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Review Article

Doxorubicin-induced testicular toxicity: possible underlying mechanisms and promising pharmacological treatments in experimental models

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ABSTRACT

Chemotherapy is considered to be the most effective intervention in cancer treatment. The first use of chemotherapy began in the 1940s, unfortunately, its use result in many serious and debilitating side effects which affect human daily activities. Doxorubicin is a powerful antineoplastic drug FDA-approved for the management of a variety of cancer types including leukemia and lymphoma. However, its clinical use is limited due to its toxic effect on different tissues including testicular toxicity which has a bad impact on the quality of life of cancer survivors. DOX-induced testicular toxicity is accompanied by defects in sperm analysis including low sperm count, low sperm motility, and high sperm abnormalities in addition to affecting steroidogenesis. This review is intended to discuss the pharmacodynamics and the pharmacokinetics of doxorubicin, besides the possible underlying mechanisms that may be contributed to the damage of testicles caused by DOX including oxidative stress, inflammation, apoptosis, and autophagy. Moreover, the assessment of post-chemotherapy testicular toxicity in animals and the promising pharmacological treatment that has been studied in animal models were discussed.

Keywords: *testicular toxicity; Doxorubicin; oxidative stress; apoptosis; inflammation.*

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1. INTRODUCTION

Chemotherapy is considered to be a cornerstone in cancer treatment **[1]**; however, patients receiving chemotherapeutic agents are often suffered from many serious side effects **[2]**. Doxorubicin (DOX) is a powerful chemotherapeutic agent FDA-approved for the management of many types of cancer including acute leukemia, and lymphomas besides many solid tumors including breast cancer and some types of lung cancer **[3–5]**. However, its use is limited due to its serious toxicity to many organs including cardiotoxicity, nephrotoxicity, and cognitive impairment as well as testicular toxicity **[6-8]**. Around 160,000 children are diagnosed with cancer each year and it has been reported that a male infant has a 1 in 300 chance of being diagnosed with cancer by the age of 20 **[9]**. Interestingly, over the past few decades, the rate of treating cancer in childhood has increased and now about 80% of children survive following treatment **[10, 11]**. It has been reported that chemotherapy is gonadotropic and may lead to infertility, which cause a bad impact on the quality of life of cancer survivors **[12, 13]**. Now

it has been recommended by the American Society of Clinical Oncology to discuss the reproductive risk of cancer treatment with patients' prepubertal boys, adolescents, and adult men' before starting chemotherapy **[13]**.

Currently, the mechanism involved in DOX-induced testicular damage is not yet fully intelligible **[14]**. The previous study reported that it includes oxidative stress resulting in lipid peroxidation and cellular apoptosis **[15]**. Moreover, a previous study reported that DOX treatment leads to increasing many inflammatory mediators **[16]**. Autophagy is another mechanism responsible for DOX-induced organ toxicity **[17, 18]**, where DOX has been found to upregulate autophagy-related genes **[19]**. In addition, it was found that DOX has a direct testicular toxicity effect, it has been reported that DOX results in lipid biosynthesis defects which inhibit steroidogenesis in the testis and also it results in DNA damage and mutation **[20]**. In this review, we will discuss the possible mechanisms of DOX-induced testicular toxicities and the promising pharmacological treatment in experimental models.

2. Pharmacodynamics of doxorubicin as a chemotherapeutic agent

DOX is the most powerful anti-cancer drug used in the treatment of many types of malignancies. Many studies proved that DOX exert its antineoplastic activity by inhibiting DNA, RNA, and protein synthesis, leading ultimately to cell death by intercalating into the DNA helix and/or binding covalently to proteins involved in DNA replication and transcription **[21, 22]**. Many studies considered DOX as a topoisomerase II poison **[23, 24]**. DOX undergoes a one-electron reduction through a variety of oxidoreductases, which include, xanthine oxidase, NADPH cytochrome P450 reductase (CPR), nitric oxide synthase, and NADH dehydrogenase **[25-27]**. The process results in the transfer of an electron from reduced nucleotides, which results in the formation of a semiquinone radical form. Molecular oxygen (O_2) can form superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) that interact with various macromolecules by nonenzymatic semiquinone radical re-oxidation as shown in **(Fig.1) [28]**.

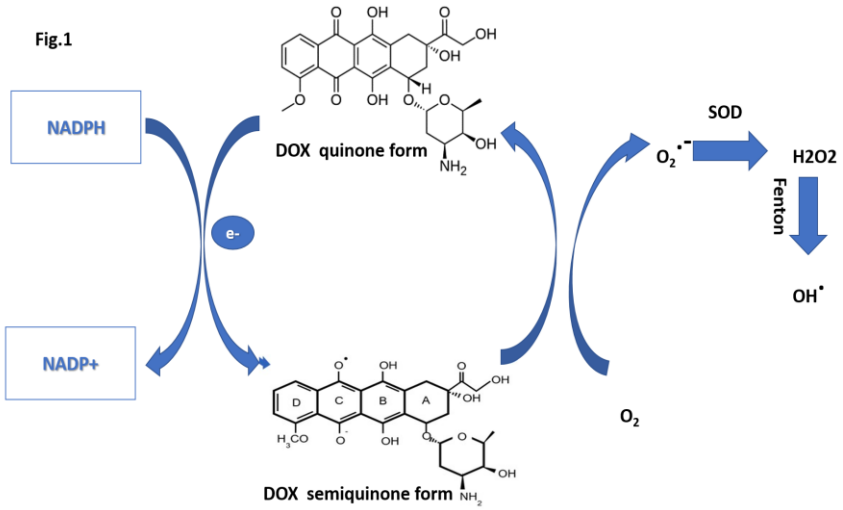

Fig. 1. A redox cycling of doxorubicin

3. Doxorubicin pharmacokinetics

3.1. Absorption

Doxorubicin has low oral bioavailability due to poor oral absorption because DOX is considered to be the substrate of both cytochrome p450 metabolic enzymes and P-glycoprotein efflux pump so it is administrated intravascularly **[29]**.

3.2. Distribution

DOX undergoes triphasic plasma clearance when it is infused intravenously. This makes DOX distribution half-life to be 3-5 min which indicates cells uptake the drug rapidly. DOX takes a longer time to be eliminated from tissues and its uptake is due to the that the terminal half-life of DOX is 24-36 h. **[30]**. DOX tends to accumulate mostly in the liver as it is the organ of metabolism besides, the concentration of DOX in the bone marrow and white blood cells is 200-500 times higher than in the plasma. The rapid distribution of DOX into tissues leads to a rapid drop in DOX levels in the blood. The lipophilic nature and DNA intercalating and binding characteristics enable DOX to penetrate tissues in a very effective manner and also remain inside the cells **[31]**.

3.3. Metabolism

The elimination of 50% of DOX from the body is in its original form. The metabolism of dox is carried out by many metabolic enzymes such as the aldo/keto reductase superfamily, cytochrome p450 [**32],** and carbonyl reductases **[33]**. Dox metabolites are present in vivo in five forms including DOX-semiquinone, DOX hydroxyaglycone, DOXol aglycone, doxorubicinol (DOXol), and DOX deoxyglucose, and **[34-36]**.

3.4. Elimination

DOX is characterized by rapid clearance from the plasma and concentrates in the tissues. Urinary excretion is considered low, rarely responsible for more than 10% of the administered dose on the other side, biliary excretion is high. Dose reduction is very important in patients with hepatic dysfunction as plasma concentrations of DOX and its metabolites are markedly increased and the rate of elimination is greatly decreased in the presence of severe liver impairment **[37]**.

4. Mechanism involved in DOX-induced testicular toxicities

DOX exerts its anticancer activity by interfering with the negative supercoiling of DNA by inhibiting the topoisomerase II enzyme. However, the mechanisms involved in testicular toxicities may differ from that responsible for anticancer activity **[15]**. In this review, we will discuss the potential possible mechanism that may be contributed to the damage of the testes caused by DOX **(Fig. 2)**.

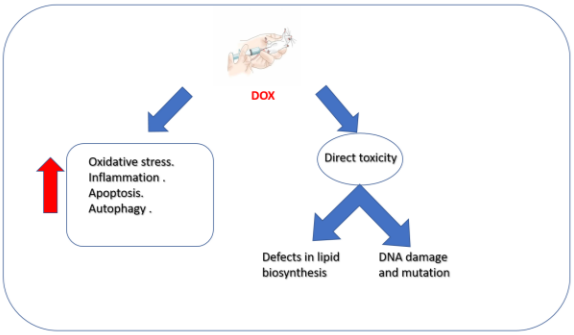

Fig. 2. Summarized possible underlying mechanisms that may be involved in DOX-induced testicular toxicity

4.1. Oxidative stress

DOX anti-tumor activity results in to increase production of free radicals and suppression of the antioxidant enzymes in many tissues including the testes **[38]**. Oxidative stress leads to great damage to the sperm proteins, membranes, and DNA which contributed to affecting male fertility **[39]**. It has been reported that DOX-induced oxidative damage of DNA generated

lipid peroxidation **[40]**. There are two different pathways involved in free radical generation by DOX. The first one implicates the reduction of DOX to the corresponding DOX semiquinone by the action of several NADPH-dependent reductases. Redox cycling of DOX-derived quinine semiquinone produces superoxide radicals in the presence of oxygen. The second pathway involves non-enzymatic mechanisms with iron. Redox reaction occurs between ferric and DOX leading to the production of the ferrous-DOX free radical complex. This complex reduces oxygen to hydrogen peroxide and other active oxygen species **[8]**.

4.2. Inflammation

The expression of many inflammatory mediators has been elevated with doxorubicin treatment. It has been reported that iNOS, IL-1β, MMP-9and TNF-α iNOS levels were significantly increased in doxorubicin-treated animals **[41]**. The previous study has shown that reactive oxygen species generated by DOX result in an elevation in the level of expression of an inflammatory biomarker NFκB **[42]**.

4.3. Autophagy in testicular functions

Autophagy is a natural process that enables cells to survive under stressful conditions including nutrient shortage, but, it has been recently involved in the actual death process **[43, 44]**. In the testis, oxidative stress caused by the excessive generation of reactive oxygen species (ROS) leads to the induction of autophagy [**44]**. Previous studies have reported that autophagy has an important role in the biogenesis of acrosome **[45]** and the differentiation of spermatids during spermatogenesis **[46]**.

4.4. Apoptosis

Increased oxidative stress results in lipid peroxidation and subsequently apoptosis in spermatogonial cells **[47, 48]**. The mechanism of apoptosis is still argumentative but the critical component involved in this process may be due to direct injury of mitochondria induced by oxidative stress **[49]** or indirect mitochondrial depolarization by pro-apoptotic Bcl-2 family proteins **[50]**. A previous study has reported that two important intrinsic apoptotic pathway effectors, p53 and Apaf-1, were responsive to DOX stimulation in testes **[51]**, as reported in heart tissue **[52]**.

5. Direct testicular toxicity

5.1. Lipid metabolism

Lipids are a very important part of the reproductive system. Cholesterol is considered to be the precursor of steroid hormones. Steroidogenesis plays an important role in the synthesis of spermatogenesis hormones. The biosynthesis of testosterone from pregnenolone is carried out by Steroidogenesis enzymes including 17β hydroxysteroid dehydrogenase (17β-HSD) 3β and hydroxysteroid dehydrogenase (3β-HSD). It has been reported that DOX result in the downregulation of these enzymes **[53, 54]**. Adipocytes are the main sites for triacylglycerol storage. It has been found that DOX downregulates adipogenesis in vitro by decreasing the expression of PPARγ **[55]**. Doxorubicin inhibits spermatogenesis by causing defects in epididymal adipose tissue **[56]** which is very important for normal spermatogenesis **[57]**.

5.2. DNA damage and mutation

Au *et al*., in 1980 reported that administration of DOX result in chromosomal aberrations after three days of administration of the drug. Chromosomal abnormalities were observed. It was found that after 20 days of administration of DOX spermatogenesis was completely lost. But, after 50 days of treatment spermatogenesis was recovered with chromosomal aberrations **[58]**. DNA damage has occurred to sperms treated with DOX *ex-vivo*

[59].

6. Assessment of testicular toxicity in animal models

Animal models are considered the cornerstone in scientific research as they enable researchers to understand the toxicity, besides allowing investigation of the underlying mechanisms so can develop suitable management.

6.1. Sperm motility and count

Sperm motility and count are assessed by squeezing the seminal content taken from the epididymis gently in a sterile clean watch glass and diluted 10 times with 2.9% sodium citrate solution and thoroughly mixed to estimate the percentage of sperm progressive motility and sperm count using a hemocytometer under a light microscope with 40x objective lens according to the technique adopted by Bearden and Fuquay **[60]**. It has been reported that the administration of DOX decreases sperm motility and count **[42]**.

6.2. Evaluation of sperm abnormality

Sperm abnormalities can be detected by mixing a drop of seminal content with an equal drop of Eosin-Nigrosin stain for detection of dead and malformed sperm then examined under 90x power (objective lens) and 10x (eyepiece) of the microscope. The type and percentage of abnormal sperm were recorded **[60]**. The previous study demonstrated that DOX leads to an increase in the percentage of dead and abnormal forms of sperm **[42]**.

6.3. Effect on steroidogenesis

Serum testosterone levels, 3β-HSD, and 17β-HSD can be assessed. It has been reported that DOX decreases serum testosterone levels and steroidogenesis enzymes **[14]**.

7. Promising pharmacological treatment

Nowadays, there are no certain treatments for DOX-induced testicular toxicity. But few studies have proved that some drugs may be promising in alleviating testicular damage induced by DOX.

7.1. Zinc/alogliptin

Zinc is an important trace element in the human body involved in many biochemical and physiological processes **[61]**. Moreover, it has antioxidant activity **[62]**. Alogliptin is FDA approved for the treatment of diabetes mellitus. Previous studies have reported that male reproductive health may be affected by GLP-1 by affecting the synthesis and secretion of gonadal hormones **[63]**. A study using experimental animals has shown that the zinc/alogliptin combination may be promising in modulating testicular toxicity induced by DOX through TGF-β1/NF-κB signaling **[63]**.

7.2. Propolis

Propolis is a resinous substance used by the honeybee to seal holes **[64]**. Due to its immunoregulatory, bacteriostatic, bactericidal, and anti-inflammatory activities, it has been used widely in folk medicine **[65, 66]**. The high content of caffeic acid, caffeic acid phenethyl ester (CAPE), and polyphenolic compounds were responsible for its pharmacological treatment **[67]**. An experimental study proved that propolis extract may be effective in protecting the testis from DOX-induced toxicity without affecting the anticancer effect **[14]**.

7.3. Hesperidin

Hesperidin is a bioflavonoid used in Chinese biomedicine **[68]**. It is extracted from citrus fruits and acts as a prodrug **[69]**. It also has anti-inflammatory, antioxidant, and anti-carcinogenic properties **[70]**. A controlled experimental study has shown that hesperidin was a promising treatment for DOX-induced testicular toxicity **[15]**.

7.4. Chrysin

Chrysin is a natural flavonoid present in honey **[71]**. It also has antioxidant and anti-inflammatory properties **[72, 73]**. The previous study suggested that the co-administration of DOX and chrysin promising in preventing testicular damage caused by DOX **[74]**.

7.5. Silymarin

Silymarin (SMN) is a flavonoid found in seeds of the milk thistle. The major active substituent of silymarin is Silibinin (SBN).beside its hepatoprotective effect, it also has anti-inflammatory and anticancer activity **[75]**. Silymarin exerts its anti-oxidant effect by reacting with the reactive oxygen species (ROS) and increasing the effect of the anti-oxidant enzyme **[76]**. The previous experimental study proved that silymarin is effective in preventing DOX-induced testicular toxicity **[77]**.

Conclusion

Many experimental models have proven that DOX induces testicular damage and affects male fertility which has a bad impact on men's lives. The possible underlying mechanisms include oxidative stress, apoptosis, and autophagy. Currently, there is no specific treatment for DOX-induced -testicular toxicity. However, animal studies provided many promising pharmacological treatments that may be used in alleviating testicular toxicity induced by DOX without affecting the anti-cancer activity of DOX.

Abbreviations

DOX, doxorubicin; FDA, food and drug administration; CPR, cytochrome P450 reductase; DOXol, doxorubicinol; MMP-9, matrix metallopeptidase 9; TNF-α, tumor necrosis factor alpha; iNOS, intrinsic nitric oxide synthase; NFκB, nuclear factor kappa B; ROS, reactive oxygen species; 3β – HSD, 3β hydroxysteroid dehydrogenase; 17β – HSD, 17β hydroxysteroid dehydrogenase; PPARγ, peroxisome proliferator-activated receptor gamma, TGF-β1, transforming growth factor β1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

All authors have read and agreed to the published version of the manuscript

Availability of data and materials

Data analyzed during this study are all included in the main manuscript.

Competing interests

No competing interests were declared by the authors

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