Doxorubicin-Induced Cardiotoxicity: From Mechanisms to Development of Efficient Therapy

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Abstract

First isolated in the early 1960s, doxorubicin (DOX) is among the most effective anticancer agents ever developed. DOX has been used mainly for the treatment of breast cancer, solid tumors in children, soft tissue sarcomas, and aggressive lymphomas. However, the use of DOX may have dose-dependent cardiotoxic effects that generate changes in myocardial structure, which can develop into severe and irreversible cardiomyopathy. Here, we describe the incidence of DOX-induced cardiotoxicity (DIC); the progress made over the past four decades in understanding the molecular mechanisms of the pathogenesis of acute and chronic DIC; the current strategies for heart protection; and the major breakthroughs and challenges in basic and clinical research to the development of efficient targeted therapy for DIC.

Keywords: doxorubicin, chemotherapy, cardiotoxicity, mechanisms, pathogenesis, heart protection, targeted therapy

1. Introduction

Cardiomyopathy induced by doxorubicin (DOX) is considered an extremely serious adverse effect of oncologic treatment. It is known that this disease significantly affects the quality of patients' life who survived cancer, especially children. Since its discovery, several molecular mechanisms have been proposed to understand the pathogenesis of acute and chronic DOX-induced cardiotoxicity (DIC), including oxidative stress, iron metabolism, Ca^{2+} homeostasis dysregulation, sarcomeric structure alterations, gene expression modulation, and apoptosis. Based on these mechanisms, different strategies have been developed in order to protect the

heart during cancer treatment, including the administration of iron-chelating antioxidants and adrenergic receptor agonists. However, the use of these drugs is limited due to their adverse side effects as well as the loss of beneficial cardiac effects years after the end of the treatment. Therefore, the development of new therapies has been a great challenge for the scientific community. In this context, a new emergent strategy is cell therapy. Considering that DOX causes cardiomyocyte death, the transplant of autologous cardiomyocytes obtained through the differentiation of human induced pluripotent stem cells (iPSC) is a viable option for cardiac repair and a promising therapeutic strategy for the treatment of cardiovascular diseases, including DIC.

2. Doxorubicin

DOX (also known as adriamycin) was isolated in the early 1960s from the pigment-producing bacterium *Streptomyces peucetius var. caesius*, along with daunorubicin (DAU, also known as daunomycin and rubidomycin), and belongs to the family of anthracyclines [1–3]. Until to now, DOX remains among the most largely prescribed and effective antineoplastic agents ever developed for the treatment of a variety of adult and pediatric cancers [3–5]. Whereas DAU has been used against acute lymphoblastic and myeloblastic leukemias, DOX has been used against breast cancer, soft tissue sarcomas, childhood tumors (e.g., Wilms' tumor), leukemias, Hodgkin's and non-Hodgkin's lymphoma, and many other cancers [4, 5].

The minor differences in the chemical structure between DOX and DAU are responsible for the different spectrums of activity of these drugs. The side chain of DOX terminates with primary alcohol, while that of DAU terminates with a methyl group [3, 6]. Unfortunately, in addition to its potent antitumor effect, the use of DOX has been hampered by conventional toxicities (hematopoietic suppression, nausea, vomiting, extravasation, and alopecia), development of resistant tumor cells or toxicity in healthy tissues, especially with serious cardiac toxicity manifested by congestive cardiomyopathy [4, 7]. Over time, more than 2000 analogs were developed in an attempt to reduce the adverse effects of DOX and DAU. However, few analogs have reached the stage of clinical development and approval, such as epirubicin (EPI) and idarubicin (IDA), with DOX- and DAU-like spectrums, respectively [6]. Despite the development of new components, replacing DOX does not eliminate the risk of developing cardiotoxicity [6, 7]. Thus, DOX continues to be considered as a first-line antineoplastic drug [7].

2.1. DOX-induced cardiotoxicity: clinical aspects

Since the late 1970s, DOX-induced cardiotoxicity (DIC) has been recognized as a complication of chemotherapy [5]. The first case report in the literature was that of a 23-year-old patient with osteosarcoma, who was treated for 9 months with DOX. One month after the end of treatment, the patient died due to development of congestive heart failure [8]. A second report describes the case of an 11-year-old patient, also with osteosarcoma, who died 9.5 years after the end of chemotherapy with DOX as a result of progressive heart failure with late severity

[9]. In 1991, long-term cardiotoxic effects were identified in patients with acute lymphoid leukemia in childhood [10]. Patients with childhood cancer and those treated with DOX have a high risk of developing symptomatic cardiac events at an early stage, and this risk remains high within 30 years after treatment. In addition, it is estimated that one in eight DOX-treated patients will be afflicted with severe cardiac disease [11].

DIC manifests in several forms, ranging from asymptomatic electrocardiography (ECG)-changes to decompensated cardiomyopathy characterized by decreased left ventricular ejection fraction [4, 7]. According to their clinical manifestation, these cardiotoxic events can be classified into three types: (1) acute, occurring during or immediately after treatment; (2) early-onset chronic progressive cardiotoxicity, occurring within 1 year after exposure to chemotherapeutic treatment; and (3) late-onset chronic progressive cardiotoxicity, occurring 1 or more years after the end of treatment [11].

Acute cardiotoxicity is characterized by depression of myocardial contractility that may be reversible within 1 week when discontinuing the DOX treatment [12, 13]. In some patients, complications have already been described, such as hypotension; pericarditis; myocarditis; supraventricular, ventricular, or sinus (more common) tachycardia; ST-T wave changes; decrease in QRS complex; prolongation of QT interval; and increase in serum levels of brain natriuretic peptide and cardiac troponin [3, 12–14]. However, this type of cardiotoxicity is very rare and affects less than 1% of patients [12].

Early-onset chronic progressive cardiotoxicity is characterized by systolic or diastolic ventricular dysfunction within 1 year after the completion of DOX treatment. It can be progressive and occurs in 5–35% of the cases [11, 14, 15]. In the majority of adult patients, early cardiotoxicity is related to the development of a chronic dilated cardiomyopathy, with a decrease in the mass and wall of left ventricle. In the pediatric patient, in addition to chronic dilated cardiomyopathy, restrictive cardiomyopathy characterized by increase in the wall stiffness of the left ventricle cavity may also occur in isolated moments [11–13]. The typical manifestation of these cardiomyopathies is the progressive reduction of the ejection fraction [13]. Other events, including severe electrical conduction changes, damage to cardiac valves, and/or depression of contractility may also be observed [15].

Finally, late-onset chronic progressive cardiotoxicity is characterized by cardiac dysfunction after a latency period of 1 or more years following the completion of DOX treatment [12, 13]. In this type of cardiotoxicity, there is a period during which the patient is asymptomatic (normal cardiac function). After that, chronic dilated and/or restrictive cardiomyopathy can be manifested with subsequent development of congestive heart failure. In this case, mortality rate is more than 50% [3, 12, 13, 15].

2.2. Mechanisms of cardiotoxicity

Despite almost 60 years of research, the mechanisms to explain DIC are not completely understood. It seems to be a multistep process, with different potential pathways involved that leads to cardiomyocyte death [11, 16, 17]. Until now, the main mechanisms that have been proposed by various research groups include oxidative stress, iron metabolism, Ca^{2+}

homeostasis dysregulation, sarcomeric structure alterations, gene expression modulation, and apoptosis [4, 13, 16, 17].

2.2.1. Oxidative stress

Since the discovery of DOX, oxidative stress is the most frequently proposed mechanism to explain the complex pathophysiology of DIC [3, 5, 16]. The myocardium injury evidenced by lipid peroxidation occurs as a result of the increase of the reactive oxygen species (ROS) production, including superoxide (O_2^-) and hydroxyl radicals (OH^-) as well as other non-radicals such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2), etc. [3, 4, 11, 17, 18]. Unlike other tissues, the heart is extremely prone to oxidative damage, at least in part, due to lower levels of antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase. In addition, the chemical structure of DOX contains quinone groups that can be reduced to a semiquinone, an unstable metabolite which can react with molecular oxygen (an electron acceptor) and rapidly revert to the parent compound. This redox cycle leads to the formation of superoxide anion radicals within mitochondria, causing cardiotoxicity [11, 16–20].

The mitochondria have been identified as the main subcellular organelles injured in the heart by DIC [4, 17]. DOX is a cationic drug that binds with high affinity to cardiolipin (a phospholipid) forming nearly irreversible complex in the mitochondrial inner membrane [17, 21]. It is important to know that cardiolipin is required for the proper functioning of the electron-transport chain proteins. In this context, evidence suggests that DOX disrupts the cardiolipin-protein interface, causing more superoxide anion radicals formation [4, 22]. As a result, ROS can induce different forms of cardiomyocyte death (apoptosis or necrosis) [17]. Furthermore, the reduction of mitochondrial function causes energetic metabolism change evidenced by a decrease of the adenosine triphosphate (ATP) production, which may contribute to abnormal contraction and relaxation in the failing heart [4, 11, 23].

Other forms of DOX-induced ROS generation in the myocardium include nitric oxide synthases (NOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases pathways. These enzymes interact with DOX and induce oxidative stress [4, 16, 17].

NOS are a group of enzymes responsible for the nitric oxide (NO) production from L-arginine and oxygen [24]. The NO generation is altered by the direct binding of DOX to endothelial NOS (eNOS) reductase domain, leading to the reduction of the DOX semiquinone radical, which reacts with oxygen and produces superoxide. There is evidence to suggest that, in low DOX concentrations, eNOS signaling is the main pathway for DOX reduction. In addition, the increase of DOX-eNOS interaction completely modifies normal functioning of the enzyme (NO production) and transforms it into a potent superoxide generator [25]. DOX also affects NOS signaling by increasing eNOS transcription and protein activity in bovine aortic endothelial cells (BAEC). In this study, BAEC were pretreated with eNOS antisense oligonucleotides or antioxidants and the results showed apoptosis decrease [26]. Recently, an in vivo study has shown that the pretreatment with folic acid (FA, a modulator of eNOS) prevented DOX-induced increases in superoxide anion and attenuated DOX-induced decreases in superoxide dismutase, eNOS phosphorylation, and NO production [27]. Another study

showed a decrease in ROS generation, preservation of cardiac function, and reduction of mortality rate after acute and chronic DOX administration in the eNOS knock-out (eNOS$^{-/-}$) mice model, whereas cardiomyocyte-specific eNOS overexpression intensified the pathological response to DOX in the heart [28]. In all, these studies demonstrate the importance of eNOS signaling in DIC.

Recent evidence suggests that the other isoform called inducible NOS (iNOS) is also involved with DOX-induced oxidative stress. In some studies, iNOS transcription and expression are increased in mouse and rat hearts and isolated cardiomyocyte after DOX treatment [29–31]. In iNOS knock-out (iNOS$^{-/-}$) mice model, cell death and nitrotyrosine (NT) formation induced by DOX were mitigated. The same results were observed when selective iNOS inhibitors such as S,S'-[1,3-phenylene-bis(1,2-ethanediyl)]bis-isothiourea (1,3-PB-ITU) and L-N6-(1-iminorthyl)-lysine (L-NIL) were administered. In this study, DIC occurs due to the generation of peroxynitrite, a potent oxidant which generates secondary free radicals, including nitrogen dioxide and carbonate radical [30]. It is possible that the reduction of the peroxynitrite production using specific antioxidant(s) is a viable strategy for the decrease of DIC. In support of this view, the significant increase of superoxide radical and peroxynitrite induced by DOX observed in isolated cardiomyocytes was blunted after treatment with vitamin C (Vit C). These results suggest that Vit C provides cardioprotection by reduction of oxidative/nitrosative stress [31]. Altogether, these works thus also highlight the importance of iNOS signaling in DIC.

The activity of the third isoform, neuronal NOS (nNOS), in DOX-induced oxidative stress is poorly understood. It appears that the flavin domain is involved with DOX reduction [32]. Nevertheless, no changes were observed in nNOS transcription and protein activity after the treatment of DOX [30]. Therefore, further studies will be needed to elucidate the role of NOS isoforms as well as the therapeutic potential of their pharmacological targeting in DOX-dependent heart disease.

In relation to NADPH oxidases, also known as NOXs, recent work has identified these enzymes as important sources of myocardial ROS [33]. NADPH oxidase is a multicomponent complex that consists of membrane-bound cytochrome b-558, which is a heterodimer of gp91phox and p22phox, cytosolic regulatory subunits p47phox and p67phox, and the small GTP-binding protein Rac1 [34]. These enzymes mediate the transfer of one electron from NADPH to quinone DOX, leading to DOX semiquinone radical. As result, they can produce superoxide similar to NOSs. The semiquinone radical also reacts with hydrogen peroxide generating hydroxyl radicals [35]. An in vitro study using NADPH oxidase inhibitors (diphenyliodonium and apocynin) on H9c2 cells showed that DOX-induced apoptosis was mitigated, demonstrating NADPH oxidase is also involved in the development of cardiac toxicity induced by DOX [36]. Furthermore, there is accumulating evidence to support an important role for Nox2 NADPH oxidase (one of the seven different NADPH oxidase isoforms) in DIC, identified using Nox2-deficient (Nox2$^{-/-}$) or gp91phox knock-out (gp91$^{-/-}$) mice [33, 37–39]. DOX-induced cardiomyocyte apoptosis and atrophy, interstitial fibrosis, leukocyte infiltration, and cardiac dysfunction in wild-type (WT) mice were attenuated in Nox2$^{-/-}$ mice [33, 39]. DOX-induced superoxide production was also mitigated in this animal model [39].

Recently, Rac1 has been reported to be a key regulator of oxidative stress due to its ability to bind and activate the NADPH oxidases [34]. In this context, a study showed that the deletion of Rac1 (a subunit of the NADPH oxidases complex) in cardiomyocytes impairs DOX-induced NADPH oxidases activation, ROS generation, DNA fragmentation and apoptosis, and improves cardiac function [40]. The same results were observed when NSC23766, a RAC inhibitor, was administered. In contrast, the overexpression of Rac1 exacerbated DIC [41]. Therefore, Rac is extremely important for the regulation of DIC by NADPH oxidase/ROS-dependent pathway.

Patient's genetic susceptibility is another factor that has been considered extremely important for the understanding of NADPH oxidases-dependent cardiotoxicity. Single-nucleotide polymorphisms (SNPs) in one of the subunits of the NADPH oxidases complex have been identified in non-Hodgkin lymphoma patients. After the treatment with DOX, these patients developed acute arrhythmias and congestive heart failure. For example, the presence of SNP variants in NADPH oxidase subunit NCF4 and in the p22phox and Rac2 subunits were linked with the development of chronic and acute DIC, respectively [36]. Thus, detection of the genetic polymorphisms in NADPH oxidases complex may help to identify patients who have higher risk to develop DIC.

2.2.2. Iron metabolism

It is reported that DOX is able to alter iron metabolism due to its strong affinity for this metal, thereby forming iron-DOX complexes which, in turn, react with oxygen and trigger ROS production [42]. Thus, the researchers believed that only oxidative stress was responsible for the cardiotoxicity induced by iron-DOX complexes. However, in physiological conditions, there would not be enough free iron to interact with DOX to the extent necessary to cause cardiomyopathy [6]. On the other hand, another theory suggests that the effect of DOX on iron metabolism occurs due to the interference of this drug in the activity of proteins that transport and bind intracellular iron. For example, one of the mechanisms involves the doxorubicinol (DOXol), a metabolite of DOX, which removes iron from the catalytic Fe-S cluster of the cytoplasmic aconitase (also called iron regulatory protein 1; IRP-1), converting this enzyme to a null protein. Consequently, there is an increase in the stability of transferrin mRNA and preventing translation of iron sequestration proteins. As a result, reduction of IRP-1 causes an increase in free iron, which can lead to free radical production [43, 44]. Furthermore, a recent work reports that DOX can also interact with iron-responsive elements (IREs) of the ferritin heavy and light chains. It is known that ferritin operates as an iron transporter, reducing free iron within the cell. Accordingly, disruption of this protein eventually results in increased free iron, which in turn causes myocardium injury [45]. Another work showed iron-overload, mitochondrial damage, and mortality after DOX treatment in mice depleted of the iron regulatory gene HFE (also known as human hemochromatosis protein). The HFE protein is responsible for the regulation of circulating iron uptake [46]. Therefore, free iron accumulation within the myocardium after DOX treatment seems to be the major determinant of DIC [20].

It is important to recognize that patients undergoing chemotherapy are submitted blood transfusions and iron supplementation due to abnormal losses and nutritional status deficient,

respectively. The fact is that these procedures modify body iron stores. In addition, adult and pediatric patients with leukemia can develop a significant level of iron-overload during, and as result of, chemotherapy [46]. Thus, it is possible that the reduction of iron levels is an effective strategy to prevent DOX-induced cardiomyopathy.

2.2.3. Calcium homeostasis dysregulation

The precise control of calcium levels during the contraction-relation cycle in cardiomyocytes is extremely important for normal beat-to-beat contractile activity [47]. Unfortunately, many studies suggest that calcium homeostasis dysregulation has a major role in the pathogenesis of DIC. To date, severe mechanisms have been proposed that are responsible for an increase in calcium intracellular concentrations [4, 16]. One of the mechanisms is related to DOX metabolism, which generates a toxic metabolite, DOXol, through a reduction of its carbonyl group, capable of inhibiting the sodium-calcium exchanger channel [48]. The sodium/potassium pump of the sarcolemma is also affected by DOXol, which disrupts the sodium gradient needed for calcium to flow into the sarcolemma of a cardiomyocyte [49]. Consequently, there is an imbalance in the energetics of the myocardium and diminished systolic function [48]. Furthermore, it is reported that this secondary metabolite is more difficult to eliminate from the cardiomyocyte than the parent drug [50]. Thus, DOXol accumulation contributes significantly to the dysregulation of calcium homeostasis, leading to myocardial damage.

Moreover, normal calcium homeostasis is altered by ROS and hydrogen peroxide via disruption of normal sarcoplasmic reticulum function. This is accomplished by inhibiting the Ca^{2+}-ATPase pumps, caused by reducing the expression of SERCA2a mRNA levels and/or the direct activation of the ryanodine calcium-release channels themselves [51, 52]. In addition, a study suggests that DOX induces calcium release from the sarcoplasmic reticulum due to increasing the frequency of opening of these channels [52]. At the same time, DOX induced the inhibition of sodium-calcium channels in the plasma membrane as well as increased L-type calcium channel activation [53, 54]. DOX has also been shown to decrease the calcium storage capacity of mitochondria by specifically activating the selective CsA-sensitive calcium channel, exacerbating the calcium-overload [49]. As result, an increase of calcium cytoplasmic concentrations occurs, leading to mitochondrial dysfunction and apoptosis [55]. Therefore, the preservation of calcium homeostasis is essential to prevent DOX-induced cardiomyopathy.

2.2.4. Sarcomeric structure alterations

DIC is also accompanied by disarray and loss of myofilaments of the sarcomere. Titin is a giant protein and a key component of the cardiac sarcomeres, extending from the M-line to the Z-disk. This protein has multiple functions, from structural to regulatory [56]. Recent studies have shown that the loss of integrity or function of titin is directly related to the development of dilated cardiomyopathy [57, 58]. It is known that DOX induces rapid degradation of titin through the activation of proteolytic pathways, leading to an imbalance in the energetics of the myocardium. Furthermore, studies have shown that the degradation of titin also occurs by the activation of calpains (calcium-dependent proteases) and reported that the inhibition of this protein is responsible for preserving cardiac function after DOX treatment [59].

Another study showed that the depletion of the cardiac ankyrin repeat protein (CARP), which are important in negative regulation of cardiac genes expression, leads to marked sarcomeric disarray [60]. Taken together, these studies thus also highlight the importance of sarcomeric structure stability to prevent DIC. It is necessary to recognize that other proteins are essential for sarcomeric cytoskeleton such as α-actinin, myomesin, and nebulin, and further studies should be performed to verify the DOX effect on these proteins.

2.2.5. Gene expression modulation

Some studies suggest that DOX down-regulates cardiac muscle-specific proteins such as contractile proteins, mitochondrial proteins, sarcoplasmic reticulum proteins, and others. Suppression of the cardiac muscle gene is associated with abnormal contraction and relaxation observed after DOX treatment [3, 11]. Another study showed that DOX induces depletion of GATA-4, leading commitment of the regulation of sarcomeric proteins expression such as myosin heavy chain and troponin I [61, 62]. In addition, suppression of GATA-4 induced by DOX is also related to the induction of apoptosis, suggesting the essential role of GATA-4 in cell survival [63, 64]. Regarding mitochondrial proteins, there is evidence that the suppression of these proteins after DOX treatment results in disruption of myocardial energy production, thereby causing cardiac dysfunction [3].

On the other hand, DOX induces upregulation of endothelin-1 (ET-1) and its receptors' expression [65, 66]. An in vivo study has shown that DOX-induced cardiotoxicity was reduced when mice were pretreated with the combined endothelin A/B antagonist (bosentan). In addition, the authors suggest that the reduction of TNF-α and BAX expression, lipid peroxidation, and increased expression of GATA-4 are responsible for cardioprotective effects observed in this study [67]. However, it is unclear if combined blocking of endothelin A/B receptors is necessary or whether selective inhibition of one of the ET-1 receptors is sufficient for the observed cardioprotection. In this context, a recent study evaluated the effects of dual (bosentan) and single endothelin receptor antagonism through sitaxentan (receptor A blocker) or BQ788 (receptor B blocker). The results demonstrated more beneficial effects of cardiac function when both receptors were blocked [66]. Taken together, these data support a substantial role of endothelin-1 signaling as a mediator of DIC.

2.2.6. Apoptosis

DOX can induce apoptosis through different mechanisms, which have been extensively studied in both acute and chronic cardiotoxicity. As mentioned in this chapter, one pathway involves ROS production and oxidative mechanisms and it is accepted that both the extrinsic and intrinsic apoptotic pathways are involved [17]. Increased oxidative stress has been shown to promote apoptosis and antioxidants have been shown to inhibit this process [7]. Oxidative stress also is known to activate apoptosis-signal regulating kinase-1 (ASK1), which activates the c-Jun NH2-terminal kinase (JNK) and p38 MAPK pathways to induce apoptosis [68]. In addition, it is reported that transcription factor NF-κB activated by ROS in DOX-treated neonatal rat cardiomyocytes and myocardium exerts a proapoptotic effect via direct activation of apoptotic genes, including FasL, Fas, c-Myc, and p53 [69–71]. The

activation of p53 by superoxide and hydrogen peroxide activates Bax genes, causing apoptosis [72, 73]. At the same time, evidence indicates that there is also an increase in the production of proapoptotic proteins as a result of p53 stabilization through increased heat shock protein (Hsp)25 production due to the activation of heat shock factor 1 (HSF-1), which is induced by DOX-dependent oxidative stress. In contrast, several studies suggest that Hsp proteins, such as Hsp27, Hsp10, Hsp20, and Hsp60, are involved in the prevention of DOX-induced apoptosis and myocardial dysfunction [74, 75]. Overexpression of Hsp27 plays a beneficial role in the regulation of oxidative stress responses and maintenance of mitochondrial function [74]. Regarding Hsp10 and Hsp60, overexpression of these proteins is associated with an increase in the post-translational modification of Bcl-2 proteins, which are important for the activation of anti-apoptotic pathways [75]. In addition, it is reported that overexpression of Hsp20 inhibits DOX-triggered cardiac injury, and these beneficial effects appear to be dependent on protein kinase B (also known as Akt) activation [76]. Therefore, more studies should be performed to understand the anti- and/or proapoptotic signaling pathways activated by Hsp proteins and their relationship to DOX.

Recently, an in vitro study using cardiomyocytes derived from human induced pluripotent stem cells (CM-iPSC) showed that DOX significantly upregulated the expression of death receptors (DRs) (TNFR1, Fas, DR4, and DR5) at both protein and mRNA levels. This study also showed that spontaneous apoptosis is exacerbated by death ligands including TNF-related apoptosis inducing ligand (TRAIL) [77]. Another study reported that Toll-like receptor-2 (TLR-2) functions as a novel "death receptor" that employs the apoptotic apparatus such as FADD and caspase 8 without a conventional cytoplasmic death domain. In this study, the authors observed reduction of apoptosis in myocardium after DOX treatment in TLR-2-knock-out mice (TLR-2$^{-/-}$) when compared to wild-type mice [78]. These results demonstrate that the induction of death receptors in cardiomyocytes is probably another mechanism by which DOX causes cardiotoxicity.

DOX is also appearing to influence caspase activity. Using both rat primary cultured cardiomyocytes and rat hearts from an animal model, the study demonstrated that DOX treatment induces apoptosis through the activation of caspase-3 activity [79]. In addition, another study showed that caspase-3 can be activated after Akt and Bad phosphorylation caused by DOX-induced upregulation of Ser/Thr PP1 phosphatase [76]. In support of this view, in TLR-2-knock-out mice, DOX-induced caspase-3 activity was decreased and this effect is a result of inhibition of NF-κB activation and reduction of proinflammatory cytokine [78, 80]. In all, these data demonstrated the need to understand the molecular signaling pathways that mediate DOX-induced cardiomyocyte apoptosis. This knowledge is extremely important for the advancement and development of new approaches for the treatment and/or prevention of DIC.

2.2.7. Other emerging mechanisms: role of microRNAs

Several studies indicate DIC is associated with modulation of microRNAs due to their role in all cardiac functions, including conductance of electrical signals, heart muscle contraction, and growth [81]. It is reported that a group of microRNAs, such as miR-34a, miR-34c,

miR-208b, miR-215, miR-216b, and miR-367 are upregulated in the rat heart when increasing doses of DOX are administered. In this same condition, there is evidence that miR-21, miR-34a, miR-208a, miR-208b, miR-221, miR-222, and miR-320a are upregulated in mice myocardium [81, 82]. On the other hand, other microRNAs including Let-7 g, miR-30a, miR-30c, and miR-30e are downregulated in rat myocardium after DOX treatment, confirming the role of DOX in the modulation of microRNAs [81].

Recently, the effects of DOX on the expression of miR-21 were examined in rat H9C9 cardiomyocytes. This study showed that overexpression of miR-21 attenuated DOX-induced apoptosis, whereas knocking down its expression increased DOX-induced apoptosis. In addition, the authors suggest that miR-21 protects cardiomyocytes by modulating the anti-proliferative factor, B cell translocation gene 2 (BTG2) [83]. Furthermore, the effects of DOX on expression of miR-208a were investigated in Balb/C mice hearts. In this study, DOX significantly upregulated miR-208a, downregulated GATA4, and increased myocyte apoptosis. In contrast, therapeutic silencing of miR-208a recovered GATA4 and BCL-2 and decreased apoptosis [84]. DOX also induced overexpression of miR-146a, which are responsible for downregulating ErB2 receptor tyrosine kinase 4 (ErB4), a key component of neuregulin-1-ErbB signaling, resulting in apoptosis in cardiomyocytes [85].

In turn, a recent study demonstrated that the miR-30 family, which is downregulated by DOX, is involved in the modulating of β-adrenergic and mitochondrial apoptotic pathways. In this study, the authors identify GATA-6 as a mediator of DOX-associated reductions in miR-30 expression. Moreover, they showed that overexpression of miR-30 protects cardiomyocytes from DOX-induced apoptosis [86]. Therefore, these data highlight the importance of modulating microRNA expression as well as providing a novel therapeutic approach to DIC prevention.

2.3. Strategies for heart protection

Since several mechanisms are involved in the development of cardiac toxicity, different strategies are being performed to prevent DOX-induced cardiomyopathy. One of these strategies is the use of dexrazoxane (also known as ICRF-187), an adjunctive agent derivative of ethylene-diaminetetraacetic acid (EDTA), which acts as a free radical scavenger. In this case, dexrazoxane is an EDTA-like chelator that interferes with iron-mediated oxygen free radical generation and, consequently, lipid peroxidation [87, 88]. The beneficial effects of dexrazoxane have been demonstrated in murine [89, 90] and canine [91, 92] models. Further, a meta-analysis of six randomized trials that included 1013 adult and pediatric patients demonstrated significantly reduced incidence of heart failure after dexrazoxane treatment, confirming its beneficial effects [93]. In addition, cardioprotective effects have been observed in children with high-risk acute lymphoblastic leukemia (ALL) receiving chronic DOX (10 doses of 30 mg/m^2) [94, 95]. The studies concluded that treatment with dexrazoxane is justified individually when the risk of cardiac dysfunction is expectedly high [87]. Unfortunately, according to the current Food Drug Administration (FDA) approval statement and European Medicines Agency (EMA), the use of this drug as a cardioprotective is limited to women with metastatic breast cancer who have received cumulative doses of 300 mg/m^2 DOX [95, 96].

Another strategy that has been evaluated is the use of angiotensin-converting enzyme (ACE) inhibitors, including enalapril, zofenopril, and lisinopril. ACE inhibitors are commonly used in patients with heart failure as afterload-reducing agents. In addition to their features as an effective ACE inhibitor, these drugs act as antioxidant and, thus, may contribute to prevent cardiac toxicity [97]. In support of this view, recent preclinical study demonstrated that administration of enalapril attenuated DOX-induced cardiac dysfunction via preservation of mitochondrial respiratory efficiency and reduction in DOX-associated free radical generation [98]. Unluckily, in long-term survivors of childhood cancer treated with DOX, the beneficial effect of enalapril-induced improvement in left ventricle structure and function was lost after 6 or 10 years. It is important to mention that ACE inhibitors have adverse side effects and, therefore, the choice of this drug as a cardioprotective agent during cancer treatment should be carefully evaluated [99].

Another antioxidant that has already been tested against DOX-induced cardiomyopathy is vitamin E. A study has shown that vitamin E only prevents the acute effects of DOX cardiotoxicity in mice [100]. Several other antioxidants also have been tested with limited success, including vitamin C, reduced glutathione, selenorganic compound PZ51, oleanolic and ursolic acids, and ambroxol [101–105]. On the other hand, probucol, a lipid-lowering agent and potent antioxidant, provided complete protection against DOX-induced cardiomyopathy and heart failure in animal experiments without interfering with the antitumor properties of this antibiotic [106, 107]. In this case, it is extremely important that clinical trials using DOX therapy in combination with probucol are performed to determine a new preventive cardiotoxicity strategy. This approach was recently tested using a β-adrenergic receptor blocker and also an antioxidant agent called carvedilol. As result, this drug protected systolic functions of the left ventricle due to reducing DIC [108]. Therefore, the use of carvedilol may become a promising strategy to improve DIC. However, more studies are needed to assess whether the beneficial effect observed on cardiac function is preserved over the years.

In addition to the antioxidant agents, accumulating evidence indicates that cardiac α1-adrenergic receptors (α1-ARs) protect cardiomyocytes from DIC. In particular, the stimulation of α1-AR-specific agonists phenylephrine (PE) and dabuzalgron have been shown to reduce apoptosis, interstitial fibrosis, and myocardial dysfunction caused by DOX. This protective effect is associated, at least in part, with the expression of anti-apoptotic proteins of the Bcl2 family and preservation of mitochondrial function [64, 109]. Thus, further studies will be needed to elucidate the full mechanisms responsible for the cardioprotective effects observed up to now.

2.4. Breakthroughs and challenges in basic and clinical research

Although therapeutic strategies to prevent cardiomyopathy have been proposed for more than four decades, it is important to highlight that there is still no specific treatment for total recovery of the myocardial injury caused by DOX. In this case, cardiac transplantation remains a vital option for patients with end-stage heart failure due to DOX-induced cardiomyopathy [3]. However, the major problem is the long time of wait in the queue of transplant due to low donor/acceptor ratio. Statistical data show that 10–20% of the patients in the waiting queue come to death annually [110].

In this scenario, in which therapeutic options for DOX-induced cardiomyopathy are insufficient, a newly emerging strategy is cell therapy. The principle of cell therapy is to restore the function of an organ or tissue by transplanting new cells [111]. In this context, a study has shown that transplanted mouse embryonic stem cell (ESC) in DOX-induced cardiomyopathy mice model attenuated various pathological mechanisms such as: (1) cardiomyocyte apoptosis due to inhibition of phosphoinositol-3-kinase (PI3K)/Akt and ERK pathway; (2) cardiac fibrosis; (3) cytoplasmic vacuolization; and (4) myofibrillar loss [112]. Although beneficial effects have been observed in this study, the teratogenic potential of these cells represents serious limitation to their use [113, 114]. In fact, experimental models of myocardial infarction have demonstrated the formation of teratomas after the transplantation of undifferentiated ESC [115, 116]. Thus, to overcome this limitation, several studies suggest the use of already differentiated ESC in cardiomyocytes [116, 117].

Considering that DOX causes cardiomyocyte death through the activation of different molecular and pathophysiological mechanisms, this therapeutic approach seems to be promissory for future application against DIC. In support of this view, recently, our research group has shown that the transplant of cardiomyocytes derived from mouse ESC (CM-mESC) improved cardiac function and electrical activity of the mice hearts with DIC, as well as reduced the percentage of cardiomyocyte apoptosis [118].

For clinical research, cardiomyocytes derived from human induced pluripotent stem cells (CM-iPSC) are potential cell sources for cardiomyocyte transplantation therapy. The generation of induced pluripotent stem cells (iPSC) from human somatic cells through overexpression of four transcription factors (OCT4, SOX2, c-Myc, and KLF4) represented a scientific milestone opening new perspectives for treatment of heart diseases. In addition to showing the same characteristics of ECS and, thus, the ability to differentiate into cardiomyocytes, these cells are not associated with immune rejection [119, 120].

Currently, the great challenge for the scientific community is the development of pro-maturation strategies to obtain human adult cardiomyocytes in vitro, with ventricular-like phenotype based on action potential, genetic, morphological, and metabolic characteristics [121]. Once maturation is achieved, CM-iPSC are a viable option as an autologous cell source for cardiac repair and a powerful tool for treatment of cardiovascular diseases, including DIC.

3. Conclusion

DIC is an important public health concern given the fact that this disease may not to be detected for many years and remains a life-long threat. Mechanisms contributing to the development of cardiomyopathy involve (1) free radical generation; (2) alteration in iron metabolism through iron-DOX complex formation or interference in the proteins' activity that transport and bind intracellular iron; (3) increased calcium intracellular concentrations; (4) disarray and loss of myofilaments of the sarcomere; (5) gene expression modulation; (6) activation of apoptosis by different signaling pathways; and (7) modulating microRNA expression. Based on these mechanisms, a variety of strategies to prevent cardiotoxicity have been tried, including the use of iron-chelating antioxidants and adrenergic receptor agonists. However, so far, the ability of these treatments to protect the heart from DOX-induced damage has been limited. Since DOX causes cardiomyocyte death, one recent approach that has shown promise is the transplant of

CM-iPSC. In this context, the scientific community has been engaged in the establishment of pro-maturation protocols to obtain adult human cardiomyocytes in vitro. Once this challenge has been overcome, we believe that cell therapy with CM-iPSC may be a promising strategy for the development of effective therapy against DIC.

Conflict of interest

The authors declare that they have no conflict of interest.

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