized by the pentosephosphatepathway and reduce the amount of NADP/NADPH turnover. Since NADPH is an important factor to reduce oxidative stress by maintaining the reduced glutathione pool in the cell. [30], insulin might also serve as anti proapoptotic factor, this knowledge can be exploited to test if insulin also increases life span of thalassemic red blood cells. This is the second goal of the study.

Objective of the study

1. To study pro-eryptosis function of death receptor CD95 in red blood cells
2. To study effect of insulin stimulation on eryptosis in normal and thalassemic red blood cells

Scope of the study

1. To study the activation of the death receptor CD95 in red blood cells
2. To study the modulation of eryptosis by insulin and the following events in normal and thalassemic red blood cells

Benefit of the study

Information of modulation of eryptosis in thalassemic red blood cells might be exploited for the improving of the treatment program of patients suffering from thalassemia, as one complication of thalassemia is anemia, caused at least in part by enhanced eryptosis