Published in final edited form as:

Curr Drug Targets. 2018 ; 19(9): 1068–1076. doi:10.2174/1389450119666180605112235.

Pathological roles of iron in cardiovascular disease

Motoi Kobayashi¹, Tomohiro Suhara^{1,2}, Yuichi Baba^{1,3}, Nicholas K. Kawasaki¹, Jason K. Higa¹, and Takashi Matsui^{1,*}

¹Department of Anatomy, Biochemistry & Physiology, John A. Burns School of Medicine, University of Hawai'i at Manoa, Honolulu, HI

²Department of Anesthesiology, Keio University School of Medicine, Tokyo, Japan.

³Department of Cardiology and Geriatrics, Kochi Medical School, Kochi University, Kochi, Japan

Abstract

Iron is an essential mineral required for a variety of vital biological functions. Despite being vital for life, iron also has potentially toxic aspects. Iron has been investigated as a risk factor for coronary artery disease (CAD), however iron's toxicity in CAD patients still remains controversial. One possible mechanism behind the toxicity of iron is "ferroptosis", a newly described form of iron-dependent cell death. Ferroptosis is an iron-dependent form of regulated cell death that is distinct from apoptosis, necroptosis, and other types of cell death. Ferroptosis has been reported in ischemia-reperfusion (I/R) injury and several other diseases. Recently, we reported that ferroptosis is a significant form of cell death in cardiomyocytes. Moreover, myocardial hemorrhage, a major event in the pathogenesis of heart failure, could trigger the release of free iron into cardiac muscle and is an independent predictor of adverse left ventricular remodeling after myocardial infarction. Iron deposition in the heart can now be detected with advanced imaging methods, such as T2 star (T2*) cardiac magnetic resonance imaging, which can non-invasively predict iron levels in the myocardium and detect myocardial hemorrhage, thus existing technology could be used to assess. We will discuss the role of iron in cardiovascular diseases and especially with regard to myocardial I/R injury.

Keywords

myocardial infarction; ischemia-reperfusion injury; iron; reactive oxygen species; ferroptosis

Introduction

implied that high amounts of body iron in men and postmenopausal women may increase the risk of CAD [6]. However, the validity of this hypothesis is still under Iron is an essential mineral required for a variety of vital biological functions, and exists in both ferrous [Fe(II)]

*Corresponding author: Takashi Matsui, M.D. Ph.D., Department of Anatomy, Biochemistry & Physiology, John A. Burns School of Medicine, University of Hawai'i at Manoa, 651 Ilalo St., BSB #110, Honolulu, HI, 96813, U.S.A., Tel.: 808-692-1554, Fax: 808-692-1973, tmatsui@hawaii.edu.

Conflict of Interest

Motoi Kobayashi, Tomohiro Suhara, Yuichi Baba, Nicholas Kawasaki, Jason K. Higa, and Takashi Matsui declare that they have no conflict of interest.

and ferric [Fe(III)] forms in physiological conditions. Several vital processes depend on iron catalysts, including oxygen metabolism, protein production, cellular respiration, lipid metabolism, DNA synthesis, and overall metabolism [1]. However, despite being essential for life, iron also carries potentially toxic properties. These toxic properties become especially prevalent when iron is overloaded or its homeostasis is disrupted. For example, iron becomes harmful by catalyzing the overproduction of reactive oxygen species (ROS) via the Fenton reaction and Haber-Weiss reaction [2]. This overproduction of ROS overwhelms the balance between free radical formation and depletion and is defined as oxidative stress. Iron-induced oxidative stress is involved in various pathological conditions, including heart failure, cardiomyopathy, coronary artery disease (CAD) and atherosclerosis [3–5]. In the early 1980's, this led to Sullivan formulating the 'iron hypothesis', which debate.

In addition to its physiological roles, iron is also used clinically as a reagent for treating patients with cardiovascular diseases. Metallic nanoparticles made of pure metals such as silver and aluminum are widely utilized in biomedical science [7]. Recent studies showed the potential of iron oxide nanoparticles in cardiac clinical therapies such as cell labeling in stem cell therapy [8]. Iron also has potential use in the prognosis of cardiovascular disease. Many clinical studies suggest that the level of myocardial iron is a prognostic factor of heart failure following myocardial infarction (MI) [9, 10]. Advanced imaging methods such as T2 star (T2*) cardiac magnetic resonance imaging (MRI) can non-invasively detect iron levels in the myocardium [9].

Myocardial hemorrhage is a critical event in cardiovascular disease that could lead to the deposition of iron in cardiac tissue. In ST-segment elevation MI (STEMI) patients, myocardial hemorrhage is a frequent complication after successful myocardial reperfusion and is an independent predictor of adverse left ventricular (LV) remodeling [11, 12]. Moreover, a recent study using cardiac MRI showed that in post-MI patients that received reperfusion therapy, the presence of myocardial hemorrhage was associated with residual myocardial iron [10]. This finding suggests that in ischemia-reperfusion (I/R) injury, the impeded flow of red blood cells (RBCs) at obstructive lesions may cause RBCs to lyse, resulting in local accumulation of iron from hemoglobin and heme released at the focal lesion. This iron accumulation can generate excessive ROS and trigger pathological events such as inflammation [13]. If iron deposition or accumulation is a key pathological trigger for cardiovascular disease and increased risk of heart failure, it is important to understand how iron mediates pathological processes such as cell death and inflammation. Further studies into these mechanisms may yield new strategies to inhibit these mechanisms and prevent pathological effects, and could provide potential novel mechanistic targets for treating cardiovascular diseases.

Ferroptosis is an example of an iron-mediated mechanism with pathological effects on the heart. Ferroptosis is an iron-dependent form of regulated cell death that is distinct from apoptosis and other types of programmed cell death [14] (reviewed in [15, 16]). Ferroptosis is a key factor in the pathogenesis of several diseases such as hepatocellular carcinoma and renal I/R injury [17, 18]. Recently, we reported that ferroptosis is a significant form of cell death in cardiomyocytes [19]. However, the role of ferroptosis in cardiovascular diseases is

not well characterized. This review reports the pathophysiological roles of iron in the heart and iron-mediated mechanisms such as ferroptosis, with emphasis on the role of iron in I/R injury in the heart.

Cellular iron homeostasis and ROS

Intracellular iron concentrations are tightly regulated to maintain essential cellular functions such as ROS-dependent cell signaling. However, the redox properties of iron in the cell also make it an ideal catalyst for the production of toxic ROS. One such reaction is the Fenton reaction in mitochondria, which generates hydroxyl radicals (HO) [20] (Figure 1). Another example is how Fe(II) and Fe(III) also mediate lipid peroxidation by the reductive cleavage of hydroperoxides (ROOH) derived from membrane phospholipids, resulting in the formation of alkoxyl (RO) and peroxy (RO₂) radicals [2] (Figure 1). This formation of lipid radicals is also central to pathophysiological processes in ferroptosis (discussed later).

Intracellular iron concentrations and homeostasis are regulated by several key factors, including: iron regulatory proteins (IRP1 and IRP2), which are mRNA-binding proteins that strictly control intracellular iron metabolism; transferrin receptor 1 (TfR1) and divalent metal transporter 1 (DMT1), which control iron uptake; H and L ferritin subunits, which regulate iron storage; and ferroportin, which exports iron via the plasma membrane [21]. These mechanisms of controlling iron intake, storage, and export, maintain the amount of iron in the redox-active labile iron pool (LIP). The LIP is in continuous equilibrium with sites of iron utilization or storage. An imbalance among these homeostatic factors could lead to an accumulation of excess intracellular iron, increased ROS production, and induction of cell death [21].

Mitochondrial ferritin (FtMt) is an iron-storage protein and a well-characterized cytosolic H-ferritin belonging to the ferritin family [22]. FtMt is expressed in the cells of several organs such as the central nervous system, testes, thymus, kidney, and heart. However, FtMt is not expressed in the liver or spleen, which instead express high levels of cytosolic ferritin rather than FtMt. This is due to the liver and spleen also being important in iron storage and recycling [23]. Overexpression of FtMt causes redistribution of iron from the cytosol to the mitochondria [24]. Therefore, high levels of FtMt may reduce cytosolic iron while reducing both the mitochondrial LIP and cytosolic LIP, thus resulting in overall diminished ROS production [25]. Furthermore, a recent report showed that FtMt overexpression suppressed erastin-induced cell death (ferroptosis) and ROS production [26]. These studies suggest that FtMt is a potential new target molecule for treatments focusing on balancing iron homeostasis and decreasing ROS.

Iron and Ferroptosis

Apoptosis is a well-known form of regulated cell death with distinct molecular pathways and cellular features such as caspase cleavage and chromatin condensation [27]. Ferroptosis is a form of regulated cell death that is distinct from apoptosis and necroptosis [28]. In addition, ferroptosis contains unique cellular features such as mitochondrial shrinkage and increased mitochondrial membrane density [14]. Furthermore, biochemical features and gene

expression profiles of ferroptosis are distinct from other types of cell death, including apoptosis, necroptosis and autophagy (see another review article for details about major differences between them [17]). Two key pathophysiological features of ferroptosis are the inactivation of glutathione peroxidase 4 (GPx4) and accumulation of lipid peroxides [14, 29] (Figure 2), neither of which are central features to apoptosis, necroptosis, or other forms of cell death. Currently there are two categories of ferroptosis inducers that target specific proteins in the ferroptotic pathway. Class 1 ferroptosis inducers inhibit system X_c^- , whereas class 2 ferroptosis inducers inhibit GPx4 [30].

System X_c^- is a cystine/glutamate antiporter, and class 1 ferroptosis inducers reduce cystine uptake into cells, which results in ferroptotic cell death [14]. The reduction in cystine uptake also leads to decreased glutathione synthesis, resulting in a lack of substrate and inactivation of GPx4 [29]. Erastin is an example of a class 1 ferroptosis inducer that in addition to system X_c^- , also binds to and inhibits voltage-dependent anion channel (VDAC) 2/3, which also leads to cell death [31]. A previous report showed that there is no expression of system X_c^- in the heart [32], but our recent study showed that erastin still induces cell death in cardiomyocytes [19]. These findings suggest that the effects of erastin on ferroptosis in the heart may be due to the inhibition of VDAC rather than system X_c^- . Given that this alternate pathway still induces ferroptosis in the absence of system X_c^- , further studies are required to characterize the effects of erastin and other class 1 ferroptosis inducers in cardiomyocytes.

Class 2 ferroptosis inducers, such as Ras Selective Lethal 3 (RSL3), directly inhibit GPx4, increase lipid peroxidation, and induce ferroptosis [14]. To date, 8 glutathione peroxidases (GPx1-GPx8) have been identified in mammals [33]. Overexpression of mitochondrial GPx4 also protects the heart against I/R injury [34], however the effects of ferroptosis on GPx4 in the context of cardiac I/R injury are not well characterized, and further studies are needed to determine if class 2 ferroptosis inducers target GPx4 in the heart like they do in other tissues.

Circulating iron has a pivotal role in the development of ferroptosis. A study using iron chelators showed that chelation also inhibits erastin-induced ferroptosis, suggesting that circulating iron (present as Fe(III)) is necessary for ferroptosis [14] (Figure 2). Other endogenous molecules can also affect circulating iron and ferroptosis. Circulating iron binds to transferrin (TfR1), which imports Fe(III) into cells and localizes into endosomes. TfR1 expression also increases cell susceptibility to ferroptosis, suggesting that cells that store iron are also more likely to be sensitive to ferroptosis [14]. Another recent study showed that L-glutamine, which is converted into glutamate by glutaminases, is another trigger of ferroptosis [35]. The study demonstrated that a glutaminase inhibitor and deferoxamine (DFO), a chelator, also protected the heart against I/R injury in ex vivo perfused hearts [35]. This suggests that ferroptosis inhibitors in combination with chelators may protect against I/R injury by limiting the iron available for ferroptosis and thus inhibiting the molecular mechanisms of ferroptosis.

Previously, we found that cardiac mTOR (mechanistic target of rapamycin) protects the heart against I/R injury [36, 37]. mTOR is a key downstream molecule in the PI3K/Akt signaling axis and plays an important role in metabolism, cell growth and cell proliferation

in the heart and multiple other organs [38, 39]. Activation of mTOR is involved in several pathological cardiac conditions including I/R injury, hypertrophy, and diabetic cardiomyopathy [36, 37, 40]. We recently reported that mTOR protects cardiomyocytes against excess iron-induced cell death and ferroptosis [19]. We demonstrated that Fe(III), erastin (a class 1 ferroptosis inducer), or RSL3 (a class 2 ferroptosis inducer), all induced cardiomyocyte cell death and caused a significant increase in ROS production. Moreover, co-treatment with ferrostatin-1, a ferroptosis inhibitor, suppressed cell death in all conditions. We also demonstrated that mTOR is sufficient and necessary for cell survival against excess iron-induced cell death and ferroptosis by using cardiac-specific transgenic mice overexpressing mTOR and cardiac-specific mTOR knockout mice [41]. These studies indicate that mTOR-mediated cell survival pathways may protect the heart during pathological conditions that cause ferroptosis and can be a target for new anti-ferroptotic therapies in heart disease.

Iron and Atherosclerosis

Monocytes and macrophages are inflammatory cells that play key roles in the formation of atherosclerosis. Circulating monocytes enter the subendothelial space of the arterial intima and differentiate into macrophages. Macrophages are known to have two types: pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages. Both of them have been identified in atherosclerotic plaques [42]. M1 macrophages express scavenger receptors on their surface and deposit oxidatively modified lipoproteins to atheroma plaques, thus worsening atherosclerosis. On the other hand, the role of M2 macrophages in atherogenesis is complicated as they have a variety of cellular and molecular targets and effects. M2 macrophages produce anti-inflammatory cytokines such as IL-4, IL-10 and IL-13, which contribute to the deactivation of endothelial cells, Th1 lymphocytes, and other macrophage populations [43]. Animal models showed that iron-overloading conditions with persistent RBC extravasation increase proinflammatory M1 macrophage populations and perpetuate inflammation [13]. It is likely that iron accumulation from RBCs may cause chronic inflammation in vessels, resulting in exacerbation of atherosclerosis.

In human atherosclerotic lesions, H- and L- ferritin are present at high levels. Moreover, high levels of L-ferritin are observed in coronary arteries in patients with CAD, indicating that iron accumulates in atherosclerotic plaques [44]. In addition, cholesterol levels in plaque lesions associate with iron deposits [45]. While most of iron in the circulating blood exists in complex with transferrin and heme, bound non-transferrin-bound iron (NTBI) is thought as a pathological trigger during iron-overloaded states such as thalassemia, sickle cell disease or transfusional iron overload [46]. Iron in the NTBI state has an increased probability of being accessible to various plaque cell types, including endothelial cells, macrophages, and vascular smooth muscle cells. Iron can also be deposited at atherosclerotic lesions in the form of free hemoglobin (Hb), which is released when intravascular hemolysis or intraplaque hemorrhage occurs [47]. Hb is easily oxidized in the atheroma with its highly oxidative properties, resulting in the formation of metHb and ferrylHb, both of which can release heme. Heme and iron, taken together, affect the function of several types of cells, such as endothelial cells [48], smooth muscle cells [49], and macrophages [50], and contribute to low-density lipoprotein formation, and other components leading to

atherosclerosis [48]. MRI is now the established method for detection of atherosclerotic plaques. In several studies, various states of atherosclerosis can be detected on T1-weighted images, such as differentiated calcification and lipid-rich necrotic core [51, 52]. Recently, MRI T2* imaging, which can detect hemorrhage, has been used to evaluate the level of hemorrhage in several tissues (i.e. myocardium, liver and arterial wall) [53]. Additionally, mechanisms of intraplaque microhemorrhage and iron deposition were clarified by using T2* MRI in carotid atherosclerosis disease [54].

The relationship between iron and atherosclerosis was assessed using several hypercholesterolemic animal models such as hypercholesteremic rabbits [55] and apoE-deficient mice [56]. Intramuscular administration of dextran worsened the formation of atherosclerosis [57] but iron chelation using DFO inhibited atherosclerotic lesion development [58, 59]. On the other hand, several reports demonstrated that administration of iron also reduced the atherosclerotic lesion [55, 56]. Several studies reported that there is a strong positive correlation between iron stores, especially serum ferritin, and asymptomatic atherosclerosis in the carotid arteries [60, 61]. Conversely, some reports denied the association between iron stores and atherosclerosis [62]. A clinical study reported that periodic phlebotomy had no effect on the rate of mortality, myocardial infarction, or stroke in patients with peripheral artery disease [63]. To define mechanisms of iron accumulation in atherosclerotic lesions and its effects on the local environment, further studies are required.

Iron and Coronary Artery Disease

Various risk factors such as dyslipidemia, diabetes, smoking and obesity have been recognized as a predictor of atherosclerosis and CAD. Iron may possibly be another additional risk factor involved in cardiovascular disease, however the evidence for this issue is still controversial. Numerous studies have tried to verify this hypothesis, and one study that supports it showed that high-frequency blood donors exhibited lower body iron stores and lesser oxidative stress, compared with low-frequency donors [64]. In addition, high levels of iron in serum are correlated with the severity of CAD [65]. On the contrary, a systematic review using the PubMed and Cochrane Library databases showed that there is a negative association between transferrin levels and CAD [66]. A recent study reported that a higher iron status decreased CAD risk using a Mendelian randomization approach [67]. Additionally, several other studies seem to show no evidence to support this hypothesis [68, 69]. Taken together, there is not a consistent amount of evidence that corroborates the association between serum iron concentrations and CAD.

The majority of intracellular iron is located in ferritin, however some iron can be present in a loosely bound condition known as the cytosolic LIP [70]. The LIP has a pool of redox-active and chelatable iron, and its capacity promotes formation of ROS. A recent report in clinical study showed that the LIP in circulating monocytes correlates positively with the transferrin receptor/ferritin ratio, hepcidin levels, atherosclerotic progression, and arterial stiffness in patients with chronic cardiovascular disease [71]. The level of LIP in monocytes might be able to use as an indicator of atherosclerotic condition in arteries, including coronary arteries.

Iron and Myocardial Ischemia-Reperfusion Injury

In the heart, I/R injuries are a common clinical problem following percutaneous coronary intervention (PCI) or thrombolysis for acute myocardial infarction [72]. From this viewpoint of myocardial salvage, cardiologists have tried to shorten the interval between ischemia and reperfusion (door-to-balloon time). Although immediate restoration of blood flow is needed to suppress ischemic damage and ensure survival, reperfusion initiates the next phase of injury and can cause further substantial amounts of tissue damage and necrosis.

Experimental studies suggest that reperfusion injury by interventions such as PCI accounts for up to 50% of the final infarct size [73]. To date, although several clinical trials have attempted to improve and limit the cardiac damage due to reperfusion injury, no effective therapies have been established.

Many mechanisms have been thought to be associated with I/R injury, such as the production of ROS [74] and mitochondrial permeability transition pore (mPTP) formation [75]. Increased iron has been implicated in the pathology of I/R injury in a variety of organs such as the kidney [76] and lung [77]. A previous study using animal models of cardiac I/R injury suggested that activation of hypoxia inducible factor (HIF) signaling increases iron TfR1 expression, which in turn increases iron accumulation and exacerbates ROS-induced oxidative damage [78]. To suppress ROS production, several studies attempted to ascertain the efficacy of depleting iron by using iron chelators. However, the benefits of using chelation on I/R injuries are still debated. One study that reported a beneficial effect of chelators showed that the administration of DFO after I/R attenuated cardiac dysfunction in both ex vivo and in vivo [79–81]. Moreover, in clinical studies, DFO administration improved cardiac function in patients recovering from bypass surgery [82] and thalassemia [83]. On the contrary, it was reported that DFO failed to repair the cardiac damage created from I/R injuries [84].

The mitochondria may be the reason behind this difference, as they are central to the metabolic stresses that lead to cell death during I/R injury. Mitochondria are essential sources of ROS, while oxidative stress is a major cause of mitochondrial dysfunction during I/R injuries [85]. Reperfusion generates a large amount of ROS as oxygen reacts with leaked electrons to form superoxide. Within the early stage of reperfusion, intracellular pH returns from acidemia to a neutral pH because of the protons given by the sodium/hydrogen exchanger. These events contribute to the formation and opening of the mPTP, which leads to the induction of cardiomyocyte death [86]. A previous report demonstrated that increased mitochondrial iron contributes to myocardial injury in animal models and human samples [87]. The study also showed that a cell-permeable iron chelator, 2,2'-bipyridyl, protected the heart from I/R injury, but DFO, which has low cellular permeability, did not have the same protective effects. The mitochondria also have its own set of proteins that regulate its iron homeostasis. Mitochondrial ferritin is a protein that regulates iron storage [88], whereas FtMt and H-ferritin-like protein, which exists only in mitochondria and possesses ferroxidase activity, play a critical role in cell survival and ROS regulation in acute exhaustive exercise-induced myocardial injury [89]. These findings suggest that mitochondrial iron and its unique cohort of proteins that regulate mitochondrial iron are

potential therapeutic targets to treat myocardial injury, especially given the important role of mitochondria in I/R injuries.

Drugs and Therapies Targeting Iron and Myocardial Ischemia-Reperfusion Injury

Several iron uptake transport systems exist in the heart, such as TfR1, DMT1, and L-type or T-type voltage-dependent Ca^{2+} channels (LTCC or TTCC) [88, 90] (Figure 1). Transferrin highly regulates the iron uptake process, however once iron overload conditions occur, transferrin becomes saturated and toxic NTBI begins to accumulate. Although the mechanism of NTBI uptake in cardiomyocytes remains undefined, several reports showed LTCC or TTCC as potential routes for iron uptake under iron overload conditions [90]. Endosomes also play a role in iron uptake during iron overload [91]. Once iron enters myocytes, it is immediately bound by ferritin and transported to the lysosomes for long-term storage and degradation. Iron stored in lysosomes can be detected by MRI, and this method is useful in assessing myocardial iron noninvasively [9]. This technique could potentially be used to assess iron transport and homeostasis in in vivo I/R models.

The evaluation of pre- or post-treatment iron levels is very important to not only get a grasp of the disease's condition, but also determine the effectiveness of potential therapies. Serum ferritin levels are the most widely used diagnostic to assess cardiac risk, and high serum ferritin levels are associated with an increased risk for several cardiovascular diseases such as heart failure and CAD [92, 93]. However, since serum ferritin is affected by several pathological factors including infection, inflammation, ineffective erythropoiesis, ascorbate status, and hepatic condition, further studies are required to assess serum ferritin levels as a risk factor for cardiovascular diseases.

Chelation therapies are commonly used for individuals with iron overload caused by genetic disorders such as hereditary hemochromatosis. Chelation is also used for patients who receive frequent blood transfusions for genetic anemias such as beta-thalassemia major and sickle cell disease [94]. Currently, 11 FDA (Food and Drug Administration)-approved commercial chelators are available, and among them there are three specific iron chelators, DFO, deferasirox and deferiprone - all of which can eliminate cardiac iron [95]. Among them, DFO is the most widely-used non-toxic iron chelator to treat patients with beta-thalassemia and other iron overload diseases. However, since the half-life of DFO is very short, NTBI can only be suppressed for a short time after administration. For this reason, continuous administration of DFO is required to achieve effective iron chelation in the heart. DFO has yielded some positive results in treating cardiovascular disease, including studies reporting that DFO reduced mitochondrial ROS in rat cardiomyocytes [96] and improved endothelium-dependent vasodilation in patients with CAD [97]. In addition, injection of DFO after coronary artery bypass grafting shortened patients' stays in intensive care units and overall hospital stay, and also decreased lipid peroxidation [82]. On the other hand, a combination therapy of DFO and ascorbic acid did not seem to have a significant effect against myocardial I/R injury [84]. Unlike DFO, both deferasirox and deferiprone can be used for oral administration. Deferiprone has the lowest molecular weight of the three iron

chelators, and therefore can easily enter myocytes and intracellular components such as lysosomes and mitochondria [98]. Several studies support the use of deferiprone and demonstrate that it can reduce cardiac iron and protect against cardiac events when used alone or in combination with DFO [99, 100]. Deferasirox, the most recently approved oral iron chelator, has a cardioprotective effect by decreasing iron concentrations in cardiac tissue [101, 102]. Although all three iron chelators can reduce labile iron from plasma, there are few studies documenting the association and outcomes between iron chelators and I/R injury.

In addition to the three chelators discussed above, other chelators have been studied in efforts to improve post MI cardiac function and in other pathological settings. Chelation therapy using EDTA (ethylene diamine tetraacetic acid) with acute MI patients showed attenuated adverse cardiovascular outcomes [103]. Dexrazoxane, a mitochondria-permeable intracellular metal chelator, also decreased free radicals and improved the ex vivo hemodynamics of I/R injuries in rat hearts [104], while showing no protective effect on I/R injuries in pigs [105]. Further studies could take advantage of the different bioavailabilities, pharmacodynamics, and subcellular localization of chelators to select the right chelator in combination with other therapies that target the effect of iron in cardiovascular disease.

Conclusion

Iron is an essential mineral and plays pivotal roles in both normal physiological processes and pathological conditions for a variety of diseases. Starting with Sullivan's study, the relationship between iron and cardiovascular disease has been discussed for the last four decades. Although an uncontestable link between the two remains elusive, discussion on the topic continues with new studies constantly bringing new pathways and results that keep suggesting the possibility of iron as a risk factor in cardiovascular diseases. The importance of iron lies not only in its role as a catalyst in the production of ROS for cell signaling, but also as a catalyst for cell death through the production of lipid ROS. Moreover, the role of iron in the mitochondria is slowly being understood, and the management of mitochondrial iron may be a potential route in treating myocardial I/R injuries. Further studies are required in order to establish therapies that target iron more efficiently in cardiovascular diseases, especially in patients with acute MI. By studying and further elucidating mechanisms of iron homeostasis and cellular pathways involved in iron overload, future therapies could potentially target these pathways that regulate iron, and provide an additional therapeutic route as compared to chelators that directly target iron itself.

Acknowledgments

This work was supported in part by a research grant from Japan Heart Foundation, Japan (MK), the Mitsukoshi Health and Welfare Foundation, Japan (TS), a research grant from Kochi Organization for Medical Reformation and Renewal, Japan (YB) and NIH grants (P30GM103341 and P20GM113134 to TM).

Abbreviations

CAD	coronary artery disease
DFO	deferoxamine

FtMt	mitochondrial ferritin
GPx	glutathione peroxidase
Hb	hemoglobin
HIF	hypoxia inducible factor
HO⁻	hydroxyl radicals
H₂O₂	hydrogen peroxide
IL	interleukin
I/R	ischemia reperfusion
IRP	iron regulatory protein
LIP	labile iron pool
LTCC	L-type voltage-dependent Ca ²⁺ channels
LV	left ventricular
MI	myocardial infarction
mPTP	mitochondrial permeability transition pore
MRI	magnetic resonance imaging
mTOR	mechanistic target of rapamycin
NTBI	non-transferrin-bound iron
O₂⁻	superoxide
PCI	percutaneous coronary intervention
PI3K	phosphoinositide 3-kinase
RBC	red blood cell
ROOH	hydroperoxysides
RO⁻	alkoxyl radicals
RO₂⁻	peroxyl radicals
ROS	reactive oxygen species
RSL3	Ras Selective Lethal 3
STEMI	ST-segment elevation MI
TfR1	transferrin receptor 1
TTCC	T-type voltage-dependent Ca ²⁺ channels

VDAC voltage-dependent anion channel

References

- [1]. Hirst J Mitochondrial complex I. *Annu Rev Biochem* 2013;82:551–75. doi:10.1146/annurev-biochem-070511-103700 [PubMed: 23527692]
- [2]. Valko M, Jomova K, Rhodes CJ, Kuca K, Musilek K. Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol* 2016;90:1–37. doi: 10.1007/s00204-015-1579-5 [PubMed: 26343967]
- [3]. Okonko DO, Mandal AK, Missouris CG, Poole-Wilson PA. Disordered iron homeostasis in chronic heart failure: prevalence, predictors, and relation to anemia, exercise capacity, and survival. *J Am Coll Cardiol* 2011;58:1241–51. doi:10.1016/j.jacc.2011.04.040 [PubMed: 21903058]
- [4]. Davis BA, O’Sullivan C, Jarritt PH, Porter JB. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. *Blood* 2004; 104:263–9. doi:10.1182/blood-2003-08-2841 [PubMed: 15001468]
- [5]. Woollard KJ, Sturgeon S, Chin-Dusting JP, Salem HH, Jackson SP. Erythrocyte hemolysis and hemoglobin oxidation promote ferric chloride-induced vascular injury. *J Biol Chem* 2009;284:13110–8. doi:10.1074/jbc.M809095200 [PubMed: 19276082]
- [6]. Sullivan JL. Iron and the sex difference in heart disease risk. *Lancet* 1981;1:1293–4. [PubMed: 6112609]
- [7]. Goudarzi M, Mir N, Mousavi-Kamazani M, Bagheri S, Salavati-Niasari M. Biosynthesis and characterization of silver nanoparticles prepared from two novel natural precursors by facile thermal decomposition methods. *Sci Rep* 2016;6:32539. doi:10.1038/srep32539 [PubMed: 27581681]
- [8]. Wierzbinski KR, Szymanski T, Rozwadowska N, Rybka JD, Zimna A, Zalewski T, et al. Potential use of superparamagnetic iron oxide nanoparticles for in vitro and in vivo bioimaging of human myoblasts. *Sci Rep* 2018;8:3682. doi:10.1038/s41598-018-22018-0 [PubMed: 29487326]
- [9]. Carpenter JP, He T, Kirk P, Roughton M, Anderson LJ, de Noronha SV, et al. On T2* magnetic resonance and cardiac iron. *Circulation* 2011;123:1519–28. doi: 10.1161/CIRCULATIONAHA.110.007641 [PubMed: 21444881]
- [10]. Bulluck H, Rosmini S, Abdel-Gadir A, White SK, Bhuvana AN, Treibel TA, et al. Residual Myocardial Iron Following Intramyocardial Hemorrhage During the Convalescent Phase of Reperfused ST-Segment-Elevation Myocardial Infarction and Adverse Left Ventricular Remodeling. *Circ Cardiovasc Imaging* 2016;9. doi:10.1161/CIRCIMAGING.116.004940
- [11]. Ganame J, Messalli G, Dymarkowski S, Rademakers FE, Desmet W, Van de Werf F, et al. Impact of myocardial haemorrhage on left ventricular function and remodelling in patients with reperfused acute myocardial infarction. *Eur Heart J* 2009;30:1440–9. doi:10.1093/eurheartj/ehp093 [PubMed: 19346229]
- [12]. Carrick D, Haig C, Ahmed N, McEntegart M, Petrie MC, Eteiba H, et al. Myocardial Hemorrhage After Acute Reperfused ST-Segment-Elevation Myocardial Infarction: Relation to Microvascular Obstruction and Prognostic Significance. *Circ Cardiovasc Imaging* 2016;9:e004148. doi:10.1161/CIRCIMAGING.115.004148 [PubMed: 26763281]
- [13]. Sindrilaru A, Peters T, Wieschalka S, Baican C, Baican A, Peter H, et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest* 2011;121:985–97. doi:10.1172/JCI44490 [PubMed: 21317534]
- [14]. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012; 149:1060–72. doi:10.1016/j.cell.2012.03.042 [PubMed: 22632970]
- [15]. Yang WS, Stockwell BR. Ferroptosis: Death by Lipid Peroxidation. *Trends Cell Biol* 2016;26:165–76. doi:10.1016/j.tcb.2015.10.014 [PubMed: 26653790]
- [16]. Imai H, Matsuoka M, Kumagai T, Sakamoto T, Koumura T. Lipid Peroxidation-Dependent Cell Death Regulated by GPx4 and Ferroptosis. *Curr Top Microbiol Immunol* 2017;403:143–70. doi: 10.1007/82_2016_508 [PubMed: 28204974]

- [17]. Yu H, Guo P, Xie X, Wang Y, Chen G. Ferroptosis, a new form of cell death, and its relationships with tumorous diseases. *J Cell Mol Med* 2017;21:648–57. doi:10.1111/jcmm.13008 [PubMed: 27860262]
- [18]. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol* 2014;16:1180–91. doi:10.1038/ncb3064 [PubMed: 25402683]
- [19]. Baba Y, Higa JK, Shimada BK, Horiuchi KM, Suhara T, Kobayashi M, et al. Protective effects of the mechanistic target of rapamycin against excess iron and ferroptosis in cardiomyocytes. *Am J Physiol Heart Circ Physiol* 2018;314:H659–H68. doi:10.1152/ajpheart.00452.2017 [PubMed: 29127238]
- [20]. Aisen P, Enns C, Wessling-Resnick M. Chemistry and biology of eukaryotic iron metabolism. *Int J Biochem Cell Biol* 2001;33:940–59. [PubMed: 11470229]
- [21]. Gammella E, Recalcati S, Cairo G. Dual Role of ROS as Signal and Stress Agents: Iron Tips the Balance in favor of Toxic Effects. *Oxid Med Cell Longev* 2016;2016:8629024. doi: 10.1155/2016/8629024 [PubMed: 27006749]
- [22]. Levi S, Corsi B, Bosisio M, Invernizzi R, Volz A, Sanford D, et al. A human mitochondrial ferritin encoded by an intronless gene. *J Biol Chem* 2001;276:24437–40. doi:10.1074/jbc.C100141200 [PubMed: 11323407]
- [23]. Santambrogio P, Biasiotto G, Sanvito F, Olivieri S, Arosio P, Levi S. Mitochondrial ferritin expression in adult mouse tissues. *J Histochem Cytochem* 2007;55:1129–37. doi:10.1369/jhc.7A7273.2007 [PubMed: 17625226]
- [24]. Nie G, Sheftel AD, Kim SF, Ponka P. Overexpression of mitochondrial ferritin causes cytosolic iron depletion and changes cellular iron homeostasis. *Blood* 2005;105:2161–7. doi:10.1182/blood-2004-07-2722 [PubMed: 15522954]
- [25]. Campanella A, Rovelli E, Santambrogio P, Cozzi A, Taroni F, Levi S. Mitochondrial ferritin limits oxidative damage regulating mitochondrial iron availability: hypothesis for a protective role in Friedreich ataxia. *Hum Mol Genet* 2009;18:1–11. doi:10.1093/hmg/ddn308 [PubMed: 18815198]
- [26]. Wang YQ, Chang SY, Wu Q, Gou YJ, Jia L, Cui YM, et al. The Protective Role of Mitochondrial Ferritin on Erastin-Induced Ferroptosis. *Front Aging Neurosci* 2016;8:308. doi:10.3389/fnagi.2016.00308 [PubMed: 28066232]
- [27]. Matsui T, Rosenzweig A. Targeting ischemic cardiac dysfunction through gene transfer. *Curr Atheroscler Rep* 2003;5:191–5. [PubMed: 12667431]
- [28]. Dixon SJ. Ferroptosis: bug or feature? *Immunol Rev* 2017;277:150–7. doi:10.1111/imr.12533 [PubMed: 28462529]
- [29]. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014;156:317–31. doi: 10.1016/j.cell.2013.12.010 [PubMed: 24439385]
- [30]. Cao JY, Dixon SJ. Mechanisms of ferroptosis. *Cell Mol Life Sci* 2016;73:2195–209. doi:10.1007/s00018-016-2194-1 [PubMed: 27048822]
- [31]. Yagoda N, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, Fridman DJ, et al. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature* 2007;447:864–8. doi:10.1038/nature05859 [PubMed: 17568748]
- [32]. Kim JY, Kanai Y, Chairoungdua A, Cha SH, Matsuo H, Kim DK, et al. Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochim Biophys Acta* 2001;1512:335–44. [PubMed: 11406111]
- [33]. Brigelius-Flohe R, Maiorino M. Glutathione peroxidases. *Biochim Biophys Acta* 2013;1830:3289–303. doi:10.1016/j.bbagen.2012.11.020 [PubMed: 23201771]
- [34]. Dabkowski ER, Williamson CL, Hollander JM. Mitochondria-specific transgenic overexpression of phospholipid hydroperoxide glutathione peroxidase (GPx4) attenuates ischemia/reperfusion-associated cardiac dysfunction. *Free Radic Biol Med* 2008;45:855–65. doi:10.1016/j.freeradbiomed.2008.06.021 [PubMed: 18638546]
- [35]. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and Transferrin Regulate Ferroptosis. *Mol Cell* 2015;59:298–308. doi:10.1016/j.molcel.2015.06.011 [PubMed: 26166707]

- [36]. Aoyagi T, Kusakari Y, Xiao CY, Inouye BT, Takahashi M, Scherrer-Crosbie M, et al. Cardiac mTOR protects the heart against ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2012;303:H75–85. doi:10.1152/ajpheart.00241.2012 [PubMed: 22561297]
- [37]. Aoyagi T, Higa JK, Aoyagi H, Yorichika N, Shimada BK, Matsui T. Cardiac mTOR rescues the detrimental effects of diet-induced obesity in the heart after ischemia-reperfusion. *Am J Physiol Heart Circ Physiol* 2015;308:H1530–9. doi:10.1152/ajpheart.00008.2015 [PubMed: 25888508]
- [38]. Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol* 2014;15:155–62. doi:nrm3757 [pii] 10.1038/nrm3757 [PubMed: 24556838]
- [39]. Aoyagi T, Matsui T. Phosphoinositide-3 kinase signaling in cardiac hypertrophy and heart failure. *Curr Pharm Des* 2011;17:1818–24. [PubMed: 21631421]
- [40]. Song X, Kusakari Y, Xiao CY, Kinsella SD, Rosenberg MA, Scherrer-Crosbie M, et al. mTOR attenuates the inflammatory response in cardiomyocytes and prevents cardiac dysfunction in pathological hypertrophy. *Am J Physiol Cell Physiol* 2010;299:C1256–66. doi:10.1152/ajpcell.00338.2010 [PubMed: 20861467]
- [41]. Bayeva M, Khechaduri A, Puig S, Chang HC, Patial S, Blackshear PJ, et al. mTOR regulates cellular iron homeostasis through tristetraprolin. *Cell Metab* 2012;16:645–57. doi:10.1016/j.cmet.2012.10.001 [PubMed: 23102618]
- [42]. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005;5:953–64. [PubMed: 16322748]
- [43]. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010;32:593–604. doi:10.1016/j.immuni.2010.05.007 [PubMed: 20510870]
- [44]. You SA, Archacki SR, Angheloiu G, Moravec CS, Rao S, Kinter M, et al. Proteomic approach to coronary atherosclerosis shows ferritin light chain as a significant marker: evidence consistent with iron hypothesis in atherosclerosis. *Physiol Genomics* 2003;13:25–30. doi:10.1152/physiolgenomics.00124.2002 [PubMed: 12644631]
- [45]. Stadler N, Lindner RA, Davies MJ. Direct detection and quantification of transition metal ions in human atherosclerotic plaques: evidence for the presence of elevated levels of iron and copper. *Arterioscler Thromb Vasc Biol* 2004;24:949–54. doi:10.1161/01.ATV.0000124892.90999.cb [PubMed: 15001454]
- [46]. Brissot P, Ropert M, Le Lan C, Loreal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochim Biophys Acta* 2012;1820:403–10. doi: 10.1016/j.bbagen.2011.07.014 [PubMed: 21855608]
- [47]. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, et al. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med* 2003;349:2316–25. doi: 10.1056/NEJMoa035655 [PubMed: 14668457]
- [48]. Nagy E, Eaton JW, Jeney V, Soares MP, Varga Z, Galajda Z, et al. Red cells, hemoglobin, heme, iron, and atherogenesis. *Arterioscler Thromb Vasc Biol* 2010;30:1347–53. doi:10.1161/ATVBAHA.110.206433 [PubMed: 20378845]
- [49]. Moraes JA, Barcellos-de-Souza P, Rodrigues G, Nascimento-Silva V, Silva SV, Assreuy J, et al. Heme modulates smooth muscle cell proliferation and migration via NADPH oxidase: a counter-regulatory role for heme oxygenase system. *Atherosclerosis* 2012;224:394–400. doi:10.1016/j.atherosclerosis.2012.07.043 [PubMed: 22954673]
- [50]. Finn AV, Nakano M, Polavarapu R, Karmali V, Saeed O, Zhao X, et al. Hemoglobin directs macrophage differentiation and prevents foam cell formation in human atherosclerotic plaques. *J Am Coll Cardiol* 2012;59:166–77. doi:10.1016/j.jacc.2011.10.852 [PubMed: 22154776]
- [51]. Puppini G, Furlan F, Cirotta N, Veraldi G, Piubello Q, Montemezzi S, et al. Characterisation of carotid atherosclerotic plaque: comparison between magnetic resonance imaging and histology. *Radiol Med* 2006;111:921–30. doi:10.1007/s11547-006-0091-7 [PubMed: 17021689]
- [52]. Albuquerque LC, Narvaes LB, Maciel AA, Staub H, Friedrich M, Filho JR, et al. Intraplaque hemorrhage assessed by high-resolution magnetic resonance imaging and C-reactive protein in carotid atherosclerosis. *J Vasc Surg* 2007;46:1130–7. doi:10.1016/j.jvs.2007.07.041 [PubMed: 17920229]

- [53]. Tanner MA, He T, Westwood MA, Firmin DN, Pennell DJ, Thalassemia International Federation Heart TI. Multi-center validation of the transferability of the magnetic resonance T2* technique for the quantification of tissue iron. *Haematologica* 2006;91:1388–91. [PubMed: 17018390]
- [54]. Raman SV, Winner MW 3rd, Tran T, Velayutham M, Simonetti OP, Baker PB, et al. In vivo atherosclerotic plaque characterization using magnetic susceptibility distinguishes symptom-producing plaques. *JACC Cardiovasc Imaging* 2008;1:49–57. doi:10.1016/j.jcmg.2007.09.002 [PubMed: 19356405]
- [55]. Dabbagh AJ, Shwaery GT, Keaney JF Jr., Frei B. Effect of iron overload and iron deficiency on atherosclerosis in the hypercholesterolemic rabbit. *Arterioscler Thromb Vasc Biol* 1997;17:2638–45. [PubMed: 9409237]
- [56]. Kirk EA, Heinecke JW, LeBoeuf RC. Iron overload diminishes atherosclerosis in apoE-deficient mice. *J Clin Invest* 2001;107:1545–53. doi:10.1172/JCI7664 [PubMed: 11413162]
- [57]. Araujo JA, Romano EL, Brito BE, Parthe V, Romano M, Bracho M, et al. Iron overload augments the development of atherosclerotic lesions in rabbits. *Arterioscler Thromb Vasc Biol* 1995;15:1172–80. [PubMed: 7542998]
- [58]. Minqin R, Rajendran R, Pan N, Tan BK, Ong WY, Watt F, et al. The iron chelator desferrioxamine inhibits atherosclerotic lesion development and decreases lesion iron concentrations in the cholesterol-fed rabbit. *Free Radic Biol Med* 2005;38:1206–11. doi:10.1016/j.freeradbiomed.2005.01.008 [PubMed: 15808418]
- [59]. Zhang WJ, Wei H, Frei B. The iron chelator, desferrioxamine, reduces inflammation and atherosclerotic lesion development in experimental mice. *Exp Biol Med* (Maywood) 2010;235:633–41. doi:10.1258/ebm.2009.009229 [PubMed: 20463304]
- [60]. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. *Circulation* 1997;96:3300–7. [PubMed: 9396420]
- [61]. Syrovatka P, Kraml P, Hulikova K, Fialova L, Vejrazka M, Crkovska J, et al. Iron stores are associated with asymptomatic atherosclerosis in healthy men of primary prevention. *Eur J Clin Invest* 2011;41:846–53. doi:10.1111/j.1365-2362.2011.02474.x [PubMed: 21281279]
- [62]. Auer J, Rammer M, Berent R, Weber T, Lassnig E, Eber B. Body iron stores and coronary atherosclerosis assessed by coronary angiography. *Nutr Metab Cardiovasc Dis* 2002;12:285–90. [PubMed: 12616808]
- [63]. Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. *JAMA* 2007;297:603–10. doi:10.1001/jama.297.6.603 [PubMed: 17299195]
- [64]. Zheng H, Cable R, Spencer B, Votto N, Katz SD. Iron stores and vascular function in voluntary blood donors. *Arterioscler Thromb Vasc Biol* 2005;25:1577–83. doi:10.1161/01.ATV.0000174126.28201.61 [PubMed: 15961703]
- [65]. Bagheri B, Shokrzadeh M, Mokhberi V, Azizi S, Khalilian A, Akbari N, et al. Association between Serum Iron and the Severity of Coronary Artery Disease. *Int Cardiovasc Res J* 2013;7:95–8. [PubMed: 24757630]
- [66]. Das De S, Krishna S, Jethwa A. Iron status and its association with coronary heart disease: systematic review and meta-analysis of prospective studies. *Atherosclerosis* 2015;238:296–303. doi:10.1016/j.atherosclerosis.2014.12.018 [PubMed: 25544180]
- [67]. Gill D, Del Greco MF, Walker AP, Srai SKS, Laffan MA, Minelli C. The Effect of Iron Status on Risk of Coronary Artery Disease: A Mendelian Randomization Study-Brief Report. *Arterioscler Thromb Vasc Biol* 2017;37:1788–92. doi:10.1161/ATVBAHA.117.309757 [PubMed: 28684612]
- [68]. Germain M, Delage G, Blais C, Maunsell E, Decary F, Gregoire Y. Iron and cardiac ischemia: a natural, quasi-random experiment comparing eligible with disqualified blood donors. *Transfusion* 2013;53:1271–9. doi: 10.1111/trf.12081 [PubMed: 23320537]
- [69]. Menke A, Muntner P, Fernandez-Real JM, Guallar E. The association of biomarkers of iron status with mortality in US adults. *Nutr Metab Cardiovasc Dis* 2012;22:734–40. doi:10.1016/j.numecd.2010.11.011 [PubMed: 21330119]
- [70]. Kakhlon O, Cabantchik ZI. The labile iron pool: characterization, measurement, and participation in cellular processes. *Free Radic Biol Med* 2002;33:1037–46. [PubMed: 12374615]

- [71]. Risko P, Platenik J, Buchal R, Potockova J, Kraml PJ. The labile iron pool in monocytes reflects the activity of the atherosclerotic process in men with chronic cardiovascular disease. *Physiol Res* 2017;66:49–61. [PubMed: 27782743]
- [72]. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest* 2013;123:92–100. doi:10.1172/JCI62874 [PubMed: 23281415]
- [73]. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007;357:1121–3S. doi: 10.1056/NEJMr071667
- [74]. Raedschelders K, Ansley DM, Chen DD. The cellular and molecular origin of reactive oxygen species generation during myocardial ischemia and reperfusion. *Pharmacol Ther* 2012;133:230–SS. doi:10.1016/j.pharmthera.2011.11.004 [PubMed: 22138603]
- [75]. Morciano G, Giorgi C, Bonora M, Punzetti S, Pavasini R, Wieckowski MR, et al. Molecular identity of the mitochondrial permeability transition pore and its role in ischemia-reperfusion injury. *J Mol Cell Cardiol* 2015;7B:142–S3. doi:10.1016/j.yjmcc.2014.0B.01S
- [76]. Kaushal GP, Shah SV. Challenges and advances in the treatment of AKI. *J Am Soc Nephrol* 2014;2S:B77–B3. doi:10.16B1/ASN.20130707B0
- [77]. Ghio AJ. Disruption of iron homeostasis and lung disease. *Biochim Biophys Acta* 2009;1790:731–9. doi:10.1016/j.bbagen.2008.11.004 [PubMed: 19100311]
- [78]. Tang WH, Wu S, Wong TM, Chung SK, Chung SS. Polyol pathway mediates iron-induced oxidative injury in ischemic-reperfused rat heart. *Free Radic Biol Med* 200B;4S:602–10. doi: 10.1016/j.freeradbiomed.200B.0S.003
- [79]. Williams RE, Zweier JL, Flaherty JT. Treatment with deferoxamine during ischemia improves functional and metabolic recovery and reduces reperfusion-induced oxygen radical generation in rabbit hearts. *Circulation* 1991;83:1006–14. [PubMed: 1847847]
- [80]. Nicholson SC, Squier M, Ferguson DJ, Nagy Z, Westaby S, Evans RD. Effect of desferrioxamine cardioplegia on ischemia-reperfusion injury in isolated rat heart. *Ann Thorac Surg* 1997;63:1003–11. [PubMed: 9124897]
- [81]. Kobayashi S, Tadokoro H, Wakida Y, Kar S, Nordlander R, Haendchen RV, et al. Coronary venous retroinfusion of deferoxamine reduces infarct size in pigs. *J Am Coll Cardiol* 1991;18:621–7. [PubMed: 1856431]
- [82]. Paraskevaidis IA, Iliodromitis EK, Vlahakos D, Tsiapras DP, Nikolaidis A, Marathias A, et al. Deferoxamine infusion during coronary artery bypass grafting ameliorates lipid peroxidation and protects the myocardium against reperfusion injury: immediate and long-term significance. *Eur Heart J* 2005;26:263–70. doi:10.1093/eurheartj/ehi028 [PubMed: 15618054]
- [83]. Porter JB, Wood J, Olivieri N, Vichinsky EP, Taher A, Neufeld E, et al. Treatment of heart failure in adults with thalassemia major: response in patients randomised to deferoxamine with or without deferiprone. *J Cardiovasc Magn Reson* 2013;1S:38. doi:10.1186/1S32-429X-1S-38
- [84]. Chatziathanasiou GN, Nikas DN, Katsouras CS, Kazakos ND, Bouba V, Vougiouklakis T, et al. Combined intravenous treatment with ascorbic acid and desferrioxamine to reduce myocardial reperfusion injury in an experimental model resembling the clinical setting of primary PCI. *Hellenic J Cardiol* 2012;S3:19S–204.
- [85]. Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res* 2006;70:181–90. doi:10.1016/j.cardiores.2006.02.02S [PubMed: 16580655]
- [86]. Pell VR, Chouchani ET, Frezza C, Murphy MP, Krieg T. Succinate metabolism: a new therapeutic target for myocardial reperfusion injury. *Cardiovasc Res* 2016; 111:134–41. doi: 10.1093/cvr/cvw100 [PubMed: 27194563]
- [87]. Chang HC, Wu R, Shang M, Sato T, Chen C, Shapiro JS, et al. Reduction in mitochondrial iron alleviates cardiac damage during injury. *EMBO Mol Med* 2016;8:247–67. doi:10.15252/emmm.201505748 [PubMed: 26896449]
- [88]. Wood JC. Cardiac iron across different transfusion-dependent diseases. *Blood Rev* 2008;22 Suppl 2:S14–21. doi:10.1016/S0268-960X(08)70004-3 [PubMed: 19059052]
- [89]. Wu W, Chang S, Wu Q, Xu Z, Wang P, Li Y, et al. Mitochondrial ferritin protects the murine myocardium from acute exhaustive exercise injury. *Cell Death Dis* 2016;7:e2475. doi:10.1038/cddis.2016.372 [PubMed: 27853170]

- [90]. Wijarnpreecha K, Kumfu S, Chattipakorn SC, Chattipakorn N. Cardiomyopathy associated with iron overload: how does iron enter myocytes and what are the implications for pharmacological therapy? *Hemoglobin* 2015;39:9–17. doi:10.3109/03630269.2014.987869 [PubMed: 25572185]
- [91]. Sohn YS, Ghoti H, Breuer W, Rachmilewitz E, Attar S, Weiss G, et al. The role of endocytic pathways in cellular uptake of plasma non-transferrin iron. *Haematologica* 2012;97:670–8. doi: 10.3324/haematol.2011.054858 [PubMed: 22180428]
- [92]. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;22:2171–9. [PubMed: 11913479]
- [93]. Pourmoghaddas A, Sanei H, Garakyaraghi M, Esteki-Ghashghaei F, Gharaati M. The relation between body iron store and ferritin, and coronary artery disease. *ARYA Atheroscler* 2014;10:32–6. [PubMed: 24963311]
- [94]. Mobarra N, Shanaki M, Ehteram H, Nasiri H, Sahmani M, Saeidi M, et al. A Review on Iron Chelators in Treatment of Iron Overload Syndromes. *Int J Hematol Oncol Stem Cell Res* 2016;10:239–47. [PubMed: 27928480]
- [95]. Wax PM. Current use of chelation in American health care. *J Med Toxicol* 2013;9:303–7. doi: 10.1007/s13181-013-0347-2 [PubMed: 24113860]
- [96]. Philipp S, Cui L, Ludolph B, Kelm M, Schulz R, Cohen MV, et al. Desferoxamine and ethyl-3,4-dihydroxybenzoate protect myocardium by activating NOS and generating mitochondrial ROS. *Am J Physiol Heart Circ Physiol* 2006;290:H450–7. doi:10.1152/ajpheart.00472.2005 [PubMed: 16155105]
- [97]. Duffy SJ, Biegelsen ES, Holbrook M, Russell JD, Gokce N, Keaney JF Jr., et al. Iron chelation improves endothelial function in patients with coronary artery disease. *Circulation* 2001;103:2799–804. [PubMed: 11401935]
- [98]. Glickstein H, El RB, Shvartsman M, Cabantchik ZI. Intracellular labile iron pools as direct targets of iron chelators: a fluorescence study of chelator action in living cells. *Blood* 2005;106:3242–50. doi:10.1182/blood-2005-02-0460 [PubMed: 16020512]
- [99]. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, et al. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. *Blood* 2006;107:3733–7. doi:10.1182/blood-2005-07-2933 [PubMed: 16373663]
- [100]. Pepe A, Meloni A, Capra M, Cianciulli P, Prossomariti L, Malaventura C, et al. Deferasirox, deferiprone and desferrioxamine treatment in thalassemia major patients: cardiac iron and function comparison determined by quantitative magnetic resonance imaging. *Haematologica* 2011;96:41–7. doi:10.3324/haematol.2009.019042 [PubMed: 20884710]
- [101]. Cappellini MD, Porter J, El-Beshlawy A, Li CK, Seymour JF, Elalfy M, et al. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *Haematologica* 2010;95:557–66. doi:10.3324/haematol.2009.014696 [PubMed: 19951979]
- [102]. Pennell DJ, Porter JB, Cappellini MD, El-Beshlawy A, Chan LL, Aydinok Y, et al. Efficacy of deferasirox in reducing and preventing cardiac iron overload in beta-thalassemia. *Blood* 2010;115:2364–71. doi:10.1182/blood-2009-04-217455 [PubMed: 19996412]
- [103]. Lamas GA, Goertz C, Boineau R, Mark DB, Rozema T, Nahin RL, et al. Effect of disodium EDTA chelation regimen on cardiovascular events in patients with previous myocardial infarction: the TACT randomized trial. *JAMA* 2013;309:1241–50. doi:10.1001/jama.2013.2107 [PubMed: 23532240]
- [104]. Ramu E, Korach A, Houminer E, Schneider A, Elami A, Schwalb H. Dexrazoxane prevents myocardial ischemia/reperfusion-induced oxidative stress in the rat heart. *Cardiovasc Drugs Ther* 2006;20:343–8. doi:10.1007/s10557-006-0497-4 [PubMed: 17119874]
- [105]. Kamat P, Vandenberghe S, Christen S, Bongoni AK, Meier B, Rieben R, et al. Dexrazoxane Shows No Protective Effect in the Acute Phase of Reperfusion during Myocardial Infarction in Pigs. *PLoS One* 2016;11:e0168541. doi:10.1371/journal.pone.0168541 [PubMed: 28002439]

Figure 1. Intracellular iron regulation and ROS generation.

Ferric iron [Fe(III) , Fe^{3+}] is bound with transferrin (Tf) and transported via TfR1 (Transferrin receptor 1), and ferrous iron [Fe(II) , Fe^{2+}] enters the cells mostly through several transport systems, such as divalent metal transporter (DMT1) and L-type voltage-dependent Ca^{2+} channels (LTCC) [90]. Intracellular Fe(H) moves to mitochondria and generates ROS via the Fenton reaction (1). Fe(III) and Fe(II) also mediate membrane lipid peroxidation by catalyzing the reductive cleavage of hydroperoxides (ROOH) resulting in the formation of alkoxyl ($\text{RO}^\bullet$) and peroxy ($\text{RO}_2^\bullet$) radicals (2). The red lightning bolts indicate free radical oxidative damage and propagation of free radicals.

Figure 2. Signaling pathways of ferroptosis.

Class 1 ferroptosis inducers such as erastin, inhibit system X_c⁻, a cystine/glutamine antiporter, and deprive cells of glutathione. Glutathione is a necessary substrate for GPx4, which inhibits lipid peroxidation and prevents cell death. Class 2 ferroptosis inducers such as Ras Selective Lethal 3 (RSL3), directly inhibit GPx4, thus triggering the accumulation of lipid oxygen reactive species (ROS) and resulting in cell death. The inhibition of lipid peroxidation by deferoxamine (DFO), an iron chelator, or ferrostatin-1, a ferroptosis inhibitor, reduces the production of lipid ROS and attenuates cell death. The effects of erastin in cardiomyocytes is different from other cells because system X_c⁻ is not expressed in the heart. Please see the details in the text.