Chapter 3

Doxorubicin-Induced Cardiotoxicity

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Abstract

Doxorubicin (DOX) is one of the most effective antineoplastic drugs. However, its clinical use is largely limited by potential dose-dependent cardiotoxicity. To date, the mechanisms of DOX-induced cardiotoxicity remains incompletely understood. More importantly, no efficient therapeutic strategy is available to counteract DOX-induced cardiomyopathy, underscoring the importance of the prevention of this disease. In this chapter, we first describe the pathophysiology of DOX-induced cardiotoxicity. We then update the findings of molecular biology of DOX-induced cardiomyopathy including molecular mechanisms, established and putative biomarkers for early diagnosis, and potential genetic factors for prediction of susceptibility. Finally, we introduce a number of pharmaceutical measures and practical lifestyle modifications for the prevention of this disease.

Keywords: doxorubicin, cardiotoxicity, cardiac function, oxidative stress, iron accumulation, topoisomerase, autophagy, mitochondria, inflammation, calcium, cell death, microRNA, polymorphisms, antioxidant, exercise, fasting

1. Introduction

Doxorubicin (DOX), an anthracycline antibiotic produced by the fungus Streptomyces peucetius, has been proved to be one of the most effective drugs for the treatment of solid tumor and haemotological malignancies. However, the clinical use of DOX is limited by potential dose-dependent cardiotoxicity. Incidences of progressive congestive heart failure were approximately 5, 16, 26 and 48% in patients who had received a cumulative dose of 400, 500, 550 and 700 mg/m^2 of DOX, respectively [1]. DOX-induced cardiotoxicity can be acute or chronic. Acute DOX cardiotoxicity occurs within several days after administration of the drug, while chronic DOX cardiotoxicity takes place months or even years after use of DOX [2]. However,

the biological mechanisms underlying DOX cardiotoxicity is not fully understood, although multiple factors have been suggested. As a consequence, no efficacious therapeutic strategies are available to cure DOX cardiotoxicity. Therefore, the prevention of DOX cardiotoxicity is crucial for cancer patients. Currently, several pharmaceutical strategies have been used or tested clinically to prevent DOX cardiotoxicity. In addition, a number of nonpharmacological strategies have shown promising results in preclinical studies. To accomplish more successful prevention or intervention of DOX cardiotoxicity, efforts should be exerted on identification of the susceptible population on the basis of genetic variants or early diagnosis of this disease taking advantage of biomarkers. In this chapter, we first describe morphological and functional characteristics of the heart in DOX cardiotoxicity. We then update the findings regarding molecular biology of DOX cardiotoxicity. Finally, we introduce several promising pharmacological strategies and lifestyle modifications for the prevention of DOX cardiotoxicity.

2. Morphological and functional characterization

The earliest alteration of the heart in DOX cardiotoxicity is calpain-dependent degradation of a giant cardiac structural protein titin, which may predispose the heart to diastolic dysfunction [3]. Histological changes include cardiomyocyte vacuolar degeneration and myofibrillar disarray [4]. In addition, fibrosis is markedly increased in both interstitial area of myocardium and perivascular area in animal models of chronic DOX-induced cardiotoxicity [5]. At the ultrastructural level, DOX-induced cardiac damage is characterized by dilatation of sarcoplasmic reticulum, loss of the Z-band, myofibrillar dropout, marked accumulation of cytoplasmic vacuoles, damaged mitochondria, and increased numbers of autophagic vacuoles [6, 7]. These changes result in cardiomyocyte dysfunction and cell death via necrosis or apoptosis. Cell death and fibrosis lead to compromised cardiac function in DOX-induced cardiomyopathy. DOX cardiotoxicity can be diagnosed if the patients receiving DOX treatment show signs and symptoms of congestive heart failure. However, DOX cardiotoxicity is usually diagnosed on the basis of left ventricular cardiac function. Three types of criteria are widely used to diagnose DOX cardiotoxicity: (i) the left ventricular ejection fraction (LVEF) is reduced by 20% to a value >50%, (ii) the LVEF is reduced by 10% to a value <50%, and (iii) the LVEF is reduced by >10 points to a value <50% [8].

3. Cellular and molecular mechanisms

The cause of DOX cardiotoxicity is multifactorial, and the precise mechanisms remain to be elucidated. Here, we describe the major mechanisms that have been suggested to contribute to DOX cardiotoxicity. It should be pointed out that the mechanisms are not mutually exclusive. As a matter of fact, most of the factors are interconnected with each other.

3.1. Oxidative stress

Oxidative stress, caused by enhanced intracellular levels of reactive oxygen species (ROS), has long been believed to be the major mediator of DOX cardiotoxicity. The major types of

ROS include superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl free radical (HO) [9]. ROS is mainly generated through redox cycling in mitochondria [9]. However, ROS is also produced outside mitochondria by activation of pro-oxidant enzymes such as NADPH oxidase and xanthine oxidases [10]. Low level of ROS functions as signaling molecules and cell defense system. The cells have efficient antioxidant defense system to eliminate overproduced ROS and maintain ROS to physiological levels [11]. However, if the balance between ROS production and antioxidant system is disrupted in favor of ROS production, then oxidative stress occurs, which triggers a number of deleterious events including DNA damage, mitochondrial dysfunction, cell death, disrupted cellular calcium homeostasis, attenuated protein synthesis, defect in protein quality control, and mitochondrial quality control [12]. After DOX treatment, DOX is preferentially accumulated in mitochondria. As a potent electron acceptor, DOX promotes ROS generation and damages the activities of antioxidant enzymes, shifting the balance between pro-oxidant and antioxidant to the former, leading to elevated ROS levels. Excessive ROS is capable of damaging mitochondria, which in turn, produces more ROS, forming a vicious cycle called ROS-induced ROS release [13]. Given that the cardiomyoyctes are exceptionally rich in mitochondria, DOX is especially harmful to the heart. At the molecular level, the harmful effects of DOX-induced ROS are exerted primarily by its direct damage to mitochondrial genome, RNA, proteins and lipids [12]. In addition, enhanced ROS also participates in cellular signaling involved in detrimental events such as DNA damage and cell death [14].

3.2. Iron accumulation

Following DOX administration, DOX cardiotoxicity occurs through iron accumulation in mitochondria. Cardiac specific over-expression of ABCB8, a mitochondrial inner membrane protein involved in iron export, reduced iron accumulation in mitochondria and mitigated DOX cardiotoxicity [15]. Dexrazoxane, a drug approved by FDA to prevent DOX cardiotoxicity, decreased iron accumulation and ameliorate DOX-induced cardiac injuries in mice. In addition, patients with DOX cardiotoxicity showed higher levels of mitochondrial iron compared with patients with other types of cardiomyopathy or patients with normal cardiac function [15]. These studies provide convincing evidences demonstrating that iron accumulation is one of the major mechanisms involved in DOX cardiotoxicity. However, the underlying mechanisms that iron overload causes DOX cardiotoxicity remain to be clarified. Although several lines of evidences point to enhanced ROS generation by iron accumulation, a number of antioxidants fail to protect DOX cardiotoxicity in clinical settings, suggesting that other unidentified mechanisms are responsible for iron accumulation-mediated cardiac damage in DOX cardiotoxicity [16].

3.3. Topoisomerase IIβ

Type II topoisomerases (Top II) is an enzyme that generates DNA double-strand breaks, which is crucial to control the conformational changes of DNA and the entire chromosome. Mammalian cells consist of two types of Top II isoenzymes, Top IIα and Top IIβ. Top IIα is only expressed in proliferating cells, while Top-IIβ is ubiquitously expressed including postmitotic cells such as adult cardiomyocytes [17]. The antitumor activity of DOX is achieved through the formation of Top II-DOX-DNA ternary complex (also called the cleavage complex), which

increases Top II-DNA complexes and consequent DNA double-strand breaks [17]. In cardiomyocyte, Top IIβ is targeted by DOX, and the increased Top IIβ DNA cleavage complex induces DNA damage, which in turn, leads to cell death. Cardiomyocyte-specific depletion of Top IIβ conferred protection against DOX-induced DNA double-strand breaks, transcriptome changes, and heart failure [18, 19]. These data suggest that Top IIβ in cardiomyocytes plays a major role in mediating DOX-induced cardiotoxicity.

3.4. Macroautophagy dysregulation

Macroautophagy (hereafter referred to as autophagy) is a conserved pathway delivering cytoplasmic contents to lysosome for degradation and recycling [20]. Basal level of autophagy in the heart plays an essential role in the maintenance of cardiac structure and function by removing damaged protein and organelles such as mitochondria [21]. Autophagy can be either activated or suppressed in pathological conditions [22]. The significance of autophagy activation can be either beneficial or detrimental depending upon pathological settings [22]. Recent studies have shown that autophagy is dysregulated after DOX treatment in animals. However, it is controversial whether autophagy is activated or suppressed. There are studies showing that DOX treatment activates autophagy in the heart or cardiomyocytes [23–26], while others have shown conflicting results [7, 27–30]. Moreover, the significance of autophagy in DOX cardiotoxicity is still on debate. Some data are in favor of beneficial effects of autophagy in DOX cardiotoxicity [23–26], while others argue against it [27–30]. The discrepancies may be caused by the difference in animal species, cell types, methods monitoring autophagy, means of drug administration, and dosage and duration of the drug used in these studies. More recently, we and others have shown that DOX treatment stimulated autophagy initiation, while suppressed multiple subsequent steps including autophagosome formation, autophagosome maturation and lysosomal degradation [7, 27, 29, 30]. As a consequence, the autophagic flux was attenuated in DOX-induced cardiotoxicity. Inhibition of autophagic flux using UVRAG-deficient mice exacerbated DOX-induced cardiotoxicity [30]. Conversely, enhancement of autophagic flux mitigated DOX cardiotoxicity [27, 29, 30]. In addition, suppression of autophagy initiation using *beclin* $1^{+/-}$ mice ameliorated DOX cardiotoxicity [7]. The regulation of autophagy in the heart and its significance in cancer patients treated by DOX needs to be investigated. Moreover, the effects of autophagy modulation on cancer cells should be considered if autophagy is targeted for prevention of DOX cardiotoxicity.

3.5. Mitochondrial dysfunction

Mitochondria are the organelle that produces ATP, which plays an essential role in cell survival. Mitochondria are the major source of free radicals and as a consequence are vulnerable to damage caused by oxidative stress. It has been demonstrated that mitochondrial dysfunction is one of the mechanisms of DOX cardiotoxicity [12]. Under physiological conditions, mitochondrial quality is controlled by mitochondrial quality control system, which includes selective elimination of mitochondria by autophagy (also called mitophagy), mitochondrial biogenesis, and mitochondrial dynamics including mitochondrial fusion and fission [31].

Pink1-Parkin-mediated mitophagy is the most well-studied mechanism for mitophagy. Pink 1 is a serine/threonine kinase, which is normally localized in the inner membrane of mitochondria (IMM). However, in depolarized mitochondria, Pink 1 is unable to be translocated to IMM and is retained on the outer membrane of mitochondria (OMM), where Pink-1 undergoes autophosphorylation and is activated. The activated Pink-1 then recruits parkin, a cytosolic E3 ligase to the OMM. Parkin ubiquitinates the substrate proteins localized on the OMM and facilitates degradation of mitochondria by autophagy [32, 33]. DOX treatment has been shown to suppress Pink 1 and Parkin expression [34]. In addition, DOX enhances p53 expression, which promotes its interaction with Parkin and prevents Parkin translocation from cytoplasm to mitochondria [35]. Moreover, as aforementioned, DOX inhibits autophagic flux in the heart at multiple steps, which also attenuates mitochondrial degradation [7, 27, 29, 30]. Therefore, DOX treatment suppresses Pink 1-Parkin-mediated autophagy in the heart and promotes accumulation of damaged mitochondria. In addition to Pink 1-Parkin-meidated mitophagy, other mitochondria-localized proteins such as Nix, Bnip3, FUNDC1, and cardiolipin have been shown to interact with LC3 or LC3 homologs to mediate mitophagy [33]. However, the significance of Parkin-independent mitophagy mediated by these molecules remains to be elucidated in DOX cardiotoxicity.

Mitochondria are highly dynamic organelle, which continuously undergo fusion and fission to organize interconnecting networks to fulfill its function. Mitochondrial fusion and fission are essential for the maintenance of mitochondrial number and quality under stress conditions. Mitochondrial fusion allows the mixture of the contents from partially damaged mitochondria and healthy mitochondria to alleviate the stress. Mitochondrial fission separates mitochondria into two daughter mitochondria, which allows the biogenesis of new mitochondria and the removal of the damaged mitochondria via mitophagy [31]. Mitochondrial fusion is controlled by GTPase Mitofusin1 (MFN1), Mitofusin2 (MFN2), and optic atrophy factor 1 (OPA1). MFN1 and MFN2 are localized to the OMM, while OPA1 is an IMM protein. MFN1, MFN2, and OPA1 mediate the fusion of the OMM and IMM, respectively [31]. Mitochondrial fission is mainly regulated by Drp1, a large GTPase. Drp1 is recruited from cytoplasm to mitochondrial OMM during fission process. In mitochondrial OMM, Drp1 has four interacting partners, FIS1, Mff, Mid55, and Mid49 [31, 36]. Mitochondrial fusion and fission are well balanced to maintain mitochondrial number and quality under physiological conditions. In animal models of DOX cardiotoxicity, DOX treatment induces changes in the expression of mitochondrial fusion and fission proteins, which alters mitochondrial dynamics and contributes to apoptosis [37].

Mitochondrial biogenesis is the process of expansion of existing mitochondria or generation of new mitochondria. Mitochondrial biogenesis is tightly regulated to coordinate mitophagy, mitochondrial fusion and fission for the maintenance of mitochondrial mass and remodeling of dynamic interconnected mitochondrial network. DOX treatment impairs cardiac mitochondrial biogenesis as manifested by reduced mitochondrial DNA copy number and expression of regulating factors for mitochondrial biogenesis such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha, peroxisome proliferator-activated receptor alpha, and estrogen-related receptor alpha, leading to suppression of mitochondrial metabolism and ATP synthesis [38, 39].

3.6. Inflammation

A growing body of evidences has shown that cardiac inflammation contributes to DOX cardiotoxicity. DOX treatment induces increased activity of NF-κB, a key component of innate immune system, leading to enhanced levels of pro-inflammatory cytokines including IL-1β, IL-6, and TNFα [40]. Toll-like receptors (TLRs) especially TLR2 has been considered as the major mediator to activate NF-κB [40]. DOX-induced oxidative stress and damage-associated molecular pattern molecules (DAMPs) such as HMGB-1 are responsible for the activation of TLR2 [41]. In addition to TLR2, TLR9 is capable of activating NF-κB and may be engaged in cardiac inflammation in DOX-induced cardiotoxicity [42]. It has been shown that mitochondrial DNA escaped from autophagy triggers cardiac inflammation through TLR9 activation during progression of pressure-overloaded heart failure [43]. Given that autophagic flux in the heart is impaired by therapeutic dose of DOX, it is likely that TLR-9 activation is involved in inflammatory response in DOX-induced cardiotoxicity. However, studies need to be designed to address this issue.

3.7. Abnormal intracellular calcium handling

Calcium is critical for cardiac systolic and diastolic function. Calcium regulates cardiac contraction through a process called cardiac excitation-contraction coupling (EC coupling). In this process, calcium enters cytoplasm through L-type calcium channel activates ryanodine (RyR) receptor localized on the sarcoplasmic reticulum (SR) membrane, resulting in calcium-induced calcium release in the SR. The released calcium form SR stimulates cardiomyocytes to contract. Subsequently, the cytoplasmic calcium is taken up by the sarcoendoplasmic reticulum calcium transport ATPase (SERCA2) localized on the SR membrane, resulting in reduced cytoplasmic calcium concentration and cardiomyocyte relaxation [44]. DOX regulates cytoplasmic calcium levels through several mechanisms. First, DOX is able to bind RYR2 directly and enhances its open probability [45]. Second, DOX is capable of interacting with calsequestrin, a calcium binding protein localized in SR lumen, and promotes calcium release [46]. Third, DOX elevates intracellular calcium levels by binding to SERCA2A and modify its activity [47]. Fourth, DOX induces SR calcium leakage in a CAMK II-dependent manner, leading to impaired calcium handling in cardiomyocytes [48]. Finally, oxidative stress induced by DOX amplifies RYR opening and calcium release [49]. Thus, DOX regulates calcium release from SR through both oxidant-dependent and independent mechanisms, and the abnormal calcium handling contributes to DOX cardiomyopathy.

3.8. Cell death

Numerous studies have shown that DOX induces apoptosis, which contributes to cardiotoxicity. DOX stimulates ROS generation and produces oxidative stress, which activates p53. In addition, DOX itself promotes p53 activity in the heart. p53-mediated signaling stimulates apoptotic cell death of cardiomyocytes [50, 51]. Moreover, multiple lines of evidences have suggested that mitochondrial calcium is overloaded and contributes to apoptotic cell death of cardiomyocytes in DOX cardiotoxicity. As aforementioned, DOX promotes calcium release

from SR. Mitochondria, which are physically close to SR calcium release sites, uptake a portion of calcium released from SR, leading to rise in mitochondrial calcium levels. Calcium overload triggers loss of mitochondrial membrane potential, swelling of mitochondria, and ultimately rupture of OMM and leakage of cytochrome C, resulting in apoptosis of cardiomyocytes [52].

Necrotic cardiomyocyte death is also increased in DOX cardiotoxicity. Oxidative stress induced by DOX is considered as the major cause for necrosis. Oxidative stress enhances calcium release from SR and raises calcium levels in mitochondria, which induces loss of mitochondrial membrane potential, mitochondrial swelling, and ultimately mitochondrial outer membrane rupture, leading to ATP depletion [53]. In addition, oxidative stress induces mitochondrial DNA damage and mitochondrial lipid peroxidation, leading to disruption of integrity of mitochondrial structure, mitochondrial dysfunction, and ATP depletion [54]. Recently, Bnip3 has been shown to disrupt interaction of COXI and UCP3, leading to defective mitochondrial respiratory chain and cardiomyocyte necrosis in DOX cardiotoxicity [55].

4. Biomarkers and genetic factors

Currently, no effective therapy is available to cure DOX-induced cardiotoxicity. Thus, prevention become more important and should be primarily directed. Early detection is crucial for the prevention of irreversible cardiac damage. Traditional technology such as echocardiography, electrocardiogram, and angiography are not efficient for early detection of cardiac damage since cardiac dysfunction already occurs when diagnosis is made by means of aforementioned technology. Biochemical biomarkers are sensitive and ideal for early detection of cardiac damage. Two types of biomarkers, i.e., troponins and natriuretic peptides, have been established and are currently used in clinic for early diagnosis of DOX cardiotoxicity. In addition, other promising putative biomarkers have been tested.

4.1. Cardiac troponins and B-type natriuretic peptide

Cardiac troponins are a complex consisting of three regulatory proteins, i.e., troponin T (cTnT), troponin C (cTnC), and troponin I (cTnI) in cardiac muscle. cTnT and cTnI are well-established sensitive and specific biomarkers to detect myocardial damage caused by differential insults [56]. Both cTnI and cTnT have also been utilized in clinic to detect and predict cardiac damage caused by DOX [57, 58].

B-type natriuretic peptide (BNP) is a peptide prohormone, which is primarily produced in ventricles and brain. BNP is synthesized as pre-pro-BNP, which is cleaved to generate pro-BNP. Pro-BNP is further cleaved into a C-terminal biologically active form of BNP and N-terminal inactive form of NT-pro-NPs. Both NT-pro-NPs and BNP are secreted into serum and serve as sensitive biomarkers predictive of congestive heart failure [59–61]. Currently, NT-pro-NPs and BNP are used in clinic as indicators of early cardiac damage caused by DOX [62, 63].

4.2. MicroRNAs

MicroRNAs can become ideal clinical biomarkers due to their characteristics such as high stability, tissue specificity, and presence in body fluids [64]. Emerging evidences have indicated that alteration of certain microRNAs is associated with DOX cardiotoxicity and may be served as biomarkers. An in vitro study using human pluripotent stem cell-derived cardiomyocytes showed that a number of microRNAs, including miR-34a, miR-34b, miR-187, miR-199a, miR-199b, miR-146a, miR-15b, miR-130a, miR-214, and miR-424, were differentially expressed during and after DOX treatment [65]. However, the expression pattern of these microRNAs in animal models and patients receiving DOX treatment remains to be investigated. A study using a mouse model of DOX cardiotoxicity explored whether microRNAs including miR-208a, miR-133b, miR-146a, miR423-5p and miR-1 are suitable to predict cardiac damage in patients receiving DOX treatment. The results showed that miR-208a and miR-208b were not useful biomarkers for DOX cardiotoxicity since they were undetectable in the serum. MiR-133b, miR-146a, and miR423-5p were not appropriate biomarkers either since although detectable, no significant alterations were observed in cardiotoxic-patients compared with noncardiotoxic-patients. miR-1 was upregulated in patients suffering from cardiotoxicity compared with noncardiotoxic patients. Moreover, miR-1 expression levels were associated with changes of left ventricular ejection fraction. Therefore, miR-1 is a promising circulating biomarker for early detection of cardiac injury caused by DOX [66]. However, further studies should be developed to validate the putative diagnostic marker.

4.3. Genetic risk factors

The susceptibility to DOX cardiotoxicity is apparently patient dependent, suggestive of a role of genetic factors. To date, a number of gene polymorphisms associated with DOX cardiotoxicity have been identified. A German non-Hodgkin lymphoma study including 1697 enrolled patients has suggested that polymorphisms of the NAD(P)H oxidase were associated with DOX cardiotoxicity. Specifically, the 212A→G variant of NAD(P)H oxidase subunit NCF4 was associated with chronic DOX cardiotoxicity. The His72Tyr polymorphism in the p22phox subunit and the variant 7508T→A of the RAC2 subunit of NAD(P)H oxidase were associated with acute DOX cardiotoxicity [67]. Consistent with these findings, mice deficient for NAD(P)H oxidase activity were resistant to chronic doxorubicin treatment [67]. In the same study, Gly671Val variant of the doxorubicin efflux transporter multidrug resistance protein 1 (MRP1) and the Val1188Glu-Cys1515Tyr haplotype of MRP2 have been shown to be associated with acute DOX cardiotoxicity [67]. Polymorphisms of other genes that have been reported to be potentially associated with cardiotoxicity caused by DOX or DOX-based treatment include CBR3, CAT, ABCB1, ABCC1, ABBCC2, RAC2, GSTP1, CYBA, ABCC5, CASP3, MSH2, SLCO1A2, SLC28A3, FMO2, SPG7, SLC10A2, UGT1A6, ABCB4, SULT2B1, HFE, POR, HAS3, HNMT, SLC22A7, SLC22A17, RARG, and NOS3 [68]. Most of the candidate genes are related to cellular transport of DOX, oxidative stress, DOX metabolism, and DNA repair and replication. In a recent study involving a relatively small number of patients treated with DOX for breast cancer, 18 SNPs in nine genes in the HLA region (NFKBIL1, TNF-α, ATP6V1G2-DDX39B, MSH5, MICA, LTA, BAT1, and NOTCH4) and in the psoriasis

susceptibility region of HLA-C were identified to be potentially associated with DOX cardiotoxicity, implicating an important role of dysregulation of genes involved in inflammatory disease and autoimmune disorders in DOX cardiotoxicity [69]. Polymorphisms of RAAS genes, which are useful for the prediction of congestive heart failure, were not significantly associated with DOX-induced cardiotoxicity [67]. Additional studies are required to identify and functionally validate genetic variants in DOX cardiotoxicity.

5. Preventive strategy

5.1. Doxorubicin dosage and administration

Given that DOX-induced cardiotoxicity is cumulative dose-dependent, the most straightforward way to prevent DOX cardiotoxicity is to reduce the dosage utilized for patients. However, lower dosage is associated with less therapeutic efficacy [70]. Thus, alternative approaches of drug administration such as continuous infusion and liposome DOX versus bolus injection are used to prevent cardiac toxicity. Continuous infusion of DOX causes significantly less injury to the heart compared to bolus doses without compromising cancer treatment efficacy. The mechanisms are due to the changes in the distribution of DOX with reducing drug concentration in the heart and no impact on drug doses in tumor tissues [71–73]. It should be pointed out that continuous infusion does not confer cardiac protection in children with acute lymphoblastic leukemia [74]. Administration of DOX by liposome encapsulation is another effective strategy to reduce cardiotoxicity. Liposomal DOX formulation is not capable of crossing the tight gap junction of endothelial cells of blood vessels in the heart. However, in tumor tissues, the vasculature is irregular and leaky, which allows the diffusion of liposomal DOX formulation [75]. In addition, the diffused DOX accumulates in the tumor tissue due to poor lymph drainage. Both lead to selective accumulation of DOX in tumor tissues. This phenomenon is known as "enhanced permeability and retention effect," which characterizes solid tumors and is used to target tumor cells [76]. Moreover, the liposomal DOX formulations diffused into tumor tissues are prone to destabilization due to more acidic extracellular pH, release of necrotic tumor cell lipases, and inflammatory cell oxidizing agents in tumor microenvironment [76]. A number of preclinical and clinical studies have demonstrated that liposomal DOX formulation delivers relatively larger amount of DOX to tumor tissues and much less doses to the heart tissues compared to conventional DOX. Thus, the liposomal DOX formulations are more active and safer. Currently, two types of liposomal DOX formulations, i.e., pegylated (Caelyx® in Europe and Doxil® in the USA) or nonpegylated (Myocet®), have been approved as a first-line treatment for defined group of cancer patients [77]. In recent years, nanoparticle DOX delivery systems have attracted much attention due to potential increased bioavailability in tumor tissues and minimum cardiac toxicity, which hold promise as an efficient approach for the prevention of DOX cardiotoxicity [78].

DOX treatment combining with cardioprotective agents is an alternative strategy to prevent cardiotoxicity. Dexrazoxane (Zinecard, ICRF-187, ADR-529, NSC-169780), a cyclic derivative of edetic acid, is a cardioprotective agent approved by FDA to prevent DOX cardiotoxicity in

the clinic [79]. The molecular mechanisms that Dexrazoxane confers cardioprotection have previously been attributed to its iron chelating capability. However, other iron chelators fail to exert preventive effects for DOX cardiotoxicity, suggesting that iron chelation is not the major molecular basis for dexrazoxane cardioprotection. It turns out that dexrazoxane interferes with Top IIβ either through promoting Top IIβ proteasomal degradation or preventing the formation of Top IIβ-DNA cleavage complex in cardiomyocytes [79]. It should be noted that coadministration of dexrazoxane may trigger secondary malignancies in cancer patients [80]. However, this issue is still controversial and requires further investigation.

5.2. Antioxidant reagents

Considering oxidative stress has been believed to be the major mediator of DOX-induced cardiotoxicity, it is reasonable to expect that coadministration of antioxidants is capable of preventing or mitigating DOX cardiotoxicity. The antioxidants reduce intracellular ROS levels through reducing ROS generation, scavenging ROS themselves, chelating irons to inhibit HO. formation or eliminating other active molecules generated in response to ROS reaction such as lipid peroxide [81]. Although antioxidants are effective in the treatment of acute DOX cardiotoxicity in animal models, Clinically relevant animal experiments and clinical trials have suggested that among a variety of antioxidant reagents, only dexrazoxane has shown definitive effect on DOX cardiotoxicity [79]. As mentioned above, dexrazoxane ameliorates DOX cardiotoxicity likely through mechanisms independent of ROS elimination [79]. Thus, it still remains unclear whether antioxidants should be given to cancer patients during or after DOX treatment to prevent cardiotoxicity. In addition, ROS generation could be the mechanism that DOX is toxic to cancer cells, antioxidant may reduce response rate for DOX in patients, although DOX may cause cytotoxicity in cancer cells through both ROS-dependent and independent mechanisms. Further study should be conducted to address these issues.

5.3. Neurohormone blockers

Neurohormone blockers such as angiotensin II-converting enzyme inhibitors and angiotensin receptor blockers have been widely utilized in clinics to treat heart failure including DOX-induced heart failure. Angiotensin receptor blockers have been shown to prevent decline of cardiac function induced by DOX in cancer patients. The preventive effect may be related to decreased generation of oxidative stress and reduced apoptosis of cardiomyocytes [82, 83]. Thus, neurohormone blockers may be used in combination with DOX to prevent cardiac toxicity.

5.4. Exercise

In addition to pharmaceutical measure, lifestyle modifications are promising alternative strategies to counteract DOX-induced cardiomyopathy since it is practical to be introduced to patients. Several types of exercise such as chronic resistance exercise [84], chronic swimming [85], voluntary exercise [86, 87], and treadmill running [88–91] have been shown to exert beneficial effect on mitigation of cardiac structural damage and preservation of cardiac

performance in animal models of DOX cardiotoxicity. Moreover, acute exercise prior to DOX treatment protects cardiac function of breast cancer patients [92]. The protective effects of exercise on DOX-induced cardiac injury may be attributed to increased antioxidant ability, increased expression of heat shock proteins and antiapoptotic proteins, improved mitochondrial quality control, maintenance of calcium handling, and altered delivery of DOX to myocardium [90, 91, 93]. Importantly, exercise training has no effect on antitumor efficacy of DOX [94]. However, these preclinical and clinical findings need to be verified by studies involving a large cohort of patients.

5.5. Calorie restriction and fasting

Calorie restriction is beneficial for several types of cardiovascular diseases including DOX cardiotoxicity [95, 96]. However, calorie restriction is hard to sustain in the long term. Although calorie restriction mimetics are more practical in terms of sustainability, they are less accessible and cost ineffective. Fasting has been shown to exert beneficial effects on certain forms of cardiovascular diseases including age-related cardiac hypertrophy, myocardial ischemic injury, and coronary heart disease risk factors through diverse mechanisms including remodeling of mitochondrial networks, improvement of energy metabolism, reduction in signaling pathways related to survival such as insulin and insulin-like growth factor-1 signaling, decrease in mitochondrial oxidative stress, and enhancement of autophagic flux [97, 98]. Recent studies suggest that fasting also conferred cardioprotection against DOX cardiotoxicity. In animal models, short-term fasting ameliorates cardiac damage and cardiac dysfunction caused by DOX [98]. Alternate-day fasting, a type of intermittent fasting, is capable of mitigating DOX cardiotoxicity in mouse models of both acute and chronic DOX cardiotoxicity [30]. More importantly, intermittent fasting and multiple fasting cycles have recently been shown to suppress tumor growth and sensitize various tumors to chemotherapy [99, 100]. Therefore, intermittent fasting could be considered as a potential preventive or therapeutic strategy for cardiotoxicity induced by DOX. However, given that long-term fasting is harmful to health especially for cancer patients due to malnutrition problem, the procedure of intermittent fasting should be optimized under clinical supervision to improve its efficacy while minimizing side effects.

6. Conclusions

DOX is one of the most effective chemotherapeutic agents. However, potential acute or chronic irreversible cumulative cardiotoxicity limits its clinical application. It is encouraging that accumulating evidences from basic research, preclinical experiments and clinical trials provide insight into the pathophysiology and molecular mechanisms of this disease, which potentially leads to identification of novel biomarkers for early detection and establishment of preventive strategies. Moreover, emerging evidences have associated DOX cardiotoxicity with genetic risk factors. Findings in this direction will be helpful to predict tumor sensitivity to DOX treatment and susceptibility to DOX-induced cardiotoxicity of the population. As

a consequence, precise strategies may be developed and applied to individuals to achieve maximal efficacy for cancer treatment and meanwhile minimal side effects on the basis of patient-specific genetic variants.

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Conflict of interest

No potential conflict of interests were declared.

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