Cardiac complications in beta-thalassemia: From mice to men

Sirinart Kumfu[1,2,3], Suthat Fucharoen[4], Siriporn C. Chattipakorn[1,3,5] and Nipon Chattipakorn[1,2,3]

[1]Faculty of Medicine, Cardiac Electrophysiology Research and Training Center, Chiang Mai University, Chiang Mai 50200, Thailand; [2]Faculty of Medicine, Department of Physiology, Cardiac Electrophysiology Unit, Chiang Mai University, Chiang Mai 50200, Thailand; [3]Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai 50200, Thailand; [4]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom 73170, Thailand; [5]Faculty of Dentistry, Department of Oral Biology and Diagnostic Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
Corresponding author: Nipon Chattipakorn. Email: nchattip@gmail.com

Impact statement

Iron overload cardiomyopathy is a major cause of morbidity and mortality in patients with thalassemia. Since investigation of iron overload cardiomyopathy in thalassemia patients has many limitations, a search for an animal model for this condition has been ongoing for decades. In the past decades, there is no doubt that the use of β-thalassemic mice as a study model to investigate the pathophysiology of iron overload cardiomyopathy and the role of various pharmacological interventions, has shed some light in understanding this serious complication and in improving the associated cardiac dysfunction. In this review, the effects of iron overload on the hearts of β-thalassemic mice under conditions of iron overload as well as the efficacy of pharmacological interventions to combat these adverse effects on the heart are reviewed and discussed.

Abstract

Beta-thalassemia is an inherited hemoglobin disorder caused by reduced or absent synthesis of the beta globin chains of hemoglobin. This results in variable outcomes ranging from clinically asymptomatic to severe anemia, which then typically requires regular blood transfusion. These regular blood transfusions can result in an iron overload condition. The iron overload condition can lead to iron accumulation in various organs, especially in the heart, leading to iron overload cardiomyopathy, which is the major cause of mortality in patients with thalassemia. In the past decades, there is no doubt that the use of β-thalassemic mice as a study model to investigate the pathophysiology of iron overload cardiomyopathy and the role of various pharmacological interventions, has shed some light in understanding this serious complication and in improving the associated cardiac dysfunction. In this review, the effects that iron overload has on the hearts of β-thalassemic mice under conditions of iron overload as well as the efficacy of pharmacological interventions to combat these adverse effects on the heart are reviewed and discussed. The in-depth understanding of biomolecular alterations in the heart of these iron overload thalassemic mice will help give guidance for more effective therapeutic approaches in the near future.

Keywords: Beta-thalassemic mice, heart, iron overload cardiomyopathy, cardiomyocytes, thalassemia, pathophysiology

***Experimental Biology and Medicine* 2017; 242: 1126–1135. DOI: 10.1177/1535370217708977**

Introduction

Thalassemia in humans is a group of inherited disorders of hemoglobin synthesis which are characterized by the absence or reduction in amounts of one or more of the globin chains of hemoglobin.[1–3] The absence or reduction of globin chain synthesis could generate an imbalance between the subunits of hemoglobin.[1,4] Hemoglobin is mainly composed of α- and β-globin polypeptide chains which are encoded by multigene clusters which have been conserved throughout mammalian evolution.[4–6] Beta-thalassemia caused by reduced (β^+) or absent (β^0) synthesis of the beta globin chains of hemoglobin results in variable outcomes ranging from severe anemia to clinically asymptomatic individuals.[1–3] These disorders can be divided into three major groups, β-thalassemia major, intermedia and minor (carrier or trait), based on the quantity of β-globin produced and symptomatic features.[1,3] β-thalassemia patients with severe anemia often require regular blood transfusions which can result in an iron overload state.[1,7] This iron overload condition can lead to iron accumulation in various organs, especially in the heart where it leads to iron overload cardiomyopathy. This is the major cause of mortality in patients with β-thalassemia.[7,8] Since investigation of iron overload cardiomyopathy in thalassemia patients has many limitations, a search for an animal model for this condition has been ongoing for decades.

In animals, although no naturally occurring β^0-thalassemia has been observed in mice, a mouse model with β^+-thalassemia exists. The mouse β-globin gene cluster is located on chromosome 7 and has four functional β-globin

genes: *βh1* and *εy*, embryonic globin genes; and two adult globin genes, β^{major} and β^{minor}.[5,6,9] The β^{major} gene encodes for 80% and β^{minor} gene for 20% of β-globin production in adult mice.[10] Three mouse models of β-thalassemia have been developed relatively recently. First, a DNA deletion in the β-globin locus was developed which includes the whole β^{major} gene and its subsequent upstream sequences.[11,12] Mice homozygous for the deletion (Hbb^{th-1}/Hbb^{th-1}) have a survival to adulthood rate of 60%.[11,12] The heterozygous mice exhibit a mild phenotype which is related to the thalassemia trait in humans.[11,12] Second, the mouse model for β-thalassemia was created by targeted gene disruption of the mouse β^{major} gene.[12] Mice homozygous for an insertional disruption of the β^{major} gene (Hbb^{th-2}/Hbb^{th-2}) had severe anemia and did not survive after birth.[12] Finally, a mouse model for homozygous deletion of both β^{major} and β^{minor} genes (Hbb^{th-3}/Hbb^{th-3}) was developed which results in a severe form of β^0-thalassemia and the mice die perinatally. This is similar to the most severe form of Cooley anemia (β-thalassemia major) in humans.[13] The heterozygous animals with this deletion show a pathophysiology comparable to that of patients with human β-thalassemia intermedia.[13]

These β-thalassemic mouse models have been used in investigations to understand the pathophysiology of iron-overload cardiomyopathy found in patients. Currently, the model mostly used to study the heart in this condition is the heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic genotype.[14–19] Mice heterozygous for the deletion (Hbb^{th-3}/Hbb^{+}) showed characteristics typical of severe thalassemia such as: a significant decrease in hematocrit (Hct), hemoglobin (Hb), red blood cell (RBC) counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), and increased reticulocyte counts, serum bilirubin concentrations, and red cell distribution width (RDW).[13] Heterozygous animals also exhibit bone deformities and splenic enlargement due to increased hematopoiesis which is a typical picture of β-thalassemia, and a spontaneous iron overload in the spleen, liver, and kidneys in this mouse model has been found.[13]

Although TI patients did not need blood transfusions to sustain levels of anemia, several other pathogenic mechanisms remained in play.[20] Ineffective erythropoiesis results in the suppression of hepcidin levels, leading to increased intestinal iron absorption and increased release of recycled iron from macrophages within the reticuloendothelial system, resulting in hepatocyte iron loading.[21,22] Although iron accumulation in patients with TI occurs more slowly than in TM patients who have received regular blood transfusions, it can reach levels much higher than the normal threshold, especially in aged patients.[22,23] Moreover, it has been found upon autopsy that some small subgroups of older TI patients had developed myocardial siderosis.[24–26] Damage to cardiac tissues may have resulted from exposure to NTBI, without the accumulation of toxic iron species within myocytes.[27,28] This suggests that even without evidence of cardiac siderosis, TI patients may still be at risk of iron-related cardiac dysfunction. Although homozygous β-thalassemic mice with blood transfusion is the model that is related to TM patients, these mice had severe anemia and did not survive after birth. Furthermore, giving mice a blood transfusion is a very difficult process. Therefore, the heterozygous β-thalassemic mice, which were similar to TI patients, were frequently used in this research field. However, to mimic the iron overload condition as observed in thalassemia major, iron loading either orally or intraperitoneally was commonly applied to these mice.[14,29–31] This also resulted in iron overload condition (indicated by the increased plasma NTBI), followed by mitochondrial dysfunction in those mice.[29] Therefore, heterozygous β-thalassemic mice with iron overload induced by iron injection or iron feeding is a suitable model for representing an iron overload condition and their pathophysiology was similar to that in thalassemia patients with blood transfusion.

The hearts of β-thalassemic mice

In *in vitro* studies, cultured cardiomyocytes of heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice showed increased intracellular accumulation of both Fe^{2+} and Fe^{3+},[17,18] whereas the cardiac mitochondrial function and morphology were still intact.[16] A summary of reports regarding the iron accumulation and mitochondrial function in the heart of β-thalassemic mice (*in vitro* models) is shown in Table 1.

In *in vivo* models, Gelderman and colleagues demonstrated that the hearts of heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice showed increased cardiac iron accumulation at 12 months of age,[32] a state consistent with reports from *in vitro* models. However, the hearts of 5-, 6- and 7–10-month-old β-thalassemic mice did not show any changes in cardiac iron accumulation, compared with normal mice.[14,19,30,33] These findings suggest that the level of iron deposition in cardiac tissue is associated with age in thalassemic mice. In addition, the older β-thalassemic mice (7–10 months old[14,19]) showed increased heart weight when compared with normal mice, but the heart weight of

Table 1 A summary of the reports on iron accumulation and mitochondrial function in the hearts of β-thalassemic mice (*in vitro* models) under basal conditions

Mouse models	Age	Cardiac iron	Cardiac mitochondria function and morphology	Ref.
Hbb^{th-3}/Hbb^{+} (Isolated cardiac mitochondria)	3–6 months		Function & morphology; normal	16
Hbb^{th-3}/Hbb^{+} (Cultured cardiomyocytes)	9–12 months	↑ Fe^{2+} accumulation		18
Hbb^{th-3}/Hbb^{+} (Cultured cardiomyocytes)	9–12 months	↑ Fe^{3+} accumulation		17

younger β-thalassemic mice (7 weeks old[13]) did not differ from normal mice.

The blood parameters from most studies of heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice showed decreased levels of RBC, Hb, and a decreased Hct, increased levels of ROS and serum iron, but non-altered levels of plasma NTBI and MDA levels.[13–15,19,30,32] Only one study[33] has shown increased levels of plasma NTBI in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic 5-month old mice, which was inconsistent with three other studies.[14,19,30] This inconsistency might be due to the different techniques used for NTBI detection. For example, Gelderman *et al.* were detecting plasma NTBI levels at nanogram levels, whereas the other studies were limited to microgram levels.

The heart is known to be tightly controlled by the sympathetic and parasympathetic nerves of the cardiac autonomic nervous system. It has been shown that β-thalassemic mice have impaired cardiac autonomic function which is indicated by depressed heart rate variability (i.e. increased LF/HF ratio), increased stroke volume (SV) and cardiac output (CO) when compared with normal mice.[14,15,19] This indicates a high-output state in β-thalassemic mice, which is similar to that found in patients with thalassemia.[7] High-output state, as shown by increased stroke volume (SV) and cardiac output (CO), develops as a consequence of prolonged tissue hypoxia, resulting from chronic anemia in beta-thalassemia patients[34,35] and animal models.[14,29] Although thalassemia patients have high SV and CO, they still have cardiac dysfunctions such as decreased fractional shortening (FS) and ejection fraction (EF), when compared with a normal physiological state.[35,36] Moreover, when iron overload occurs in the heart, it can aggravate cardiac dysfunction, as shown by decreased SV, CO, FS and EF.[14,29,36] However, cardiac mitochondrial function and morphology in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice has been found to be normal at this basal condition, these results being consistent with an *in vitro* study.[19]

The other models of β-thalassemic mice, homozygous (Hbb^{th1}/Hbb^{th1}), ($Hbb^{d3(th)}/Hbb^{d3(th)}$) and heterozygous $\beta^{IVSII-654}$, display a greater severity of the condition than the heterozygous (Hbb^{th-3}/Hbb^{+}) model does.[37–39] These mice showed decreased levels of RBC, Hb, Hct and impaired cardiac function indicated by decreased SV, EF, and FS.[37–39] Also, these mice also had increased heart weight and cardiac iron levels even at the basal condition when compared with normal mice.[37,39] However, the study by Stoyanova *et al.* was the only one not to observe increased cardiac iron accumulation in homozygous ($Hbb^{d3(th)}/Hbb^{d3(th)}$) β-thalassemic mice.[38] This discrepancy could be due to the fact that Stoyanova *et al.* used only Prussian blue staining to determine cardiac iron accumulation, which is a less sensitive method than the atomic absorption and colorimetric methods used by other studies. Consistent with a report concerning the heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice,[19] the cardiac mitochondrial morphology in the heterozygous $\beta^{IVSII-654}$ β-thalassemic mice was still normal under basal conditions.[37]

Both *in vitro* and *in vivo* studies indicated that heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice were anemic, had increased heart weight and cardiac iron accumulation when getting old, and had measurable cardiac dysfunction.[13–19,30,32,33,37–40] Moreover, the homozygous (Hbb^{th1}/Hbb^{th1}), ($Hbb^{d3(th)}/Hbb^{d3(th)}$) and heterozygous $\beta^{IVSII-654}$ β-thalassemic mice showed a greater severity of anemia and had higher levels of cardiac iron accumulation than the heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice did, even though the mice were still young.[37–39] A summary of reports regarding cardiac iron accumulation, cardiac function, and mitochondrial function in β-thalassemic mice (*in vivo* models) is shown in Table 2. Regarding the age influence on cardiac function, in Table 2, there are two studies on Hbb^{th-3}/Hbb^{+} which showed the same results of SV and CO, because the mice were the same age in these studies.[14,19] However, in the other two studies on $Hbb^{d3(th)}/Hbb^{d3(th)}$, cardiac function could not be compared since different parameters were determined.[38,40]

The hearts of β-thalassemic mice under an iron overload condition

Under conditions of iron overload, both Fe^{2+} and Fe^{3+} iron uptake was increased in cultured cardiomyocytes from heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice.[17,18] Moreover, the iron overload condition leads to increased cardiac mitochondrial ROS production, mitochondrial depolarization, and mitochondrial swelling in isolated cardiac mitochondria from the hearts of heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice.[16] These findings suggest that the iron overload condition leads to increased iron uptake and accumulation in cardiomyocytes which then leads to mitochondrial dysfunction in these mice. Available *in vitro* reports regarding the effects of iron overload on iron uptake and mitochondrial function in the hearts of β-thalassemic mice models are summarized in Table 3.

In the *in vivo* studies, conditions of iron overload led to increased plasma NTBI and MDA levels, and increased cardiac iron accumulation without altering the heart weight in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice.[14,19,30,31,33] Also, iron overload impaired cardiac autonomic regulation as indicated by an increased LF/HF ratio, and impaired cardiac function by decreasing SV, CO, ESP, Pmax and dP/dtmax in these mice.[14,19,30] Cardiac mitochondrial dysfunction was demonstrated by increased cardiac mitochondrial ROS production, mitochondrial depolarization and swelling in the hearts of heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice under iron overload conditions.[19] All *in vivo* findings were consistent and suggested that iron overload can lead to increased cardiac iron accumulation and cardiac mitochondrial dysfunction, leading to left ventricular dysfunction in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice.[14,19,30,31,33] Available *in vivo* reports regarding the effects of iron overload on cardiac iron accumulation, cardiac function and mitochondrial function of β-thalassemic mice are summarized in Table 4. In Table 4, the two studies on Hbb^{th-3}/Hbb^{+} did not show a difference in results on cardiac function, even though there was a difference in the time of iron expose between the two studies. However, these two studies showed that there was

Table 2 A summary of the reports on cardiac iron accumulation, cardiac function and mitochondrial function of β-thalassemic mice (*in vivo* models) under basal conditions

Mouse models	Age	Blood parameter	Heart weight	Cardiac iron	Cardiac autonomic regulation	Cardiac function	Cardiac mitochondria function and morphology	Ref.
$Hbb^{th\text{-}3}/Hbb^{+}$	7 weeks	↓ RBC, Hb, Hct	↔					13
$Hbb^{th\text{-}3}/Hbb^{+}$	3–5 months	↓ Hb ↑ ROS, SI			↑ LF/HF ratio			15
$Hbb^{th\text{-}3}/Hbb^{+}$	5 months	↑ Plasma iron, NTBI ↓ RBC, Hb, Hct		↔				33
$Hbb^{th\text{-}3}/Hbb^{+}$	6 months	↓ Hb ↔ Plasma NTBI, MDA		↔				30
$Hbb^{th\text{-}3}/Hbb^{+}$	7–10 months	↔ Plasma NTBI, MDA	↑	↔	↑ LF/HF ratio	↑ SV, CO		14
$Hbb^{th\text{-}3}/Hbb^{+}$	7–10 months	↔ Plasma NTBI, MDA	↑	↔	↑ LF/HF ratio	↑ SV, CO	Function & morphology; normal	19
$Hbb^{th\text{-}3}/Hbb^{+}$	12 months	↓ Hb		↑				32
Hbb^{th1}/Hbb^{th1}	15–35 weeks	↑ Plasma iron	↑	↑				39
Heterozygous $\beta^{IVSII\text{-}654}$	–	↓ Hb, Hct ↑ ROS		↑			Morphology; normal	37
$Hbb^{d3(th)}/Hbb^{d3(th)}$	14 weeks					↓ SV ↔ CO		38
$Hbb^{d3(th)}/Hbb^{d3(th)}$	15 months	↓ RBC, Hb, Hct	↑	↔		↓ EF, FS		40

RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; ROS: reactive oxygen species; SI: serum iron; NTBI: non-transferrin bound iron; MDA: malondialdehyde; LF/HF: low frequency/high frequency; SV: stroke volume; CO: cardiac output; EF: ejection fraction; FS: fractional shortening.

Table 3 A summary of the reports on the effects of iron overload on iron uptake and mitochondrial function in the hearts of β-thalassemic mice (*in vitro* models)

Mouse models	Age	Iron overload	Cardiac iron	Cardiac mitochondria function and morphology	Ref.
$Hbb^{th\text{-}3}/Hbb^{+}$ (Isolated cardiac mitochondria)	3–6 months	FAC 0-5 μg/ml + 1 mM AA (Fe^{2+}), 5 min		↑ ROS & Swelling Mitochondrial depolarization Morphology; swelling	16
$Hbb^{th\text{-}3}/Hbb^{+}$ (Cultured cardiomyocytes)	9–12 months	FAC 20 μg/ml + 1 mM AA (Fe^{2+}), 48 h	↑ Fe^{2+} uptake		18
$Hbb^{th\text{-}3}/Hbb^{+}$ (Cultured cardiomyocytes)	9–12 months	FAC 20 μg/ml (Fe^{3+}), 48 h	↑ Fe^{3+} uptake		17

FAC: ferric ammonium citrate; AA: ascorbic acid; ROS: reactive oxygen species.

a progression in cardiac iron accumulation which correlated to the increased time of iron exposure.

The effects of pharmacological interventions on the hearts of β-thalassemic mice under iron overload conditions

Recently, several studies have investigated novel ways of treating iron overload cardiomyopathy besides the currently used iron chelator therapy in β-thalassemic mice models. Studies on cultured cardiomyocytes of heterozygous ($Hbb^{th\text{-}3}/Hbb^{+}$) β-thalassemic mice showed that treatment with a T-type calcium channel (TTCC) blocker and an iron chelator could prevent Fe^{2+} uptake into cardiomyocytes, whereas blockers of transferrin receptor1 (TfR1), divalent metal transporter1 (DMT1) and L-type calcium channel (LTCC) could not.[18] However, blockers of TfR1, DMT1, LTCC and TTCC could not prevent Fe^{3+} uptake into these cells.[17] These findings suggest that TTCC is an important pathway for Fe^{2+} uptake in a cultured thalassemic cardiomyocyte model. Reports also demonstrated other alternative pathways which could play a major role in Fe^{3+} uptake in thalassemic cardiomyocytes. Normally, mitochondrial calcium uniporter (MCU) mediates calcium uptake into the mitochondria. However, in the isolated cardiac mitochondria of heterozygous ($Hbb^{th\text{-}3}/Hbb^{+}$)

Table 4 A summary of the reports on the effects of iron overload on cardiac iron accumulation, cardiac function and mitochondrial function of β-thalassemic mice (*in vivo* models)

Mouse models	Age	Iron overload	Blood parameter	Heart weight	Cardiac iron	Cardiac autonomic regulation	Cardiac function	Cardiac mitochondria function and morphology	Ref.
Hbb^{th-3}/Hbb^{+}	7 weeks	Iron dextran i.p. injection (20 mg/day), 2 weeks		↔	↑				31
Hbb^{th-3}/Hbb^{+}	2 months	Feeding with iron diet (0.2% ferrocene), 2 months	↑ Plasma NTBI & MDA		↑	↑ LF/HF ratio			30
Hbb^{th-3}/Hbb^{+}	3 months	0.2 ml packed RBCs transfused every 3 days (five transfusions) 15 days	↑ Plasma iron & NTBI ↑ RBC,Hb, Hct		↑				33
Hbb^{th-3}/Hbb^{+}	3–6 months	Feeding with iron diet (0.2% ferrocene), 3 months	↑ Plasma NTBI & MDA	↔	↑	↑ LF/HF ratio	↓ SV, CO, ESP, Pmax, dP/dtmax		14
Hbb^{th-3}/Hbb^{+}	3–6 months	Feeding with iron diet (0.2% w/w) ferrocene, 4 months	↑ Plasma NTBI & MDA	↔	↑	↑ LF/HF ratio	↓ SV, CO, ESP, Pmax, dP/dtmax	↓ Mitochondria function Morphology; swelling	19

RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; NTBI: non-transferrin bound iron; MDA: malondialdehyde; LF/HF: low frequency/high frequency; SV: stroke volume; CO: cardiac output; ESP: end systolic pressure; Pmax: maximum pressure; dP/dtmax: maximum dP/dt; i.p.: intra-peritoneal.

β-thalassemic mice, it was shown that MCU acts as a major portal for Fe^{2+} entry into cardiac mitochondria.[16] By using the MCU blocker, ROS production and mitochondrial depolarization could be effectively prevented, and mitochondrial swelling caused by Fe^{2+} overload attenuated.[16] Future clinical studies are needed to warrant the clinical usefulness of MCU blockers in thalassemia patients. Available *in vitro* reports regarding the effects of pharmacological intervention on iron uptake and mitochondrial function in the hearts of β-thalassemic mice (models) under iron overload conditions are summarized in Table 5.

Several *in vivo* studies showed that treatment with either LTCC blockers (verapamil, nifedipine, amlodipine), a TTCC blocker (efonidipine), a DMT1 blocker (ebselen) or an antioxidant (curcuminoid) effectively reduced plasma NTBI levels and cardiac iron deposition, and improved cardiac autonomic regulation and left ventricular function in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice under conditions of iron overload with the same efficacy as commercial iron chelators (deferoxamine, deferasirox or deferiprone).[14,19,30] No treatments had any effect on the heart weights of heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice under iron overload conditions.[14,19,31,33] Moreover, treatment with either an LTCC blocker (amlodipine) or a TTCC blocker (efonidipine) led to improved mitochondrial function in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice under iron overload conditions to the same degree as three commercial iron chelators (deferoxamine, deferasirox or deferiprone).[19] Treatment with apo-transferrin also decreased plasma iron levels, plasma NTBI levels, and cardiac iron deposition in heterozygous (Hbb^{th-3}/Hbb^{+}) β-thalassemic mice even though they were under a basal condition.[33] All findings in the *in vivo* studies suggested that a TTCC blocker, a LTCC blocker, a DMT1 blocker, an antioxidant and apo-transferrin could be beneficial for treatment of an iron overloaded state, especially for iron overload cardiomyopathy in β-thalassemia models. Available *in vivo* reports regarding the effects of pharmacological intervention on cardiac iron accumulation, cardiac function and mitochondrial function of β-thalassemic mice under iron overload conditions are summarized in Table 6.

Cardiac complications in beta-thalassemia patients

Thalassemic cardiomyopathy and arrhythmia, caused by cardiac iron overload, are the most important complications of iron overload in beta-thalassemia patients and are responsible for 71% of deaths, globally, in these patients.[41] In the early stages, patients are usually asymptomatic and symptoms are related to the degree of ventricular impairment.[42] Cardiac arrhythmias, including premature ventricular contraction, ST-T changes, QT prolongation, and second- and third-degree atrioventricular block have been reported in iron overload beta-thalassemia patients.[43–46] In view of cardiac complications, serum ferritin concentrations have been shown to correlate poorly with all stages of cardiac dysfunction.[35,47] Non-transferrin-bound iron (NTBI) seems to be the most accurate parameter among serum iron markers; however, it is commonly used only

Table 5 A summary of the reports on the effects of pharmacological interventions on iron uptake and mitochondrial function in the heart of β-thalassemic mice (*in vitro* models) under conditions of iron overload

Mouse models	Age	Iron overload	Intervention	Cardiac iron	Cardiac mitochondria function and morphology	Ref.
Hbb^{th-3}/Hbb^{+} (Isolated cardiac mitochondria)	3–6 months	FAC 0-5 μg/ml + 1 mM AA (Fe^{2+}), 5 min	MCU blocker (Ru360) 10 μM		↓↓ ROS & Swelling Restoration mitochondrial depolarization Morphology; normal	16
			mPTP blocker [Cyclosporin A (CsA)] 5 μM		↓ ROS ↔ Swelling Mitochondrial depolarization Morphology; swelling	
Hbb^{th-3}/Hbb^{+} (Cultured cardiomyocytes)	9–12 months	FAC 20 μg/ml + 1 mM AA (Fe^{2+}), 48 h	Efonidipine 0–20 μM, DFO 0–20 μg/ml	↓ Fe^{2+} uptake		18
			Anti-TfR 0–10 μg/ml, Ebselen 0–25 μM, Verapamil 0–80 μM	↔ Fe^{2+} uptake		
Hbb^{th-3}/Hbb^{+} (Cultured cardiomyocytes)	9–12 months	FAC 20 μg/ml (Fe^{3+}), 48 h	DFO 0–20 μg/ml	↓ Fe^{3+} uptake		17
			Efonidipine 0–20 μM, Anti-TfR 0–10 μg/ml, Ebselen 0–25 μM, Verapamil 0–80 μM	↔ Fe^{3+} uptake		

FAC: ferric ammonium citrate; AA: ascorbic acid; ROS: reactive oxygen species; DFO: deferoxamine; TfR: transferrin receptor; MCU: mitochondrial calcium uniporter; mPTP: mitochondrial permeability transition pore.

in research studies.[48–53] Accurate assessments of cardiac dysfunction and/or cardiac iron status are currently based on imaging techniques. The T2-star magnetic resonance imaging (MRI-T2*) has been demonstrated as evaluating cardiac iron status accurately and allows the early detection of global ventricular dysfunction.[47] In addition, it can be used for monitoring myocardial iron levels during iron chelation therapy.[54,55] In beta-thalassemia major patients with normal myocardial T2* (>20 ms), the right ventricular (RV) and left ventricular (LV) ejection fraction (EF) was found to be within the normal range.[47,56] It has been shown that lower myocardial T2* values (<20 ms) are associated with an increase in RV and LV dysfunction,[47,56] whereas an improvement in myocardial T2* results in improvement in LV and RV EF in beta-thalassemia major patients.[54,55] There was a linear relationship between RV and LV EF.[56] Clinical findings were consistent with *in vivo* reports in β-thalassemic mice, suggesting that iron overload can lead to increased cardiac iron accumulation and cardiac dysfunction in β-thalassemic mice.[14,19,30,31,33]

An altered sympathovagal balance, as shown by depressed heart rate variability, was found in thalassemia major patients.[51,57–59] Heart rate variability parameters were reduced even in β-thalassemia major patients without evident cardiac siderosis (T2* >20 ms)[57] and preclinical stages of heart disease.[58] The studies show that reduction in heart rate variability may start before cardiac iron loading and preclinical stages of cardiac disease in β-thalassemia major and non-transfusion-dependent thalassemia patients.[50–53,57,58] β-thalassemic mice had impaired cardiac autonomic function which was indicated by depressed heart rate variability.[14,15,19] This was consistent with and showed a correlation with the results that were found in β-thalassemia patients. These findings suggest that cardiac autonomic dysregulation may precede clinical signs of iron overload cardiomyopathy.

Iron chelation is the main therapy available to treat conditions of iron overload. The therapy uses the parenteral iron chelator deferoxamine (DFO), and the oral iron chelators deferiprone (DFP) and deferasirox (DFX). Many studies have shown that DFO, DFP and DFX can cause a reduction in myocardial iron and improve cardiac function in β-thalassemia patients.[55,60–65] However, it has been suggested that combined chelation therapy be used in some iron overload β-thalassemia patients who have shown no improvement when treated with a single iron chelator, due to its increased efficacy.[62–65] At this time, new evidence indicates a greater efficacy for iron chelation when an iron chelator was combined with an antioxidant such as vitamin E and N-acetylcysteine (NAC).[66,67] Combined iron chelator plus NAC shows greater efficacy than vitamin E in reducing DNA damage in children β-thalassemia patients.[66] Currently, only the LTCC blocker has supporting evidence from clinical studies showing the improved cardiac function and decreased cardiac iron level in β-thalassemia patients; however, the study was only carried out using a small population.[68] Fernandes *et al.* showed that amlodipine reduces cardiac iron overload patients with

Table 6 A summary of the reports on the effects of pharmacological interventions on cardiac iron accumulation, cardiac function, and mitochondrial function of β-thalassemic mice (*in vivo* models) under iron overload conditions

Mouse models	Age	Iron overload	Intervention	Blood parameter	Heart weight	Cardiac iron	Cardiac autonomic regulation	Cardiac function	Cardiac mitochondria function and morphology	Ref.
Hbb^{th-3}/Hbb^{+}	7 weeks	Iron dextran i.p. injection (20 mg/day), 2 weeks	DFO (125 μg/g BW), 7 days		↔	↔				31
			Deferiprone (80 μg/g BW), 7 days		↔	↓				
Hbb^{th-3}/Hbb^{+}	2 months	Feeding with i ron diet 0.2% ferrocene, 2 months	Curcuminoid (Cur; 200 mg/kg/day), 2 months	↓ Plasma NTBI & MDA		↓	↓ LF/HF ratio			30
			Deferiprone (DFP; 50 mg/kg/day), 2 months	↓ Plasma NTBI, ↔ MDA		↓	↓ LF/HF ratio			
Hbb^{th-3}/Hbb^{+}	3 months	–	300 mg/kg/d (i.p.) Apo-transferrin, 60 days	↓ Plasma iron & NTBI ↑ RBC,Hb, Hct		↓				33
Hbb^{th-3}/Hbb^{+}	3–6 months	Feeding with iron diet 0.2% ferrocene, 3 months	DFO (42 mg/kg/day) Verapamil (10 mg/kg/day) Nifedipine (5 mg/kg/day) Efonidipine (4 mg/kg/day) Ebselen (5 mg/kg/day), 1 month	↓ Plasma NTBI ↓ Plasma MDA (except Ver & Nife)	↔	↓	↓ LF/HF ratio	↑ SV, CO, ESP, Pmax, dP/dtmax		14
Hbb^{th-3}/Hbb^{+}	3–6 months	Feeding with iron diet 0.2% ferrocene, 4 months	DFO (42 mg/kg/day) Deferiprone (75 mg/kg/day) Deferasirox (30 mg/kg/day) Efonidipine (4 mg/kg/day) Amlodipine (5 mg/kg/day), 1 month	↓ Plasma NTBI ↓ Plasma MDA (except amlodipine)	↔	↓	↓ LF/HF ratio	↑ SV, CO, ESP, Pmax, dP/dtmax	↑Mitochondria function Morphology; normal	19

RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; NTBI: non-transferrin bound iron; MDA: malondialdehyde; LF/HF: low frequency/high frequency; SV: stroke volume; CO: cardiac output; ESP: end systolic pressure; Pmax: maximum pressure; dP/dtmax: maximum dP/dt; i.p.: intra-peritoneal; DFO: deferoxamine.

β-thalassemia major; however, again only a small sample size was included in the study.[69] Only one case report showed that treatment with verapamil plus DFO improved cardiac function in an early stage of iron overload cardiomyopathy.[68] Larger and longer cohort studies are needed to verify these findings. Although treatment with LTCC blockers, TTCC blockers, DMT1 blockers or antioxidants were successful in animal models, large cohort clinical studies are needed to warrant their use in patients. The proposed mechanism responsible for these benefits could be due to the fact that iron chelators are effective in removing excess iron accumulation in cells and calcium channel blockers are expected to be particularly effective early on in iron overload by reducing tissue iron uptake and preventing oxidative stress occurring during the disease progression.

Conclusions

Information obtained from studies into the pathophysiologic mechanisms of iron overload on the heart in thalassemia mice have led us to better understand the clinical pictures of iron overload cardiomyopathy. In addition to the traditional pathways of iron uptake into cardiomyocytes, these studies using thalassemia mouse models have allowed us to discover novel pathways which could potentially cause iron overload cardiomyopathy, and ultimately may lead to the development of new strategies and drugs to prevent this lethal complication in thalassemia.

Authors' contributions: SK and NC collected experimental data and reviewed all articles cited in the paper; SK and NC wrote the main text; SK, SF, SCC, and NC revised the paper. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

This work is supported by grants from the Thailand Research Fund MRG5980222 (SK) and RTA (SCC), the NSTDA Research Chair Grant from the National Science and Technology Development Agency Thailand (NC), and Chiang Mai University Center of Excellence Award (NC).

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Rund D, Rachmilewitz E. Beta-thalassemia. *N Engl J Med* 2005;**353**:1135–46
2. Clegg JB, Weatherall DJ. Molecular basis of thalassaemia. *Br Med Bull* 1976;**32**:262–9
3. Cao A, Galanello R. Beta-thalassemia. *Genet Med* 2010;**12**:61–76
4. Weatherall DJ, Clegg JB. Molecular genetics of human hemoglobin. *Annu Rev Genet* 1976;**10**:157–78
5. Jahn CL, Hutchison CA 3rd, Phillips SJ, Weaver S, Haigwood NL, Voliva CF, Edgell MH. DNA sequence organization of the beta-globin complex in the BALB/c mouse. *Cell* 1980;**21**:159–68
6. Whitelaw E, Tsai SF, Hogben P, Orkin SH. Regulated expression of globin chains and the erythroid transcription factor GATA-1 during erythropoiesis in the developing mouse. *Mol Cell Biol* 1990;**10**:6596–606
7. Kremastinos DT, Farmakis D, Aessopos A, Hahalis G, Hamodraka E, Tsiapras D, Keren A. Beta-thalassemia cardiomyopathy: history, present considerations, and future perspectives. *Circ Heart Fail* 2010;**3**:451–8
8. Lekawanvijit S, Chattipakorn N. Iron overload thalassemic cardiomyopathy: iron status assessment and mechanisms of mechanical and electrical disturbance due to iron toxicity. *Can J Cardiol* 2009;**25**:213–8
9. Li Q, Peterson KR, Fang X, Stamatoyannopoulos G. Locus control regions. *Blood* 2002;**100**:3077–86
10. Russell ES. Hereditary anemias of the mouse: a review for geneticists. *Adv Genet* 1979;**20**:357–459
11. Skow LC, Burkhart BA, Johnson FM, Popp RA, Popp DM, Goldberg SZ, Anderson WF, Barnett LB, Lewis SE. A mouse model for beta-thalassemia. *Cell* 1983;**34**:1043–52
12. Shehee WR, Oliver P, Smithies O. Lethal thalassemia after insertional disruption of the mouse major adult beta-globin gene. *Proc Natl Acad Sci U S A* 1993;**90**:3177–81
13. Yang B, Kirby S, Lewis J, Detloff PJ, Maeda N, Smithies O. A mouse model for beta 0-thalassemia. *Proc Natl Acad Sci U S A* 1995;**92**:11608–12
14. Kumfu S, Chattipakorn S, Chinda K, Fucharoen S, Chattipakorn N. T-type calcium channel blockade improves survival and cardiovascular function in thalassemic mice. *Eur J Haematol* 2012;**88**:535–48
15. Incharoen T, Thephinlap C, Srichairatanakool S, Chattipakorn S, Winichagoon P, Fucharoen S, Vadolas J, Chattipakorn N. Heart rate variability in beta-thalassemic mice. *Int J Cardiol* 2007;**121**:203–4
16. Kumfu S, Chattipakorn S, Fucharoen S, Chattipakorn N. Mitochondrial calcium uniporter blocker prevents cardiac mitochondrial dysfunction induced by iron overload in thalassemic mice. *Biometals* 2012;**25**:1167–75
17. Kumfu S, Chattipakorn S, Fucharoen S, Chattipakorn N. Ferric iron uptake into cardiomyocytes of beta-thalassemic mice is not through calcium channels. *Drug Chem Toxicol* 2013;**36**:329–34
18. Kumfu S, Chattipakorn S, Srichairatanakool S, Settakorn J, Fucharoen S, Chattipakorn N. T-type calcium channel as a portal of iron uptake into cardiomyocytes of beta-thalassemic mice. *Eur J Haematol* 2011;**86**:156–66
19. Kumfu S, Chattipakorn SC, Fucharoen S, Chattipakorn N. Dual T-type and L-type calcium channel blocker exerts beneficial effects in attenuating cardiovascular dysfunction in iron-overloaded thalassaemic mice. *Exp Physiol* 2016;**101**:521–39
20. Musallam KM, Taher AT, Rachmilewitz EA. beta-thalassemia intermedia: a clinical perspective. *Cold Spring Harb Perspect Med* 2012;**2**:a013482
21. Origa R, Galanello R, Ganz T, Giagu N, Maccioni L, Faa G, Nemeth E. Liver iron concentrations and urinary hepcidin in beta-thalassemia. *Haematologica* 2007;**92**:583–8
22. Taher A, El Rassi F, Isma'eel H, Koussa S, Inati A, Cappellini MD. Correlation of liver iron concentration determined by R2 magnetic resonance imaging with serum ferritin in patients with thalassemia intermedia. *Haematologica* 2008;**93**:1584–6
23. Taher AT, Musallam KM, El-Beshlawy A, Karimi M, Daar S, Belhoul K, Saned MS, Graziadei G, Cappellini MD. Age-related complications in treatment-naive patients with thalassaemia intermedia. *Br J Haematol* 2010;**150**:486–9
24. Voskaridou E, Douskou M, Terpos E, Papassotiriou I, Stamoulakatou A, Ourailidis A, Loutradi A, Loukopoulos D. Magnetic resonance imaging in the evaluation of iron overload in patients with beta thalassaemia and sickle cell disease. *Br J Haematol* 2004;**126**:736–42
25. Lombardo T, Tamburino C, Bartoloni G, Morrone ML, Frontini V, Italia F, Cordaro S, Privitera A, Calvi V. Cardiac iron overload in thalassemic patients: an endomyocardial biopsy study. *Ann Hematol* 1995;**71**:135–41
26. Au WY, Lam WW, Chu WW, Tam S, Wong WK, Lau J, Yeung YM, Liu HS, Liang R. Organ-specific hemosiderosis and functional correlation in Chinese patients with thalassemia intermedia and hemoglobin H disease. *Ann Hematol* 2009;**88**:947–50
27. Glickstein H, El RB, Link G, Breuer W, Konijn AM, Hershko C, Nick H, Cabantchik ZI. Action of chelators in iron-loaded cardiac cells: accessibility to intracellular labile iron and functional consequences. *Blood* 2006;**108**:3195–203
28. Taher AT, Musallam KM, Wood JC, Cappellini MD. Magnetic resonance evaluation of hepatic and myocardial iron deposition in

transfusion-independent thalassemia intermedia compared to regularly transfused thalassemia major patients. *Am J Hematol* 2010;**85**:288–90
29. Kumfu S, Chattipakorn SC, Fucharoen S, Chattipakorn N. Effects of iron overload condition on liver toxicity and hepcidin/ferroportin expression in thalassemic mice. *Life Sci* 2016;**150**:15–23
30. Thephinlap C, Phisalaphong C, Lailerd N, Chattipakorn N, Winichagoon P, Vadolas J, Fucharoen S, Porter JB, Srichairatanakool S. Reversal of cardiac iron loading and dysfunction in thalassemic mice by curcuminoids. *Med Chem* 2011;**7**:62–9
31. Yatmark P, Morales NP, Chaisri U, Wichaiyo S, Hemstapat W, Srichairatanakool S, Svasti S, Fucharoen S. Iron distribution and histopathological characterization of the liver and heart of beta-thalassemic mice with parenteral iron overload: Effects of deferoxamine and deferiprone. *Exp Toxicol Pathol* 2014;**66**:333–43
32. Gardenghi S, Marongiu MF, Ramos P, Guy E, Breda L, Chadburn A, Liu Y, Amariglio N, Rechavi G, Rachmilewitz EA, Breuer W, Cabantchik ZI, Wrighting DM, Andrews NC, de Sousa M, Giardina PJ, Grady RW, Rivella S. Ineffective erythropoiesis in beta-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. *Blood* 2007;**109**:5027–35
33. Gelderman MP, Baek JH, Yalamanoglu A, Puglia M, Vallelian F, Burla B, Vostal J, Schaer DJ, Buehler PW. Reversal of hemochromatosis by apotransferrin in non-transfused and transfused Hbbth3/+ (heterozygous B1/B2 globin gene deletion) mice. *Haematologica* 2015;**100**:611–22
34. Wood JC. Cardiac complications in thalassemia major. *Hemoglobin* 2009;**33**:S81–6
35. Aessopos A, Farmakis D, Hatziliami A, Fragodimitri C, Karabatsos F, Joussef J, Mitilineou E, Diamanti-Kandaraki E, Meletis J, Karagiorga M. Cardiac status in well-treated patients with thalassemia major. *Eur J Haematol* 2004;**73**:359–66
36. Marsella M, Borgna-Pignatti C, Meloni A, Caldarelli V, Dell'Amico MC, Spasiano A, Pitrolo L, Cracolici E, Valeri G, Positano V, Lombardi M, Pepe A. Cardiac iron and cardiac disease in males and females with transfusion-dependent thalassemia major: a T2* magnetic resonance imaging study. *Haematologica* 2011;**96**:515–20
37. Sanyear C, Butthep P, Nithipongvanich R, Sirankapracha P, Winichagoon P, Fucharoen S, Svasti S. Cardiomyocyte ultrastructural damage in beta-thalassaemic mice. *Int J Exp Pathol* 2013;**94**:336–42
38. Stoyanova E, Trudel M, Felfly H, Garcia D, Cloutier G. Characterization of circulatory disorders in beta-thalassemic mice by noninvasive ultrasound biomicroscopy. *Physiol Genomics* 2007;**29**:84–90
39. Van Wyck DB, Tancer ME, Popp RA. Iron homeostasis in beta-thalassemic mice. *Blood* 1987;**70**:1462–5
40. Stoyanova E, Cloutier G, Felfly H, Lemsaddek W, Ah-Son N, Trudel M. Evidence for a novel mechanism independent of myocardial iron in beta-thalassemia cardiac pathogenesis. *PLoS One* 2012;**7**:e52128
41. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Origa R, Piga A, Romeo MA, Zhao H, Cnaan A. Survival and complications in thalassemia. *Ann N Y Acad Sci* 2005;**1054**:40–7
42. Carpenter JP, He T, Kirk P, Roughton M, Anderson LJ, de Noronha SV, Sheppard MN, Porter JB, Walker JM, Wood JC, Galanello R, Forni G, Catani G, Matta G, Fucharoen S, Fleming A, House MJ, Black G, Firmin DN, St Pierre TG, Pennell DJ. On T2* magnetic resonance and cardiac iron. *Circulation* 2011;**123**:1519–28
43. Kaye SB, Owen M. Cardiac arrhythmias in thalassaemia major: evaluation of chelation treatment using ambulatory monitoring. *Br Med J* 1978;**1**:342
44. Engle MA, Erlandson M, Smith CH. Late cardiac complications of chronic, severe, refractory anemia with hemochromatosis. *Circulation* 1964;**30**:698–705
45. Detterich J, Noetzli L, Dorey F, Bar-Cohen Y, Harmatz P, Coates T, Wood J. Electrocardiographic consequences of cardiac iron overload in thalassemia major. *Am J Hematol* 2012;**87**:139–44
46. Russo V, Rago A, Papa AA, Nigro G. Electrocardiographic presentation, cardiac arrhythmias, and their management in beta-thalassemia major patients. *Ann Noninvasive Electrocardiol* 2016;**21**:335–42
47. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;**22**:2171–9
48. Breuer W, Ermers MJ, Pootrakul P, Abramov A, Hershko C, Cabantchik ZI. Desferrioxamine-chelatable iron, a component of serum non-transferrin-bound iron, used for assessing chelation therapy. *Blood* 2001;**97**:792–8
49. Jakeman A, Thompson T, McHattie J, Lehotay DC. Sensitive method for nontransferrin-bound iron quantification by graphite furnace atomic absorption spectrometry. *Clin Biochem* 2001;**34**:43–7
50. Koonrungsesomboon N, Tantiworawit A, Phrommintikul A, Saekho S, Srichairattanakool S, Chattipakorn N. Heart rate variability for early detection of iron overload cardiomyopathy in beta-thalassemia patients. *Hemoglobin* 2015;**39**:281–6
51. Rutjanaprom W, Kanlop N, Charoenkwan P, Sittiwangkul R, Srichairatanakool S, Tantiworawit A, Phrommintikul A, Chattipakorn S, Fucharoen S, Chattipakorn N. Heart rate variability in beta-thalassemia patients. *Eur J Haematol* 2009;**83**:483–9
52. Silvilairat S, Charoenkwan P, Saekho S, Tantiworawit A, Phrommintikul A, Srichairatanakool S, Chattipakorn N. Heart rate variability for early detection of cardiac iron deposition in patients with transfusion-dependent thalassemia. *PLoS One* 2016;**11**:e0164300
53. Wijarnpreecha K, Siri-Angkul N, Shinlapawittayatorn K, Charoenkwan P, Silvilairat S, Siwasomboon C, Visarutratna P, Srichairatanakool S, Tantiworawit A, Phrommintikul A, Chattipakorn SC, Chattipakorn N. Heart rate variability as an alternative indicator for identifying cardiac iron status in non-transfusion dependent thalassemia patients. *PLoS One* 2015;**10**:e0130837
54. Anderson LJ, Westwood MA, Holden S, Davis B, Prescott E, Wonke B, Porter JB, Walker JM, Pennell DJ. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol* 2004;**127**:348–55
55. Smith GC, Alpendurada F, Carpenter JP, Alam MH, Berdoukas V, Karagiorga M, Ladis V, Piga A, Aessopos A, Gotsis ED, Tanner MA, Westwood MA, Galanello R, Roughton M, Pennell DJ. Effect of deferiprone or deferoxamine on right ventricular function in thalassemia major patients with myocardial iron overload. *J Cardiovasc Magn Reson* 2011;**13**:34
56. Alpendurada F, Carpenter JP, Deac M, Kirk P, Walker JM, Porter JB, Banya W, He T, Smith GC, Pennell DJ. Relation of myocardial T2* to right ventricular function in thalassaemia major. *Eur Heart J* 2010;**31**:1648–54
57. Alp A, Ozdogan O, Guloglu CC, Turker M, Atabay B. Heart rate variability in beta-thalassaemia major with or without cardiac siderosis. *Cardiol Young* 2014;**24**:263–7
58. Gurses D, Ulger Z, Levent E, Aydinok Y, Ozyurek AR. Time domain heart rate variability analysis in patients with thalassaemia major. *Acta Cardiol* 2005;**60**:477–81
59. Kardelen F, Tezcan G, Akcurin G, Ertug H, Yesilipek A. Heart rate variability in patients with thalassemia major. *Pediatr Cardiol* 2008;**29**:935–9
60. Pennell DJ, Porter JB, Cappellini MD, Chan LL, El-Beshlawy A, Aydinok Y, Ibrahim H, Li CK, Viprakasit V, Elalfy MS, Kattamis A, Smith G, Habr D, Domokos G, Roubert B, Taher A. Deferasirox for up to 3 years leads to continued improvement of myocardial T2* in patients with beta-thalassemia major. *Haematologica* 2012;**97**:842–8
61. Pennell DJ, Berdoukas V, Karagiorga M, Ladis V, Piga A, Aessopos A, Gotsis ED, Tanner MA, Smith GC, Westwood MA, Wonke B, Galanello R. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. *Blood* 2006;**107**:3738–44
62. Pennell DJ, Carpenter JP, Roughton M, Cabantchik Z. On improvement in ejection fraction with iron chelation in thalassemia major and the risk of future heart failure. *J Cardiovasc Magn Reson* 2011;**13**:45
63. Pepe A, Meloni A, Rossi G, Cuccia L, D'Ascola GD, Santodirocco M, Cianciulli P, Caruso V, Romeo MA, Filosa A, Pitrolo L, Putti MC, Peluso A, Campisi S, Missere M, Midiri M, Gulino L, Positano V,

Lombardi M, Ricchi P. Cardiac and hepatic iron and ejection fraction in thalassemia major: multicentre prospective comparison of combined deferiprone and deferoxamine therapy against deferiprone or deferoxamine monotherapy. *J Cardiovasc Magn Reson* 2013;**15**:1

64. Lai ME, Grady RW, Vacquer S, Pepe A, Carta MP, Bina P, Sau F, Cianciulli P, Maggio A, Galanello R, Farci P. Increased survival and reversion of iron-induced cardiac disease in patients with thalassemia major receiving intensive combined chelation therapy as compared to desferoxamine alone. *Blood Cells Mol Dis* 2010;**45**:136–9
65. Wongjaikam S, Kumfu S, Chattipakorn SC, Fucharoen S, Chattipakorn N. Current and future treatment strategies for iron overload cardiomyopathy. *Eur J Pharmacol* 2015;**765**:86–93
66. Ozdemir ZC, Koc A, Aycicek A, Kocyigit A. N-acetylcysteine supplementation reduces oxidative stress and DNA damage in children with β-thalassemia. *Hemoglobin* 2014;**15**:1–6
67. Rachmilewitz EA, Weizer-Stern O, Adamsky K, Amariglio N, Rechavi G, Breda L, Rivella S, Cabantchik ZI. Role of iron in inducing oxidative stress in thalassemia: can it be prevented by inhibition of absorption and by antioxidants? *Ann N Y Acad Sci* 2005;**1054**:118–123
68. Sugishita K, Asakawa M, Usui S, Takahashi T. A case of iron overload cardiomyopathy: beneficial effects of iron chelating agent and calcium channel blocker on left ventricular dysfunction. *Int Heart J* 2009;**50**:829–38
69. Fernandes JL, Sampaio EF, Fertrin K, Coelho OR, Loggetto S, Piga A, Verissimo M, Saad ST. Amlodipine reduces cardiac iron overload in patients with thalassemia major: a pilot trial. *Am J Med* 2013;**126**:834–7