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Chapter 10

ROLE OF ANTIOXIDANT AND IRON CHELATING THERAPIES IN THALASSEMIA PATIENTS

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ABSTRACT

Thalassemia could be regarded as an "oxidative stress" disorder because its pathogenesis inevitably contributes to anemia and iron overload conditions, typically leading to oxidative damage of cells and organs in the patients. Over decades, alleviation strategies of iron-induced oxidative stress, either by antioxidant supplementation or iron chelation, have been extensively studied. Although antioxidants are not able to increase hemoglobin concentration, they exert preventive effects by direct scavenging of free radicals, modulating gene expression of proteins involved in oxidative cascade, up-regulating the activities of cellular antioxidant enzymes, and chelating metal ions. The iron toxicity is mainly caused by non-transferrin bound iron (NTBI) formed after transferrin saturation which is highly reactive and uncontrollable uptake into cells. During iron-chelating programs, the decreased levels of serum ferritin and NTBI, and the clearance of iron loaded in various organs such as heart, liver, and endocrine glands have been reported with variable degree of efficacy and side effects. In this article, we summarize the central role of oxidative stress in thalassemia pathogenesis and also discuss the therapeutic effects of potential antioxidants such as vitamin E, curcuminoids, catechins, and coenzyme Q10 based on emerging data from several animal studies and clinical trials. Furthermore, the importance of iron chelators is also highlighted. Finally, the concept of combined therapies between antioxidants and iron chelators has been evaluated with the goal to reduce side effects and increase treatment efficacy, which results in improved quality of life and increased life expectancy in the iron-overloaded thalassemia patients.

INTRODUCTION

Thalassemia is a group of inherited anemia caused by mutations in globin genes resulting in quantitative defect of the corresponding subunits followed by insufficient hemoglobin (Hb) synthesis. Generally, thalassemia is named according to 2 major systems: (1) clinical severity: classified as thalassemia minor *asymptomatic to mild anemia*, thalassemia intermedia *transfusion-independent*, and thalassemia major *transfusion-dependent*; and (2) genotype: classified as α- and β-thalassemia which are characterized by the mutations occurred in α- and β-globin genes, respectively. Frequently, thalassemic allele combines with other structural Hb variants (qualitative defect) resulting in genotypic diversity. Moreover, thalassemic patients also show a remarkable phenotypic heterogeneity despite having the same genotype [1].

The etiology of thalassemia is the imbalanced synthesis of α- and β globins. The excess unpaired globins in hematopoietic cells develop anemia and iron overload, which are the characteristics of thalassemia (pathophysiological changes are discussed later). In the presence of insufficient antioxidant defense, iron-catalyzed reactive oxygen species (ROS) cause oxidative damage to cellular biomolecules and eventually lead to organ dysfunction, which is associated with morbidity and mortality of thalassemic patients. Therefore, administration of antioxidants and iron chelators have been proposed to be potentially supportive therapies in diseases associated with iron overload and oxidative stress such as thalassemia. This chapter discusses the role of oxidative stress in pathophysiology of thalassemia, and the protective role of antioxidants and iron chelators in the patients. Also, we summarize information obtained from the key clinical studies of both antioxidants and iron chelators.

PATHOPHYSIOLOGY OF THALASSEMIA

The pathophysiology of thalassemia is summarized in Figure 1. The primary iron overload is developed since excess unpaired globins are denatured, aggregated, and dissociated to form intraerythrocytic iron species including hemichrome, Heinz bodies, inclusion bodies, free heme, and free iron [2]. A great amount of ROS are formed by Fenton reaction, accumulate, and oxidize intracellular biomolecules in mature red blood cells (RBC) and erythroid progenitor cells resulting in accelerated hemolysis and ineffective erythropoiesis, the main causes of anemia state in thalassemia.

Accelerated hemolysis is predisposed by oxidative abnormality of RBC membrane proteins and phospholipids. Partially oxidized band 4.1 protein affects the generation of spectrin-actin-band 4.1 skeleton complexes with decreased 50% efficiency [3], whereas oxidized band 3 proteins are recognized as "neo-antigen" activating complement-mediated phagocytosis by macrophages in spleen and bone marrow [4]. Oxidative membrane also increases intracellular Ca^{2+} and several enzyme activities are regulated by calcium concentration inside cells. Increased intracellular Ca^{2+} activates scramblase and inhibits translocase resulting in exposure of PS to RBC outer leaflet, recognized by specific receptors on macrophages' membrane as a signal for phagocytosis. Calcium also activates calpain enzyme facilitating membrane blebbing and RBC vesiculation [5].

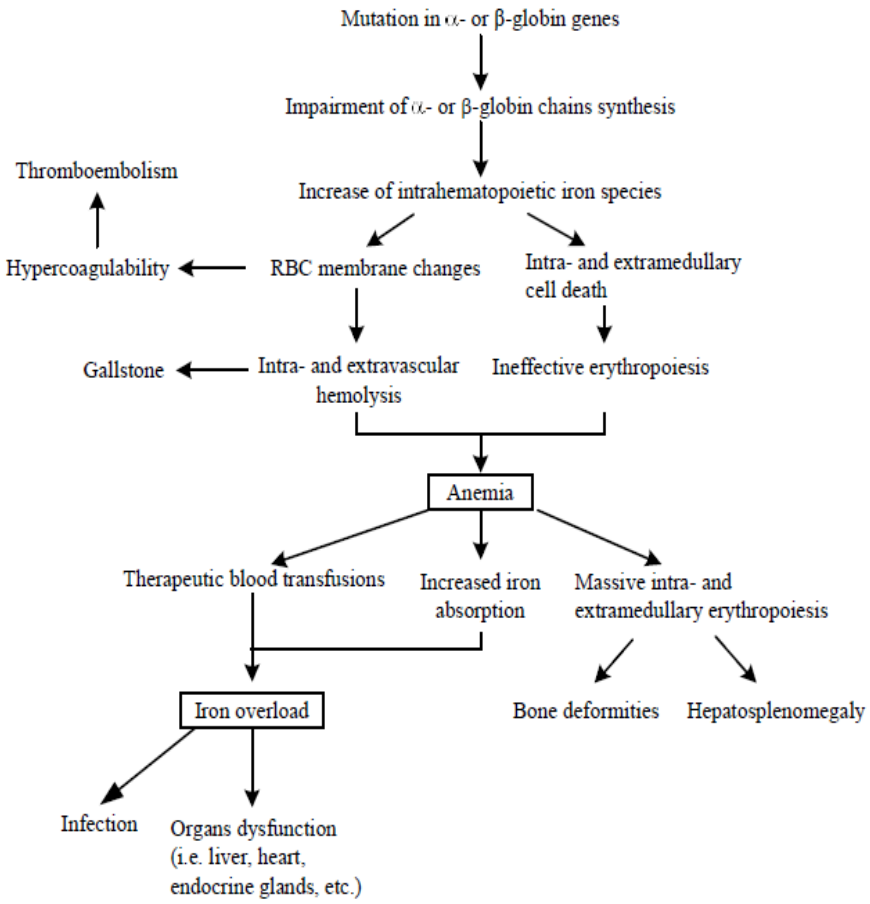

Figure 1. The pathophysiology of thalassemia.

The significant increase of PS-positive RBC and vesicles in thalassemia patients may play an important role in hypercoagulability by several mechanisms including triggering platelet activation [6], elevating activities of coagulation factors [5], enhancing self-aggregation of RBC and vesicles [7], and promoting RBC-endothelial adherence [8].

Ineffective erythropoiesis in thalassemia is characterized by high levels of either erythroid precursors or their apoptotic cells at the polychromatophilic stage [9]. The bone marrow of thalassemic patients had 5-6 folds erythroid precursors and 15 folds apoptotic cells levels higher than that of normal subjects [10]. The exact mechanism is still unclear, but it has been proposed that the deposition of unpaired globins and free iron species may be responsible for enhanced apoptosis since the degree of ineffective erythropoiesis correlates well with α/β globins ratio [11]. The possible mechanisms of apoptosis reported so far include extracellular PS exposure [2], Fas-Fas ligand interaction [12], the unfolded protein response pathway [13], and autophagy [14]. The knowledge of erythropoiesis is still limited and needs to be further investigated especially the uncharacterized factors that control erythropoiesis. This may lead to novel strategies improving ineffective erythropoiesis in thalassemia.

Profound anemia is accompanied with high risk of gallstones (hyperbilirubinemia) [15], massive erythropoiesis, and secondary iron overload. As a compensatory response to anemia, the erythropoietin (EPO) level is dramatically increased and drives massive erythropoiesis either within bone marrow or extramedullary sites, resulting in bone deformities and hepatosplenomegaly [2]. The skeleton is changed, particularly facial bone known as "thalassemic face", and metabolic bone diseases such as osteopenia, osteomalacia, and osteoporosis could develop [16, 17]. Splenomegaly can accelerate RBC destruction and splenectomy is a standard treatment in those cases. Although the anemia state is improved in splenectomized patients, they may suffer from new set of complications such as postsplenectomy infection and thrombosis [18].

Thalassemic patients are subjected to secondary iron overload by increased gastro-intestinal iron absorption and blood transfusions. Once the iron influx exceeds the binding capacity of transferrin, it forms non-transferrin bound iron (NTBI), labile plasma iron (in plasma, LPI), and labile iron pool (intracellular, LIP) [19]. Uncontrolled uptake of NTBI and LPI causes oxidative damage to organs (especially heart, liver, and endocrine glands). These adversely affect organ functions and cause serious complications including hepatic or renal diseases, cardiac dysfunction, severe infection, diabetes, arthropathy, hypothyroidism, hypogonadism, and other endocrine disorders [20].

Oxidative Stress in Thalassemia

Oxidative stress occurs when pro-oxidants surpass the capacity of antioxidant defense. The pro-oxidants in thalassemic RBC are ROS produced by iron-catalyzed reactions including superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). These oxidants, particularly $OH^{\bullet}$, can damage cells by interacting with lipids, proteins, and nucleic acids. Direct ROS determination using flow cytometry was documented and thalassemic RBC showed significantly higher ROS levels than in normal subjects [21, 22]. ROS is highly unstable and the flow cytometry data express ROS level in arbitrary relative units (fluorescence intensity); therefore, various studies evaluate the degree of oxidative stress by detecting the levels of oxidative products instead.

So far, several oxidative products have been generally accepted as potential biomarkers of oxidative stress. The products include malondialdehyde (MDA) [23], protein carbonyl groups [24], and 8-hydroxy-2'-deoxyguanosine (8OHdG) [25]. Patients with thalassemia suffer from oxidative stress shown as significantly higher levels of MDA [26], F2-isoprostanes [27], protein carbonyl groups [28], ortho- or meta-tyrosine [29], and DNA adducts [30].

To protect cells from ROS-induced oxidative damage, cellular antioxidant defense consisting of enzymes and non-enzymes interact as a network. In normal conditions, superoxide anions from the electron transport chain are converted to H_2O_2 catalyzed by superoxide dismutase (SOD), and then H_2O_2 is further converted to water and oxygen by catalase (only in peroxisome) or glutathione peroxidase (GPX) with the expense of reduced glutathione (GSH), which in turn is oxidized to glutathione disulfide (GSSG) and recycled to its reduced form by glutathione reductase (GR). Oxidative metabolites including peroxides are conjugated with GSH by glutathione S-transferase (GST) and excreted from the body.

In thalassemia, excess iron from both uncontrolled uptake of plasma iron (NTBI, LPI) and unpaired globins generates $OH^{\bullet}$ from H_2O_2 by two sequential reactions:

(1) $Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$
(2) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$
Net reaction: $O_2^{\bullet-} + H_2O_2 \rightarrow OH^{\bullet} + OH^- + O_2$

Increased ROS production shifts the pro-oxidant/antioxidant balance in favor of an oxidative state. In response to the altered redox state, several antioxidant and detoxifying enzymes such as SOD, GPX, GST, glutamate-cysteine ligase, thioredoxin reductase, and metallothionein are upregulated [31]. Our previous study showed significantly increased SOD and GPX activities accompanied with decreased GSH levels in red blood cells of patients with β-thalassemia/Hb E [26], and similar results were found in other thalassemia types [32-34]. The activities of GST and glutamate-cysteine ligase (the rate-limiting enzyme in GSH synthesis) in thalassemic RBC were approximately 6-fold and 2-fold higher than normal control, respectively [34, 35].

Dietary antioxidants including micronutrients and plant-derived polyphenols also participate in cellular redox maintenance. In thalassemia, antioxidant micronutrients (i.e. vitamin A, C, E, carotenoids, zinc, selenium, and copper) rapidly neutralize the intracellular ROS pool resulting in dramatically decreased circulation levels [36, 37]. Therefore, antioxidant supplementation is recommended to increase body antioxidant capacity.

Iron Overload in Thalassemia

Patients with thalassemia major develop severe anemia and require regular blood transfusions. Therefore, the main source of iron overload is iron recycled from the massive destruction of the patient's own or transfused RBC. According to Angelucci formula [38], only 40 units of transfusion (200 mL blood per unit, 0.5 mg iron/mL blood) are required to reach the threshold of iron-induced organ dysfunction (7 mg iron/g dry liver weight). Whereas, the source of loaded iron in thalassemia intermedia is diet [39]. The rate of iron absorption in patients with thalassemia intermedia is approximately 3-4 times higher than normal subjects [40]. Iron gradually accumulates and the patients develop clinical symptoms of iron overload after the third decade of life.

To compensate for anemia, erythropoiesis rate is elevated resulting in greatly increased iron demand for Hb production. Generally, iron is supplied from dietary, senescent RBC recycle, and iron store which all are regulated by hepcidin, a peptide hormone synthesized in the liver and secreted into circulation. Hepcidin regulates extracellular iron concentration by binding to ferroportin (the only known iron-export channel expressed on the cell membrane of duodenal enterocytes, splenic macrophages, and hepatocytes); followed by inducing internalization, degradation, and reducing iron efflux [41]. Upon reaching iron demand, hepcidin expression returns to its basal level.

Ineffective erythropoiesis does not reach the optimal level of mature RBC from their immature pool. Therefore, the circulating hepcidin level is always low in thalassemic patients resulting in increased iron efflux followed by transferrin saturation, NTBI formation, and iron accumulation in organs. An *ex vivo* study identified growth differentiation factor 15 (GDF15)

and twisted gastrulation protein-1 (TWSG1) as erythroblast-derived hepcidin suppressors in thalassemic model [42]. Overexpression of TWSG1 and GDF15 in thalassemic models was reported with unknown mechanism. However, GDF15 and TWSG1 may cooperatively work in hepcidin suppression which the possible mechanism had been proposed by Tanno *et al.* [43]. Increased hepcidin expression could be a strategy to reduce iron loading in thalassemia.

Among thalassemic complications, iron-induced cardiac diseases account for more than 70% of deaths [44]. Recently, cardiac iron is clinically assessed using Magnetic Resonance Imaging (MRI) by measuring myocardial T2* parameter (normal, >20 ms; mild/moderate iron load, 8 to 20 ms; severe iron load, <8 ms) [45], and this parameter is related to left ventricular dysfunction [46]. In practice, the MRI technique is difficult to access due to its high diagnostic costs, expensive equipment and maintenance, and limited technologists in developing countries. The challenge is to discover biomarkers indicating precise cardiac iron loading from non-invasive specimens and easily accessed at acceptable cost. This will help clinicians to earlier monitor and better manage the treatment of thalassemic patients with iron-induced heart disease.

Table 1. Mechanisms underlying antioxidant effect of dietary antioxidants

Dietary antioxidants	Mechanisms	*References*
Curcuminoids	Free radicals scavenging via β-diketone group and hydroxyl/methoxyl groups on phenyl rings	[50]
	Upregulation of transcriptional factor Nrf2, resulting in elevated expression of glutamate-cysteine ligase (the rate-limiting enzyme of GSH synthesis) and antioxidant enzymes	[51]
	Iron chelating via β-diketone moiety	[54]
	Modulating expression of iron-regulatory proteins, thereby decreasing the translation of ferritin, increasing the degradation of transferrin receptor 1 mRNA, and suppressing hepcidin expression	[54]
Catechins	Free radicals scavenging via tri-hydroxyl groups on the B ring and galloyl moiety on the C ring	[66]
	Iron chelating via hydroxyl and gallate groups on the B ring and C ring, respectively	[68]
	Downregulation of pro-oxidant enzymes such as inducible nitric oxide synthase (iNOS) and xanthine oxidase	[64]
	Upregulation of antioxidant enzymes such as SOD, catalase, and GPX	[64]
	Regeneration of vitamin E	[67]
Vitamin E	Free radicals scavenging and inhibiting chain reactions of lipid peroxidation via hydroxyl group	[73]

ANTIOXIDANT SUPPLEMENTATION

Antioxidants are molecules that can inhibit the oxidation processes of other molecules. Patients with thalassemia inevitably face high oxidative stress with low or depleted antioxidant micronutrients [47]. Antioxidant supplementation at appropriate doses could neutralize free radicals and reduce their oxidative consequences. Here we summarize the mechanism underlying the antioxidant effect of dietary antioxidants (Table 1) and the studies conducted in thalassemic models.

Curcumin

Curcumin (*Curcuma longa* Linn) is a natural herb mainly cultivated in India, China, Southeast Asia, and other tropical countries. Curcuminoids are the active polyphenols extracted from dried rhizomes and consist of curcumin (~77%), demethoxycurcumin (~17%), and bisdemethoxycurcumin (~3%) [48]. The therapeutic activities of curcuminoids include antioxidant, anti-inflammatory, antitumor, antirheumatic, hypoglycemia, antiamyloid, and anti-ischemic [49].

Curcuminoids decrease ROS levels by direct donating electron to superoxide anion and hydroxyl radical which their radical-scavenging activity is derived from the β-diketone group and the hydroxyl/methoxyl groups on phenyl rings [50]. Moreover, curcuminoids increase GSH synthesis by upregulating transcriptional factor Nrf2, facilitating Nrf2 translocation to nucleus, and stimulating the expression of Nrf2/antioxidant response element (ARE) target genes including glutamate-cysteine ligase (the rate-limiting enzyme of GSH synthesis) and antioxidant enzymes [51]. Moreover, a recent study demonstrated that increased expression of fetal hemoglobin (HbF) can be induced by the Nrf2/ARE signaling pathway in K562 cell line [52], thus curcuminoids may be a potential HbF inducer.

Our previous study showed that curcuminoids can alleviate oxidative stress in thalassemic patients [26]. The patients were administered 500 mg of curcuminoids daily for 12 months, antioxidants and oxidative stress parameters were improved as shown by decreased RBC MDA and increased GSH levels. Increased GSH and decreased GSSG levels alter the cellular redox state resulting in decreased activities of redox-sensitive antioxidant enzymes (SOD and GPX), relative to their baseline values. Although serum ferritin levels were not changed, the levels of serum NTBI were significantly decreased.

Curcumin is a bidentate chelator which the formation of Fe^{3+}-curcumin complex occurs via β-diketo moiety [53]. It shows a moderate iron chelating property with pFe^{3+} = 16.6 (negative logarithm value of free Fe^{3+} concentration at pH 7.4, 10 μM ligand, and 1 μM Fe^{3+}; the higher the value, the higher the iron-chelating activity) [54]; relative to deferiprone (pFe^{3+} = 20) [55], and deferoxamine (pFe^{3+} = 26) [56]. An *in vitro* study showed the synergistic effect of curcuminoids and deferiprone (DFP) on NTBI chelation in thalassemic plasma in dose- and time-dependent manner [57]. *In vivo* study, plasma NTBI and lipid peroxidation were significantly decreased in thalassemic mice after co-administration of curcuminoids (200 mg/kg/day) and DFP (50 mg/kg/day) for 2 months [53]. Curcuminoids also showed the removal effect of iron accumulated in hepatocytes [53] and cardiomyocytes [58] in thalassemic mice. Besides the direct iron chelating property, curcuminoids showed another

mechanism of iron regulation by inactivating the activities of iron-regulatory proteins in iron-overloaded mice, thereby decreasing ferritin translation, increasing the degradation of transferrin receptor 1 mRNA, and interestingly, suppressing hepcidin expression [54].

The major limitation of curcuminoids is their low solubility in water resulting in low bioavailability, as shown by extremely low level (0.006±0.005 μg/mL) found in human serum at 1 hour after a single dose of 2 g curcumin [59]. There are a number of studies aiming to improve the bioavailability of curcuminoids such as liposome encapsulation [60], concomitant supplementation with piperine (inhibitor of hepatic and intestinal glucuronidation) [59], and a self-emulsifying drug delivery system [61]. Enhanced bioavailability requires further investigation of the optimal dose since curcuminoids have been found to exert pro-oxidant activities at a high concentration [62]. The effect of curcuminoids in combination with other antioxidants has not been investigated in thalassemia; although both curcuminoids and vitamin E synergistically decreased lipid peroxidation and protein carbonylation in hypothyroid rats [63]. Thus, exploration of the combined therapy of curcuminoids and other antioxidants/iron chelators is encouraged.

Green Tea

Green tea is derived from tea (*Camellia sinensis*) consisting of catechins as the most abundant flavonoids (80-90% of total), which may mainly contribute to the beneficial effects of green tea. The backbone of catechin is one of the benzene rings (the A ring) condensed with a *dihydropyran* heterocycle (the C ring) which carry a hydroxyl group on carbon 3 and another benzene ring (the B ring) on carbon 2. All principal catechins in green tea extract including (-)-epigallocatechin-3-gallate (EGCG, 48-55%), (-)-epigallocatechin (EGC, 9-12%), (-)-epicatechin gallate (ECG, 9-12%), and (-)-epicatechin (EC, 5-7%) [64]; contain multiple hydroxyl groups contributing to the antioxidant effect of green tea. Besides antioxidant, tea catechins also possess anti-inflammatory, anti-hypertensive, anti-microbial, anti-diabetic, and anti-cancer activities [64].

Catechins directly scavenge various ROS and reactive nitrogen species including the superoxide anion, singlet oxygen, hydroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite [65]. EGCG is the most efficient radical scavenger relative to other catechins since it has more hydroxyl groups (3', 4', and additional 5' position on the B ring) and a galloyl moiety on the C ring [66], and these structures also contribute to metal chelation including Fe^{3+}. Catechins can down-regulate pro-oxidant enzymes (i.e. inducible nitric oxide synthase and xanthine oxidase), and up-regulate antioxidant enzymes (i.e. SOD, catalase, and GPX) [64]. Moreover, an *in vitro* study showed the ability of catechins to regenerate vitamin E during oxidation of human low-density lipoproteins [67].

Green tea extracted by microwave heating contained high amount of catechins (particularly EGCG) and showed dose- and time-dependent removal of plasma NTBI resulting in significantly decreased plasma MDA and RBC oxidative stress parameters in *in vitro* studies [68]. Similar results were also found in iron-overloaded mice [69] and thalassemic mice [70]. Their bioavailability in humans is low with the plasma concentration of catechins only 0.2%-2.0% of the oral dose at 90 minutes and not detectable at 24 hours after administration [71]. Clinical studies with thalassemic patients are needed to evaluate whether this amount is sufficient to exert an antioxidant effect. Previous study showed high

instability of EGCG at intestinal pH (pH 8.5) which can be improved in the presence of ascorbic acid, quercetin, and selenium [72]. Thus, frequent consumption of tea alone or with a dietary source of ascorbic acid, quercetin, and selenium may increase their bioavailability. The optimal daily dose of tea should be investigated because the pro-oxidant effect of catechins was observed at >10 μM/L concentration [64].

Vitamin E

Vitamin E is an important hydrophobic antioxidant which is well-documented in preventing lipid peroxidation and oxidation of plasma lipoproteins. The hydroxyl group of vitamin E neutralizes lipid peroxyl radicals (LOO•) to yield lipid hydroperoxide and vitamin E radicals (Vit E-O•) which can be reduced by vitamin C or other reducing agents (i.e. retinol, ubiquinol) [73]. Thus, a vitamin C supplement is needed to restore vitamin E in its reduced form.

In thalassemia, depletion of antioxidant micronutrients including vitamin E in RBC and plasma is well-documented and several studies indicated its protective role in the oxidation of fatty acids in the RBC membrane, thereby leading to reducing oxidative hemolysis. Administration of vitamin E to both splenectomized and nonsplenectomized β-thalassemia intermedia for 3 months significantly reduced serum MDA and ROS levels in RBC, lymphocytes, and polymorphonuclear neutrophils (PMN), whereas GSH levels and platelet reactivities were improved in splenectomized patients [21, 74, 75].

However, vitamin E supplementation did not increase hemoglobin level in patients. Pharmacokinetics of vitamin E is still controversial with highly variable degrees of absorption in individuals [73], which causes difficulty in estimating an effective dose in clinical studies and may lead to vitamin E toxicity including bleeding disorders.

Other Antioxidants

Other antioxidants treated in thalassemic models in the past decade are briefly reported here. An *in vitro* study showed the antioxidant role of erythropoietin by increased GSH, decreased ROS and lipid peroxidation, and declined PS exposure on thalassemic RBC membrane resulting in reduced hemolysis and phagocytosis [76].

N-acetylcysteine amide (AD4) showed superior antioxidant effects over its precursor, N-acetylcysteine (NAC) [77]. In *In vitro* model, thalassemic RBC and platelets treated with AD4 significantly increased GSH and decreased ROS levels, similar to the *in vivo* study of thalassemic mice injected with AD4 at 150 mg/kg.

Coenzyme Q10 (CoQ10) is self-synthesized hydrophobic antioxidant and it intervenes initiation and propagation steps of lipid peroxidation. Our previous study showed extremely low levels of CoQ10 in patients with β-thalassemia/Hb E; supplementation with daily 100 mg CoQ10 for 6 months significantly increased CoQ10 levels in plasma, decreased RBC MDA, and decreased activities of antioxidant enzymes, implying improved oxidative stress in patients [78].

Additionally, a number of antioxidants such as mangiferin [79], indicaxanthin [80], and fermented papaya [81] were also evaluated for their activities in thalassemic models.

Unfortunately, none of these supplements was found to completely neutralize excess ROS and improve hematological parameters especially hemoglobin concentration. Since iron overload is responsible for massive ROS generation, iron chelation may represent a preventive strategy against high oxidative stress in thalassemia.

IRON CHELATION

Typically, iron chelation is established in multi-transfused thalassemic patients within the first 2-3 years of transfusion or when serum ferritin is more than 1000 μg/L in order to significantly increase the life expectancy of patients [82]. It is more beneficial for patients if iron chelation can be started before iron accumulation in tissues since the iron present in the circulation is easier to remove than in tissues, particularly in heart. Cardiac iron is much more difficult and slower to remove compared to circulating iron and hepatic iron [83]. Furthermore, plasma NTBI and labile iron rapidly rebound after stopping chelation therapy [82], thus "stop-start" behavior is undesirable.

Currently, there are 3 iron chelators clinically used for the treatment of diseases associated with iron overload: deferoxamine, deferiprone, and deferasirox. Hundreds of references regarding iron chelating in thalassemia have been listed on PubMed. Information of each chelating agent and their significant findings are summarized.

Deferoxamine

Deferoxamine (DFO) has been a commercial iron chelator since the late 1970s. DFO is a hexadentate iron chelator (1:1 binding molar ratio) in which the iron-DFO complex is highly stable and secreted in urine, thus minimizing side effects for long-term use. The most common side effects are skin reactions (pain or swelling) at injection site; whereas ocular and auditory disturbance, and skeletal changes are likely to be developed in patients who take high doses for a long time [82].

For standard dosing, DFO is prescribed to be subcutaneously infused with a portable pump continuously for 8-12 hours at night, 5-7 days per week at 20-60 mg/kg/day dose [84]. DFO has a short half-life (20-30 minutes) with steady-state concentration in plasma less than 10 μM and its level is undetectable within 2-3 minutes after stopping infusion [82]. Since its molecule is high molecular weight and highly hydrophilic, this prolongs DFO uptake into most cell types. Two-third of patients (65%) with thalassemia major who were maintained on DFO chelation showed myocardial siderosis (T2* < 20 ms) of which 13% were severe cases (T2* < 8 ms) [46]. Therefore, only a few studies of DFO monotherapy were performed in the past 5 years.

Deferiprone

Deferiprone (DFP) is a bidentate iron chelator (3:1 binding molar ratio). It is a small hydrophobic molecule which can be easily absorbed. This oral chelator has intermediate

plasma half-life (2-3 hours), so it is usually given at 25 mg/kg, 3 times daily (total 75 mg/kg/day) [85]. Pharmacokinetic studies showed peaks of plasma DFP concentration within 1 hour after oral administration and then, the liver rapidly inactivated DFP by glucuronidation which was further excreted by the kidneys (mean 0.53 mg/kg/day from daily dose of 75 mg/kg) [86]. The common side effects of DFP are gastrointestinal symptoms (nausea, vomiting, or abdominal pain) and arthralgia [82].

A 3-year prospective trial showed the efficiency of DFP (75 mg/kg daily, orally taken every 8 hours) on treatment of 21 patients with thalassemia major, 42% decreased liver iron concentration (LIC, $p<0.005$) and 40% decreased serum ferritin levels [87]. After an additional 4.6 years of treatment with the same dose, the progression of liver fibrosis was detected from liver biopsy in 78% of patients, although their LIC decreased only 26% from the beginning of treatment. Their final serum ferritin was 2830±491 µg/L which is higher than the threshold (>2500 µg/L) that iron-overloaded patients increase risk of cardiac disease. No significant changes of hematological parameters were observed during 7.5 years of treatment [88]. Whereas, there was little or no change of the amount of liver fibrosis in a 7-year trial of DFO chelation in iron-overloaded patients with homozygous thalassemia [89]. The rationale behind this may be the difference of iron-binding stoichiometry. Three molecules of DFP are required to bind 1 atom of iron, whereas DFO binds to iron only at 1:1 ratio to form ferroxamine which is highly inert. Under suboptimal concentration of DFP, iron binds partially to DFP and unoccupied binding sites are able to increase ROS production which may be responsible not only for hepatic fibrosis, but also the toxicity in RBC and myocytes [88].

For cardiac improvement, DFP monotherapy showed superior ability to reduce cardiac iron and improve cardiac function by increasing either left- or right ventricular ejection fraction in prospective randomized trials [90, 91]. In combination with DFO, DFP acts synergistically to enhance NTBI chelation by trapping and transferring iron from tissues into the circulation, donating iron to DFO to form ferroxamine, and facilitating urinary excretion [92]. Recently, combined therapy between DFP and DFO has been extensively studied in various clinical trials with the expectation of greater efficiency and minimizing the frequency of subcutaneous DFO infusion. Compared to DFO monotherapy, DFO and DFP treatment for one year significantly removed cardiac iron and improved LVEF in thalassemic patients with mild-moderate myocardial iron loading (myocardial T2* 8-20 ms) [46], and severe myocardial iron loading (T2* < 8 ms) [83]. The improvements of impaired heart function were also supported by other trials in Greece [93] and Italy [15].

Deferasirox

Deferasirox (DFX) is an oral chelator approved by the US Food and Drug Administration (FDA) in 2005 for treatment of iron overload. DFX is a tridentate iron chelator (2:1 binding molar ratio) with intermediate iron-chelating capacity between DFO and DFP. For administration, it is provided as a suspension with the standard dose at 20-40 mg/kg/day only once daily (long plasma half-life of 11-16 hours) [85]. The common side effects of DFX are skin rashes, gastrointestinal symptoms, diarrhea, and mild abnormality of hepatic and renal function [85]. However, continuous monitoring is highly recommended since severe side

effects including severe hepatic and renal dysfunction, and peptic ulcers may be developed in long-term treatment.

So far, the ESCALATOR and EPIC trials have been the two largest clinical trials of DFX monotherapy; especially more than 1000 thalassemic patients were recruited in the latter study. Their experimental results suggested the DFX dose ≥ 30 mg/kg/day as the optimum dose to treat heavily iron-overloaded patients, supported by significantly decreased liver iron and serum ferritin [94-96]. Moreover, this dose also showed cardiac improvement by significantly decreased myocardial iron with unchanged LVEF after 1 year treatment in thalassemic patients with myocardial siderosis [97].

CONCLUSION

Patients with thalassemia syndromes inevitably undergo high oxidative stress caused by primary and secondary iron overload and they suffer from oxidative-induced complications. No monotherapy of antioxidants or iron chelators can completely normalize oxidative stress in patients. Recently, combined antioxidants are significantly less interesting as no clinical trials have been reported, in contrast to a huge amount of data from several completed or ongoing clinical trials of combined iron chelator therapies. However, the experimental results vary greatly among studies depending on various factors including dose and administration schedule, inclusion/exclusion criteria of subject recruitment, follow-up periods, and differential outcome parameters. Systematically comparative studies are required with the goal of finding the best regime for different degrees of iron overload. Also, pharmacokinetic or genetic studies are encouraged to determine the factors contributing to variable drug/antioxidant response. This will benefit for future treatment where individually optimal strategies are required because of phenotypic heterogeneity among thalassemic patients.

REFERENCES

[1] Weatherall, DJ. Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. *Nat Rev Genet*, 2001; 2, 245-55.

[2] Rund, D; Rachmilewitz, E. Beta-thalassemia. *N Engl J Med*, 2005; 353, 1135-46.

[3] Advani, R; Sorenson, S; Shinar, E; Lande, W; Rachmilewitz, E; Schrier, SL. Characterization and comparison of the red blood cell membrane damage in severe human alpha- and beta-thalassemia. *Blood*, 1992; 79, 1058-63.

[4] Pantaleo, A; Giribaldi, G; Mannu, F; Arese, P; Turrini, F. Naturally occurring anti-band 3 antibodies and red blood cell removal under physiological and pathological conditions. *Autoimmune Rev*, 2008; 7, 457-62.

[5] Zwaal, RF; Schroit, AJ. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood*, 1997; 89, 1121-32.

[6] Ruf, A; Pick, M; Deutsch, V; Patscheke, H; Goldfarb, A; Rachmilewitz, EA, et al. In-vivo platelet activation correlates with red cell anionic phospholipid exposure in patients with beta-thalassaemia major. *Br J Haematol*, 1997; 98, 51-6.

[7] Chen, S; Eldor, A; Barshtein, G; Zhang, S; Goldfarb, A; Rachmilewitz, E, et al. Enhanced aggregability of red blood cells of beta-thalassemia major patients. *Am J Physiol*, 1996; 270, H1951-6.

[8] Hovav, T; Goldfarb, A; Artmann, G; Yedgar, S; Barshtein, G. Enhanced adherence of beta-thalassaemic erythrocytes to endothelial cells. *Br J Haematol*, 1999; 106, 178-81.

[9] Mathias, LA; Fisher, TC; Zeng, L; Meiselman, HJ; Weinberg, KI; Hiti, AL, et al. Ineffective erythropoiesis in beta-thalassemia major is due to apoptosis at the polychromatophilic normoblast stage. *Exp Hematol*, 2000; 28, 1343-53.

[10] Centis, F; Tabellini, L; Lucarelli, G; Buffi, O; Tonucci, P; Persini, B, et al. The importance of erythroid expansion in determining the extent of apoptosis in erythroid precursors in patients with beta-thalassemia major. *Blood*, 2000; 96, 3624-9.

[11] Srinoun, K; Svasti, S; Chumworathayee, W; Vadolas, J; Vattanaviboon, P; Fucharoen, S, et al. Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice. *Haematologica*, 2009; 94, 1211-9.

[12] De Maria, R; Testa, U; Luchetti, L; Zeuner, A; Stassi, G; Pelosi, E, et al. Apoptotic role of Fas/Fas ligand system in the regulation of erythropoiesis. *Blood*, 1999; 93, 796-803.

[13] Lithanatudom, P; Leecharoenkiat, A; Wannatung, T; Svasti, S; Fucharoen, S; Smith, DR. A mechanism of ineffective erythropoiesis in beta-thalassemia/Hb E disease. *Haematologica*, 2010; 95, 716-23.

[14] Lithanatudom, P; Wannatung, T; Leecharoenkiat, A; Svasti, S; Fucharoen, S; Smith, DR. Enhanced activation of autophagy in beta-thalassemia/Hb E erythroblasts during erythropoiesis. *Ann Hematol*, 2011; 90, 747-58.

[15] Origa, R; Galanello, R; Perseu, L; Tavazzi, D; Domenica Cappellini, M; Terenzani, L, et al. Cholelithiasis in thalassemia major. *Eur J Haematol*, 2009; 82, 22-5.

[16] Haidar, R; Musallam, KM; Taher, AT. Bone disease and skeletal complications in patients with beta thalassemia major. *Bone*, 2011; 48, 425-32.

[17] Skordis, N; Toumba, M. Bone disease in thalassaemia major: recent advances in pathogenesis and clinical aspects. *Pediatr Endocrinol Rev*, 2011; 8 Suppl 2, 300-6.

[18] Cadili, A; de Gara, C. Complications of splenectomy. *Am J Med*, 2008; 121, 371-5.

[19] Rachmilewitz, EA; Weizer-Stern, O; Adamsky, K; Amariglio, N; Rechavi, G; Breda, L, et al. Role of iron in inducing oxidative stress in thalassemia: Can it be prevented by inhibition of absorption and by antioxidants? *Ann N Y AcadSci*, 2005; 1054, 118-23.

[20] Borgna-Pignatti, C; Rugolotto, S; De Stefano, P; Zhao, H; Cappellini, MD; Del Vecchio, GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica*, 2004; 89, 1187-93.

[21] Pfeifer, WP; Degasperi, GR; Almeida, MT; Vercesi, AE; Costa, FF; Saad, ST. Vitamin E supplementation reduces oxidative stress in beta thalassaemia intermedia. *Acta Haematol*, 2008; 120, 225-31.

[22] Amer, J; Fibach, E. Chronic oxidative stress reduces the respiratory burst response of neutrophils from beta-thalassaemia patients. *Br J Haematol*, 2005; 129, 435-41.

[23] Del Rio, D; Stewart, AJ; Pellegrini, N. A review of recent studies on malondialdehyde as a toxic molecule and biological marker of oxidative stress. *NutrMetabCardiovasc Dis*, 2005; 15, 316-28.

[24] Dalle-Donne, I; Rossi, R; Giustarini, D; Milzani, A; Colombo, R. Protein carbonyl groups as biomarkers of oxidative stress. *ClinChimActa*, 2003; 329, 23-38.

[25] Halliwell, B. Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? *Am J ClinNutr*, 2000; 72, 1082-7.

[26] Kalpravidh, RW; Siritanaratkul, N; Insain, P; Charoensakdi, R; Panichkul, N; Hatairaktham, S, et al. Improvement in oxidative stress and antioxidant parameters in beta-thalassemia/Hb E patients treated with curcuminoids. *ClinBiochem*, 2010; 43, 424-9.

[27] Matayatsuk, C; Lee, CY; Kalpravidh, RW; Sirankapracha, P; Wilairat, P; Fucharoen, S, et al. Elevated F2-isoprostanes in thalassemic patients. *Free RadicBiol Med*, 2007; 43, 1649-55.

[28] Trombetta, D; Gangemi, S; Saija, A; Minciullo, PL; Cimino, F; Cristani, M, et al. Increased protein carbonyl groups in the serum of patients affected by thalassemia major. *Ann Hematol*, 2006; 85, 520-2.

[29] Matayatsuk, C; Poljak, A; Bustamante, S; Smythe, GA; Kalpravidh, RW; Sirankapracha, P, et al. Quantitative determination of ortho- and meta-tyrosine as biomarkers of protein oxidative damage in beta-thalassemia. *Redox Rep*, 2007; 12, 219-28.

[30] Meerang, M; Nair, J; Sirankapracha, P; Thephinlap, C; Srichairatanakool, S; Arab, K, et al. Accumulation of lipid peroxidation-derived DNA lesions in iron-overloaded thalassemic mouse livers: comparison with levels in the lymphocytes of thalassemia patients. *Int J Cancer*, 2009; 125, 759-66.

[31] Rahman, I; Adcock, IM. Oxidative stress and redox regulation of lung inflammation in COPD. *Eur Respir J*, 2006; 28, 219-42.

[32] Prasartkaew, S; Bunyaratvej, A; Fucharoen, S; Wasi, P. Comparison of erythrocyte antioxidative enzyme activities between two types of haemoglobin H disease. *J ClinPathol*, 1986; 39, 1299-303.

[33] Kassab-Chekir, A; Laradi, S; Ferchichi, S; Haj Khelil, A; Feki, M; Amri, F, et al. Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. *ClinChimActa*, 2003; 338, 79-86.

[34] Kukongviriyapan, V; Somparn, N; Senggunprai, L; Prawan, A; Kukongviriyapan, U; Jetsrisuparb, A. Endothelial dysfunction and oxidant status in pediatric patients with hemoglobin E-beta thalassemia. *Pediatr Cardiol*, 2008; 29, 130-5.

[35] Chiang, WL; Hsieh, YS; Yang, SF; Lu, TA; Chu, SC. Differential expression of glutathione-S-transferase isoenzymes in various types of anemia in Taiwan. *ClinChimActa*, 2007; 375, 110-4.

[36] Nasr, MR; Ali, S; Shaker, M; Elgabry, E. Antioxidant micronutrients in children with thalassaemia in Egypt. *East Mediterr Health J*, 2002; 8, 490-5.

[37] Livrea, MA; Tesoriere, L; Pintaudi, AM; Calabrese, A; Maggio, A; Freisleben, HJ, et al. Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood*, 1996; 88, 3608-14.

[38] Kohgo, Y; Ikuta, K; Ohtake, T; Torimoto, Y; Kato, J. Body iron metabolism and pathophysiology of iron overload. *Int J Hematol*, 2008; 88, 7-15.

[39] Fibach, E; Rachmilewitz, EA. The role of antioxidants and iron chelators in the treatment of oxidative stress in thalassemia. *Ann N Y AcadSci*, 2010; 1202, 10-6.

[40] Fiorelli, G; Fargion, S; Piperno, A; Battafarano, N; Cappellini, MD. Iron metabolism in thalassemia intermedia. *Haematologica*, 1990; 75 Suppl 5, 89-95.

[41] Nemeth, E; Tuttle, MS; Powelson, J; Vaughn, MB; Donovan, A; Ward, DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, 2004; 306, 2090-3.

[42] Tanno, T; Bhanu, NV; Oneal, PA; Goh, SH; Staker, P; Lee, YT, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nat Med*, 2007; 13, 1096-101.

[43] Tanno, T; Porayette, P; Sripichai, O; Noh, SJ; Byrnes, C; Bhupatiraju, A, et al. Identification of TWSG1 as a second novel erythroid regulator of hepcidin expression in murine and human cells. *Blood*, 2009; 114, 181-6.

[44] Ginzburg, Y; Rivella, S. {beta}-thalassemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. *Blood*, 2011.

[45] Tanner, MA; Galanello, R; Dessi, C; Smith, GC; Westwood, MA; Agus, A, et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. *Circulation*, 2007; 115, 1876-84.

[46] Tanner, MA; Galanello, R; Dessi, C; Westwood, MA; Smith, GC; Nair, SV, et al. Myocardial iron loading in patients with thalassemia major on deferoxamine chelation. *J CardiovascMagnReson*, 2006; 8, 543-7.

[47] Claster, S; Wood, JC; Noetzli, L; Carson, SM; Hofstra, TC; Khanna, R, et al. Nutritional deficiencies in iron overloaded patients with hemoglobinopathies. *Am J Hematol*, 2009; 84, 344-8.

[48] Goel, A; Kunnumakkara, AB; Aggarwal, BB. Curcumin as "Curecumin": from kitchen to clinic. *BiochemPharmacol*, 2008; 75, 787-809.

[49] Hatcher, H; Planalp, R; Cho, J; Torti, FM; Torti, SV. Curcumin: from ancient medicine to current clinical trials. *Cell Mol Life Sci*, 2008; 65, 1631-52.

[50] Ak, T; Gulcin, I. Antioxidant and radical scavenging properties of curcumin. *ChemBiol Interact*, 2008; 174, 27-37.

[51] Lee, JS; Surh, YJ. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett*, 2005; 224, 171-84.

[52] Macari, ER; Lowrey, CH. Induction of human fetal hemoglobin via the NRF2 antioxidant response signaling pathway. *Blood*, 2011; 117, 5987-97.

[53] Thephinlap, C; Phisalaphong, C; Fucharoen, S; Porter, JB; Srichairatanakool, S. Efficacy of curcuminoids in alleviation of iron overload and lipid peroxidation in thalassemic mice. *Med Chem*, 2009; 5, 474-82.

[54] Jiao, Y; Wilkinson, Jt; Di, X; Wang, W; Hatcher, H; Kock, ND, et al. Curcumin, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator. *Blood*, 2009; 113, 462-9.

[55] Buss, JL; Torti, FM; Torti, SV. The role of iron chelation in cancer therapy. *Curr Med Chem*, 2003; 10, 1021-34.

[56] Benassi, R; Ferrari, E; Grandi, R; Lazzari, S; Saladini, M. Synthesis and characterization of new beta-diketo derivatives with iron chelating ability. *J InorgBiochem*, 2007; 101, 203-13.

[57] Srichairatanakool, S; Thephinlap, C; Phisalaphong, C; Porter, JB; Fucharoen, S. Curcumin contributes to in vitro removal of non-transferrin bound iron by deferiprone and desferrioxamine in thalassemic plasma. *Med Chem*, 2007; 3, 469-74.

[58] Thephinlap, C; Phisalaphong, C; Lailerd, N; Chattipakorn, N; Winichagoon, P; Vadolus, J, et al. Reversal of cardiac iron loading and dysfunction in thalassemic mice by curcuminoids. *Med Chem*, 2011; 7, 62-9.

[59] Shoba, G; Joy, D; Joseph, T; Majeed, M; Rajendran, R; Srinivas, PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med*, 1998; 64, 353-6.

[60] Rogers, N; Stephenson, M; Kitching, A; Horowitz, J; Coates, P. Amelioration of renal ischaemia-reperfusion injury by liposomal delivery of curcumin to renal tubular epithelial and antigen presenting cells. *Br J Pharmacol*, 2011.

[61] Yan, YD; Kim, JA; Kwak, MK; Yoo, BK; Yong, CS; Choi, HG. Enhanced Oral Bioavailability of Curcumin via a Solid Lipid-Based Self-Emulsifying Drug Delivery System Using a Spray-Drying Technique. *Biol Pharm Bull*, 2011; 34, 1179-86.

[62] Banerjee, A; Kunwar, A; Mishra, B; Priyadarsini, KI. Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. *ChemBiol Interact*, 2008; 174, 134-9.

[63] Subudhi, U; Das, K; Paital, B; Bhanja, S; Chainy, GB. Alleviation of enhanced oxidative stress and oxygen consumption of L-thyroxine induced hyperthyroid rat liver mitochondria by vitamin E and curcumin. *ChemBiol Interact*, 2008; 173, 105-14.

[64] Babu, PV; Liu, D. Green tea catechins and cardiovascular health: an update. *Curr Med Chem*, 2008; 15, 1840-50.

[65] Khan, N; Mukhtar, H. Tea polyphenols for health promotion. *Life Sci*, 2007; 81, 519-33.

[66] Cabrera, C; Artacho, R; Gimenez, R. Beneficial effects of green tea--a review. J Am CollNutr, 2006; 25, 79-99.

[66] Zhu, QY; Huang, Y; Tsang, D; Chen, ZY. Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *J Agric Food Chem*, 1999; 47, 2020-5.

[68] Srichairatanakool, S; Ounjaijean, S; Thephinlap, C; Khansuwan, U; Phisalpong, C; Fucharoen, S. Iron-chelating and free-radical scavenging activities of microwave-processed green tea in iron overload. *Hemoglobin*, 2006; 30, 311-27.

[69] Ounjaijean, S; Thephinlap, C; Khansuwan, U; Phisalapong, C; Fucharoen, S; Porter, JB, et al. Effect of green tea on iron status and oxidative stress in iron-loaded rats. *Med Chem*, 2008; 4, 365-70.

[70] Saewong, T; Ounjaijean, S; Mundee, Y; Pattanapanyasat, K; Fucharoen, S; Porter, JB, et al. Effects of green tea on iron accumulation and oxidative stress in livers of iron-challenged thalassemic mice. *Med Chem*, 2010; 6, 57-64.

[71] Nakagawa, K; Okuda, S; Miyazawa, T. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma. *BiosciBiotechnolBiochem*, 1997; 61, 1981-5.

[72] Gawande, S; Kale, A; Kotwal, S. Effect of nutrient mixture and black grapes on the pharmacokinetics of orally administered (-)epigallocatechin-3-gallate from green tea extract: a human study. *Phytother Res*, 2008; 22, 802-8.

[73] Traber, MG; Stevens, JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radic*Biol Med*, 2011; 51, 1000-13.

[74] Tesoriere, L; D'Arpa, D; Butera, D; Allegra, M; Renda, D; Maggio, A, et al. Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce

oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. *Free Radic Res*, 2001; 34, 529-40.

[75] Unchern, S; Laoharuangpanya, N; Phumala, N; Sipankapracha, P; Pootrakul, P; Fucharoen, S, et al. The effects of vitamin E on platelet activity in beta-thalassaemia patients. *Br J Haematol*, 2003; 123, 738-44.

[76] Amer, J; Dana, M; Fibach, E. The antioxidant effect of erythropoietin on thalassemic blood cells. *Anemia*, 2010; 2010, 978710.

[77] Amer, J; Atlas, D; Fibach, E. N-acetylcysteine amide (AD4) attenuates oxidative stress in beta-thalassemia blood cells. *BiochimBiophysActa*, 2008; 1780, 249-55.

[78] Kalpravidh, RW; Wichit, A; Siritanaratkul, N; Fucharoen, S. Effect of coenzyme Q10 as an antioxidant in beta-thalassemia/Hb E patients. *Biofactors*, 2005; 25, 225-34.

[79] Pardo-Andreu, GL; Sanchez-Baldoquin, C; Avila-Gonzalez, R; Yamamoto, ET; Revilla, A; Uyemura, SA, et al. Interaction of Vimang (Mangiferaindica L. extract) with Fe(III) improves its antioxidant and cytoprotecting activity. *Pharmacol Res*, 2006; 54, 389-95.

[80] Tesoriere, L; Allegra, M; Butera, D; Gentile, C; Livrea, MA. Cytoprotective effects of the antioxidant phytochemical indicaxanthin in beta-thalassemia red blood cells. *Free Radic Res*, 2006; 40, 753-61.

[81] Fibach, E; Tan, ES; Jamuar, S; Ng, I; Amer, J; Rachmilewitz, EA. Amelioration of oxidative stress in red blood cells from patients with beta-thalassemia major and intermedia and E-beta-thalassemia following administration of a fermented papaya preparation. *Phytother Res*, 2010; 24, 1334-8.

[82] Porter, JB; Shah, FT. Iron overload in thalassemia and related conditions: therapeutic goals and assessment of response to chelation therapies. *Hematol OncolClin North Am*, 2010; 24, 1109-30.

[83] Tanner, MA; Galanello, R; Dessi, C; Smith, GC; Westwood, MA; Agus, A, et al. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. *J CardiovascMagnReson*, 2008; 10, 12.

[84] Hershko, C; Konijn, AM; Link, G. Iron chelators for thalassaemia. *Br J Haematol*, 1998; 101, 399-406.

[85] Neufeld, EJ. Update on iron chelators in thalassemia. *Hematology Am Soc Hematol Educ Program*, 2010; 2010, 451-5.

[86] Collins, AF; Fassos, FF; Stobie, S; Lewis, N; Shaw, D; Fry, M, et al. Iron-balance and dose-response studies of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in iron-loaded patients with sickle cell disease. *Blood*, 1994; 83, 2329-33.

[87] Olivieri, NF; Brittenham, GM; Matsui, D; Berkovitch, M; Blendis, LM; Cameron, RG, et al. Iron-chelation therapy with oral deferipronein patients with thalassemia major. *N Engl J Med*, 1995; 332, 918-22.

[88] Olivieri, NF; Brittenham, GM; McLaren, CE; Templeton, DM; Cameron, RG; McClelland, RA, et al. Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. *N Engl J Med*, 1998; 339, 417-23.

[89] Barry, M; Flynn, DM; Letsky, EA; Risdon, RA. Long-term chelation therapy in thalassaemia major: effect on liver iron concentration, liver histology, and clinical progress. *Br Med J*, 1974; 2, 16-20.

[90]Pennell, DJ; Berdoukas, V; Karagiorga, M; Ladis, V; Piga, A; Aessopos, A, et al. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. *Blood*, 2006; 107, 3738-44.

[91] Smith, GC; Alpendurada, F; Carpenter, JP; Alam, MH; Berdoukas, V; Karagiorga, M, et al. Effect of deferiprone or deferoxamine on right ventricular function in thalassemia major patients with myocardial iron overload. *J CardiovascMagnReson*, 2011; 13, 34.

[92] Evans, P; Kayyali, R; Hider, RC; Eccleston, J; Porter, JB. Mechanisms for the shuttling of plasma non-transferrin-bound iron (NTBI) onto deferoxamine by deferiprone. *Transl Res*, 2010; 156, 55-67.

[93] Kattamis, A; Papassotiriou, I; Palaiologou, D; Apostolakou, F; Galani, A; Ladis, V, et al. The effects of erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major. *Haematologica*, 2006; 91, 809-12.

[94] Cappellini, MD; Porter, J; El-Beshlawy, A; Li, CK; Seymour, JF; Elalfy, M, et al. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *Haematologica*, 2010; 95, 557-66.

[95] Taher, A; El-Beshlawy, A; Elalfy, MS; Al Zir, K; Daar, S; Habr, D, et al. Efficacy and safety of deferasirox, an oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia: the ESCALATOR study. *Eur J Haematol*, 2009; 82, 458-65.

[96] Taher, A; Elalfy, MS; Al Zir, K; Daar, S; Al Jefri, A; Habr, D, et al. Importance of optimal dosing >/=30 mg/kg/d during deferasirox treatment: 2.7-yr follow-up from the ESCALATOR study in patients with beta-thalassaemia. *Eur J Haematol*, 2011.

[97] Pennell, DJ; Porter, JB; Cappellini, MD; El-Beshlawy, A; Chan, LL; Aydinok, Y, et al. Efficacy of deferasirox in reducing and preventing cardiac iron overload in beta-thalassemia. *Blood*, 2010; 115, 2364-71.