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REVIEW

# Aspects related to oxidative stress-mediated toxicity of doxorubicin during chemotherapy treatment

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## Abstract

**Objective:** This study aimed to describe the main toxic effects mediated by oxidative stress associated with treatment with doxorubicin in scientific research articles available in the literature. **Material and Methods:** This study employed a descriptive review methodology applied to the literature. For the theoretical scientific background, we used the electronic PubMed search engines. **Conclusion:** The toxicity of chemotherapy treatment with doxorubicin causes damage in various organs of patients who are in uninterrupted treatment with this antineoplastic agent. Anthracycline-induced cardiotoxicity has been investigated to a great degree and is especially indicated as the principal side effect. Therefore, care needs to be given to other damage caused by this medication as important as myocardial toxicity, such as renal, pulmonary and liver toxicity, among others. There is a need for further studies to prevent or even encounter a way to control the damage caused by these toxicities in various tissues.

**Keywords:** hemotherapy, doxorubicin, oxidative stress, side effects, toxicity.

## INTRODUCTION

Chemotherapeutic agents are important for the treatment of tumors, but since they do not discriminate healthy cells from malignant, they are toxic to all cells in division, with the accumulation of these agents occurring in healthy tissues causing serious clinical toxicity [1]. Several side effects originating from treatments with chemotherapeutic agents have been described, most associated with the interaction of the oxidative metabolism of antineoplastic agents with blood cells[2].

Doxorubicin (DOX) is an anthracycline drug used to treat a variety of cancers[3], and it is already clear that its mechanism of action consists of interference with the DNA of the cancer cell, mainly through interactions with DNA topoisomerase II[4].

In relation to both anticarcinogenic and toxicity effects, it is widely accepted that oxidative stress and the production of free radicals are involved in DOX toxicity[1]. The oxidative stress status of a cell is characterized by excessive production of reactive oxygen species (ROS) and/or a reduction in antioxidant defenses causing an imbalance in the normal metabolism of oxygen of an organism[5]. In spite of DOX being one of the most widely used anticancer agents, its use is limited due to severe damage to tissues such as heart, kidney, lung, liver and skeletal muscle[1].

Chemotherapy with DOX has, among other things, the characteristic of inducing acute vascular toxicity reducing gonadal blood volume and blood flow and femoral artery blood flow, compromising the blood vessel wall which may cause cardiovascular complications and the long-term progression of diseases such as atherosclerosis. To clarify this mechanism of vascular toxicity may be essential for the discovery of biological keys necessary to reduce the possible complications caused by treatment with DOX in survivors of cancer[6].

Within this context, the aim of this study was to describe the principal toxic effects associated with oxidative stress during treatment with DOX in scientific research articles available in the literature.

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## MATERIAL AND METHODS

This study employed a descriptive review methodology applied to the literature. For the theoretical scientific background, we used the electronic PubMed search engines (http://www.ncbi.nlm.nih.gov/pubmed/). Using the keywords "doxorubicin", we found 45,151 articles published during the period of 1971 until September 2011, with 3,575 review studies. Using the combination of keywords "adverse effects, doxorubicin, oxidative stress" showed 3,040 results, where the first article was published in 1972, with 948 reviews. The articles found were published in English and Portuguese, including original articles and reviews. The articles selected to compose this systematic review were in accordance with their relevance and human and experimental application, using the most recent results.

### Pharmacology of doxorubicin and the generation of free radicals

Chemically, DOX is 5,12-Naphthacenedione, 10-((3-amino-2,3,6-trideoxy-α-L-ribo-hexopyranosyl)oxy)--7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1--methoxy-, hydrochloride, (8S-*cis*). DOX is a red-orange crystalline powder, soluble in water, belonging to the class of anthracyclines, a group of antineoplastic drugs discovered in the 1960s that corresponds to one of the most effective chemotherapeutic agents ever developed against cancer[7].

The chemical structure of DOX (Figure 1) consists of a tetracyclic ring with adjacent quinone-hydroquinone groups in rings B and C, a methoxy substituent at C-4 in ring D and a short side chain at C-9 with a carbonyl at C-13. The sequence of rings A, B, C and D corresponds to the anthraquinone nucleus of anthracyclines. To date, DOX has been widely used in the treatment protocols of breast cancers, childhood solid tumors, soft tissue sarcomas and aggressive lymphomas[8].

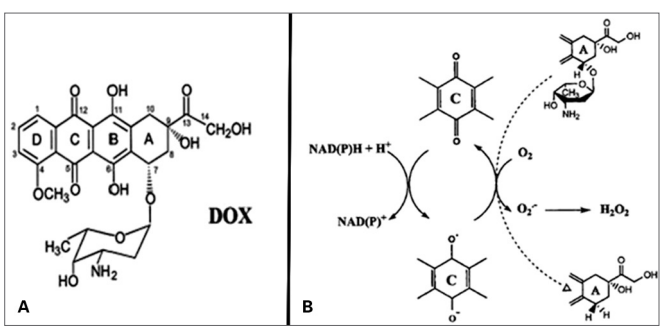


**Figure 1.** A: Chemical structure of doxorubicin; B: redox cycle Source: Minotti et al.[8].

The mechanisms of action of DOX as an antineoplastic agent include intercalation into DNA leading to inhibited synthesis of macromolecules, generation of free radicals with damage to DNA and lipid peroxidation, DNA alkylation, DNA cross-linking, separation of the DNA helices, direct effects on the cell membrane altering its fluidity, topoisomerase II inhibition and induction of apoptosis depending on the concentration that chemotherapy is able to reach the site of action[9]. It has also been described as an immunomodulatory mechanism consisting of its ability to trigger the process of caspase-dependent cell death in tumor cell lines[10].

The addition of one electron to the quinone moiety of ring C of DOX results in the formation of a semiquinone form that quickly regenerates the quinone form, by reducing molecular oxygen to ROS, such as hydrogen peroxide and superoxide anion in the presence of NAD(P) H-oxidoreductases (cytochrome P450, mitochondrial NADH dehydrogenase, xanthine dehydrogenase and endothelial nitric oxide synthase). The redox cycle of DOX is still accompanied by the release of iron ions from intracellular stores, resulting in the reaction of the chemotherapeutic agent with the metal ions released (in a 3:1 ratio) and interaction with hydrogen peroxide and the formation of hydroxyl radicals[11-13].

Such evidence has been supported by a series of studies that demonstrated the formation of free radicals during exposure to DOX in rat glioblastoma cells of, human cervical adenocarcinoma cells, mammary culture lines MCF-7 and acute lymphocytic leukemia murine cells[9,14,15]. However, this cytotoxic mechanism has been challenged[16].

Pharmacokinetic studies performed in patients with different types of tumors have shown that DOX presents a multiphase profile after intravenous injection. After intravenous administration of the usual dose of 60 to 75 mg/m$^2$, the initial half-life of distribution is approximately 5 minutes with a terminal half-life of elimination of 20 to 48 hours. It features extensive tissue binding and its main active metabolite, doxorubicinol, has plasma protein binding of approximately 70%. Elimination is predominantly via biliary excretion and oxidative metabolism[17].

Due to its electron configuration, free radicals present as unstable molecules, short half-life and high reactivity with components of biological systems such as lipids, proteins and DNA[18]. As a consequence of the action of radicals in these structures, there are the phenomena of lipid peroxidation, protein carbonilation and DNA oxidation.

### Adverse effects of doxorubicin treatment on humans

Since the 1970s, cardiotoxicity resulting from use of DOX has been described[19]. However, this adverse effect is only one of many presented by this anthracycline. These effects are described in the literature as pulmonary toxicity[20], hepatotoxic and nephrotoxic effects[21], suggesting systemic damage not only located in the tumor.

DOX is converted into its semiquinone form within the cardiomyocytes by the P450 system and flavin-containing monooxygenase[22]. Experimental studies suggest that one of the mechanisms by which cardiomyocytes target the action of DOX is by reducing the levels of catalase, superoxide dismutase (SOD) and glutathione peroxidase in cardiomyocytes after chemotherapy by promoting superoxide anion- and hydrogen peroxide- induced apoptosis[23] and in addition, tissue injury due to the large numbers of mitochondria present in heart tissue[24]. Glutathione (GSH) depletion of cardiomyocytes has also been described as a mechanism of oxidative stress-mediated toxicity of DOX[25].

Moreover, the cell death process appears to be preceded by severe deregulation in superoxide anion--mediated iron homeostasis and semiquinone form of DOX[26,13], which reaches the center of the ferritin molecule and promotes the release of ions in the form of $Fe^{2+}$ that as $Fe^{3+}$ rapidly reacts with DOX and gives rise to toxic complexes to cardiomyocytes[27].

The myocardial toxicity induced by DOX can be expressed at any stage of chemotherapy, even months or years after its end[28]. Dilated cardiomyopathy by DOX is usually related to the cumulative dose (> 500 mg per $m^2$) and has an incidence of approximately 1.7% starting from the first month of the last dose of chemotherapy received[29].

Part of the toxicity of DOX has been attributed to oxidative stress signaling via cytokines, especially TNF-α, a mediator described as a modulator of cardiac failure in several diseases. It is described that the increased production of reactive species leading to increased expression of TNF-α in cardiomyocytes via activation of NF-kB[22], thus perpetuating the inflammatory response in the host through the production of other proinflammatory cytokines such as IL-6[30].

Other toxic effects of DOX include acute events as stomatitis, neutropenia, thrombocytopenia, generalized infections, liver abnormalities, hematological toxicity, nausea and myelosuppression. In the long term, some patients may develop leukemia secondary to treatment, necrosis at the injection site, liver injury, reversible alopecia, hyperpigmentation, hypersensitivity and neurotoxicity[31].

Recent studies have shown that there is the presence of immediate systemic oxidative stress in patients treated with DOX, with oxidative cell damage and development of anemia besides the obvious showed decreased antioxidant capacity due to the drop in levels of reduced glutathione (GSSG) and total plasma antioxidant capacity[2]. It was also demonstrated that treatment with DOX promotes important immunological abnormalities in patients with advanced breast cancer immediately after infusion[32], suggesting that the modification of the immune response may be associated with increased oxidative stress and tissue damage also reported during breast cancer chemotherapy with DOX.

Some studies affirm that the most serious problem caused by DOX is cardiotoxicity[33] and demonstrate that the loss of cardiac function is commonly a result of this treatment[34]. As previously described, the production of reactive oxygen species and nitrogen is one of the mechanisms by which DOX exerts its antitumor effect[35]. Based on this assumption, there are several studies describing the involvement of reactive species produced through the use of DOX as cause of cardiac toxicity in various models. Based on results of electrocardiograms, cardiotoxicity was described as a result of treatment with this anticancer agent, in which transient abnormalities can be observed in ST-T waves, supraventricular tachyarrhythmias and ventricular extrasystoles[19].

Although the efficiency is demonstrated in the early stages of treatment with DOX, its continued infusion may lead to the development of drug resistance, requiring the subsequent use of higher doses to achieve sufficient therapeutic effect[34]. As already described, oxidative damage to the lung induced by DOX, in the form of dose-dependent lesion in the lung tissue, may be one of the pathogenic factors of pulmonary dysfunction.

To minimize or even avoid the side effects caused by treatment with DOX, numerous strategies have been used, such as administration of the chemotherapeutic agent slowly (6 to 72 hours) using cumulative doses in levels considered safer, periodic monitoring of the cardiac function by supplementary tests (e.g., echocardiography, angiocardiography, endomyocardial biopsy, etc.) and the use of drugs that may act as myocardial protectors (e.g., probucol, dexrazoxane, etc.)[28,35].

**Experimental and *in vitro* evidence**

Experimental evidence corroborates the relationship between oxidative stress and DOX-mediated toxicity. Evidence from animal studies reveals the involvement of reactive species such as superoxide anion, hydrogen peroxide and hydroxyl radicals[36,37]. Additionally, the treatment of rat cardiomyocytes in culture with DOX was demonstrated to cause, besides lipid peroxidation, an increase in LDH release and changes in glucose transport to those cells[38]. Decreased antioxidant activity and increased apoptosis in rat myocardial cells were observed both *in vivo*[39] and *in vitro*[40]. In rats treated with DOX, in addition to the decrease in cardiac mitochondrial respiration rate there is also an increase in the protein carbonyl content in cardiac tissue[41]. A recent study also conducted in rats showed consistent results that pre-treatment with zofenopril can prevent DOX-induced cardiotoxicity[41]. Other toxic effects were also observed during experimental chemotherapy with DOX, including nephrotoxicity[21,39,42,44,45] and lipid peroxidation of pulmonary cells[20].

## PERSPECTIVES AND CONCLUSIONS

The toxicity of chemotherapy treatment with doxorubicin causes damage to various organs of patients who are in uninterrupted treatment with this antineoplastic agent, therefore causing significant side effects mediated by oxidative stress. Anthracycline-induced cardiotoxicity has been investigated to a great degree and is especially indicated in the literature as the principal side effect. Therefore, care needs to be given to other damage caused by this medication as important as myocardial toxicity, such as renal, pulmonary and liver toxicity, among others. There is a need for further studies to prevent or even encounter a way to control the damage caused by these toxicities in various tissues avoiding future complications.

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