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Anemia Caused by Oxidative Stress

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1. Introduction

Anemia is considered to be one of the major health problems. According to the World Health Organization, about 30 percent of people throughout the world suffer from anemia. The most common cause of anemia is iron deficiency; however, recent work has shown that reactive oxygen species (ROS) of erythrocytes are one of the principal causative factors of anemia. Elevation of ROS in erythrocytes could occur either by activation of ROS generation or by suppression of antioxidative/redox system. When erythrocytes experience an excessive elevation of ROS, oxidative stress develops. ROS are known to contribute to the pathogenesis of several hereditary disorders of erythrocytes, including sickle cell anemia, thalassemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency.
Deficiency of antioxidant enzymes such as superoxide dismutase 1 (SOD1) or peroxiredoxin II (Prx II) induces elevation of oxidative stress in erythrocytes and causes anemia, while deficiency of catalase or glutathione peroxidase does not. In addition to the abnormalities of antioxidant enzymes, some transcription factors such as p45NF-E2 or Nrf2 can cause anemia. In this chapter, I provide some evidence of the involvement of oxidative stress in anemia.

2. Oxidative stress-mediated destruction of erythrocytes

2.1 Cellular oxidative stress and anti-oxidative system

Under normal physiological conditions, there is a balance between the ROS and the defense system of antioxidant enzymes and antioxidants, which prevents or limits oxidative damage. ROS are produced as a result of intracellular metabolic activity. During this process, ROS such as superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet$OH) are produced, even in healthy individuals. Oxidative stress is the result of an imbalance between oxidants and antioxidants. Increased pro-oxidants and/or decreased antioxidants trigger a cascade of oxidative reactions. Oxidative stress can damage specific molecular targets (lipids, proteins, nucleotides, etc.), resulting in cell dysfunction and/or death. Enzymes that participate in ROS production include xanthine oxidase (XO), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, nitric oxide synthase (NOS), cytochrome P450, cyclo-oxygenase (COX), and lipoxygenase. Major defence mechanisms against ROS include enzymatic (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), peroxiredoxin (Prx)) as well as non-enzymatic systems (reduced glutathione (GSH), ubiquinols, uric acid, vitamins C and E, flavonoids, carotenoids).

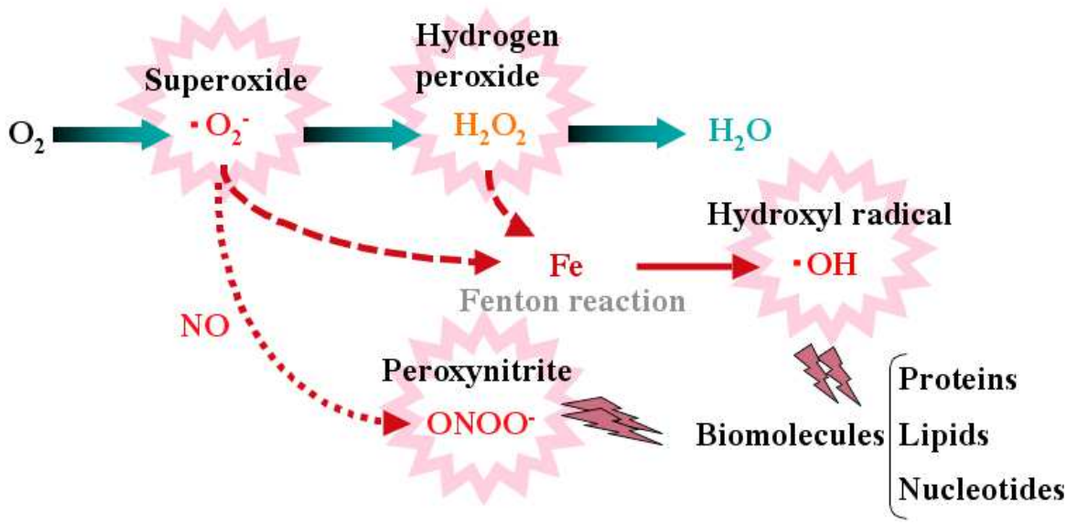

Fig. 1. Reactive oxygen species (ROS) generated in the cell.

2.2 Oxidation of erythrocyte membrane caused by ROS

During the binding of oxygen to form oxy-hemoglobin (oxy-Hb), one electron is transferred from iron to the bound oxygen forming a ferric-superoxide anion complex. The shared electron is normally returned to the iron when oxygen is released during deoxygenation. However, the electrons can remain and transform oxygen into superoxide anions. In this process, iron is left in the ferric state and Hb is transformed into methemoglobin (met-Hb). The autoxidation of Hb occurs spontaneously and transforms 0.5–3% of Hb into met-Hb per day. In addition to this physiological process, met-Hb can be produced by endogenous oxidants, such as H_2O_2, nitric oxide (NO), and hydroxyl radicals. Since met-Hb cannot bind oxygen, this is the first step in the formation of harmful hemichromes (Rice-Evans & Baysal, 1987). In normal conditions, spontaneous production of met-Hb from autoxidation and conversion of met-Hb back to Hb are in balance. However, in pathological conditions, increased oxidative stress or impaired antioxidant defence will enhance production of met-Hb and generation of ROS. Hemichrome formation depends on the amount of met-Hb formed and is accelerated by ROS such as superoxide or H_2O_2. Superoxide produced by one electron reduction of oxygen would reduce ferri-hemichrome to ferro-hemichrome. In the Fenton reaction, ferro-hemichrome catalyzes decomposition of H_2O_2 to hydroxyl radical. Hydroxyl radical is an extremely reactive free radical that can react with various biomolecules such as membrane lipids. Peroxidation of membrane lipids, most notably the polyunsaturated fatty acids arachidonic acid and linoleic acid, generates a wide array of molecules, such as lipid hydroperoxides, which are secondary lipid peroxidation products (for example, malondialdehyde and 4-hydroxynonenal, HNE). Lipid peroxidation products can damage membrane structure with the formation of membrane pores, alter water permeability, decrease cell deformability, and enhance IgG binding and complement activation. Finally, disruption of the normal asymmetrical distribution of membrane phospholipids occurs. This may enhance exposure of phosphatidylserine (PS) on the outer cell surface. Erythrocytes that have PS exposed on the outer surface are recognized and engulfed by macrophages with PS-specific receptors, resulting in their degradation (Carrell et al., 1975; Hebbel, 1985; Nur et al., 2011).

At the same time, ROS can be used for killing harmful microorganisms. However, ROS not only participate in pathogen killing but also induce activation of inflammatory mediators and production of adhesion molecules and membrane damage. The increased intra- and extra-erythrocytic oxidative stress induces lipid peroxidation and membrane instability, contributing to accelerated hemolysis. Increased levels of hydroperoxides cause erythrocyte membrane damage and deformity and, ultimately, lead to cell death.

2.3 Band 3-mediated erythrocyte removal

Band 3, also termed the anion exchanger, is a major erythrocyte membrane protein, constituting 25% of the total erythrocyte membrane protein. It has two independent domains: the membrane-spanning domain, which catalyzes anion exchange and contains the antigenic determinants recognized by naturally occurring antibodies (NAbs), and the cytoplasmic domain (Pantaleo et al., 2008). A very important feature of hemichrome/free heme/iron damage is its non-random occurrence in space. The highly damaging feature of hemichromes is their tight association with the cytoplasmic domain of band 3, which, following their binding, leads to band 3 oxidation and clusterization. These band 3 clusters show increased affinity for NAbs, which activate complement and finally trigger phagocytosis-mediated erythrocyte removal. This band 3/hemichrome complex was found not only in pathological conditions in which oxidative stress in erythrocytes is thought to be elevated, but also in senescent erythrocytes (Arese et al., 2005).

3. Relationship between human hereditary anemia and oxidative stress

3.1 Sickle cell disease

Sickle cell disease (SCD) is a hemoglobinopathy clinically characterized by chronic hemolysis. Chronic activation and damage of endothelial cells by sickle erythrocytes, heme, polymorphonuclear neutrophils (PMNs), and inflammatory mediators contribute to progressive microvascular damage in all organs, including the brain, lungs, and kidneys. SCD is an inherited disorder of hemoglobin synthesis. SCD has the same single base pair mutation (GAG to GTG, Glu to Val) in the β globin molecule of sickle cells (HbS).

Chronic oxidative stress constitutes a critical factor in endothelial dysfunction, inflammation, and multiple organ damage in SCD (Nur et al., 2011). There are several causes of oxidative stress in SCD. Major sources of ROS in SCD are thought to be the (i) enhanced rate of HbS auto-oxidation, (ii) increased xanthine oxidase activity in SCD aortic endothelium, and (iii) higher number of leucocytes, which produce twice the fluxes of superoxide in SCD (Wood & Granger, 2007).

Endothelial dysfunction in patients with SCD has been related to inflammation, high levels of production of ROS and reactive nitrogen species, and erythrocyte adhesion to blood vessel walls. There have been several studies showing that patients with SCD have a high level of oxidative damage, assessed through lipid peroxidation. In turn, oxidative stress is associated with chronic hemolysis. Sickle erythrocytes have a high frequency of phosphatidyl serine exposure, which is due to oxidative stress, suggesting that oxidative stress might play a role in intravascular hemolysis. Hypertension in patients with SCD was found to be related to ROS, which can directly deactivate endothelial nitric oxide synthase (eNOS), reducing nitric oxide (NO) levels, an important vasodilator (Rusanova et al., 2010).

3.2 Thalassemia

The thalassemia syndrome is one of the most common genetic disorders affecting a single gene or gene cluster. The various thalassemia disorders are caused by insufficient production of one of the two types of globin chains that constitute the hemoglobin tetramer. In α thalassemia , α globin production is reduced or absent, and in β thalassemia, β globin production is impaired. The α and β thalassemias are characterized by the presence of a pool of unpaired hemoglobin chains. While α or β hemoglobin chains are stable when part of the $\alpha_2\beta_2$ hemoglobin tetramer, the unpaired α or β hemoglobin chains are unstable and subject to high rates of auto-oxidation. The auto-oxidation of the unpaired hemoglobin chains leads to the generation of superoxide and H_2O_2, and subsequent release of globin-free heme and iron (Bunn, 1967; Shinar & Rachmilewitz, 1990). β thalassemic erythrocytes exhibit a significant decrease in the NADPH/NADP ratio similar to that seen in severe G6PD-deficiency anemia (see next chapter). As a consequence of this decrease, both catalase activity and GSH concentration are decreased (Scot, 2006). Expression of peroxiredoxin (Prx) II, an antioxidant enzyme that detoxifies H_2O_2, is increased in β thalassemic mouse erythrocytes (Matte et al., 2010). These findings indicate that this high expression of PrxII has a compensatory effect against elevated oxidative stress in thalassemic erythrocytes (Rund & Rachmilewitz, 2005).

3.3 Glucose-6-phosphate dehydrogenase (G6PD) deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency was first discovered in African-American subjects. The fact that it seemed to be limited to one ethnic group suggested that it has a genetic basis. Because it was shown that transmission was generally from mother to son, it became apparent that G6PD deficiency is an X-linked disorder. G6PD deficiency is one of the glycolytic enzymopathies that frequently cause hemolytic anemia. G6PD deficiency is the most common erythrocyte enzyme defect, affecting over 400 million people (Beutler, 2008).

G6PD deficiency is mainly caused by point mutations in the G6PD gene. About 140 mutations have been described: most are single base changes, leading to amino acid substitutions (Cappellini & Fiorelli, 2008). The most frequent clinical manifestations of G6PD deficiency are neonatal jaundice and acute hemolytic anemia, which is usually triggered by an exogenous agent. Some G6PD variants cause chronic hemolysis, leading to congenital non-spherocytic hemolytic anemia. The appearance of Heinz bodies both in vivo and in vitro in G6PD-deficient cells and their inability to protect their GSH against drug challenge suggested that a major component of the hemolytic process is the inability of the erythrocytes to protect sulfhydryl groups against oxidative stress (Fig. 2, Cohen & Hochstein, 1961). However, it has been shown that, in mice, targeted disruption of the gene encoding glutathione peroxidase has little effect on oxidation of hemoglobin of murine cells challenged with peroxides.

G6PD provides erythrocytes with important protection against oxidative stress. G6PD is a key regulatory enzyme of the pentose phosphate pathway (also called hexose monophosphate shunt), which is essential for the supply of reduced NADPH. NADPH enables cells to counterbalance oxidative stress that can be triggered by several oxidant agents, and to preserve the reduced form of glutathione. NADPH is pivotal to the cellular antioxidative defence systems in most organisms. Since erythrocytes do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defence against oxidative damage is dependent on G6PD.

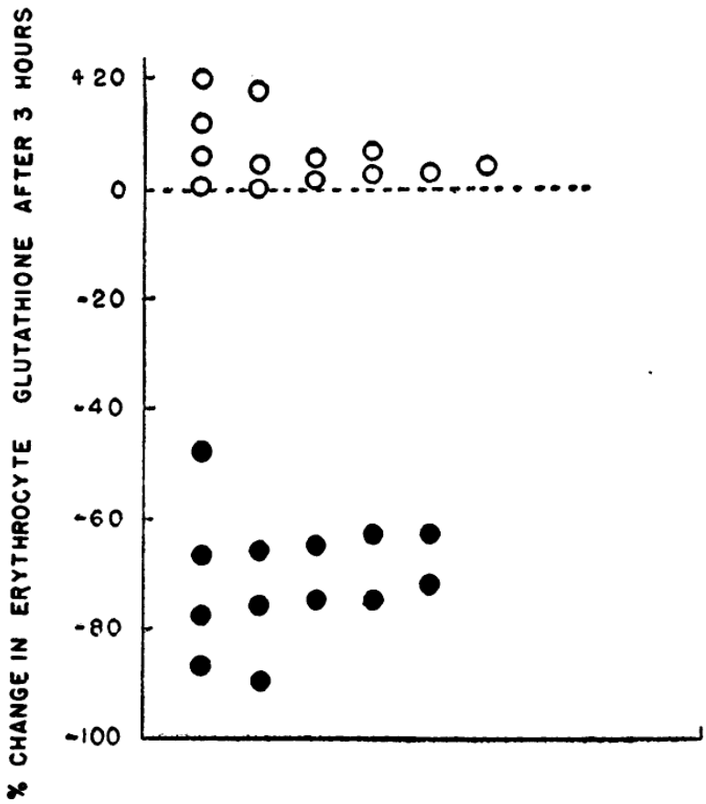

Fig. 2. Percentage change in reduced glutathione (GSH) in erythrocytes of 13 individuals with G6PD deficiency (solid circles) and 13 individuals with normal G6PD activity (open circles) after 3 hours of hydrogen peroxide diffusion (Cohen & Hochstein, 1961).

4. Animal model of anemia

4.1 Deficiency of antioxidant enzymes

4.1.1 Superoxide dismutase 1 (SOD1) deficiency

Among the known antioxidative proteins, superoxide dismutase (SOD) is thought to play a central role because of its ability to scavenge superoxide anions, the primary ROS generated from molecular oxygen in cells (Fridovich, 1995). SOD1-deficient mice have been generated by several groups. Unexpectedly, SOD1-deficient mice grow normally but develop female infertility (Ho et al., 1998; Matzuk et al., 1998), cochlear hair cell loss (McFadden et al., 1999), and vascular dysfunction (Didion et al., 2002). Adding to these phenotypes, SOD1-deficient mice exhibit severe anemia, even in infant mice (Iuchi et al., 2007; Starzyński et al., 2009). Anemia appears to be caused by shortened lifespan of erythrocytes. Increased ROS due to SOD1 deficiency makes their erythrocytes vulnerable to oxidative stress. In addition to SOD1 deficiency, GPx activity and protein levels of GPx1 were significantly lower in erythrocytes. Since GPx1 protein is prone to oxidative inactivation, oxidized GPx1 would be removed by the protease that degrades oxidized proteins in erythrocytes.

While most mammalian cells possess two intracellular SOD isoforms to protect against ROS, erythrocytes lack mitochondria and, as a result, carry only the SOD1 protein to scavenge superoxide anions. Erythrocytes of SOD1-deficient mice, therefore, face severe oxygen toxicity compared with other tissues. Erythrocytes that are hyperoxic bind oxygen in the

lungs (~21%), and release oxygen in peripheral tissues, which are relatively hypoxic (~2%). Thus, erythrocytes undergo cyclic exposure to hyperoxic and hypoxic environments, generating large amounts of superoxide via auto-oxidation of hemoglobin.
The continuous destruction of oxidized erythrocytes in SOD1-deficient mice appears to induce the formation of autoantibodies against certain erythrocyte components, for example, carbonic anhydrase II, and the immune complex is deposited in the kidney glomeruli. Therefore, these mice exhibit autoimmune hemolytic anemia (AIHA)-like symptoms when they reach old age. This pathophysiological symptom is thought as a secondary effect of elevated oxidative stress in SOD1-deficient erythrocytes.

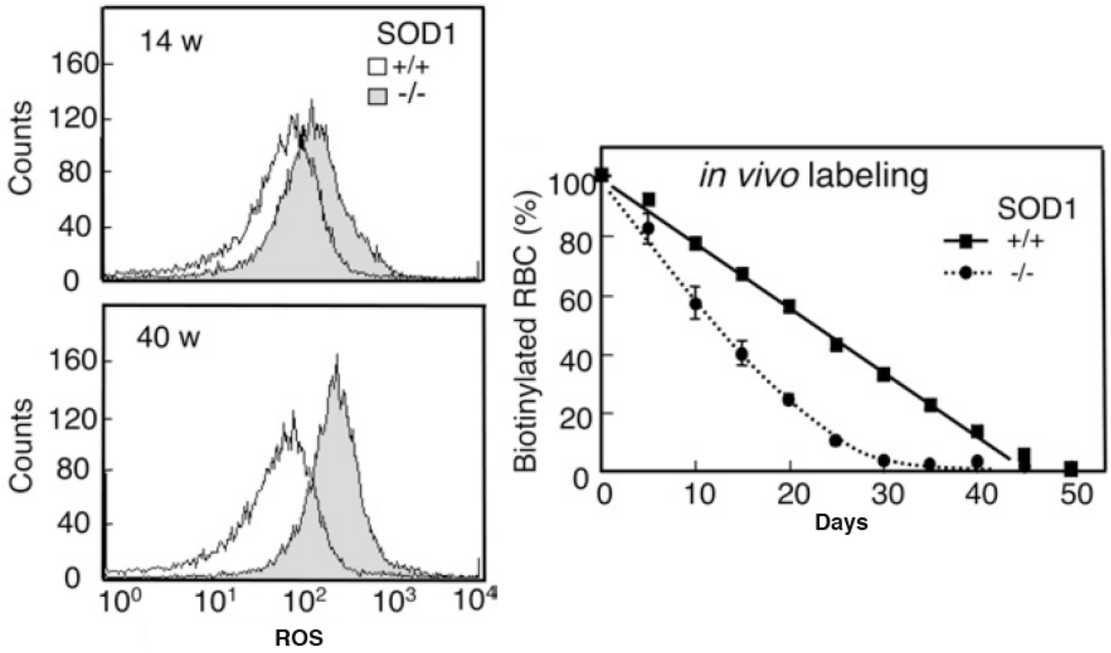

Fig. 3. Elevated ROS (left panels) and shortened lifespan (right panel) of erythrocytes in SOD1-deficient mice (Iuchi et al., 2007).

4.1.2 Catalase deficiency

Human erythrocytes contain large amounts of catalase. While the catalase and NADPH/GSH/GPx system is very important for disposal of H_2O_2 in human erythrocytes, genetic deficiencies of catalase do not predispose erythrocytes to peroxide-induced destruction (Jacob et al., 1965). Mice lacking the catalase gene develop normally (Ho et al., 2004). A link between catalase deficiency and anemia has not been reported.

4.1.3 Glutathione peroxidase-1 (GPx1) deficiency

The role of glutathione peroxidase in erythrocyte anti-oxidant defense was examined using erythrocytes from mice with genetically engineered disruption of the glutathione peroxidase-1 (GPx1) gene. Because GPx1 is the sole glutathione peroxidase in erythrocytes, all erythrocyte GSH peroxidase activity was eliminated. Oxidation of hemoglobin and membrane lipids was determined during oxidant challenge from cumene hydroperoxide and H_2O_2. As a result, no difference was detected between wild-type erythrocytes and GPx1–deficient erythrocytes,

even at high levels of H_2O_2 exposure. Thus, GPx1 appears to play little or no role in the defense of erythrocytes against exposure to peroxide (Johnson et al., 2000).

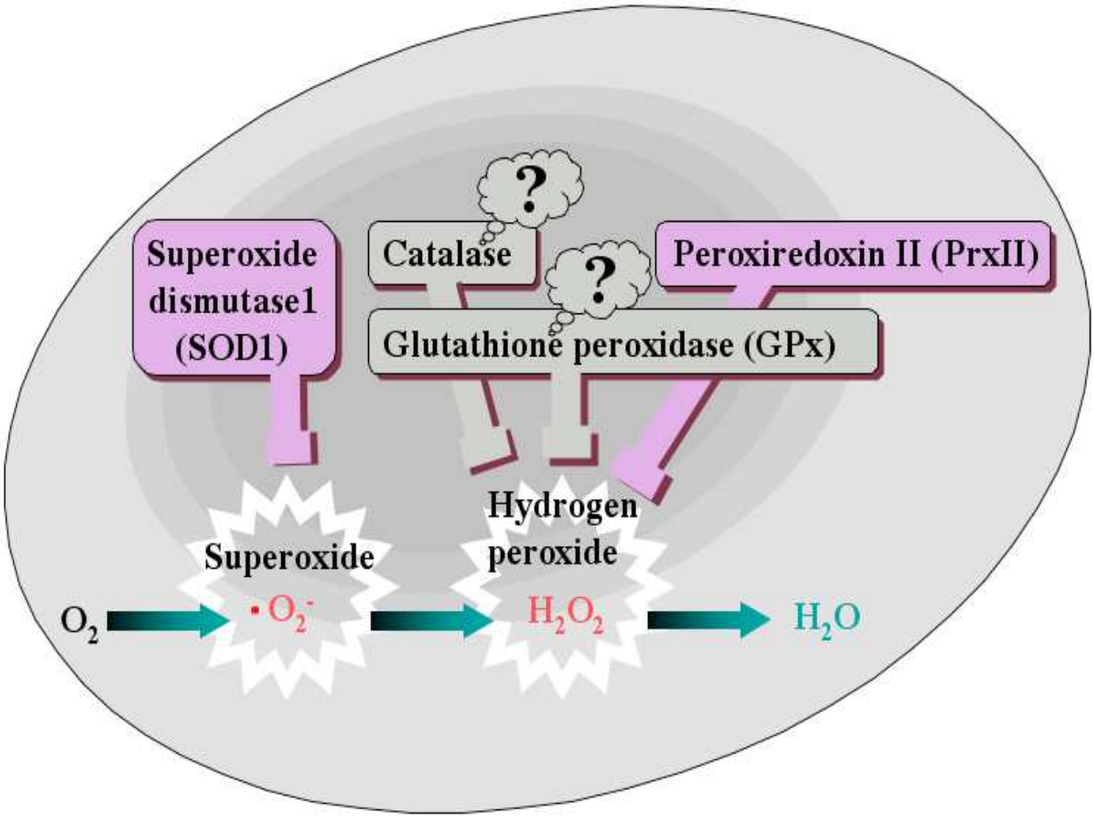

Fig. 4. Do SOD1 and PrxII actually work in erythrocytes?

4.1.4 Peroxiredoxin II (PrxII) deficiency

A number of proteins also protect cells against oxidative stress. SOD, GPx, and catalase are commonly known antioxidant enzymes and have been extensively characterized. Recently, a new family of antioxidative proteins, collectively referred to as Prxs (peroxiredoxins), have been identified. Six distinct gene products are known in the Prx family in mammals (Fujii & Ikeda, 2002). Thioredoxin-dependent peroxidase activity appears to be common to most Prx family members, and in addition, other divergent biological functions have been elucidated for individual Prx members. However, the most well-characterized function of Prx family members is the ability to modulate hydrogen peroxide signaling in response to various stimuli (Rhee et al., 2005).

Mice deficient in Prx II, which is abundantly expressed in all types of cells, were healthy in appearance and fertile. However, they had splenomegaly caused by the congestion of red pulp with hemosiderin accumulation. Erythrocytes from these mice contained markedly higher levels of ROS. The Prx II-deficient mice had significantly decreased hematocrit levels, but increased reticulocyte counts and erythropoietin levels, indicative of a compensatory action to maintain hematologic homeostasis in the mice (Lee et al., 2003).

For a long time, it was considered that catalase and GPx constitute the erythrocyte defense against H_2O_2, and there has been continuous debate about which of these is more significant

(Cohen & Hochstein, 1963; Gaetani et al., 1989; Gaetani et al., 1996). Until recently, little attention has been paid to the antioxidant role of Prxs in erythrocytes, even though Prx II is the third most abundant cytoplasmic erythrocyte protein. These mice possess fully functional catalase and GPx. Erythrocytes also possess PrxI and PrxVI, although at lower levels than PrxII. It is reported that PrxII expression and content were markedly increased in erythrocytes from β thalassemic mouse models compared with those in wild-type mice (Matte et al., 2010). This indicates that PrxII has a non-redundant function in protecting healthy erythrocytes against oxidative damage and plays a crucial role even in pathological conditions.

4.2 Deficiency of transcription factor

In many cases, transcriptional activation of genes that play an important role in detoxification of xenobiotics and defense against oxidative stress is mediated partly by the antioxidant response element (ARE). For example, AREs have been found in promoter sequences of genes including nicotinamide adenine dinucleotide phosphate–quinone oxidoreductase, heme oxygenase, glutathione-S-transferases, and glutamylcysteine synthetase (Favreau et al., 1995; Inamdar et al., 1996; Jaiswal et al., 1994; Mulcahy et al., 1995; Prestera et al., 1995). The ARE consensus sequence is very similar to the NF-E2–like sequence of the β globin locus control region, which was found to be essential for globin gene expression. Multiple proteins can interact with the NF-E2 consensus sequence. The cap 'n' collar (CNC)–bZIP factor family of proteins was identified from searches for proteins that bind and activate the NF-E2 site of the β-globin locus control region. This multiple-protein family includes p45NF-E2, NF-E2–related factor (Nrf)1, and Nrf2. The similarities among CNC family members are most notable in the basic-DNA binding region and another homology domain (Moi et al., 1994).

4.2.1 p45NF-E2 deficiency

p45NF-E2 is a member of the cap 'n' collar (CNC)-basic leucine zipper family of transcriptional activators that is expressed at high levels in various types of blood cells. It plays a crucial role in megakaryocyte maturation and platelet biogenesis. Mice with disruption of p45NF-E2 have severe platelet deficiency due to defective megakaryocyte maturation. In addition, p45NF-E2 knockout mice exhibit anemia characterized by the presence of hypochromic erythrocytes and reticulocytosis. Erythrocytes from p45NF-E2–deficient mice are sensitive to oxidative stress. Erythrocytes from p45NF-E2–deficient mice accumulated high levels of free radicals when exposed to oxidants, and this correlated with increased formation of met-Hb and loss of membrane deformability. In addition, severe anemia developed in p45NF-E2 deficient mice treated with oxidative-stress–inducing drugs, and mutant erythrocytes had decreased survival.

Because CNC factors may represent an important class of regulators of antioxidant gene expression by means of the ARE, one possibility is that p45NF-E2 is involved in regulating oxidative stress-response genes in erythrocytes. It is possible that a compensated hemolytic state contributes to the erythroid abnormalities observed in p45NF-E2 knockout mice.

4.2.2 Nrf2 deficiency

The NF-E2-related factor 2 (Nrf2) transcription factor regulates genes related to ROS scavenging and detoxification. Although Nrf2 is expressed widely and is important for cellular

antioxidant potential, Nrf2 knockout mice develop and grow normally (Chan et al., 1996). Young Nrf2 knockout mice are not anemic, whereas targeted disruption of either NF-E2 or Nrf1 resulted in anemia. In aged mice, however, disruption of Nrf2 causes regenerative immune-mediated hemolytic anemia due to increased sequestration of damaged erythrocytes. Splenomegaly and spleen toxicity in Nrf2-deficient mice raised the possibility of hemolytic anemia and splenic extramedullary hematopoiesis in Nrf2-deficient mice. Nrf2-deficient erythrocytes are highly sensitive to H_2O_2-induced hemolysis in vitro, further suggesting that Nrf2-deficient erythrocytes are highly susceptible to stress. In addition, Nrf2-deficient erythrocytes showed increased met-Hb formation after incubation with high concentrations of H_2O_2, suggesting that Hb in Nrf2-deficient erythrocytes is more easily oxidized than that in Nrf2 WT erythrocytes (Lee et al., 2004). A unique feature of the Nrf2-ARE pathway (the programmed cell life pathway) (Li et al., 2002) is that it coordinately up-regulates many protective detoxification and antioxidant genes, which can synergistically increase the efficiency of the erythrocyte defense system against oxidative stress.

4.2.3 Nrf1 deficiency

Nrf1 knockout mice have also been reported to develop anemia in early stages of embryo, and they die in utero (Chan et al., 1998). Nrf1 knockout mice have abnormal fetal liver erythropoiesis as a result of a defect in the fetal liver microenvironment specific for erythroid cells. Anemic phenotype of Nrf1-deficient mice is not due to the oxidative stress in erythrocytes, but due to abnormal erythropoiesis.

5. Relationship between hereditary sideroblastic anemia and SOD2 deficiency

5.1 Hereditary sideroblastic anemia

Iron overload is a feature of human disorders including sideroblastic anemia (SA). Excess iron is toxic because it can catalyze the generation of ROS that damage cellular molecules. Some genetic lesions have been identified as causes of hereditary or acquired SA. As defined genetic lesions, dysfunction in one of the mitochondrial metabolic pathways has been observed: heme synthesis, iron homeostasis and transport, or electron transport. These lesions result in abnormal use of erythroid mitochondrial iron, causing pathologic iron deposition (Napier et al., 2005). Identified lesions affect nuclear-encoded mitochondrial proteins or the mitochondrial genome. The heme biosynthetic pathway was identified as a primary cause of SA. However, other pathways, including mitochondrial oxidative phosphorylation and iron–sulfur cluster biosynthesis, were also identified as primary defects in SAs. They may secondarily affect heme metabolism (Rouault & Tong, 2005; Martin, 2006). Two X-linked sideroblastic anemias (XLSAs) exist, one caused by mutations of an erythroid-specific form of the heme biosynthetic enzyme aminolevulinic acid (ALA)–synthase 2 (ALAS2) (Cotter et al., 1994), and one caused by mutation of a putative mitochondrial iron-transport protein, ATP-binding cassette, member 7 (ABC7) (Allikmets et al., 1999).

5.2 SOD2-deficiency anemia

SOD2-deficiency anemia is another example of mitochondrial dysfunction resulting in an erythroid-specific SA-like phenotype. Although genetically deficient mice of SOD1 have a normal lifespan along with exhibiting an anemic phenotype (Iuchi et al., 2007), inactivation of SOD2 results in embryonic or neonatal lethality. Because of its mitochondrial location, SOD2 is the principal defense against the toxicity of superoxide generated by oxidative

phosphorylation in mitochondria. The SOD2-deficient phenotype is associated with pathologic evidence of mitochondrial injury and oxidative damage to biomolecules, as well as severe damage to cardiac muscle. Hematopoietic stem cell-specific SOD2-deficient mice were generated by transplantation of SOD2-deficient mouse HSCs, and sideroblastic anemia-like symptom was the major phenotype in the transplanted animals (Friedman et al., 2001). This model suggests that oxidative stress in mitochondria affects the reduced heme biogenesis and accumulation of iron deposits in erythroid cells, and plays an important role in the pathogenesis of sideroblastic anemia.

It is interesting to note that peroxiredoxin II (Prx II), a member of the thioredoxin peroxidase family, was decreased in SOD2-deficient cells, but showed an increase with antioxidant treatment (Friedman et al., 2004). Knockout of Prx II causes hemolytic anemia (Lee et al., 2003) with evidence of increased oxidative damage to mature RBCs. This suggests that Prx II may be an important target of oxidative damage in SOD2-deficient cells. These findings suggest that mitochondrial dysfunction with excessive ROS production and with excess iron accumulation plays a critical role in causing SA.

6. Therapeutic supplementation of antioxidant for anemia

6.1 N-acetylcysteine (NAC) and its derivatives

N-acetylcysteine (NAC) is one of the precursors of GSH. In vitro and animal studies have demonstrated that treatment of blood cells with NAC increases the intracellular concentration of the reduced form of GSH and decreases oxidative stress (Amer et al., 2006; Nur et al., 2011). Treatment of sickle cell patients with NAC at a dose of 2,400 mg per day increased intracellular GSH and reduced dense cell formation (Pace et al., 2003). NAC is also effective for antioxidant enzyme-deficient mice. Administration of NAC (1.0% in drinking water) to SOD1-deficient mice significantly suppressed ROS in erythrocytes and partly improved the anemia (Iuchi et al., 2007).

Recently, several new derivatives of NAC have been developed. Among these new agents, the amide form of NAC, N-acetylcysteine amide (AD4), in which the carboxylic group is neutralized, is more lipophilic and has better membrane permeability than NAC. AD4 is also effective for its antioxidant effects. In vitro treatment of blood cells from β thalassemic patients with AD4 elevated the reduced glutathione (GSH) content of erythrocytes, platelets, and polymorphonuclear leukocytes, and reduced their ROS. These effects resulted in significantly reduced sensitivity of thalassemic erythrocytes to hemolysis and phagocytosis by macrophages. Intra-peritoneal injection of AD4 to β thalassemic mice reduced the parameters of oxidative stress. The superiority of AD4, compared with NAC, in reducing oxidative stress markers in thalassemic cells both in vitro and in vivo has been demonstrated (Amer, J. et al., 2008).

6.2 Vitamin E

Vitamin E, a fat-soluble antioxidant, has been identified as an essential erythropoietic factor for certain species of animals. Treatment with vitamin E increased the number of colony forming units of erythroid precursors, enhanced erythropoiesis, and thus corrected the experimentally induced anemia in an animal model. Results of some of clinical trials suggested that vitamin E might prevent some types of human anemia due to its putative role in promoting erythropoiesis, enhancing the stability of erythrocyte membrane proteins and lipids, and reducing oxidative stress-induced erythrocyte injury. Supplementation of vitamin E was tried

for patients with some types of inherited hemolytic anemia. Some of these trials have shown that there was an improvement in hemolysis, as evidenced by longer erythrocyte lifespan, in elevated hemoglobin level, and decreased reticulocyte count (Corash et al., 1980; Hafez et al., 1986). On the other hand, some groups indicated no change in hematologic status after treatment with high doses of vitamin E (Newman et al., 1979; Johnson et al., 1983).

In patients suffering from homozygous β thalassemia, supplementation of vitamin E was effective in reducing plasma levels of lipid peroxidation end products and a significant improvement in the hemoglobin levels (Das et al., 2004). A 4- to 8-week-long supplementation with vitamin E given to children with various types of thalassemia was shown to decrease H_2O_2-mediated erythrocyte hemolysis and increase the resistance to oxidative damage. Supplementation with vitamin E in children suffering from sickle cell anemia was shown to reduce the percentage of sickled erythrocytes, increased resistance of erythrocytes to lysis, and enhanced blood hemoglobin concentration (Jilani & Iqbal, 2011).

7. Conclusion

In recent years, many studies have implicated oxidative stress in anemia complicated with some infectious diseases. For example, malaria infection results in decreased antioxidant enzymes and substances such as catalase, GPx, SOD, GSH, ascorbate, and plasma tocopherol. The development of new antioxidant drugs with a function based on ROS reduction might constitute a promising tool not only for hereditary anemia but also for the control of the infection-mediated anemia.

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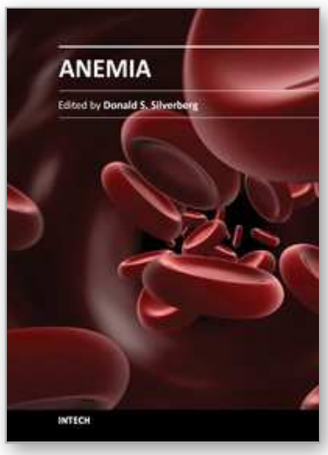

Anemia

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This book provides an up- to- date summary of many advances in our understanding of anemia, including its causes and pathogenesis, methods of diagnosis, and the morbidity and mortality associated with it. Special attention is paid to the anemia of chronic disease. Nutritional causes of anemia, especially in developing countries, are discussed. Also presented are anemias related to pregnancy, the fetus and the newborn infant. Two common infections that cause anemia in developing countries, malaria and trypanosomiasis are discussed. The genetic diseases sickle cell disease and thalassemia are reviewed as are Paroxysmal Nocturnal Hemoglobinuria, Fanconi anemia and some anemias caused by toxins. Thus this book provides a wide coverage of anemia which should be useful to those involved in many fields of anemia from basic researchers to epidemiologists to clinical practitioners.

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