Chapter

Overview on the Side Effects of Doxorubicin

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Abstract

Doxorubicin is an anthracycline antibiotic extracted from the bacterium Streptomyces peucetius. Its cytotoxic effect produced by intercalating with DNA causing breakdown of DNA strand which causes cancer cell apoptosis. Despite being an effective anticancer agent it causes several crucial side effects like carditoxicity, neuropathy, hepatotoxicity, nephrotoxicity, alopecia, typhlitis, myelosuppression, neutropenia, anaemia, thrombocytopenia, nausea, and diarrhoea were caused mainly due to the inability to distinguish between cancer cells and normal cells. This chapter mainly focuses on doxorubicin's side effects, current understanding of the molecular mechanisms, and management and preventive strategies of doxorubicin's cardiotoxicity during the treatment of various type of cancer.

Keywords: doxorubicin, nephrotoxicity, neurotoxicity, hepatotoxicity, cardiotoxicity

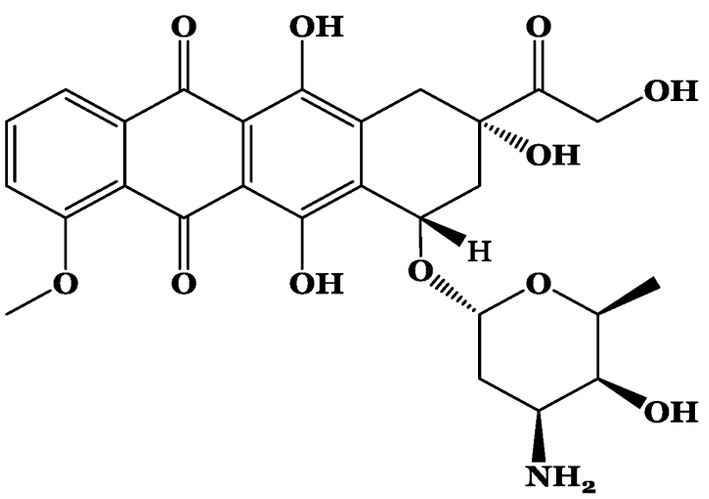
1. Introduction

1.1 Historical background

In the 1950s Italian based company; Farmitalia research laboratory began a research program in finding the anticancer compounds from soil-based microbes. In the process of research collected a soil sample from the castle named as castle Del monte, which was built in the 13th century. The collected soil sample contains new strains of bacterial species and isolated from it. The separated microbe recognised with the name Streptomyces peucetius which is typically produces a significant red pigment. The antibiotic produced from this bacterium discovered to be efficient in treating the tumours especially solid tumours while researching on mice. Since a group of French scientists found the same compound about at the same time, they agreed to call the antibiotic daunorubicin, referring to the two nations. In which, Dauni refers to the pre-Roman tribe who inhabited the position in Italy where the species of bacteria were isolated and ruby represents the colour in Italy. The clinical trials of daunorubicin were began in 1960s and confirmed as successful in treating the lymphoma and acute leukaemia [1, 2]. After a short note of the time, in 1967 daunorubicin was discovered to be cause fatal cardiotoxicity. Then, by using nitroso-N-methyl urethane, the Italian research company mutated the strains of Streptomyces peucetius and developed a new strain of bacterial species that produces 14-hydroxylated daunorubicin, also known as Adriamycin (named after the Adriatic Sea), then changed its name to doxorubicin, which has a strong therapeutic index, but cardiac toxicity remains [3].

1.2 Doxorubicin chemical structure and properties

Doxorubicin (DOX) is an anthracycline antibiotic structurally similar to Daunorubicin as natural anti-cancer antibiotic used in cancer treatment. Its anti-cancer effect produced intercalating with DNA and this will inhibit DNA transcription and replication; and by binding to the topoisomerase II enzyme and inhibit the resealing of the DNA fragments. The presence of sugar moiety attached to the anthracycline ring further enhances the binding to phosphate and sugar moieties in to DNA. This led to stops the proliferation of cancer cells in the host [4]. Besides, the presence of quinone moiety apart from contributing the cytolytic ability by generating the intermediate radicals, which further react with the oxygen and forms superoxide ions and these ions also shows a high tendency towards the damaging the cell membranes causes a dose-dependent the cardiac myopathy [5, 6].



The Doxorubicin is mainly used in case of patients suffering from Breast cancer, ovarian cancers, lung cancers, bladder cancers, leukaemia (acute lymphoblastic leukaemia, acute myeloid leukaemia) and AIDS-related Kaposi's sarcoma and various solid tumours. DOX also used in combination with other agents in case of bone sarcomas, soft tissue sarcomas, uterus cancer, endoblastoma cancer, cervix cancer, pancreatic cancer, Ewing's sarcoma, mesothelioma, multiple myeloma, Wilms tumour and in neuroblastoma [7, 8].

2. Doxorubicin side effects

2.1 Hepatotoxicity

Liver is one of the essential organs of the body; it plays a major role in metabolism and detoxification of several drugs. This can explains why liver is the primary body organ affected by chemotherapy. Despite being cytostatic and cytotoxic effects on cancer cells DOX documented to accumulate in the various tissues include liver cells. In humans, it is estimated as 50% of DOX eliminated in un-exchanged form, the remainder dose metabolised through hydroxylation, semiquinone formation [9]. The major pathway for biotransformation of DOX is catalysed by the NADPH-dependent carbonyl reductase, Nitric oxide synthase, cytochrome P-450 reductase, aldo-keto reductase enzymes. The hydroxylation occurs at C-13 carbon in group commonly reaction referred as electron reduction forms the secondary alcohol metabolites [10–13].

The metabolized intermediates in the presence of oxygen converted to carbonyl moieties resulting in generation of Superoxide anions and hydrogen peroxides causes peroxidation of lipids in membranes of cell, aggregation of proteins (**Figure 1**) [13–15].

The regenerative capacity of liver is more can cure the damage caused by various agents such as DOX, which causes damage and decreases the regeneration of liver cells by increasing the oxidative stress due to the radical generation by oxidation in hepatocytes. The generated radical causes decrease in GSH levels, damages in DNA and also act as secondary metabolites in in many metabolic pathways which includes in cell proliferation and cell death [16–18]. To overcome such situations liver employs the efflux mechanisms, the efflux of DOX is achieved through from liver by ATP dependent ABC proteins (P-glycoprotein) which increase the efflux of the intracellular DOX and maintain the homeostasis. The mechanism uses large quantity of energy but with the presence of the DOX in liver cells decreases the ATP production and increases the ADP and Pi within the cells [19–21]. Due to this effect sometimes liver cells can't able to regenerate from DOX induced effects and causes hepatotoxicity.

2.2 Nephropathy

Besides maintaining the homeostasis by regulating the body fluids, kidneys work to reabsorb the low concentrations general constituents in the body and also remove the foreign substances like drugs or other kinds of agents. For this kind of reasons kidneys considered as metastatic organs of human beings [22]. The regenerative capacity of the kidneys is low when compared to the liver and highly susceptible to

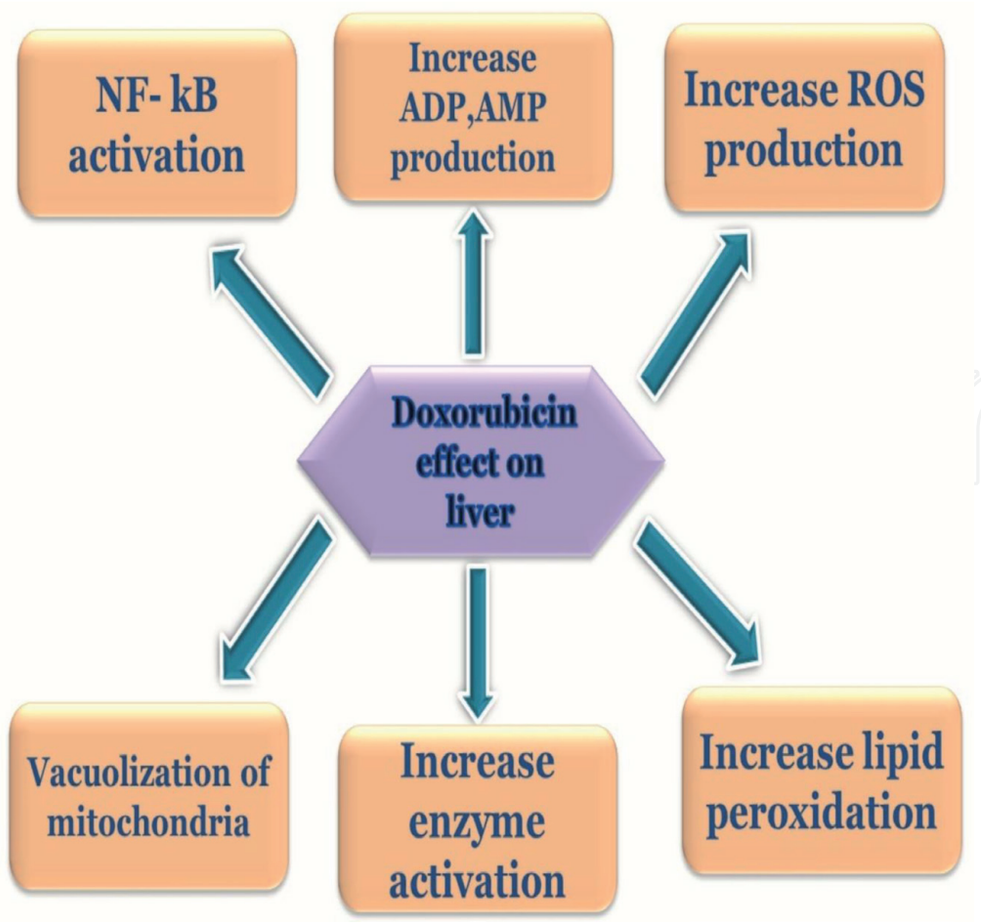


Figure 1.
DOX mediated effects on the liver.

epithelial degeneration occurs at renal glomerulus where the filtration occurs may lead to the glomerulosclerosis [23].

DOX interferes with the glandular podocytes of the kidney and cause nephropathy the most accepted mechanism behind the nephropathy is an accumulation of proteinuria in the kidney by the local passage of leaked proteins [23]. Increase in the structural changes in nephrons causes hypertension, steroid resistance, high incidents of renal failure and glomerular vacuolization, inflammation, tubular dilation, intestinal fibrosis, permeability differences in the glomerulus, and certain conditions like hypoalbuminemia, dyslipidemia, hypercoagulation, size differences in kidney most likely observed [24]. A study conducted on the DOX effect on the mitochondria by the Lebrecht suggested that DOX interfere the mitochondrial mtDNA in the kidney with ROS produced from it and accelerating the damaging of the nephron. Another study reports suggesting that DOX forming an iron-mediated anthracycline complex, which produces the ROS led to an increase in the oxidative lesions in the cells causing damage to the critical cellular components [25, 26].

The decreasing the levels of the GSH (Glutathione), vitamin E levels and other natural oxidant levels production from the liver cells enhances the nephropathic conditions which may initially affect the Bowman's capsule thickness and the glomerular tuft of the nephron. The study conducted by Rook et al. [26] Reported as Angiotensin-converting enzyme is said to be one of the responsible factors for tissue damage triggered by the DOX therapy. The ACE is causing the pro-inflammatory, pro-fibrotic effects which make interference in the kidney and nephrons to maintain the glomerular pressure and filtration rate of blood [27, 28]. The cases of nephropathy and proteinuria are rare in humans susceptibility towards such condition based on the genetic makeup of the individual.

2.3 Neurotoxicity

The brain is the largest and most complex organ in the human body contains about 100 billion neurons with 1 trillion established connections throughout the body. DOX is not able to transfer through the blood–brain barrier (BBB), therefore DOX effects against the brain via indirect way [29, 30]. These effects include: depression, anxiety, decrease in motor functions, haemoglobin levels, perception skills affected, and menopausal status, visuospatial skills are affected through cancer chemotherapy. The recovery of the cognitive functions may take up to a year [31]. The DOX mediate increase TNF-α level (inflammatory cytokines produced by the macrophages/monocytes during the acute inflammation involved in many signalling pathways) in the brain at cortex and hippocampus of mice [32–34]. The mitochondrial activity, glutathione-S-transferase, GSH levels, and MnSOD levels in the brain are decreased and increase in levels of 4-hydroxynoneal (HNA), thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA) and increase in levels of protein carbonyl groups [35–38], which causes increase the oxidative stress in the brain cells and further led to cause cell damage.

The MnSOD levels in the brain generally detoxify the oxygen free radicals, inactivated by the Nitric oxide (NO). The DOX indirectly increased the concentration of NO by overexpressing the Nitric oxide synthase enzyme [37]. A study conducted on the NOS dependent brain injury with DOC reinforcing the nitric oxide tissue damage [38]. The mitochondrial activity is very important in the brain because it is a powerhouse of cells (energy production) brain uses 20% of body glucose for energy production to conduct and maintain the regular activities [39]. The DOX induces generation of MDA, TBARS, and HNA which cause the decrease the mitochondrial activities. A study conducted on the DOX-induced toxicity on rats with 10 mg/kg dose, the rats died between 10 and 50 days with observed

light microscopic studies reveals that specific changes in the ganglionic cells of the peripheral nervous system [40].

2.4 Cardiomyopathy

This side effect found to be a dose-dependent on DOX. The DOX-induced cardiotoxicity occurs acutely and chronically. The acute effects occur within one week period the patient may experience arrhythmia, hypotension, and super ventricular tachycardia. These abnormalities are generally reversible in a noticeable period [41]. The chronic effects are shown in only 1.7% of patients with a 50% mortality rate [42, 43]. The chronic effect of DOX such as congestive heart failure reported in a study, when the patients are treated with dose 500–550 mg/m^2 in more than 4% of patients when treated with the dose is 551–600 mg/m^2 18% of patients cause the CHF, and almost 35% of patients observed with CHF when treated with >601 mg/m^2 [44, 45].

A study conducted by the Zordosky and EI-kadi on DOX-induced toxicity reported as the induction of Brain natriuretic peptides, atrial natriuretic peptides genes, monooxygenases, cytochrome P genes and hypertrophy markers responsible for the xenobiotics and certain endogenous substances [46]. The inductions of these genes are cause cardiac hypertrophy leading to heart failure and altered the arachidonic acid mechanisms. A study reported the DOX effects based on the concentrations, at low concentrations DOX dose (o.5–1 μM) causes the alterations in structural proteins (includes sarcomeric myosin, nuclear lamina), plasma membrane blebbing (causes change in cell shape), and mitochondrial depolarization and fragmentation. At high concentration causes (5–50 μM) causes the cytoplasm vacuolization, swelling of mitochondrial cells, promote the cellular alterations (**Figure 2**) at the cellular and nuclear membranes [47]. The DOX reportedly binds

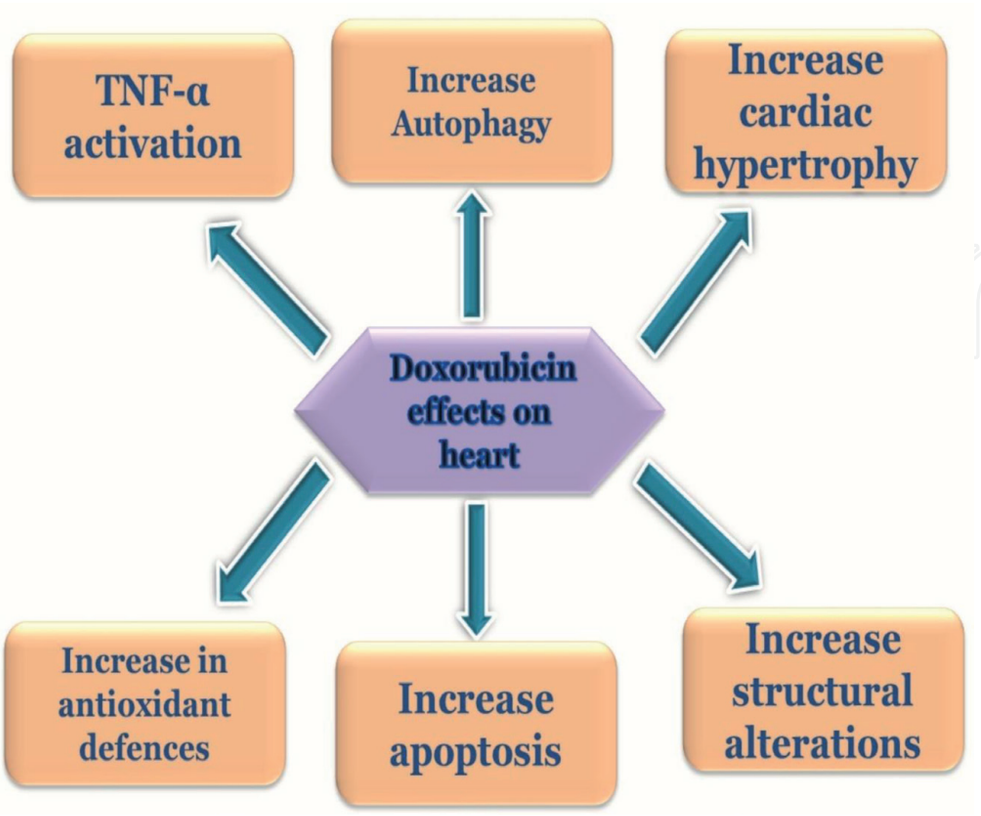


Figure 2.
DOX-mediated effects on the Heart.

to the cardiolipin (a mitochondrial inner membrane component), which raises the accumulation of the DOX inside the mycoplasma when compared to the other body cells. The high concentration existence of the NADPH dehydrogenase inside the mitochondria initiates the redox reaction in the complex and promotes the production of the Reactive oxygen species. Myocytes are generally having low levels of anti-oxidants when compared to the other tissue cells, considerably DOX shows enhanced effects on the heart and cause toxicity [48–50].

The antioxidant level differences were observed in rats under DOX treatment based on the age differences, younger Fischer rats contain more levels of antioxidants when compared to old Fischer rats. A recent study stated the involvement of the Toll-like receptor TLR-4 (a specific receptor in the immune system generally recognise the multiple bacterial antigens and plays a major role in the maturation of the phagosomes) [51]. The increase in TLR-4 expression in the DOX-induced Cardiomyocytes, when studied the cardiomyopathic cells in humans and animals. The deficiency of TLR shows decreased in lipid peroxidation and nitrotyrosine levels in cardiomyopathic cells. The other study on the glutathione peroxidase 1 (GPx) enzyme is present in both cytosol and mitochondria play a major role in the detoxification. The study conducted with the insertion of DOX on non-GPx and wild type mice, the results showed based on the study on myocytes of the non-GPx mice having the high concentration of the DOX deposits in cells, when compared to wild type mice [52–54].

The oxidative stress is a major cause for the exhibiting the cardiotoxicity, involved the generation of higher amounts of ROS cause the cellar alterations and damage are referred to as oxidative stress. The ROS is countered by the anti-oxidant system in the body, in cancer patients under the DOX chemotherapy observed the decreased the levels of GSH, TRAP levels in the body. The ROS is generation is catalysed by NADPH oxidase enzyme [55, 56].

$$\begin{gathered} O_2 + e^- \rightarrow \dot{O}_2^- \\ 2O_2 + NADPH \rightarrow 2O_2^{\bullet -} + NADP^+ + H^+ \end{gathered} \tag{1}$$

In mitochondrial cells, the same reaction is mediated by NADH –ubiquinone oxidoreductase enzyme.

$$2O_2 + NADH + H^+ \rightarrow O_2^{\cdot -} + NAD^+ + 2H^+ \tag{2}$$

The generated oxygen radical undergo dimutation with hydrohen molecules and forms hydrogen peroxide reaction is mediated by the SOD enzyme [55].

$$2O_2^{\cdot -} + 2H \xrightarrow{SOD} H2O2 + O_2 \tag{3}$$

The generated less active hydrogen peroxide is removed by the enzymes like catalase, glutathione peroxidase.

$$\begin{gathered} H2O2 \xrightarrow{Catalase} 2H2O + O2 \\ 2GSH + H2O2 \xrightarrow{GPx} GSSG + 2H2O \end{gathered} \tag{4}$$

The generated oxygen radicals combine with the H2O2 and form the highly active hydroxyl radicals. The H2O2 also reacted with the ferrous ions resulting in the formation of ferric ions and reactive hydroxyl radicals [55].

$$\begin{gathered} O_2^{\cdot -} + \mathbf{H2O2} \rightarrow OH^- + OH^{\cdot} + O_2 \\ Fe^{2+} + H2O2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \\ Fe^{3+} + O_2^{\cdot -} \rightarrow Fe^{2+} + O_2 \end{gathered} \tag{5}$$

Under stress conditions, oxygen radical facilitates the ferrous iron from the ferric ion. The iron, under normal conditions sequestered within the ferritin (a globular protein and forms the nanocage with the metal-protein complexes) but with regards to DOX when converted to its semi-quinone form complexes with iron-free radical and converted to DOX forms while generating the oxygen free radical. The generated complexes block the iron-free regulating proteins (IRP), and then these IRPs bound to the iron-responsive elements in mRNA ferritin. The tremendous amounts of free iron releases and gain complexes with the DOX. This specific condition magnifies the production of ROS in cells [57–59].

The ROS acts as secondary signalling molecules shows direct effects on the lipids, proteins, DNA, and RNA in various pathways involved in cell proliferation, cell death and maintain the homeostasis. It is domineering to maintain the levels of ROS in the body, in case of the heart the effect is maximum by ROS due to lack of efficient levels of anti-oxidants in myocytes. The conditions such as cellular hypertrophy, alterations in the gene expressions, ventricular remodelling, the extracellular matrix of the mitochondria transformation, calcium transient perturbation and cell death activation such kinds of pathological changes may be observed in myocytes lead the death of cells.

2.5 Mechanisms involved at different levels of cardio toxicity

2.5.1 Cellular hypertrophy

A particular disorder marked by an increase in cell size and volume. The abnormality shows an improvement in the degree of protein synthesis, increased in the organisation of sarcomere (contractile muscle fibre unit). At molecular level induction of hypertrophy associated genes are triggered by the DOX treatment which are alpha myosin heavy chain, ventricular myosin light chain-2, and atrial natriuretic peptide genes [60]. The main signalling cascades of the hypertrophy are tyrosine kinases, PI3K/Akt [61], and NF-κB [62, 63], protein kinase C (PKC), mitogen-activated protein kinases (AMPK [64]; ERK1/2 [65], p38 [66], and JNK) which are increased in DOX therapy induce cellular hypertrophic conditions [67].

2.5.2 ECM remodelling

Extra cellular matrix is a molecular network consisting of glycol conjugates, proteins, glycosaminoglycans and adhesive receptors that associate with each other and forms frame network, where cells reside on them [68]. The ECM frame work is present in all tissues it continuously shifts in quantitative and qualitative terms on a daily basis. In case of myocytes ECM is essential for attachment, alignment and orientation facilities the cellular contractions in myocytes. Changes in the ECM of the heart found in DOX treatments, the symptoms of DOX are related to the activation of the Membrane Metalloproteinase enzymes MMP-2 & 9 in 4 weeks of treatment [69]. Changes in MMP-2 activate the Akt channels; suppress the superoxide dismutase enzyme, which raises the amount of superoxide levels, and induce caspase-3 and all other agents together promote remodelling and apoptosis [70].

2.5.3 Impaired cardiac contraction

The heart cells (cardiomyocytes) composed of myofibrils with typical contraction and relaxation. Pump and propel the blood to systemic circulation. Myofibrils contain multiple contractile units called sarcomere, which have actin and myosin filaments. In a calm state, actin is coated in tropomyosin and protects

the myosin-binding sites. The troponin and tropomyosin are attached when the calcium enters into the cytosol from the sarcoplasmic reticulum; calcium binds to the troponin and the position of the tropomyosin and troponin changes resulting in shortening of the sarcomere. That specific condition termed as cardiac contraction controlled by calcium influx and myofilaments. DOX could affect the transcription and expression of the specific proteins [71]. Transcription factor-like GATA4 for the regulation of sarcomeric synthesis and cardiac differentiation and survival of myocytes. DOX-induced ROS decreases binding function, disrupts sarcomere structure, contractile reduction and myofibrillar deterioration [72]. DOX is believed to interact with calcium homeostasis by modifying the ion pump and modifying the ion channel movement, resulting in lipid peroxidation. ROS quickly targets the fatty acids of the membrane lipids and disrupts the mitochondrial calcium channels by increasing the activity of the voltage-sensitive L-type calcium channels on the cell membrane resulting in accumulation of calcium [73]. Calcium overload throughout cytosol, Causes the disruption in the contraction and relaxing of cardiomyocytes.

2.5.4 Cell death

The general apoptosis is a process where a cell commits to suicide, damage to genetic material, protein, cellular organelles that beyond the repair would trigger the suicide to save the energy and resources. Apoptosis firmly regulated process involves intrinsic mitochondrial apoptosis, extrinsic death receptor pathways [74]. The mitochondrial pathway relays on the Trans membrane potential is a key indicator of membrane permeability. It is assumed that permeability can be either permeability-dependent or independent of the pore transition [PT]. The PT pore consists of the adenine nucleotide translocator, matrix protein cyclophilin D, and voltage-dependent anion channel. The opening of the PT pore activates the dissipation of the proton gradient produced by electron transport, resulting in the uncoupling of oxidative phosphorylation. The opening of the PT pore also allows water to penetrate the mitochondrial matrix, resulting in the swelling of the intermembrane space and the rupturing of the outer membrane allowing the release of apoptogenic proteins. Released proteins include cytochrome c, apoptosis-inducing factor and endonuclease G. Cytochrome c in conjunction with apoptosis protease activating factor (APAF-1) and pro-caspase 9 forms an apoptosome, which in turn activates effector caspases that collectively facilitate the execution of apoptosis. Due to decrease in the number of normal cardiomyocytes is significantly reduced, the heart failed to pump the blood sequentially ventricular remodelling and death of myocytes [75].

The death receptor pathway involves the binding of death ligands such as FasL, TNF-α to their respective membrane-bound receptors. The bonded ligands signals to various proteins mediate the cascade, which leads to apoptosis of the cell [76]. In cancer therapy, DOX-induced ROS activates the p^{38}, p^{53} and NF-kB pathways resulting in the differences in pro- and anti-apaptonic signalling imbalance, such imbalance cause release of cytochrome C from mitochondrial membrane proteins, subsequently lead to apoptosis of cell [77, 78].

2.5.5 Autophagy

Autophagy is a method of restoring or repairing the destroyed cells. It is a self-degrading mechanism (survival mechanism) to maintain a balance of life in response to dietary stress, energy depletion. Autophagy destroys malformed proteins, weakened organelles, and other cell infections, which can be unique or non-specific, but processes are not completely thought out. Under diseased

environments, autophagy either facilitates cell death or induces cell death depending on the demands of different people [79, 80]. In DOX-based therapy toxicity mediated autophagy by suppressing GTAT4 expression and activating S6K1, this plays a direct and indirect role in autophagy control. Autophagy varies due to species differences; autophagy dependent on DOX is increased in mice but decreased in autophagy has been seen in mouse cases [81–84]. The autophagy achieved in DOX therapy via several mechanisms, such as ATG 5 & 12 is the inhibitors of the Bcl-2 family, which regulate the cytochrome release from the mitochondria. Cytochrome C releases the caspase-9 lead to the autophagosome, can regulate the apoptosis. In some other studies, autophagy reduces the DOX-induced cardiotoxicity by decreasing mitochondrial ROS formation.

2.6 Diagnosis

The DOX-induced cardiomyopathy consists of a complete examination of the cardiovascular system for detecting the symptoms, such as S3 gallop and elevated jugular vapour pressure, T wave impairments; low voltage QRS complexes are measured.

- Electrocardiography combined with Doopler studies used to study early diagnostic symptoms of the cardiac myopathy through the measure of latero-ventricular dysfunction.

- Radionuclide ventriculography used to access the latero-ventricular systolic and diastolic function. Observes the cardiac adrenergic denervation occurs in case of doxorubicin induced cardiomyopathy.

- Metaiodobenzylguanidine based nuclear imaging can be employed to assess cardiac adrenergic denervation occurred trough the DOX based cardiomyopathy.

- The DOX treated patients are sensitive to the indium labelled monoclonal antimyosin antibodies (myosin an ATP dependent superfamily of motor proteins major role in muscle contraction and motility) used to detect the cardiac myopathy, myocarditis, chagas heart disease ischemic myocardium, and kawasaki heart disease [85].

- The measurement of the cardiac enzymes and neurohormones are used for detecting the heart failure but not a characteristic feature of the DOX-induced cardiomyopathy [86].

- The presence of endomyocardial biopsy is the best route for detecting the DOX-induced diseases, according to the grade of biopsy severity of the disease is measured [87, 88]. It is invasive and requires experience for recognising the results become a disadvantage for this technique.

3. Management & preventive strategies for doxorubicin cardiotoxicity

The DOX has an extreme side effect like cardiotoxicity, but is still in use because of its efficacy in the treatment of cancers. Toxicity can be avoided in several ways. Many studies have shown that cardioprotective agents can achieve a reduction in cardiotoxicity. A recent research on HSP-20 (heat-shock protein) has shown that the

protection of Akt activity prevents the cardiotoxic effect caused by DOX [89–92]. Different kind of agents is used to control the DOX effects such as Dexrazoxane (DZR); it contains bisdioxopiperazine rings falling under alpha-amino acid and the derivative compound also known as cardioxane or Totect or Zincard. A promising compound that activates after hydrolysis and resembles the EDTA structure after conversion makes complexes with Iron and reduces the incidence of anthracycline-iron complexes, thus preventing ROS generation in myocytes. Dexrazoxane has also been known to contain the Topoisomerase II enzyme function and inhibit the tumour cell growth. Used mainly for the activities of iron-chelating agent, cardiac protection, anti-neoplastic activities, and chemo protection. Indirectly active in chromatin remodelling complexes by activating vitamin D receptors. DZR is often known to provide up-regulation of the ERK and Akt pathways to guard against cardiomyopathy [93–96] but DZR is not approved for routine use in patients with metastatic cancer and other forms of cancer, as stated by the American Clinical Oncology Society [97, 98]. DOX was analysed in association with DZR for 10 years in women with breast cancer [99]. No, people suffer from heart disease over the time and there are no records of adverse effects with respect to the heart.

Diuretics are used to avoid signs of systemic and pulmonary ventricular obstruction, and medications dependent on β-adrenergic receptors are used depending on the type of systolic heart problem [100]. Metoprolol is safe and effective in the treatment of cardiac myopathy [97], angiotensin II is also recommended for advanced heart disease cases, and low-dose isosorbide dinitrate substituted angiotensin inhibitor medication is favoured and hydralazine is favoured for cardiomyopathic myopathy.

The successful release of DOX at a particular site of operation is another form of preventive step. Like liposomal dosage formulations, the specified delivery mechanism passively decreases the impact caused by non-cancerous cells. For liposomes drug interaction with blood and cancer cells, structural characteristics such as vesicle size, pharmacokinetic characteristics such as stability and pharmacodynamic characteristics such as plasma clearance are important. Tumour cells have conditions that favour high-level depositions, because newly developed cells have microvasculature-permeable vessels, which contain poor lymphatic drainage, low levels of lipase enzymes and other oxidising agents. Due to these features of cancer cells shows aggregation. Once liposomes enter the tumour cells the differences in the intestinal pH favours the release of drugs constituents. The pH of cancer cells is differ from other normal cells because of this the drug is preferentially released in tumour cells and avoid the toxicity in non-cancer cells. The recently reported formulation of polyethylene glycol-coated liposomal doxorubicin (PLD) shows better pharmacokinetics relative to general formulations and has fewer side effects [101]. A phase clinical trial of 50 mg/m2 PLD administration in patients with carcinoma with a demonstrated history of platinum-based chemotherapy at intervals of 4 weeks reported low toxicity. The other formulation like poly (ethylene oxide)-b-poly (e-caprolactone-DOX) [PEO-b- P(CL-DOX)] prevents the premature release outside of the tumor cells [102].

The development of analogues is another possible strategy for reducing the toxicity [96], in the case of anthracyclines nuclear targeted and Non-nuclear targeted are two kinds of strategies concerned in the development of non-toxic chemotherapeutic agents. Analogues such as Methoxymorpholinyl doxorubicin (MMDX), sabarubicin and *N*-Benzyladriamycin-14-valerate now under development to reduce the toxicity caused by DOX. In which, MMDX is nuclear-targeted analogue activated by the liver enzyme cytochrome P450 3A and metabolize into a cytotoxic metabolite and degrades slowly [103]. Based on gene therapy expression of cytochrome enzyme activity increased, cytochrome increases the therapeutic

potency of the DOX. The sabarubicin (disaccharide analogue) is also another nuclear targeted molecule that has improved efficiency especially used in case of lung and gynaecological cancer [104]. This stimulates the NF-kB transcription factor, which happens earlier as DNA is involved with multiple tumorogenesis, regulating the expressions of differentiation, variations, cell adhesion and apoptosis [105]. N-Benzyladriamycin-14-valerate is a non-nuclear target molecule obtained by modification of the C-3 amino group and the C-14 position [106]. The compound has comparable activity to DOX but is theoretically more effective than DOX by activating the protein kinase enzyme resulting in cardio-protective activity.

4. Conclusion

Even DOX used for treating several types of cancers as a result of its wide range of pharmacological activities, but at the same time it causes a wide range of side effects. The major side effects caused by DOX are: carditoxicity, neuropathy, hepatotoxicity, nephrotoxicity, alopecia, typhlitis, myelosuppression, neutropenia, anaemia, and thrombocytopenia. DOX increasing the oxidative stress, decrease the GSH, vitamin E levels, and activates the NF-kB levels causes' hepatotoxicity. Besides, it interferes with the glandular podocytes of the kidney and cause nephropathy. Also, it induces generation of MDA, TBARS, and HNA which decrease the mitochondrial activities and increase in ROS generation causes cell necrosis. Moreover, it causes induction of brain natriuretic peptides, atrial natriuretic peptides genes, mono oxygenases, cytochrome P genes; binds to the cardiolipin, the increase in TLR-4 expression, generation of ROS led to several pathological changes in myocytes causes cardiomyopathy. Several strategies are made to manage and decrease DOX's cardiotoxicity effects, includes a change in the dosage forms for efficacious delivery systems, administration along with anti-oxidants, DZR, diuretics and β-adrenergic agents, and development of different analogues for increasing the efficiency of DOX.

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Conflict of interest

The author declares no conflict of interest.

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References

[1] Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, et al. Doxorubicin: the good, the bad and the ugly effect. Current medicinal chemistry. 2009 Sep 1;**16**(25):3267-3285

[2] Wani SH. Lone SA. Cancer: Diseases. Educreation Publishing; 2018 Nov 10

[3] Danesi R, Fogli S, Gennari A, Conte P, Del Tacca M. Pharmacokinetic-pharmacodynamic relationships of the anthracycline anticancer drugs. Clinical Pharmacokinetics. 2002 May 1;**41**(6):431-444

[4] Danesi R, Fogli S, Gennari A, Conte P, Del Tacca M. Pharmacokinetic-pharmacodynamic relationships of the anthracycline anticancer drugs. Clinical Pharmacokinetics. 2002 May 1;**41**(6):431-444

[5] Song Y, Buettner GR. Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. Free Radical Biology and Medicine. 2010 Sep 15;**49**(6):919-962

[6] Chatterjee K, Zhang J, Tao R, Honbo N, Karliner JS. Vincristine attenuates doxorubicin cardiotoxicity. Biochemical and biophysical research communications. 2008 Sep 5;**373**(4):555-560

[7] Available from: http://chemocare.com/chemotherapy/drug-info/doxorubicin.aspx [accessed on 2020-10-11].

[8] Alghorabi AA, Kabel AM, Elmaaboud MA. Doxorubicin: Insights into Dynamics, Clinical Uses and Adverse Effects. J. Cancer Res. 2019;**7**:17-20

[9] Hanna AD, Lam A, Tham S, Dulhunty AF, Beard NA. Adverse effects of doxorubicin and its metabolic product on cardiac RyR2 and SERCA2A. Molecular Pharmacology. 2014 Oct 1;**86**(4):438-449

[10] Maton A, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, et al. Human biology and health. Englewood Cliffs, New Jersey, US: Prentice Hall; 1993

[11] Shirani K, Yousefsani BS, Shirani M, Karimi G. Protective effects of naringin against drugs and chemical toxins induced hepatotoxicity: A review. Phytotherapy Research. 2020 Feb;**17**

[12] Zhao X, Jin Y, Li L, Xu L, Tang Z, Qi Y, et al. MicroRNA-128-3p aggravates doxorubicin-induced liver injury by promoting oxidative stress via targeting Sirtuin-1. Pharmacological Research. 2019 Aug 1;**146**:104276

[13] Yang XL, Fan CH, Zhu HS. Photo-induced cytotoxicity of malonic acid [C60] fullerene derivatives and its mechanism. Toxicology in vitro. 2002 Feb 1;**16**(1):41-46

[14] Forrest GL, Gonzalez B, Tseng W, Li X, Mann J. Human carbonyl reductase overexpression in the heart advances the development of doxorubicin-induced cardiotoxicity in transgenic mice. Cancer research. 2000 Sep 15;**60**(18):5158-5164

[15] Gavelová M, Hladíková J, Vildová L, Novotná R, Vondráček J, Krčmář P, et al. Reduction of doxorubicin and oracin and induction of carbonyl reductase in human breast carcinoma MCF-7 cells. Chemico-biological interactions. 2008 Oct 22;**176**(1):9-18

[16] Ahmed OM, Abdul-Hamid MM, El-Bakry AM, Mohamed HM, Abdel Rahman ES. Camellia sinensis and epicatechin abate doxorubicin-induced hepatotoxicity in male Wistar rats via

their modulatory effects on oxidative stress, inflammation, and apoptosis. J Appl Pharm Sci. 2019 Apr;**9**:30-44

[17] Injac R, Perse M, Obermajer N, Djordjevic-Milic V, Prijatelj M, Djordjevic A, et al. Potential hepatoprotective effects of fullerenol C60 (OH) 24 in doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. Biomaterials. 2008 Aug 1;**29**(24-25):3451-3460

[18] Lu H, Zhu ZG, Yao XX, Zhao R, Yan C, Zhang Y, et al. Hepatic preconditioning of doxorubicin in stop-flow chemotherapy: NF-κB/IκB-α pathway and expression of HSP72. World Journal of Gastroenterology: WJG. 2005 Apr 14;**11**(14):2136

[19] Smuder AJ. Exercise stimulates beneficial adaptations to diminish doxorubicin-induced cellular toxicity. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2019 Nov 1;**317**(5):R662-R672

[20] Songbo M, Lang H, Xinyong C, Bin X, Ping Z, Liang S. Oxidative stress injury in doxorubicin-induced cardiotoxicity. Toxicology letters. 2019 Jun 1;**307**:41-48

[21] Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. European journal of medicinal chemistry. 2015 Jun 5;**97**:55-74

[22] S Lahoti T, Patel D, Thekkemadom V, Beckett R, D Ray S. Doxorubicin-induced in vivo nephrotoxicity involves oxidative stress-mediated multiple pro-and anti-apoptotic signaling pathways. Current neurovascular research. 2012 Nov 1;9(4):282-295.

[23] Okuda S, Oh Y, Tsuruda H, Onoyama K, Fujimi S, Fujishima M. Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. Kidney international. 1986 Feb 1;**29**(2):502-510

[24] Fogo AB. Mechanisms of progression of chronic kidney disease. Pediatric nephrology. 2007 Dec 1;**22**(12):2011-2022

[25] Wang Y, Wang YP, Tay YC, Harris DC. Progressive adriamycin nephropathy in mice: sequence of histologic and immunohistochemical events. Kidney international. 2000 Oct 1;**58**(4):1797-1804

[26] Rook M, Lely AT, Kramer AB, van Goor H, Navis G. Individual differences in renal ACE activity in healthy rats predict susceptibility to adriamycin-induced renal damage. Nephrology Dialysis Transplantation. 2005 Jan 1;**20**(1):59-64

[27] Khames A, Khalaf MM, Gad AM, Abd El-raouf OM, Kandeil MA. Nicorandil combats doxorubicin–induced nephrotoxicity via amendment of TLR4/P38 MAPK/NFκ-B signaling pathway. Chemico-biological interactions. 2019 Sep 25;**311**:108777

[28] Korbet SM, Schwartz MM, Lewis EJ. Primary focal segmental glomerulosclerosis: clinical course and response to therapy. American journal of kidney diseases. 1994 Jun 1;**23**(6):773-783

[29] Jansen C, Miaskowski C, Dodd M, Dowling G, Kramer J. Potential mechanisms for chemotherapy-induced impairments in cognitive function. InOncology nursing forum 2005 Nov 1 (Vol. 32, No. 6, p. 1151). Oncology Nursing Society.

[30] Ferreira A, Neves P, Gozzelino R. Multilevel impacts of Iron in the brain: the cross talk between neurophysiological mechanisms, cognition, and social behavior. Pharmaceuticals. 2019 Sep;**12**(3):126

[31] Tangpong J, Cole MP, Sultana R, Joshi G, Estus S, Vore M, et al. Adriamycin-induced, TNF-α-mediated central nervous system toxicity. Neurobiology of Disease. 2006 Jul 1;**23**(1):127-139

[32] Brezden CB, Phillips KA, Abdolell M, Bunston T, Tannock IF. Cognitive function in breast cancer patients receiving adjuvant chemotherapy. Journal of Clinical Oncology. 2000 Jul 14;**18**(14):2695-2701

[33] Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, Skalla K, et al. Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. Journal of Clinical Oncology. 2002 Jan 15;**20**(2):485-493

[34] Tangpong J, Cole MP, Sultana R, Estus S, Vore M. St. Clair W, Ratanachaiyavong S, St. Clair DK, Butterfield DA. Adriamycin-mediated nitration of manganese superoxide dismutase in the central nervous system: insight into the mechanism of chemobrain. Journal of Neurochemistry. 2007 Jan;**100**(1):191-201

[35] Park ES, Kim SD, Lee MH, Lee HS, Lee IS, Sung JK, et al. Protective effects of N-acetylcysteine and selenium against doxorubicin toxicity in rats. Journal of veterinary science. 2003 Aug 1;**4**(2):129-136

[36] Joshi G, Sultana R, Tangpong J, Cole MP, St Clair DK, Vore M, et al. Free radical mediated oxidative stress and toxic side effects in brain induced by the anticancer drug adriamycin: insight into chemobrain. Free radical research. 2005 Jan 1;**39**(11):1147-1154

[37] Joshi G, Hardas S, Sultana R. St. Clair DK, Vore M, Butterfield DA. Glutathione elevation by γ-glutamyl cysteine ethyl ester as a potential therapeutic strategy for preventing oxidative stress in brain mediated by in vivo administration of adriamycin: Implication for chemobrain. Journal of Neuroscience Research. 2007 Feb 15;**85**(3):497-503

[38] Öz E, İlhan MN. Effects of melatonin in reducing the toxic effects of doxorubicin. Molecular and Cellular Biochemistry. 2006 Jun 1;**286**(1-2):11-15

[39] Mohanty A, Tiwari-Pandey R, Pandey NR. Mitochondria: the indispensable players in innate immunity and guardians of the inflammatory response. Journal of Cell Communication and Signaling. 2019 Sep;**1**:1-6

[40] Gorini S, De Angelis A, Berrino L, Malara N, Rosano G, Ferraro E. Chemotherapeutic drugs and mitochondrial dysfunction: focus on doxorubicin, trastuzumab, and sunitinib. Oxidative medicine and cellular longevity. 2018 Oct;**2018**

[41] Mancilla TR, Iskra B, Aune GJ. Doxorubicin-Induced Cardiomyopathy in Children. Comprehensive Physiology. 2011 Jan 17;**9**(3):905-931

[42] Arcamone F, Franceschi G, Penco S, Selva A. Adriamycin (14-hydroxydaunomycin), a novel antitumor antibiotic. Tetrahedron letters. 1969 Jan 1;**10**(13):1007-1010

[43] Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. New England Journal of Medicine. 1998 Sep 24;**339**(13):900-905

[44] Von Hoff DD, Layard MW, Basa P, DAVIS Jr HL, Von Hoff AL, Rozencweig M, Muggia FM. Risk factors for doxorubicin-lnduced congestive heart failure. Annals of internal medicine. 1979 Nov 1;**91**(5):710-717

[45] Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a

retrospective analysis of three trials. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2003 Jun 1;**97**(11):2869-2879

[46] Zordoky BN, El-Kadi AO. Induction of several cytochrome P450 genes by doxorubicin in H9c2 cells. Vascular pharmacology. 2008 Oct 1;**49**(4-6):166-172

[47] Sardão VA, Oliveira PJ, Holy J, Oliveira CR, Wallace KB. Morphological alterations induced by doxorubicin on H9c2 myoblasts: nuclear, mitochondrial, and cytoskeletal targets. Cell biology and toxicology. 2009 Jun 1;**25**(3):227-243

[48] Goormaghtigh E, Huart P, Praet M, Brasseur R, Ruysschaert JM. Structure of the adriamycin-cardiolipin complex: role in mitochondrial toxicity. Biophysical Chemistry. 1990 Apr 1;**35**(2-3):247-257

[49] Childs AC, Phaneuf SL, Dirks AJ, Phillips T, Leeuwenburgh C. Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity, and Bcl-2: Bax ratio. Cancer research. 2002 Aug 15;**62**(16):4592-4598

[50] Odom AL, Hatwig CA, Stanley JS, Benson AM. Biochemical determinants of adriamycin® toxicity in mouse liver, heart and intestine. Biochemical Pharmacology. 1992 Feb 18;**43**(4):831-836

[51] Riad A, Bien S, Gratz M, Escher F, Heimesaat MM, Bereswill S, et al. Toll-like receptor-4 deficiency attenuates doxorubicin-induced cardiomyopathy in mice. European journal of heart failure. 2008 Mar;**10**(3):233-243

[52] Esworthy RS, Ho YS, Chu FF. TheGpx1Gene encodes mitochondrial glutathione peroxidase in the mouse liver. Archives of biochemistry and biophysics. 1997 Apr 1;**340**(1):59-63

[53] Li T, Singal PK. Adriamycin-induced early changes in myocardial antioxidant enzymes and their modulation by probucol. Circulation. 2000 Oct 24;**102**(17):2105-2110

[54] Sazuka Y, Tanizawa H, Takino Y. Effect of adriamycin on the activities of superoxide dismutase, glutathione peroxidase and catalase in tissues of mice. Japanese journal of cancer research. 1989 Jan;**80**(1):89-94

[55] Angsutararux P, Luanpitpong S, Issaragrisil S. Chemotherapy-induced cardiotoxicity: overview of the roles of oxidative stress. Oxidative medicine and cellular longevity. 2015 Oct;**2015**

[56] Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenetics and genomics. 2011 Jul;**21**(7):440

[57] Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, et al. Involvement of nuclear factor-κB and apoptosis signal-regulating kinase 1 in G-protein–coupled receptor agonist–induced cardiomyocyte hypertrophy. Circulation. 2002 Jan 29;**105**(4):509-515

[58] Minotti G, Recalcati S, Mordente A, Liberi G, Calafiore AM, Mancuso C, et al. The secondary alcohol metabolite of doxorubicin irreversibly inactivates aconitase/iron regulatory protein-1 in cytosolic fractions from human myocardium. The FASEB Journal. 1998 May;**12**(7):541-552

[59] Minotti G, Mancuso C, Frustaci A, Mordente A, Santini SA, Calafiore AM, et al. Paradoxical inhibition of cardiac lipid peroxidation in cancer patients treated with doxorubicin. Pharmacologic and molecular reappraisal of anthracycline

cardiotoxicity. The Journal of clinical investigation. 1996 Aug 1;**98**(3):650-661

[60] Izumiya Y, Kim S, Izumi Y, Yoshida K, Yoshiyama M, Matsuzawa A, et al. Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II–induced cardiac hypertrophy and remodeling. Circulation Research. 2003 Oct 31;**93**(9):874-883

[61] Xin Y, Bai Y, Jiang X, Zhou S, Wang Y, Wintergerst KA, et al. Sulforaphane prevents angiotensin II-induced cardiomyopathy by activation of Nrf2 via stimulating the Akt/GSK-3ss/Fyn pathway. Redox Biology. 2018 May 1;**15**:405-417

[62] Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, et al. Involvement of nuclear factor-κB and apoptosis signal-regulating kinase 1 in G-protein–coupled receptor agonist–induced cardiomyocyte hypertrophy. Circulation. 2002 Jan 29;**105**(4):509-515

[63] Izumiya Y, Kim S, Izumi Y, Yoshida K, Yoshiyama M, Matsuzawa A, et al. Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II–induced cardiac hypertrophy and remodeling. Circulation Research. 2003 Oct 31;**93**(9):874-883

[64] Lue Y, Gao C, Swerdloff R, Hoang J, Avetisyan R, Jia Y, et al. Humanin analog enhances the protective effect of dexrazoxane against doxorubicin-induced cardiotoxicity. American Journal of Physiology-Heart and Circulatory Physiology. 2018 Sep 1;**315**(3):H634-H643

[65] Wu W, Chordia MD, Hart BP, Kumarasinghe ES, Ji MK, Bhargava A, et al. Macrocyclic MEK1/2 inhibitor with efficacy in a mouse model of cardiomyopathy caused by lamin A/C gene mutation. Bioorganic & medicinal chemistry. 2017 Feb 1;**25**(3):1004-1013

[66] Zuo G, Ren X, Qian X, Ye P, Luo J, Gao X, et al. Inhibition of JNK and p38 MAPK-mediated inflammation and apoptosis by ivabradine improves cardiac function in streptozotocin-induced diabetic cardiomyopathy. Journal of Cellular Physiology. 2019 Feb;**234**(2):1925-1936

[67] Zhen J, Yu H, Ji H, Cai L, Leng J, Keller BB. Neonatal murine engineered cardiac tissue toxicology model: Impact of dexrazoxane on doxorubicin induced injury. Life Sciences. 2019 Dec 15;**239**:117070

[68] Kwon SH, Pimentel DR, Remondino A, Sawyer DB, Colucci WS. H2O2 regulates cardiac myocyte phenotype via concentration-dependent activation of distinct kinase pathways. Journal of molecular and cellular cardiology. 2003 Jun 1;**35**(6):615-621

[69] Rabinovich-Nikitin I, Love M. Kirshenbaum LA. Cardiovascular Research: Inhibition of MMP prevents doxorubicin-induced cardiotoxicity by attenuating cardiac intracellular and extracellular matrix remodelling; 2020 Jul 10

[70] Zhao Y, McLaughlin D, Robinson E, Harvey AP, Hookham MB, Shah AM, et al. Nox2 NADPH oxidase promotes pathologic cardiac remodeling associated with Doxorubicin chemotherapy. Cancer research. 2010 Nov 15;**70**(22):9287-9297

[71] Jeyaseelan R, Poizat C, Wu HY, Kedes L. Molecular Mechanisms of Doxorubicin-induced Cardiomyopathy Selective suppression of reiske iron-sulfur protein, adp/atp translocase, and phosphofructokinase genes is associated with atp depletion in rat cardiomyocytes. Journal of Biological Chemistry. 1997 Feb 28;**272**(9):5828-5832

[72] Aries A, Paradis P, Lefebvre C, Schwartz RJ, Nemer M. Essential role

of GATA-4 in cell survival and drug-induced cardiotoxicity. Proceedings of the National Academy of Sciences. 2004 May 4;**101**(18):6975-6980

[73] Arai M, Yoguchi A, Takizawa T, Yokoyama T, Kanda T, Kurabayashi M, et al. Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca2+-ATPase gene transcription. Circulation Research. 2000 Jan 7;**86**(1):8-14

[74] Lavrik IN, Krammer PH. Regulation of CD95/Fas signaling at the DISC. Cell Death & Differentiation. 2012 Jan;**19**(1):36-41

[75] Sawyer DB, Peng X, Chen B, Pentassuglia L, Lim CC. Mechanisms of anthracycline cardiac injury: can we identify strategies for cardioprotection? Progress in cardiovascular diseases. 2010 Sep 1;**53**(2):105-113

[76] Kotamraju S, Konorev EA, Joseph J, Kalyanaraman B. Doxorubicin-induced apoptosis in endothelial cells and cardiomyocytes is ameliorated by nitrone spin traps and ebselen role of reactive oxygen and nitrogen species. Journal of Biological Chemistry. 2000 Oct 27;**275**(43):33585-33592

[77] Nitobe J, Yamaguchi S, Okuyama M, Nozaki N, Sata M, Miyamoto T, et al. Reactive oxygen species regulate FLICE inhibitory protein (FLIP) and susceptibility to Fas-mediated apoptosis in cardiac myocytes. Cardiovascular Research. 2003 Jan 1;**57**(1):119-128

[78] Lien YC, Noel T, Liu H, Stromberg AJ, Chen KC, Clair DK. Phospholipase C-δ1 Is a Critical Target for Tumor Necrosis Factor Receptor–Mediated Protection against Adriamycin-Induced Cardiac Injury. Cancer research. 2006 Apr 15;**66**(8):4329-4338

[79] Lu L, Wu W, Yan J, Li X, Yu H, Yu X. Adriamycin-induced autophagic cardiomyocyte death plays a pathogenic role in a rat model of heart failure. International journal of cardiology. 2009 May 1;**134**(1):82-90

[80] Dimitrakis P, Romay-Ogando MI, Timolati F, Suter TM, Zuppinger C. Effects of doxorubicin cancer therapy on autophagy and the ubiquitin-proteasome system in long-term cultured adult rat cardiomyocytes. Cell and tissue research. 2012 Nov 1;**350**(2):361-372

[81] Kawaguchi T, Takemura G, Kanamori H, Takeyama T, Watanabe T, Morishita K, et al. Prior starvation mitigates acute doxorubicin cardiotoxicity through restoration of autophagy in affected cardiomyocytes. Cardiovascular Research. 2012 Dec 1;**96**(3):456-465

[82] Smuder AJ, Kavazis AN, Min K, Powers SK. Doxorubicin-induced markers of myocardial autophagic signaling in sedentary and exercise trained animals. Journal of Applied Physiology. 2013 Jul 15;**115**(2):176-185

[83] Zhang YY, Meng C, Zhang XM, Yuan CH, Wen MD, Chen Z, et al. Ophiopogonin D attenuates doxorubicin-induced autophagic cell death by relieving mitochondrial damage in vitro and in vivo. Journal of Pharmacology and Experimental Therapeutics. 2015 Jan 1;**352**(1):166-174

[84] Shabalala S, Muller CJ, Louw J, Johnson R. Polyphenols, autophagy and doxorubicin-induced cardiotoxicity. Life Sciences. 2017 Jul 1;**180**:160-170

[85] Wakasugi S, Fischman AJ, Babich JW, Callahan RJ, Elmaleh DR, Wilkinson R, et al. Myocardial substrate utilization and left ventricular function in adriamycin cardiomyopathy. Journal of Nuclear Medicine. 1993 Sep 1;**34**(9):1529-1535

[86] Suzuki T, Hayashi D, Yamazaki T, Mizuno T, Kanda Y, Komuro I, et al. Elevated B-type natriuretic peptide levels after anthracycline administration. American heart journal. 1998 Aug 1;**136**(2):362-363

[87] Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. Progress in cardiovascular diseases. 2007 Mar 1;**49**(5):330-352

[88] Bristow MR, Sageman WS, Scott RH, Billingham ME, Bowden RE, Kernoff RS, et al. Acute and chronic cardiovascular effects of doxorubicin in the dog: the cardiovascular pharmacology of drug-induced histamine release. Journal of cardiovascular pharmacology. 1980 Sep 1;**2**(5):487-516

[89] Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. Progress in cardiovascular diseases. 2007 Mar 1;**49**(5):330-352

[90] ZHU YH, MA TM, Wang X. Gene transfer of heat-shock protein 20 protects against ischemia/reperfusion injury in rat hearts 1. Acta Pharmacologica Sinica. 2005 Oct;**26**(10):1193-1200

[91] Fan GC, Zhou X, Wang X, Song G, Qian J, Nicolaou P, et al. Heat shock protein 20 interacting with phosphorylated Akt reduces doxorubicin-triggered oxidative stress and cardiotoxicity. Circulation research. 2008 Nov 21;**103**(11):1270-1279

[92] 87Kim KH, Oudit GY, Backx PH. Erythropoietin protects against doxorubicin-induced cardiomyopathy via a phosphatidylinositol 3-kinase-dependent pathway. Journal of Pharmacology and Experimental Therapeutics. 2008 Jan 1;**324**(1):160-169

[93] Marty M, Espie M, Llombart A, Monnier A, Rapoport BL, Stahalova V. Multicenter randomized phase III study of the cardioprotective effect of dexrazoxane (Cardioxane®) in advanced/metastatic breast cancer patients treated with anthracycline-based chemotherapy. Annals of Oncology. 2006 Apr 1;**17**(4):614-622

[94] van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane database of systematic reviews. 2011;**6**

[95] In U, Hensley LM, Hagerty KL, Kewalramani T, Green DM, Meropol NJ, et al. American Society of Clinical Oncology 2008 clinical practice guideline update: use of chemotherapy and radiation therapy protectants. Journal of Clinical Oncology. 2008;**27**:127-132

[96] Testore F, Milanese S, Ceste M, de Conciliis E, Parello G, Lanfranco C, et al. Cardioprotective effect of dexrazoxane in patients with breast cancer treated with anthracyclines in adjuvant setting. American journal of cardiovascular drugs. 2008 Jul 1;**8**(4):257-263

[97] Shaddy RE, Olsen SL, Bristow MR, Taylor DO, Bullock EA, Tani LY, et al. Efficacy and safety of metoprolol in the treatment of doxorubicin-induced cardiomyopathy in pediatric patients. American heart journal. 1995 Jan 1;**129**(1):197-199

[98] Lotrionte M, Palazzoni G, Natali R, Comerci G, Abbate A, Di Persio S, et al. Appraising cardiotoxicity associated with liposomal doxorubicin by means of tissue Doppler echocardiography end-points: Rationale and design of the LITE (Liposomal doxorubicin–Investigational chemotherapy–Tissue Doppler imaging Evaluation) randomized pilot study. International journal of cardiology. 2009 Jun 12;**135**(1):72-77

[99] Wildiers H, Jurcut R, Ganame J, Herbots L, Neven P, De Backer J, et al. A pilot study to investigate the feasibility and cardiac effects of pegylated liposomal doxorubicin (PL-DOX) as adjuvant therapy in medically fit elderly breast cancer patients. Critical reviews in oncology/hematology. 2008 Aug 1;**67**(2):133-138

[100] Arnold JM, Howlett JG, Ducharme A, Ezekowitz JA, Gardner MJ, Giannetti N, et al. Canadian Cardiovascular Society Consensus Conference guidelines on heart failure–2008 update: best practices for the transition of care of heart failure patients, and the recognition, investigation and treatment of cardiomyopathies. Canadian Journal of Cardiology. 2008 Jan 1;**24**(1):21-40

[101] Culty M, Nguyen HA, Underhill CB. The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. The Journal of cell biology. 1992 Feb 15;**116**(4):1055-1062

[102] Lewis AD, Lau DH, Durán GE, Wolf CR, Sikic BI. Role of cytochrome P-450 from the human CYP3A gene family in the potentiation of morpholino doxorubicin by human liver microsomes. Cancer research. 1992 Aug 15;**52**(16):4379-4384

[103] Baldwin A, Huang Z, Jounaidi Y, Waxman DJ. Identification of novel enzyme–prodrug combinations for use in cytochrome P450-based gene therapy for cancer. Archives of biochemistry and biophysics. 2003 Jan 1;**409**(1):197-206

[104] Michael JB, Tannock IF. Lysosomes, lysosomal enzymes and cancer. Advances in Cancer Research. 1993;**60**:269-291

[105] Roca-Alonso L, Castellano L, Mills A, Dabrowska AF, Sikkel MB, Pellegrino L, Jacob J, Frampton AE, Krell J, Coombes RC, Harding SE. Myocardial MiR-30 downregulation triggered by doxorubicin drives alterations in β-adrenergic signaling and enhances apoptosis. Cell death & disease. 2015 May;6(5):e1754-.

[106] Ma ZG, Dai J, Yuan YP, Bian ZY, Xu SC, Jin YG, et al. T-bet deficiency attenuates cardiac remodelling in rats. Basic Research in Cardiology. 2018 May 1;**113**(3):19