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STATE-OF-THE-ART REVIEW

Molecular Mechanisms and Therapeutic Targeting of Ferroptosis in Doxorubicin-Induced Cardiotoxicity

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HIGHLIGHTS

- **Ferroptosis is a novel form of regulated cell death driven by iron overload, loss of antioxidant defenses, and lipid peroxidation.**
- **Ferroptosis involves the interplay of a variety of metabolic processes, including iron, glutathione, and lipid metabolism.**
- **Preclinical evidence supports a pathophysiologic role of ferroptosis in the pathogenesis of doxorubicin-induced cardiotoxicity.**
- **Modulation of ferroptosis with specific inhibitors may provide new therapeutic opportunities for doxorubicin-induced cardiotoxicity.**

SUMMARY

Ferroptosis, an iron-dependent form of regulated cell death, has received increasing attention for its pathophysiologic contribution to the onset and development of doxorubicin-induced cardiotoxicity. Moreover, modulation of ferroptosis with specific inhibitors may provide new therapeutic opportunities for doxorubicin-induced cardiotoxicity. Here, we will review the molecular mechanisms and therapeutic promise of targeting ferroptosis in doxorubicin-induced cardiotoxicity. (J Am Coll Cardiol Basic Trans Science 2024;9:811-826) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

As a member of the anthracycline family, doxorubicin (DOX) serves as a chemotherapeutic agent in a large variety of solid tumors and hematologic malignancies.[1-3] However, serious dose-dependent adverse effects of DOX on hearts greatly limit its clinical application.[4] Specifically, a 5% incidence of heart failure is related to a cumulative dose of 400 mg/m^2. Higher doses of DOX lead to an exponential rise in risks of heart failure, with 16%, 26%, and 48% incidences at doses of 500, 550, and 700 mg/m^2, respectively.[5] Clinically, DOX cardiotoxicity can be acute and chronic. Acute cardiotoxicity is characterized by transient left ventricular dysfunction, supraventricular arrhythmia, and electrocardiographic abnormalities.[4] By contrast, chronic cardiotoxicity is manifested by cardiac enlargement; ventricular dilation; progressive decline of cardiac function; and, ultimately, heart failure.[6,7]

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ABBREVIATIONS AND ACRONYMS

5-ALA = 5-aminolevulinic acid

Acot1 = acyl-coenzyme A thioesterase 1

ACSL4 = acyl-coenzyme A synthase long-chain family member 4

Alas1 = 5′-aminolevulinate synthase 1

AMPK = adenosine monophosphate–activated protein kinase

DFO = deferoxamine

DMT1 = divalent metal transporter 1

EGCG = epigallocatechin-3-gallate

Fer-1 = ferrostatin-1

FUNDC2 = FUN14 domain-containing 2

GPX4 = glutathione peroxidase 4

HMGB1 = high mobility group box 1

HO1 = heme oxygenase 1

IRE = iron response element

IRP = iron regulatory protein

LC/MS = liquid chromatography/mass spectrometer

LIP = labile iron pool

LOX = lipoxygenase

LPCAT3 = lysophosphatidylcholine acyltransferase 3

METTL14 = methyltransferase-like 14

NAC = *N*-acetyl cysteine

NCOA4 = nuclear receptor coactivator 4

Nrf2 = nuclear factor-erythroid 2-related factor 2

PRMT4 = protein arginine methyltransferase 4

RCD = regulated cell death

RTA = radical-trapping antioxidant

SLC39A14 = solute carrier family 39 member 14

STAT3 = signal transducer and activator of transcription 3

TEMPO = 2,2,6,6-tetramethylpiperidinyl-1-oxyl

TFR1 = transferrin receptor protein 1

Morphologically, DOX evokes cardiomyocyte atrophy and cell loss, interstitial fibrosis, and cardiomyocyte microvacuolization.[8] Currently, dexrazoxane is the only U.S. Food and Drug Administration (FDA)-approved drug for the treatment of DOX cardiotoxicity, through several mechanisms including iron chelation, attenuation of oxidative stress, and inhibition of DNA damage.[8,9] In addition, other therapies include alteration of the dosing regimen, application of antioxidants, liposomal formulation of DOX, and heart failure medications (eg, beta blockers and renin-angiotensin-aldosterone inhibitors).[10] Nonetheless, these medications are less likely targeting specific molecular pathways involved in the pathogenesis of DOX cardiotoxicity. In this context, it is important to explore alternative therapeutic modalities.

To date, several cellular and molecular mechanisms have been postulated for the pathogenesis of DOX-induced cardiotoxicity, including mitochondrial dysfunction, DNA damage response, and oxidative stress.[6,11] In addition, various forms of regulated cell death (RCD), such as pyroptosis, necroptosis, and ferroptosis, are implicated in the pathogenesis of DOX-induced cardiotoxicity.[12,13] Unlike other types of RCD, ferroptosis is an iron-dependent, regulated, nonapoptotic cell death that morphologically features cell swelling, condensed mitochondria, distorted mitochondria crista, and increased mitochondrial membrane densities. Ferroptosis is driven by inhibition of glutathione-dependent peroxidase glutathione peroxidase 4 (GPX4) and peroxidation of polyunsaturated fatty acid (PUFA)-containing phospholipids.[14,15] Therefore, the initiation of ferroptosis encompasses 3 major pillars of metabolism: iron, phospholipids, and glutathione (GSH). Over the past 5 years, an increasing number of studies have suggested a crucial pathophysiologic role for ferroptosis in the progression of DOX-induced cardiotoxicity.[16-18] Multiple studies have depicted that DOX leads to an increase in cardiac iron levels, lipid reactive oxygen species (ROS), and ferroptosis, contributing to myocardial injury.[16-18] Moreover, various pharmacologic inhibitors targeting ferroptosis have surfaced and have exhibited efficacy in the prevention and treatment of DOX-induced cardiotoxicity in preclinical models.[16-20] In this review, we summarize the molecular mechanisms in the regulation of ferroptosis in cardiomyocytes, including iron homeostasis, GSH synthesis, and lipid metabolism. Furthermore, we review recent findings regarding dysregulated iron metabolism and ferroptosis in the pathogenesis of DOX-induced cardiotoxicity and discuss newly identified putative targets of ferroptosis for DOX-induced cardiotoxicity.

MOLECULAR AND METABOLIC MECHANISMS OF FERROPTOSIS IN THE HEART

Cells undergoing ferroptosis display morphologic, genetic, and metabolic hallmarks distinct from other forms of RCD.[21] Notably, the complex interplay among various pathways involved in iron, GSH, and lipid metabolism converge to participate in regulation of the initiation and execution of ferroptosis, particularly in cardiomyocytes (**Figure 1**).[14] Here, the roles of these molecular and metabolic pathways in mediating ferroptosis are discussed.

IRON METABOLISM AND FERROPTOSIS. Cellular iron intake is driven by binding of iron-bound transferrin (Fe^{3+}) to its receptor transferrin receptor protein 1 (TFR1) and subsequent endocytosis in endosomes.[22] The endosome is acidified by vacuolar adenosine triphosphatase, resulting in the reduction of Fe^{3+} to Fe^{2+} by the 6-transmembrane epithelial antigen of prostate 3 (STEAP3). Then, Fe^{2+} is released into the cytoplasm through divalent metal transporter 1 (DMT1).[23] In addition, the solute carrier family 39 member 14 (SLC39A14) serves as an Fe^{2+} transporter for iron intake.[24] Within the cytoplasm, labile iron pool (LIP) can be used in cellular processes or stored for later use through binding with ferritin, including ferritin heavy chain (FTH) and ferritin light chain (FTL).[25] Ferritin-bound iron is degraded by nuclear receptor coactivator 4 (NCOA4)-mediated autophagy (also called ferritinophagy).[26] The ferritinophagy-mediated degradation of lysosomal ferritin then results in the release of iron,[27] which is exported to the cytosol through lysosomal DMT1.[28] Ferroportin (FPN) is the only known mammalian iron exporter, mediating the export of intracellular Fe^{2+} to extracellular space.[29] FPN-deficient mice exhibited severely disturbed iron homeostasis.[30] Notably, imbalance among iron intake, storage, and export can affect cell susceptibility to lipid peroxidation and ferroptosis. Under aerobic conditions, accumulated cellular iron, particularly labile Fe^{2+}, directly reacts with intracellular oxidants to generate cytotoxic hydroxyl radicals or peroxide radicals

through the Fenton reaction, resulting in lipid peroxidation and ferroptosis.[31]

GLUTATHIONE METABOLISM AND FERROPTOSIS. Notably, GSH depletion, cysteine or cysteine deficiency, and inactivation of GPX4 are shown to induce ferroptosis.[32] The antioxidant GSH is a tripeptide composed of cysteine, glutamic acid, and glycine, among which cysteine serves as the rate-limiting precursor in GSH synthesis.[14] Normally, most cells obtain cysteine primarily from the cystine-glutamate antiporter system Xc^- (composed of SLC7A11 and SLC3A2).[33] SLC7A11 is primarily involved in the transporter activity, whereas SLC3A2 is a chaperone protein to stabilize SLC7A11 and ensure appropriate membrane localization.[14] The system Xc^- imports extracellular cystine and exports intracellular glutamate at a 1:1 ratio, and the imported cystine is then converted to cysteine through a nicotinamide adenine dinucleotide phosphate-consuming reduction reaction in the cytoplasm,[34] ultimately resulting in GSH synthesis. Erastin acts on Xc^- to inhibit cystine import, prompting GSH depletion and ferroptosis.[35]

GPX4, together with GSH as an essential cofactor, catalyzes the reduction of lipid hydroperoxides into lipid alcohols, thereby preventing cell membrane damage.[36] Specifically, GPX4 reduces lipid-OOH (hydroperoxide) into lipid-OH (alcohol), thereby retarding generation of lipid-O• (alkoxy radicals) from lipid-OOH.[37] Previous evidence has revealed that GPX4 overexpression suppresses ferroptosis driven by RSL3, whereas GPX4 deletion increases sensitivity to ferroptosis.[38] Furthermore, supplementation with GPX4 inhibitors (eg, RSL3, ML162, and FIN56) directly evokes ferroptosis by inactivating GPX4.[39] These findings suggest critical antioxidant defensive properties of Xc^-/GSH/GPX4 against ferroptosis in cardiomyocytes.

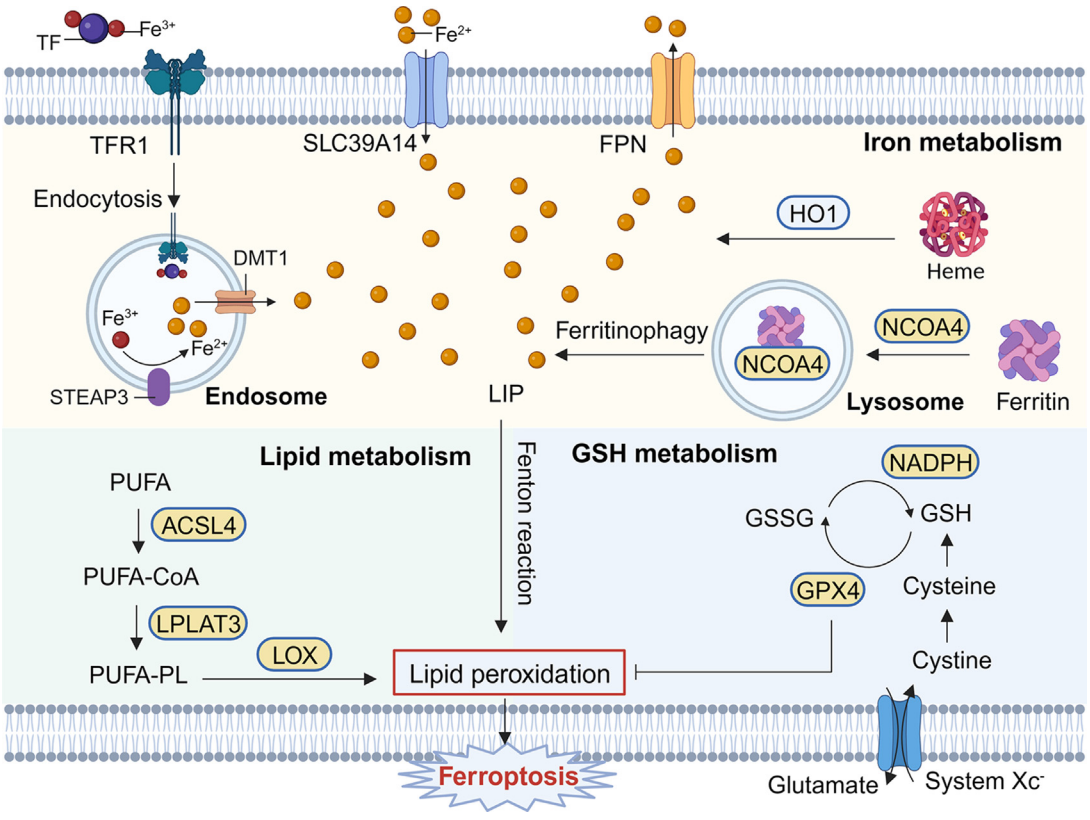
LIPID METABOLISM AND FERROPTOSIS. In mammalian cells, polyunsaturated fatty acid (PUFA)-containing phospholipids are the main substrates of lipid peroxidation and ferroptosis.[40] Accumulation of PUFA-containing phospholipid hydroperoxides, such as cardiolipin, phosphatidylcholine, and phosphatidylethanolamine, is evident in ferroptosis. In particular, the enzyme acyl-coenzyme A (CoA) synthase long-chain family member 4 (ACSL4) involved in catalyzing the formation of PUFA-CoA functions as the key driver for ferroptosis.[41] Up-regulation of ACSL4 enhances PUFA-containing phospholipids and cell susceptibility to ferroptosis.[42] Therefore, inhibition of ACSL4 protects cells against RSL3-induced ferroptosis.[42]

Another crucial enzyme, lysophosphatidylcholine acyltransferase 3 (LPCAT3), an ACSL4 downstream signal, catalyzes the esterification into phosphatidylethanolamine of PUFA-CoA to form PUFA-phosphatidylethanolamines.[43] Knockdown of LPCAT3 results in the inhibition of ferroptosis.[44] Lipoxygenases (LOXs), a family of iron-containing enzymes, directly drive oxidation of PUFAs and PUFA-containing phospholipids in cellular membranes.[45] LOXs play a crucial role in lipid peroxidation and ferroptosis.[46] Several inhibitors of LOXs, such as flavonoids and the vitamin E family, contribute to the inhibition of ferroptosis.[47]

FERROPTOSIS IN DOX-INDUCED CARDIOTOXICITY

Recent evidence suggested a key role for ferroptosis in the pathogenesis of both acute and chronic DOX cardiotoxicity.[16-18] In 2019, Fang et al[16] examined the contributions of multiple forms of programmed cell death in acute cardiotoxicity by assessing various cell death inhibitors on survival in DOX-treated mice. Their results demonstrated that inhibition of ferroptosis using ferrostatin 1 (Fer-1) significantly reduced DOX-induced mouse mortality.[16] In contrast, survival of DOX-treated mice was unaffected by other cell death inhibitors, including apoptosis, necroptosis, and autophagy.[16] In addition, DOX evoked an overt rise in cardiac iron levels, lipid ROS, and ferroptosis biomarkers.[16] Moreover, this study further elaborated that mitochondria serve as the target of heme oxygenase 1 (HO1)-mediated release of free iron, leading to lipid peroxidation and ferroptosis.[16] A subsequent report also denoted a key role for mitochondria-dependent ferroptosis in DOX-induced cardiotoxicity.[17] Of note, this study unveiled significant down-regulation of GPX4 expression in the heart in response to DOX, resulting in excessive lipid peroxidation.[17] Furthermore, GPX4 overexpression and knockdown in mice significantly prevented and exacerbated, respectively, DOX-evoked cardiac dysfunction.[17] Similarly, DOX triggered iron overload and inhibited the Xc^-/GSH/GPX4 pathway in heart tissues of mice in a chronic DOX cardiotoxicity model.[18] Collectively, these findings suggest ferroptosis might persist during DOX treatment and contribute to DOX-induced cardiotoxicity. Here, we will specifically discuss the role and mechanism of essential metabolic pathways mediating ferroptosis in DOX-induced cardiotoxicity, including iron, glutathione, and lipid metabolism (**Figures 2 and 3**).

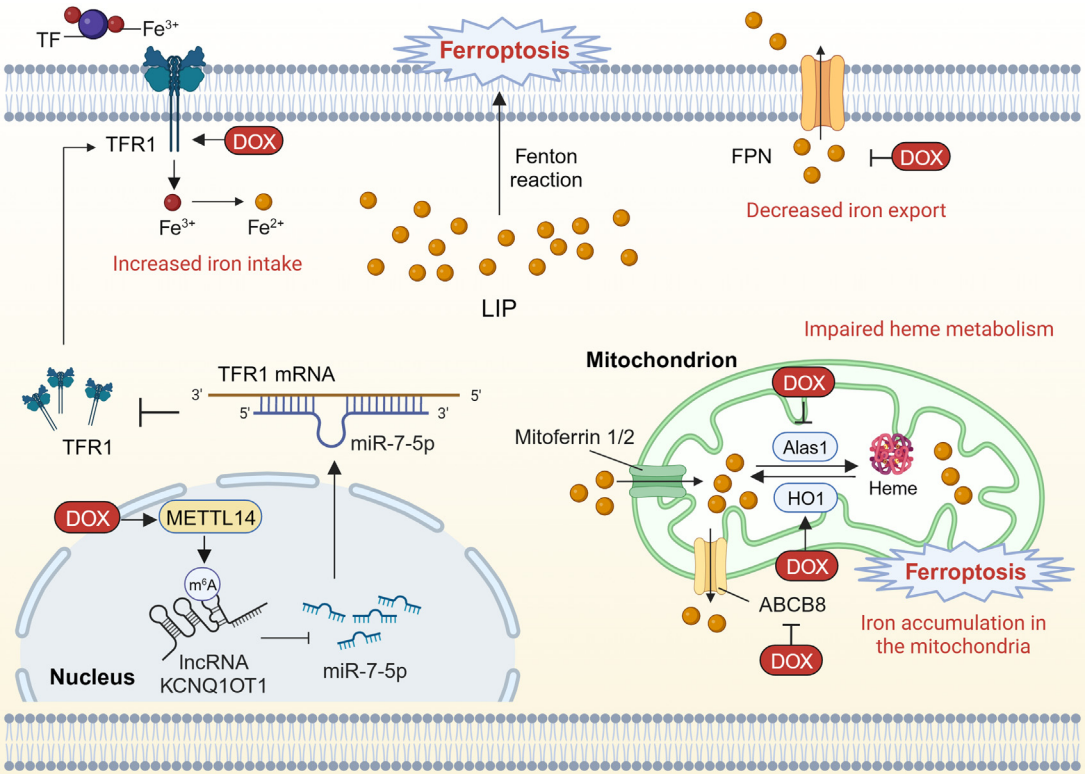
FIGURE 1 Molecular and Metabolic Mechanisms Implicated in Ferroptosis



Ferroptosis is triggered primarily by iron-dependent lipid peroxidation, which is mainly regulated by iron, lipid, and glutathione (GSH) metabolism. Iron metabolism, including many aspects such as iron import, storage, export, and release, plays an important role in regulating ferroptosis. The ferric iron (Fe^{3+}) is bound with transferrin (TF) and transported into cells by transferrin receptor protein 1 (TFR1). The ferrous iron (Fe^{2+}) is imported into cells via metal transporter solute carrier family 39 member 14 (SLC39A14). Moreover, heme oxygenase 1 (HO1)–mediated heme degradation and nuclear receptor coactivator 4 (NCOA4)–mediated ferritinophagy can lead to iron release and increase the labile iron pool (LIP), thereby inducing ferroptosis through the Fenton reaction. In the lipid metabolic pathway, the activation of acyl-coenzyme A synthase long-chain family member 4 (ACSL4), lysophospholipid acyltransferase 3 (LPLAT3), or lipoxygenase (LOX) contributes to lipid peroxidation and ferroptosis. The system Xc^-/GSH/glutathione peroxidase 4 (GPX4) serves as a canonical ferroptosis-suppressing pathway. The uptake of cystine is mediated by the cystine-glutamate antiporter (system Xc^-), which leads to GSH biosynthesis. GPX4, which uses GSH as a cofactor, mitigates lipid peroxidation by catalyzing phospholipid hydroperoxides to the corresponding alcohols.
DMT1 = divalent metal transporter 1; FPN = ferroportin; GSSG = glutathione disulfide; PUFA = polyunsaturated fatty acid; PUFA-CoA = coenzyme A-activated polyunsaturated fatty acid; PUFA-PL = polyunsaturated fatty acid-containing phospholipid; STEAP3 = 6-transmembrane epithelial antigen of prostate 3.

IRON OVERLOAD AND DOX-INDUCED CARDIOTOXICITY. Preclinical and clinical relevance for iron overload and DOX-induced cardiotoxicity. Although the precise molecular mechanism underlying the pathogenesis remains unclear, multiple findings have favored a detrimental role for iron overload in the aggravation of DOX-induced cardiotoxicity.[48,49] DOX decreased the viability of cultured cardiomyocytes, and iron supplementation using ferric ammonium citrate treatment accentuated cardiomyocyte death in a concentration-dependent manner.[50] Such observations were also confirmed in animal models treated with DOX. Systemic iron overload in mice induced by genetic modification or a high iron diet was shown to increase susceptibility to DOX-induced cardiotoxicity.[48,49] By contrast, iron-deficient diet-fed mice displayed a decreased risk and increased survival compared to control counterparts.[16] These findings have depicted that the modulation of cardiac iron levels might represent a clinically effective strategy for the prevention and treatment of DOX-induced cardiotoxicity.

FIGURE 2 Dysregulated Iron Metabolism in Doxorubicin-Induced Ferroptosis

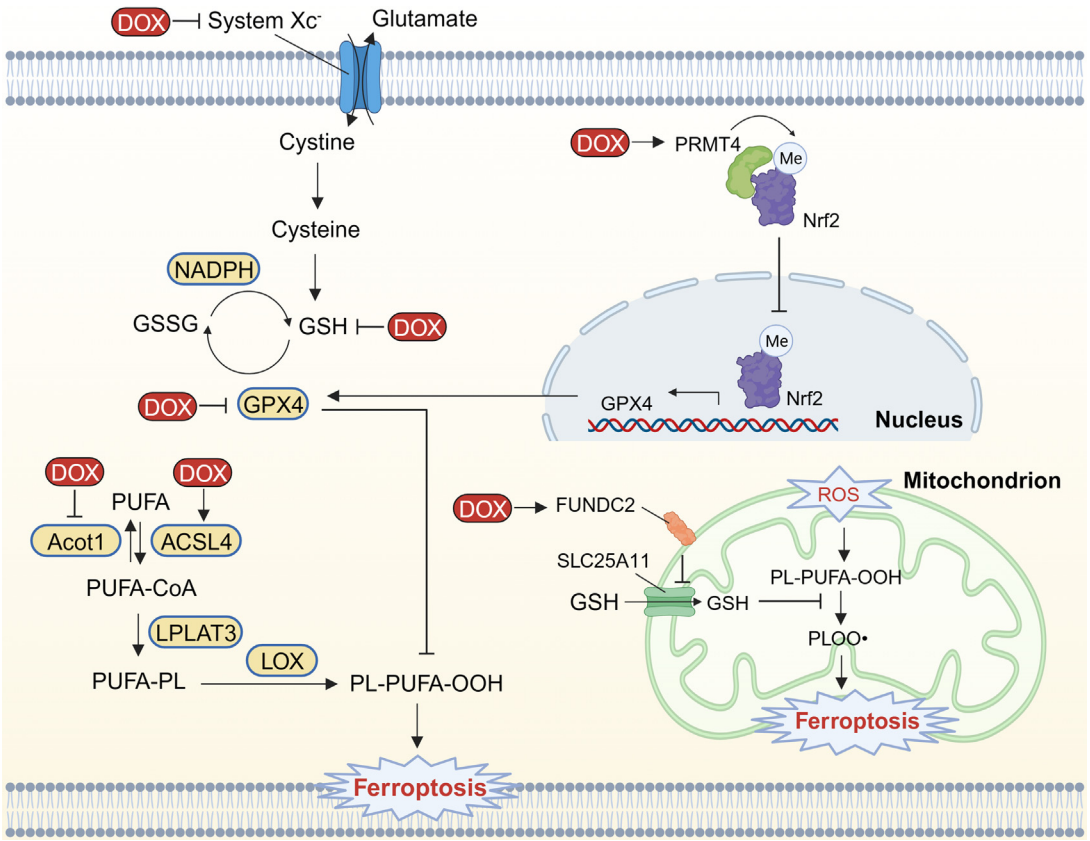


Doxorubicin (DOX) triggers cardiomyocyte iron overload by up-regulating transferrin (TF) receptor protein 1 (TFR1)-mediated iron import and down-regulating ferroportin (FPN)-mediated iron export. Mechanistically, DOX up-regulates methyltransferase-like 14 (METTL14), promoting N^6-methyladenosine (m^6A) modification and stability of long noncoding RNA (lncRNA) KCNQ1OT1. KCNQ1OT1 inhibits miR-7-5p, leading to TFR1 induction. Moreover, DOX inhibits mitochondrial iron exporter ABCB8 and impairs heme metabolism (increased degradation and reduced synthesis), leading to mitochondrial iron overload. Alas1 = 5′-aminolevulinate synthase 1; HO1 = heme oxygenase 1; LIP = labile iron pool.

The *HFE* gene encodes hereditary hemochromatosis protein, which binds to TFR1 to negatively regulate iron uptake. A previous study showed that *HFE* knockout led to a significant increase of iron level in the hearts of DOX-treated mice.[49] This finding indicated that mutations in the *HFE* gene resulted in iron overload in cardiomyocytes and higher susceptibility of cardiomyocytes to DOX-induced ferroptosis and cardiotoxicity.[49] Consistently, a recent study confirmed that the mutation in *HFE* H63D represents one important marker to identify high-risk patients for DOX-induced cardiotoxicity.[51] In another study, among survivors of high-risk acute lymphoblastic leukemia, patients with the mutation in *HFE* C282Y showed an increased severity of DOX-induced cardiotoxicity, as indicated by higher levels of cardiac troponin T, worsened left ventricular function, and lower left ventricular thickness.[52] Moreover, previous evidence indicated that carriers of the *HFE* C282Y mutation display a higher risk of myocardial infarction and cardiovascular death compared with noncarriers.[53,54] Therefore, the iron-regulated gene *HFE* polymorphism (H63D and C282Y) might clinically represent a biomarker for an increased risk of DOX-induced cardiotoxicity. Collectively, these preclinical and clinical findings have supported a pivotal role of iron overload in the development of DOX-induced cardiotoxicity.

Molecular mechanisms of iron overload and ferroptosis in DOX-induced cardiotoxicity. ***Increased iron intake and decreased iron export.*** Recent advances have demonstrated that DOX induces overt changes in several proteins involved in iron intake (TFR1), storage (ferritin, including FTH and FTL), and export (FPN) within cardiomyocytes.[55-58] Several studies have revealed that cardiomyocytes or hearts from DOX-treated mice exhibited elevated TFR1 and FTH levels and reduced FPN protein expression.[55-58] An

FIGURE 3 Dysregulated Glutathione and Lipid Metabolism in Doxorubicin-Induced Ferroptosis



In the lipid metabolic pathway, doxorubicin (DOX) up-regulates acyl–coenzyme A synthase long-chain family member 4 (ACSL4) and down-regulates acyl-coenzyme A thioesterase 1 (Acot1), which promotes the synthesis of polyunsaturated fatty acid (PUFA)–containing phospholipid (PUFA-PL) and the initiation of ferroptosis. In the glutathione (GSH) metabolic pathway, DOX leads to a reduction of solute carrier family 7A member 11 subunit (SLC7A11, a subunit of system Xc^-), GSH, and glutathione peroxidase 4 (GPX4), which promotes lipid peroxidation and ferroptosis. Mechanistically, DOX promoted the protein arginine methyltransferase 4 (PRMT4)-mediated methylation (Me) of nuclear factor-erythroid 2-related factor 2 (Nrf2), which restricts Nrf2 nuclear translocation and GPX4 transcription. In addition, DOX impedes the transport of GSH into mitochondria by promoting the interaction of FUN14 domain-containing 2 (FUNDC2) and SLC25A11.
GSSG = glutathione disulfide; LPLAT3 = lysophospholipid acyltransferase 3; PL-PUFA-OOH = polyunsaturated fatty acid-containing phospholipid hydroperoxides; PLOO· = phospholipid peroxyl radical; ROS = reactive oxygen species.

earlier study demonstrated a crucial role for TFR1 in DOX-evoked iron accumulation.[57] In this study, treatment with the specific anti-TFR1 antibody significantly inhibited DOX-induced iron uptake, ROS production, and cell death in bovine aortic endothelial cells.[57] To decipher how DOX up-regulated TFR1 expression, a recent report noted that DOX up-regulated methyltransferase-like 14 (METTL14), thus promoting N^6-methyladenosine (m^6A) modification and the stability of the long noncoding RNA KCNQ1OT1.[58] KCNQ1OT1 suppressed expression of miR-7-5p, thereby leading to an increased TFR1 level. Along the same line, disruption of the METTL14/KCNQ1OT1/miR-7-5p axis attenuated DOX-induced iron accumulation, lipid ROS, and ferroptotic cell death.[58]

Impaired cellular iron homeostasis regulated by the iron regulatory protein (IRP)-iron response element (IRE) system has recently been involved in the progression of DOX-induced cardiotoxicity.[55,59] Through binding to IRE, IRP1 and IRP2 up-regulate TFR1 and down-regulate ferritin and FPN expression to maintain intracellular iron levels.[60] Notably, binding of IRPs to IRE is regulated by iron-sulfur (Fe-S) clusters, which are synthesized in the mitochondria using ferrous iron.[61] When cellular iron level rises, Fe-S is increased and attaches to IRP1, which loses its IRE-binding capacity to function as a

cytosolic aconitase.[62] Furthermore, IRP2 is ubiquitinated and degraded via the Fe-S cluster sensitive F-box/LRR-repeat protein 5 (FBXL5) ubiquitin ligase protein complex.[63,64] Inactivation of the IRP-IRE system reduces TFR1-mediated iron uptake and increases ferritin-mediated iron storage and FPN-mediated iron export. By contrast, inhibition of the Fe-S clusters may trigger compensatory iron uptake, mitochondrial iron overload, and ferroptosis.[65] A recent study revealed that DOX induced p53 activation, which binds to Park7, to favor ubiquitination and degradation of Park7.[55] Park7 counteracted iron overload by negative regulation of the Fe-S cluster-IRP-IRE cascade in healthy cardiomyocytes.[55] In this context, overexpression of Park7 or knockout of p53 significantly restored iron homeostasis and inhibited cardiac ferroptosis.[55] Taken together, DOX induces impairment of IRP/IRE system, thereby leading to iron overload and ferroptosis by promoting iron intake and decreasing iron export.

Iron accumulation in the mitochondria. Mitochondrial iron overload is a critical potential mechanism for the pathogenesis of DOX-induced cardiotoxicity. A previous study showed that heart biopsy specimens from patients with end-stage DOX-induced cardiomyopathy exhibited excess iron accumulation in the mitochondria as compared to the hearts from healthy individuals or patients with cardiomyopathy from non-DOX-related causes.[66] Iron accumulates in the mitochondria of DOX-treated cardiomyocytes as a result of inhibition of the mitochondrial iron exporter ABCB8.[66] To this end, ABCB8 overexpression or mitochondrial iron chelation using dexrazoxane protects against DOX-induced cardiotoxicity.[66] In another study, DOX treatment was shown to up-regulate cardiac mitochondrial ferritin expression, resembling its cytoplasmic equivalent for free iron storage.[67] Genetic deletion of mitochondrial ferritin sensitized cardiomyocytes to DOX-induced iron toxicity and ferroptosis.[67] Altogether, these findings favor a crucial role of mitochondrial iron in the induction of ferroptosis and progression of DOX-induced cardiotoxicity.

Increased heme degradation and impaired heme synthesis. Recent evidence has revealed that DOX not only induces heme degradation to release free iron but also impairs heme synthesis to use iron, leading to iron overload and ferroptosis in mitochondria.[16,19,68] Using RNA sequencing analysis, a previous study demonstrated that levels of HO1 were increased via nuclear factor-erythroid 2-related factor 2 (Nrf2) activation in a mouse model of DOX-induced cardiotoxicity.[16] HO1 catalyzed heme degradation and the release of iron in mitochondria. In line with these findings, increased intracellular iron levels were also associated with degradation of high mobility group box 1 (HMGB1)-mediated heme.[19] Knockdown of HMGB1 was shown to decrease the ferroptosis markers (Ptgs2, malondialdehyde [MDA], and 4-hydroxynoneal [4-HNE]) and myocardial injury in the hearts of DOX-treated rats.[19] These findings indicate that excessive heme degradation evoked by DOX contributes to cardiac iron overload and ferroptosis.

Interestingly, a recent report showed that DOX led to a significant decline in 5′-aminolevulinate synthase 1 (Alas1), the rate-limiting enzyme in heme synthesis,[68] leading to iron use impairment, iron overload, and ferroptosis in mitochondria in cardiomyocytes.[68] In this context, Alas1 overexpression or administration of 5-aminolevulinic acid (5-ALA), the product of Alas1, inhibited cardiac iron accumulation and lipid peroxidation, thus mitigating DOX-induced ferroptosis.[68] These findings would support a convincing role for impaired heme metabolism (synthesis and degradation) in DOX-induced iron overload and ferroptosis. Collectively, **Figure 2** highlights the role of dysregulated iron metabolism in DOX-induced ferroptosis and cardiotoxicity.

GLUTATHIONE METABOLISM AND DOX-INDUCED CARDIOTOXICITY. In 2020, Tadokoro and colleagues examined the role of GSH in DOX-induced cardiomyocyte ferroptosis, given the critical role for GSH as a regulator and cofactor for GPX4.[17] Interestingly, DOX led to a significant reduction of the ratio of GSH/GSSG in cultured cardiomyocytes. Furthermore, DOX significantly down-regulated GPX4 and triggered excessive lipid peroxidation through DOX-Fe^{2+} complex in mitochondria, resulting in mitochondria-dependent ferroptosis. In this context, DOX-induced cardiac impairments were overtly ameliorated and exacerbated with GPX4 overexpression and GPX4 heterodeletion, respectively.[17] These findings favor essential roles for GSH/GPX4 dysregulation in the pathogenesis of DOX-induced cardiotoxicity (**Figure 3**) and consolidated ferroptosis as the predominant form of cell death in DOX-induced cardiotoxicity.

Recent advances have explored the mechanism by which GSH/GPX4 is decreased in response to DOX challenge.[20,69,70] The mitochondrial ubiquitin ligase (MITOL) may serve as a key regulator for GSH homeostasis to inhibit lipid peroxide accumulation and ferroptosis in cardiomyocytes.[69] DOX evoked a drop in MITOL in cultured cardiomyocytes. Down-regulation of MITOL resulted in an integrated stress response pathway and accelerated Chac1-dependent

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GSH degradation, leading to decreased mitochondrial GPX4 expression. Therefore, cardiac-specific MITOL-knockout mice exhibited severe cardiac dysfunction following DOX challenge.[69] In addition, Nrf2 serves as a transcription factor to up-regulate GPX4 expression, which exerts an antiferroptotic effect in DOX-induced cardiotoxicity.[71,72] A recent study showed that Nrf2 could be methylated by the protein arginine methyltransferase 4 (PRMT4), thus restricting its nuclear translocation to suppress GPX4.[20] Therefore, PRMT4 was shown to increase ferroptosis markers in DOX-treated murine models, and the effects were mitigated by PRMT4 knockout. Finally, chronic DOX exposure down-regulated SLC7A11, a key component of the cystine/glutamate antiporter for GSH synthesis,[70] leading to decreased GSH synthesis to prevent GPX4 from scavenging lipid peroxides.[18] DOX also compromised histamine/histamine 1 receptor signaling, leading to inactivation of signal transducer and activator of transcription 3 (STAT3) accompanied with a reduction of SLC7A11, GSH/GSSG ratio, and GPX4 expression.[70] Therefore, histamine deficiency accelerated DOX-induced cardiac ferroptosis and aggravated DOX-induced cardiotoxicity, while supplementation of exogenous histamine reversed DOX-induced cardiac dysfunction and ferroptosis. These findings suggest that DOX induces a reduction of SLC7A11/GSH/GPX4 expression, consequently triggering cardiomyocyte ferroptosis and DOX-induced cardiotoxicity.

Interestingly, recent advances have demonstrated an essential role for mitochondrial GSH (mitoGSH) in the regulation of DOX-induced ferroptosis and cardiotoxicity.[73] Normally, mitochondria account for 10% to 15% of total cellular GSH, although mitochondria do not produce GSH.[74] GSH is negatively charged at physiologic pH and impermeable to mitochondria. To enter the mitochondrial matrix, GSH only depends on the mitochondrial carrier proteins, such as SLC25A11.[75-77] Earlier studies have demonstrated that mitoGSH plays a critical role in iron homeostasis and Fe-S cluster assembly inside mitochondria.[78,79] Similarly, it also serves as a cofactor for GPX4 to attenuate lipid peroxidation,[80] and mitochondrial GPX4 was found to protect against DOX-induced cardiotoxicity.[17] These findings suggest a potential role of mitoGSH in cardiac ferroptosis. A recent study noted that FUN14 domain-containing 2 (FUNDC2), a mitochondrial outer membrane protein, regulated ferroptosis and contributed to progression of DOX-induced cardiotoxicity.[73] Mechanistically, FUNDC2 interacted with and decreased the stability of SLC25A11 to regulate mitoGSH levels.[73] These findings have depicted that FUNDC2 contributes to ferroptotic stress by regulating mitoGSH. In this context, preventing mitoGSH depletion and ferroptosis may represent a therapeutic strategy of protection against DOX-induced cardiotoxicity.

LIPID METABOLISM AND PEROXIDATION IN DOX-INDUCED CARDIOTOXICITY. In 1983, Demant et al[81] first reported that DOX may produce oxidized phospholipids. These investigators noted that DOX exposure to submitochondrial particles from pig heart triggered a drop in major phospholipid content (including cardiolipin, phosphatidylcholine, and phosphatidylethanolamine), which was interpreted as oxidative degradation of phospholipids.[81] A recent study examined oxidized phospholipid species using liquid chromatography/mass spectrometry in cardiomyocytes treated with DOX.[82] The results showed that DOX induced generation of oxidized non-fragmented and fragmented species of phosphatidylcholines.[82] Moreover, recent findings depicted that lipid peroxides, such as 4-hydroxynoneal (4-HNE) and malondialdehyde (MDA) accompanied with ferroptosis were significantly increased in hearts and cultured cardiomyocytes challenged with DOX.[20,73]

As mentioned earlier, accumulation of cellular iron, particularly the labile ferrous iron (Fe^{2+}), reacts with cellular oxidants to generate cytotoxic hydroxyl radicals through the Fenton reaction, leading to ferroptosis.[83] DOX can be combined with Fe^{3+} to form the DOX-Fe^{3+} complex, which transforms to the DOX-Fe^{2+} complex through both enzymatic or nonenzymatic reactions.[84] The DOX-Fe^{2+} complex reacts with oxygen to produce a superoxide anion radical ($O2\bullet^-$), which is transformed into hydrogen peroxide (H_2O_2) through a disproportionation reaction.[83] H_2O_2 can react with the DOX-Fe^{2+} complex to generate a hydroxyl radical ($OH\bullet$). Under the action of the DOX-Fe^{2+} complex, $OH\bullet$, and O_2, PUFA (eg, arachidonic acid and adrenic acid) undergoes lipid peroxidation.[83] Furthermore, DOX can extract Fe^{3+} directly from ferritin to form the DOX-Fe^{3+} complex even in the absence of free iron, leading to lipid peroxidation.[85] In this context, administration of specific iron chelators effectively attenuated cardiomyocyte lipid peroxidation.[84]

Recently, DOX was shown to prompt peroxidation of PUFA-containing phospholipids through regulation of key enzymes[86-88] (**Figure 3**). Among these, ACSL4 converts PUFAs to the acetylated form and is considered a critical driver of cardiac ferroptosis, because ACSL4 increases PUFA content in phospholipids and renders the cell more susceptible to ferroptosis.[42] A recent study showed that DOX up-regulated ACSL4 en route to lipid peroxidation and

TABLE 1 Small-Molecule Modulators of Ferroptosis Used in DOX-Induced Cardiotoxicity

Therapeutic Target	Drug	Model	Mechanism	Cardiac Effects	Ref. #
Iron metabolism	Dexrazoxane	Mouse and rat	Suppresses iron overload	↑ Cardiac function ↑ Mitochondrial function ↓ Lipid ROS	16,19,55
	Deferoxamine	Mouse and rat	Suppresses iron overload	↑ Cardiac function ↑ Mitochondrial function ↓ Lipid ROS	55,89-92
	Mito-FerroGreen	NRVMs	Reduces Fe^{2+} in mitochondria by converting Fe^{2+} to Fe^{3+}	↓ Lipid ROS ↑ Cell viability	17
	Zinc protoporphyrin IX	Mouse	Inhibits HO1-mediated heme degradation to reduce iron levels	↓ Cardiac fibrosis ↓ Lipid ROS	16
	5-Aminolevulinic acid	Mouse	Promotes heme synthesis to promote iron use	↑ Cardiac function ↓ Cardiac injury	68
Lipid peroxidation	Ferrostatin 1	Mouse and rat	Suppresses lipid peroxidation	↑ Cardiac function ↑ Mitochondrial function ↓ Lipid ROS	16,17,19,20,55,87
	MitoTEMPO	Mouse	Scavenges mitochondrial ROS to inhibit lipid peroxidation	↓ Cardiac injury ↓ Cardiac remodeling ↓ Lipid ROS	16
	Ethoxyquin	Mouse	Suppresses lipid peroxidation	↑ Cardiac function ↓ Cardiac injury ↓ Myocardial fibrosis ↓ Lipid ROS	93
GSH metabolism	*N*-acetyl cysteine	NRVMs	Restores GSH level to synthesis GPX4	↑ Cell viability ↑ GPX4 level	69
	GSH	NRVMs	Restores GSH level to synthesis GPX4	↑ GPX4 level	69
Nrf2 activation	Resveratrol	Mouse	Activates p62/Nrf2 pathway	↑ Cardiac function ↓ Cardiac remodeling ↓ Lipid ROS	94

DOX = doxorubicin; GPX4 = glutathione peroxidase 4; GSH = glutathione; HO1 = heme oxygenase 1; MitoTEMPO = mitochondria-targeted 2,2,6,6-tetramethylpiperidinyl-1-oxyl; Nrf2 = nuclear factor-erythroid 2-related factor 2; NRVM = neonatal rat ventricular cardiomyocytes; ROS = reactive oxygen species.

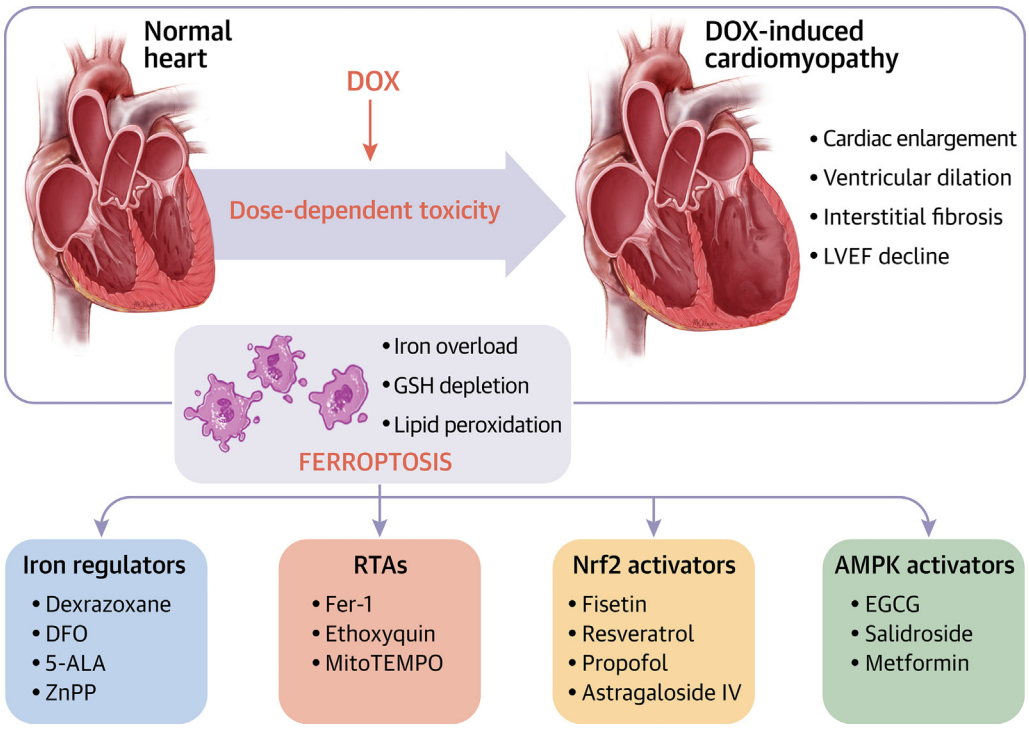
subsequent ferroptosis.[86] Acyl-CoA thioesterase 1 (Acot1) is a critical enzyme in fatty acid metabolism that catalyzes the reaction of fatty acyl-CoA to CoA and free fatty acids.[87] Therefore, Acot1 catalyzes the opposite lipid metabolism process mediated by ACSL4 and produces antiferroptotic capacity. DOX was shown to overtly down-regulate Acot1 in cardiomyocytes, leading to alterations in fatty acid composition and lipid peroxidation.[87] Thus, overexpression of Acot1 exhibited protection against ferroptosis, whereas Acot1 knockdown sensitized cardiomyocytes to ferroptosis and aggravated DOX-induced cardiotoxicity.[87] To the best of our knowledge, little is known with regard to how DOX affects ACSL4 or Acot1. Further studies are warranted to explore the cardiac lipid metabolic response of DOX, with a special focus on PUFA (eg, arachidonic acid) metabolism.

FERROPTOSIS AS A PROMISING TREATMENT TARGET FOR DOX-INDUCED CARDIOTOXICITY

Given the key role of ferroptosis in the pathogenesis of DOX-induced cardiotoxicity, targeting these metabolic pathways in ferroptosis appears to be a promising therapeutic target for DOX-induced cardiotoxicity. In this section, we will summarize a variety of drugs that suppress the ferroptosis pathway and discuss the application of these drugs in preclinical models and clinical settings of DOX-induced cardiotoxicity (**Table 1**, **Central Illustration**).

IRON CHELATORS. Dexrazoxane. Given that ferroptosis is an iron-dependent RCD, it is not surprising that ferroptosis can be attenuated by iron chelation.[35] To date, the iron chelator dexrazoxane is the only FDA-approved medication for the management of DOX-induced cardiotoxicity in patients with cancer.[6] **Table 2** summarizes the clinical evidence of dexrazoxane in the prevention and treatment of anthracycline cardiotoxicity. A previous study has shown that suppression of ferroptosis is a critical mechanism through which dexrazoxane exerts cardioprotective effects in DOX-induced cardiotoxicity.[16] In another independent study, DOX-induced cardiac ferroptosis was found to be mediated by HMGB1, because knockdown of HMGB1 overtly inhibited ferroptosis.[19] Dexrazoxane

CENTRAL ILLUSTRATION **Therapeutic Targets in Doxorubicin-Induced Ferroptosis and Cardiotoxicity**



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Doxorubicin (DOX) causes a dose-dependent cardiotoxicity manifested by cardiac enlargement, ventricular dilation, interstitial fibrosis, and decline of left ventricular ejection fraction (LVEF). Ferroptosis serves as a core mechanism and participates in the progression of DOX-induced cardiotoxicity. In this context, multiple natural and pharmaceutical compounds have been shown to inhibit DOX-induced ferroptosis and cardiomyopathy, including iron regulators, radical-trapping antioxidants (RTAs), nuclear factor-erythroid 2-related factor 2 (Nrf2) activators, and adenosine monophosphate-activated protein kinase (AMPK) activators. 5-ALA = 5-aminolevulinic acid; DFO = deferoxamine; EGCG = epigallocatechin-3-gallate; Fer-1 = ferrostatin 1; GSH = glutathione; MitoTEMPO = mitochondria-targeted 2,2,6,6-tetramethylpiperidinyl-1-oxyl; ZnPP = zinc protoporphyrin IX.

attenuated DOX-induced cardiac dysfunction and ferroptosis by reversing the up-regulation of HMGB1.[19] In addition, dexrazoxane also regulated iron metabolism-related protein in cardiomyocytes and mitigated the increase of HO1 induced by daunorubicin.[109] Therefore, dexrazoxane could inhibit heme degradation and the release of free iron (the conversion of heme iron to nonheme iron), which decreased labile iron content in cardiomyocytes. Notably, in addition to iron chelation and inhibition of ferroptosis, dexrazoxane may also prevent DOX cardiotoxicity through other mechanisms, including alleviation of oxidative stress and suppression of cardiac topoisomerase IIβ (Top2β, a key mediator of DOX-induced DNA damage).[8,9]

Although dexrazoxane attenuates DOX-induced cardiotoxicity clinically and experimentally, clinical application of dexrazoxane is encountering several challenges. Dexrazoxane may increase the risk of secondary solid tumors and acute myeloid leukemia in children.[110-112] Moreover, the FDA restricts the use of dexrazoxane for only those patients with metastatic breast cancer receiving cumulative doses of more than 300 mg/m^2 of DOX.[6] Therefore, further studies are required to discern the clinical application of dexrazoxane in patients receiving DOX chemotherapy.

Deferoxamine and other iron chelators. Deferoxamine (DFO) is a widely used iron chelator that chelates excess intracellular Fe^{2+}, thereby alleviating

TABLE 2 Clinical Studies of Dexrazoxane in the Treatment of Anthracycline Cardiotoxicity

Patients	Group Design and Sample Size	Cumulative Anthracycline Dose	Major Study Outcome	Ref. #
T-ALL or L-NHL	DOX (30 mg/m^2 bolus infusion) with (n = 273) or without (n = 264) dexrazoxane (10:1[a])	360 mg/m^2	Cardiac function (eg, mean LVFS, wall thickness) was significantly better in patients with dexrazoxane therapy after 3 years.	95
Children with cancer	Anthracycline alone (n = 123) or combined with dexrazoxane (n = 135)	Up to 500 mg/m^2	Cardiotoxicity rate was significantly higher in the anthracycline-only group vs the anthracycline plus dexrazoxane group.	96
Osteosarcoma	DOX (37.5 mg/m^2 infusion) plus dexrazoxane (10:1[a]) (n = 242)	450-600 mg/m^2	Dexrazoxane did not compromise the antitumor effects of DOX or increase risk of secondary malignancy.	97
T-ALL/lymphoma, intermediate-high-risk HL, low-risk HL	DOX: 25-30 mg/m^2 infusion with (n = 507) or without (n = 501) dexrazoxane (10:1[a])	100-360 mg/m^2	Dexrazoxane did not compromise long-term survival and was not associated with mortality from cardiovascular causes.	98
AML	Anthracycline (n = 16) alone or combined with dexrazoxane (n = 28)	Not applicable	LVEF and shortening fraction *z*-scores were significantly higher in dexrazoxane patients than patients treated with anthracycline chemotherapy alone.	99
ALL	DOX (30 mg/m^2 infusion) with (n = 105) or without (n = 100) dexrazoxane (10:1[a])	300 mg/m^2	cTnT and NT-proBNP levels were significantly higher in patients treated with DOX alone than patients treated with dexrazoxane.	100
Advanced breast cancer and soft tissue sarcomas	Epirubicin (160 mg/m^2 by intravenous bolus) with (n = 59) or without (n = 62) dexrazoxane (1,000 mg/m^2)	Not applicable	Decrease in LVEF from baseline was significantly greater in the epirubicin group than the dexrazoxane group.	101
Advanced breast cancer	Anthracycline-based chemotherapy with (n = 85) or without (n = 79) dexrazoxane	Not applicable	Patients treated with dexrazoxane had fewer cardiac events and a lower incidence of congestive heart failure.	102
Advanced breast cancer	DOX (50 mg/m^2) and other antitumor drugs with (n = 76) or without (n = 74) dexrazoxane (1,000 mg/m^2)	300-1,000 mg/m^2	Dexrazoxane permitted greater doses of DOX to be administered to patients with advanced breast cancer with greater safety.	103
Advanced breast cancer	DOX (50 mg/m^2) and other antitumor drugs with dexrazoxane (10:1[a] or 20:1[a]; n = 489) or placebo (n = 519)	Not applicable	Dexrazoxane had a significant cardioprotective effect as measured by LVEF changes.	104
Advanced breast cancer	Epirubicin-based chemotherapy with (n = 78) or without (n = 82) dexrazoxane	Not applicable	Dexrazoxane protected against epirubicin-induced cardiotoxicity and did not affect noncardiac toxicity and clinical activity of epirubicin.	105
Pediatric sarcoma	DOX (70 mg/m^2 infusion) with (n =18) or without (n = 15) dexrazoxane (20:1[a])	210-410 mg/m^2	Dexrazoxane-treated patients had lower incidences of subclinical cardiotoxicity, had a smaller decrease in LVEF, and received a higher median cumulative dose of DOX.	106
ALL, HL, or osteosarcoma	DOX with (n = 775) or without (n = 1,028) dexrazoxane	100-1,170 mg/m^2	Dexrazoxane did not adversely affect long-term mortality, cardiovascular mortality, event-free survival, or risk of second cancers.	107
Advanced soft-tissue sarcoma	DOX (75 mg/m^2 infusion) with or without dexrazoxane (total N = 504)	Up to 600 mg/m^2	DOX can be treated at >450 mg/m^2 with a low cardiotoxicity rate in the context of dexrazoxane coadministration.	108

[a]Dose ratio of dexrazoxane:anthracycline.

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; cTnT = cardiac troponin T; DOX = doxorubicin; HL = Hodgkin lymphoma; L-NHL =lymphoblastic non-Hodgkin lymphoma; LVEF = left ventricular ejection fraction; LVFS = left ventricular fractional shortening; NT-proBNP =N-terminal pro–B-type natriuretic peptide; T-ALL = T-cell acute lymphoblastic leukemia.

DOX-induced cardiac ferroptosis and dysfunction.[55,89-92,113] Moreover, DFO is considered as a protective drug for mitochondrial permeability transition pore.[113] In this context, DFO weakens the opening of Ca^{2+}-dependent pores induced by the iron-DOX complex and decreases the Fe^{2+} uptake in the mitochondria. However, the protective effects of DFO in DOX-induced cardiotoxicity are still controversial. A previous study showed that DFO failed to provide effective protection against DOX-induced cardiotoxicity, whereas dexrazoxane significantly attenuated DOX-induced cardiac dysfunction and mitochondrial iron accumulation.[66] This might be explained by the fact that dexrazoxane can directly enter mitochondria in cardiomyocytes and decrease mitochondrial iron accumulation, whereas DFO cannot enter the mitochondria. This phenomenon is further supported by the notion that Mito-Ferro-Green, which reduces Fe^{2+} via conversion to Fe^{3+}, prevents DOX-induced lipid peroxidation and ferroptosis in cultured cardiomyocytes.[17] Notably, the cardioprotective benefits of DFO against DOX-

induced cardiotoxicity require a narrow window for dosage, because a slight deviation would diminish the beneficial outcome.[114] Further studies are warranted to explore the dose and clinical efficacy of DFO and other iron chelators in DOX-induced cardiotoxicity.

FER-1 AND OTHER RADICAL-TRAPPING ANTIOXIDANTS. As a radical-trapping antioxidant (RTA), Fer-1 is a first-generation ferroptotic inhibitor that reduces the accumulation of cytosolic and lipid ROS.[35] Following the initial report on its antiferroptotic properties, Fer-1 has been used in various disease models, including DOX-induced cardiotoxicity.[16,17,19,20,55,87] A previous study demonstrated that Fer-1 protects against cardiac damage without affecting cardiac iron levels in a DOX-treated mouse model.[16] A subsequent study showed that Fer-1 also inhibited DOX-induced cell death in cultured cardiomyocytes.[17] In addition, Fer-1 was shown to up-regulate expression of FTH1 to promote iron storage, leading to a decreased Fe^{2+} level and suppressed ferroptosis in DOX-induced cardiotoxicity.[19]

Other common RTAs used in DOX-induced cardiotoxicity include 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO) and MitoTEMPO.[115] The latter is being developed as a mitochondria-targeted TEMPO to scavenge mitochondrial superoxide.[115] MitoTEMPO was shown to inhibit DOX-induced cardiac lipid peroxidation, ferroptotic cell death, and cardiac dysfunction in mice.[16] Interestingly, TEMPO only mildly attenuates DOX-induced ferroptosis and cardiotoxicity. Ethoxyquin, a lipophilic RTA widely used for food preservation, was found to ameliorate DOX-induced lipid peroxidation and ferroptosis, thereby preventing DOX-induced cardiac dysfunction in mice.[93] These findings suggest that cardiac ferroptosis and mitochondrial lipid peroxidation coordinate to contribute to the progression of DOX-induced cardiotoxicity.

GSH PRECURSORS. Cysteine imported via system Xc^- is the rate-limiting precursor for GSH biosynthesis. The addition of cystine or cysteine to cell culture media effectively suppresses ferroptosis in vitro.[116] *N*-acetyl cysteine (NAC) is an antioxidant and improves cysteine bioavailability. Multiple studies have shown that NAC preserves cardiovascular homeostasis, including protection against DOX-induced cardiotoxicity.[117,118] Recently, the antiferroptosis property of NAC was demonstrated in the context of system Xc^- inhibition or cysteine depletion-induced ferroptosis.[119] To the best of our knowledge, only one study consolidated the antiferroptotic property of NAC in DOX-induced cardiotoxicity.[69] In that study, MITOL knockdown triggered lipid peroxidation and ferroptosis and promoted susceptibility to DOX toxicity in cardiomyocytes by reducing GSH contents. NAC treatment significantly inhibited cardiomyocyte ferroptosis and reduced DOX-induced cardiomyocyte injury.[69] These findings denote the clinical promises of NAC in DOX-induced cardiotoxicity.

NRF2 ACTIVATORS. Nrf2 is a critical transcription factor in antioxidant response by the regulation of multiple genes to counteract oxidative stress.[120] Recent advances have shown that Nrf2 is a key transcriptional regulator of a variety of antiferroptotic genes,[120] such as *GPX4*, *SLC7A11*, *FTH*, *FTL*, and *FPN*. Therefore, Nrf2 is involved in the prevention of lipid peroxidation, the accumulation of free iron, and ferroptosis.[120] A recent study showed that the E3 ubiquitin ligase TRIM21 inhibited the Nrf2-mediated antioxidant pathway, and TRIM21 knockout mice are protected against DOX-induced cardiotoxicity and cell death.[121] Furthermore, Nrf2 is activated by the deacetylation of Sirt1, whereas cardiac Sirt1 deficiency exacerbated DOX-induced ferroptosis and cardiac injury through Nrf2 signaling.[122] Recently, several natural compounds have been shown to attenuate DOX-induced cardiotoxicity via inhibition of ferroptosis through Nrf2 activation, including fisetin, resveratrol, propofol, and astragaloside IV.[71,72,94,123] Thus, activation of Nrf2 offers therapeutic promises for the management of DOX-induced cardiotoxicity by inhibition of ferroptosis.

OTHER COMPOUNDS REGULATING FERROPTOSIS IN DOX CARDIOTOXICITY. Several medications targeting heme metabolism also suppress ferroptosis and DOX-induced cardiotoxicity.[16,68] For instance, zinc protoporphyrin IX, a competitive inhibitor of HO1, has been shown to decrease DOX-induced iron accumulation and cardiac ferroptosis in mice by preventing heme degradation.[16] Likewise, administration of 5-ALA, a primary substrate for heme synthesis, to both mice and cultured cardiomyocytes significantly suppressed iron overload, lipid peroxidation, and ferroptosis, thereby retarding the progression of DOX-induced cardiotoxicity.[68] Therefore, inhibiting heme degradation and promoting heme synthesis may be effective in preventing DOX-induced iron accumulation and ferroptosis.

The energy fuel adenosine monophosphate-activated protein kinase (AMPK) has been shown to negatively regulate ferroptosis by suppressing fatty acid synthesis.[124,125] Recently, several compounds have displayed antiferroptotic properties through the activation of AMPK in mouse DOX-induced cardiotoxicity models.[126,127] Epigallocatechin-3-gallate

(EGCG) is an active polyphenol compound extracted from green tea and shows multiple biological activities, including antioxidant, anti-inflammatory, antitumor, and antiarteriosclerotic activities.[126] EGCG was shown to ameliorate DOX-induced ferroptosis and cardiotoxicity by up-regulating AMPKα2, activating adaptive autophagy, and maintaining mitochondrial function.[126] In another study, salidroside, a phenylpropanoid glycoside derived from Rhodiola rosea, inhibited DOX-induced ferroptosis through AMPK activation, maintenance of mitochondrial function, and fatty acid metabolism.[127] Therefore, AMPK activators represent a therapeutic approach to inhibit DOX-induced cardiac ferroptosis and dysfunction.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Mounting evidence has supported an important role for ferroptosis in the pathogenesis of DOX-induced cardiotoxicity. However, several questions remain unanswered before the clinical application of ferroptosis-targeted drugs. First and foremost, although ferroptosis inhibitors exhibited cardioprotective effects in DOX-induced cardiotoxicity, these agents might also boost the risk of tumor growth and metastasis. Currently, few studies have examined the role of ferroptosis inhibition in animal models with concurrent cancer and cardiotoxicity. Notably, ferroptosis inducers have been reported to act synergistically with classical cancer therapy (eg, chemotherapy, immunotherapy, and radiotherapy) to inhibit tumor growth in mice.[128] For example, erastin, a ferroptosis inducer, enhanced the sensitivity of acute myeloid leukemia cells to chemotherapeutic drugs, including DOX.[129] In fact, clinical use of the iron chelator dexrazoxane leads to higher risks of secondary solid tumors in children[110-112] because of suppression of the topoisomerase IIα (Top2α) isoform expressed in cancer cells.[9] Therefore, it is imperative to identify heart- or cardiomyocyte-targeted ferroptotic inhibitors for the treatment of DOX-induced cardiotoxicity and other cardiovascular diseases. Second, can we identify reliable serum biomarkers for ferroptosis in DOX-induced cardiotoxicity? Currently, the ferroptosis biomarkers (eg, MDA, 4-HNE) used in preclinical studies are nonspecific and may also present in other forms of cell death. The lack of ferroptosis-specific biomarkers limits the development of ferroptosis-targeted therapy. Third, it is important to explore the roles of ferroptosis in a variety of cardiac cell types (eg, immune cells, fibroblasts, and endothelial cells) in the setting of DOX cardiotoxicity. Fourth, when and how do ferroptosis and other types of cell death (eg, apoptosis, necroptosis, pyroptosis) occur in the onset and development of DOX-induced cardiotoxicity? A recent study showed that ferroptosis is the predominant RCD during the late phase of myocardial ischemia/reperfusion injury.[130] However, few studies have delineated ferroptosis in DOX-induced cardiotoxicity. Finally, although ferroptotic inhibitors are widely tested in animal models, clinical trials using ferroptosis-specific inhibitors (except dexrazoxane) to treat DOX cardiotoxicity are still lacking.

In conclusion, ferroptosis denotes iron-dependent RCD, characterized by iron overload, GSH depletion, and lipid peroxidation. Current advances indicate a crucial role for ferroptosis in the pathogenesis of DOX-induced cardiotoxicity. Inhibition of ferroptosis has been shown to be effective in the treatment of DOX-induced cardiotoxicity in preclinical studies. Therefore, a better understanding for the precise role of ferroptosis is important for clinical translation in the management of DOX-induced cardiotoxicity.

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