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Review article

Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies

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ABSTRACT

The utility of anthracycline antineoplastic agents in the clinic is compromised by the risk of cardiotoxicity. It has been calculated that approximately 10% of patients treated with doxorubicin or its derivatives will develop cardiac complications up to 10 years after the cessation of chemotherapy. Oxidative stress has been established as the primary cause of cardiotoxicity. However, interventions reducing oxidative stress have not been successful at reducing the incidence of cardiotoxicity in patients treated with doxorubicin. New insights into the cardiomyocyte response to oxidative stress demonstrate that underlying differences between in vitro and in vivo toxicities may modulate the response to superoxide radicals and related compounds. This has led to potentially new uses for pre-existing drugs and new avenues of exploration to find better pharmacotherapies and interventions for the prevention of cardiotoxicity. However, much work still must be done to validate the clinical utility of these new approaches and proposed mechanisms. In this review, the authors have reviewed the molecular mechanisms of the pathogenesis of acute and chronic doxorubicin-induced cardiotoxicity and propose potential pharmacological interventions and treatment options to prevent or reverse this specific type of heart failure.

Contents

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1. Introduction

Doxorubicin is a secondary metabolite of *Streptomyces peucetius* var. *Caesius*, along with daunorubicin, epirubicin, and idarubicin, and belongs to the family of anthracyclines. These are well-established and highly effective anti-neoplastic agents, used to treat several adult and pediatric cancers, such as solid tumors, leukemia, lymphomas and breast cancer. The successful use of doxorubicin has been hampered by toxicities such as hematopoietic suppression, nausea, vomiting, extravasation, and alopecia, yet the most feared side-effect is cardiotoxicity. The onset of this cardiotoxicity may be delayed until as many as 10–15 years after cessation of chemotherapy. It is characterized by a broad spectrum of symptoms ranging from asymptomatic electrocardiography (ECG)-changes, to pericarditis and decompensated cardiomyopathy. While the probability of developing cardiomyopathy is largely dose-dependent [1], cardiotoxicity may occur at low doses due to increased individual susceptibility [2].

Gender difference has been mentioned as one of the risk factors in the toxic effects of doxorubicin. Lipshultz SE et al. [3] reported that female had more severe cardiotoxicity with more depressed contractility. Several other groups demonstrated also a significant higher risk in subclinical cardiotoxicity in female compared with male by using multivariate analysis [4–6]. Another risk factor is age, for example, elders older than 65 years or children younger than 4 years are at an increased risk [7–9] for doxorubicin-induced cardiotoxicity. Simultaneous administration of other cardiotoxic drugs, mediastinal radiotherapy, and cumulative dose of doxorubicin can correlate with an increased risk of doxorubicin-induced cardiomyopathy [2]. In addition, chronic conditions such as hypertension, diabetes mellitus, liver disease, and previous cardiac disease can also contribute to an increased risk of cardiotoxicity [8]. It should be noted that at least one epidemiological study has disputed the validity of these risk factors [10]. Earlier investigations tend to use clinical symptoms of heart failure as their end point [11], while more recent studies use a variety of functional and biochemical endpoints, such as decreased ejection fraction or cardiac troponin-T measurements, to define doxorubicin-induced cardiomyopathy [10].

Since the deleterious effects of doxorubicin on the heart are often not detected until years after cessation of the chemotherapy [7,12], the greatest impact is on pediatric cancer survivors. About 60% of pediatric cancer patients will be given an anthracycline [13] and 10% of these patients will develop symptomatic cardiomyopathy up to 15 years after the end of chemotherapy [8]. The difference in time-of-onset suggests that different mechanisms may be involved in doxorubicin-induced cardiomyopathy. Treatment protocols to protect the heart without diminishing the drug's anti-tumor activity are urgently needed. In this review we explore the molecular mechanisms of doxorubicin-induced cardiotoxicity and provide an extensive overview of current and potential pharmacological interventions and treatment options to prevent or reverse this specific type of heart failure.

2. Molecular mechanism

Multiple mechanisms are involved in doxorubicin induced heart failure. Doxorubicin-induced cardiomyopathy is strongly linked to an increase in cardiac oxidative stress, as evidenced by reactive oxygen species (ROS) induced damage such as lipid peroxidation, along with reduced levels of antioxidants and sulfhydryl groups. Myofibrillar deterioration and intracellular calcium dysregulation are also important mechanisms commonly associated with doxorubicin-induced cardiac toxicity. Not only are cardiomyocytes a target of doxorubicin induced apoptosis, but endothelial cells are also affected, as indicated by caspase activation and internucleosomal DNA degradation. Furthermore, the cardiac toxicity associated with doxorubicin administration is mediated, at least in part, by changes in the high-energy phosphate pool, endothelin-1 levels, and disturbances of myocardial adrenergic signaling. All of these molecular mechanisms of cardiotoxicity are explored in greater detail below.

2.1. Oxidative stress

The induction of free radical production is the best described major mechanism through which doxorubicin injures the myocardium [14–16]. The heart's unique vulnerability to oxidative stress [17] has given this aspect of doxorubicin-induced cardiomyopathy an overwhelming prominence in the literature. Over the past thirty years, the understanding of how free radicals are generated and how they damage the heart has evolved from a purely chemical reaction to a molecular understanding of how enzymes such as nitric oxide synthases (NOS) and NAD(P)H oxidase interact with doxorubicin and induce oxidative stress.

2.1.1. Mitochondrial dependent ROS

The mitochondria are the most extensively and progressively injured subcellular organelles of doxorubicin-induced cardiotoxicity. One reason for this may be due to the fact that the cationic drug doxorubicin is retained in the mitochondrial inner membrane by forming a nearly-irreversible complex with cardiolipin [18]. The proteins of the electron-transport chain require cardiolipin binding to function properly, and it has been argued that since doxorubicin disrupts the cardiolipin–protein interface, more superoxide (O_2^-) formation occurs [19]. Other membrane proteins, such as those responsible for the transfer of carnitine, can also be adversely affected by doxorubicin, contributing to the decrease in mitochondrial function [20]. It is quite plausible that these events disrupt mitochondrial (and therefore cellular) metabolism, since mitochondria produce more than 90% of the ATP utilized by cardiomyocytes [21]. This functional disruption leads to ultrastructural pathologic changes such as mitochondrial swelling and myelin figures within the mitochondria [22]. However, the studies showing doxorubicin-induced myocardial dysfunction were often performed with supraclinical concentrations of doxorubicin [23,24]. Yet clinical doses of doxorubicin can also directly affect mitochondrial function, but the effects are less severe. In a rat model of chronic doxorubicin-induced cardiomyopathy, it was found that long-chain-fatty acid oxidation in cardiac mitochondria is significantly decreased, while glucose metabolism is increased, indicating an overall shift from an aerobic to anaerobic metabolic state [25]. Although this shift in metabolism is a common feature of heart failure, doxorubicin induced oxidative stress could induce signaling to cause the shift in metabolism from long-chain fatty acid metabolism to glucose when metabolic genes are transcriptionally suppressed.

Suliman et al. [26] indicate that doxorubicin treatment affects mitochondrial gene expression. This group used transgenic mitochondrial reporter mice to demonstrate that doxorubicin suppresses cardiac mitochondrial metabolism and biogenesis, resulting in apoptosis. This could be reversed by allowing the mice to inhale low levels of carbon monoxide (CO), which activates the genes required for mitochondrial biosynthesis and by upregulating levels of nuclear encoded heme oxygenase (HO). This indicates that doxorubicin also interferes with both nuclear and mitochondrial transcriptional regulation [27].

Upregulation of manganese superoxide dismutase (MnSOD) has been shown to enhance cell survival in the presence of doxorubicin through its role as a free radical scavenger in mitochondria [28]. The compound calceolarioside protects from Dox-induced apoptosis by upregulation of several superoxide dismutases (SOD) and heme oxygenase, and preserving mitochondrial membrane potential [29]. In addition, overexpression of Gpx1, a cytosolic and mitochondrial enzyme that reduces hydrogen peroxide (H_2O_2) and fatty acid hydroperoxides, protects mice hearts against acute doxorubicin-induced cardiotoxicity and prevents impairment of mitochondrial respiration and inhibition of complex I activity [30].

It is well-known that mitochondria play an important role in the pathogenesis of doxorubicin-induced cardiomyopathy. Prevention of mitochondria dysfunction will prevent myocardial alteration and subsequently make better cardiac outcome. However, further experiments to unravel specific function of mitochondria in this pathogenesis are needed.

2.1.2. NOS dependent ROS

Vasquez-Vivar et al. [31] demonstrated that the binding of doxorubicin to eNOS reductase domain results on O_2^- generation. The one-electron reduction by eNOS forms the doxorubicin semiquinone radical, which reduces oxygen to generate O_2^-. The importance of the reductase domain (and not of the oxygenase domain) of eNOS involved in doxorubicin reduction is stressed by the observation that this reaction is not dependent on Ca^{2+}, is not inhibited by L-NAME, and is attenuated by diphenyliodonium (NADPH inhibitor). They also found that at low doxorubicin concentrations, eNOS is the focus of doxorubicin reduction. The interaction between drug and enzyme implies that, at increasing doxorubicin concentrations, eNOS will be transformed from a nitric oxide (NO) to a superoxide generator. Therefore, it is possible that eNOS inhibition will have far-reaching consequences in terms of cardiotoxicity. Furthermore, doxorubicin-induced apoptosis is linked to the redox activation of doxorubicin by eNOS. Kalivendi et al. [32] demonstrated that doxorubicin treatment causes an increase in eNOS transcription and protein activity in bovine aortic endothelial cells and pre-treatment with antisense eNOS mRNA causes a decrease in doxorubicin-induced apoptosis.

Uncoupling of eNOS is already known to be a major contributor to pressure-overload induced heart failure, [33] and there is some evidence that eNOS-dependent ROS formation also does have a role in doxorubicin-induced myocardial dysfunction. Indeed, Neilan et al. [34] demonstrated that genetic disruption of eNOS transcription protects against the doxorubicin-induced cardiac dysfunction, injury and mortality via a mechanism that does not require induction of cardioprotective genes such as COX-2, HO-1, Bcl-xL, and GATA-4. In addition, they demonstrated that cardiomyocyte-specific overexpression of eNOS exacerbates the pathological response to doxorubicin in the heart [34]. Furthermore, doxorubicin-induced levels of cardiac ROS synthesis were greatest in eNOS-transgenic mice and least in knockout mice [34]. In human studies, endothelium-dependent and -independent vasodilation was significantly attenuated within 30 min of doxorubicin administration and was accompanied by a significant decrease in serum nitrate levels. These human results are consistent with dysfunctional eNOS activity after doxorubicin administration, especially in vascular beds [35].

The role of inducible nitric oxide synthase (iNOS) in the pathogenesis of doxorubicin-induced cardiac dysfunction has been more controversial. Deficiency of iNOS has been reported both to enhance and protect against [36] doxorubicin-induced cardiac toxicity [22]. Neilan et al. [34] did not detect an increase in iNOS mRNA levels in an acute model of doxorubicin cardiotoxicity, although this finding appears to depend on the model, as other papers have shown that iNOS mRNA levels increase in the presence of doxorubicin [36]. It appears as though the cardioprotective effects of iNOS are due to the generation of NO, while the cardiotoxic effects are due to the induction of peroxynitrites that are generated when NO reacts with O_2^-. Furthermore, peroxynitrites are known to damage DNA, activating Poly (ADP-ribose) polymerase, leading to an energetic imbalance and eventual cell death [36].

The role of the third isoform, neuronal nitric oxide synthase (nNOS), in doxorubicin-induced cardiomyopathy is less understood. This isoform can also catalyze one-electron reduction of doxorubicin and the flavin domain is suggested to have an important role in this reduction [37]. However, the role of nNOS in doxorubicin induced cardiotoxicity has been minimized because there are no changes in myocardial mRNA-expression of nNOS after doxorubicin administration [38]. In conclusion, from three NOS isoforms, eNOS is the most important player in doxorubicin-induced cardiomyopathy. Deterioration in the myocardium due to doxorubicin is attenuated in eNOS KO mice or following eNOS inhibition, establishing the significant role of eNOS in doxorubicin-induced ROS generation.

2.1.3. NAD(P)H-dependent ROS

Recently, Deng et al. [39] demonstrated that doxorubicin and NAD(P)H can produce O_2^- in the absence of any enzymatic activity, although this is a minor source of O_2^- radicals at best. However, they also showed that gp91phox (the catalytic domain) knockout mice were resistant to cardiotoxicity from chronic doxorubicin treatment, unlike wild-type mice [39]. The importance of the NAD(P)H complex in the development of doxorubicin induced cardiomyopathy has recently been confirmed pharmacologically in vitro using NAD(P)H inhibitors on cultured cell lines and in experiments where inhibitors of NAD(P)H activity were found to enhance cell survival [40].

Understanding a patient's genetic susceptibility to doxorubicin is one factor to consider. NAD(P)H is such a large polypeptide complex, some researchers have theorized that single-nucleotide polymorphisms (SNP) in any one of the subunits might make the NAD(P)H complex more vulnerable to doxorubicin. Wojnowski et al. [41] were able to correlate the development of doxorubicin-induced cardiotoxicity with polymorphisms of the NAD(P)H oxidase complex in non-Hodgkin lymphoma patients. Chronic doxorubicin-induced cardiotoxicity was associated with an SNP variant of the NAD(P)H oxidase subunit NCF4, which is responsible for down regulation of the NAD(P)H complex, while acute cardiotoxicity was associated with SNPs in the p22phox and Rac2 subunits [41]. Therefore, these genetic polymorphisms in NAD(P)H oxidase might be used as a screening tool in the future to detect individual patients at a higher risk of developing doxorubicin-induced cardiotoxicity. Yet polymorphisms in many other genes may be important in susceptibility. For example, Blanco et al. demonstrated a correlation between the development of doxorubicin cardiomyopathy and the CBR3 V244M, variant of the carbonyl reductase domain, an enzyme involved in doxorubicin's metabolism [42].

2.1.4. Fe–DOX complex

Doxorubicin–iron complexes have been known since 1980, when the first studies demonstrated that doxorubicin had a strong affinity for iron [43], and that the iron complex could cause lipid peroxidation through its interactions with the negatively-charged membranes [44]. Doxorubicin reduction in the presence of free iron also sets up a cycle for free radical generation (redox recycling) and the

metabolite doxorubicinol is known to interact with thiol groups on proteins, compounding the damages to the cell [45] (Fig. 1). However, the free iron content of most cells is very low including cardiomyocytes. In physiological conditions, there would not be enough free iron to couple with doxorubicin to the extent necessary to cause cardiomyopathy [23]. More recent studies have suggested that the effects of doxorubicin on iron metabolism are not mediated by doxorubicin–iron interactions, but rather via the proteins that sequester and bind intracellular iron. One such mechanism involves the doxorubicinol metabolite forming complexes with the Fe–S group the cytoplasmic aconitase/IRP-1 (iron regulatory protein), thereby enhancing the stability of transferrin mRNA and preventing translation of iron sequestration proteins [46]. The subsequent decrease in IRP-1 leads to an increase in free iron, which could perpetuate the cycle of free radical generation. Interference with iron sequestration therefore remains a critical component of doxorubicin-induced cardiotoxicity. Miranda et al. [47] observed increased susceptibility to doxorubicin-induced cardiotoxicity in mice depleted of the iron regulatory gene *HFE*. In humans, this defective gene is responsible for hereditary hemochromatosis. Feeding iron-rich chow for 10 to 14 weeks to rats also resulted in a profound increase in doxorubicin-induced cardiotoxicity [48]. Both of these studies emphasize the critical role of iron in the pathogenesis of doxorubicin-induced cardiotoxicity.

There is a wide variability in body iron stores in patients undergoing cancer chemotherapy due to abnormal blood losses, blood transfusions, iron supplementation, and nutritional status in these patients. Adult and pediatric patients undergoing treatment for leukemia and other malignancies can develop a significant level of iron overload during, and as a result of, chemotherapy and bone marrow transplantation [49]. If clinical studies confirm the strategic role of iron as an independent variable in the pathogenesis of doxorubicin-induced cardiotoxicity, the simple and relatively inexpensive screening tests for total body iron stores and presence of *HFE* mutation may appear useful.

Regardless of the source, an excess of ROS in the myocardium is clearly detrimental. In addition to overwhelming cardiomyocytes' enzymatic defenses, ROS can also alter gene expression through their interactions with regulatory proteins. ROS can affect the function of membrane-bound proteins such as the G-proteins, via lipid peroxidation. ROS can alter a protein's tertiary structure by oxidizing S – S bonds. Perhaps, most critically for the myocardium, ROS can induce the release of Ca^{2+} ions [50].

In spite of the many studies pointing to the role of oxidative stress in causing cardiomyopathy, it must be noted that, thus far, the administration of simple antioxidants such as vitamin E do not seem to have much of a cardioprotective effect against doxorubicin [51]. The reasons for this are still unclear, but it is becoming apparent that modifying the cellular response to ROS may be more effective than trying to eliminate them. Although it is clear that oxidative stress is an instigator for the development of doxorubicin induced cardiomyopathy, the effects of doxorubicin on other cellular events in the myocardium may also play a role in the development of cardiomyopathy, and contribute to the differences in how individuals respond to chemotherapy.

2.2. Apoptosis

It is generally accepted that the oxidative stress evoked by doxorubicin activates apoptotic signaling leading to cardiomyocyte apoptosis [52], and that both the extrinsic and intrinsic apoptotic-pathways are involved [24,53]. An overview of all apoptotic pathways involved in doxorubicin-induced cardiotoxicity is given in Fig. 2. It has also become apparent that doxorubicin can induce apoptosis via mechanisms that do not directly involve ROS production and oxidative stress, although this point is complicated by the fact that apoptosis itself also generates free radicals. An example of the difficulties in disentangling oxidative stress from apoptosis can be found in studies examining the interactions of heat-shock factor 1 (HSF-1), heat-shock protein 25 (Hsp 25), and p53. In a doxorubicin model, oxidative stress activates HSF-1, which acts to produce more Hsp25, which stabilizes p53 and increases the production of pro-apoptotic proteins [54].

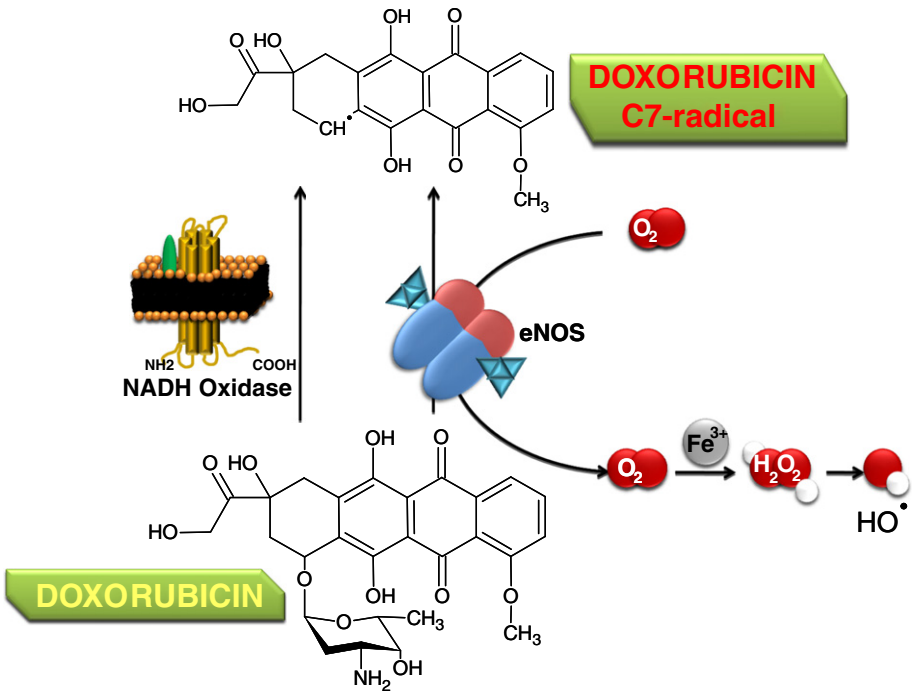

Fig. 1. Molecular transformations of doxorubicin. Doxorubicin can be reduced to a semiquinone (not depicted) by NADPH oxidase or eNOS. This semiquinone undergoes a further transformation to a C7 free radical, which can interact with molecular oxygen, as well as other intracellular molecules, most notably lipids. NADPH oxidase and eNOS oxidize NADPH or FAD/FMN as their electron donors, respectively; NADPH supplies two electrons and FAD/FMN can each supply one electron. Because doxorubicin is a potent electron acceptor, it can "steal" the electrons away from the normal reactions of generating $HB_{2B}OB_{2B}$ and NO. One of the mechanisms through which dexrazoxane is believed to act is by chelating free iron, thereby reducing the reactants for molecular oxygen.

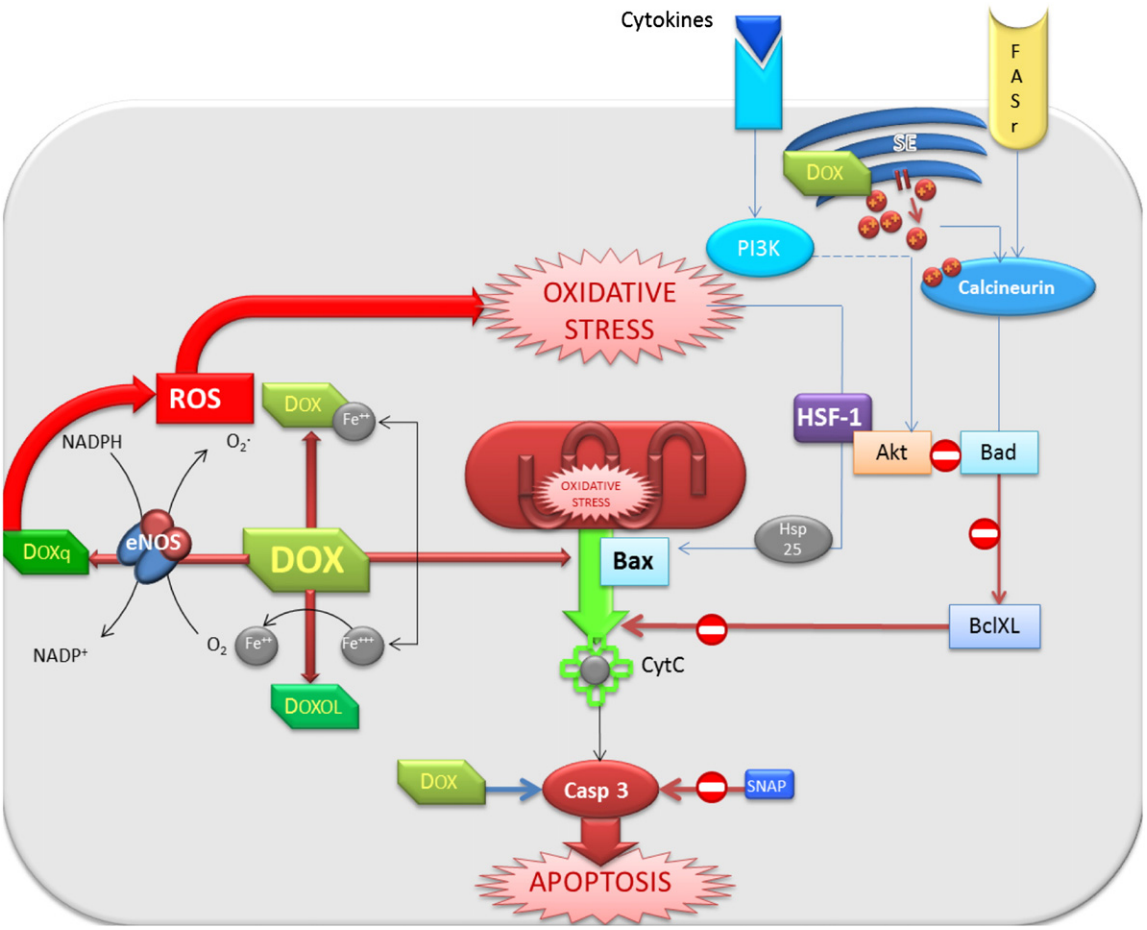

Fig. 2. Interaction between doxorubicin with the various apoptotic pathways in the cardiomyocyte. The left side of the figure shows how doxorubicin begins to generate ROS and the dissociation of the eNOS into monomers. Doxorubicin also enters the mitochondria, causing the release of cytochrome C oxidase, and also prolongs the opening time of calcium channels in the sarcoplasmic reticulum, which activates calcineurin. Akt phosphorylation inhibits Bad activation and is one of the main anti-apoptotic pathways. Oxidative stress activates HSF-1 and produces more Hsp25, which increases the pro-apoptotic proteins. Dox: doxorubicin, Doxol: doxorubicinol, Doxq: doxorubicin semiquinone, ROS: reactive oxygen species, SE: sarcoplasmic reticulum, HSF: heat-shock factor, Hsp: heat-shock protein, CytC: cytochrome C, and Casp3: caspase 3.

The heat-shock family of proteins has a distinct role in these processes. These proteins are well-established as molecular chaperones, acting to stabilize their client proteins involved in anti-apoptotic signaling by preventing their dephosphorylation, ubiquitination, and degradation [55]. Liu et al. [53] demonstrated that overexpression of Hsp27, known for its cardioprotective effect against ischemia/reperfusion injury, also prevents doxorubicin-induced apoptosis and myocardial dysfunction, due to the protective role of Hsp27 in the regulation of oxidative stress responses and maintenance of mitochondrial function. Overexpression of Hsp10 and Hsp60 likewise results in increased post-translational modification of Bcl-2 proteins, shifting the balance toward anti-apoptotic signaling, possibly through their effects as molecular chaperones [55].

Hsp20 enhances the maintenance of Akt phosphorylation, one of the main cell survival pathways. [56]. In addition, some heat shock proteins can be secreted into the extracellular space and into the bloodstream, where they can act as ligands for toll-like receptors (TLRs). It is likely that they act as ligands for other receptors as well, but, as of this writing, the most information is available about TLRs. It has been shown that signaling of Hsp60 can be blocked by antibodies to these peptides, as well as by TLR-2 antagonists [57], whereas Hsp70 interacts with TLR-4 [58]. Thus far, the roles of TLR-2 and TLR-4 in doxorubicin-induced cardiomyopathy have been partially established. TLR-2-mediated signaling through the pro-inflammatory nuclear-factor κB (NFκB) pathway is involved in cytokine production, apoptosis, and cardiac dysfunction after doxorubicin treatment in vivo, and the abolition of the receptor in knock-out mice preserves cardiac function and prevents apoptosis [59]. TLR-4 has likewise been shown to act through the NFκB pathway; knockout mice demonstrate improved cardiac function after doxorubicin administration over their wild-type counterparts [60]. Although this pathway appears to be relatively specific for doxorubicin-induced apoptosis in many cells, it is not specific for the myocardium. For example, the activation of NFκB by doxorubicin has also been demonstrated in endothelial cells and the kidney [61,62]. Moreover, the activation of NFκB signaling is not specific to TLRs, and the modulation of the pathway initiates the transcription and translation of many proteins, including inflammatory cytokines, which might also be involved in apoptosis.

Caspase activity can also be influenced by doxorubicin. It was demonstrated that caspase-3 activation and therefore apoptosis is associated with doxorubicin administration in vivo [63], but the results were not duplicated in isolated cardiomyocytes [64]. The results seem to be system-dependent, or require input from multicellular tissue, as both caspase-dependent and -independent mechanisms of cell death have been documented [65]. It is difficult to determine whether or how doxorubicin directly influences caspase activity, as many pathways can contribute to the activation of caspase-dependent apoptosis. The administration of the NO donor S-nitrosyl-*N*-acetyl-penicillamine (SNAP) produces an anti-apoptotic effect by suppression of caspase activity via S-nitrosylation in cardiomyocytes treated with doxorubicin [66]. Blocking volume-sensitive chloride channels has also been shown to prevent caspase-3 dependent apoptosis in doxorubicin toxicity [67].

2.3. Intracellular calcium dysregulation

Doxorubicin-induced cardiotoxicity is also accompanied by an increase in intracellular calcium levels. Dysregulation of intracellular calcium concentrations is both a result and a cause of ROS-generation. Doxorubicin-mediated ROS generation and apoptosis can be inhibited by using a Ca^{2+} chelator [68]. The ROS and H_2O_2 generated through mechanisms described above alter normal calcium homeostasis in a variety of muscle cell types via disruption of normal sarcoplasmic reticulum function. This is accomplished by inhibiting the Ca^{2+} ATPase pump e.g. by reducing the expression-levels of SERCA2a mRNA resulting in impaired Ca^{2+} handling [69] and/or by directly activating the ryanodine calcium-release channels themselves. Doxorubicin has been shown to induce the release of calcium from the sarcoplasmic reticulum by increasing the probability that the channel adopts the open state [70]. The work of Saeki et al. [71] suggests that the ryanodine channel has several sites for binding doxorubicin, and that the binding occurs regardless of whether the channel is open or closed. Doxorubicin has also been shown to inhibit the sodium–calcium exchanger channel in the sarcolemma [72], as well as increase the activity of the L-type calcium channel [73]. In in vitro studies in skeletal muscle cells, H_2O_2 can modify key thiol groups on the ryanodine Ca^{2+}-release channels in the sarcoplasmic reticulum [74]. Taken together, this suggests that calcium dysregulation has a major role in the pathogenesis of doxorubicin-induced cardiomyopathy.

Like other caspases, caspase-12 also activates apoptotic pathways, but its activation is specific to signals of distress from the sarcoplasmic reticulum, namely calpain dysregulation [75]. Calpains are calcium-dependent proteases that are activated by calcium; because much of the intracellular calcium in the cardiomyocyte is contained in the sarcoplasmic reticulum, oxidative stress can result in calcium leakage, calpain activation, and caspase-12 cleavage. Experimental results in a rat model show that this is one of the mechanisms activated by doxorubicin [76]. However, it is still uncertain how large the role of this system is in doxorubicin-mediated cardiotoxicity. Furthermore, doxorubicin cardiomyopathy is associated with myofibrillar deterioration [77], which may also be a consequence of calpain activation [78]. Calpains are known to degrade titin, one of the largest proteins and a key component of the cardiac sarcomeres. Inhibition, or perhaps more accurately, prevention of calpain activity could help maintain these critical contractile structures, supported by the work of Lim et al. [78] where calpain inhibition preserved cardiac function after doxorubicin exposure.

In addition to the direct effects of excessive calcium, doxorubicin enhances the sensitivity of the mitochondria to intracellular calcium. The mitochondria of cells isolated from rats treated with doxorubicin have a decreased ability to retain calcium, exhibiting a calcium-dependent calcium release that is not seen in mitochondria from rats treated with saline [79]. Administration of ruthenium red, a non-specific inhibitor of Ca^{2+} uniporter, is cytoprotective in Sprague–Dawley rat cardiomyocytes from an acute doxorubicin model [80]. Increase in intracellular calcium is not the only cause of mitochondrial calcium dysregulation, but doxorubicin does in addition affect mitochondrial calcium transport, which contributes to the rise in intracellular calcium levels.

2.4. Changes in the high-energy phosphate pool

Impairment of the cardiac energy homeostasis is one of the main manifestations of doxorubicin-induced mitochondrial impairment and apoptosis. Mitochondrial damage impairs the ability to generate adenosine triphosphate (ATP). The depletion of ATP decreases the affinity of Hsp90 for ErbB2, a cardioprotective protein that is upregulated in rat myocardium after doxorubicin therapy [81]. In conditions where ATP may be depleted, erbB2 levels will drop, as HSP90 cannot maintain its chaperone role [82]. Since erbB2 is coupled to various GPCRs, this transactivation to the pro-survival ERK1/2 pathways is impaired [83]. This could explain why the cardiotoxicity of trastuzumab, an anti-ErbB2 antibody used to treat some breast cancers, and doxorubicin are synergistic [84]. Decreases in ATP levels can also arise from the activation of apoptotic pathways and calcium-dependent proteases, which consume ATP [78]. Energy expenditures to replace damaged proteins can also be immense, especially if they are as large as titin, which is degraded by indiscriminately activated calpain, Therefore, changes in the high-energy phosphate pool appear to come about mostly as a result of the processes described earlier rather than directly from doxorubicin itself.

Both acute and chronic consequences of doxorubicin administration include compromised mitochondrial function, as measured by respiration and reduced generation of high-energy phosphates with lowered ratios of phosphocreatine-to-creatine (PCr/Cr), PCr-to-ATP (PCr/ATP), and ATP-to-ADP (ATP/ADP) [85–87]. Tokarska-Schlattner et al. [85] demonstrated in a Langendorff rat-model that acute cardiac dysfunction caused by clinically relevant concentrations of doxorubicin may impair energy signaling via the energy sensor AMP-activated protein kinase (AMPK),which is consistent with a fast inhibition of fatty acid oxidation [88] and impaired mitochondrial function.

Creatine kinase (CK) acts as a modulator of the energy reservoir by converting creatine to phospho-creatine, using ATP as a substrate. This system is not notably affected in acute doxorubicin-induced cardiotoxicity, as the ATP/ADP ratio remains fairly constant [89]. However, CK is vulnerable to radical-mediated molecular damage, known to accumulate over time, inactivating CK isoenzymes to further impair the function of the sarcomeric mitochondrial CK-isoenzyme [90,91]. In a mouse-model of doxorubicin-induced cardiomyopathy, Mihm et al. [91] found significant inactivation of myofibril CK (MM-CK), which is a vulnerable target unique to doxorubicin-induced peroxynitrite generation. Oxidative damage to CK can also be enhanced by the presence of ferrous iron [90], which is another consequence of doxorubicin administration.

2.5. Endothelin-1

Endothelin-1 (ET-1) signaling increases cell survival signaling in cardiomyocytes, which explain its upregulation in heart failure [92]. ET-1 expression and the expression of its receptors are increased in the myocardium of rats with congestive heart failure [93], in patients with idiopathic dilated cardiomyopathy [94], and in patients treated with doxorubicin [95]. However, the benefit of activating pro-survival pathways is counteracted by the vasoconstrictive effects of endothelin-1 on the vasculature [96]. In the vasculature, the activity of ET-1 is balanced by the effects of NO [97], but in patients treated with doxorubicin, NO production is impaired.

Recently, Bien et al. [98] demonstrated in mice that pre-treatment with the combined ETA/Endothelin B (ETB) antagonist bosentan significantly reduced doxorubicin-induced cardiotoxicity with preservation of myocardial contractility. This beneficial effect was associated with reduced TNF-α and BAX expression, lipid peroxidation and increased expression of GATA-4 [98]. Similar results were observed with the ETA/ETB antagonist LU420627, but not the selective ETB antagonist LU135252 in animals over-expressing endothelin-1 in cardiomyocytes [99]. Molecular studies indicate that ETB agonists can increase NO production. These data support a substantial role of endothelin-1 signaling through the ETA receptor as a mediator of doxorubicin cardiotoxicity. However, no corresponding clinical data in patients with doxorubicin-induced heart failure are available at the moment to confirm these experimental data.

2.6. Extracellular matrix remodeling

Both cellular and extracellular factors do have an important role in the complex process of myocardial remodeling. Significant alterations in the structure and composition of the extracellular matrix contribute to the development of heart failure [100]. Doxorubicin inhibits

the transcription and translation of collagenase/matrix metalloproteinase 1 (MMP-1) in tumor cells, decreasing tumor cell mobility. It has since been shown that doxorubicin has the opposite effect on the heart, enhancing production of matrix metalloproteinases-2 and -9 (MMP-2, MMP-9) [101,102]. This is believed to contribute to cardiomyopathy by weakening the collagenous matrix against which the cardiomyocytes work and contributing to pathological remodeling. Both MMP-2 and MMP-9 activities are enhanced by doxorubicin-induced ROS generation, although Spallarosa et al. [102] show that MMP-2 levels depend on NADPH oxidase levels. Tissue-inhibitor of metalloproteinase-3 (TIMP, the family which includes MMP-2 and MMP-9) also decreased after doxorubicin administration [103], which is consistent with the apparent increase in MMP-2 and MMP-9 activity in earlier studies.

2.7. Other mechanisms

Other novel cytotoxic mechanisms have been explored and while they are not very well characterized, research suggests that they are also not trivial.

2.7.1. COX-2 inhibitors

Co-administration of diclofenac sodium, a cyclooxygenase-2 (COX-2) inhibitor, to rats receiving doxorubicin aggravates doxorubicin-induced myocardial apoptosis by accompanying increases in serum lactate dehydrogenase, increases in cardiac thiobarbituric acid reactive substances (lipid peroxidation indicator) and catalase expression, possibly to counteract the damage caused by oxidative stress [104]. Induction of COX-2 is believed to be cardioprotective through the induction of molecules such as prostacyclins [105]. The induction of COX-2 activity occurs concomitantly with doxorubicin administration in a rat model, and administration of PGI_2, a downstream product of COX-2 activity, could prevent injury to the myocardium. Administration of a COX-2 inhibitor prevents the enzyme from generating prostacyclins, removing a cardioprotective mechanism [105].

2.7.2. Neuregulin signaling

Neuregulin is a small paracrine peptide that signals through the ErbB family of tyrosine kinase receptors, activating cell survival pathways. The results of a very recent study by Horie et al. [106] demonstrated that acute doxorubicin cardiotoxicity is associated with an increase in miRNA-146a, specific for downregulation of the ErbB4 protein, and that doxorubicin-induced increases in miR-146a levels are dose-dependent. The downregulation of ErbB4 is accompanied by increased levels of apoptosis, as indicated by decreased Akt signaling and increased caspase-3 cleavage. Interestingly, the authors do not detect a change in ErbB2 mRNA or protein levels, after acute doxorubicin administration. This is in contrast to what is seen in a chronic rat model, where doxorubicin exposure induces upregulation of ErbB2 protein, not mRNA, most likely through the upregulation of HSP90, erbB2's chaperone [81]. ErbB4 levels are unchanged in this same model.

2.7.3. Ceramide accumulation

It is known that ceramide accumulation in the cell membrane triggers apoptosis via caspase-3 activation, although the exact mechanism with respect to how this occurs remains unclear. Ceramide has been implicated in two separate pathways relating to doxorubicin-induced cardiomyopathy, one involving mitochondrial L-carnitine. When L-carnitine was applied to isolated adult rat cardiomyocytes in the presence of clinically relevant doxorubicin concentrations, ceramide levels returned to nearly baseline levels while sphingomyelin, one of the precursors of ceramide, did not decrease [107]. This was correlated with L-carnitine inhibition of sphingomyelinase and prevented doxorubicin-induced apoptosis of cardiac myocytes [107]. The other pathway involves the volume-sensitive chloride ion channel [I(Cl, vol)]. Electrophysiological measurements on isolated cardiac myocytes show that doxorubicin administration (1 μM, clinically relevant) is accompanied by a current, that is characterized as I(Cl, vol) based on external chloride sensitivity, and occurs simultaneously with cell shrinkage and volume decrease, one of the hallmarks of apoptosis [108].

2.7.4. Cannabinoid signaling

In a rat model of doxorubicin cardiotoxicity, administration of cannabinoid-1 (CB1) receptor antagonists improved the degree of cardiac dysfunction [109]. The same group showed that administration of the agonist anandamide or HU210 activates apoptotic pathways and amplifies ROS and reactive nitrogen species production that is already induced by doxorubicin therefore compounding the damage [110]. It should be noted that one study purports to refute these data and suggests that anandamide preserves cardiac function in doxorubicin cardiotoxicity, measured by pressure measurements and fractional shortening [111].

3. Symptoms and diagnostic tools

Doxorubicin-induced cardiotoxicity may be divided into acute, subacute and late forms. Acute cardiotoxicity starts within 24 h of the infusion and includes ECG abnormalities such as atypical ST-T changes, reduced QRS voltages, sinus tachycardia, premature supraventricular and ventricular complexes, QT interval prolongation, and, rarely, acute myocardial ischemia. In spite of this, the prognosis is fairly good at this stage. These electrocardiographic changes are usually associated with few symptoms, but may also be completely asymptomatic, and usually resolve spontaneously within several hours or weeks after the completion of chemotherapy in most patients. The golden standard for detection of acute doxorubicin-induced cardiotoxicity is endomyocardial biopsy of the right ventricle because of its high sensitivity and specificity [112]. Endomyocardial tissue from the right ventricle will show typical histopathological changes, including vacuolization of the cytoplasm, while by electron microscopy, the common findings are loss of myofibrils and distention of the sarcoplasmic reticulum and T-tubules. The biopsy is scored and uses a scale where a 1 (<5% of cells show typical changes), 1.5 (5-15%), 2 (16-25%), 2.5 (26–35%) and 3 (>35%) grade scale [113]. A biopsy score of 2.5 or higher is a strong indicator that doxorubicin therapy should be terminated [113]. However, endomyocardial biopsy is rarely used, because the procedure is considered high risk, although it is not. Acute doxorubicin-induced cardiotoxicity occurs in up to 40% of the patient population. Tests for acute cardiomyopathy include monitoring ECG abnormalities, assessing adrenergic denervation and energy metabolism through radionuclide scanning, and using cardiac biomarkers such as cardiac troponin-T to assess cellular injury [114].

Sub-acute cardiotoxicity is rather rare, appears several weeks or months (as late as 30 months) after the last dose of anthracycline and its most frequent manifestation is myo/pericarditis [115]. Microscopically, severe interstitial myocardial edema without a cellular infiltrate is seen. The chronic form may not become evident until as many as 4 to 20 years after the last administration of doxorubicin, and is associated with progressive myocardial dysfunction. It has been demonstrated that the mortality rate in these patients is 50% after five years [114]. Sporadic, spontaneous reversal of severe LV dysfunction has been reported, usually after an acute-onset of the symptoms [116,117].

Early detection of myocardial damage might enable implementation of preventive measures that could reduce the likelihood of further development to ventricular decompensation, but as of this writing, the best preventative measure is to limit doxorubicin chemotherapy.

Serial echocardiographic monitoring is generally used for cardiotoxicity detection [12]. Evaluation of left ventricular systolic function using ejection fraction, or fractional shortening by echocardiography, as well as, nuclear ventriculography, will detect development of

cardiomyopathy. However, these are insensitive and still inaccurate markers of early doxorubicin injury [118,119], as the guidelines for terminating doxorubicin are based on a preset decrease in left ventricular ejection fraction [120]. Several other radionuclide-based tests are currently being developed; one of them, technetium99m-labeled annexin V seems the most promising. In early animal tests, the increase in Tc99 uptake correlates well with the degree of doxorubicin-induced cardiomyopathy [120] and the method is sensitive enough to detect dose-dependent doxorubicin-mediated cell death in a rat model, even before echocardiography detected systolic dysfunction [121]. However, no clinical validation of this latter diagnostic tool is yet available.

Furthermore, in a rodent model of doxorubicin-induced cardiomyopathy, it has been demonstrated that 2-dimensional radial strain echocardiography, a novel method of assessing myocardial function that is based on measuring myocardial deformation using speckle tracking from B mode images, can be useful in the early detection of doxorubicin cardiac injury. Strain is a measure of myocardial deformation, which is an intrinsic mechanical property. The reduction in radial strain is associated with the degree and the onset of histologic indices of myocardial injury. In this way, myocardial dysfunction can be detected using radial strain earlier than with standard assessment of ventricular function [122]. Low-dose dobutamine stress echocardiography, which detects myocardial contractile reserve, (5–10 μg/kg/min) can also be used in the clinic as a safe and sensitive indicator of diminished myocardial function [123,124]. Other indices of diastolic function such as isovolumic relaxation period, early peak mitral flow velocity and the ratio of early/atrial mitral peak flow velocity [125,126] were found to be useful in earlier detection of doxorubicin cardiomyopathy, but their practical applicability in the clinical setting is limited due to considerable individual variability.

Since doxorubicin disrupts cardiac myocyte cell membranes, biomarkers can be used to assay for the presence and extent of myocyte injury. Lipshultz et al. [127] demonstrated the utility of cardiac troponin T (cTnT) as a possible quantifier for acute doxorubicin-induced myocardial injury. Other potential markers include plasma levels of circulating natriuretic peptides, such as atrial-type and brain-type natriuretic peptides (ANP and BNP, respectively), which are elevated in left ventricular dysfunction and heart failure. Levels of these proteins were significantly elevated in a subgroup of patients treated with doxorubicin who had cardiac dysfunction, compared with healthy controls or patients with normal cardiac function [128,129]. Cardiac biomarkers can be good indicators of doxorubicin-induced myocardial injury and can provide useful diagnostic information, especially when used in combination with assessment of left ventricular function.

4. Therapeutic and preventive possibilities

The challenge for the future is to design protocols that are cardioprotective for both the short-term and long-term effects of doxorubicin, preferably without long-term administration and without hindering the antitumor activity of the drug. An overview of the possible therapeutic strategies to reduce doxorubicin-induced cardiotoxicity is shown in Fig. 3.

The most effective tool to prevent doxorubicin-induced cardiotoxicity is modulating the dosage. It has been reported in several studies, that a lower weekly dosage, or even continuous infusion, will permit adequate solid tumor suppression while limiting initial

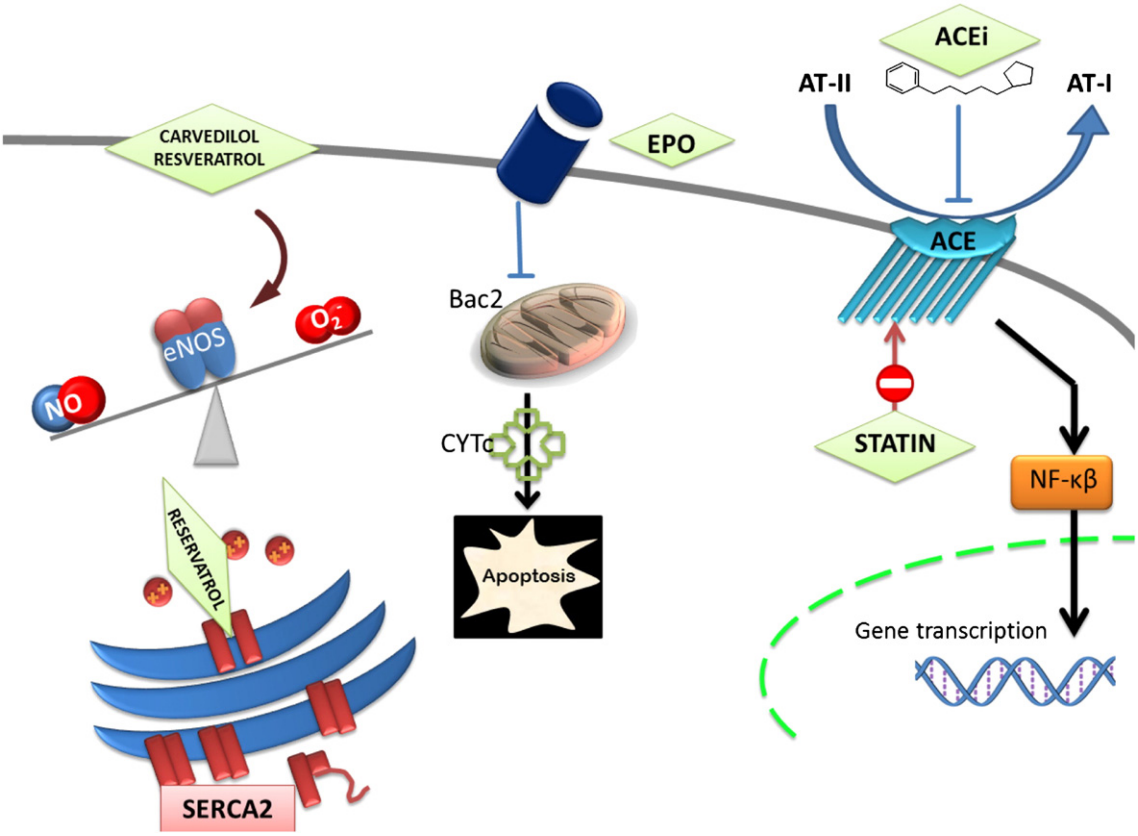

Fig. 3. Pharmacologic interventions. The known activities of the newer targets and the drugs that have been mentioned are depicted here. Receptor signaling through the β-adrenergic receptor and angiotensin II receptor is cardioprotective. Carvedilol enhances transcription of calcium-channel mRNA, while resveratrol inhibits the improper release of calcium. Resveratrol and carvedilol (and, presumably, other β-blockers as well) prevent dissociation of the eNOS dimer. Erythropoietin directly inhibits apoptosis. Statins appear to inhibit signaling through small G-proteins. EPO: erythropoietin, ACEi: angiotensin-converting enzyme inhibitors, AT: angiotensin, eNOS: endothelial nitric oxide synthase, NO: nitric oxide, O_2^-: superoxide, and CYTc: cytochrome C.

damage to the myocardium [130,131]. Maximal antineoplastic efficacy of doxorubicin depends not only upon the concentration, but also on the amount of time the drug is present in the body.

Dexrazoxane is the most studied cardioprotective adjuvant for doxorubicin chemotherapy. Although it was initially introduced as an antineoplastic agent in its own right, due to its ability to interfere with topoisomerase II activity, several studies have demonstrated possible cardioprotective properties, partially due to the hydrolyzed metabolite that chelates free iron [132,133]. In addition, it has also been shown to prevent depletion of mitochondrial DNA in a chronic rat-model of doxorubicin-induced cardiotoxicity [134].

The clinical trials examining the role of dexrazoxane as a cardioprotectant by Lipshultz et al. [127] demonstrated in doxorubicin-treated children with acute lymphoblastic leukemia that dexrazoxane therapy reduced myocardial injury, as indicated by decreased serum troponin T. Moreover, in a relatively large population of children with acute lymphoblastic leukemia ($n = 205$), dexrazoxane was not associated with an increased risk of a second malignant event [135], as suggested earlier [136] and is therefore recommended to be administered with doxorubicin-containing pediatric regimens [135]. The clinical practice guidelines of 2008 suggests using a 10:1 ratio of dexrazoxane-to-anthracycline, administered 15–30 min prior to doxorubicin administration, but it should be mentioned that the optimal dose of dexrazoxane has not been empirically established [137]. The American Society of Clinical Oncology does not endorse the routine use of dexrazoxane with anthracycline chemotherapy, although they do note exceptions for situations where the cumulative dose of anthracyclines approaches or exceeds 300 mg/m^2 [137].

Another cardioprotective agent is monoHER, the main constituents of flavonoids Venoruton (O-(β-hydroxyethyl)-rutosides) [138]. Pretreatment with monoHER protects against doxorubicin-induced cardiotoxicity in both an in vivo and ex vivo mouse model of chronic doxorubicin-induced cardiotoxicity [139,140]. In vitro and in vivo experiments have shown that monoHER did not interfere with the antitumor effect of doxorubicin [141]. High doses of monoHER (>1500 mg/m^2) are indicated for potentiating the effect of antitumor and low doses for cardioprotection effect [142]. Further clinical investigations are needed to assert monoHER's dose–response characteristics.

Beta-adrenergic antagonists and angiotensin-converting enzyme (ACE) inhibitors are the keystrokes of standard heart failure therapy. Kalay et al. [143] demonstrated that left ventricle diameters remained constant and diastolic function was better preserved after doxorubicin-treatment in patients receiving carvedilol, compared with placebo. Georgakopoulos et al. [144], demonstrated that metoprolol, a β-blocker without antioxidative properties, failed to give cardioprotection in lymphoma-treated doxorubicin patients. Early additions of β-blockers along with angiotensin-converting enzyme inhibitors have also been demonstrated to improve myocardial contractility in doxorubicin-induced cardiotoxicity, but the exact mechanism is still poorly understood [145]. In patients with congestive heart failure after epirubicin treatment, the administration of ACE inhibitors (enalapril or ramipril) restored systolic function after the relief from digoxin or administration of a diuretic is stopped [146]. This benefit was independently observed with both zofenepril and captopril [147–149]. Furthermore, the degree of improvement is the same whether a receptor antagonist or an ACE-inhibitor is used [150]. These indicate that some aspect of angiotensin signaling could be involved in alleviating doxorubicin-induced cardiomyopathy.

Erythropoietin (EPO) is commonly used for treating anemia in patients who have undergone chemotherapy by restoring red blood cell production. EPO has the potential to act as a cardioprotective agent against doxorubicin-induced apoptosis and cardiomyopathy, especially when administered prophylactically [151]. Clinical trials with epoetin alfa in patients with advanced-stage Hodgkin's lymphoma showed an improvement in doxorubicin-induced anemia and no difference between placebo and treatment groups in cancer treatment outcome, which means the combination does not affect the cell-killing ability from doxorubicin [152].

Preventive administration of sildenafil, a phosphodiesterase 5 inhibitor, can attenuate cardiomyocyte apoptosis, preserve the mitochondrial membrane potential, maintain myofibrillar integrity, prevent ST-interval prolongation, and left ventricular dysfunction in a mouse model of doxorubicin-induced chronic cardiotoxicity [153]. These effects are NOS-dependent and establish the significant role of mitochondrial K_{ATP} channel opening in sildenafil induced cardioprotection. The authors hypothesize that pretreatment with sildenafil helps to maintain mitochondrial integrity by augmenting cellular mechanisms mediated by NO/cyclic GMP [153]. Other studies demonstrated the combination between sildenafil and doxorubicin increased cell-killing effect of doxorubicin in cancer cells [154] and improved cardiac function [155]. These effects could be due to the combination that sildenafil and doxorubicin produce greater amounts of ROS in cancer cells. On the other hand, this combination produced less ROS in normal cells [156]. Future clinical studies addressing these interesting mechanisms are required and worth pursuing.

Tatlidede et al. [157] showed that resveratrol, a broad antioxidant, pretreatment for two weeks in acute doxorubicin treatment significantly decreased ROS generation, improved glutathione, SOD and catalase activity, which subsequently generate better cardiac function. It stands to reason that pretreating patients with resveratrol can be cardioprotective for doxorubicin-induced cardiotoxicity, but so far, no large clinical trials have been done.

The beneficial effect of cardiac resynchronization therapy (CRT) on doxorubicin-induced heart failure in patients who are non-responders to pharmacologic therapy, and meet criteria for resynchronization device implantation has been observed in a pilot-trial [158]. This study demonstrated improved left ventricular ejection fraction, reduced end-diastolic dimensions, reduced heart failure symptoms, and improved function [158]. While the number of patients was very limited, all were highly responsive to CRT in this study (9 to 24 months). The long-term sustainability of the observed beneficial response to CRT remains unknown at the moment.

Lastly, Ward et al. [159] conclude that transplantation is an acceptable treatment option for pediatric patients with intractable cardiac failure secondary to anthracycline therapy. They demonstrated that survival is comparable with ISHLT Registry data for all pediatric heart recipients and that tumor recurrence after transplantation is rare, even with immunosuppression [159]. Current guidelines regarding the duration of a cancer-free interval before listing should be re-examined on an individual basis, with input from the oncologist and consideration of type of tumor, stage, grade, and response to initial therapy [159].

5. Conclusion

Doxorubicin-induced cardiomyopathy is an important public health concern because it may not be detected for many years and remains a life-long threat. This is of particular importance in children who may survive for decades after successful antineoplastic treatment. There is strong evidence that the surveillance of patients treated with anthracyclines should be prolonged to more than 10 years to define the real impact of anthracyclines on the myocardium, especially when these patients have received a co-administration of other agents that may induce cardiac damage such as trastuzumab, paclitaxel, etoposide, teniposide, the vinca alkaloids, fluorouracil, cytarabine, amsacrine, cladribine, asparaginase, tretinoin and pentostatin. The search for cardioprotective agents will continue to rely on increasing our understanding of the mechanisms of doxorubicin-induced cardiotoxicity and how to counteract them.

The take home messages of this review are that there are some strategies that will reduce this cardiovascular side effect. First, lower doses of doxorubicin, continuous infusion, or different formulations

may be less cardiotoxic [160]. Second, co-administration of doxorubicin and other cardiotoxic chemotherapeutic agents, such as trastuzumab will aggravate the cardiotoxic effects [161]. Third, in clinical situation, dexrazoxane can be considered to reduce the cardiotoxicity in patients who have received a cumulative dose of doxorubicin $>300\ mg/m^2$ [137]. In addition, according to European Society of Cardiology, the cardiovascular status of cancer patients must be adequately monitored and when they develop heart failure, the treatments for doxorubicin-induced cardiotoxicity will follow the standard guideline for heart failure [162]. Currently, no clear guidelines are available for treating doxorubicin-induced cardiotoxicity in adults, emphasizing the need to detect doxorubicin-induced cardiotoxicity as early as possible to limit/prevent subsequent damage.

Despite the predominant role of ROS in doxorubicin-induced cardiotoxicity, many of the potential protectants do not appear to affect the production of free radicals at all, but instead seem to alter the cellular response to ROS. Increasingly, more researchers are moving away from efforts to minimize or eliminate ROS, and targeting the cellular mechanisms that cause apoptosis instead. This shift in approaching the problem may lead to interesting applications of older drugs, as well as a more systems biology-oriented approaches to develop solutions against doxorubicin-induced cardiotoxicity.

Disclosure statement

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