This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Thalassemias, disorders of hemoglobin synthesis

By

Suwimol Jetawattana

Department of Radiation Oncology

Free Radical and Radiation Biology

The University of Iowa

Iowa City, IA 52242-1181

For 77:222, Spring 2005

5 May 2005

Abbreviations:

Epo: erythropoietin; **Fe^{2+}**: ferrous iron; **Fe^{3+}**: ferric iron; **GSH**: reduced glutathione; **Hb**: hemoglobin; **H_2O_2**: hydrogen peroxide; **LDL**: low density lipoprotein; **MDA:** malondialdehyde; **MnSOD**: manganese superoxide dismutade; **O_2**: oxygen; **$O_2^{\bullet -}$**: superoxide anion radical; $^{\bullet}$OH: hydroxyl radicals; **PMN**: polymorphonuclear neutrophil

Outline **Page**

Abstract

Thalassemias are a group of hereditary diseases of abnormal hemoglobin synthesis where the normal hemoglobin protein is produced in lower amounts than usual. These conditions cause varying degrees of anemia, which can range from insignificant to life threatening. Thalassemia can be classified according to the deficient globin chain, alpha or beta thalassemia. Patients with severe anemia who receive regular blood transfusions become iron overloaded, which increases damaging free radical activity and lowers antioxidant levels in their bodies. Several investigators have provided evidence supporting the hypothesis that oxidative stress plays an important role in development of clinical complications in thalassemia. Increasing antioxidant activity is then expected to bring oxidative stress down to minimal level. Therefore, it is important to understand the mechanisms of free radical-induced oxidative stress damage in thalassemia before using it as a potential tool in diagnosis and treatment of the disease.

Introduction

Thalassemia is described as a heterogenous group of inherited anemias characterized by a reduced or absent amount of hemoglobin. Thalassemia can be classified according to the deficient globin chain, alpha or beta thalassemia. The symptoms vary from relatively mild anemia to life-threatening. Many studies have shown that reactive oxygen species are generated in increased amounts in thalassaemic red cells. Conditions such as rupture of erythrocytes, iron overload, and depletion of antioxidants in tissues (e. g. blood circulation) are considered in the promotion of oxidative stress. This implies the possible alteration of redox status in thalassaemic patients, which may adversely affect their health. Specific treatments for thalassemia are employed based on many factors such as age of the patients and severity of the disease. Currently, studies on the correlation between lipid peroxidation and plasma levels of antioxidants such as vitamin A, C, and E as well as correlations with antioxidant enzymes to hemoglobin disorders have been reported (5, 12, 13). An effective form of gene therapy and stem cell transplantation are now being used to improve conventional treatment and enhanced the prognosis of thalassaemia (20, 22,23). The goal of this paper is to provide information about thalassemia and will focus on its correlation with oxidative stress.

Normal structure and expression of globin gene clusters

Human hemoblobin is a heterotetramer protein, compose of two alpha and two beta subunits as shown in **Figure 1**. Each subunit contains a heme group, an iron containing compound that binds to oxygen. The synthesis of hemoglobin is controlled by two developmentally regulated multigene clusters: the alpha-like globin cluster on chromosome 16 and the beta-like globin cluster on chromosome 11 as demonstrated in **Figure 2**. In healthy

persons, the synthesis of alpha and beta globin chains is finely balanced during terminal erythroid differentiation but the mechanism of balanced expression is unknown [2].

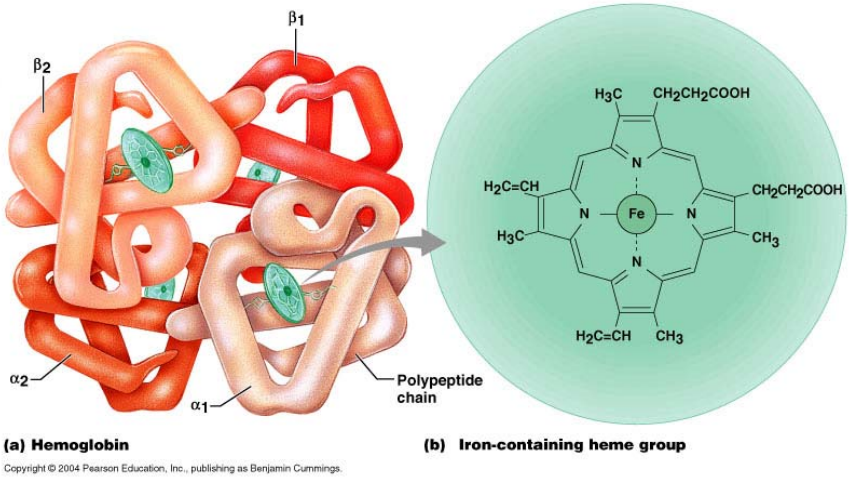

Figure 1. Hemoglobin structure[1].

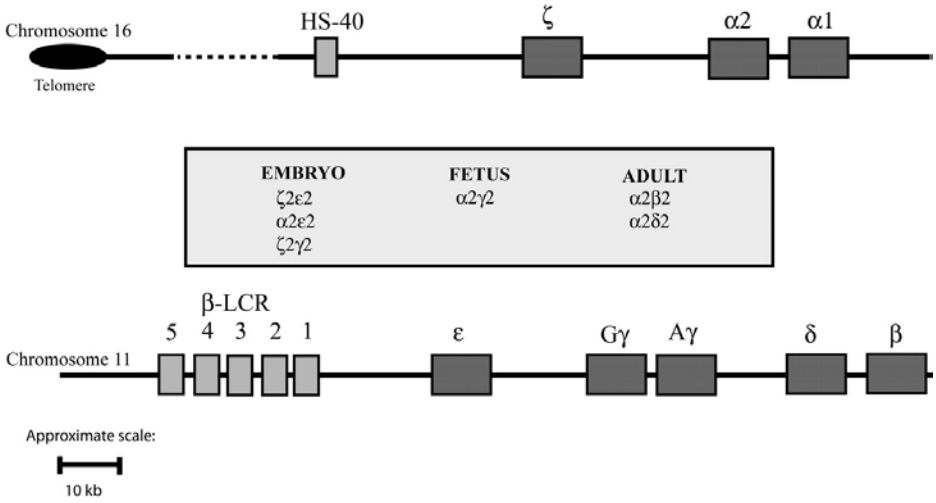

Figure 2. **Schematic representation of the human globin gene clusters**. The α-like globin cluster (top) is situated near the telomeric region of the short arm of chromosome 16 and includes the ζ, α2, and α1 globin genes, which are under the control of an upstream remote regulatory region, HS-40 (so-called because it is a DNase I hypersensitive site located approximately 40 kb upstream of the 5' end of the globin gene). The β-like globin cluster (bottom) is interstitial and located on the short arm of chromosome 11; expression of the genes in this cluster is under the control of a group of remote regulatory elements/DNase I hypersensitive sites collectively known as the locus control region (LCR). Pseudogenes and the globin gene, whose functional significance is unknown, are not represented. To make visualization of them easier, the globinlike genes are represented over a larger space of genome than they actually span [2].

[1] http://www.people.virginia.edu/~dp5m/phys_304/figures/hemoglobin.jpg access on Apr 25, 2005

Normal Hemoglobins [1]

A normal human hemoglobin pattern is expressed as A2A. Hemoglobin A is the designation for the major hemoglobin protein that exists after birth that is a tetramer with two alpha chains and two beta chains ($\alpha_2\beta_2$). Hemoglobin A2 is a minor component (less than 3%) of the hemoglobin found in red cells after birth and consists of two alpha chains and two delta chains ($\alpha_2\delta_2$). The beta protein is not expressed before birth and the gamma hemoglobin (HbF) is the predominant hemoglobin protein found only during fetal development. Hemoglobin F is a tetramer molecule of two alpha chains and two gamma chains ($\alpha_2\gamma_2$). The genes for HbF and HbA are closely related and exist in the same gene cluster on chromosome 11.

Classefication of thalassemia

Beta thalassemia

Thalassemia was first recognized clinically in 1925 when Thomas Cooley described a syndrome of anemia, splenomegaly, and bony deformities among Italian descents [1]. Beta thalassemia or Cooley's anemia is caused by a change in the gene for the beta globin component of hemoglobin. The symptoms of beta thalassemia can range from moderate to severe, depending in part on the exact genetic change underlying the disease. Beta thalassemia major is diagnosed on clinical symptoms of severe anemia that can begin within months after birth. The severe anemia can result in severe lethargy, paleness, and insufficient growth and development if left untreated. Other characteristic physical complications such as heart failure and enlargement of liver and spleen can dramatically decrease life-expectancy. Beta thalassemia can be identified before symptoms have developed as early as the newborn period by a screening test and thus the baby can be started on ongoing blood transfusion therapy as needed. On the other hand, a condition of mild to moderate Cooley's anemia that periodically requires blood transfusions is

described as beta thalassemia intermedia while beta thalassemia minor (also called thalassemia trait) may cause no symptoms, but changes in the blood do occur.

Alpha thalassemia

Alpha thalassemia is the result of reduction in the synthesis of the alpha globin chains. Two main types of alpha thalassemia are described as alpha thalassemia major and hemoglobin H disease. Alpha thalassemia major is a very serious disease of severe anemia characterized by hypochromic microcytic anemia that begins even before birth. Most affected babies do not survive full gestation or die shortly after birth. In contrast, patients with HbH disease can experience events of hemolytic anemia, which is caused by the rapid breakdown of the red blood cells. These events are thought to be triggered by various environmental causes, such as infection and/or exposure to certain chemicals. Hemoglobin H disease is milder than beta thalassemia and does not generally require transfusion therapy [1]. However, the combination of the very low production of alpha chains and destruction of red cells in HbH disease can produce a severe, life-threatening anemia.

Genetic and molecular basis for thalassemia

The inheritance is recessive in nearly all types of thalassemia. The rare exception is most commonly found in beta thalassemia where globin gene mutations exhibit a dominant pattern of inheritance in which only one gene needs to be altered in order to see disease expression[2].

Globin synthesis in beta thalassemia

Healthy persons make the beta globin component of normal adult hemoglobin, HbA, from two normal copies of the beta globin gene, which is located on chromosome 11. The main pathophysiologic feature of beta thalassemia is the accumulation of unpaired alpha globin chains in erythrocyte precursors and red blood cells. This accumulation alters cell membrane function

[2] http://www.healthatoz.com/healthatoz/Atoz/ency/thalassemia.jsp accessed on Apr 25, 2005

and results in ineffective erythropoiesis and early cell destruction. In contrast to alpha thalassemia, most of the beta thalassemia syndromes are caused by mutations affecting gene regulation or expression rather than gene deletion [1]. The mutations are designated as β^0 where no beta globin is produced while only small fraction of the normal amount of beta globin is produced with a β^+ mutation. When one normal beta globin gene and one beta thalassemia mutation are present, it is said that a person carries the beta thalassemia trait, which is thought not to cause health problems.

There are other thalassemia-like mutations that can affect the beta globin gene. Hemoglobin E is a hemoglobin variant which has structural defect in hemoglobin molecule resulting from a single nucleotide base substitution in the hemoglobin beta chain. The combination of HbE and beta thalassemia manifests a condition more severe than is seen with either HbE trait or beta-thalassemia trait. Large deletions around and including the beta globin gene can lead to delta/beta thalassemia or hereditary persistence of fetal hemoglobin (HPFH). Clinical manifestations of the delta/beta thalassemia trait behave very similarly to beta thalassemia trait, while the HPFH trait is not likely to cause hemoglobin disease when co-inherited with other beta globin mutation or a second thalassemia.

Globin synthesis in alpha thalassemia

Alpha thalassemia occurs when there is a reduction in the synthesis of the alpha globin chains of adult hemoglobin (HbA, $\alpha 2\beta 2$) relative to beta globin synthesis. Two alpha thalassemia phenotypes are recognized; one is associated with a total absence of alpha globin synthesis and is designated α^0 thalassemia while the other phenotype, designated as α^+ thalassemia, is only a reduction in alpha globin synthesis [1]. The severe imbalance between the alpha chain and beta chain production causes an accumulation of beta chains inside the red blood

cells. The excess beta chains then form homotetramers (HbH, β4) that are characterized by erythrocyte inclusions and can be detected by supravital staining, chromatography, or gel electrophoresis. These tetramers of beta globin subunits do not transport oxygen properly, making it functionally useless to the cell. Moreover, HbH protein damages the membrane that surrounds the red cell, accelerating cell destruction. The α^0 thalassemia phenotype is caused by several deletions affecting both alpha globin genes. Some of the α^+ thalassemias result from a deletion involving one of the two alpha globin genes. The other result from nondeletion mutations, give rise to structurally abnormal hemoglobin and limit alpha gene expression. Interactions of the mutations causing deficient alpha globin synthesis produce a spectrum of phenotypes that can be grouped into four clinical syndromes [1]. In each syndrome, the severity of symptoms correlates closely with the deficiency of alpha globin chains relative to beta chains. The loss of one alpha globin structural gene diminishes the production of the alpha protein only slightly. This condition is very close to normal and a person with this condition is called a silent carrier. The syndrome of alpha thalassemia minor is characterized by the two-gene deletion that produces a condition with small red blood cells, but anemia is mild or absent. The three-gene deletion of alpha thalassemia produces a serious hematological problem with severe anemia and often requires blood transfusions to survive. The loss of all four alpha genes causes the gamma chains produced during fetal life to form homotetramers hemoglobin Bart's. Most individual with four-gene deletion alpha thalassemia die in utero or shortly after birth.

In addition, abnormalities of hemoglobin synthesis may also arise as a secondary manifestation of another disease. Acquired alpha thalassemia is reported to be associated with a myelodysplastic syndrome (MDS) and other hematologic malignancies such as x-linked alpha thalassemia mental retardation syndrome (ATR-X). Two molecular mechanisms for acquired

alpha thalassemia are now recognized: acquired deletion of the alpha globin gene cluster limited to the neoplastic clone and, more commonly, inactivating somatic mutations of the trans-acting chromatin-associated factor ATRX. This causes dramatic down regulation of the alpha globin gene expression. The relationship between ATRX mutations that lead to reduced expression of alpha globin gene and a thalassemia through epigenetic mechanisms is still unclear [2].

Prevalence and geographic distribution of thalassemia [1]

The thalassemias are among the most common genetic diseases worldwide. Both alpha and beta thalassemia have been described in individuals of almost every ancestry, but the conditions are more common among certain ethnic groups. Beta thalassemia trait is seen most commonly in people with the following ancestry: Mediterranean (including North African, and particularly Italian and Greek), Middle Eastern, Indian, African, Chinese, and Southeast Asian (including Vietnamese, Laotian, Thai, Singaporean, Filipino, Cambodian, Malaysian, Burmese, and Indonesian). All types of alpha thalassemia disease are most common among people of Southeast Asian and Chinese descent. Unaffected carriers of all types of thalassemia traits do not experience health problems. Many different mutant alleles of globin genes have been selected for many generations to reach high frequencies in tropical and subtropical regions of the world. Coincidentally, these mutations increased the likelihood that carriers would survive malaria infection and allowed survivors to pass the mutation onto their offspring. Thus the trait became established throughout areas where malaria is common. The geographic distribution of thalassemia trait population increased when populations migrated. However, it is difficult to obtain accurate prevalence for various types of thalassemia within different populations due to limitations in diagnostic testing, as well as the fact that many studies have focused on small, biased hospital populations.

Free radicals and oxidative stress in thalassemia

Oxidative stress, from the generation of reactive oxygen species (ROS), is believed to play a role in the pathophysiology of thalassemia. One study has reported that significant ROS and lipid peroxidation were found to be higher, and GSH lower in beta thalassemic red blood cells compared to their normal counterparts [6, 7]. Similar results were found in polymorphonuclear neutrophils (PMN) and platelets obtained from beta thalassemia patients which indicated a state of oxidative stress [8]. In addition, the conditions such as rupture of erythrocytes, iron overload and depletion of antioxidants in tissues and blood circulation have been reported to be common in beta thalassemia [3, 4]. The levels of cellular antioxidant vitamin such as vitamin A, C and E, as well as the activities of enzymatic antioxidants such as catalase, glutathione peroxidase, and glutathione reductase, were found to be considerably lower in thalassemic patients compared to normal subjects. These results suggest a major consumption of antioxidants under iron overload from continuous blood transfusions or oxidative stress in thalassemia [12, 13, 14]. It has also been reported that superoxide dismutase and catalase activities increased significantly in beta thalassemia patients seminal plasma but total antioxidant status values were unaltered. However, seminal lipoperoxidation did increase. These results also suggested an oxidative stress in semen of these patients and it could have contributed to the impairment of sperm motility [15]. These imply the possible alteration of redox status in thalassemic patients, which may unfavorably affect their health.

In order to prevent severe anemia and allow for normal growth and development, patients with beta thalassemia major usually receive regular blood transfusions on a monthly basis. While transfusions can prevent many of the complications of severe anemia, the body is unable to eliminate the iron overload that accompanies each transfusion. This excess iron deposits in

tissues and organs, resulting in damage and organ failure [1]. The mechanism of iron-induced organ dysfunction was reported to be part of a free radical-mediated process [11]. Additional chelation therapy, usually with the iron-binding agent desferrioxamine (Desferal), is needed to help the body get rid of excess iron and to prevent death from complications by tissue iron toxicity. The peroxidative status generated by iron overload in thalassemia patients was confirmed when free and total malondialdehyde (MDA) and nontransferrin-bound iron levels were found to be higher in transfused beta thalassemia major patients than in untransfused beta thalassemia intermedia patients. In transfused beta thalassemia major patients, the free MDA levels were positively correlated with serum iron, while the total MDA positively correlated with nontransferrin-bound iron. However, a negative correlation was observed between the total peroxyl radical-trapping antioxidant parameter and nontransferrin-bound iron determined by spectrophotometry. The marked amounts of free MDA were also rapidly formed despite the chelation therapy. In contrast, no significant correlations between free or total MDA and the total peroxyl radical-trapping antioxidant parameter or nontransferrin-bound iron were observed in untransfused beta thalassemia intermedia patients [16]. The elevated level of endogenous hemin, a denaturative product of hemoglobin (iron (III)- protoporphyrin IX), was reported to be a reliable cause of oxidative stress in blood circulation of beta thalassemia/HbE disease and may contribute a major pro-oxidant in blood circulation [5]. Oxidative stress, therefore, is thought to be an important mechanism for development of clinical complications.

Role of free radical reactions and mechanisms of injury

It is generally accepted that iron, released from macromolecules which normally sequester it, represents the source of iron-catalyzed oxidative stress, such as DNA and protein oxidation, and lipid peroxidation. Under physiological conditions, iron is not available to catalyze the conversion of molecular oxygen to the highly reactive radical species by Fenton chemistry, because ferric iron (Fe^{3+}) is bound to proteins which prevent it from participating in reactions that could lead to cell injury [3]. One of the mechanisms involved in the oxidative stress of thalassemia diseases is due to the key reactions involving oxidation and reduction of hemoglobin that take place in the presence of hydrogen peroxide (H_2O_2) and superoxide anion radicals ($O_2^{\bullet-}$) according to Fenton-like reactions as shown in **reactions (1)** and **(2)**:

$$\text{Haem-}Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad \textbf{(1)}$$

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2 \quad \textbf{(2)}$$

In general, the O_2 carried by hemeproteins is only bound directly to the ferrous iron with an oxidation state of two (Fe^{2+}). In the presence of H_2O_2, ferrous iron and H_2O_2 are going to react to generate ferric iron (Fe^{3+}) and highly reactive hydroxyl radicals ($^{\bullet}OH$). Addition of a reducing agent, such as ascorbate, leads to a cycle which increases the damage to biological molecules.

According to the Haber–Weiss reactions, consisting of the following reactions shown in **reactions (4)** and **(5)**, $^{\bullet}OH$ and molecular oxygen can be formed. R**eaction (4)** can be catalyzed by Fe^{3+} and is a possible source of $^{\bullet}OH$; however, its rate constant is negligible.

$$O_2^{\bullet-} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2 \quad \textbf{(4)}$$

$$^{\bullet}OH + H_2O_2 \rightarrow O_2^{\bullet-} + H_2O + H^+ \quad \textbf{(5)}$$

It was demonstrated that the release of Fe^{3+} from the porphyrin ring increased as a function of O_2 concentration as shown in **reactions (6)** and **(7)**:

$$\text{Heme-Fe}^{2+} + O_2 \rightarrow \text{Heme-Fe}^{3+} + O_2^{\bullet -} \quad \textbf{(6)}$$

$$\text{Heme-Fe}^{3+} \rightarrow Fe^{3+} + \text{iron-free Heme} \quad \textbf{(7)}$$

The above oxidation and subsequent ejection of Fe^{3+} from the porphyrin ring could also be done by $^{\bullet}OH$, as shown in **reactions (8)** and **(9).** When Fe^{3+} is released, it could generate reactive oxygen species that would, in turn, damage cellular compartments [17]. Thus, iron supplements should be avoided by patients with thalassemia unless iron deficiency is diagnosed.

$$\text{Heme-Fe}^{2+} + {}^{\bullet}OH \rightarrow \text{Heme-Fe}^{3+} + OH^{-} \quad \textbf{(8)}$$

$$\text{Heme-Fe}^{3+} \rightarrow Fe^{3+} + \text{iron-free Heme} \quad \textbf{(9)}$$

In addition, since the modification of low density lipoprotein (LDL) was observed in patients with the severe hemolytic anemia beta thalassemia [9], it was suggested that under oxidative stress extracellular chains of normal hemoglobin (HbA) can trigger oxidation of LDL in the presence of H_2O_2. The modified LDL particles observed in beta thalassemia may reflect lipoprotein oxidation by alpha chains in circulation. A possible explanation of the high peroxidative activity of alpha chains is their ability to undergo appreciable autoxidation yielding hydrogen peroxide as a byproduct, enhancing LDL peroxidation [10].

Interestingly, in order to investigate the role of oxidant injury in the pathophysiology of human thalassemias, one study has shown that the apoptotic programs turned on in beta thalassemic erythroid precursor cells were not triggered by oxidative damage but were dependent on activation of FAS/FAS-Ligand cell surface interaction. The authors suggested that the destruction of thalassemic erythroid precursors may involve different mechanisms other than oxidant injury that cause red blood cells hemolysis [18].

Future research directions

The goal for future research in thalassemia is not only to introduce new strategies of diagnosis and treatment of thalassemia but also to discover ways to prevent the occurrence of oxidative damage in thalassemic patients. Current work deals with specific complications in thalassemia patients such as endocrine, cardiopulmonary, thrombophilic, and osteopenic problems, as well as iron overload. The effectiveness of oral iron-chelating drugs are being developed and tested, which could greatly simplify treatment of this disease. Studies conduced on in oxidative status and correlations between antioxidants can also provide insights accomplishing this purpose. In addition, the thalassemias are likely to benefit from specific gene therapy designed to overcome the adversity in the synthesis of a specific globin chain [19].

Bone marrow and stem cell transplantation

Specific treatments for thalassemia are employed based on many factors such as age of the patients and severity of the disease. Red blood cell transfusion, splenectomy, iron chelation are among the general methods used for thalassemia treatments [1]. A major event in the area was the discovery that allogeneic bone marrow (stem cell) transplantation could succeed in severely affected thalassemia patients [19]. However, bone marrow transplants have cured some cases of thalassemia but they are not widely used due to less successful of the methods [20]. Currently, scientists are investigating the use of umbilical cord cell transplantation[3]. Cord blood is an excellent source of stem cells that have remarkable potential to develop into many different cell types in the body. If it continues to be successful, this could eliminate the need for long-term transfusion therapy or iron chelation treatment.

[3] http://web1.tch.harvard.edu/cfapps/A2ZtopicDisplay.cfm?Topic=Beta%20Thalassemia access Apr 25, 2005

Oxygen-regulated gene therapy

Many concepts of gene therapy have been proposed[4]. Gene therapy may involve inserting a normal beta or alpha globin gene into the patient's stem cells. The new alpha or beta globin genes should be designed to insert with appropriate copy number into the hematopoietic stem cells, but they should be expressed in normal amounts only in erythroid precursors and not in any of the other nine stem-cell-derived blood cell lines [19]. Presently, significant advances have been made toward this goal using lentiviral vectors to obtain high-level expression of complex globin gene cassettes. Therapeutic correction in murine models of both beta-thalassemia and sickle cell anemia has been achieved using this approach [22, 23]. However, further refinement of the gene therapy approach in thalassemia requires more animal modeling, particularly with primate models, before planning a phase one human study. Another form of gene therapy may concern using drugs or other methods to reactivate the patient's genes for fetal hemoglobin (HbF). Then they can make the blood cells of patients with thalassemia produce more fetal hemoglobin to compensate for their deficiency of adult hemoglobin. Initial studies of rare individuals with genetic traits that allow them to produce only fetal hemoglobin show that they are generally healthy, demonstrating that fetal hemoglobin can be a fine substitute for adult hemoglobin [21].

Recently, the development of a physiologically regulated gene therapy that can deliver physiologically regulated expression of Epo has been demonstrated. One of the study reported that the continuous delivery of high amounts of autologous erythropoietin, *via* a recombinant adeno-associated virus-cytomegalovirus vector into skeletal muscles, induced a sustained stimulation of beta minor globin synthesis and a stable improvement of erythropoiesis in the beta thalassemic mouse model [24]. However, the relevance of the study to chronic anemia has been

[4] http://www.icomm.ca/geneinfo/thalass.htm access on Apr 25, 2005

limited because the Epo gene has been delivered to beta thalassemic mice. Recently, the potential of genetic delivery of the Epo gene in a more relevant model of chronic anemia was performed. It was reported that an intramuscular delivery of the constructed promoter, Oxford Biomedica hypoxia response element (OBHRE), used in treating homozygous erythropoietin-SV40 T antigen (Epo-TAg(h)) mice with relative erythropoietin deficiency, gives rise to physiologically regulated erythropoietin secretion. The long-term delivery of Epo expression can correct the hematocrit level in anemic mice to a normal physiologic level that stabilized without resulting in polycythemia (an expanded red cell mass) but had no significant effect on the hematocrit of control mice. The OBHRE-Epo-treated Epo-TAg(h) mice also shown a significant reversal of cardiac hypertrophy, which is a common adaptive response in patients with chronic state of anemia. This establishes that a hypoxia regulatory mechanism similar to the natural mechanism can be achieved, and it makes Epo gene therapy more attractive and safer in clinical settings [25].

Antioxidant therapy

It should be noticed that many studies are now focusing on the correlations between antioxidant activities and severity of the disease in thalassemic patients. It has been reported that oxidative stress-induced changes in thalassemic erythrocytes can be attenuated by vitamin E [13]. N-Acetylcysteine amide, a novel cell-permeating thiol was reported to restore cellular glutathione and protect human red blood cells from oxidative stress [26]. Oxidative stress is greatly increased in thalassemia because of the prolonged exposure to iron accumulation. Thus, the measurement of ROS or other pro-oxidant activity levels in thalassemic patient's serum will be useful for the management of the disease in preventing oxidative damage by appropriate antioxidant treatment.

Experimental design to test the hypothesis

From the evidence of several studies, it is clearly indicated that oxidative stress, at least some parts, plays an important role in pathophysiologic status of thalassemia disease. In order to prove this hypothesis and explore the potential combination therapies that would allow for attenuation of ROS in thalassemia, some of the following experiment designs might provide promising results for further investigation.

Experiments using animal models

1. In order to reveal and understand whether free radical initiated oxidative damage plays an important role in thalassemia, the research strategy should directly measure the levels of free radials and oxidative damage in blood components of normal and thalassemic mice. The correlations between free radical levels and oxidative damage will provide a clue underlying pathophysiology of the disease.
2. More information underlying the mechanism of oxidative damage in thalassemia is needed. A long-term study of free radicals exposure to normal and thalassemic mice may help to determine the adverse effects of a certain levels of free radicals that develop severity of anemia and other complications.
3. It might be useful to measure the levels of free radicals, oxidative products *in vivo* in thalassemia transgenic mice at different age to see whether free radicals and oxidative stress levels are influenced by age.
4. A decrease in antioxidant enzyme has been detected in thalassemic patients suggesting that a deficient activity of antioxidant enzymes may play important role in at lease some thalassemia case. Increasing antioxidant activity will hopefully reduce oxidative stress down to minimal level. For this particular study, the overexpression of antioxidant

enzymes such as MnSOD and catalase in mice will provide a rational basis for development of gene therapy as a method of protection against free radical-induced oxidative stress in thalassemic mice.

Experiments using human model

1. The mechanism of oxidative damage in thalassemia is likely to be complex, involving iron overload. Utilizing a case-control study design, determine the status of antioxidant, lipid peroxides in the presence or absence of iron chelating agent might provide information of mechanism which contributes to iron overload.
2. Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Preliminary studies have found that oral supplements of 200 to 600 IU per day of vitamin E reduce free radical damage to red blood cells in thalassemia patients [27], while an *in vitro* study has shown that propionyl-L-carnitine protects red blood cells of thalassemia patients against free radical damage [28]. These results suggest that providing appropriate nutritional supplements to the patients may prevent any complications from hemolytic red blood cells including significantly decreased need for blood transfusions. Therefore, it might be useful to investigate whether various kinds of supplementary antioxidants, such as vitamin C, E, beta-carotene, and the resultant antioxidant plasma status of these nutrients are associated with regression of oxidative damage of red blood cells.

Summary

The fundamental abnormality in thalassemia is impaired production of either the alpha or beta hemoglobin chain. Several theories of pathogenesis and severity of thalassemia disease have emerged including free radicals induce oxidative stress. Patients with severe thalassemia who receive regular blood transfusions become iron overloaded, which increases damaging free radical activity and lowers antioxidant levels in their bodies. General treatment strategies of thalassemia are blood transfusion and iron chelation. Besides the promising treatment of stem cell transplantation and gene therapy, an imbalance in the antioxidant protective mechanisms leading to oxidative stress in blood components suggests a new era of antioxidant therapy for thalassemia disease. However, more attempts to put forth the evidence for involvement of free radicals in pathophysiology of thalassemia and the potential of treatment with antioxidant and scavenger substances are still needed and required a combination of a multitude of disciplines.

References

1.Lukens JN. (1993) The thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In: Lee GR, ed. Wintrobe's Clinical Hematology 9th edition Vol. 1. Lea & Febiger, PA Press; 1102-1145.

2. Steensma DP, Gibbons RJ, Higgs DR. (2005) Acquired α-thalassemia in association with myelodysplastic syndrome and other hematologic malignancies. *Blood.* **105**(2): 443-452.

3. Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, D'Arpa D, D'Anna R, Bongiorno A. (1996) Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood.* **88**(9):3608-3014.

4. Luca C, Filosa A, Grandinetti M, Maggio F, Lamba M, Passi S. (1999) Blood antioxidant status and urinary levels of catecholamin metabolites in ß-thalassemia. *Free Radic Res*. **30**:453-462.

5. Phumala N, Porasuphatana S, Unchern S, Pootrakul P, Fucharoen S, Chantharaksri U. (2003) Hemin: a possible cause of oxidative stress in blood circulation of beta halassemia/hemoglobin E disease. *Free Radic Res*. **37**(2):129-135.

6. Amer J, Goldfarb A, Fibach E. (2004) Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. *Cytometry A*. **60**:73-80.

7. Amer J, Fibach E. (2004) Oxidative status of platelets in normal and thalassemic blood. *Thromb Haemost*. **92:**1052-1059.

8. Amer J, Fibach E. (2005) Chronic oxidative stress reduces the respiratory burst response of neutrophils from beta-thalassaemia patients. *Br J Haematol*. **129**(3):435-441.

9. Altamentova SM, Marva E, Shaklai N. (1997) Oxidative interaction of unpaired hemoglobin chains with lipids and proteins: a key for modified serum lipoproteins in thalassemia. *Arch Biochem Biophys.* **345**:39-46.

10. Altamentova SM, Shaklai N. (1998) Oxidative stress in beta-thalassemia: hemoglobin alpha-chains activate peroxidation of low density lipoproteins. *Biofactors.* **8**:169-172.

11. Bartfay WJ, Bartfay E. (2000) Iron-overload cardiomyopathy: evidence for a free radical-mediated mechanism of injury and dysfunction in a murine model. *Biol Res Nurs.* **2**(1):49-59.

12. Kassab-Chekir A, Laradi S, Ferchichi S, Haj Khelil A, Feki M, Amri F, Selmi H, Bejaoui M, Miled A. (2003) Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. *Clin Chim Acta*. **338**(1-2):79-86.

13. Das N, Das Chowdhury T, Chattopadhyay A, Datta AG. (2004) Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. *Pol J Pharmacol*. **56**:85-96.

14. Cheng ML, Ho HY, Tseng HC, Lee CH, Shih LY, Chiu DT. (2005) Antioxidant deficit and enhanced susceptibility to oxidative damage in individuals with different forms of alpha-thalassaemia. *Br J Haematol*. **128**(1):119-127.

15. Carpino A, Tarantino P, Rago V, De Sanctis V, Siciliano L. (2004) Antioxidant capacity in seminal plasma of transfusion-dependent beta-thalassemic patients. *Exp Clin Endocrinol Diabetes*. **112**(3):131-134.

16. Cighetti G, Duca L, Bortone L, Sala S, Nava I, Fiorelli G, Cappellini MD. (2002) Oxidative status and malondialdehyde in beta thalassaemia patients. *Eur J Clin Invest.* **32** Suppl 1:55-60.

17. Anastassopoulou J, Anifantakis B, Anifantakis ZA, Dovas A, Theophanides T. (2000) The role of free radical reactions with haemoglobin and thalassaemia. *J Inorg Biochem*. **79**:327-329.

18. Schrier SL, Centis F, Verneris M, Ma L, Angelucci E. (2003) The role of oxidant injury in the pathophysiology of human thalassemias. *Redox Rep*. **8**(5):241-245.

19. Schrier SL, Angelucci E. (2005) New strategies in the treatment of the thalassemias. *Annu Rev Med*. **56**:157-171.

20. Gaziev J, Lucarelli G. (2005) Stem cell transplantation for thalassaemia. *Reprod Biomed Online*. **10**(1):111-115.

21. Persons DA, Tisdale JF. (2004) Gene therapy for the hemoglobin disorders. *Semin Hematol.* **41**(4):279-286.

22. Puthenveetil G, Scholes J, Carbonell D, Qureshi N, Xia P, Zeng L, Li S, Yu Y, Hiti AL, Yee JK, Malik P. (2004) Successful correction of the human beta-thalassemia major phenotype using a lentiviral vector. *Blood*. **104**(12):3445-3453.

23. Imren S, Fabry ME, Westerman KA, Pawliuk R, Tang P, Rosten PM, Nagel RL, Leboulch P, Eaves CJ, Humphries RK. (2004) High-level beta-globin expression and preferred intragenic integration after lentiviral transduction of human cord blood stem cells. *J Clin Invest*. **114**(7):953-962.

24. Bohl D, Bosch A, Cardona A, Salvetti A, Heard JM. (2000) Improvement of erythropoiesis in beta-thalassemic mice by continuous erythropoietin delivery from muscle. *Blood.* **95**(9):2793-2798.

25. Binley K, Askham Z, Iqball S, Spearman H, Martin L, de Alwis M, Thrasher AJ, Ali RR, Maxwell PH, Kingsman S, Naylor S. (2002) Long-term reversal of chronic anemia using a hypoxia-regulated erythropoietin gene therapy. *Blood*. **100**:2406-2413.

26. Grinberg L, Fibach E, Amer J, Atlas D. (2005) N-acetylcysteine amide, a novel cell-permeating thiol, restores cellular glutathione and protects human red blood cells from oxidative stress. *Free Radic Biol Med*. **38:**136-145.

27. Suthutvoravut U, Hathirat P, Sirichakwal P, Sasanakul W, Tassaneeyakul A, Feungpean B. (1993) Vitamin E status, glutathione peroxidase activity and the effect of vitamin E supplementation in children with thalassemia. *J Med Assoc Thai*. **76** Suppl 2:146-152.

28. Palmieri L, Ronca F, Malengo S, Bertelli A. (1994) Protection of beta-thalassaemic erythrocytes from oxidative stress by propionyl carnitine. *Int J Tissue React*. **16**(3):121-129.