Minireview

Iron overload cardiomyopathy: Using the latest evidence to inform future applications

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Impact Statement

Iron overload cardiomyopathy (IOC) is the main cause of death in iron overload patients. Although iron chelation therapy is the standard treatment for this condition, combining it with other therapeutic approaches may improve the clinical outcomes. There is growing evidence to demonstrate promising results associated with the use of calcium channel blockers, antioxidants, mitochondrial dynamic modulators, and cell death inhibitors. This review provides comprehensive information regarding these potential therapeutic strategies against IOC.

Abstract

Iron overload can be the result of either dysregulated iron metabolism in the case of hereditary hemochromatosis or repeated blood transfusions in the case of secondary hemochromatosis (e.g. in β-thalassemia and sickle cell anemia patients). Under iron overload conditions, transferrin (Tf) saturation leads to an increase in non-Tf bound iron which can result in the generation of reactive oxygen species (ROS). These excess ROS can damage cellular components, resulting in the dysfunction of vital organs including iron overload cardiomyopathy (IOC). Multiple studies have demonstrated that L-type and T-type calcium channels are the main routes for iron uptake in the heart, and that calcium channel blockers, given either individually or in combination with standard iron chelators, confer cardioprotective effects under iron overload conditions. Treatment with antioxidants may also provide therapeutic benefits. Interestingly, recent studies have suggested that mitochondrial dynamics and regulated cell death (RCD) pathways are potential targets for pharmacological interventions against iron-induced cardiomyocyte injury. In this review, the potential therapeutic roles of iron chelators, antioxidants, iron uptake/metabolism modulators, mitochondrial dynamics modulators, and inhibitors of RCD pathways in IOC are summarized and discussed.

Keywords: Iron overload cardiomyopathy, iron chelator, oxidative stress, mitochondrial dynamics, regulated cell death

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Introduction

Hereditary hemochromatosis (HH) is a group of genetic disorders in which defects of iron metabolism result in iron overload through increased intestinal iron absorption.[1] However in secondary hemochromatosis, repeated blood transfusions are the main cause of iron overload, often seen in patients with transfusion-dependent thalassemia (TDT) or sickle cell disease.[2] In addition, in mild or moderate secondary hemochromatosis which is non-transfusion dependent, patients can develop iron overload from increased intestinal iron absorption secondary to ineffective erythropoiesis.[1,2] Under iron overload conditions, the non-transferrin bound iron (NTBI) can be deposited in many vital tissues, leading to the generation of highly toxic reactive oxygen species (ROS) via the Fenton reaction.[3] Cardiac iron accumulation culminates in IOC, which is a common cause of death in iron overload patients.[3] The excess ROS can damage cellular and organellar (in particular, mitochondrial) macromolecules, including membrane lipids, proteins, and nucleic acids, leading to organ dysfunction.[3] Although iron chelation therapy remains the standard treatment for iron overload patients, inadequate chelation and harmful side effects are of concern, and mortality rates in iron overload patients receiving iron chelation are still high.[4] Therefore, fundamental insights regarding the cardiac iron uptake mechanisms and the pathophysiology of iron toxicity in cardiomyocytes are crucial to the devising of novel therapeutic strategies to achieve better clinical outcomes for iron overload patients.

Mechanisms involved in iron-mediated cardiomyocyte damage

Under the physiological condition, iron is an essential element involved in numerous metabolic and biological

processes including cell growth, cellular respiration and metabolism, and DNA synthesis and repair; however, an excessive level of iron can result in tissue damage due to oxidative stress.[5,6] Normally, dietary iron, an inorganic iron (ferric [Fe^{3+}] ion), is reduced to the ferrous ion (Fe^{2+}) by a ferrireductase (Dcytb, duodenal cytochrome b reductase) at the apical surface of the brush border and then taken up into enterocytes via divalent metal transporter-1 (DMT1).[5,6] The iron then enters the blood circulation via the iron exporter ferroportin which is regulated by hepcidin.[5,6] Hephaestin oxidizes the released iron to ferric ions, which then bind to plasma transferrin (Tf) to form the Tf-bound iron complex, which transports iron into several cells via transferrin receptors (TFRs) for use in cellular metabolism or storage in the iron storage protein ferritin.[5,6] In general, cellular iron homeostasis is regulated by IRPs/IREs (iron regulatory proteins/iron-responsive elements) system which ensures adequate iron supply for cell needs without cellular toxicity.[7] IRPs are cytosolic proteins which can bind to IREs in the 3′ or 5′ untranslated regions (UTRs) of mRNAs encoding proteins involved in iron uptake, storage, export, and also enzymes for heme synthesis and iron-sulfur protein subunit (Fe-S) in mitochondrial electron transport chain.[7] When IRPs bind to IREs at the 5′UTR of target mRNAs, IRPs can inhibit the translation of those target mRNAs, including iron storage protein (ferritin), iron export protein (ferroportin), enzyme for heme synthesis (5-aminolevulinate synthase; ALAS) as well as iron-sulfur protein subunit in the mitochondrial electron transport chain (mitochondrial aconitase and succinate dehydrogenase). In contrast, when IRPs bind to IREs at the 3′UTR of target mRNAs, it enhances the expression via blocking the mRNAs degradation that include iron import proteins (TfR1 and DMT1).[7] In summary, when cell is in the stage of iron depletion, IRPs will bind to the IREs of target mRNAs to enhance iron import and reduce iron export and storage to maintain optimal intracellular iron concentrations. When the levels of iron are higher than the physiological level, reduced IRPs binding to IREs will happen, resulting in increased iron export and storage protein expression, and decreased iron import protein expression.[7] However, under the condition of chronic iron overload such as severe hemochromatosis diseases, this mechanism is not sufficient to prevent iron toxicity in those diseases. Under iron overload conditions, the excess of iron in the blood circulation triggers Tf saturation, leading to the appearance of NTBI which can be deposited in many vital organs.[5,6] Many studies have demonstrated that L-type and T-type calcium channels (LTCC and TTCC) are the main routes for cardiac iron uptake under iron overload conditions.[8–11] Recently, this study demonstrated that lipocalin-2 (LCN-2) and its receptor were essential for Fe^{3+} uptake into iron-overloaded cultured cardiomyocytes.[12] However, cardiac iron overload is rarely observed in HH patients, which is characterized by iron overload in different organs including the liver and pancreas.[5] In animal models with HH, it was found that cardiomyocytes can protect themselves by increasing iron export under iron overload conditions.[5] However, when systemic iron overload is severe (ferritin $\geqslant$1000 μg/L), cardiac iron overload can be observed, but this is still at a more reduced rate in comparison to other organs.[5]

The excess intracellular free iron causes increased production of highly toxic radicals, produced via the Fenton reaction, which can induce damage to DNA, proteins, and membrane lipids.[3] Cardiac excitation–contraction coupling-related proteins are highly sensitive to iron overload toxicity, leading to impaired cardiac function as seen in IOC, and iron excess in the conduction system also leads to arrhythmias.[5] Mitochondrial dysfunction through cardiac mitochondrial iron overload is also a major cause of IOC since the homeostasis of mitochondrial iron is crucial for the preservation of overall cardiac iron homeostasis.[5] Evidence suggests that cytosolic iron can enter the mitochondria via mitoferrin 2 (Mfrn2) and mitochondrial calcium uniporter (MCU) to be used for synthesizing enzyme of heme synthesis as well as Fe-S proteins in the mitochondrial electron transport chain.[7] Here, iron can be exported via ATP-binding cassette subfamily B member 8 (ABCB8).[5–7] The excess iron in the mitochondria can be stored in the mitochondrial ferritin (mtFT), which is the defense mechanism protein observed during cardiac injury.[5,6] In addition, frataxin (FXN) is the important protein in mitochondria that involves in iron metabolism including mitochondrial iron storage protein, and acts as an iron chaperone for Fe-S and heme biosynthesis in the mitochondria.[7] The deficiency of frataxin causes Friedreich's Ataxia which is an inherited disease that causes severe neurodegeneration. The excess iron in mitochondria can be stored in mtFT, which is the defense mechanism protein response during cardiac injury.[5,6] Under conditions of iron overload, the excess intracellular iron can be taken up into the mitochondria which generates oxidative stress in mitochondria, as indicated by increased ROS production, depolarization of mitochondrial membrane potential and mitochondrial swelling, leading to mitochondrial dysfunction.[13,14] Mitochondrial membrane depolarization under iron overload conditions leads to the release of cytochrome, resulting in activation of the caspase cascade and apoptosis.[15] In addition, iron overload has been found to cause mitochondrial dynamic imbalance by promoting mitochondrial fission and suppressing mitochondrial fusion.[13] Intracellular and mitochondrial iron overload have also been observed in association with the cardiotoxicity caused by myocardial infarction (MI) and doxorubicin (DOX).[6] In the past decades, iron dysmetabolism has been observed in association with MI which is characterized by increased iron import via TFR and reduced ability for iron sequestration into ferritin in cardiomyocytes in the chronic phase of the disease.[6] In the case of DOX, a chemotherapeutic agent widely used to treat various cancers, cardiotoxicity is one of its serious side effects.[6] In the animal models treated with DOX, DOX-induced iron overload is caused by the increase in non-heme iron resulting from heme degradation.[16] Moreover, both *in vitro* and *in vivo* data show that iron overload induced by DOX is concentrated in mitochondria, leading to mitochondrial iron overload.[6,16] However, the actions of DOX on cellular iron metabolism are quite complex as although it causes increased iron and oxidative stress in mitochondria, it reduces iron import and enhances the ability of ferritin to bind free iron in the cytoplasm and mitochondria. Additional mechanisms involving inflammation, fibrosis, and several cell death pathways can account for the pathophysiologic processes of iron-induced cardiac

damage that leads to heart failure (HF).[3] Inflammation is associated with increased pro-inflammatory cytokine levels including tumor necrosis factor alpha (TNF-α) which can promote pathological cardiac remodeling (hypertrophy and fibrosis).[17] A previous study showed that forkhead box protein O 1 (FOXO1) was involved in the development of cardiac hypertrophy and also the regulation of calcium ion homeostasis, protein synthesis, autophagy, and apoptosis in cardiomyocytes.[18] Sirtuin 1 (SIRT1) is an enzyme that deacetylates the transcription factors involved in cellular regulation, and responses to stress.[19] Under conditions of oxidative stress, it has been shown that SIRT1 can regulate FOXO-mediated transcription via nicotine adenine dinucleotide (NAD)-dependent deacetylation.[19] In both acquired and genetic IOC rodents models, it has been demonstrated that iron overload increased the acetylation of FOXO1 and nuclear accumulation with an inverse correlation to changes in the SIRT1 levels of the heart and liver.[20,21]

Regulated cell death (RCD) is an important process that maintains organismal homeostasis and also plays a pivotal role in pathologic conditions.[22] In the past decade, the type of RCD in the heart which has been studied the most is apoptosis which is believed to contribute to the pathogenesis of IOC.[15,23] A previous study found that iron overload induced caspase-3 activation resulting in cardiac apoptosis via mitochondria-dependent pathways in HL-1 cardiomyocytes.[15] It has also been found that the mitochondrial dysfunction associated with iron overload can lead to the activation of the caspase-dependent apoptotic pathway in an iron-overloaded mouse model.[13,24] Furthermore, necroptosis (programmed necrosis) also plays a role in the pathophysiological process of IOC by induced cell death via the TNF-α activated receptor-interacting serine/threonine-protein kinase 1/3 (RIPK1/3) pathway.[25] Although pyroptosis is a pro-inflammatory pathway of programmed cell death, as is necroptosis, there is no evidence to indicate the involvement of pyroptosis in iron overload models. In addition, the recently identified iron-dependent programmed cell death, ferroptosis, has been found to participate in the pathophysiological process of IOC.[25,26] Nevertheless, studies regarding the role of cardiac mitochondrial dynamics, necroptosis, and ferroptosis under iron overload conditions are still limited.

In this review, the evidence of the potential uses of iron chelators, antioxidants, iron uptake and metabolism modulators, mitochondrial dynamic modulators, and several cell death pathway inhibitors in the treatment in IOC is evaluated and discussed. Although clinical trials have already begun into gene therapy to improve erythropoiesis for the treatment of TDT patients, their effects on IOC are not included in this review and will need to be evaluated in the future.[27] The aim of our review is to provide therapeutic target insights into how future studies can inform and identify better treatments for IOC patients. The pathophysiologic mechanism-based therapeutic approaches for treatment of IOC are summarized in Figure 1. Several therapeutic targets for pharmacological interventions in IOC are discussed below and proposed in Figure 1 including modulation of iron absorption via hepcidin and ferroportin, promotion of iron clearance by iron chelators, blockading against cardiac iron entry through calcium channels and lipocalin-2, attenuation of oxidative stress by antioxidants, modulation of mitochondrial dynamics, and inhibition of several RCD pathways.

Therapeutic targets in IOC

Systemic and cardiac iron

The main iron chelators used in standard chelation therapy for iron overload patients are deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX).[4] The differences between the three drugs include routes of administration, pharmacokinetics profiles (e.g. half-life), and adverse effects. DFO has a 30-min half-life and must be administered by continuous subcutaneous or intravenous infusion.[4] DFP and DFX are oral chelators with longer half-lives (2–3 h and 8–16 h, respectively).[4] Important adverse effects are local reactions at the site of infusion for DFO, agranulocytosis for DFP, and a decrease in renal function for DFX.[4]

Previous evidence has shown that iron chelation improves cardiac function, prevents arrhythmias, and reduces mortality in secondary iron overload patients, especially TDT patients.[28–32] Previous studies have demonstrated decreased cardiac morbidity and mortality, as well as reduced cardiac iron deposition among iron overload patients treated with DFP when compared with DFO.[32,33] DFP has also been shown to be more effective than DFX in lowering cardiac iron deposition and improving cardiac function.[28,29] Although the total iron excretion achieved by DFP is reportedly less than that by DFO and DFX, DFP could have a more significant cardioprotective effect due to its ability to cross the cell membrane.[28,29] Although each of these three chelators generally reduces cardiac iron deposition and improves ventricular function, these may not be accomplished in some patients receiving a single iron chelator.[4] Therefore, various combinations of iron chelators have been commonly used to treat iron overload (especially in TDT) patients with left ventricular dysfunction.[30,31] In comparison with DFO alone, a combination of DFO with DFP has been shown to reduce myocardial iron deposition further and to improve ventricular and endothelial function.[30,31] However, there are still relatively few clinical studies that test the cardioprotective efficacy of other combined chelator regimens (DFO plus DFX or DFP plus DFX).[34,35] Since cardiac complications are still common and remain a major cause of mortality in iron overload patients who have already received iron chelation,[4,32] novel therapeutic approaches are warranted.

Calcium channels

In addition to iron chelation, new therapeutic strategies in IOC are focused on the blockade of LTCC and TTCC, both of which are capable of transporting iron into cardiomyocytes under iron overload conditions.[8–11] Calcium channel blockers (CCBs) are widely used as antihypertensive drugs since they inhibit Ca^{2+} influx in vascular smooth muscle cells, thus causing vasodilation.[36] Several studies have demonstrated that CCBs also exert beneficial effects on the iron-overloaded heart.[8–11,37–39] In *in vitro* and *ex vivo* studies, efonidipine (a TTCC blocker)[9,40] or verapamil and amlodipine (LTCC blockers)[15] could prevent iron uptake into cardiomyocytes

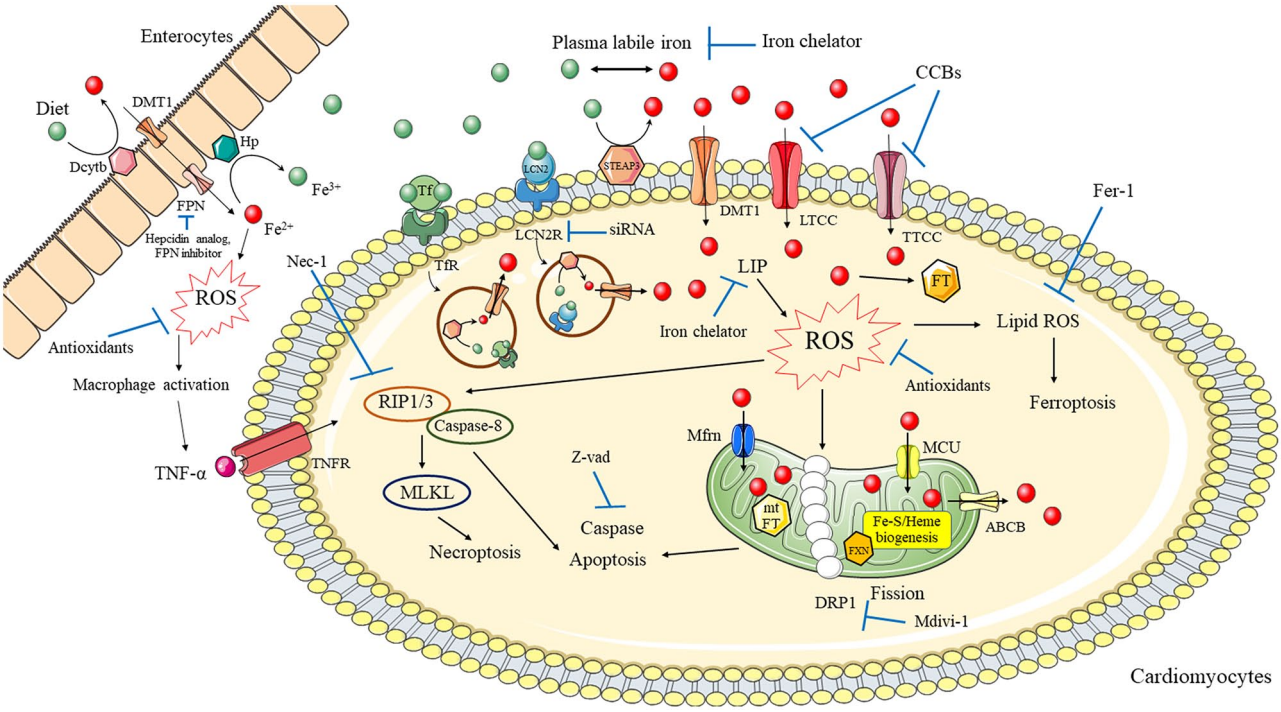

Figure 1. Pathophysiological mechanism-based therapeutic approaches for treatment of iron overload cardiomyopathy. Dietary iron uptake through enterocytes into blood circulation via the iron exporter ferroportin which is regulated by hepcidin. Excess iron, both from intestinal absorption and blood transfusion, can potentially be taken up into cardiomyocytes via LTCC, TTCC, and LCN-2 under iron overload conditions. Cytosolic iron can enter the mitochondria via Mfrn and MCU for synthesized protein of heme synthesis and Fe-S proteins for electron transport chain which can regulate by FXN, while iron can be exported via ABCB8. The excess iron in mitochondria can be stored in the mitochondrial ferritin (mtFT). Iron overload causes cardiomyocyte injury via alteration of cardiac mitochondrial dynamics, impairment of cardiac mitochondrial function, and induction of several regulated cell death pathways including apoptosis, ferroptosis, and necroptosis. Several clinical strategies have been proposed including modulation of iron absorption via enhanced hepcidin levels, promotion of iron clearance by iron chelators, blockading against cardiac iron entry by calcium channel blockers, and attenuation of oxidative stress by antioxidants. Evidence from preclinical research indicates several novel potential targets for pharmacological interventions and continues to reveal additional mechanistic insights regarding iron overload cardiomyopathy including the inhibition of Fe^{3+} uptake via LCN-2, the use of mitochondrial dynamics modulators, and the inhibitors of several regulated cell death pathways. (A color version of this figure is available in the online journal.)

ABCB: ATP-binding cassette subfamily B member; CCBs: calcium channel blockers; Dcytb: duodenal cytochrome b; DMT1: divalent metal transporter; DRP1: dynamin-related protein 1; Fer-1: ferrostatin-1; Fe-S: iron-sulfur; FPN: ferroportin; FT: ferritin; FXN: frataxin; Hp: hephaestin; LCN2: lipocalin 2; LCN2R: lipocalin 2 receptor; LIP: labile iron pool; LTCC: L-type calcium channel; MCU: mitochondrial calcium uniporter; Mdivi-1: mitochondrial division inhibitor-1; Mfrn: mitoferrin; MLKL: mixed lineage kinase domain-like protein; mtFT: mitochondrial ferritin; Nec-1: necrostatin-1; RIPK1/3: receptor-interacting protein kinase 1/3; ROS: reactive oxygen species; siRNA: silencing RNA; STEAP3: six-transmembrane epithelial antigen of the prostate 3; Tf: transferrin; TfR: transferrin receptor; TNF-α: tumor necrosis factor alpha; TNFR: tumor necrosis factor receptor; TTCC: T-type calcium channel; Z-vad: Z-VAD-FMK.

and the Langendorff-perfused isolated heart[39] under iron overload conditions. In several rodent models and thalassemic mice with iron overload, treatment with either LTCC blockers (amlodipine, nifedipine, and verapamil) or a TTCC blocker (efonidipine) resulted in a significant decrease in Fe^{2+} uptake, an attenuated deposition of cardiac iron, and an improvement in cardiac mitochondrial function, resulting in improved cardiac function.[8–11,37–39] All findings suggested that LTCC and TTCC blockers could provide cardioprotective effects under iron overload conditions. Therefore, clinical studies are needed to warrant the potential use of CCBs on preventing cardiac iron deposition and left ventricular dysfunction in iron overload patients. Currently, some clinical data on LTCC blockers are available; however, no clinical data on TTCC blockers are available to support the findings from preclinical studies.

The results from clinical studies supported the preclinical data which demonstrated that treatment with a combination of an iron chelator (DFO, DFP, DFX, and DFO + DFP) and a LTCC blocker (amlodipine) resulted in a decrease in cardiac iron levels, as verified by cardiac magnetic resonance imaging T2* (MRI T2*); however, this was without any improvement in left ventricular ejection fraction (LVEF) in β-thalassemia major patients.[41–43] Only one case report on patients with IOC exists to date which demonstrated that treatment with DFO plus verapamil improved left ventricular function.[44] However, the applicability of CCBs may be limited in the patients in an advanced phase of systolic cardiac dysfunction due to their negative inotropic effects, and some side effects including gastrointestinal (GI) upset, dizziness, and swollen ankles are noted.[45] Since all these reports were based on a short observation period and a small sample size, larger prospective studies with long duration of follow-up are needed to warrant the use of a combination of a CCB and iron chelator in IOC patients. Moreover, although TTCC blockers have shown cardioprotective effects in animal models with iron-induced cardiotoxicity,[8–10] further clinical studies are needed to confirm these beneficial effects in human subjects.

Lipocalin-2

Lipocalin-2 (LCN2), or neutrophil gelatinase-associated lipocalin (NGAL), is a protein originally found in human

neutrophils which confers an aspect of innate immunity by sequestrating iron from bacteria, thus preventing their growth.[46] Cardiomyocytes have been shown to express LCN-2 receptors (LCN-2R) and produce LCN-2, which were found to be significantly increased in both experimental and clinical HF.[47,48] Previous studies have demonstrated that LCN-2 levels were significantly increased in cardiomyocytes of post-MI rats[48] and also increased in the serum of patients who had MI, chronic HF,[48,49] or β-thalassemia.[50] In addition, cultured cardiomyocytes exposed to LCN-2 showed increased intracellular iron levels and apoptosis.[51] Since LCN-2 levels in both serum and the heart are often significantly increased in HF and thalassemia patients, it has been proposed that LCN-2 and LCN-2-R potentially play a role in iron transport into cardiomyocytes in IOC. A recent study demonstrated that downregulation of LCN-2 and LCN-2 receptors using siRNA improved cardiomyocyte viability via decreasing iron uptake, improving mitochondrial dynamics, and reducing apoptosis in the Fe^{3+} overload condition, while treatments with either an LTCC or TTCC blocker showed similar beneficial effects on those parameters in Fe^{2+} overload scenarios.[12] These findings suggested that LCN-2 and LCN-2R play roles in Fe^{3+} uptake, while LTCC and TTCC were essential for Fe^{2+} uptake into the cardiomyocytes under iron overload conditions. Thus, in addition to CCBs that are potentially effective against cardiac Fe^{2+} toxicity, the inhibition of cardiac Fe^{3+} uptake via LCN-2/LCN-2R could potentially be therapeutically beneficial in IOC. Since LCN-2 acts on the bacteriostatic proteins by binding to the iron-loaded siderophores required for bacterial growth, the inhibition of these proteins under iron overload conditions may let the patients be more susceptible to infection. However, due to the limited number of investigations available, further preclinical and clinical studies are needed to support these findings.

Hepcidin and ferroportin

Other potential therapeutic targets to prevent organ iron overload are the modulated iron regulatory proteins, which include hepcidin and ferroportin. Hepcidin, is an iron regulatory hormone, primarily synthesized by hepatocytes, which regulates the entry of iron into the circulation by downregulating the iron exporter ferroportin, thus inhibiting enterocyte iron absorption and macrophage iron release.[52] Matriptase-2, or transmembrane protease serine 6 (TMPRSS6), is an enzyme mainly expressed in the liver that inhibits the expression of hepcidin.[52] Several studies have demonstrated that the increased hepcidin levels induced by TMPRSS6 siRNA[53,54] or antisense oligonucleotides[55] and minihepcidins[56] could decrease iron overload and anemia in an NTDT mouse model. Likewise, an oral ferroportin inhibitor (VIT-2763) reduced liver iron and improved anemia in an NTDT mouse model, and also transiently decreased serum iron and Tf saturation in a phase-1 study in healthy volunteers.[57] However, since iron overload in the TDT model is mainly from repeated blood transfusions rather than increased GI iron uptake, the beneficial effects of hepcidin activators or ferroportin inhibitors in TDT patients are still unclear. In addition, information regarding the manipulative effects of hepcidin or ferroportin on IOC is still lacking.

Oxidative stress

The excess accumulation of iron leads to increased generation of ROS which is a major underlying basis of disease and known to damage several cellular components.[3] Previous studies demonstrated that sex steroids and sexual dimorphism also play a role in cardiac iron metabolism in IOC both in experimental animal and human models.[58,59] Males were shown to be more susceptible to iron overload-mediated organ injury than females, leading to worsening clinical outcomes in β-thalassemia, sickle cell anemia, and primary hemochromatosis patients.[58,59] However, the relationship between androgens and estrogens and the proteins involved in cardiac iron overload is quite complex and will need to be further evaluated in future studies. Sex hormones are known to affect both the antioxidant status and the response to oxidative stress under conditions of iron overload.[58,59] Increased lipid peroxidation and antioxidant depletion has been demonstrated in iron overload patients, and oxidative stress in males was enhanced more than in females, which could also contribute to the increased susceptibility to iron overload pathologies in males.[58,59] Moreover, it has been shown that ovariectomized female mice lost their antioxidant defense, resulting in aggravated iron-induced myocardial injury and enhanced severe cardiac dysfunction under iron overload conditions, whereas 17β-estradiol therapy rescued cardiac injury in male iron-overloaded mice.[58] These findings suggested that estrogen therapy could mediate the beneficial effects in a male IOC model.

Previous studies have shown that antioxidant compounds could decrease oxidative stress and iron deposits in vital tissues, and improve organ function in iron-overloaded rodent models.[24,60–62] The possible mechanisms used by antioxidant therapies such as N-acetylcysteine (NAC), silymarin (plant flavonoid), and curcumin that provide these beneficial effects and attenuate the organ dysfunction induced by iron overload are probably related to their antioxidant abilities and iron-chelating properties.[24,60–62] Resveratrol is a plant polyphenol compound which has a wide range of biological properties including antioxidant, cardioprotective, neuroprotective, anti-inflammatory, and anticancer activities.[63] In previous studies, the genetic hemochromatosis (hemojuvelin [HJV] knockout mice) and acquired (secondary iron overload) murine models of iron overload showed increased myocardial and liver iron deposition, oxidative stress, inflammation, fibrosis, and apoptosis, resulting in IOC in addition to hepatic iron overload in these models.[20,21,64] Resveratrol supplementation attenuated oxidative stress, reversed pathological hypertrophy, and fibrosis leading to normalized cardiac and liver function in both acquired and genetic iron overload rodent models.[20,21,64] Moreover, resveratrol suppressed iron-mediated pro-oxidant and pro-fibrotic effects in human and mouse cardiomyocytes and cardiofibroblasts, outcomes which showed a correlation with the reduction in iron-induced myocardial oxidative stress and fibrosis in the acquired and genetic iron-overloaded rodent models.[20] In addition, iron overload increased FOXO1 acetylation and nuclear accumulation had an inverse correlation to changes in SIRT1 levels of the heart and liver, whereas resveratrol therapy reversed this effect through its pleiotropic antioxidant properties.[20,21] Based upon these beneficial effects of

resveratrol without any toxic side effects, resveratrol treatment could be potentially useful in reversing the clinical burden of IOC.[20,21]

In addition to antioxidant monotherapy, the combination of an antioxidant and an iron chelator could provide enhanced and increased therapeutic benefits to improve organ function by decreasing tissue iron accumulation and reduce oxidative stress in iron overload conditions. A combination of vitamin C with an iron chelator was shown to significantly increase urinary iron excretion (UIE),[65,66] and increase LVEF[67] in idiopathic hemochromatosis, beta-thalassemia major (β-TM) and adult non-thalassemic patients with transfusional iron overload, and also improve the biochemical and histopathological cardiac changes in iron-overloaded rats.[68] However, the use of vitamin C has been raised as a serious concern since it may induce iron toxicity by altering the Fe^{3+} ion to Fe^{2+}, the toxic form, which leads to increased enterocyte iron absorption and increased iron release from the macrophages of the reticuloendothelial system.[69] Interestingly, a combination of NAC and the iron chelator DFP was shown to exert greater efficacy than either as a monotherapy in restoring cardiac and brain function by reducing iron deposition in the tissues, oxidative stress, and apoptosis in iron-overloaded rodent models.[24,62,70] In clinical studies, NAC combined with an iron chelator also reduced oxidative stress markers in blood cells from thalassemia patients[71] and significantly reduced serum oxidative stress, increased Hb levels, and also reduced DNA damage in children with β-TM.[72]

There is an accumulation of evidence to demonstrate that curcuminoids could decrease plasma NTBI both in iron-overloaded thalassemic mice[73] and thalassemic patients,[74] and also improve cardiac function in those mice. Furthermore, a combination of tetrahydrocurcumin and DFP was shown to exert a greater efficacy than either as a monotherapy in reducing systemic iron overload, oxidative stress, and vascular dysfunction in iron-overloaded mice.[61] Curcumin combined with an iron chelator could also reduce oxidative stress, but did not improve anemia or iron status in β-TM patients.[75] Interestingly, the iron chelator DFP combined with idebenone (CoQ10 analog) therapy was shown to decrease apoptosis, improve heart hypertrophy, and may possibly improve neurological function, but further studies are needed.[76,77] Treatment with vitamin E alone also increases antioxidants and reduces oxidative stress in beta-thalassemia intermedia patients.[78,79] Moreover, an iron chelator combined with vitamin E effectively reduced oxidative stress, decreased GPx activity, and improved anemia in β-TM patients.[72,80] In addition, DFP combined with a cocktail of antioxidants (curcuminoids + NAC) or (vitamin E + NAC) improved anemia, and reduced iron overload status as well as oxidative stress in hemoglobin E (HbE)-β-thalassemia patients.[81] Another interesting clinical report pertains to silymarin. Several studies have revealed that an iron chelator combined with silymarin effectively improved systemic and liver iron status, as well as liver function, in β-TM patients without any improvement in anemia.[82,83]

All of these findings suggest that the combination of iron chelators and antioxidant cocktails could provide greater efficacy than either as a monotherapy in reducing oxidative stress, systemic iron status, and tissue iron accumulation in iron overload patients. Although a combination of an iron chelator and antioxidants has beneficial effects on the heart in reducing cardiac iron accumulation and oxidative stress, most studies have not validated the effects of those therapeutic approaches on left ventricular function status. Further large-scale clinical studies are needed to validate the beneficial effects of these combinations, especially on cardiac function.

Apoptosis

Apoptosis is a classic form of programmed cell death that has two signaling pathways, intrinsic and extrinsic.[84] The intrinsic pathway is mitochondria-dependent and is activated by various intracellular stimuli, resulting in excessive stress which activates the caspase-dependent apoptotic pathway.[84] The extrinsic pathway is initiated by the death ligands binding to their receptors, for example, TNF-α. This then activates the caspases (caspase-3, 6, and 7) leading to the activation of the mitochondria-mediated intrinsic apoptotic pathway.[84] Apoptosis is known to be involved in the pathophysiology of several diseases, and the pan-caspase inhibitor z-VAD-FMK has been shown to exert beneficial effects in several pathological disease models, including cardiovascular disease and neurodegeneration.[85–87] Moreover, inhibition of caspase by z-VAD-FMK alleviates myocardial ischemia/reperfusion (I/R) injury in rodent models.[86,87] All these findings suggest that apoptotic inhibition by pan-caspase inhibitors would be an effective strategy in the attenuation of pathological cardiac conditions. However, the effects of caspase inhibitors in IOC have never been investigated and still need further investigation.

Ferroptosis

Ferroptosis is a form of iron-dependent RCD which was discovered more recently.[25,26] Excess iron metabolism can lead to the accumulation of lipid peroxidation products resulting in cellular damage.[25,26] Previous studies have already documented that ferroptosis plays a role in the pathophysiology of many diseases, including neurodegenerative disorders, renal failure, and cardiac I/R injury.[26,88] However, the study of the impact of ferroptosis on IOC is still limited. Although ferroptosis has been shown in other cell types including fibroblasts and hepatocytes,[89,90] only one study showed that ferroptosis in cardiomyocytes was induced by excess iron.[91] Currently, there is only one *in vivo* study into ferroptosis inhibitors which found that they decreased cardiomyopathy in mice with sickle cell disease.[92] Recently, ferroptosis has been associated with IOC induced by DOX and I/R in mice.[16] This effect is confirmed by increased cellular iron mainly in mitochondria and increased lipid peroxidation, which was worsened after receiving a high iron diet.[16] Treatment with an antioxidant that targeted mitochondria (MitoTEMPO) significantly rescued DOX-induced cardiomyopathy, supporting oxidative damage in mitochondria as a major mechanism in ferroptosis-induced IOC.[16] In addition, inhibition of ferroptosis by ferrostatin-1 rescued heart failure conditions induced by DOX and I/R in mice.[16] Although ferroptosis contributes to the pathophysiological process of IOC,[89–91]

studies supporting the beneficial effects of ferroptosis inhibitors in IOC are still limited and further investigation in this area is needed.

Necroptosis

Necroptosis or programmed necrosis is also a type of RCD pathway that is mediated by death receptors.[93] Activation of TNF receptors leads to the activation of RIPK1 and RIPK3 interaction, resulting in the promotion of phosphorylation of the mixed-lineage kinase domain-like (MLKL) protein, causing an inflammatory response then triggering necroptosis.[93] Previous studies demonstrated that the suppression of necroptosis by necroptosis inhibitor Nec-1 could alleviate myocardial I/R injury in mice, rats, and pigs.[94–96] Furthermore, the combined treatment of necroptosis inhibitor Nec-1 and apoptosis inhibitor zVAD-FMK could reduce the infarct size and cause a greater impact on the RIPK1-mediated necroptosis pathway.[96] Currently, the effects of Nec-1 on IOC have not been investigated but do warrant further investigation.

Mitochondrial dynamics

Mitochondria are dynamic organelles, the dynamics including mitochondrial fusion and fission processes, fission being regulated by dynamin-related protein-1 (Drp-1), and fusion by mitofusin-1 and -2 (Mfn-1 and Mfn-2) and optic atrophy type 1 (OPA1).[97] Imbalance in mitochondrial dynamics has been demonstrated in iron overload conditions.[13] Under pathological conditions, excess Drp1-activated mitochondrial fission results in apoptosis; thus, attenuation of mitochondrial fission could be a promising therapeutic target.[98] However, currently there is only one relevant study which reports an imbalance of cardiac mitochondrial dynamics indicated by an increased cardiac Drp-1/Mfn-2 ratio in both wild-type and thalassemic mice under conditions of IOC.[13]

In past decades, the modulators of mitochondrial dynamics including mitochondrial fission inhibitor 1 (Mdivi-1) and a mitochondrial fusion promoter (M1) have been shown to exert cardioprotective and neuroprotective effects in several models of cardiac and brain ischemia.[98–100] Moreover, Mdivi-1 has been shown to protect against neuronal injury via Drp1-independent action by attenuating oxidative stress and mitochondrial membrane depolarization.[98] Previous studies demonstrated that Mdivi-1 effectively attenuates mitochondrial ROS production and dynamic imbalance, resulting in reduced cardiac cell death and improved mitochondrial function, as well as cardiac function in both normal rats and prediabetic rats during I/R injury.[99] Previous studies also found that the promotion of mitochondrial fusion during cardiac I/R injury attenuated cardiac mitochondrial dynamic imbalance and improved cardiac function in prediabetic rats[99] and diabetic cardiomyopathy rats.[100] Therefore, the inhibition of mitochondrial fission or the promotion of mitochondrial fusion might be potential therapeutic strategies for the attenuation of cardiac pathology. Since there are limited studies investigating the effects of the modulators of mitochondrial dynamics, Mdivi-1 and M1, on IOC, further studies are needed to warrant their use in the future.

Conclusions

Iron chelation therapy is the gold standard for treating iron overload patients, but the side effects of its long-term use are still a concern. There is increasing evidence to demonstrate that there is an opportunity to improve the clinical outcomes when an iron chelator is combined with other types of pharmacological interventions, including CCBs, LCN-2 siRNA, hepcidin activators, antioxidants, cell death inhibitors, and mitochondrial dynamics modulators. Currently, there is evidence to show that combining an iron chelator with either CCBs or antioxidants has more beneficial effects in IOC than monotherapy. However, other combinations should be further investigated to warrant their use in the future and more clinical studies to support their use for cardioprotective effects in IOC are needed. All associated fundamental concepts, which are illustrated in Figure 1, may contribute to improved therapeutic strategies in the near future to improve clinical outcomes in patients with IOC.

AUTHORS' CONTRIBUTIONS

All authors wrote and approved the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Pietrangelo A. Hereditary hemochromatosis–a new look at an old disease. *N Engl J Med* 2004;**350**:2383–97
2. Wood JC. Cardiac iron across different transfusion-dependent diseases. *Blood Rev* 2008;**22**:S14–21
3. Murphy CJ, Oudit GY. Iron-overload cardiomyopathy: pathophysiology, diagnosis, and treatment. *J Card Fail* 2010;**16**:888–900
4. Brittenham GM. Iron-chelating therapy for transfusional iron overload. *N Engl J Med* 2011;**364**:146–56
5. Vela D. Keeping heart homeostasis in check through the balance of iron metabolism. *Acta Physiol (Oxf)* 2020;**228**:e13324
6. Ravingerova T, Kindernay L, Bartekova M, Ferko M, Adameova A, Zohdi V, Bernatova I, Ferenczyova K, Lazou A. The Molecular Mechanisms of Iron Metabolism and Its Role in Cardiac Dysfunction and Cardioprotection. *Int J Mol Sci* 2020;**21**:7889
7. Rouault TA. The role of iron regulatory proteins in mammalian iron homeostasis and disease. *Nat Chem Biol* 2006;**2**:406–14
8. 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

9. 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
10. 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
11. Oudit GY, Sun H, Trivieri MG, Koch SE, Dawood F, Ackerley C, Yazdanpanah M, Wilson GJ, Schwartz A, Liu PP, Backx PH. L-type Ca2+ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. *Nat Med* 2003;**9**:1187–94
12. Kumfu S, Siri-Angkul N, Chattipakorn SC, Chattipakorn N. Silencing of lipocalin-2 improves cardiomyocyte viability under iron overload conditions via decreasing mitochondrial dysfunction and apoptosis. *J Cell Physiol* 2021;**236**:5108–20
13. Khamseekaew J, Kumfu S, Wongjaikam S, Kerdphoo S, Jaiwongkam T, Srichairatanakool S, Fucharoen S, Chattipakorn SC, Chattipakorn N. Effects of iron overload, an iron chelator and a T-Type calcium channel blocker on cardiac mitochondrial biogenesis and mitochondrial dynamics in thalassemic mice. *Eur J Pharmacol* 2017;**799**:118–27
14. 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
15. Chen MP, Cabantchik ZI, Chan S, Chan GC, Cheung YF. Iron overload and apoptosis of HL-1 cardiomyocytes: effects of calcium channel blockade. *PLoS ONE* 2014;**9**:e112915
16. Fang X, Wang H, Han D, Xie E, Yang X, Wei J, Gu S, Gao F, Zhu N, Yin X, Cheng Q, Zhang P, Dai W, Chen J, Yang F, Yang HT, Linkermann A, Gu W, Min J, Wang F. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci USA* 2019;**116**:2672–80
17. Sun M, Chen M, Dawood F, Zurawska U, Li JY, Parker T, Kassiri Z, Kirshenbaum LA, Arnold M, Khokha R, Liu PP. Tumor necrosis factor-alpha mediates cardiac remodeling and ventricular dysfunction after pressure overload state. *Circulation* 2007;**115**:1398–407
18. Yu W, Chen C, Cheng J. The role and molecular mechanism of FoxO1 in mediating cardiac hypertrophy. *ESC Heart Fail* 2020;**7**:3497–504
19. Kobayashi Y, Furukawa-Hibi Y, Chen C, Horio Y, Isobe K, Ikeda K, Motoyama N. SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. *Int J Mol Med* 2005;**16**:237–43
20. Das SK, Wang W, Zhabyeyev P, Basu R, McLean B, Fan D, Parajuli N, DesAulniers J, Patel VB, Hajjar RJ, Dyck JR, Kassiri Z, Oudit GY. Iron-overload injury and cardiomyopathy in acquired and genetic models is attenuated by resveratrol therapy. *Sci Rep* 2015;**5**:18132
21. Das SK, DesAulniers J, Dyck JR, Kassiri Z, Oudit GY. Resveratrol mediates therapeutic hepatic effects in acquired and genetic murine models of iron-overload. *Liver Int* 2016;**36**:246–57
22. Orrenius S, Nicotera P, Zhivotovsky B. Cell death mechanisms and their implications in toxicology. *Toxicol Sci* 2011;**119**:3–19
23. Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, Kitsis RN. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J Clin Invest* 2003;**111**:1497–504
24. Kumfu S, Khamseekaew J, Palee S, Srichairatanakool S, Fucharoen S, Chattipakorn SC, Chattipakorn N. A combination of an iron chelator with an antioxidant exerts greater efficacy on cardioprotection than monotherapy in iron-overload thalassemic mice. *Free Radic Res* 2018;**52**:70–9
25. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, Antonov AV, Arama E, Baehrecke EH, Barlev NA, Bazan NG, Bernassola F, Bertrand MJM, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Boya P, Brenner C, Campanella M, Candi E, Carmona-Gutierrez D, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Cohen GM, Conrad M, Cubillos-Ruiz JR, Czabotar PE, D'Angiolella V, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, DeBerardinis RJ, Deshmukh M, Di Daniele N, Di Virgilio F, Dixit VM, Dixon SJ, Duckett CS, Dynlacht BD, El-Deiry WS, Elrod JW, Fimia GM, Fulda S, Garcia-Saez AJ, Garg AD, Garrido C, Gavathiotis E, Golstein P, Gottlieb E, Green DR, Greene LA, Gronemeyer H, Gross A, Hajnoczky G, Hardwick JM, Harris IS, Hengartner MO, Hetz C, Ichijo H, Jaattela M, Joseph B, Jost PJ, Juin PP, Kaiser WJ, Karin M, Kaufmann T, Kepp O, Kimchi A, Kitsis RN, Klionsky DJ, Knight RA, Kumar S, Lee SW, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, Lopez-Otin C, Lowe SW, Luedde T, Lugli E, MacFarlane M, Madeo F, Malewicz M, Malorni W, Manic G, Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ* 2018;**25**:486–541
26. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B, 3rd, Stockwell BR. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012;**149**:1060–72
27. Makis A, Voskaridou E, Papassotiriou I, Hatzimichael E. Novel therapeutic advances in beta-thalassemia. *Biology (Basel)* 2021;**10**:546
28. Berdoukas V, Farmaki K, Carson S, Wood J, Coates T. Treating thalassemia major-related iron overload: the role of deferiprone. *J Blood Med* 2012;**3**:119–29
29. Kwiatkowski JL. Management of transfusional iron overload—differential properties and efficacy of iron chelating agents. *J Blood Med* 2011;**2**:135–49
30. Chuang TY, Li JP, Weng TF, Wu KH, Chao YH. Combined chelation with high-dose deferiprone and deferoxamine to improve survival and restore cardiac function effectively in patients with transfusion-dependent thalassemia presenting severe cardiac complications. *Ann Hematol* 2020;**99**:2289–94
31. Tanner MA, Galanello R, Dessi C, Smith GC, Westwood MA, Agus A, Pibiri M, Nair SV, Walker JM, Pennell DJ. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. *J Cardiovasc Magn Reson* 2008;**10**:12
32. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Piga A, Romeo MA, Zhao H, Cnaan A. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. *Blood* 2006;**107**:3733–7
33. Anderson LJ, Wonke B, Prescott E, Holden S, Walker JM, Pennell DJ. Comparison of effects of oral deferiprone and subcutaneous desferrioxamine on myocardial iron concentrations and ventricular function in beta-thalassaemia. *Lancet* 2002;**360**:516–20
34. Aydinok Y, Kattamis A, Cappellini MD, El-Beshlawy A, Origa R, Elalfy M, Kilinc Y, Perrotta S, Karakas Z, Viprakasit V, Habr D, Constantinovici N, Shen J, Porter JB, Investigators H. Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. *Blood* 2015;**125**:3868–77
35. Voskaridou E, Komninaka V, Karavas A, Terpos E, Akianidis V, Christoulas D. Combination therapy of deferasirox and deferoxamine shows significant improvements in markers of iron overload in a patient with beta-thalassemia major and severe iron burden. *Transfusion* 2014;**54**:646–9
36. Elliott WJ, Ram CV. Calcium channel blockers. *J Clin Hypertens (Greenwich)* 2011;**13**:687–9
37. Crowe S, Bartfay WJ. Amlodipine decreases iron uptake and oxygen free radical production in the heart of chronically iron overloaded mice. *Biol Res Nurs* 2002;**3**:189–97
38. Otto-Duessel M, Brewer C, Wood JC. Interdependence of cardiac iron and calcium in a murine model of iron overload. *Transl Res* 2011;**157**:92–9
39. Tsushima RG, Wickenden AD, Bouchard RA, Oudit GY, Liu PP, Backx PH. Modulation of iron uptake in heart by L-type Ca2+ channel modifiers: possible implications in iron overload. *Circ Res* 1999;**84**:1302–9
40. 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
41. Eghbali A, Kazemi H, Taherahmadi H, Ghandi Y, Rafiei M, Bagheri B. A randomized, controlled study evaluating effects of amlodipine addition to chelators to reduce iron loading in patients with thalassemia major. *Eur J Haematol* 2017;**99**:577–81
42. Fernandes JL, Loggetto SR, Verissimo MP, Fertrin KY, Baldanzi GR, Fioravante LA, Tan DM, Higa T, Mashima DA, Piga A, Coelho OR, Costa FF, Saad ST. A randomized trial of amlodipine in addition to

standard chelation therapy in patients with thalassemia major. *Blood* 2016;**128**:1555–61
43. Khaled A, Salem HA, Ezzat DA, Seif HM, Rabee H. A randomized controlled trial evaluating the effects of amlodipine on myocardial iron deposition in pediatric patients with thalassemia major. *Drug Des Devel Ther* 2019;**13**:2427–36
44. 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
45. Russell RP. Side effects of calcium channel blockers. *Hypertension* 1988;**11**:II42–14
46. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 2002;**10**:1033–43
47. Ding L, Hanawa H, Ota Y, Hasegawa G, Hao K, Asami F, Watanabe R, Yoshida T, Toba K, Yoshida K, Ogura M, Kodama M, Aizawa Y. Lipocalin-2/neutrophil gelatinase-B associated lipocalin is strongly induced in hearts of rats with autoimmune myocarditis and in human myocarditis. *Circ J* 2010;**74**:523–30
48. Yndestad A, Landro L, Ueland T, Dahl CP, Flo TH, Vinge LE, Espevik T, Froland SS, Husberg C, Christensen G, Dickstein K, Kjekshus J, Oie E, Gullestad L, Aukrust P. Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure. *Eur Heart J* 2009;**30**:1229–36
49. Emans ME, Braam B, Diepenbroek A, van der Putten K, Cramer MJ, Wielders JP, Swinkels DW, Doevendans PA, Gaillard CA. Neutrophil gelatinase-associated lipocalin (NGAL) in chronic cardiorenal failure is correlated with endogenous erythropoietin levels and decreases in response to low-dose erythropoietin treatment. Kidney Blood Press Res 2012;**36**:344–54
50. Roudkenar MH, Halabian R, Oodi A, Roushandeh AM, Yaghmai P, Najar MR, Amirizadeh N, Shokrgozar MA. Upregulation of neutrophil gelatinase-associated lipocalin, NGAL/Lcn2, in beta-thalassemia patients. *Arch Med Res* 2008;**39**:402–7
51. Xu G, Ahn J, Chang S, Eguchi M, Ogier A, Han S, Park Y, Shim C, Jang Y, Yang B, Xu A, Wang Y, Sweeney G. Lipocalin-2 induces cardiomyocyte apoptosis by increasing intracellular iron accumulation. *J Biol Chem* 2012;**287**:4808–17
52. Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. *Adv Nutr* 2017;**8**:126–36
53. Schmidt PJ, Racie T, Westerman M, Fitzgerald K, Butler JS, Fleming MD. Combination therapy with a Tmprss6 RNAi-therapeutic and the oral iron chelator deferiprone additively diminishes secondary iron overload in a mouse model of beta-thalassemia intermedia. *Am J Hematol* 2015;**90**:310–3
54. Schmidt PJ, Liu K, Visner G, Fitzgerald K, Fishman S, Racie T, Hettinger JL, Butler JS, Fleming MD. RNAi-mediated reduction of hepatic Tmprss6 diminishes anemia and secondary iron overload in a splenectomized mouse model of beta-thalassemia intermedia. *Am J Hematol* 2018;**93**:745–50
55. Casu C, Aghajan M, Oikonomidou PR, Guo S, Monia BP, Rivella S. Combination of Tmprss6- ASO and the iron chelator deferiprone improves erythropoiesis and reduces iron overload in a mouse model of beta-thalassemia intermedia. *Haematologica* 2016;**101**:e8–11
56. Preza GC, Ruchala P, Pinon R, Ramos E, Qiao B, Peralta MA, Sharma S, Waring A, Ganz T, Nemeth E. Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. *J Clin Invest* 2011;**121**:4880–8
57. Richard F, van Lier JJ, Roubert B, Haboubi T, Gohring UM, Durrenberger F. Oral ferroportin inhibitor VIT-2763: first-in-human, phase 1 study in healthy volunteers. *Am J Hematol* 2020;**95**:68–77
58. Das SK, Patel VB, Basu R, Wang W, DesAulniers J, Kassiri Z, Oudit GY. Females are protected from iron-overload cardiomyopathy independent of iron metabolism: key role of oxidative stress. *J Am Heart Assoc* 2017;**6**:1–23
59. Das SK, Zhabyeyev P, Oudit GY. Role of sex steroids and sexual dimorphism on cardiac iron metabolism in iron-overload cardiomyopathy. *Transl Res* 2014;**163**:141–4
60. Navidi-Shishaone M, Mohhebi S, Nematbakhsh M, Roozbehani S, Talebi A, Pezeshki Z, Eshraghi-Jazi F, Mazaheri S, Shirdavani S, Gharagozloo M, Moaeidi BA. Co-administration of silymarin and deferoxamine against kidney, liver and heart iron deposition in male iron overload rat model. *Int J Prev Med* 2014;**5**:110–6
61. Sangartit W, Pakdeechote P, Kukongviriyapan V, Donpunha W, Shibahara S, Kukongviriyapan U. Tetrahydrocurcumin in combination with deferiprone attenuates hypertension, vascular dysfunction, baroreflex dysfunction, and oxidative stress in iron-overloaded mice. *Vascul Pharmacol* 2016;**87**:199–208
62. Sripetchwandee J, Wongjaikam S, Krintratun W, Chattipakorn N, Chattipakorn SC. A combination of an iron chelator with an antioxidant effectively diminishes the dendritic loss, tau-hyperphosphorylation, amyloids-beta accumulation and brain mitochondrial dynamic disruption in rats with chronic iron-overload. *Neuroscience* 2016;**332**:191–202
63. Salehi B, Mishra AP, Nigam M, Sener B, Kilic M, Sharifi-Rad M, Fokou PVT, Martins N, Sharifi-Rad J. Resveratrol: a double-edged sword in health benefits. *Biomedicines* 2018;**6**:91
64. Das SK, Zhabyeyev P, Basu R, Patel VB, Dyck JRB, Kassiri Z, Oudit GY. Advanced iron-overload cardiomyopathy in a genetic Murine model is rescued by resveratrol therapy. *Biosci Rep* 2018;**38**:BSR20171302
65. Conte D, Brunelli L, Ferrario L, Mandelli C, Quatrini M, Velio P, Bianchi PA. Effect of ascorbic acid on desferrioxamine-induced urinary iron excretion in idiopathic hemochromatosis. *Acta Haematol* 1984;**72**:117–20
66. Hussain MA, Green N, Flynn DM, Hoffbrand AV. Effect of dose, time, and ascorbate on iron excretion after subcutaneous desferrioxamine. *Lancet* 1977;**1**:977–9
67. Jensen PD, Olsen N, Bagger JP, Jensen FT, Christensen T, Ellegaard J. Cardiac function during iron chelation therapy in adult non-thalassaemic patients with transfusional iron overload. *Eur J Haematol* 1997;**59**:221–30
68. Emara AM, El Kelany RS, Moustafa KA. Comparative study of the protective effect between deferoxamine and deferiprone on chronic iron overload induced cardiotoxicity in rats. *Hum Exp Toxicol* 2006;**25**:375–85
69. Freedman MH. Management of beta-thalassemia major using transfusions and iron chelation with deferoxamine. *Transfus Med Rev* 1988;**2**:161–75
70. Wongjaikam S, Kumfu S, Khamseekaew J, Sripetchwandee J, Srichairatanakool S, Fucharoen S, Chattipakorn SC, Chattipakorn N. Combined iron chelator and antioxidant exerted greater efficacy on cardioprotection than monotherapy in iron-overloaded rats. *PLoS ONE* 2016;**11**:e0159414
71. Amer J, Atlas D, Fibach E. N-acetylcysteine amide (AD4) attenuates oxidative stress in beta-thalassemia blood cells. *Biochim Biophys Acta* 2008;**1780**:249–55
72. Ozdemir ZC, Koc A, Aycicek A, Kocyigit A. N-acetylcysteine supplementation reduces oxidative stress and DNA damage in children with beta-thalassemia. *Hemoglobin* 2014;**38**:359–64
73. 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
74. 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
75. Nasseri E, Mohammadi E, Tamaddoni A, Qujeq D, Zayeri F, Zand H. Benefits of curcumin supplementation on antioxidant status in beta-thalassemia major patients: a double-blind randomized controlled clinical trial. *Ann Nutr Metab* 2017;**71**:136–44
76. Elincx-Benizri S, Glik A, Merkel D, Arad M, Freimark D, Kozlova E, Cabantchik I, Hassin-Baer S. Clinical experience with deferiprone treatment for friedreich ataxia. *J Child Neurol* 2016;**31**:1036–40
77. Velasco-Sanchez D, Aracil A, Montero R, Mas A, Jimenez L, O'Callaghan M, Tondo M, Capdevila A, Blanch J, Artuch R, Pineda M. Combined therapy with idebenone and deferiprone in patients with Friedreich's ataxia. *Cerebellum* 2011;**10**:18
78. Tesoriere L, D'Arpa D, Butera D, Allegra M, Renda D, Maggio A, Bongiorno A, Livrea MA. 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
79. 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
80. Rashidi M, Aboomardani M, Rafraf M, Arefhosseini SR, Keshtkar A, Joshaghani H. Effects of vitamin E and zinc supplementation on antioxidants in beta thalassemia major patients. *Iran J Pediatr* 2011;**21**:8–14
81. Yanpanitch OU, Hatairaktham S, Charoensakdi R, Panichkul N, Fucharoen S, Srichairatanakool S, Siritanaratkul N, Kalpravidh RW. Treatment of beta-thalassemia/hemoglobin E with antioxidant cocktails results in decreased oxidative stress, increased hemoglobin concentration, and improvement of the hypercoagulable state. *Oxid Med Cell Longev* 2015;**2015**:537954
82. Hagag AA, Elfrargy MS, Gazar RA, El-Lateef AE. Therapeutic value of combined therapy with deferasirox and silymarin on iron overload in children with beta thalassemia. *Mediterr J Hematol Infect Dis* 2013;**5**:e2013065
83. Moayedi B, Gharagozloo M, Esmaeil N, Maracy MR, Hoorfar H, Jalaeikar M. A randomized double-blind, placebo-controlled study of therapeutic effects of silymarin in beta-thalassemia major patients receiving desferrioxamine. *Eur J Haematol* 2013;**90**:202–9
84. Verbrugge I, Johnstone RW, Smyth MJ. SnapShot: extrinsic apoptosis pathways. *Cell* 2010;**143**:1192
85. Ma J, Qiu J, Hirt L, Dalkara T, Moskowitz MA. Synergistic protective effect of caspase inhibitors and bFGF against brain injury induced by transient focal ischaemia. *Br J Pharmacol* 2001;**133**:345–50
86. Mersmann J, Zacharowski PA, Schmitz I, Zacharowski K. Caspase inhibitor zVAD.fmk reduces infarct size after myocardial ischaemia and reperfusion in rats but not in mice. *Resuscitation* 2008;**79**:468–74
87. Mocanu MM, Baxter GF, Yellon DM. Caspase inhibition and limitation of myocardial infarct size: protection against lethal reperfusion injury. *Br J Pharmacol* 2000;**130**:197–200
88. Linkermann A, Skouta R, Himmerkus N, Mulay SR, Dewitz C, De Zen F, Prokai A, Zuchtriegel G, Krombach F, Welz PS, Weinlich R, Vanden Berghe T, Vandenabeele P, Pasparakis M, Bleich M, Weinberg JM, Reichel CA, Brasen JH, Kunzendorf U, Anders HJ, Stockwell BR, Green DR, Krautwald S. Synchronized renal tubular cell death involves ferroptosis. *Proc Natl Acad Sci USA* 2014;**111**:16836–41
89. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and transferrin regulate ferroptosis. *Molecular Cell* 2015;**59**:298–308
90. Fang S, Yu X, Ding H, Han J, Feng J. Effects of intracellular iron overload on cell death and identification of potent cell death inhibitors. *Biochem Biophys Res Commun* 2018;**503**:297–303
91. Baba Y, Higa JK, Shimada BK, Horiuchi KM, Suhara T, Kobayashi M, Woo JD, Aoyagi H, Marh KS, Kitaoka H, Matsui T. Protective effects of the mechanistic target of rapamycin against excess iron and ferroptosis in cardiomyocytes. *Am J Physiol Heart Circul Physiol* 2018;**314**:H659–68
92. Menon AV, Liu J, Tsai HP, Zeng L, Yang S, Asnani A, Kim J. Excess heme upregulates heme oxygenase 1 and promotes cardiac ferroptosis in mice with sickle cell disease. *Blood* 2021;**1**:1–15
93. Zhu H, Sun A. Programmed necrosis in heart disease: molecular mechanisms and clinical implications. *J Mol Cell Cardiol* 2018;**116**:125–34
94. Dmitriev YV, Minasian SM, Demchenko EA, Galagudza MM. Study of cardioprotective effects of necroptosis inhibitors on isolated rat heart subjected to global ischemia-reperfusion. *Bull Exp Biol Med* 2013;**155**:245–8
95. Smith CC, Davidson SM, Lim SY, Simpkin JC, Hothersall JS, Yellon DM. Necrostatin: a potentially novel cardioprotective agent. *Cardiovasc Drugs Ther* 2007;**21**:227–33
96. Koshinuma S, Miyamae M, Kaneda K, Kotani J, Figueredo VM. Combination of necroptosis and apoptosis inhibition enhances cardioprotection against myocardial ischemia-reperfusion injury. *J Anesth* 2014;**28**:235–41
97. Forte M, Schirone L, Ameri P, Basso C, Catalucci D, Modica J, Chimenti C, Crotti L, Frati G, Rubattu S, Schiattarella GG, Torella D, Perrino C, Indolfi C, Sciarretta S, Italian Society of Cardiology Working group on Cellular and Molecular Biology of the Heart. The role of mitochondrial dynamics in cardiovascular diseases. *Br J Pharmacol* 2021;**178**:2060–76
98. Ruiz A, Alberdi E, Matute C. Mitochondrial division inhibitor 1 (mdivi-1) protects neurons against excitotoxicity through the modulation of mitochondrial function and intracellular Ca(2+) signaling. *Front Mol Neurosci* 2018;**11**:31–16
99. Maneechote C, Palee S, Kerdphoo S, Jaiwongkam T, Chattipakorn SC, Chattipakorn N. Modulating mitochondrial dynamics attenuates cardiac ischemia-reperfusion injury in prediabetic rats. *Acta Pharmacol Sin* 2022;**43**:26–38
100. Ding M, Liu C, Shi R, Yu M, Zeng K, Kang J, Fu F, Mi M. Mitochondrial fusion promoter restores mitochondrial dynamics balance and ameliorates diabetic cardiomyopathy in an optic atrophy 1-dependent way. *Acta Physiol (Oxf)* 2020;**229**:e13428