Molecular basis of anthracycline-induced cardiotoxicity

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Abstract

Anthracyclines are highly effective chemotherapeutic agents used to treat a wide variety of malignancies. Cardiotoxicity is a well recognized side effect of anthracycline therapy that limits the total amount of drug administered and can cause heart failure in some patients. Most experimental data support oxidative stress as the etiology of anthracycline-induced cardiotoxicity. This review will discuss the effect of anthracycline cardiotoxicity on human hearts and present insight into the cellular and subcellular mechanisms believed to be responsible for the lethal myocyte injury associated with anthracycline cardiotoxicity.

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Keywords: Anthracycline, cardiotoxicity, cardiac muscle, oxidative stress, apoptosis, mechanism

Introduction

Anthracyclines are highly effective chemotherapeutic agents used to treat a wide variety of common malignancies, including breast cancer, Hodgkin's lymphoma, non Hodgkin lymphoma, and acute leukemia. The advent of anthracycline in chemotherapy regimens has resulted in dramatic increases in cancer survival, especially in childhood malignancies, with increases in survival of up to 80% being reported since the introduction of the drug. However, cardiotoxicity is a well recognized side effect of anthracycline treatment that limits the total amount of drug administered and can cause heart failure in some patients. Early retrospective studies from the 1970s demonstrated that anthracycline toxicity and heart failure were dose-related, with the incidence of complications increasing sharply when the cumulative dose exceeded 550 mg/m^2 of body surface area. The incidence of heart failure was approximately 4% when the cumulative dose was between 500 and 550 mg/m^2, but increased to 18% when the dose was increased to 551–600 mg/m^2 and to as much as 36% when the total dose was at least 601 mg/m^2 [1,2]. The prognosis of the cardiomyopathy, once developed, is grave, and it is difficult to predict which individuals will develop heart failure on a case-by-case basis. Children treated with anthracycline can develop cardiotoxicity even when exposed to cumulative doses well below that believed to be safe in adults [3]. The problem of anthracycline-induced cardiotoxicity is considerable and will continue to grow, because more than 50% of long-term survivors of childhood cancer have received at least one treatment with anthracycline during their chemotherapy.

Mechanism of cardiotoxicity

A possible pathway for anthracyclin-induced cardiac dysfunction, described here, is summarized in *Figure 1*.

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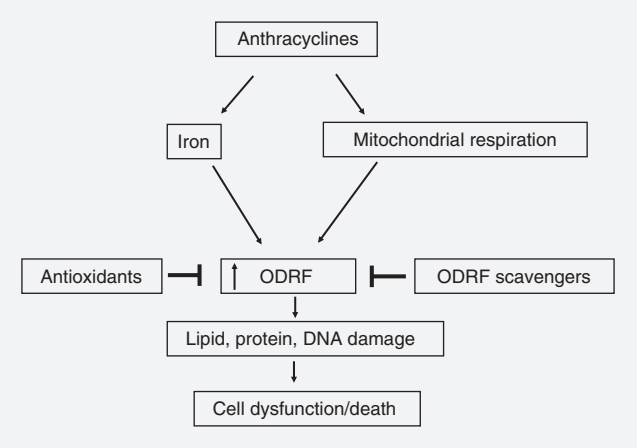

Figure 1. Proposed pathway of anthracycline-induced cardiac dysfunction/death. ODFR, oxygen-derived free radicals.

Most experimental studies have pointed to oxidative stress as the primary cause of anthracycline-induced cardiotoxicity. Evidence to support this hypothesis is derived from studies showing that the administration of anthracycline causes oxidative stress in the heart and that protection from cell death is afforded by administration of antioxidants [4]. The oxidative stress is believed to be secondary to the generation of oxygen-derived free radicals.

Coupled with the increase in free-radical generation, adult myocytes are terminally differentiated cells with highly oxidative metabolism and limited oxidative defenses [5]. Many experimental studies have shown success in limiting anthracycline-induced injury through manipulations designed to increase antioxidant defenses. For example, in cultured cardiac myocytes, administration of amifostine, trolox, 5-aminosalicyclic acid, or α-phenyl-tert-butyl nitrone (all antioxidants) before administration of anthracycline reduced indices of oxidative stress and myocyte injury [6,7]. In small-animal models, the degree of anthracycline-induced injury was also reduced by pretreatment with the antioxidants thymoquinone, butylated hydroxyanisole, and probucol [8]. The major antioxidant enzymes that protect myocytes from oxidative injury include superoxide dismutase (SOD), glutathione *S*-transferase (GST), catalase, and glutathione peroxidase. Transgenic overexpression of both SOD and catalase has been shown to be cardioprotective [9,10], as has overexpression of thioredoxin, a redox-sensitive protein [11]. We have shown in cardiac myoblasts that regulated increased expression of GST protects against anthracycline-induced cell death [4]. In that study, controlled overexpression of the α4-isoform of GST reduced oxidative stress, as measured by DFDA fluorescence, and both oncotic and apoptotic cell death. These results also support the concept that anthracycline-induced cell death is closely correlated with increased oxidative stress.

Anthracyclines induce the generation of oxygen-derived free radicals through two main pathways: a non-enzymatic pathway that utilizes iron [12,13], and an enzymatic mechanism using the mitochondrial respiratory chain. Iron is an important cofactor in the generation of many toxic free-radical species, catalyzing the Haber–Weiss reaction. Iron chelation reduces the generation of free radicals and therefore has been studied in many experimental systems, and in some human clinical trials, for its ability to reduce anthracycline-induced cardiotoxicity. Small clinical trials have shown that iron chelation allowed for the administration of greater doses of anthracycline and reduced the number of patients developing heart failure [14,15]. Meta-analysis of a number of clinical trials examining one of the more promising chelating agents, dexrazoxane, has shown a reduction in the incidence of heart failure in some patients with advanced cancer [16]. However, dexrazoxane has serious myelosuppressive side effects and may reduce the efficacy of antitumor drugs, which may limit its wider therapeutic use [16–19].

The enzymatic pathway of free-radical generation involves mitochondria. Anthracyclines have a high affinity for cardiolipin, a phospholipid that is enriched in the inner mitochondrial membrane; this affinity may allow for anthracyclines to concentrate inside myocytes [20]. The specific mechanism(s) by which this occurs is not entirely known, but includes the possibilities, first, that anthracycline-induced mitochondrial damage may cause respiratory chain defects which would allow for continued production of free radicals and, secondly, that mitochondrial damage may result in the release of cytochrome *c*, which is integral to the pathway for the induction of apoptosis. Other investigators have hypothesized that targeting the myocardial energetic network is part of anthracycline-induced cardiotoxicity [21]. This hypothesis is based on data showing that anthracycline causes significant reductions in high-energy

phosphate concentrations in heart and myocytes. Anthracycline is also believed to reduce the activity of respiratory complexes and to compromise the function of the adenine nucleotide translocator or the voltage-dependent anion channel, or both; these are proteins important in the generation and transport of ATP from the mitochondria to the cytosol, where it is utilized for many cellular functions. It is also possible that anthracycline-induced alterations in gene expression may preferentially affect metabolic enzymes, including enzymes from both oxidative metabolism and glycolysis [21].

Free radicals cause direct damage to several different types of macromolecules, including proteins [22], lipids [23], and DNA, through the generation free-radical chain reactions, direct intercalation into DNA, or both. A large part of the antitumor effects of anthracyclines may occur by irreversible damage to the tumor-cell DNA. If this is the case, it is not surprising that anthracyclines may also cause cardiotoxicity through a similar mechanism. It has been reported that oxidative stress can cause base modifications in myocyte nuclear DNA under conditions of ischemia-reperfusion [24] or depletion of antioxidant defenses [25]. Anthracyclines bind avidly to DNA in the nucleus of tumor cells, forming adducts that interfere with protein binding and therefore replication and transcription [26]. DNA damage is well known to activate the p53 pathway. Activation of p53 results in translocation of the protein to the nucleus, where it induces transcriptional changes in proteins that prevent cell division (ie, p21) and cause apoptosis [27]. Alternatively, once a cell sustains DNA damage, DNA repair pathways are activated. Such repair enzymes excise oxidized bases before replication [28], remove oxidized bases from the nucleotide pool [28], or remove oxidized bases from DNA after replication [29]. Because myocytes are terminally differentiated cells, repair pathways may play an important part in the outcome of administration of anthracycline to the heart.

Recent data from our laboratory suggest that DNA damage may have an important role in anthracycline-induced cardiotoxicity [30]. Doxorubicin (a well known anthracycline) induces oxidative DNA damage, with specific lesions including oxidized pyrimidines and 8-hydroxyguanine. DNA oxidation was followed by activation of p53 and loss of mitochondrial membrane potential in cultured myocytes. Chemical inhibition of p53 prevented activation of p53, the loss of mitochondrial membrane potential, and doxorubicin-induced cell death, but did not prevent DNA damage. In addition, in contrast to the purely oxidative injury produced by hydrogen peroxide, the DNA damage resulting from anthracycline administration is *not* repairable. These results suggest that DNA damage plays an important early part in anthracycline-induced cardiotoxicity through a pathway that involves p53 and the mitochondria.

Summary

Anthracyclines are highly effective chemotherapeutic agents that have substantially increased survival for cancer patients, but use of which is limited by dose-related cardiotoxicity. Anthracycline-induced cardiotoxicity is believed to be secondary to oxidative stress. Recent data indicate that early anthracycline-induced DNA damage is different from the DNA damage induced by a pure oxidative stress, and may represent a novel therapeutic target that may reduce cardiotoxicity or lead to better therapeutic regimens for patients with cancer. ■

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