

Review

Endothelial Dysfunction as a Determinant of Trastuzumab-mediated Cardiotoxicity in Patients with Breast Cancer

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Abstract. *Background: Breast cancer is the most common cancer in females in the UK and has greater severity in patients who overexpress human epidermal growth receptor 2 (HER2) proteins in the breast tissue. Trastuzumab is a humanised monoclonal antibody and is targeted towards blocking the HER2 pathway and effectively reduces the recurrence of breast cancer and associated mortality. However, trastuzumab is also associated with an increased risk of cardiotoxicity which likely results from inhibition of the HER2 pathway. Under normal conditions HER2 pathways help maintain the integrity of the myocardial contractile elements, as well as the coronary vasculature, but trastuzumab inhibits these survival pathways and increases the risk for congestive heart failure (CHF). In the present review, we summarise the pathways that are implicated in the development of CHF in patients receiving trastuzumab. We also highlight the role of trastuzumab-mediated endothelial dysfunction and CHF.*

Breast cancer is the most common cancer in the UK, accounting for 31% of all new cancer cases in females. One of the biological markers of the severity of breast cancer is overexpression of human epidermal growth receptor-2 (HER2) protein in the breast tissue. In approximately 25-30% of patients with breast cancer, HER2 is overexpressed (1). Trastuzumab is a humanised monoclonal antibody targeted towards blocking the HER2 pathway (2). Trastuzumab effectively reduces the recurrence of cancer by 50% and mortality by 33% (2-5). However, trastuzumab is also associated with an increased risk for cardiotoxic effects (6). The exact mechanisms by which trastuzumab causes

cardiotoxicity have received little attention to date; however, proposed mechanisms suggest that interference in HER4 and HER2 survival pathways leads to over-production of reactive oxygen species (ROS) which eventually causes apoptosis of cardiomyocytes (6, 7). This depletion in the number of cardiomyocytes leads to a drop in left ventricular ejection fraction (LVEF) which clinically manifests as congestive heart failure (CHF).

The purpose of the present review is to discuss the mechanisms implicated in the development of trastuzumab-mediated cardiotoxicity, with a specific focus on factors that may affect the vasculature.

HER2 and Breast Cancer Outcome

In normal breast tissue, HER2 is involved in growth, repair and reproduction of breast cancer cells, however, in some patients, polymorphisms in the gene encoding HER2 results in over-production of HER2 receptors leading to uncontrolled cell growth (HER2 positivity) (8). A number of studies have reported that HER2-positive breast tumours are associated with poorer overall and disease-free survival (1, 9). This poor outcome is partly attributed to an increased metastatic potential of HER2-positive cells in terms of invasion and survival after migration into the site of metastasis (10). There is also evidence to suggest that patients with HER2-positive disease may have increased resistance to common anticancer treatments, such as chemotherapy and radiation therapy (10).

The Importance of HER2 in the Myocardium

The human epidermal growth receptors are tyrosine-kinase receptors consisting of four isoforms: HER1, HER2, HER3, and HER4 (11). The HER2 isoform plays a critical role in cell survival and is involved in the embryogenesis of the heart (12). During cellular stress (such as hypoxia and oxidative stress) a protein called neuregulin, which is released by endothelial cells located in the coronary

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microvasculature and endocardium (both are structures located close to cardiomyocytes), binds to HER4 receptors which dimerize with HER2 receptors and activate cell survival pathways (13). The survival pathways inhibit cellular apoptosis by increasing cellular transcription factors (13), reduce ROS from mitochondrial respiration *via* activation of protein kinase B (14), and stimulate production of endothelial nitric oxide synthase (eNOS) which produces the cardioprotective molecule nitric oxide (NO) also *via* activation of protein kinase B (15).

In addition, the integrity of cardiomyocytes is dependent on HER2 signaling, as it helps maintain the contractile elements (*i.e.* the sarcomere) of cardiac muscle cells (16). Interestingly, neuregulin is thought to play a major role in regulating homeostasis of the cardiovascular system, and as such, the inhibition of its effects could result in a wide range of cardiac complications, including injury to the endothelium – termed endothelial dysfunction (ED) (17). Figure 1 summarises the key components of the HER2 survival pathway, as well as the implications for the heart when HER2 receptors are inhibited.

The Effect of HER2 Inhibition on Cardiomyocytes

Inhibition of HER2 signaling with trastuzumab reduces the cardioprotective effects of HER2-mediated survival pathways, leading to cardiotoxicity and CHF (Figure 1) (7). Many of the adverse effects occur *via* the accumulation of ROS from the mitochondria of contracting cardiac cells, which cause apoptosis of cardiomyocytes (6). However, oxidative stress from accumulated ROS also augments levels of angiotensin II which is a potent vasoconstrictor. It is proposed that in turn, angiotensin II down-regulates the production of neuregulin in the cardiac microvasculature, causing a further reduction in HER2-mediated survival pathways (18). Moreover, angiotensin II activates nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) which releases further ROS and therefore perpetuates the adverse effects on cardiac tissue (19, 20). NADPH is also a major source of ROS in vascular endothelial cells and increases the risk for ED (21).

Adjuvant Chemotherapy and HER2 Inhibition

Patients treated with anthracyclines (such as doxorubicin) and trastuzumab appear to have greater incidence of CHF than patients treated with trastuzumab only (27% and 5% respectively) (2, 22). The suggested explanation for this finding is that in patients who are exposed to anthracyclines, HER2 survival pathways are activated to counteract the stress to cardiomyocytes from antracycline treatment, but subsequent administration of trastuzumab blocks these survival pathways, exerting further stress on the cardiac tissue and adversely-affecting cardiomyocytes, leading to CHF (7).

HER2 Inhibition and CHF

In patients with breast cancer receiving trastuzumab, LVEF may decrease following treatment, resulting in CHF. In the general population, an LVEF of less than 40% is usually the threshold for classifying heart failure, but in breast cancer, patients are not advised to start trastuzumab if pre-treatment LVEF is less than 55% (23). In patients who are already receiving trastuzumab treatment, a reduction in LVEF of more than 10% or an LVEF of less than 50% is the threshold to halt treatment. Monitoring of cardiotoxicity is performed using an echocardiogram and is repeated at 3-month intervals according to National Institute for Health and Clinical Excellence guidelines (23).

In the general population, CHF, can be caused by a variety of factors (*e.g.* reduction in the number of cardiomyocytes), but abnormalities in the coronary vasculature are thought to play a significant role in the pathogenesis of CHF (24). In particular, narrowing of the coronary and peripheral microvasculature reduces blood flow to the myocardium and increases total peripheral resistance. This serves to increase left ventricular intracavitory pressure and myocardial workload is subsequently increased (24). When coupled with reduced myocardial perfusion, cardiac function will be compromised. Indeed, poor coronary microvascular blood flow is related to the development of severe CHF and even death in patients with existing CHF (25). In patients receiving trastuzumab, changes in the myocardium are not related to drug dose, are not known to cause structural changes to cardiomyocytes, and are reversible (7). This suggests that trastuzumab might exert adverse effects on the coronary and peripheral vasculature rather than just on cardiomyocytes, and this could then transiently impair myocardial function and increase the risk for CHF. In the next section, we provide an overview of the vascular endothelium and discuss how inhibition of HER2 pathways could contribute to ED in CHF.

Potential Role of HER2 Inhibition in ED and CHF

The endothelium is the innermost lining of the vasculature and controls endothelial function by responding to various neurohumoral stimuli and vasoactive factors which influence vasomotion, thrombosis, platelet aggregation and inflammation (15). Damage to the endothelium disrupts vascular homeostasis and results in ED, which is an early indicator of atherosclerosis (26). The endothelium releases a number of different vasoactive factors which help regulate vasomotor tone and can either promote or prevent atherosclerosis (15). NO is the most crucial of these molecules and was first identified in 1980 by Furchtgott and Zawadzki (27). NO is an endothelium-dependent vasodilator of the underlying smooth muscle and plays an important role in the maintenance of basal vasodilator tone of the blood vessels (28). NO is formed under the influence of the

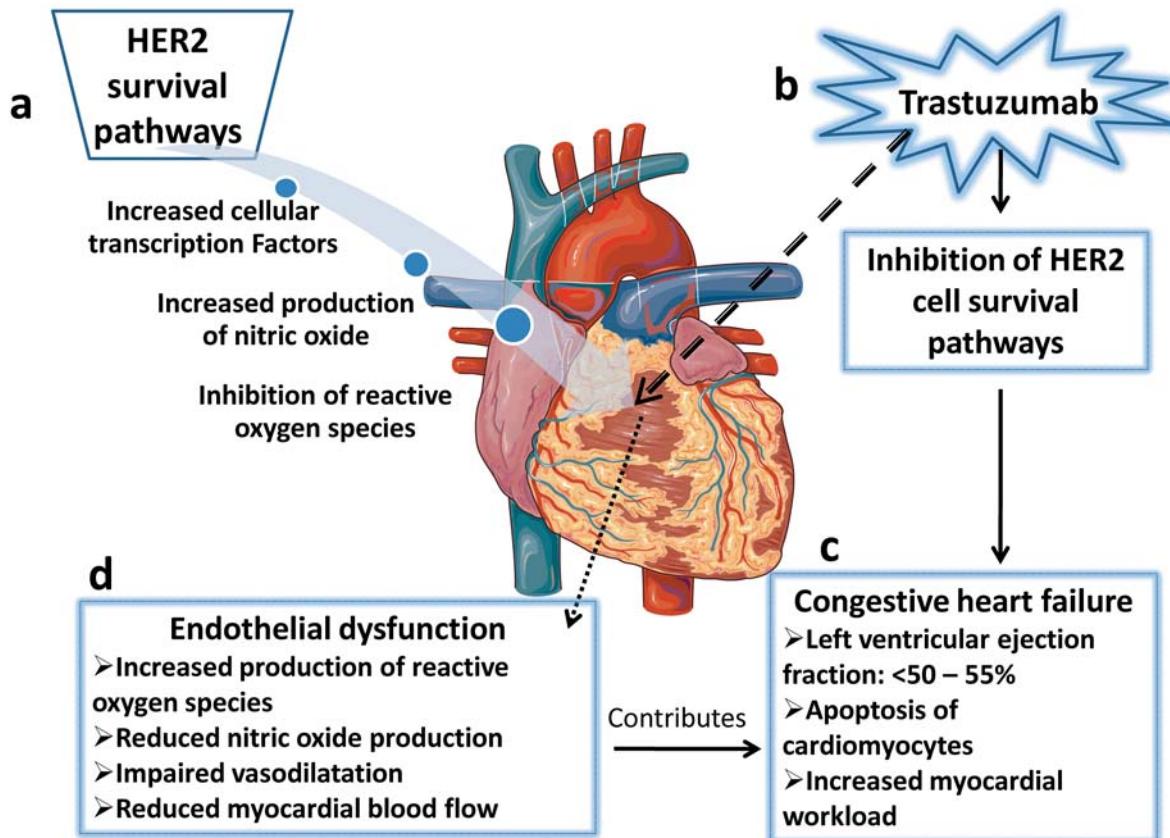


Figure 1. a: The HER2 pathways increase cellular transcription factors which prevent apoptosis of cardiomyocytes. Nitric oxide increases vasodilatation of the coronary blood vessels allowing an increase in myocardial blood flow. HER2 signaling also helps to regulate the integrity of the sarcomeres in cardiomyocytes. b: Trastuzumab inhibits HER2 cell survival pathways and may also contribute to endothelial dysfunction. c: Trastuzumab is not indicated in patients with a pre-treatment left ventricular ejection fraction of $<55\%$, and should be halted if it is $<50\%$ when treatment has commenced. Apoptosis of cardiomyocytes reduces contractile efficiency, and this increases myocardial workload. d: Inhibition of HER2 pathways can affect the vasculature by increasing production of reactive oxygen species, which injure the endothelium and reduce the production of nitric oxide. This causes impaired vasodilatation along with a concomitant decrease in myocardial blood flow. Endothelial dysfunction is a well-established contributor to congestive heart failure.

enzyme eNOS, which is constitutively expressed in endothelial cells (29), and helps maintain a cardioprotective environment within the vessel (15). A reduction in eNOS expression is a major contributor to ED (30), but other factors, such as a reduction in co-factors involved in NO production (31), as well as accumulation of ROS (21), also contribute to ED. One of the earliest signs of a reduction in NO levels is an impairment in vasodilatory function (15).

In CHF, ED results from a reduction in NO bioavailability, which predisposes the vessel to atherosclerosis. The mechanisms which damage cardiomyocytes due to HER2 inhibition also potentiate damage to the vasculature primarily through a reduction in NO levels. For example, increase in ROS which causes apoptosis of cardiomyocytes (6), also causes a reduction in NO bioavailability, thus compromising endothelial function (21). In addition, increase in angiotensin

II levels as a result of HER2 inhibition can stimulate vascular smooth muscle cells to directly increase the formation of ROS (21), which may compromise endothelial function (32, 33). Finally, trastuzumab inhibits the actions of neuregulin, which is involved in promoting NO production in the coronary microvasculature (17).

A reduction in NO bioavailability has a number of implications for the onset of CHF. Firstly, reduced vasodilatation of the peripheral arteries increases systemic vascular resistance, resulting in increased afterload. Secondly, reduced vasodilatation of the coronary arteries results in poor myocardial perfusion (34), and collectively these two states will increase cardiac workload, resulting in myocardial ischaemia, which if not halted, will lead to myocardial damage. Several studies have reported that ED is an important contributor to CHF (25, 35-37).

It is clear that ED contributes to the pathogenesis of CHF, and that the mechanisms resulting from the inhibition of HER2 survival pathways in patients with breast cancer not only affect cardiomyocytes, but also affect the vasculature. However, to our knowledge, there are no studies that have examined the effect of trastuzumab on vascular function and morphology in patients with breast cancer. We hypothesise that the cardiotoxic effects of trastuzumab could be due to impairments in endothelial function in the coronary vasculature. Further prospective studies are warranted to explore this hypothesis in greater detail.

Summary

The present review highlights that inhibition of HER2 survival pathways results in increased ROS production, leading to cardiac dysfunction due to apoptosis of cardiomyocytes, and ED in the coronary vasculature. The production of ROS appears to be a unifying mechanism for both abnormalities. Further studies utilising assessments of endothelial function will help provide greater insight on the effects of trastuzumab on NO bioavailability and occurrence of CHF. This may lead to targeted-treatment of vascular dysfunction, which can help reduce trastuzumab-related cardiotoxicity in patients with breast cancer.

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