Chapter 13

Mitochondrial Dysfunction Associated with Doxorubicin

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Additional information is available at the end of the chapter

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Abstract

Cancer prevalence is scaling up each year. Anthracycline groups are still the best chemotherapeutic agent. The most popular anticancer drug in the group is doxorubicin (DOX). Unfortunately, DOX has potent toxicity on noncancerous tissues, e.g., heart, kidneys, etc. However, it is well documented that the severest toxicity of the drug affects heart tissue. Of course, some reasons have been suggested why and/or how the heart is so vulnerable to toxicity. The primary mechanism responsible for DOX's cardiospecific toxicity remains unidentified so far; however, mitochondrial dysfunction induced by DOX is now considered one of the leading reasons for DOX's toxicities and undesired side effects. Mitochondrial reactive oxygen production in the heart is a significant contributor to developing mitochondrial dysfunction-exposed DOX based on a variety of evidence. The objective of this review chapter is to critically evaluate and highlight the role of mitochondria in the development of DOX-induced cardiotoxicity.

Keywords: doxorubicin, cardiotoxicity, bioenergy stress, heart, mitochondrial toxicity, mitochondrial membrane potential

1. Introduction

This chapter gives the state of the art on doxorubicin (DOX) toxicity in mitochondria, particularly heart tissue. It is well known that cancer is a global threat to human health. The therapeutic effect of the drug is still below expectations in satisfaction. The drug that is the subject of the current chapter is a good chemotherapeutic against various soft solid cancer types. However, its toxic effect on noncancerous tissue, especially heart tissue, has not been ruled out. Therefore, even cancer patient can accomplish to fight cancer; they

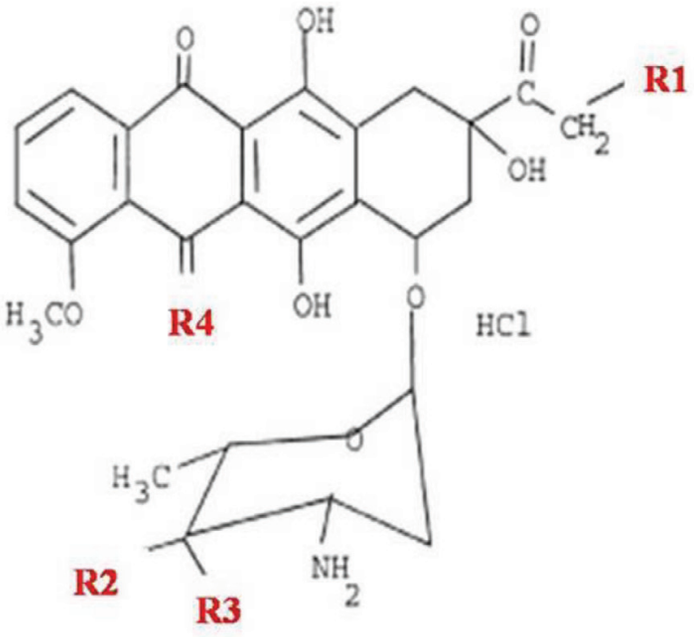
have still kept going to fight various severe diseases related to chemotherapeutic agents. The clinic utilization of drug is limited due to its side effects. When the molecular mechanism of the chemotherapeutic drug's side effects is clarified, it will be possible to manage all side effects. Hopefully, DOX may be able to improve the lifespan of cancer patients, which is why the purpose of the present chapter is to summarize its toxic effects on cardiac mitochondria.

2. Cancer: a modern epidemic

Cancer has a very high mortality and morbidity rate worldwide [1, 2] and is scaling up every year. In 2012 14 million people were diagnosed with cancer and 8.2 million people died due to cancer and cancer-associated diseases [3]. It is estimated that these figures will double by the year 2030 [4]. Patients are treated with radiotherapy, chemotherapy, and combination therapy. However, radiotherapy has been reported to be toxic and chemotherapy has been suggested to partially reduce the number of side effects. So, a chemotherapeutic agent is preferred to cure cancer [2]. Chemotherapy considerably enhances the survival rate of cancer patients. But, it is recognized to lead to side effect such as cardiovascular disease after growing survival population of a cancer patient with chemotherapy [5]. Therefore, cardiovascular diseases induced by chemotherapy are associated with high morbidity and mortality. The issue of heart damage caused by chemotherapy is a top priority due to the elevated cancer population treated with chemotherapy [6]. Anthracycline antibiotic groups are one of chemotherapeutic agents that is widely used in the treatment of solid and hematological cancer [7]. It has been indicated that the 5-year survival rate of childhood cancer patients was 30% before the discovery of group agents. However, this rate is around 80% today [8]. Still, extensive studies report that the group causes cardiotoxicity [6].

3. Anthracycline chemotherapy agents

Anthracycline antibiotics include DOX, daunorubicin, epirubicin, and idarubicin (**Figure 1**) [9]. DOX and daunorubicin are natural syntheses from *Streptomyces*, although epirubicin and idarubicin are synthetic derivatives from natural products [10]. Anthracyclines have a very high survival rate (~75%) within childhood cancer patients [11]. The drugs have been well recognized as a potential treatment against hematological cancers, including leukemias, lymphomas, solid carcinomas, and sarcomas [10, 12]. All these drugs have been reported to cause cardiotoxicity, classified as an acute and chronic effect [10]. Intracellular anthracycline tends to accumulate in the nucleus at the drug-sensitive cancer cell. However, the drug-resistant cancer cells have been outlined to find the chemotherapic agent at the cytoplasm [10]. Although some mechanism is proposed to explain the molecular structure, including oxidative stress, mitochondrial DNA (mtDNA) damage, etc., the molecular base of anthracycline on noncancerous tissue is still a mystery [12]. Because DOX is the most toxic drug in its class this chapter will evaluate only DOX toxicity, in particular heart damage [10].



	R1	R2	R3	R4
Doxorubicin	**OH**	**H**	**OH**	**O**
Daunorubicin	**H**	**H**	**OH**	**O**
Epirubicin	**OH**	**OH**	**H**	**O**
5-Iminodaunorubicin	**H**	**H**	**OH**	**NH**

Figure 1. The chemical differences between anthracycline antibiotics. Modified from Berthiaume et al. [13].

4. Doxorubicin: anticancer antibiotics

DOX was discovered by Farmitalia Research Laboratories, and they gave it the name Adriamycin after the Adriatic Sea [14]. So, DOX is also known as Adriamycin [15], discovered from *Streptomyces peucetius* (*Streptomyces peucetius* var. *caesius*) in 1967 [16–18]; however, some studies said it was discovered in 1969 [4, 13], and its clinical utilization began in the 1970s [13] after approved in 1974 by the US Food and Drug Administration [19]. DOX is a nonselective class-I anthracyline antibiotic [20]. It has positively charged groups, mannose amine, so that the drug can efficiently bind to a negatively charged molecule, such as nucleic acid. The standard cure is in the drug range 10–50 mg/m^2 [18].

DOX has been widely used in the treatment of human and nonhuman tumors, including leukemia [15], lymphomas, soft tissue sarcomas, and solid cancer [21], e.g., breast tumors, osteosarcomas, Kaposi's sarcoma, Hodgkin's and non-Hodkin's lymphomas [14, 22], thyroid and lung carcinomas, stomach, breast, bone, and ovarian cancers [23]. DOX is used for the treatment of solid childhood tumors too, such as non-Hodgkin's lymphomas, Hodgkin's disease, and soft tissue sarcomas [22].

DOX has been administered by intravenous infusion [13]. Peak plasma concentration and half-life have been reported to be 5–15 μmol/L and 20–30 h, respectively [13]. Another study, however, stated that the peak plasma concentration of patients treated with DOX is between 2 and 6 μM after bolus injection, but typically 1–2 μM [24]. DOX is reported to be very low when bound to plasma proteins [25]. The plasma clearance of DOX is measured between 324 and 809 mL/min/m^2, dominantly by biliary excretion; the maximum volume is around 809–1214 L/m^2. Moreover, the half-life of the drug is around 5 min, which means that reuptake velocity is very high for tissues. However, elimination velocity is slow within the range 20–48 h [26]. After injection, DOX is disseminated to the heart, liver, kidneys, and intestine [25].

4.1. Doxorubicin's chemical structure

The chemical structure of DOX is {(7*S*, 9*S*)-7-[(2*R*, 4*S*, 5*S*, 6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl] oxy-6, 9, 11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8, 10-dihydro-7*H*-tetracene-5, 12-dione (**Figure 2**). Due to structural specifications with a tetracycline moiety containing a quinone and a conjugated amino sugar residue, DOX can undergo metabolic modification by enzymes dominantly in the liver and kidneys during the elimination process. Some oxidoreductase enzymes, especially nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome P450 reductases at the endoplasmic reticulum (ER), nicotinamide adenine dinucleotide (NADH) dehydrogenase (complex I) at the electron transport chain (ETC), and cytosolic xanthine oxidase, have been suggested to play an important role in DOX elimination. The oxidoreductase enzyme can convert DOX to its semiquinone form by using molecular oxygen [13].

DOX's structure includes a glycoside group with anthraquinone moiety. The structure is responsible for its antineoplastic activity and also its toxicity [14]. DOX contains a tetracyclic ring with two quinone-hydroquinones and daunosamine. Though the tetracyclic sugar is nonsoluble in water, daunosamine sugar is soluble in water. DOX has been produced in a derivative form, e.g., daunorubicin. The difference between DOX and daunorubicin is only in the hydroxyl groups. Even though there is a slight difference between the drugs, their

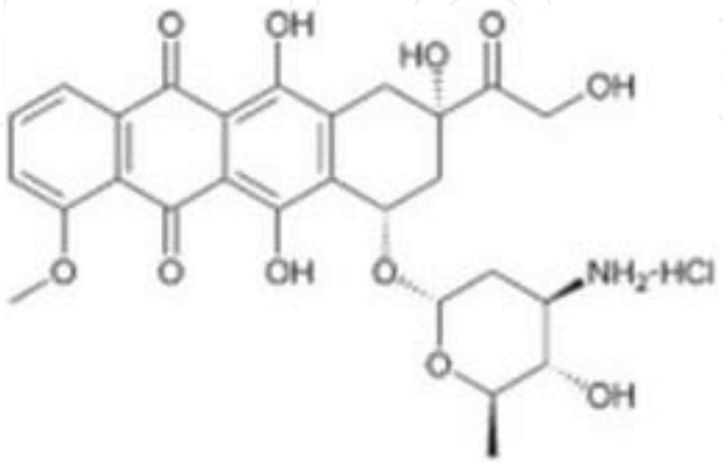


Figure 2. The chemical structure of doxorubicin antibiotics. From Imstepf et al. [27].

activities very are different to each other. How DOX intercalates DNA is related to the drug's chemical structure, including its chromophore's hydroxyl and daunosamine sugar's amino groups [22] (**Figure 2**).

4.2. The mechanism of doxorubicin's anticancer activity

It is agreed that the mechanism of its anticancer activity and its toxic impact follow different molecular mechanisms [13, 28, 29]. The anticancer activity of DOX relies on the interaction of the cell nucleus, mitochondria, and membranes. There are a number of reasons that explain the antineoplastic efficiency of the drug [17]:

1. DOX intercalates the DNA double strand, causing DNA replication and protein synthesis inhibition.
2. Reactive oxygen species (ROS) are produced, leading to the destruction of DNA and elevation of lipid peroxidation.
3. DNA is cross-linked and subject to alkylation.
4. DNA strands become obstructed and divided and there is helices activity.
5. The membrane structure is affected.
6. Prevention of topoisomerase II (TOPII) results in elevation of DNA damage [17].

The anticancer effect of DOX is associated with intercalation of the DNA strands, regulatory protein, covalent binding to DNA, and condensation of histone protein. However, its toxic impact on tissue does not rely on DNA impact [15].

The therapeutic effects of DOX have been associated with binding and intercalation of DNA strands, resulting in the destruction of replication and transcription of DNA by topoisomerase inhibition [4, 13, 30]. The reason why enzymes are so crucial is because TOPII has a role to play in modulating the DNA superhelical state [31], relaxing accumulated positive supercoils, and unlinking intertwined DNA strands. Thus, proteins are vital for complete DNA replication [22].

Also, DOX's cardiotoxicity has been related to disrupting TOPIIβ [30]. DOX selects toxic cardiac mitochondria through selective accumulation and redox cycling. However, free DOX enters the nuclei of cancer cells without entering mitochondria, which causes lack of mitochondrial pathway therapy [31]. Besides nuclear DNA, DOX intercalates with the mtDNA double helix, binds to a protein, and has a role in DNA replication and transcription as well [23].

To give more detailed knowledge on drug intercalation, DOX is one of the great anticancer drugs that kills cancerous cells by interaction with the cells' DNA; it also produces covalent adducts, resulting in inhibition of DNA synthesis by DNA polymerase blocking. DOX could also interfere with DNA and TOPIIα, finally forming a TOPIIα/DOX/DNA complex. The interruption of DNA and TOPIIα by DOX causes DNA breakage and cell death. A special

situation has been reported whereby heart tissue does not contain TOPIIα, but expresses TOPIIβ [20]. So, the TOPIIβ/DOX/DNA complex could only occur in the heart tissue [20, 28]. It is strongly supported that DOX's cardiotoxicity associates with TOPIIβ based on a TOPIIβ knockout mice study. When DNA damage occurs for some reason, e.g., by using DOX, the ataxia/telangiectasia-mutated protein is activated to trigger tumor suppressor protein p53. Activation of p53 by DOX has been indicated to elevate ROS production, double-strand DNA damage, and apoptotic cell death as well. Furthermore, another effect of p53 addresses one of the cardioprotective transcription factors, known as GATA-4. AMP-activated protein kinase (AMPK) plays the role of energy sensor to maintain enough available energy levels. If energy status drops too low by enhancing ROS production, and intracellular Ca^{2+} accumulates, AMPK can be activated and/or its phosphorylation can be elevated [20].

4.3. The risk factors of doxorubicin's toxicity

DOX utilization is limited due to its toxic effect [27]. There are well-established factors that pointed to an increase in DOX-related heart damage. These factors are total cumulative dose, one course or a day's full dose, radiation, especially mediastinal, age, gender, other cardiotoxic drugs or chemicals, cardiovascular illness, and liver pathologies [17].

One of the riskiest factors for toxicity is the drug's cumulative dose [4]. Extensive reports are available in the literature. The mortality of congestive heart failure-induced DOX is around 50% at higher than a 500 mg/m^2 cumulative dose [17]. This side effect is related to its dose, which is reported to be between 75 and 1095 mg/m^2, and the median dose of its toxicity is around 390 mg/m^2 [10]. The risk of developing toxicity on noncancerous tissue has been reported to enhance the cumulative dose, e.g., at 400 mg/m^2 with 3–5% [4, 10, 20], 550 mg/m^2 with 7–26% [4, 10], 700 mg/m^2 with 18–48% [4, 10, 20], or 950 mg/m^2 with 50% [10]. Furthermore, the risk of toxicity has been reported to enhance at 550 mg/m^2 [10]. When mice are given a total of 71 mg/m^2 DOX, the risk of heart damage is almost 100% [20]. Also, cancer type determines the risk ratio. For example, around 20% of lung cancer patients treated with DOX have been reported to develop heart failure [32]. DOX, an anthracycline antibiotic, therapy has been indicated to develop side effects in almost 35% of patients [33]. Its clinical utilization is, therefore, limited because many tissues become toxic when patients are treated with a 550–600 mg/m^2 cumulative dose [15]. DOXs toxicity can reach a 50% mortality rate at the highest cumulative dose [14].

The detrimental effect of DOX is related to its dosage and treatment duration. Its utilization is recognized to develop into cachexia and cardiotoxic impact over time [4]. DOX can even cause cardiomyopathy after years of treatment [4]. Cardiomyopathy has been claimed to occur following final DOX treatment of 0–231 days (median 23 days) and final daunorubicin treatment of 9–192 days (median 60 days). Cardiotoxicity due to DOX therapy has been seen even after 20 years [10]. In other words, its toxic effect is mostly dose and time dependent [13]. DOX-induced cardiotoxicity causes death in 50% of patients within 2 years [34].

DOX is used not only for childhood cancer patient treatment, but also for adults cancer patients [35]. So, age is an important factor in the development of cardiotoxicity of DOX [9]. For example, it is reported that patients over 65 years and under 4 years treated with DOX are more susceptible to cardiomyopathy. Children, adolescents, and the elderly treated with

DOX are at high risk of developing cardiac damage. It seems that in children DOX causes some stem cells to vanish, including pluripotent, undifferentiated, and cardiac stem cells. The decreased stem cell ability in the heart by DOX results in decompensating for the decline of cardiac mass induced by the drug's treatment. However, DOX has been shown to accumulate in cardiac tissue in elderly patients, resulting in reduced blood flow in the heart [17].

Another factor associated with DOX toxicity is gender [36]. Unusually, female cancer patients treated with DOX have higher mortality vs. male cancer patients, but females develop cardiovascular disease 10 years later than males. However, after the menopause, females become more vulnerable than males at the same age [37].

4.4. Cytotoxic effect of doxorubicin on noncancerous tissues

DOX is widely used for cancer therapy [38]. However, it has been recognized to have a toxic effect on noncancerous tissue such as the heart [13, 39], liver, kidneys [22], as well as the brain [40], and its poisonous effect is related to its dose [38]. This is why the drug's use for cancer treatment is limited based on its undesired impact on healthy tissue. Unfortunately, the mechanism of its toxic effect on noncancerous tissue has been not understood so far [38, 39].

DOX side effect symptoms are nausea, vomiting, alopecia, myelosuppression, stomatitis, and gastrointestinal disturbances [14], which are typical of cytotoxic chemotherapic agents [28]. The soft side effects of drugs include nausea, fever, and vomiting. Nausea, fever, and vomiting appear after DOX therapy as soft side effects. However, hypotension, arrhythmias, tachycardia, and congestive heart failure are also described after treatment as severe undesired side effects [17].

DOX's cytotoxicity includes two molecular mechanisms: intercalation of nuclear DNA and elevation of ROS production [41]. A cancer cell's DNA replication is well known to be faster than normal cells [41]. If DOX generates normal levels of ROS, it might selectively destroy heart pump function [41]. The most accepted mechanism leading to DOX's toxicity is oxidative stress, which causes damage to membrane lipid peroxidation products and decreases antioxidants as well. The most severe ROS generation by DOX is in the heart vs. other organs or tissues, e.g., kidneys, liver, etc. [10]. Extensive research has been suggested as to why DOX's cardiotoxicity relies on oxidative stress, mitochondrial dysfunction, and mitochondrial energy-forming disruption [16]. DOX treatment after 3 h has been reported to cause oxidative stress, lipid peroxidation, as well as lipid aldehydes in cardiac tissue [42]. Selective toxicity of the heart by DOX will explain these reasons. There is strong evidence supporting a critical role of oxidative stress on DOX's toxicological effect, though the molecular mechanism of its toxicity is still a mystery. According to study results from animal and human tissue, DOX disrupts the myofibril, mitochondrial membrane [13].

DOX's anticancer activity is associated with intercalation to DNA by decreasing TOPII activity after double-strand breakage, resulting in alleviation of DNA replication and protein synthesis. However, it is accepted that DOX's toxicity and anticancer efficiency are different from each other. The cytotoxic effect of DOX is produced by a mechanism such as ceramide synthesis by CREB3L1 activation, oxidative damage to DNA, losing mitochondrial membrane potential (MMP), caspase-3 activation, and p53 and c-Jun NH2-terminal kinase (JNK) activations. Nuclear factor-kappa B (NF-κB), a proapoptotic factor, could participate in DOX's cytotoxicity [33].

The clinical utilization of DOX is limited because of its toxic impact, especially on heart tissues, e.g., heart failure, cardiomyopathy [20]. The mortality rate of congestive heart failure induced by DOX is estimated at around 20%. There is no explanation for how DOX causes its toxicity on noncancerous tissue. However, it is thought to be multiple and complex mechanisms, nitrosative and nitrative stress, DNA damage, dysregulation of metabolites, and inflammation [16] involving DOX's toxicity, eventually triggering apoptotic cell loss [43]. The dysfunction of energy production has played a critical role in the development of both acute and chronic DOX toxicity and is related to time-dependent mitochondrial dysfunction [43]. Also, there is limited knowledge of its toxic mechanism, including disruption of calcium homeostasis by activation of calcium-dependent kinases, phospholipases, proteases [15], myofibrillar disruption, apoptotic cell death, as well as mitochondrial dysfunction. The mitochondrial toxic effect of DOX relates to the generation of ROS, destroying energy production [44]. The impact on its mitochondrial toxicity is caused by blocking the ETC associated with cardiolipin, which is an inner mitochondrial membrane protein [44]. DOX's toxicity is mainly associated with enhancing mitochondrial ROS production and decreasing mitochondrial biogenesis [38].

However, its clinical utility is limited due to irreversible myocardial damage and dysfunction. Apoptosis mediated by DOX contributes to heart failure. DOX's main intracellular target is mitochondria, causing mitochondrial damage and ROS elevation, and initiating apoptosis [21]. So, DOX gives rise to degrading contractile proteins [29]. However, limited knowledge of how mitochondrial dysfunction triggers cardiac apoptotic cell death-mediated DOX is still a mystery. Therefore, further studies are needed to increase knowledge [21].

DOX has detrimental effects, classified as acute and chronic abnormalities, including arrhythmias, heart failure, and ventricular dysfunction. The primary issue for DOX therapy is to overcome and minimize its toxic effect without altering its therapeutic impact on cells with cancer. Knowledge of its detrimental effect remains a mystery. There are, however, disorders that may explain its side effect, such as mitochondrial dysfunction and ROS production. Mitochondria have an essential function, including energy metabolism, cellular apoptosis, and cell death pathways, apoptosis, and necrosis [35]. The cardiotoxicity of DOX relies on its dosage. For example, electrocardiologic abnormalities have been reported to occur at a low dose, although dilated cardiomyocytes and congestive heart failure have been reported at a high dose [13]. After left ventricular end diastolic pressure and left ventricular ejection fraction are suppressed, DOX dilates cardiomyopathy because of the decline in heart pump function. Besides cardiomyopathy, DOX also leads to the development of cardiac remodeling, including cytoplasmic vacuolization, myofibrillar clutter, or sarcoplasmic reticulum (SR) swelling. This is why further studies are required to evaluate DOX's toxic effects on noncancerous tissue [4]. There is no defined specific therapy to cope with DOX's cardiomyopathy yet, except receiving traditional treatment of congestive heart failure, e.g., angiotensin converting enzyme blockers, etc. [24].

Based on our best knowledge of the mechanisms associated with apoptosis, oxidative stress, and mitochondrial dysfunction, to avoid undesired toxicity it has been suggested to use some form of antioxidant. Unfortunately, antioxidant therapy has failed to accomplish the drug's toxicity effect in many tissues, particularly the heart and liver according to clinical data [39]. This is why any approaches to use the drug clinically may reduce its toxic effects on noncancerous mass. Therefore, further studies are needed to evaluate the molecular mechanism of DOX's toxicity [45].

5. Cardiospecific toxicity of doxorubicin

The most severe toxic effect of DOX is on the heart [17]. This toxic effect is related to mitochondria because DOX targets cellular mitochondria, resulting in mitochondrial damage and cell death [20]. Cardiomyocytes are differentiated and nondividing cells, so they would not be a direct target of the drug since it blocks DNA replication and synthesis [28]. Therefore, cardiomyocytes have an insufficient regenerative ability after significant injury [46, 47]. In case of severe damage, the majority of heart muscle functions can be terminally lost. DOX could selectively oxidize mtDNA associated with heart failure [28]. For some reasons the most severe detrimental effect of DOX is seen as heart based. These reasons are:

1. The heart contains a high volume of mitochondria per cardiomyocyte.
2. There is a high affinity for cardiolipin in the mitochondrial inner membrane of the heart.
3. Existing cardiospecific NADH dehydrogenase results in elevated ROS production.
4. There is lower antioxidant capacity in the cardiac tissue. The opening of the mitochondrial permeability transition (MPT) pore initiates apoptosis by releasing a proapoptotic factor, such as cytochrome, SMAC/DIABLO, and the apoptosis-inducing factor (AIF). MPT can form with the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocase (ANT) matrix chaperon cyclophilin D (Cyp D). The open probabilities of MPT can be enhanced by DOX, so mitochondriopathy has been related to DOX's cardiotoxicity [23].

Cardiomyocytes contain high mitochondrial density, and one cardiomyocyte occupies 40–45% of mitochondria [21, 34]. The organelle has a function to maintain standard cardiac capacity due to a high-demanding, high-energy substrate for contractile function [21]. DOX accumulates in mitochondria 100 times more than plasma [34]. After binding DOX, cardiolipin loses the cofactor role in mitochondrial enzymes [34].

DOX tends to accumulate in the nucleus and mitochondria. In heart tissue, mitochondria make up around 50% of its volume [48]. DOX has a high affinity to bind the inner mitochondrial membrane and is collected on the matrix side [3]. One of DOX's similarities in the inner mitochondrial membrane is cardiolipin, which has a much higher affinity vs. other lipids in mitochondria (around 80 times). Phosphatidylethanolamine and cardiolipin are adaptors in the hexagonal (HII) phase in existent divalent cations, e.g., DOX, leading to changes in fluidity and functionality of mitochondrial membranes. DOX inactivates mitochondrial lipid-dependent enzymes, such as NADH dehydrogenase, cytochrome-*c* oxidase, and cytochrome-*c* reductase. DOX binds to cardiolipin, causing inactivation of complex I–III. DOX and NADH/NADH dehydrogenase incubations have been suggested to reduce sequestration at the SR by around 80% [48]. Also, mitochondrial TOPI is also found to relate to anthracyline-based cardiac toxicity [11].

The heart's mitochondria have two NADH dehydrogenases. One, known as cytosolic or intermembranous, is located at the outer surface of the inner mitochondrial membrane. However, the other one, known as matrix NADH dehydrogenase, is placed at the matrix surface of the inner mitochondrial membrane. Complex I relates to cytosolic NADH dehydrogenase as a

function to capture the electrons from the mitochondrial cytosol to the electron transport system (ETS). Moreover, cytosolic NADH dehydrogenase probably participates in DOX-induced heart toxicity. The molecular weight of DOX is around 600 Da. So, DOX with a hydrophilic structure could smoothly transit from the outer membrane to the mitochondrial cytosol. However, it is difficult to pass through an inner mitochondrial membrane with a lipoidal structure. Therefore, DOX cannot reach the matrix NADH dehydrogenase. This is why DOX is almost impossible to convert its semiquinone form at most cell types, e.g., renal or hepatic tissues and tumor cells as well. On the other hand, heart tissue contains cytosolic NADH dehydrogenase at mitochondria. This is why DOX can be converted to its semiquinone form, leading to oxidative stress by transferring one electron to molecular oxygen [10]. Furthermore, the semiquinone form can produce dihydroquinone via itself by deletion of the sugar moiety to make its aglycone form. The primary metabolites are suggested to be of aglycone form because the form can easily pass through the inner membrane due to its lipoidal structure. In this way, the major form could substitute coenzyme-Q10 and block complex I and II as well at around a 100 μM concentration. Thus, this results in dissociation of coenzyme-Q10 from mitochondria. This is why the plasma coenzyme-Q10 level is increased in cancer patients receiving DOX therapy and decreased in heart tissue as well. The aglycone form of DOX could deliver electrons to an oxygen molecule, enhancing the superoxide radical. Superoxide dismutase at mitochondria can serve to convert hydrogen peroxide (H_2O_2) to hydroxyl radicals and water, which is why heart tissue is susceptible to oxidative stress produced by ETS and the DOX semiquinone form as well. The other detrimental effect of the aglycone form breaks energy synthesis from mitochondria due to the substitution of coenzyme-Q10 acting as a potent antioxidant. Aglycone derivatives of DOX lose the anticancer impact of the drug because it does not bind to DNA [10].

Excess electrons generated are captured by oxidizing agents, such as oxygen, and the cardiac tissue has a very high oxygen consumption rate [36]. Heart tissue needs more energy to maintain contractile function and cell survival, which is why cardiomyocytes have substantial mitochondrial volume. The mechanism of DOX's toxicity is still a mystery. However, many studies have suggested the association between ROS and reactive nitrogen species (RNS) with their side effects [20] (**Figure 3**). In other words, the heart has been extensively exposed to oxidative stress. The reason for this is due to the enormous volume of mitochondria and weak antioxidant defense in the tissue [17]. The heart contains low-level catalase enzymes; in addition, DOX immediately inactivates selenium-dependent glutathione (GSH)-peroxidase-1 and cytosolic Cu–Zn superoxide dismutase enzymes after therapy [17, 36, 42]. DOX is claimed to have a univalent redox potential of around −320 mV [17]. This fact can be combined with information that a high proton concentration might have potential to enhance mitochondrial ROS production [49]. Based on this potential, DOX is a suitable substrate for certain oxidoreductase enzymes, which are NADPH-dependent cytochrome P450 reductase, NADH dehydrogenase, and xanthine oxidase. DOX is highly reduced by complex I, resulting in semiquinone. It is well determined to have DOX affinity to cardiolipin with phospholipids. Cardiolipin acts as a cofactor for respiratory chain enzymes, e.g., cytochrome-c oxidase and NADH cytochrome-c oxidoreductase [17].

The semiquinone and molecular oxygen reaction is very fast ($k = 10^8\ M^{-1}\ s^{-1}$). Semiquinone and H_2O_2 can be catalyzed under very low oxygen conditions. Cholesterol is a crucial element to determine the localization and/or association of the drug. If cholesterol is high, DOX can

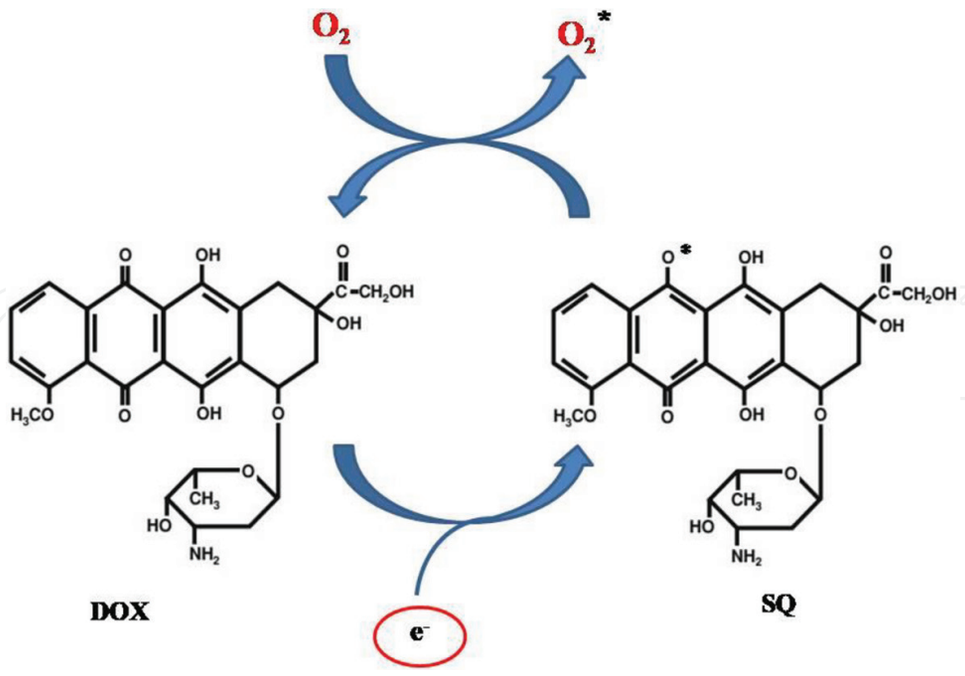


Figure 3. The formation of a semiquinone radical (SQ) from doxorubicin (DOX) by capturing one electron, which causes auto-oxidation in existing molecular oxygen, resulting in superoxide radical formation. Modified from Carvalho et al. [17].

lower its binding to the membrane. This knowledge is vital when the inner mitochondrial membrane is thought not to contain cholesterol. So, cholesterol and DOX or DOX's derivatives as semiquinone compete with binding of the hydrophobic region of the mitochondrial membrane. There are reasons why mitochondrial lipid peroxidation is high. The first reason is that the outer mitochondrial layer produces more ROS. The second reason is that the inner mitochondrial membrane is a very rich nonsaturated fatty acid. The third reason is that cardiolipin exists as 18% of total lipids in the mitochondria. So, DOX has a very high affinity for cardiolipin [48]. DOX tends to accumulate in mitochondria; therefore, mitochondrial ROS and RNS can be produced [28]. Elevation of ROS causes the enhancement of NF-κB and inducible nitric oxide synthase (iNOS) [28]. This process could trigger a positive feedback. iNOS also initiates to form ROS, which will be looked at in another section of this chapter.

5.1. The acute toxic effect of doxorubicin

It is well known that DOX's toxicity is based on its cumulative dose. It is reported that DOX could be lethal when mice are treated with DOX as a single dose of 12.5–25 mg/kg or two 15 mg/kg doses. Thus, the survival rate of drug treatment is between 40 and 0% at lower and higher doses, respectively [10]. DOX's toxicity has been classified as acute and chronic. Its acute effect occurs when patients receive drug treatment and has been reported to show transient arrhythmias, hypotension, and pericarditis. However, chronic DOX's results are evident even years after treatment and give rise to more severe damage, including congestive heart failure and dilated cardiomyopathy [43].

Acute toxicity has been seen by electrocardiographic (ECG) alternation as suppression of myocardial contractile function [10]. Another myocardial dysfunction induced by the acute DOX

effect is diastolic dysfunction after therapy. Although there are no severe symptoms of diastolic dysfunction, it is becoming a very crucial issue for chronic DOX therapy due to concomitant systolic dysfunction [24]. The signs are transient electrophysiological alternations, including sinus tachycardia, supraventricular, and reversible arrhythmias, ST- and T-wave alternations, prolonged QT interval, QRS voltage decline, and flattening of the T wave, predicted at 11% within all cases [14, 17]. Some symptoms have been reported to appear rarely but are more severe, e.g., pericarditis, myocarditis, and acute left ventricular failure [17]. Also, one of the previous studies indicated that DOX led to pericardial, peritoneal, and pleural effusion [49]. The other severe side effects are hyperpigmentation of the skin veins used for drug injection, stomatitis, and myelosuppression [18]. Moreover, other acute effects cause loss of body, heart, and liver weights and also enhance lipid peroxidation [10]. Acute drug-exposure is suggested to cause ROS generation from complex I at ETC in mitochondria [13], as well as the initiation of apoptosis [50]. With this knowledge, our previous studies have shown that antioxidant supplementation might be an excellent candidate to moderate DOX's toxicity [51–56].

Interestingly, a transient DOX effect has been reported to shift mitochondrial dynamics to fission at the heart. However, acute DOX therapy at the liver tissue is reported to decrease fusion, but not alter fission. This means that a decrease in fusion leads to an increase in mitochondrial fragmentation in the liver. DOX is said to improve mitophagy at the organ. When mitochondrial fusion and mitophagy occur, mitochondrial content reduces. DOX also causes a decrease in citrate synthase. Acute DOX has been mentioned not to change proliferating cell nuclear antigen, which means that mitochondrial fission does not accompany cell proliferation [39].

However, all acute toxic effects are transient, occur within the first 24 h after drug therapy, and are spontaneously ameliorated [10]. Sometimes an acute DOX effect can transiently appear and disappear within a few minutes to a week. Acute DOX is prevalent in cancer patients receiving DOX therapy at around 20–30% [17]. The chronic toxic effects of DOX cause cardiomyopathy and congestive heart failure [10].

5.2. The chronic toxic effect of doxorubicin

The chronic toxic effect of DOX results in an irreversible defect in cardiomyopathy, congestive heart failure. A dose of DOX at 430–600 mg/m^2 given to 50–60% of patients has been reported to develop left ventricular failure. However, a cumulative dose of DOX at 300 mg/m^2 has been shown to increase heart failure by almost 2%. Moreover, causing heart failure induced by DOX is quickly enhanced after a 550 mg/m^2 dose [17], which is a limited dosage because it induces irreversible toxicity [48]. To see the chronic effects of the drug takes a year of therapy. However, rapid treatment still leads to damaged heart tissue [17]. Other toxicities of DOX have been reported to be palmar–plantar erythrodysesthesia (also known as a hand–foot syndrome) [48] and typhlitis [57]. DOX's toxic impact on tissue is associated with:

1. A high affinity to bind membrane lipid-dependent pH, resulting in membrane alternation of lipid structure by lipid peroxidation [48].
2. Production of a semiquione structure [48].

3. Bioalkylation at C7 aglycone by metabolic activation, resulting in alkylation and destruction of DNA [48].

4. A high affinity for iron (both ferric and ferrous forms) and copper so the drug can reduce activation through metal chelating effects, leading to free radical formation [48].

Chronic DOX therapy leads to heart failure; cardiomyopathy has been reported to be associated with oxidative stress and mitochondrial dysfunction [58]. Mitochondria are essential organelles that synthesize ATP with a total of four membrane-associated complexes: complex I, II, III, and IV. The amount of ATP production is related to the complexes' activities (**Figure 3**). For example, high activity produces more ATP, although low activity has the opposite effect. DOX is toxic to mitochondria; all complexes could be inhibited, leading to energy stress. A recent study result showed that cryptotanshinone treatment, which is obtained from *Salvia miltiorrhiza* root, could reverse the toxic compound effect of DOX, except complex II (succinate dehydrogenase) by elevation of MMP, resulting in enhanced ATP formation [58]. How the increase in MMP by cryptotanshinone treatment occurs can be explained by a decline in free radicals, particularly superoxide anion. Since the increase in oxidative stress destroys the reduction/oxidation balance in mitochondria, DOX has been well accepted to elevate ROS generation [58]. So, the cryptotanshinone mitigates the imbalance, eventually increasing both MMP and ATP production [58]. However, lengthy drug exposure is reported not to be related to drug interaction with ETC enzymes, but the molecular mechanism of extended DOX treatment to produce ROS is so far not well understood. So, further studies are required to evaluate the production of ROS induced by lengthy DOX treatment [13].

DOX has been reported to cause severe histological and electrophysiological (electrocardiogram) alternations of cardiac tissue related to cardiomyopathy, and also creatine phosphokinase elevation at 450 mg/m^2 cumulative dose. In contrast to acute studies, body weight gain has been reported in animal research with chronic DOX therapy. Histopathological and electrophysiological, including flattened-inverted T wave and declining QRS voltage, alternations have appeared in chronic DOX therapy [10]. Chronic DOX's toxicity led to more severe arrhythmias, including sudden death [59]. High blood pressure was reported after DOX treatment [60]. Histopathological variation of DOX's cardiotoxicity is observed as myofibrillar loss, sarcoplasmic swelling, cytoplasmic, myelin, and mitochondrial vacuolization, and crystal degeneration in mitochondria [23]. The acute and chronic toxic effects of DOX are summarized in **Table 1**.

5.3. The mechanism of reactive oxygen species production of doxorubicin

Under the standard physiologic condition, ROS can be by synthesis only 1–5% of oxygen consumption [11]. The most acceptable hypothesis of DOX's toxicity is extensive ROS production [4]. The reason for elevation by DOX is associated with its accepting and donating electrons. DOX contains a hexose sugar with tetracycline having quinone and hydroquinone moieties, which are part of the capture electron, producing semiquinone. A superoxide radical can be provided by semiquinone from an oxygen molecule. A superoxide radical does not have a

Acute DOX treatment	Chronic DOX treatment
1. Transient arrhythmias	1. More serious arrhythmias
2. Transient hypotension	2. Hypertension
3. Alternation of ECG	3. More severe ECG alternation
4. ROS production	4. High ROS production
5. Transient histopathological alternation	5. Permanent histopathological alternation
6. Induction of apoptosis	6. Induction of apoptosis
7. Alteration of mitochondrial dynamics	7. Mitochondrial dysfunction

Table 1. Comparison of some parameters of acute and chronic doxorubicin toxicities.

potentially harmful effect. However, superoxide radicals can be transformed by superoxide dismutase converting to H_2O_2; this is called a Fenton or Haber–Weiss reaction, and the highly toxic hydrogen radical can be produced from H_2O_2. DOX can be reduced in some intracellular enzymes, e.g., xanthine oxidase and microsomal NADPH-cytochrome P450 reductase that expresses in almost all cells. Mitochondrial NADH dehydrogenase, which mediates to produce ROS when DOX is present, is not present in other tissues, except cardiac tissue. This is why DOX is highly toxic to heart tissue because it causes ROS to elevate [10].

Given more detailed knowledge regarding its structure and radical formation, DOX can be reduced at the C13 position from doxorubicinol. Although DOX can be transformed to doxorubicinone at its daunosamine sugar by acid-catalyzed hydrolysis, doxorubicinol can also undergo the same acid-catalyzed hydrolysis to form doxorubicinolone. Both can then experience protonation at C7, resulting in the formation of 7-deoxydoxorubicinone and 7-deoxydoxorubicinolone, respectively, by deletion of the sugar. After double reducing DOX, a tautomer of C7 deoxyaglycone, that is, C-7-quinone-methide, is produced. C7-quinone-methide can connect to DNA and form free radicals [22].

The drug can form ROS via two pathways: the first is iron dependent, and the second is redox cycling, which is catalyzed by NADPH oxidoreductases [30]. DOX has one of the paths, which produces ROS, and is mediated by iron (Fe). According to the Haber–Weiss reaction, the superoxide radical formed by DOX could be transformed into H_2O_2, and then a hydroxyl radical can be produced by H_2O_2 in existing iron. Another way is for DOX to directly interplay, resulting in a ferro (Fe^{2+}) to ferric (Fe^{3+}) form of abundant ROS [28].

Oxidative stress produced by DOX relies on nitric oxide synthase (NOS) and nicotinamide adenine dinucleotide phosphate-oxidase (NOX). NOX and/or NOS can transform DOX to its semiquinone form, causing oxidative stress. When nitric oxide is produced by NOS, peroxynitrite, reactively oxidizing DNA, proteins, and lipids are produced as by-products. Moreover, two isoforms of NOS, namely endothelial NOS and inducible NOS (iNOS), have been reported to play a role in DOX's toxicity to produce RNS. Besides NOS, DOX can synthesize radicals by complexing with iron to produce hydroxyl radicals, which are also very dangerous for cells and can have a detrimental effect on DNA, proteins, and especially lipids [20].

DOX's ROS production effect is capable of transferring one electron to oxygen resulting in superoxide radicals. So, DOX oxidizes complex I of ETC [44]. DOX contains a quinine moiety, so it can reduce one electron catalyzed by NADPH, resulting in production of semiquinone free radicals. The semiquinone can undergo oxidation by molecular oxygen to superoxide oxygen radicals [30, 38].

The reason why mitochondrial ROS production is crucial is because it could amplify its detrimental effect by triggering intracellular signal pathways. According to one previous study, mitogen-activated kinases (MAPK) have participated in DOX's cardiotoxicity by ROS production [5]. Research has suggested that cardiotoxicity induced by DOX involves p-JNK, the p-ERK1/2 [61], as well as p38 [5]. Based on our previous studies, the renin–angiotensin system also crosstalks with DOX's toxicity [62, 63]. However, we need to investigate which intracellular signal pathways are potentially involved in DOX's toxicity.

5.4. Apoptotic cell death induced by doxorubicin

Apoptosis plays a role in developmental and homeostatic mechanisms. So, uncontrolled apoptosis relies on an illness, e.g., cancer [64]. This is why apoptosis, known as programmed cell death [65], has a role in the development of cancer and cancer treatment [2]. Apoptotic pathways start as intrinsic or mitochondrial and extrinsic stimulus stimulated by the cell death receptor [64]. There are many ways to initiate the intrinsic apoptotic path, particularly nutrient deficiency, genotoxic damage induced by cytotoxic chemotherapies, and radiation [64].

Extensive research has been conducted on DOX's apoptotic pathways. The evidence supports a significant role of oxidative stress induced by DOX. The difficulties in determining DOX's apoptotic pathways are related to the drug's dosage, route [26], and duration of treatment [9]. It is almost impossible to explain a single, unique apoptotic pathway induced by DOX [26]. However, literature data have suggested that DOX has been reported to trigger apoptosis in both pathways, intrinsic or mitochondrial and extrinsic [57, 66].

The pathways are controlled under pre- and proapoptotic factors. Apoptosis can be triggered by proapoptotic factors such as Bax or Bak activation by BH3-only protein, BIM, and also BID. When Bax and Bak become oligomerized, mitochondrial outer membrane permeabilization occurs and results in releasing cytochrome-*c* to the cytosol. Then the apoptosome can be formed by cytochrome-*c* with apoptotic protease-activating factor-1 (APAF-1), resulting in a triggering caspase cascade, including caspase-3 (**Figure 4**). In contrast to proapoptotic factors, e.g., Bcl-2, Bcl-XL can prevent apoptotic pathways maintaining monomeric Bax/Bak or BH3-only proteins [64]. Caspase-8 participates in extrinsic pathways, whereas caspase-3 and -9 have a role in the intrinsic route [2].

Bax activation releases cytochrome-*c* by the mitochondrial permeability transition pore (PMT) activation, resulting in APAF-1 activation [26]. After the apoptosome complex is formed by APAF-1, cytochrome-*c*, dATP, and caspase-9, procaspase-3 can be transformed into its activated form by the apoptosome [67]. Alternatively, DOX can facilitate apoptosis through mitochondrial p53 by depolarizing MMP. Recently published data are suggests that p53 elevation by DOX treatment influences Bcl-2 decline and Bax expression [26, 67].

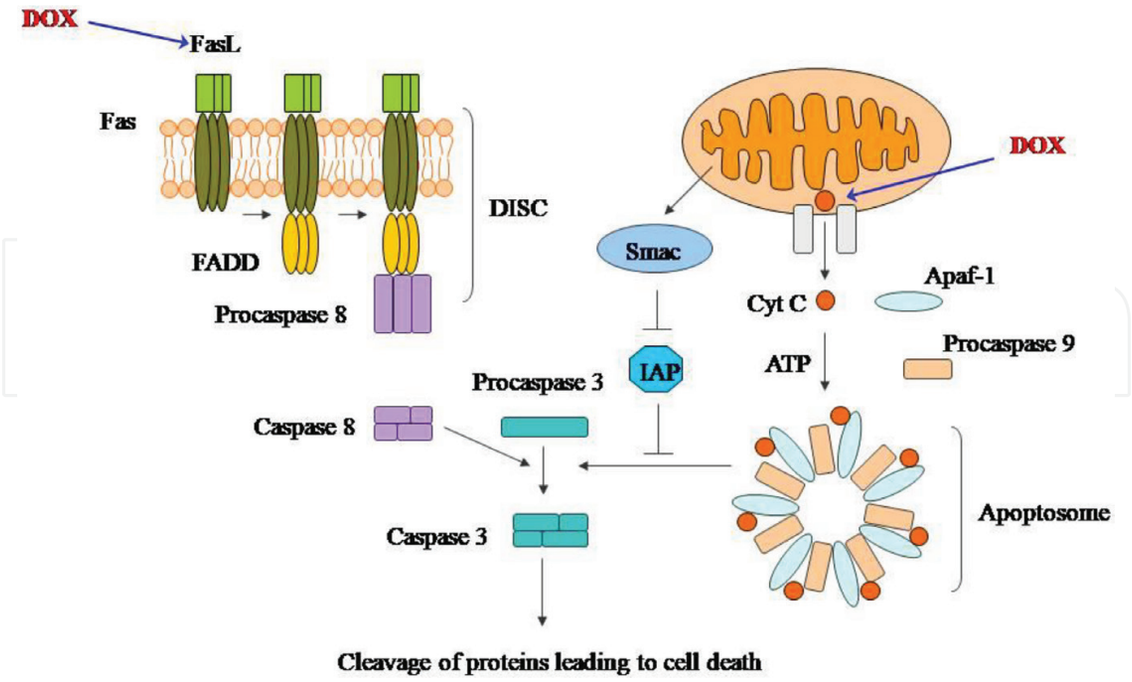


Figure 4. Apoptotic cell death by doxorubicin (DOX). Cyt C: Cytochrome-*c*. Modified from Meredith et al. [22].

DOX's toxicity is mainly thought to relate to ROS enhancement and TOPII inhibition [68]. Therefore, the therapy improves ROS production as described in the previous section. Cell death is stimulated based on transforming DOX to a semiquinone radical via complex I [15]. However, the heart has cardioselective external NADH dehydrogenase, which is a kind of alternative complex I, resulting in a long DOX redox cycle [14]. When semiquinone reverses to produce DOX, oxygen converts to a superoxide anion free radical. This superoxide radical can be scavenged by GSH, creating its oxidation form, glutathione disulfide [15]. It must be remembered that cardiac tissue has less antioxidant capacity than other tissues [14]. A decline in GSH causes oxidation of thiol groups in proteins, including MPT, resulting in depolarization of MMP. Enhancing MMP gives rise to decreased ATP production, and release of proteins from mitochondria to the cytosol, such as cytochrome-*c*. So, releasing cytochrome-*c* can trigger apoptosis and/or necrosis. Eventually, this causes cell loss [15]. DOX, therefore, leads to decreased heart muscle thickness [64] due to apoptotic cell death.

DOX's toxicity on mitochondria is dose and time dependent. The mitochondrial toxicity of DOX has been observed from 2 to 13 weeks at a low dose. Moreover, mitochondria play an essential role in the regulation of calcium homeostasis. Under the standard physiological condition, there is little impact of mitochondria on calcium homeostasis. However, the mitochondrial function of calcium homeostasis is essential under pathological circumstances to decrease cytosolic calcium. Calcium efflux into mitochondria causes MMP to depolarize. DOX's effect on calcium homeostasis in mitochondria is based on inhibition of the inward calcium flux and exaggeration of the release of calcium from mitochondria by swelling of the mitochondria–calcium-dependent pathways through MPT, resulting in enhanced calcium concentration at the cytosol [15]. Cytosolic calcium concentration at 30 nM is tightly controlled by several pumps, channels, and exchangers [69]. Besides initiating its role in

apoptosis, calcium homeostasis has a crucial function for cell metabolism. Several mitochondrial dehydrogenases, e.g., pyruvate dehydrogenase, isocitrate dehydrogenase, oxoglutarate dehydrogenase, and glycerol-3-phosphate dehydrogenase, are controlled by intracellular calcium concentration. As a result, the influx of calcium to mitochondria plays a central role in the regulation of cell metabolism. Therefore, any reason for preventing calcium entering mitochondria might also cause a drop-off in bioenergy production [69]. Furthermore, DOX at a low dose gives rise to a decline in the calcium storage capacity of mitochondria. The decline in mitochondria calcium storage capacity by DOX exaggerates its dosage. Eventually, the effects of DOX lead to the dissipation of MMP [15]. The other way that DOX disrupts both intracellular calcium homeostasis and mitochondrial calcium loading is via connexin 43 (Con-43). Con-43 is one of the essential gap junction proteins, and plays a role in the regulation of mitochondrial function. So, when Con-43 is blocked by a gap junction blocker it releases cytochrome-*c* and induces apoptosis. According to a recent study, DOX treatment enhanced Con-43 from cytosol to mitochondria through heat shock protein 90 and translocase of the outer membrane 20 pathways [70].

MPT pore, one of the redox-sensitive proteins, has a function to regulate mitochondrial tasks [15]. It is thought that the pore consists of many proteins; however, this has been not fully understood yet [13]. So far, MPT is believed to contain VDAC [17], ANT [13], and Cyp D (also called Cyp F). The most crucial elements of the pore have been claimed to be Cyp D [17]. MPT is controlled by creatine kinase (CK), hexokinase, the Bcl-2 family, and peripheral-type benzodiazepine receptors [17]. DOX reduces mitochondrial calcium loading capacity based on triggering of the MPT pore [17]. So, oxidative stress through complex I [15], dissipation of MMP, and loss of mitochondrial calcium capacity trigger MPT, resulting in enhanced inner mitochondrial membrane permeability, and eventually augmentation of small molecules less than 1.5 kDa due to the opening of nonselective protein pores [13, 17]. Besides loss of MMP, opening the pore triggers apoptosis by releasing cytochrome-*c* and another apoptotic factor from mitochondria to cytosol and subsequent activation of caspase pathways [13]. This is why the opening of MPT initiates apoptosis by releasing cytochrome-*c* or SMAC/DIABLO. DOX prompts the opening of MPT through oxidation of thiol residues in mitochondrial proteins. The other way of initiating apoptosis by DOX is to delete GATA-4, which is a transcriptional factor encoding Bcl-XL antiapoptotic genes preventing mitochondrial function and integrity. Anthracycline also blocks AKT phosphorylation, resulting in GSK3β activation, leading GATA-4 suppression in the nucleus. DOX causes bioenergetic stress by reducing mitochondrial ATP production and damaging CK isoenzymes and AMPK [17]. DOX is shown to change Bax and Bcl-2 protein levels as well [13]. Moreover, MPT opening gives rise to mitochondria-related osmotic swelling and structural detriment. The MPT formation is needed to clarify, so further studies are needed to evaluate MPT structure [17].

ER and mitochondrial dysfunction have been reported to include and follow the same apoptotic pathways [67, 71]. DOX also triggers apoptosis by ER dysfunction through activation of an ER stress sensor and transcription factor 6 [21]. Moreover, a study also found that apoptosis-related ER stress by DOX is instigated to elevate Ca^{2+}, calpain-1 protein level, and caspase-12, which is a marker of ER stress [67]. Cardiac damage mediated by DOX can be merged with lysosome dysfunction causing autophagic flux as well. DOX damages mitochondria by tending to accumulate in it, triggering apoptotic cell death [21].

Extrinsic pathways involve death receptors, their ligand interaction, e.g., Fas/FasL, and then caspase-8 activation [57]. DOX also uses the extrinsic pathways for instigating apoptosis by elevation of Fas protein levels, caspase-8, and BID [67] (**Figure 4**). Even so, DOX's leading approach to initiate apoptosis is through intrinsic, called mitochondrial, pathways. The outer membrane of mitochondria has a central role in the natural apoptotic route because it has pro- and preapoptotic factors. The elevation of ROS and depolarization of MMP by DOX release proapoptotic factors to the cytosol, e.g., cytochrome-*c*. p38, p53, Bax, and caspase-3 have also been suggested to participate in the induction of apoptosis. p53 enhances the permeability of the outer membrane to release proapoptotic factors, such as Bax [57]. DOX has been reported to increase p53 in the nucleus and mitochondria from the heart. So, p53 localization is thought to associate with mtDNA. However, there is limited knowledge available of nuclear and mitochondrial p53 localization by DOX in cardiac tissue. DOX has been suggested to elevate 8-hydroxydeoxyguanosine (8-OHdG) and p53 levels in mitochondria within 3 and 24 h. Cytochrome-*c* release is an assessment of cytosolic/mitochondrial cytochrome-*c*. DOX enhances the ratio of heart tissue by around 35%. It can trigger apoptosis through p53 stabilization by MAPK [72].

The MAPK family has extracellular signal-regulated kinases (ERK), p38 MAPK, and JNK [73]. While ERK1/2 predominantly operates cell proliferation, JNK and p38 participate in cell death pathways. DOX has been shown to kill prostate cancer cells by phosphorylation of p38 and JNK [74]. One of the MAPKs is p38, which has a pivotal role in cell growth, apoptosis, and inflammation. The apoptotic role of p38 depends on cell type, stimuli, or isoform activation of p38, which has four isoforms: p38α, β, γ, and δ. One study showed that DOX triggers apoptosis at the MCF-7 breast cancer cell line by elevation of caspase-3 and caspase-9 during 24 h of treatment [65]. So, p38 is one of the intrinsic pathway activators dependent on cellular stress, mitochondrial dysfunction, and caspase activation [57]. ERK1/2 probably has a role in the activation of caspase-3, Bax, p53, and cytochrome-*c* release. Moreover, ERK1/2 could contribute external apoptotic pathways at the caspase-8 level [75]. ERK1/2 could also phosphorylate p53. So, DOX activates apoptosis by the p53-dependent activation of caspases-2, -3, -8, -9, and -12 [66]. The release of caspase-12 activates caspase-3 [66].

Through extrinsic (receptor-mediated) or intrinsic (mitochondrial) pathways. Both pathways have a role in the trigger of apoptosis as upstream (initiator) caspase, e.g., caspase-8 and -9, and downstream (effector) caspase, e.g., caspase-3, -6 and -7 [76]. When MMP is depolarized and opened, mitochondrial apoptotic factors are released such as cytochrome-*c* and AIF to the cytosol [73]. Cytochrome-*c* can contain an apoptosome formation with APAF-1, caspase-9. Caspase-3 can be activated from both pathways [73]. The human fibroblast cell was used in one of the previous studies and reported that DOX at 3 μM concentration causes apoptosis through caspase-3, -7, and -9 by ROS [76]. DOX has been said to stimulate apoptosis via caspase-3-dependent pathways. The bcl-2 protein family is shown to play a role in apoptosis in cardiomyocytes as expected. Also, Bcl-2 and Bax can affect the MPT pore [68] (**Figure 4**).

DOX also stimulates apoptosis by an AIF. There are three sides of AIF: a NAD binding, FAD binding, and C-terminal. AIF is located at the intermembrane space or weakly binded to inner mitochondrial membrane and exhibits NADH oxidase activity. AIF can be released to the cytosol via PMT and translocate to the nucleus by poly (ADP-ribose) polymerase-1, resulting

in stimulation of chromatin condensation and DNA breakage, eventually triggering apoptosis by caspase-independent pathways. The other function of AIF is to repair and mature mitochondrial complex I and peroxide scavenging activities. Elevation of cytosolic AIF leads to release of cytochrome-*c*, resulting from depolarization of MMP. Why the molecular mechanism of DOX toxicity is so crucial is based on its effective utilization of therapy against cancer. This is why finding an effective therapy to counteract its toxicity, especially of the heart, will give hope to cancer patients treated with DOX to overcome nondesired effects. The other mechanism of cell death mediated with DOX decreases GATA, controlling apoptosis through antiapoptotic Bcl-X gene activation [77].

6. Mitochondrial dysfunction induced by doxorubicin

Mitochondria have a role in regulating cell death or survival under cell stress or damage. The organelle has its own genome encoding 37 genes, of which 13 are complex I, III, IV; complex II is encoded by nuclear DNA [22]. So, mitochondrial dysfunction is associated with disease and aging as well.

Besides its nuclear effect, DOX has been reported to cause mitochondrial dysfunction, energy stress via disruption of the ETC [4]. It is well recognized that mitochondrial bioenergetics mechanism disruption has been thought to play an essential role in the development of the drug's toxicity, especially its cardiotoxicity. Adequate ATP production is not just necessary to maintain contractile function, it is also crucial for protein synthesis, the protein quality control function of ER, cytoskeletal function, and clearing cellular waste from lysosomes as well [4]. Moreover, DOX's mitochondrial effect is shown to change ultrastructure, swelling, and oxidative capacity. Furthermore, DOX tends to accumulate in nuclei and mitochondria vs. plasma [43]. All this is needed to explain why or how DOX selectively targets mitochondria in noncancerous tissue rather than cancerous tissue. One reason is that cancer has been reported to alter a cell's metabolic activation. A healthy cell produces energy by oxidative phosphorylation in mitochondria. However, a cancer cell synthesizes its energy by the glycolytic pathway, known as the Warburg effect. Enhancing glycolytic activity could be multifactorial, relying on mtDNA damage, oxidative phosphorylation defect, mitochondrial dysfunction, etc. [78]. Another reason could be that DOX is more toxic to mitochondria in noncancerous cells than in cancerous cells. Moreover, DOX could alter mitochondrial function in noncancerous and cancerous cells, resulting in different apoptotic pathways [79].

6.1. The acute mitochondrial toxic effect of doxorubicin

The acute toxic effect of DOX on mitochondria has been reported to rely on its dose, especially redox cycling and ETC blocking. A low concentration of DOX treatment has been reported to have minimal alternation to ATP production and MMP, resulting from enhancing hydroxyl radical (*OH), H_2O_2, and oxygen consumption. Although up to 160 μM DOX concentration has been emphasized, redox cycling is the primary process to augment ROS production; ETC blocking is the primary source of ROS manufacture at densities higher than 160 μM. Until it reaches a threshold, which means 480 μM, mitochondrial toxicity is progressively enhanced.

Eventually, mitochondrial collapse is inevitable, resulting in MMP dissipation, improvement in ROS production, inhibition of ATP production, and also oxygen utilization. A dose of 1 mg/kg of DOX has been reported to increase superoxide radicals within 2 h, although a 37 mg/m^2 dose, which equals to 5–30 μM mitochondrial concentration, has the same effect on human beings [44].

6.2. The chronic mitochondrial toxic effect of doxorubicin

The heart, skeletal muscle, and brain are reasonably active organs [14], so energy demand is very high. This is why the majority of energy production is based on mitochondria for maintaining heart function [80]. Therefore, organ failure can develop because of damage to mitochondrial function [14].

Mitochondria have a role in regulating cell survival and death, proliferation, and calcium and redox homeostasis. It is reported that the inner mitochondrial structure is different from tissue to tissue, based on their metabolic activities; even the structure is different in the same tissue [14]. For example, heart tissue has been indicated to have two types of mitochondria. One is located near the T tubule and SR, and the other is firmly located at a contractile component of myocytes. The dynamic of the organelle is limited due to its close relation with the cytoskeleton [14]. So, these two mitochondria present different features, including dynamic organization, calcium accumulation, functional capacity, and localization. Subsarcolemmal mitochondria are situated at the plasma membrane and have a role in supplying ATP to the ion pump. However, intermyofibrillar mitochondria are situated between the myofibrillar structure and produce energy for the contraction/relaxation function of the tissue [80].

Mitochondria have two shapes or morphologies: long and filamentous (fused) and short and punctuated (fragmented). The form is vital to respond to damage. For example, fused mitochondria are suggested for counteracting apoptosis, while fragmented mitochondria are susceptible to apoptosis [81]. Mitochondria have two structures: long tubules and small round vesicles [82].

Mitochondria are dynamic organelles and perform trafficking, fusion, and fission, called mitochondrial dynamics [14, 21, 83]. The importance of mitochondrial dynamics is that mitochondria have to divide from existing organelles and proliferate via growth [83]. There is a balance between the fission and fusion processes. If the balance shifts towards fission, it causes damage, including mitochondrial fragmentation, mitophagy, a rise in oxidative stress, and cell death [21]. This equilibrium could relate to a number of factors, e.g., cell conditions such as stress, cell compartmentation such as neuronal axons or dendrites, and mitochondrial function as well. The dynamic is crucial for mitochondrial morphogenesis [82]. The importance of dynamics is to scale up because of its role in mitochondrial function, apoptosis, or aging [83]. DOX also leads to senescence of cardiomyocytes by inhibition of Akt Ser473 phosphorylation [21].

Mitochondrial morphological alternation is claimed to provide some idea about DOX's degree of toxicity. For example, the morphological shift at organelles is displayed at the beginning of DOX toxicity on heart tissue. Mitochondria have a homeostatic balance between

fission (divide) and fusion. Fission could cause mitophagy, apoptosis, and cell proliferation. However, fusion provides a homogeneous network of mitochondria. Imbalance of the mitochondrial dynamic causes it to lose cell function, e.g., when the shift towards fission could initiate apoptotic cell death due to severe ROS production. In contrast, the change towards fusion would increase mitochondrial dysfunction because of extinguishment of the mitophagy mechanism. The dynamic provides healthy, functional mitochondria and cells [39].

To maintain its function the heart prefers to metabolize fatty acid in mitochondria and peroxisomes via β-oxidation due to its high demand energy [14]. Mitochondrion starts energy production by the tricarboxylic acid cycle (TCA) in the ETS. Mitochondrial ATP is synthesized by ETS steps. Although TCA elements are placed in the mitochondrial matrix, except for succinate dehydrogenase, ETS elements having a spherical shape are present at the mitochondrial inner membrane and project to the mitochondrial matrix. The space between the inner and outer layer is called the intermembrane space or mitochondrial cytosol. A molecule with hydrophilic structure can transit the inner membrane as a requirement of the transport system. The outer membrane of mitochondria can pass through almost all particles less than 10,000 Da [10]. The respiratory chain is under the control of four complexes: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome-*c* reductase), and complex IV(cytochrome-*c* oxidase) [34] (**Figure 5**). Complex V is ATP synthase [11]. After synthesis, ATP can be transferred from the inner mitochondrial membrane to the cytosol through ANT and the outer membrane VDAC. MPT induction opens the nonselective pores permitting the diffusion of a 1.5 kDa small molecule [14]. Electrons can be relocated from complex I (NADH dehydrogenase) and II (succinate dehydrogenase) to coenzyme Q10 with a quinine structure as DOX. Then, particles are moved to complex III, cytochrome-*c*, and complex IV. Eventually, oxygen can capture the particle, resulting in water synthesis (**Figure 5**). All ETS elements associated with the enzyme are placed near the matrix surface of the inner mitochondrial membranes. It has been reported that complex I and II could not reach the particles in the mitochondrial cytosol from any organ and any cancer cells, except the heart. The mitochondria of cardiac tissue has two position on the outer surface of inner mitochondrial membrane and faces the matrix [10]. However, overexposed DOX causes mitochondrial dysfunction, consisting of decreasing state 3 respiration, complex I, and ANT activities. Moreover, lengthy DOX treatment increases susceptibility to calcium, resulting in dissipation of MMP at low and high concentrations [13].

Bioenergetics failure may be primarily a mechanism of DOX cardiotoxicity [13]. So, the other molecular base of DOX toxicity on noncancerous tissue relies on the destruction of mtDNA. Oxidative stress leads to damage to mtDNA, especially heart tissue. Additionally, DOX represents the harmful effect of mtDNA much more than nuclear DNA. If the integrated knowledge that the mtDNA repair system is fragile vs. the nuclear DNA system, DOX is understood to be highly toxic to mitochondria [10]. The mitochondrial genome has 13 subunits of ETC codes, which are almost all of the ETC complex, except complex II, a succinate dehydrogenase encoded by nuclear DNA [13]. mtDNA has also been encoded by mitochondrial ribosomal and transfer RNA [10]. DOX damages mtDNA via elevation of ROS [13]. Oxidative damage of DNA has been evaluated by using 8-OHdG formation. After DOX treatment, 8-OHdG has been reported to reach a peak value at 24 h, but a baseline value at 14 days [13]. The chronic

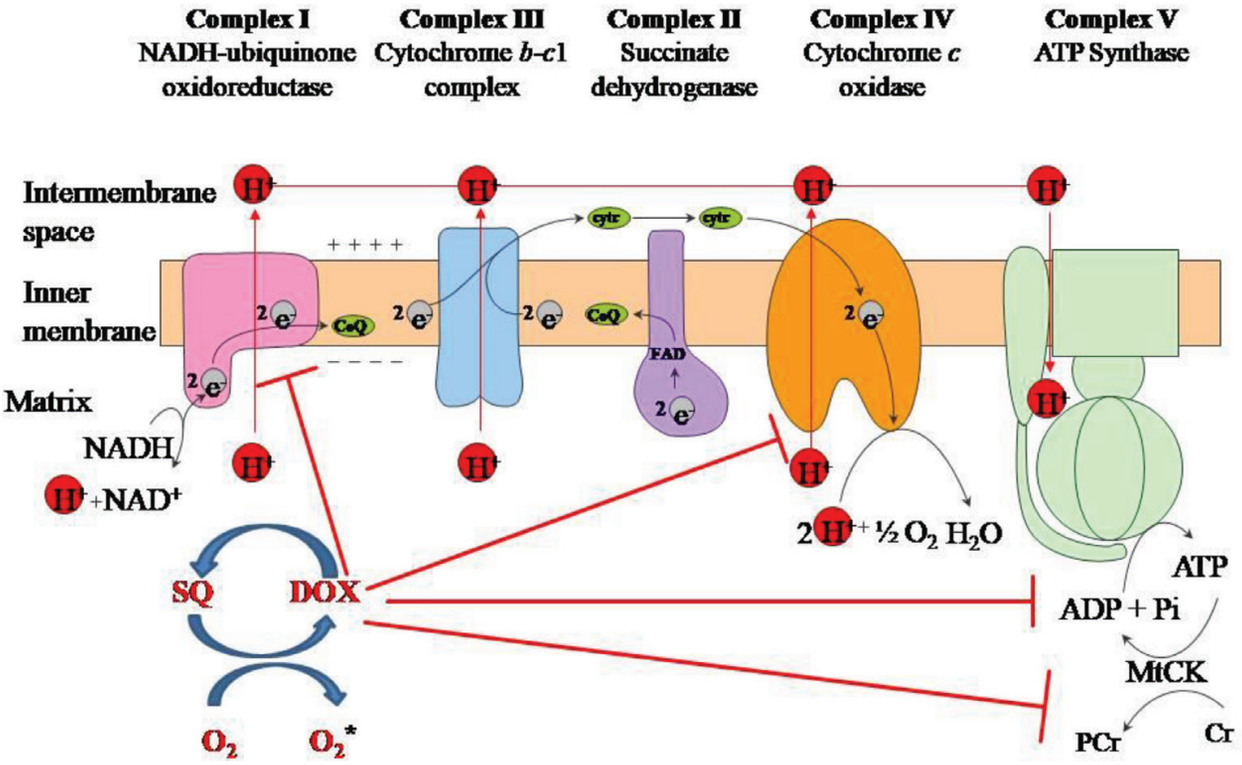


Figure 5. The effect of doxorubicin and its derivate on the electron transport system and mitochondrial energy production. Modified from Govender et al. [34].

effect of DOX on mitochondria has been reported to appear when it destroys mtDNA [44], mainly developing mtDNA deletion. The prevalence of the elimination has been reported to be between 33 and 80% at a low and high dose of DOX, respectively [10]. When DOX oxidizes mtDNA, mitochondria can no longer produce high-energy substrate, resulting from destroying to reproduce mtDNA [10]. This alternation is explained by DNA repair and elimination of damage to the genomic material, which changes or eliminates the protein function. Alternation or disappearance of mitochondrial protein function elevates ROS formation as well [13]. At this moment, we should take time to diagnose DOX's chronic cardiotoxic effect, e.g., heart failure, dilated cardiomyopathy, and congestive heart failure [10]. Moreover, mitochondrial complex I activity has been claimed to inhibit isolated mitochondria from cardiac tissue, but not hepatic tissue by chronic DOX therapy for 28 weeks. This notion has given rise to the thought that the drug's toxicity in mitochondria is cardioselective [10]. One study suggested that endurance exercises reduce DOX toxicity based on modulation of state 3 alternation at mitochondria. Also, the study reported that apoptosis induced by DOX could be counteracted by endurance exercises giving rise to a decline in apoptotic factors, such as Bax or Bax/Bcl-2 ratio. Moreover, DOX alters the ultrastructure of heart tissue by mitochondrial destruction, including damaging cristae and vacuoles, and causing distension and abnormal size and shape [80]. Mainly, the outer mitochondrial membrane plays a role in the transduction of signals, e.g., apoptotic [82]. Endurance exercises have been suggested to reverse the ultrastructural alternation, e.g., a rise in glycogen storage, and enhance cytosolic and mitochondrial sodium oxide dismutase. Due mostly to the sensitive oxidative stress of MPT, elevation of antioxidant by endurance exercises leads to decreased apoptosis induced by DOX [80].

Another chronic drug mechanism is associated with cardiolipin. DOX has high affinity for cardiolipin; therefore, DOX–cardiolipin complex formation interrupts the standard oxidative phosphorylation mechanism. DOX can be transformed into the semiquinone form by NADPH reductases in mitochondria. The opening of MTP led to swelling of mitochondria and depolarizing mitochondria membrane potential, structural and cytoskeleton disorganization, and mtDNA injury. Peroxisome proliferator-activated receptor-*c* coactivator-1α (PGC-1α) has a crucial role in mitochondrial biogenesis and oxidative metabolism [16]. Also, alternative posttranslational modification could participate in the alteration of mitochondrial function. Posttranslational modification through acetylation and deacetylation from lysine residues play a crucial role in regulating mitochondrial function. Mitochondrial proteins are modulated posttranslationally as sirtuin enzymes, sirtuin-3, -4, and -5, by deacetylation. Posttranslational modification can occur under redox stress and nutrient flux. Mitochondria express mostly sirtuin-3 and nuclear NAD^+-dependent histone deacetylase [34].

6.3. Bioenergetics dysfunction induced by doxorubicin

Mitochondria have sources of bioenergetics and ROS as well. This is why mitochondria become the target of multiple factors such as drugs, including DOX, and environmental compounds. PGC-1α plays an essential role in the regulation of mitochondrial function, including production of bioenergy and energy homeostasis. PGC-1α can regulate gene transcription, including mitochondrial services such as NRF-1, PPARa, and ERRa, which modulate and are divided into three groups of metabolic enzymes in the TCA cycle: antioxidant enzymes, other mitochondrial protein components of the ETC complexes, and mitochondrial transcription factor A (TFAM) [38].

When mitochondrial dysfunction occurs, i.e., enhancing ROS, reduction of ATP synthesis, a complex transcriptional network, including PGC-1α can be triggered to maintain cellular homeostasis. PGC-1α can transcript three groups of genes, which are mentioned above. Although PGC-1α's effect on three groups is suggested to have a minor impact at a low mitochondrial toxic concentration to alter ROS and ATP production, its effect on the groups is essential at a high level of mitochondrial toxins. It is reported that DOX's toxic effect is related to increasing mitochondrial ROS production and the most destructive impact of DOX appears in cardiac tissue due to its accumulation in cardiac cells [38].

It is well known that heart tissue contains a high cell volume of mitochondria, almost 35% due to the requirement of energy supply because of maintaining the contraction function of the tissue [15]. The heart produces energy requirement by using β-oxidation of fatty acid in mitochondria. The cardiotoxicity of DOX might be related to swelling and destroy bioenergy from the organelle and myofibril of cardiac tissue. DOX also increases oxidative stress, resulting in enhancing mitochondria dysfunction. The other mechanism of DOX on mitochondrial malfunction is reported to be associated with the dissipation of mitochondrial ETC at different levels. So, NADH and succinate oxidase in cardiac tissue have been shown to be blocked by DOX treatment. Also, DOX separates complex I from ETC, resulting in the elevation of oxidative damage by producing semiquinone free radicals. Moreover, DOX might inhibit stage-3 and stage-4 respiration. The other mechanism for blocking mitochondrial function is related to the prevention of Mg-dependent F0F1-ATPase in muscle, including heart and

skeletal muscle. The other mechanism associates with DOX's structure since DOX has a high affinity to bind cardiolipin, which is one of lipids and locates at the inner mitochondrial membranes. DOX's toxicity on mitochondria is related to its dose and is time dependent. Mitochondrial toxicity of DOX has been observed from 2 to 13 weeks at a low dose. Moreover, mitochondria play an essential role in the regulation of calcium homeostasis. Under normal physiological conditions, there is little impact of mitochondria on calcium homeostasis. However, the mitochondrial function of calcium homeostasis is essential under pathological circumstances to decrease cytosolic calcium. So, calcium efflux into mitochondria depolarizes MMP. DOX's effect on calcium homeostasis at mitochondria is based on inhibition of the inward calcium flux and exaggeration of the release of calcium from mitochondria by swelling of the mitochondria–calcium-dependent pathways through MPT, resulting in enhancing calcium concentration at the cytosol. Furthermore, DOX at a low dose gives rise to a decline in the calcium storage capacity of mitochondria. DOX attenuates mitochondria calcium storage capacity exaggerated at its high dose, which means it is related to dose. Eventually, the effects of DOX lead to the dissipation of MMP. The mitochondrial permeability pore is highly sensitive to MMP and regulated by the redox status of mitochondria. DOX also opens MPT in the manufacture of ROS through complex I, which is mentioned above (**Figure 5**). Thus, DOX's MPT effect might be based on the prevention of ANT, an element of the MPT pore complex. It is interesting to note that DOX-treated cardiomyocytes have fewer ATP levels (~30%) vs. normal cardiomyocytes [15].

It is suggested that ROS produced by mitochondria has a critical role in DOX's cardiotoxicity. Hepatic tissue plays a role in detoxification by the cytochrome P450 enzyme in the ER, which also occurs in DOX's redox cycling. However, this bioreductive activation of DOX by cytochrome P450 in the heart is much less than in the liver. So, ROS production and DOX redox cycling in the ER in the heart are negligible [13]. Because it is continuously working, the heart needs ATP by β-oxidation in mitochondria. Therefore, cardiomyocytes contain tremendous mitochondrial density (around 25–30%) vs. the other cell types. This is why ROS production by mitochondria is significant compared to the other compartments of cardiomyocytes in heart tissue [13]. DOX has been reported to have high affinity to bind to cardiolipin from inner mitochondria. Also, DOX inhibits complexes II–IV from ETS [13]. Another suggestion is that DOX affects complex I, II, III, and IV [42]. DOX can also alter mitochondrial membrane organization but not physically interact with mitochondrial enzymes. According to isolated mitochondria and in vivo studies, DOX can capture an electron from complex I, resulting in a decline in oxidative phosphorylation and elevated oxygen consumption [13].

DOX elevates the nonphosphorylating rate of oxygen consumption (state 4) and decreases phosphorylation-linked oxygen consumption (state 3). Therefore, DOX triggers the manufacture of superoxide. Also, these impacts lead to declining ATP synthesis. Energy stress induced by DOX eventually prefers other pathways for producing ATP, e.g., glycolysis. However, glycolytic ATP does not have sufficient energy to maintain cell function. The metabolic switch is a requirement to make alternations such as enhancing glucose transporter type 1 (GLUT1) transportation to plasmalemma. So, glucose uptake increases by using GLUT1 within 1 h of DOX treatment [13].

Some compensating mechanism is recommended to modulate energy supply to maintain cellular function, e.g., CK or AMPK. Studies have shown how to decrease ATP and phosphocreatine (PCr). One μM DOX concentration has indicated a decline of ~50% ATP production within 24 h. Why is the reduction essential to utilize around 90% ATP synthesized by mitochondria? When mitochondrial function is destroyed, heart or tissue function is automatically affected by low energy supply. DOX's mitochondrial effect is shown to change the ultrastructure, swelling, and oxidative capacity as well. Although DOX inhibits state 3, state 4 is activated by the drug. DOX is highly bound to cardiolipin, one of the anionic phospholipids from the inner membrane of mitochondria. When DOX binds to cardiolipin, enzymes from respiration and oxidation, e.g., cytochrome-*c*, might be inactive due to the alternation of the lipid environment. After binding cardiolipin to DOX, cardiolipin-related proteins such as cytochrome-*c* and mitochondrial creatine kinase (MtCK) are released from the mitochondrial inner membrane to the cytosol. Besides the indirect effect of that enzyme, DOX also has a direct effect on these enzymes. The mitochondrial impact of DOX can amplify its toxic effect by using the target organelle's enzyme system. DOX inactivates NADH dehydrogenase, cytochrome P-450 reductase, and xanthine oxidase. Moreover, DOX tends to accumulate in nuclei and mitochondria vs. plasma. The heart mainly utilizes fatty acid to generate energy by β-oxidation. So, DOX also destroys β-oxidation by inhibiting of consumption of palmitate, a long chain fatty acid, through impairment of carnitine palmitoyltransferase I (CPTI) and/or its substrate L-carnitine. DOX elevates glycolysis as a compensatory response to a decline in fatty acid oxidation. However, some studies' results show a decrease in both fatty acid and glucose oxidation by using cell line and rat models. Why glucose utilization is decreased after DOX treatment is explained by two theories. One is that DOX might reduce glucose supply. It is reported that DOX treatment initially increases glycolysis (~50%; within 1 h of exposure to the drug), but later depresses it sharply. The second explanation is that DOX reduces phosphofructokinase (PFK) activity, one of the rate-limiting enzymes of glycolysis. So, one reason for DOX's low-energy generation is that it disrupts cell metabolism tissue. The other reason is the effects of DOX's CK system. There are two CK isoforms in the heart: cytosolic and mitochondrial (MtCK). CK can easily produce PCr from creatine. Transformation of creatine to PCr closely binds between energy generation and utilization. MtCK, an octameric, is accompanied by ANT) and VDAC (porin), so transforming energy by the generation of oxidative phosphorylation to the cytoplasm. However, cytosolic CK isoform (MM-, MB-, BBCK), a dimeric, links to both energy manufacture by glycolysis and energy consumption, including actomyosin ATPase at myofibrils, the Ca^{2+}-ATPase at the SR, Na^{+}, and K^{+}-ATPase in the sarcolemma [43]. DOX inactivates the entire CK system, especially MtCK. MtCK's effects dissociate its structure from octamer to dimer, resulting in dissociation from the mitochondrial inner membrane. Moreover, cardiac MtCK has been reported to be more sensitive to DOX than ubiquitous MtCK, leading to selective toxicity in heart tissue. The inactivation of DOX on MtCK has been indicated to be linked to the drug's dose. At a low dose below 100 μM, MtCK became inactive because of DOX's redox modification from its cysteine residues. Its high dose, however, depresses MtCK due to ROS production. Furthermore, DOX and MtCK have been indicated to have a common feature, i.e., they tend to attach to an inner mitochondrial membrane. So, the feature provides high DOX concentration around the MtCK. Additionally,

when DOX is activated by peroxidase/H_2O_2, CK inhibition via DOX accelerates. The inhibition link to oxidative and nitrous stress means that CK is very vulnerable to the redox status of cells. Even a μM DOX concentration has been reported to lead to dimerization of MtCK and augments inhibition and dimerization at a 20 μM concentration. Also, it is indicated that total CK activity has been noticed to reduce (by nearly 20%) for DOX treatment compared to 20 μM concentrations. Even under this circumstance, CK can still maintain its function due to a compensatory mechanism that causes to reduce muscle-type CK (MCK) (a myofibrillar isoform) and elevate brain-type CK (BCK; a fetal isoform) that is raised by heart failure or cardiac hypertrophy. It is important to know that CK shift is reported to be within 1 h at 2 μM DOX treatment. So, CK system dysfunction might probably participate in DOX-mediated heart failure. MtCK inhibition by dimerization not only causes energy transfer from mitochondria to the cytosol but also affects the mitochondrial respiratory chain. Moreover, inhibition destroys the three-modal interaction between MtCK, ANT, and DAC, which means that MtCK plays a role in MPT. So, destruction of modal interaction could trigger apoptosis as well. Besides programmed cell death, myofibrillar CK functionally integrates with the sarcoplasmic Ca^{2+} pump (SERCA). When a CK defect occurs, cytosolic Ca^{2+} balance is destroyed, leading to defects in contraction and relaxation coupling due to Ca^{2+} accumulation. Ca^{2+}accumulation could trigger apoptosis as well. This is why dysfunction of CK causes innate apoptosis in two ways. When energy disruption occurs such as CK dysfunction, AMPK is activated to regain energy balance. AMPK is one of the sensory energy proteins that compensates for shifting from ATP to ADP and/or AMP. It means that AMPK is highly sensitive to a ratio of AMP/ATP and oxidative stress as well. Under energy stress, AMPK changes the metabolic activity of cells to increase ATP synthesis by elevation of fatty acid oxidation, glycolysis, and a decline in ATP utilization. All these processes are crucial to surviving cells to maintain protein, lipid, and carbohydrate manufacture. It is reported that DOX inhibits AMPK, resulting in energy stress. In a study by using isolated heart, DOX at 2 μM, which is the plasma peak value of the patients treated with the drug, was reported to cause to plume AMPK and acetyl-CoA carboxylase proteins after 1-h perfusion. Therefore, further study is needed to evaluate the mechanism. However, it is suggested that DOX causes energy and oxidative stress in both reactive and nitrogen stress. AMPK inhibition means that DOX leads to a change in metabolic activity of cells by a decline in fatty acid oxidant. How the fatty acid oxidant decreases relates to enhancing acetyl-CoA carboxylase, resulting in CPTI by malonyl-CoA, eventually leading to a decrease in mitochondrial fatty acid oxidation. Besides the decline of mitochondrial fatty acid oxidation, AMPK inhibition also causes to reduce glycolysis by decreasing of PFK and glucose uptake as well. Under physiological conditions, energy stress is expected to activate AMPK [43].One study showed that AMPK, glucose, and fatty acid is related to gene and protein expressions, and acetyl-CoA carboxylase have been decreased by DOX in males more than in females. AMPK is also a crucial function for cardiolipin synthesis and remodeling. By AMPK, PGC-1α/β modulates cardiolipin synthesis as well [36].

6.4. Doxorubicin's effect on myocardial energy metabolism

It is well known that heart tissue contains a high cell volume of mitochondria nearly (25–35%) [3, 15] due to the requirement of energy supply to maintain the contraction function of the

tissue [84]. The heart produces an energy requirement by using the β-oxidation of fatty acid in mitochondria [15]. Adequate ATP production is not just significant to maintain contractile function, but is also crucial for protein synthesis, controlling the protein quality function of ER, cytoskeletal function, and to clear the cellular waste from lysosomes. This is why DOX destroys energy production systems and has been reported to destroy the protein degradation function, resulting in overwhelming the ER and mitochondria [4].

The impact of DOX on bioenergetics and oxidative stress might partially be associated with its structure because it has a high affinity to bind to cardiolipin, which is one of the lipids and locates at the inner mitochondrial membrane [13, 43]. ROS production by mitochondria might be a significant contributor to the drug's toxicity in heart tissue [13]. This is why mitochondria is one of the targets of multiple factors such as drugs including DOX and environmental compounds. There are defined control systems that maintain healthy, functional mitochondria. One of the systems is PGC-1α, which plays an essential role in the regulation of mitochondrial function, including production of bioenergy and energy homeostasis. PGC-1α can regulate gene transcription, including mitochondrial functions such as NRF-1, PPARa, and ERRa, which modulate and divided three groups in metabolic enzymes in the TCA cycle: antioxidant enzymes, other mitochondrial protein components of the ETC complexes, and TFAM [38]. When mitochondrial dysfunction occurs, i.e., enhancing ROS and reducing ATP synthesis, a complex transcriptional network, including PGC-1α, can be triggered to maintain cellular homeostasis. PGC-1α can transcribe three groups of genes, which are mentioned above. Although PGC-1α has an effect on three groups it is suggested to have a minor impact at a low mitochondrial toxic concentration to alter ROS and ATP production; its effect on the groups is important at high concentration of mitochondrial toxins. It is reported that DOX's toxic effect is related to increasing mitochondrial ROS production and the most destructive impact of DOX can appear in cardiac tissue due to its accumulation in cardiac cells [38]. One study has noticed that DOX led to a decrease in PGC-1α and its related genes, including NRF1, TFAM, SOD2, CS, VDAC, and COXIV. PGC-1α is phosphorylated by AMPK, and is also modulated by acetylation by SIRT1. The posttranslational modification of PGC-1α is a potent mechanism for mitochondrial function by oxidative stress and apoptosis. So, SIRT1 is suggested to decline mitochondrial dysfunction and cardiotoxicity induced by DOX [16]. The other control system is SIRT-3 which is one of NAD-dependent deacetylases and places at mitochondria. Deacetylase plays a crucial role in maintaining healthy mitochondrial function by deacetylation of metabolic, apoptotic, and ROS-production enzymes. Also, SIRT-3 has been shown to enhance Foxo-3a, which is associated with an antioxidant mechanism. Thus, SIRT-3 closes the MPT by blocking Cyp D activity. SIRT-3 has a modulating effect on cardiac hypertrophy via strengthening the LKB1 event, which is one of the upstream kinases of AMPK. One study has reported that SIRT-3 is decreased by DOX treatment due to the rise in ROS production and mitochondrial dysfunction. So, reducing SIRT-3 leads to elevation to ROS and HIF1α stabilization, which is an essential factor to shift metabolism from β-oxidation of fatty acid to glycolysis (the Warburg effect) [85]. Further, the inhibition of protein or slicing of the HIF1α gene has been suggested to decrease drug resistance against DOX [86].

It is well known that the heart can produce ATP from fatty acid. However, metabolic shift is developed from fatty acid to glucose due to a compensating energy demand under pathologic

conditions. So, the expression of peroxisome proliferator-activated receptor gamma is also affected by the drug, resulting in decline in adipogenesis and destruction of glucose intake via glucose transporter type 4 (GLUT4) [34]. In contrast, according to previous study results, 1 h after 1 μM of DOX was given to cultured adult rat cardiac cells, sarcomeric titin protein was reported significantly to degrade via the calpain-dependent mechanism. DOX increases glucose uptake by GLUT1 into plasma membrane [24], which is a requirement of the metabolic switch in the first hours of treatment [13]. However, it is reported that GLUT4 was not affected by DOX [24]. Also, DOX impairs PFK, which is a rate-limiting glycolytic flux [24, 34]. Energy stress induced by DOX eventually prefers the other pathways for producing ATP, e.g., glycolysis. However, glycolytic ATP does not have sufficient energy to maintain cell function [13]. The cardiotoxicity of DOX might relate to swelling and destroy bioenergy from the organelle and myofibril of cardiac tissue. DOX also increases oxidative stress, resulting in enhancing mitochondrial dysfunction [15].

The other mechanism of DOX on mitochondrial dysfunction is reported to be associated with dissipation of mitochondrial ETC at different levels. So, NADH and succinate oxidase in cardiac tissue has been shown to be blocked by DOX treatment. Also, DOX separates complex I from ETC, resulting in elevated oxidative damage by producing semiquinone free radicals [15]. Also, DOX inhibits complexes II–IV from ETS [13]. DOX disrupts complex I, III, and IV, and is especially susceptible to complex I and IV [34]. Specifically, DOX decreases the content of the complex I NDUFB8 subunit and the ATP synthase ATP5A subunit [87]. The opinion of others is that DOX can alter mitochondrial membrane organization but not physical interaction with any mitochondrial enzymes [13]. In other words, DOX affects all complexes from I to IV [42]. There is a discrepancy between DOX's effects on the stage of respiration. One of the studies suggested that DOX might inhibit state 3 and state 4 respiration [15]. Although others have reported that DOX impedes state 3, which is phosphorylation-linked oxygen consumption, it is activated by the drug [13, 43]. Therefore, DOX triggers superoxide manufacturing. Also, this impact led to declining ATP synthesis [13]. The other mechanism for blocking the mitochondrial function is related to prevention of Mg-dependent F0F1-ATPase in muscle, including heart and skeletal muscle [15].

The ATP pool of cardiac tissue is reported to be 5 mmol/kg wet heart weight. When the demand for energy in the heart is increased, PCr (concentration around 10 mmol/kg wet heart weight) can compensate for the requirement. PCr can be transformed by CK, one of the energy reservoir regulators [34]. Interestingly, total ATP decline due to DOX treatment on cardiomyocytes vs. normal, healthy cardiomyocytes is reported to be ~30% [15]. Some compensating mechanism is recommended to modulate energy supply to maintain cellular function, e.g., CK or AMPK. Studies have shown that ATP and/or PCr decrease by using the drug. One μM DOX concentration has indicated a decline of ~50% ATP production within 24 h [43]. PCr is destroyed by DOX treatment due to the accumulation of ferrous iron by the drug. So, DOX declines both ATP and PCr. Children treated with DOX for 4 years have been reported to have decreased PCr/ATP ratios of around 20% [34]. Why the decline is vital to utilize around 90% ATP synthesized by mitochondria is because heart or tissue function is automatically affected by low energy supply when mitochondrial function is destroyed [43].

The other mechanism of energy dysfunction due to DOX treatment is AMPK destruction [87]. It is well known that the heart mainly utilizes fatty acid to generate energy by β-oxidation [43]. DOX inhibits fatty acid β-oxidation and myocardial function as well, but enhances glucose intake though AMPK phosphorylation [88]. AMPK inhibition means that DOX leads to a change in the metabolic activity of cells by declining fatty acid oxidant, particularly palmitate consumption. How the fatty acid oxidant decreases relates to enhancing acetyl-CoA carboxylase by AMPK inhibition, resulting in a decline in CPTI and/or its substrate L-carnitine by malonyl-CoA, eventually leading to a decrease in mitochondrial fatty acid oxidation [43]. Under physiological conditions, energy stress activates AMPK [43]. DOX elevates glycolysis as a compensatory response to a decline in fatty acid oxidation. However, some study results showed a decrease in both fatty acid and glucose oxidation by using cell line and rat models. Why glucose utilization is reduced after DOX treatment is explained by two theories. One is that DOX might minimize glucose supply. It is reported that DOX treatment initially increases glycolysis (~50%; within 1 h of exposure to the drug), but later causes it to depress sharply. The second explanation is that DOX leads to reduced PFK activity, one of the rate-limiting enzymes of glycolysis [43].

When energy disruption occurs such as CK dysfunction, AMPK is activated to regain energy balance. AMPK is one of the sensory energy proteins that compensates for shifting from ATP to ADP and/or AMP. It means AMPK is highly sensitive to a ratio of AMP/ATP and oxidative stress. Under energy stress, AMPK changes the metabolic activity of cells to increase ATP synthesis by elevation of fatty acid oxidation, glycolysis, and a decline in ATP utilization. All these processes are crucial to the surviving cell by maintaining proteins, lipids, and the manufacture of carbohydrate. It is reported that DOX inhibits AMPK, resulting in energy stress [43]. Another study noticed that AMPK can be inactivated with a 2 μM concentration of DOX. This explains how DOX can change substrate utilization to produce energy [24]. There is no clearly understood process as to how AMPK is inhibited. Therefore, further study is needed to evaluate the mechanism. However, it is suggested that DOX causes energy and oxidative stress in both reactive and nitrogen stress [43]. Kinase is also a crucial function in cardiolipin synthesis and remodeling. By AMPK, PGC-1α/β modulates cardiolipin synthesis as well [36]. Moreover, DOX could destroy desmin interaction with mitochondria, resulting in triggering apoptosis [89]. When our knowledge of AMPK, cardiolipin, DOX, and PGC1α/β are superimposed, it can easily be understood that DOX-induced cardiac mitochondrial toxicity is more complex and multifactorial. Metabolic dysfunction induced by DOX might also relate to gender [36].

One pathway of DOX's low-energy generation disrupts cell metabolism tissue. DOX destroys CK as an energy shuttle and storage system, AMPK as an energy-sensing and signaling system [24], and the channel of ATP and PCr from mitochondria to the cytosol. PCr and creatine can regulate the ATP/ADP ratio [14]. This is why the mechanism of DOX's mitochondrial energy dysfunction can be explained by DOX's cardiac cell metabolism CK system effects [43]. CK in the heart has two isoforms. One is located free at the cytosol (cytosolic CK (cCK)), and the other is bound to sarcoplasmic or mitochondrial membranes [14, 43]. cCK has two subtypes: muscle-type MCK and brain-type BCK. Also, MtCK has two subtypes known as sarcomeric MtCK (sMtCK) that exist only in the heart and skeletal muscles and ubiquitous MtCK (uMtCK) that is present in other organs and tissues, such as the brain, spermatozoa,

and skin. Cardiac cCK has two forms as a homodimer (MMCK and/or BBCK) or heterodimer (MBCK), which is a cardiac-specific form [24]. According to this knowledge, it is said that MBCK can usually be determined as an indicator of a heart attack. sMtCK is the mainly octameric form [24] and places the outer intermembrane space and mitochondrial cristae between membranal protein ANT in the inner membrane and VDAC in the outer layer [24]). MtCK has high affinity to cardiolipin [14] and the outer surface of the inner mitochondrial membrane [24]. It must not be forgotten that DOX has a great relationship with cardiolipin. So, one of DOX's targets is MtCK. Moreover, DOX oxidizes MtCK at cysteine residues. Besides oxidation, DOX leads to inactivation of MtCK, resulting in enhanced embryonic CK isoform expression [14].

MtCK can efficiently produce PCr from creatine [24, 43]. Transformation of creatine to PCr firmly binds between energy generation and utilization. MtCK, an octameric, accompanies ANT, and VDAC (porin) [43]. ANT can transfer ADP to matrix space. Then, ADP resynthesizes ATP through oxidative phosphorylation. However, PCr can be sent to the cytosol via VDAC [24]. PCr is utilized by cCK to maintain the subcellular local ATP/ADP ratio [24].

Although DOX inactivates all CK, MtCK is especially destroyed [43] by the drug through dissociation of its structure from octamer to dimer [24, 43] or infusion of binding MtCK at mitochondrial membranes, such as cardiolipin [24]. Moreover, cardiac MtCK has been reported to be more sensitive to DOX than uMtCK, leading to selective toxicity in heart tissue. The inactivation of MtCK by DOX is linked to the drug's dosage. At a low dose below 100 μM, MtCK's inactivation occurs because of DOX's redox modification from its cysteine residues. Its high treatment, however, depresses MtCK due to ROS production. Furthermore, DOX and MtCK have been indicated to have a standard feature, they tend to attach an inner mitochondrial membrane, providing high DOX concentration around the MtCK. Additionally, when DOX is activated by peroxidase/H_2O_2, CK inhibition via DOX accelerates. The inhibition is linked to oxidative and nitrous stress, which means that CK is very vulnerable to the redox status of cells. Even a 2 μM DOX concentration has been reported to lead to dimerization of MtCK (ordinarily octameric), and augment the inhibition and dimerization at a 20 μM intensity. Also, it is indicated that total CK activity has been noticed to reduce (by nearly 20%) for DOX treatment concentrated at 20 μM. Under this circumstance, CK has still been maintaining its function due to a compensatory mechanism, which reduces MCK (a myofibrillar isoform) and high BCK (a fetal isoform) that is elevated by heart failure or cardiac hypertrophy. It is vital to know that CK shift is reported to be within 1 h at 2 μM of DOX. So, CK system dysfunction might probably participate in DOX-mediated heart failure. MtCK inhibition by dimerization not only causes energy transfer from mitochondria to the cytosol but also influences the mitochondrial respiratory chain. Moreover, this inhibition destroys the three-modal interaction between MtCK, ANT, and VDAC, which means that MtCK plays a role in MPT. So, damage to the modal interaction could first trigger apoptosis. Besides programmed cell death, myofibrillar CK functionally integrates with SERCA. When a CK defect occurs, cytosolic Ca^{2+} balance is destroyed, leading to defects in contraction and relaxation coupling due to Ca^{2+} accumulation. Ca^{2+}accumulation could also trigger apoptosis. This is why dysfunction of CK causes innate apoptosis in two ways [43].

Besides the CK shuttle, the malate–aspartate shuttle (MAS) has a role in declining traffic equivalents between mitochondria and the cytosol. Moreover, TCA and MAS have demonstrated to be associated with each other physically. This interaction provides a direct reason for metabolic alternation of the mitochondrial matrix to the cytosol. MAS suppression in the heart has been proposed to reduce mitochondrial respiration before cardiac damage, thereby declining oxidative injury. A cancer cell produces energy by glycolysis known as the Warburg effect. So, it is suggested that MAS inhibition might be an excellent candidate for overt DOX toxicity, without affecting the anticancer drug's effects [29].

DOX directly affects oxidative phosphorylation enzymes, e.g., NADH dehydrogenase, Rieske iron sulfur protein, succinate dehydrogenase, cyclooxygenase, CK, carnitine palmitoyltransferase, fatty acid β-oxidation-related enzymes, as well as the translocation of phosphate and pyruvate to the mitochondrial matrix. DOX reduces fatty acid β-oxidation by blocking fatty acid transfer protein to mitochondria, resulting in an increase in the pyruvate dehydrogenase complex, which is a rate-limiting enzyme of glycolysis. The other effect of DOX metabolism is that the triosephosphate isomerase enzyme essential for glucose metabolism can be inhibited by the treatment [14]. The anthracyline causes mitochondrial dysfunction, e.g., displacing α-enolase from mitochondria [30]. ATP synthesis is decreased in both the cytosol and mitochondria by DOX [90].

7. Conclusion

It is impossible to ignore DOX therapy from cancer patients' treatment due to its inevitable chemotherapeutic efficiency on a variety of cancers. Unfortunately, there is limited knowledge available on DOX's cardiotoxicity, particularly mitochondriopathy. This is why the molecular clarifying mechanism of DOX's myocardial and mitochondrial toxicities will hopefully overcome the side effects and increase the survival rate of cancer patients as well. Therefore, further studies are needed to evaluate the detrimental effects of DOX on mitochondria to restore its limited utilization in cancer patients' therapy.

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