Chapter

Oxidative Stress and Iron Overload in β-Thalassemia: An Overview

Nadia Maria Sposi

Abstract

In β-thalassemia, the erythropoietic process is markedly altered, and the lack or reduced synthesis of β-globin chains induces an excess of free α-globin chains within the erythroid cells. Aggregation, denaturation, and degradation of these chains lead to the formation of insoluble precipitates causing damage to the red blood cell membrane. One of the major consequences in this genetic disorder is iron overload due to ineffective erythropoiesis and premature hemolysis in the plasma and in major organs such as heart, liver, and endocrine glands. The chapter describes the etiology of iron accumulation, the role of hepcidin in regulating increased iron absorption, and the pathophysiology resulting from excess of "free iron" and discusses new ways to decrease the iron overload and to neutralize its deleterious effects in the tissues other than iron chelation.

Keywords: oxidative stress, iron overload, β-thalassemia

1. Introduction

β-thalassemias are a group of hereditary blood disorders characterized by the reduced or absent synthesis of β-globin chains representing one of the most common autosomal recessive disorders worldwide. It is prevalent in the Mediterranean countries, the Middle East, and Southeast Asia, as well as countries along the Americas, coincidental with the occurrence of malaria. Carriers of β-thalassemia genes are considered relatively protected against malaria parasite. At present, because of vast population migration and intermarriage between different ethnic groups, β-thalassemia is also common in North and South America, Northern Europa, Australia, and the Caribbean. As a consequence of the reduced or absent synthesis of β-globin chains, there is an excess on α-globin chains that are instable and precipitate in red blood cell precursors causing abnormal cell maturation and their premature destruction in the bone marrow (ineffective erythropoiesis). Red blood cells that survive to reach the peripheral circulation are prematurely destroyed in the spleen. The break down products of Hb, heme, and iron catalyze chemical reactions that generate free radicals, including reactive oxygen species (ROS), which in excess are toxic, causing damage to vital organs such as the heart and liver and the endocrine system [1]. More than 300 different point mutations cause β-thalassemia. They are inherited in a multitude of genetic combinations responsible for clinical manifestations extremely diverse, spanning a broad spectrum from the transfusion-dependent state of thalassemia major (TM) to the asymptomatic state of heterozygous carriers for β^0 or β^+ (thalassemia trait). β-thalassemia intermedia requires only periodic blood transfusion,

while β-thalassemia minor does not require a specific treatment. One of the major consequences in this genetic disorder is iron overload due to multiple blood transfusions, ineffective erythropoiesis, and premature hemolysis in the plasma. Cardiomyopathy is the most common cause of death in transfusion-dependent thalassemia patients as a consequence of iron overloading. Thanks to the significant improvement in therapy, patients with β-thalassemia may reach an advanced age. This is associated with clinical symptoms that are the consequence of the disease itself and the treatment modalities. The aim of this chapter is that to give a complete picture of current knowledge on the etiology of iron accumulation, the role of hepcidin in regulating increased iron absorption, and the pathophysiology resulting from excess of "free iron." It will also be explored whether there are ways to decrease the iron overload and to neutralize its deleterious effects in the tissues other than iron chelation (for an extensive revision, see Refs. [1–5]).

2. Iron overload in β-thalassemia

In β-thalassemia, as well as in other acquired and hereditary hemolytic anemia, iron overload is a common and serious complication and represents a major cause of morbidity and premature mortality in these patients. Hemoglobin instability, frequent blood transfusion, and increased iron absorption from the gastrointestinal tract represent the main causes of iron overload in β-thalassemia. Iron deposition occurs in visceral organs (mainly in the heart, liver, and endocrine glands), causing tissue damage and ultimately organ dysfunction and failure. Iron homeostasis depends on a coordinated regulation of molecules involved in the import of this element and those exporting it out of the cells. In particular, the iron status reflects the balance among iron uptake from the diet, its storage and mobilization, and its utilization [1]. Normally, 1–2 mg of iron is absorbed from the diet per day, with an equivalent amount lost by the turnover of gastrointestinal tract epithelial cells. In β-thalassemia and other transfusion-dependent anemias, iron overload may accumulate in relatively short time because there are no physiologically regulated means of iron excretion. Iron is essential for several vital biological processes. It regulates enzymatic activity and oxidation-reduction reactions playing a pivotal role in proliferation and cell survival. Iron ensures the transport of oxygen and the catalysis of reactions involved in electron transfer, DNA synthesis, and nitrogen fixation. However, it is also highly toxic due to its ability to react with oxygen and catalyze the production of reactive oxygen species (ROS). In solution, iron can exit in two states of oxidation, Fe (II) and Fe (III), and is very poorly soluble at physiological pH, especially when it is in the oxidized form Fe (III). Living organisms have thus developed many proteins to carry iron in biological fluids and transport it through cellular membranes and to store it in a non-toxic and easily mobilizable form [2, 6–8]. Iron is bound to transferrin in the plasma, but the iron overload in β-thalassemia patients saturates the ability of the transferrin iron transport system, leading to nontransferrin bound iron (NTBI) and labile plasma iron (LPI) starting to circulate in plasma and subsequently becoming deposited inside the susceptible cells [9, 10]. Rather than using the transferrin receptor, NTBI enters cells by nontransferrin pathways [1, 11]. Long-term uptake and accumulation of NTBI and LIP, its redox active component, lead to increate levels of storage iron and labile cellular iron [12]. Tissues susceptible to iron accumulation by this mechanism include the liver, endocrine system, and myocardium [13]. When the magnitude of the cellular LIP exceeds the capacity of the cell to synthesize new ferritin molecule, a critical concentration is reached that can generate reactive oxygen species (ROS). ROS produced by the metabolism of NTBI play a central role in inducing cellular

dysfunction, apoptosis, and necrosis [14]. A variety of ROS, most notably hydroxyl radicals, increase lipid peroxidation and organelle damage, leading to cell death and fibrogenesis mediated by transforming growth factor β-1 (TGF-beta-1) [15]. An underappreciated effect of iron overload is increased the infection risk that is a high cause of mortality in β-thalassemia patients [16]. The LIP has been suggested as a low-molecular-weight intermediate or transitory pool between extracellular iron and intracellular firmly bound iron [17]. The intracellular LIP is redox active, catalyzing the Fenton and Haber-Weiss reactions that generate ROS [18]. Excess ROS are cytotoxic through their interaction with cellular components, such as DNA, proteins, and lipids, causing damage to vital organs [19].

3. Strategies to remove iron in excess

β-thalassemia is a significant health problem in various areas of the world due to its frequency and severity. The standard treatment of β-thalassemia is currently based on transfusion therapy, iron chelation, and, in rare cases, splenectomy. This has led to an increased survival and amelioration of the quality of life, although many patients continue to be affected by cardiac disease and other clinical complications, e.g., developed endocrine failure and delayed pubertal maturation. The only approach that may lead to a definitive cure for β-thalassemia is represented by allogenic hemopoietic stem cell transplantation, but the need to control transplant-related complications and the requirement for matched donors make this option not available to most patients. Thus, the main therapeutic option for the majority of patients remains to be supportive care in the form of blood transfusion combined with chelation therapy [2]. The function of iron chelators is that to remove excess iron from the plasma and the cells by binding the labile and chelatable iron, thus facilitating its excretion through the urine and feces. Deferoxamine was the first iron chelator to be used clinically and is given by a slow, continuous, subcutaneous, overnight infusion through a portable pump. Its side effects are minimal, but its mode of administration results in low compliance [1]. Deferasirox presents several side effects [1, 2]. Neutropenia is the main potential complication of deferiprone, the first effective oral iron chelator in removing excess iron from the organs and from the heart. The use of a combination of chelators leads to an improvement in the efficacy of chelation therapy: deferiprone may mobilize iron from tissues into the circulation, while deferoxamine binds and facilitates its excretion in the urine (the "shuttle mechanism") [1]. An additional potential approach to reduce iron overload is the downregulation of transferrin receptor 1 (TfR1) by administration of exogenous iron-free (apo) transferrin. In addition to free iron, some iron-containing compounds, due to hemolysis, are elevated in the plasma of β-thalassemia patients. They are free hemin and hemoglobin and are of considerable toxicity [1, 2]. These compounds are neutralized by their scavengers: hemopexin for free hemin and haptoglobin for free hemoglobin. These proteins are reduced in β-thalassemia patients, leaving free, un-neutralized hemin, and hemoglobin. The administration of hemopexin and haptoglobin may be suggested to reduce iron toxicity.

4. Strategies to modulate iron absorption

The discovery of hepcidin has led to an important advancement in the understanding of iron metabolism. Hepcidin is a key regulator of whole body iron homeostasis originally identified from urine as an antimicrobial peptide produced in the hepatocytes [20]. Mutations in the human HAMP gene or targeted deletion of the

HAMP gene in mice result in massive iron overload [21]. Conversely, high levels of hepcidin lead to decreased iron absorption and iron-restricted anemias indicating that hepcidin is a negative regulator of iron transport into plasma. Many experimental data suggested that the hepcidin could be the regulator of iron absorption and recycling acting principally or solely by binding to ferroportin, the only known cellular iron exporter. The systemic iron homeostasis is controlled by hepcidin-ferroportin interaction: hepcidin binds to ferroportin and induces its internalization and degradation, thus regulating the distribution of iron in the body. When hepcidin concentration increases, hepcidin binds to ferroportin, causing its phosphorylation, internalization, ubiquitylation, sorting through the multivesicular body pathway, and degradation in lysosomes, and iron is retained within the cells in cytoplasmic ferritin [22–25]. The expression of hepcidin is regulated by different stimuli at the transcriptional level: hypoxia, iron deficiency, erythroid expansion, and anemia are all negative regulators of hepcidin expression, while transferrin receptor 2 (TfR2), the membrane isoform of hemojuvelin (HJV), IL-6, iron, and the hemochromatosis protein HFE are all positive regulators of hepcidin transcription [2]. In β-thalassemia, in spite of iron overload, hepcidin production is generally low, and consequently, iron absorption is high. The process of differentiation and maturation of erythroid precursors is markedly altered in β-thalassemia (ineffective erythropoiesis). An excess of free α-globin chains within the red blood cells is the consequence of the reduced or lack synthesis of β-globin chains. Aggregation, denaturation, and degradation of these chains lead to the formation of insoluble precipitates that cause oxidative membrane damage within the red blood cell and developing erythroblasts (**Figure 1A**) [26]. Ineffective erythropoiesis is accompanied by a massive iron overload, due to an increase in iron absorption by the gastrointestinal tract and to frequent blood transfusions. Nevertheless, iron overload occurs also in patients who have not received transfusions such as patients suffering from thalassemia intermedia [27, 28]. If iron was a dominant regulator, patients with β-thalassemia should express very high levels of hepcidin in serum in order to decrease intestinal iron absorption. By contrast, the levels of hepcidin are very low in these patients, suggesting that the ineffective erythropoiesis alone is able to suppress the synthesis of hepcidin in spite of the presence of a severe iron overload [25, 29–31]. Transfusions of erythrocyte partially rerelieved suppression of hepcidin, but transfusions add large amounts of exogenous iron and lead to iron overload. Hepcidin mRNA expression in the HepG2 cell line by serum from β-thalassemia patients suggested the existence of a negative erythropoietic regulator of hepcidin expression [32]. The nature of this humoral factor is still uncharacterized but may include growth differentiation factor (GDF-15), twisted gastrulation protein homolog 1 (TWSG1), soluble transferrin receptor, and erythroferrone, which are all overproduced by the proliferating erythroid precursors (**Figure 2**). Controlling absorption of iron may be beneficial to the administration of synthetic hepcidin or of agents that increase its expression. Hepcidin agonists or stimulators of hepcidin production are being developed for the treatment or prevention of iron overload in hepcidin deficiency states, including hereditary hemochromatosis and β-thalassemia [33]. The rationale for the use of hepcidin agonists is justified by two principal observations: first, the phlebotomy is an expensive and effective treatment for iron overload that is acceptable to must but not all patients affected by hereditary hemochromatosis; second, iron-loading anemias cannot be treated in this way and require iron chelation therapy, which is not well tolerate by many patients. Hepcidin agonists are agents that replace hepcidin activity or stimulate its endogenous production and, in both hereditary hemochromatosis and iron loading anemia, could prevent iron accumulation by redistributing iron from parenchymal tissues to macrophages where iron is less toxic [34, 35].

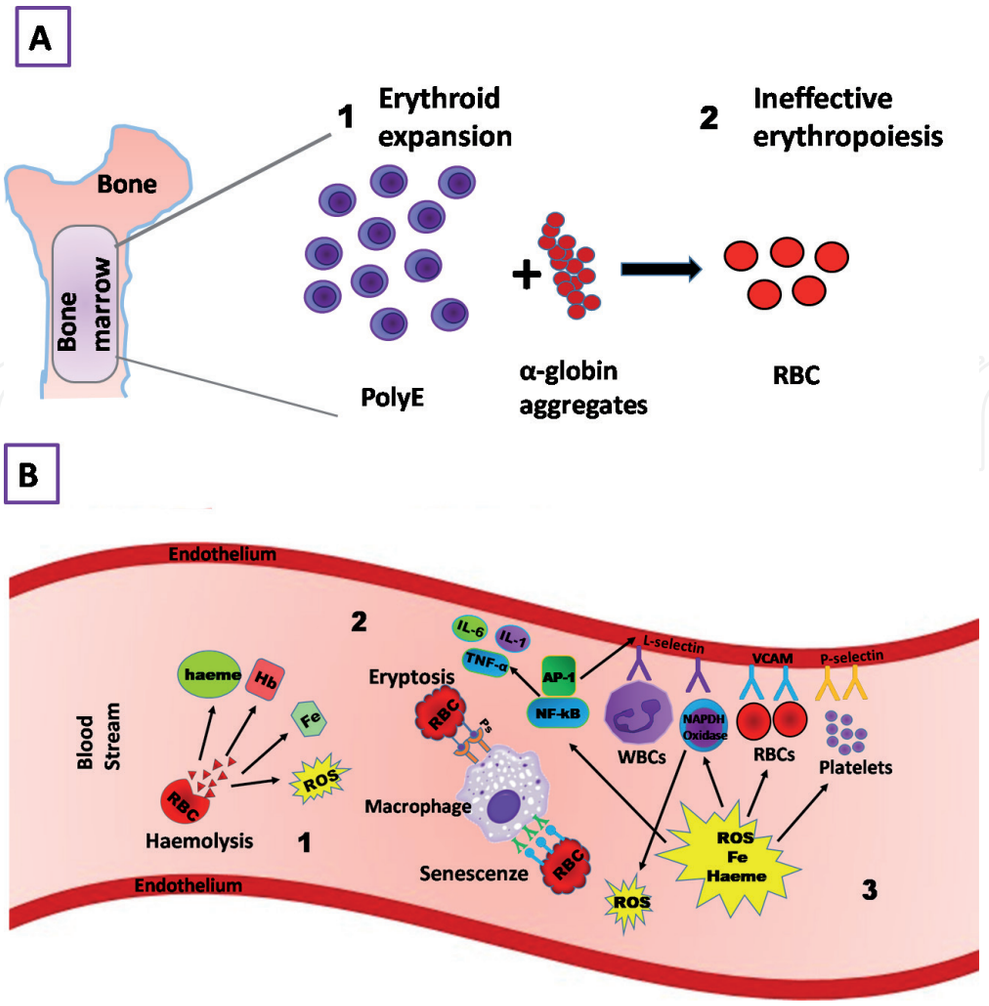

Figure 1.
(A) Erythroid expansion and ineffective erythropoiesis represent oxidative events in the bone marrow. (B). Oxidative events in the circulation: (1) hemolysis leads to hemoglobin release in the plasma. Autoxidation of free hemoglobin produces ROS, free heme, and iron; (2) eryptosis and senescence: two different mechanisms of endocytosis of red blood cells (RBCs) by macrophages; and (3) membrane oxidative damage by ROS, free heme, and iron: activation of NF-κB and AP-1 by ROS and heme increases the production of pro-inflammatory cytokines (IL-1, IL-6, and TNFα) and adhesion molecules on the endothelium. Activated leucocytes generate more ROS by their NAPDH oxidase, creating a loop of oxidative stress and inflammation.

TMPRSS6 suppression could be an alternative approach to increase hepatic synthesis of hepcidin. It is a transmembrane serine protease (matriptase-2) that normally suppresses the synthesis of hepcidin by deactivating hemojuvelin (HJV) [36]. Data showed that the deletion of TMPRSS6 gene in mouse model increased hepcidin expression resulting in anemia improvement, ineffective erythropoiesis, and splenomegaly reducing and decreased iron loading [37]. An improvement in anemia and iron overload has been showed in mice and in preclinical studies using antisense oligonucleotides or small interfering RNAs (siRNA9 decreasing TMPRSS6) [38, 39]. The somministration of exogenous transferrin, through the downregulation of TfR1, increased erythroid precursor enucleation and improved terminal erythroid differentiation and maturation in β-thalassemic mice [40, 41]. Recently, a new iron metabolism regulating factor produced in erythroblasts in response to erythropoietin, ERFE (erythroferrone), was identified. In murine models with β-thalassemia intermedia, ERFE is highly expressed and mediates hepcidin suppression and contributes to iron overload. On the contrary, a deficiency of ERFE leads to an increase in hepcidin expression, a significant reduction in iron overload, and a slight improvement of erythropoietic indices [42]. All these data indicate that the inhibition of ERFE may be a future target with therapeutic potential in diseases

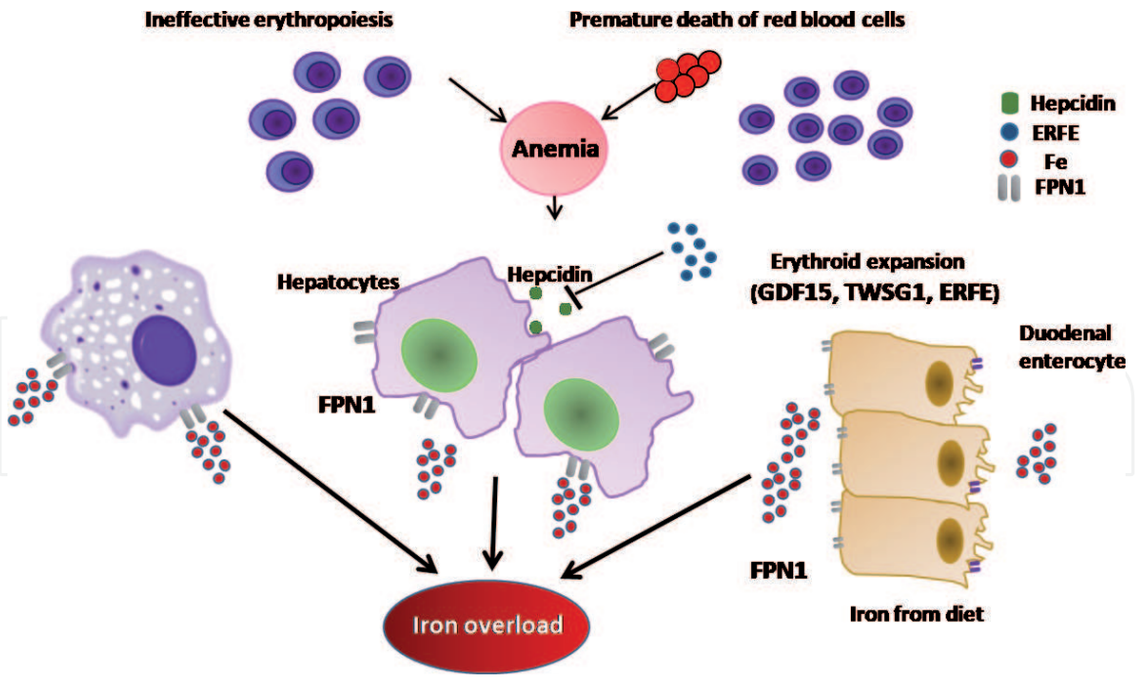

Figure 2.
Dysregulation of iron homeostasis in β-thalassemia disease. Ineffective erythropoiesis and premature death of red blood cells are the main cause of anemia in β-thalassemia patients. Erythropoietin production induced by anemia causes an increase in erythropoiesis activating secretion of erythroid factors such as GDF15, TWSG1, and ERFE. The suppression of hepcidin expression caused by an excess of eryhtroid factors leads to an increase in intestinal iron absorption, release of iron from the liver, and reticuloendothelial system. All these lead to iron overload. GDF15: Growth differentiation factor 15; TWSG1: Twisted-gastrulation 1; ERFE: Erythroferrone.

with ineffective erythropoiesis and iron overload as β-thalassemia. Agents targeting hepcidin expression are more likely to be beneficial to patients with NTDT than those with TDT because transfusional iron overload is not mediated by low hepcidin levels. However, mini-hepcidins and TMRSS6 inhibitors can be evaluated for use in patients with TDT because improvement in erythropoiesis could potentially reduce transfusion requirements [43]. All discussed novel agents merit further evaluation of efficacy and safety in both preclinical and clinical development studies.

5. Oxidative stress in β-thalassemia

Oxidative stress plays a major role in pathophysiology of β-thalassemia, although it is not the primary etiology of disease. The cell oxidative status depends on the equilibrium between oxidants and anti-oxidants. The reactive oxygen species (ROS) are oxidants produced mainly as byproducts of cellular respiration, while reduced glutathione is an example of anti-oxidants. A balance between oxidants and anti-oxidants is crucial for normal physiology. ROS are utilized from the cells as regulators in many physiological processes, including proliferation and differentiation of the erythroid precursors. Oxidative stress ensues in many pathological processes when the balance between oxidants and anti-oxidants is broken, as it occurs in β-thalassemia. Excess ROS cause cytotoxicity by binding to cell components such as DNA, proteins, and membrane lipids [19]. In β-thalassemia, the main consequence of the unstable Hb_s and iron overload is the oxidative stress. It mediates many of symptoms due to oxidative damage to red blood cells, leukocytes (recurrent infections), platelets (hypercoagulable state), as well as in heart, liver, and the endocrine glands (**Figures 1A** and **B**) [19, 44–46]. Endogenous and exogenous antioxidants may ameliorate the oxidative stress in β-thalassemia. They act scavenging and inactivating ROS and correcting their damage to cellular components. We introduce

many antioxidants by nutrition. For example, a moderate wine consumption and a "Mediterranean diet" are thought to have a protective effect due to their high contents of antioxidants [47, 48]. Antioxidants can also be taken as food additives, or as crude extracts, such as preparation of papaya fermented and curcumin, either as pure compounds such as vitamins C and E and Q10 [49]. An improvement in many parameters of oxidative stress by using such additives in β-thalassemia was observed, but a clear clinical benefit, such as reducing transfusion dependence, was less successful. A combination of drugs affecting both the oxidative stress and the iron overload can give an effective outcome. Forkhead-box-O3 (Foxo3) is a critical transcription factor that protects the cell from oxidative stress by upregulating antioxidant enzymes during early stages of erythropoiesis [50]. At early stages, Foxo3 is phosphorylated by proteins of the EPOR-P13K/AKT/mTOR signaling pathway and is translocated out of the nucleus, where it remains inactivated. At late stages, Foxo3 is relocated into the nucleus, gets activated, and induces the production of antioxidants that neutralize ROS to allow efficient erythropoiesis [1, 36, 51, 52]. In mice with β-thalassemia intermedia, downregulation of Foxo3, as a result of persistent activation of EPOR-p13K/AKT/mTOR pathway, was observed. Inactivation of Foxo3 leads to oxidative damage in late erythroblasts and plays a significant role in the process of ineffective erythropoiesis [53]. β-thalassemia patients could be beneficial in improving anemia by activation of Foxo3 as a potential inducer of HbF. However, the function of Foxo3 in hemoglobinopathies has yet to be elucidated. A remarkable improvement in erythroid cell maturation, production of β-globin chains, and anemia has been observed following the use of rapamycin, an mTOR inhibition, in mice with β-thalassemia intermedia [53]. In another study, rapamycin increased α-globin expression and HbF production in cultured erythroid precursors from patients with β-thalassemia intermedia [54, 55]. Similar findings were reported with the use of another Foxo3 activating agent, resveratrol (3,5,4′-trihydroxy-trans-stilbene), a non-flavonoid polyphenol that upregulates antioxidant enzymes in mice with β-thalassemia intermedia [56]. Metformin, an approved drug for diabetes type 2 and a Foxo3 inducer, has been investigated as an HbF inducer in an ongoing phase 1 clinical trial in patients with sickle cell anemia and nontransfusion-dependent thalassemia (NTDT; NCT02981329) [57]. All these agents are in preclinical studies and need further evaluation. Then, further laboratory and clinical investigations are required in this field. A factor required for the initiation of translation through the binding of tRNA to the ribosomes is the eukaryotic initiation factor 2 (eIF2). It is regulated by a mechanism involving phosphorylation at its α-subunit by heme-regulated eIF2a kinase (HRI) in the erythroid precursors. Stress, as heme deficiency and oxidative stress during the late stage of erythroid differentiation, activates HRI that coordinates the synthesis of heme and globin. It was demonstrated that the phosphorylated α-subunit of eIF2 turned on the activating transcription factor 4 (ATF4) to diminish oxidative stress in erythroid precursors [58–60]. A selective inhibitor of eIF2aP dephosphorylation as salubrinal augmented the HRI signaling pathway and reduced the production of hemichromes in β-thalassemia erythroid precursors [59]. In another study, salubrinal increased HbF production with a concomitant decrease of HbA in differentiating human CD34 cells by a post-transcriptional mechanism [61]. Thus, manipulation of the HRI-eIF2aP signaling pathway could represent a new approach for the treatment of β-thalassemia.

An antioxidant protein that scavenges and inactivates ROS is the peroxiredoxin-2 (Prx2), essential during erythropoiesis. The expression of this protein is upregulated both murine and human β-thalassemia indicating that the oxidative stress induces peroxiredoxin-2 as a novel cytoprotective response in β-thalassemic erythropoiesis [62, 63]. Heme oxygenase (HO-1) is an enzyme that catalyzes the degradation of heme in response to stress, such as oxidative stress or hypoxia, both

of which occur in β-thalassemia. In EPO-dependent fetal liver erythropoietic cells from β-thalassemic mice, the expression of HO-1 was augmented. The administration of tin protoporphyrin IX, an HO-1 inhibitor, improved ineffective erythropoiesis and Hb levels and decreased spleen size and liver iron [64, 65].

6. Potential role of antioxidants in β-thalassemia

Various antioxidant enzyme systems are activated by the oxidative stress to protect the body tissues from its damaging effects in β-thalassemia patients. These antioxidants include superoxide dismutase (SOD), catalase, glutathione (GSH), thioredoxin (Trx), and ferritin. Superoxide (O_2^-) is the first reactive radical produced, and this radical can be neutralized by SOD. There are three distinct SODs: SOD1 (cu/Zn-SOD) is present in cytoplasm, whereas SOD2 (Mn-SOD) is present in the mitochondria, and SOD3 is almost exclusively extracellular [66, 67]. Each of these distinct SODs performs a specific function in human cells. In β-thalassemia, major patients higher levels of erythrocyte superoxide dismutase and glutathione peroxidase (GPx) as well as higher plasma malondialdehyde (MDA) were observed as compared to healthy controls [68]. Iron overload through repeated blood transfusions and subsequent oxidative stress produced by reactive oxygen species may be the cause of increased levels of MDA. The rise in SOD and glutathione peroxidase may occur as a result of compensatory mechanisms in response to oxidative stress [44]. Neutralization of O_2^- produces H_2O_2, which can be metabolized into nontoxic products by a catalase and glutathione peroxidase (GPx) in conjunction with glutathione. Location of GPx depends on the subtype, whereas catalase is present in peroxisomes [67]. The stability of the cellular and subcellular membranes depends mainly on glutathione peroxidase, and the protective antioxidant effect of glutathione peroxidase depends on the presence of selenium. In patients with β-thalassemia, major was confirmed the peroxidative status generated by iron overload and the high increase in serum ferritin, iron, plasmatic thiobarbituric acid reactive substances (TBARS), SOD, and glutathione peroxidase activity, while the vitamin E and zinc concentration decreased in these patients [44, 69]. Glutathione (GSH) is present in nearly all cells in the body and is present in high levels in organs with high oxygen consumption and energy production, e.g., the brain [67, 70]. Glutathione, in conjunction with its oxidized form (GSSG), plays a major role in controlling cellular redox state. The ubiquitous thioredoxin system also plays an important role in maintaining the cell's redox state [67, 71]. Finally, ferritin is considered an endogenous antioxidant as it performs the important function of sequestering potentially toxic labile iron. When endogenous antioxidants are unable to neutralize oxidative stress, as in β-thalassemia, exogenous antioxidants can be used to augment the antioxidant system of the body. Iron metabolism underlies the dynamic interplay between oxidative stress and antioxidants in many pathophysiological processes. Iron overload can affect redox state, and not only this condition can be restored to physiological conditions using iron chelation, but also the addition of antioxidants to these treatment regimens can be a viable therapeutic approach for attenuating tissue damage induced by oxidative stress (**Table 1**), (**Figure 3**, [72–74]). Vitamin A (β-carotene), vitamin C, vitamin E (α-tocopherol), polyphenols, and other bioactive plant-derived compounds are effective exogenous antioxidants that also regulate iron metabolism. At the transcriptional level, antioxidant enzymes are regulated by the transcription factor Nrf2, which binds to the antioxidant response element (ARE) in the target gene's promoter region. Nrf2 is believed to be phosphorylated by protein kinase C (PKC), which causes the transcription factor to translocate to the nucleus, where it activates ARE-containing genes [67, 75], ultimately leading

Antioxidant	Mechanisms of iron regulation	Sources of antioxidants
Curcumin	• Potent flavonoid antioxidant • Iron chelator • Redox state modulator • Decreased iron levels • Attenuated lipopolysaccharide (LPS)-induced oxidative stress-related inflammation • Activated hepatic IRPs and TfR1, repressed hepatic hepcidin and ferritin synthesis	Curcumin is a bright yellow chemical produced by *Curcuma longa* plants
Quercetin	• Decreased hepatic iron levels • Reduced iron-related damage • Increased BMP6, intranuclear SMAD4, SMAD4 binding to the HAMP promoter, and hepcidin expression	Vegetables, leaves, grains, red onions, kale, red wine, and tea
Flavonoid-rich extract of orange and bergamot juice	• Decreased ROS production and membrane lipid peroxidation by iron chelation in iron-overload A549 cells and activation of antioxidant catalase enzyme	Citrus fruits
Genistein	• Reduced inflammation induced by ethanol and oxidative stress in mice • Increased HAMP promoter activity in both zebrafish and human hepatocytes via Stat3- and Smad4-dependent process	Lupin, fava beans, soy beans, kudzu, psoralea, *Maackia amurensis*, and *Flemingia vestita*
Silymarine	• Iron-chelating properties	*Silybum marianum* extract
Ferulic acid	• Decreased iron-induced oxidative stress, reduced liver injury, and ROS production • Increased hepatic antioxidant and mitochondrial membrane potential	Vegetables, popcorn, bamboo shoots, cereals (bran, wheat, and barley grain)
Resveratrol	• Reduced myocardial damage by modulating vascular cell function, low density lipoprotein (LDL) oxidation, and platelet aggregation	Skin of grapes, blueberries, raspberries, mulberries, peanuts, and red wine

Table 1.
Mechanisms of iron regulation by antioxidants.

to the neutralization of free radicals and the attenuation of oxidative damage [76]. **Table 1** summarizes the flavonoids and other antioxidants that regulate both iron homeostasis and redox state, in some cases via independent mechanisms. Flavonoids are present in a wide variety of plants and represent the most common class of polyphenols, organic chemicals that protect the plant from ultraviolet radiation, pathogens, and effects of oxidative stress, making them suitable for therapeutic purposes [77, 78]. Examples of flavonoids include quercetin, cathechins, curcumin, and kaempferol, which are abundant in fruits, vegetables, legumes, red wine, and green tea. Curcumin is a potent flavonoid antioxidant that can chelate iron in addition to modulating redox state [79]. A flavonoid-rich extract of orange and bergamot juice has been shown to chelate iron in iron-overload A549 cells and to activate the antioxidant enzyme catalase, leading to a decrease in ROS production and membrane lipid peroxidation [80]. It is a promising candidate for regulating both oxidative stress and iron homeostasis. Quercetin can reduce hepatic iron deposition in mice

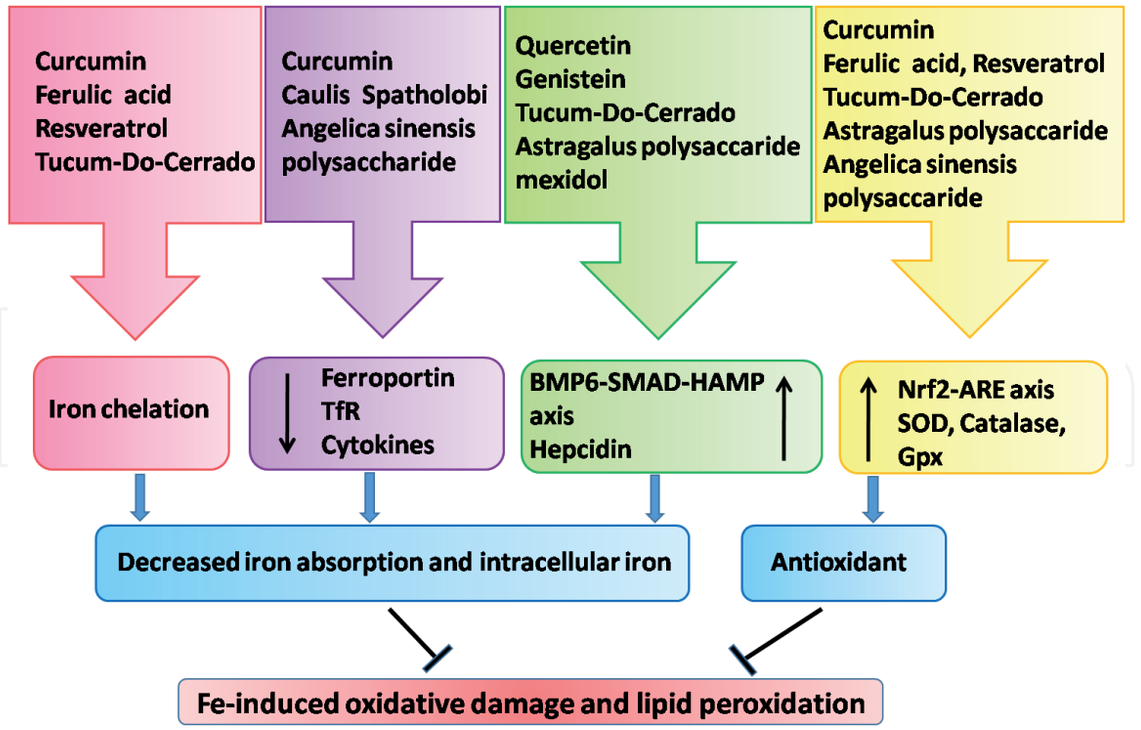

Figure 3.
Summary of the mechanisms regulating iron and oxidative stress by antioxidants. BMP6-SMAD-HAMP: Bone morphogenetic factor-mothers against decapentaplegic homolog-hepcidin antimicrobial peptide; GPx: Glutathione peroxidase;Nrf2-ARE: Nuclear factor erythroid 2-related factor 2-antioxidant response element; SOD: Superoxide dismutase.

that were exposed to either ethanol or excess iron and increase BMP6, intranuclear SMAD4, SMAD4 binding to the HAMP promoter, and hepcidin expression, leading to decreased hepatic iron levels and reduced iron-related damage [81]. Another potent antioxidant is genistein. It reduces inflammation induced by ethanol and oxidative stress in mice [82] and, similar to quercetin, increases HAMP promoter activity in both zebrafish and human hepatocytes via Stat3- and Smad4-dependent process [83]. Silymarin, another flavonoid, is present in milk thistle plant extract and may have iron-chelating properties [84]. It is safe, well tolerated, cost-effective alternative to currently available iron chelation therapies for treating patients with β-thalassemia [84]. Ferulic acid is present in a wide variety of plants, and the antioxidant effects are believed to be mediated via the neutralization of free radicals [85]. The antioxidant effects of resveratrol may prevent adverse changes that lead to cardiovascular disease by modulating vascular cell function, low density lipoprotein (LDL) oxidation, and platelet aggregation, thereby reducing myocardial damage [86, 87]. Both vitamin A and vitamin C have well-established antioxidant properties that are mediated via the attenuation of oxidative damage [88]. Vitamin A and β-carotene increase hepcidin and TfR expression and intestinal iron absorption, reduce inflammatory signaling and ferroportin expression, increase intracellular ferritin levels, and release intracellular trapped iron [89–91]. Vitamin C reduces Fe^{3+} to Fe^{2+} and inhibits hepcidin expression [92]. In recent years, research for new therapies based on plant-derived compounds has developed considerably. This is to maximize the benefits of plant phytochemicals and avoid the adverse effects often associated with synthetic pharmaceutical agents [93]. Several plant extracts, such as tucum-do-cerrado, astragalus, *Angelica sinensis*, *Caulis Spatholobi*, *Scutellaria baicalensis*, and others, have been studied for their putative effects on iron homeostasis and oxidative stress. The results obtained are very promising (for esaustive review, see Ref. [76]).

7. Conclusions

Alteration in iron homeostasis is associated with oxidative stress and inflammation. Many bioactive antioxidants and plant-derived phytochemicals can regulate iron homeostasis, inflammation, and oxidative stress. Nevertheless, the majority of data collected to date are derived from in vitro and animal experiments, and further studies are needed in order to evaluate the efficacy of these phytochemicals as a natural substitute for pharmaceutical agents. This is very important because many pharmaceutical agents are associated with adverse side effects.

Author details

Nadia Maria Sposi
Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Rome, Italy

*Address all correspondence to: nadia.sposi@iss.it

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