Feature Review

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Anthracyclines/trastuzumab: new aspects of cardiotoxicity and molecular mechanisms

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Anticancer drugs continue to cause significant reductions in left ventricular ejection fraction resulting in congestive heart failure. The best-known cardiotoxic agents are anthracyclines (ANTHs) such as doxorubicin (DOX). For several decades cardiotoxicity was almost exclusively associated with ANTHs, for which cumulative dose-related cardiac damage was the use-limiting step. Human epidermal growth factor (EGF) receptor 2 (HER2; ErbB2) has been identified as an important target for breast cancer. Trastuzumab (TRZ), a humanized anti-HER2 monoclonal antibody, is currently recommended as first-line treatment for patients with metastatic HER2$^+$ tumors. The use of TRZ may be limited by the development of drug intolerance, such as cardiac dysfunction. Cardiotoxicity has been attributed to free-iron-based, radical-induced oxidative stress. Many approaches have been promoted to minimize these serious side effects, but they are still clinically problematic. A new approach to personalized medicine for cancer that involves molecular screening for clinically relevant genomic alterations and genotype-targeted treatments is emerging.

Active anticancer drugs: ANTHs and TRZ

In the study of cancer, interest has grown in topoisomerase II (TOP2) following the discovery that it is targeted by active anticancer drugs such as DOX. DOX and derivatives are highly active anticancer agents in many different clinical settings, and the identification of a crucial target of these drugs was a major landmark in the pharmacology of anticancer drugs [1]. One approach that has frequently been used in clinical trials is to combine several different anticancer drugs. The rationale for this combination was originally based on the hypothesis that these drugs act on different pharmacological targets with additive actions. A new generation of antitumor drugs has been shown to be mediated by protein kinases and to act on a family of tyrosine kinase receptors. Various therapies that target these receptors have been approved for the treatment of several cancers (lung, breast, renal cell carcinoma, and melanoma). Members of the EGF family of transmembrane receptors (ErbB family) are potent mediators of normal cell growth and development. The ErbB family consists of four closely related type 1 transmembrane tyrosine kinase receptors: the EGF receptor (EGFR; also known as HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4). Members of the EGFR family are frequently overexpressed in solid tumors. Many of the tumor-expressed targets for therapeutic antibodies are growth factor receptors. By blocking ligand binding and/or signaling through these receptors, monoclonal antibodies may serve to normalize growth rates, induce apoptosis, and/or help sensitize tumors to chemotherapeutic agents. TRZ, pertuzumab, and ado-TRZ emtansine are monoclonal antibodies that target the extracellular domain and are used for the treatment of ErbB2-positive breast cancer [2]. The importance of ErbB2 signaling in cardiac physiology soon became evident by the discovery that some breast cancer patients treated with TRZ (Herceptin, anti-ErbB2), an inhibitor of HER2 signaling, develop synergistic cardiac dysfunction, particularly when TRZ is combined with DOX. Some of these anticancer agents have associated cardiotoxicities and can, at least in some patients, cause symptomatic congestive heart failure (CHF) and, in others, asymptomatic left ventricular dysfunction, which is a greater risk in young cancer survivors treated with ANTH. Several mechanisms have been suggested to be associated with the pathogenesis of ANTH-induced cardiotoxicity. Oxidative stress, ion dysregulation, and modifications of the cardiac-specific gene expression cooperate in inducing cardiomyopathy. We review here what is known about the basic mechanisms of cardiotoxicity of cancer therapies with ANTH and TRZ. The identification of targets that mediate cardiotoxicity can also help to guide future drug development.

Oxidative stress and redox signaling

Sources and metabolism of reactive nitrogen and oxygen species (RNOS): reactive oxygen species (ROS) and reactive nitrogen species (RNS)

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Redox signaling is part of the normal physiology of all cells, and plays a significant role in pathophysiological responses. Cellular (reduction–oxidation) redox environment refers to the reduction potential or reducing capacity in cellular compartments. The redox state refers to the ratio of the interconvertible oxidized and reduced forms of a specific redox couple. Cellular redox status is regulated by the balance between cellular oxidant and reductant levels. Oxidative and reductive stress can trigger redox cascades, and the environment of the cell can determine if a cell will proliferate, differentiate, or die [3]. Any imbalance between oxidants and reductants causes oxidative or reductive stress, which triggers cell damage or aberrant signaling, leading to dysregulation. Oxidative stress occurs during biological processes including cardiovascular disease, atherosclerosis, diabetes, cancer, inflammation, and apoptosis [4].

Free radicals have emerged as important regulators of many physiological and pathological processes through the redox process. High levels of free radical production that overwhelm cellular antioxidant defense systems may damage biomolecules and deregulate signaling pathways.

Free radicals can be defined as molecules or compounds containing one or more unpaired electrons which confer a great degree of reactivity to free radicals. Radicals derived from oxygen (ROS) and nitrogen (RNS: derived from nitric oxide: NO) are the largest class of radical species generated in living systems. RNOS are products of cell metabolism and have either beneficial or deleterious effects depending on the concentration reached in the area of the cells [5,6]. RNOS include superoxide ($O_2^{\bullet -}$), the hydroxyl radical ($^{\bullet}OH$), carboxyl radical ($CO_2^{\bullet -}$), nitric oxide ($^{\bullet}NO$), and ($^{\bullet}NO_2$), as well as the non-radical species hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and carbon monoxide (CO).

The major RNS is endothelium-derived nitric oxide: $^{\bullet}NO$. Under physiological conditions, in the presence of substrate (L-Arginine) and cofactors (tetrahydrobiopterin: BH_4) endothelial nitric oxide synthase (eNOS) produces $^{\bullet}NO$, which is a potent gaseous mediator widely accepted as a key determinant of endothelial function [7]. Of the ROS generated in cells, the major sources of $O_2^{\bullet -}$ include nicotinamide dinucleotide phosphate (NADPH) oxidases, xanthine oxidases and cyclooxygenases (COXs). In addition, the endothelial and neuronal NO synthases (eNOS and nNOS, respectively), both cytochrome p450 reductase-like enzymes, can produce large amounts of ROS when deprived of their key cofactor tetrahydrobiopterin or their substrate L-arginine [7,8]. Of the many ROS-generating enzymes, NADPH oxidase, of which there are 7 homologs (termed Nox 1–5, Duox 1,2), appears to be particularly important in cardiovascular disease [9]. Activation of NADPH oxidases may result from the stimulation of several cell surface receptors, such as the angiotensin II receptor, which is particularly important in hypertension and heart failure. Upon stimulation by angiotensin II, the activity of NADPH oxidases is increased in endothelial and smooth muscle cells, suggesting that in the presence of an activated renin–angiotensin system (either local or circulating), dysfunction due to increased vascular production of superoxide anions is to be expected [10,11].

NO has potent vasodilator, anti-inflammatory, and antithrombotic properties [12,13]. The free radical $^{\bullet}NO$ has a half-life of only a few seconds in an aqueous environment. $^{\bullet}NO$ reacts with molecular oxygen and ROS to generate a range of oxidation products. One well-characterized RNS-forming reaction is that of $^{\bullet}NO$ with superoxide ($O_2^{\bullet -}$), which occurs at nearly diffusion-limited rates to produce peroxynitrite: $ONOO^-$. The latter is itself strongly oxidizing and, when protonated, undergoes homolytic scission to produce $^{\bullet}OH$ and $^{\bullet}NO_2$. Additional reactive radicals derived from other endogenous molecules such as CO and hydroperoxyl can be formed in living systems [14]. Under physiological conditions, scavengers or antioxidants can attenuate the toxic effects of ROS and RNS. An antioxidant can be defined as any substance that, when present at very low concentrations compared to those of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate. The non-enzymatic antioxidants can be classified further into directly acting antioxidants (e.g., scavengers and chain-breaking antioxidants) and indirectly acting antioxidants (e.g., chelating agents). Antioxidant vitamins (i.e., vitamins C, E, A, and folic acid) are some of the main defense mechanisms of the body's non-enzymatic antioxidant systems. The functions of endogenous antioxidant vitamins are very important via the temporal and spatial monitoring of oxidative metabolic processes [15,16]. Oxidative stress is associated with dysfunction of the mitochondria and endoplasmic reticulum, thus inducing apoptosis and protein misfolding. Mitochondria are endowed with an elaborate and well-defined multilevel antioxidant defense system of enzymes and non-enzymes to quench ROS. The scavenging system includes the matrix Mn superoxide dismutase (SOD), glutathione (GSH), and thioredoxin systems, as well as peroxidases and catalases. Redox homeostasis is crucial for cell viability and normal cell function. Balance is maintained by two major cellular antioxidant systems, namely the glutathione system and the thioredoxin system [17,18].

Several other antioxidants (*N*-acetylcysteine: NAC, lipoic acid) have been shown to reduce lipid peroxidation products [19–21]. Antioxidant defense enzymes such as heme oxygenase 1 (HO-1), catalases, SODs, peroxiredoxins, and GSH peroxidases (GPXs) have been shown to significantly decrease lipid peroxidation products. Glutathione, an endogenous scavenger, exists in both reduced (GSH) and oxidized (GSSG) states. The balance between GSH/GSSG and pyridine nucleotides [NADH/NAD, NAD(P)H/NADP] determines the cellular redox status and the level of oxidative stress [22]. Thioredoxin-dependent peroxiredoxins are the first-line defense against ROS and peroxynitrite ($ONOO^-$) and regulate H_2O_2-mediated signal transduction. H_2O_2 can act as either a destructive oxidant or as a second messenger [23].

Oxidative stress is able to induce deleterious modifications to a variety of cellular compounds: DNA, lipids, and proteins. ROS can mediate an indirect attack on endogenous molecules by reacting with cellular membrane phospholipids, and this results in the generation of secondary reactive intermediates [24]. Some intermediary metabolites are electrophilic. The nature of electrophilic stress and its role is distinct from and can be functionally