Breast Cancer Treatment and Adverse Cardiac Events: What Are the Molecular Mechanisms?

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Key Words

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Abstract

Cardiotoxicity associated with breast cancer treatment is an important concern in the oncology clinic. Different types of anti-cancer therapies have recorded high rates of cardiac dysfunction in treated patients. Cardiac dysfunction linked to anthracyclines – one of the most common conventional chemotherapies – has extensively been described and several mechanisms have been proposed, although their mode of action is not fully understood even in cancer cells. The mediation of cardiac damage by reactive oxygen species stress is a recent hypothesis that has attracted a lot of interest, since it might explain the tissue-specific toxic effects of anthracyclines in the heart. Regarding molecular targeted tyrosine kinase inhibitors used in patients with human epidermal growth factor receptor type 2+ tumours (e.g., trastuzumab, lapatinib), it is the blockage of survival pathways required for a normal heart development and function that seems to lead to cardiac pathology. Both types of breast cancer treatment appear to trigger cardiotoxicity synergically, being patients under adjuvant therapy closely monitored. Given the complex nature of heart failure and of the path-

ways altered by anti-cancer drugs, global gene expression regulation is key in the heart disease process. MicroRNAs have been demonstrated to be small molecules with big roles as essential gene expression modulators. The great potential of microRNAs as biomarkers in the cardio-oncology field needs to be further explored before new microRNA-based diagnostic and therapeutic tools can be developed.

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Breast cancer is the commonest cancer in females, being responsible for 16% of all cancer cases worldwide and for 31% in the UK. The available treatment options can be classified into two groups: conventional therapies (i.e. chemotherapy and radiotherapy) which aim to have a greater effect on cancer cells without being specifically directed to them, and newer molecular targeted therapies, which are designed to recognize determined cancer cell markers in order to spare normal cells [1, 2].

Anthracycline-Based Chemotherapy: Doxorubicin

Doxorubicin (DOX), also known by its commercial name Adriamycin, was the first anthracycline (ANT) antibiotic to be isolated from *Streptomyces peucetius* during

the 1960s, together with danorubicin. Since its discovery and despite constant efforts to develop improved ANTs, DOX is still the most widely used antibiotic in the clinic, providing high anti-tumour efficacy against numerous types of cancer [3]. As a consequence of the lack of specificity, patients receiving DOX treatment present several adverse effects, with cardiomyopathy leading to heart failure (HF) being the main long-term complication [4, 5]. This serious side effect has been shown to remain as a late-onset problem, affecting patients up to 20 years after completion of ANT treatment in a long-term follow-up study [6].

The exact mechanisms of anti-cancer activity of ANTs are poorly understood and further research is needed before a well-defined mode of action can be agreed. Current evidence suggests that it is of a multifactorial and complex nature [7], with several cellular processes having been described as affected by DOX: (1) inhibition of DNA and RNA synthesis through intercalation in nucleic acids [8, 9]; (2) generation of reactive free radicals after DOX-redox cycling [10, 11]; (3) interference with DNA helicases [12]; (4) induction of DNA strand breaks through type II topoisomerase inhibition [7, 11, 13]; (5) apoptosis, p53 dependent or independent [14]; (6) growth arrest [15].

In a retrospective study including 399 DOX-treated patients, HF was diagnosed even in patients without a previous history of cardiac conditions. It affected 4% of the patients treated with 500–550 mg/m² DOX, 18% of those treated with 551–600 mg/m² DOX and 36% of patients treated with higher doses. Due to the appearance of adverse cardiac events in a dose-dependent manner, 500 mg/m² has been established as the maximum safe dose of DOX [16]. Apart from determining the concentration limits to ensure the safest possible use of DOX, a variety of prevention and management measures are followed for cancer patients undertaking ANT-based regimes [16, 17]. Early diagnosis of DOX-induced cardiotoxicity is essential, since once patients develop advanced-stage HF, their prognosis is not favorable. This is due to the lack of specific available treatments, given that DOX-induced cardiac damage cannot be reverted by conventional therapies [18].

In terms of cardioprotection, new compounds have been tested with the aim of more specific drug delivery. In recent years, pegylated liposomal DOX has emerged as an interesting and effective alternative to conventional DOX, presenting a different pharmacokinetic profile and reduced cardiotoxicity in clinical trials [19, 20]. The myocardium is less affected by these modified ANTs because larger lipidic complexes are not able to diffuse through

the heart and other tissues, releasing most free DOX into the tumour, which has a looser endothelium [21]. Even more specific variants of DOX are currently under investigation, i.e. the recognition of estrogen receptor, one of the strategies for a targeted delivery [22].

Mechanisms of DOX-Induced Cardiotoxicity: Reactive Oxygen Species Stress Hypothesis

In contrast to fast-dividing cancer cells, adult cardiomyocytes are quiescent cells. This important fact suggests that the mechanisms underlying DOX tumour and cardiac cytotoxicity are likely to be different. The existence of differential mechanisms of cell death induction in cancer cells and in the myocardium would facilitate the development of less cardiotoxic ANTs [17].

The generation of free radicals seems to play a vital role in DOX-associated cardiotoxicity [10, 23–25]. Although mammalian tissues have protective resources against oxidative stress, the heart has been shown to present remarkably low levels of enzymes such as catalase, superoxide dismutase and glutathione peroxidase, all of which help minimizing reactive oxygen species (ROS) toxicity. The reduced levels of antioxidant enzymes in the heart in comparison to other tissues may explain the tissue-specific toxicity associated with DOX [26].

Cardiomyocytes are very rich in mitochondria, which contain enzymes that mediate a 1-electron reduction in ANTs into a free radical semiquinone form shortly after they enter the cell. This reaction produces high concentrations of ROS, such as superoxide anion (O₂⁻) or hydrogen peroxide (H₂O₂) [3]. Some research suggests that DOX not only generates new damaging free radicals, but also contributes to a decrease in endogenous antioxidant levels. Altogether, this leads to oxidative stress that will cause cytotoxicity through multiple mechanisms (fig. 1) [26–28].

The explanation of DOX-induced cardiotoxicity through oxidative stress pathways has become a very popular hypothesis in the last decade and has been supported by several studies demonstrating cardioprotective effects when administering antioxidants in combination with DOX in vivo, without interfering with anti-tumour efficacy [25, 27, 29].

There is a considerable amount of evidence indicating that mitochondria are highly susceptible to damage by generation of superoxide radicals. Mitochondrial dysfunction has been shown after DOX treatment in vitro, with cytochrome c release leading to caspase-9-mediated

Fig. 1. Oxidative stress causing adverse effects in cardiomyocytes following DOX metabolism. After redox cycling of ANTs (i.e. DOX) in the mitochondria, the generation of free oxygen radicals damages the cell through diverse mechanisms. These include: DNA damage, alteration of gene expression, sarcomere degradation with deregulation of Ca^{2+} levels, and mitochondrial dysfunction [21].

caspase 3 activation and resulting in apoptosis [30, 31]. The fact that a $\text{p53}^{-/-}$ mouse model did not fully evade DOX-induced cardiac damage supports the idea that ROS might be able to directly trigger p53-independent apoptotic pathways [32].

Molecular Targeted Therapy: Tyrosine Kinase Inhibitors

Advances in medical knowledge and new technologies have enabled the development of molecular targeted therapies, the main advantage of which is that they are more specifically directed to cancer cells, being thus less harmful for healthy cells. In the context of breast cancer, the human epidermal growth factor receptor type 2 (HER2), also known as erbB2 [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)], was described to be overexpressed in 20–30% of the patients. HER2 overexpression was associated with particularly aggressive forms of disease and poorer prognosis. Since this was recorded, HER2 has attracted many efforts in drug discovery for breast cancer therapy [33–35].

Different types of tyrosine kinase inhibitors have successfully reached the market and are approved by the Food and Drug Administration. Trastuzumab and lapatinib are the two most studied and clinically applied. Trastuzumab (Herceptin®) is a humanized monoclonal antibody that binds to the extracellular domain of the HER2, impairing its activation and downstream cascade of survival signals. Apart from the direct inhibition of the HER2 pathway, trastuzumab is thought to contribute to

tumour cell death also via antibody-directed cell cytotoxicity [36]. On the other hand, lapatinib (Tyverb®) is a small molecule capable of inhibiting both HER2 and epidermal growth factor receptor (so-called HER1) by interacting with their intracellular tyrosine kinase (tyrK) domain (fig. 2). HER1 and HER2 overexpressing tumours undergo apoptosis or growth arrest after treatment with lapatinib [37].

Both trastuzumab and lapatinib are able to block the same signalling pathway – mediated by Akt and MAP kinases (ERK1/2) – and during respective clinical trials proved to be efficient anti-cancer treatments for breast cancer patients. Maximum therapeutic results were achieved when combining them with ANT-based chemotherapy. In fact, HER2 blockers are normally used as chemotherapy adjuvants for second-line therapy [37, 38].

In spite of inhibiting the same pathway, different rates of cardiotoxicity are observed upon trastuzumab and lapatinib treatment. Although some cardiac events were recorded, lapatinib was better tolerated than trastuzumab during clinical trials [37, 39]. Regarding ANT-combined regimes, 27% of patients under trastuzumab + DOX developed cardiac dysfunction, while the incidence for lapatinib + DOX remained as low as 2.2% [38, 40]. The mechanisms responsible for this difference are not yet well understood, but are likely to be related to the nature of each molecule. Although the antibody-directed cell cytotoxic activity of trastuzumab is the main obvious differential mode of action, it does not seem to be responsible for the higher cardiotoxicity. An intriguing mechanism to explore is the induction of mitochondrial dysfunction by trastuzumab while lapatinib seems to have a protective effect on such organelle [41, 42]. This would be

Fig. 2. Trastuzumab and lapatinib inhibition of the HER family members. HER2 belongs to a family of growth factor receptors with intracellular tyrK domains (HER1–HER4). Although HER2 is the only member that does not have a known ligand, it forms dimers with other HERs acting as a co-receptor. The humanized monoclonal antibody trastuzumab recognizes the extracellular domain of HER2, preventing dimerization with other members. The small inhibitor lapatinib blocks the tyrK domains of both HER2 and HER1 (epidermal growth factor receptor, EGFR). The subsequent inhibition of the downstream signalling pathway prevents the translocation into the nucleus of the activated factors that usually stimulate transcription of survival and proliferation-promoting genes.

an interesting hypothesis to link to the mitochondrial dysfunction triggered by DOX in a putative synergic way, given that combined therapy results in higher cardiotoxicity risk.

Neuregulins are an essential family of growth factors during development which bind to HER3 and HER4. Neuregulin-1 (NRG1, also known as heregulin or neu differentiation factor) has been particularly described to be required for an adequate heart formation and function [43, 44]. Moreover, the lack of erbB2 (HER2) and erbB4 (HER4) receptors is also associated with the same cardiac

defects during embryogenesis [45–47]. Given the requirement of a functional HER2 in the heart and the stimulation of HER2–HER3/4 dimerization by NRG1, administration of such growth factor has been considered as an option to counteract HER2-targeted therapy toxicity and prevent HF [48, 49]. Interestingly, NRG1 administration to adult rat cardiomyocytes was protective of DOX-induced toxicity by reducing oxidative stress. This may explain why an increased cardiotoxicity is recorded when combining ANT-based chemotherapy with HER2 inhibitors (fig. 3) [50].

Role of MicroRNAs in Heart Disease

MicroRNAs (miRNAs) are abundant endogenous short (approximately 22 nucleotides, nt) non-coding RNA molecules that play an important and evolutionary conserved regulatory role in gene expression, affecting up to one third of the human genome [51]. miRNAs act mainly at a post-transcriptional level, basepairing with the 3' untranslated region of target messenger RNAs [52]. The resulting impairment of protein synthesis from target messenger RNAs will determine different effects in key biological processes such as development, differentiation, metabolism, proliferation and growth [53].

Regardless of the primary cause – including toxicity derived from cancer treatment – most cardiac conditions convert to HF in the later stage. Generally, progression to HF starts with remodelling of the ventricles – mainly the left ventricle – in order to achieve a functional compensation in the damaged heart. This process is tightly regulated and involves important changes in gene expression, such as re-activation of fetal genes (e.g., the switch from the α to the fetal β form of the myosin heavy chain), and myocyte hypertrophic growth [54]. Eventually, this compensatory hypertrophy causes myocardial wall stress and contractile dysfunction, leading to a final stage of failure where the heart is not able to pump enough blood to the organism. Importantly, also other cardiac cell types like fibroblasts and endothelial cells play an active role in cardiac remodelling [55]. It has only recently been described how important cardiomyocyte loss through apoptosis is in the development of HF, suggesting that there are still numerous relevant mechanisms involved to be yet elucidated [56].

Although miRNA implications have been extensively studied in cancer, the importance of these short regulatory RNAs within the cardiovascular system has only been highlighted in recent years [57]. Spatiotemporal ex-

Fig. 3. Cardiotoxicity of DOX and HER2 inhibitors in combination. **a** When DOX is administered alone, HER2 is active and able to dimerize with other HER members upon recognition of NRG1, subsequently triggering survival-promoting pathways in the cell. **b** When trastuzumab (TRZ) or lapatinib (LAP) are combined with DOX, HER2/NERG1-mediated counteraction of cardiac toxicity is lost.

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Fig. 4. Progression of HF. Pathological hypertrophy is the result of ventricular remodelling, which follows damage to the heart through a variety of injuries (ischaemia, pressure/volume overload, myocardial infarction, exposure to toxic agents, and others). After this hypertrophic phase, the enlarged myocardium starts

losing viable cardiomyocytes, being affected by progressive fibrosis and failing to contract properly. All these events finally lead to HF. Gene expression regulation is key during the pathological process of HF, and miRNA signatures vary from the healthy heart as the disease progresses, being essential regulatory elements.

pression of miRNA in the different tissues has been demonstrated to influence development and evolution, and the heart is not an exception [58]. In fact, impairment of miRNA biogenesis is demonstrated to result in embryonic lethality with poor myocardium formation [59]. The contribution of miRNA to a normal heart development and function is not surprising, since cardiogenesis, ventricular remodelling and HF are all highly complex processes. miRNAs constitute an additional level of gene expression regulation that has a high impact on the gene

expression pattern of both the healthy and diseased heart, modulating aberrant signatures involved in heart disease (fig. 4).

Different studies have recently identified particular miRNA signatures associated with several cardiac conditions, although the majority of research focuses on hypertrophy and HF. Van Rooij [60], for instance, described an miRNA expression pattern in the heart under stress. On the other hand, Thum et al. [61] showed a miRNA sub-signature shared by the fetal and the failing heart,

which correlates with the re-expression of fetal genes in the failing heart. Another example of research in the field is a big profiling study by Ikeda et al. [62] of different types of heart disease where altered miRNA expression in the dysfunctional myocardium was confirmed for humans. The most studied and well-characterized miRNAs in the heart are: (1) miR-1 (the most abundant cardiac miRNA), described to be involved in arrhythmias and down-regulated in HF patients [63, 64]; (2) miR-133 and miR-195 and their role in hypertrophy [60, 65, 66]; and (3) miR-208, which is encoded in an intronic region of the α form of the myosin heavy chain and governs a feedback loop that results in impaired contractility when deregulated [67]. Other miRNAs such as miR-29 and miR-499 have also been shown to have relevant functions associated with heart disease [68, 69]. Not much is known regarding miRNA implications in the particular case of heart disease following cancer treatment. So far, only one paper reports the up-regulation of an miRNA (miR-146a) upon DOX treatment in cardiomyocytes, being cells more resistant to DOX when artificially reducing miR-146a expression in vitro [70].

All the studies proving detrimental consequences in cardiac function upon up-/down-regulation of certain miRNAs have highlighted the potential of miRNAs both as diagnostic and therapeutic tools. Moreover, the fact that these small RNAs seem to be actively secreted to and stable in the bloodstream has attracted a lot of interest in trying to use circulating miRNAs as disease biomarkers [71]. This would be highly beneficial for patients with cardiovascular conditions, since it would allow a rapid and non-invasive diagnosis and monitoring. In terms of therapeutic applications, the main challenge is engineering both systemic stability and, most importantly, specificity of the miRNA-targeting drug, given that the same molecule is likely to have different effects depending on the tissue. Furthermore, miRNAs are known to have multiple targets. Although this may lead to a synergic therapeutic effect when an miRNA governs the expression of several genes of the same pathway, the benefit might be lost to 'off-target' effects [72]. These issues need to be carefully addressed before miRNA-based therapies can reach the market for safe clinical applications.

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