American Journal of BioMedicine
AJBM 2018;6 (3): 242-258
Research Article Doi: 10.18081/2333-5106/015-10/631-643

Cardioprotective Role of Trans Retinoic Acid in Trastuzumab-Induced Cardiotoxicity Through Modulation of Erk2 Signalling Pathway

Jun Fan, Cheng Yang [1], Dong-Qing Chin [1], Alexis Romanos [2], Eva Wang [2], Monica M. Vargas[1], Hao Cheng [1*]

Abstract

Breast cancer is the most frequent cancer in women, and it is increasingly identified in younger women. The involvement of several oncogenic pathways, including ErbB2, ErbB3, and phosphoinositide 3-kinase (PI3K), has led to the transformation of the ErbB2 receptor in most of the aggressive breast cancers. Chemotherapy and radiation therapy have been used to treat these patients. Moreover, chemicals used in these treatments have numerous side effects, and cardiotoxicity is one of the more critical and studied adverse effects. Cardiotoxicity often involves a dyssynchrony of the contractile cycle in the myocardium and especially mitosis, which is an underlying terminal feature. Trans retinoic acid (tRA), known to be a differentiating molecule, has many protective effects such as anti-differentiation and anti-proliferative effects in several cancers. However, the complete influence of tRA on tyrosine kinase signaling in relation to ErbB2 is still unknown. We are therefore interested in its influence on the ErbB family. The objectives of this study are to investigate the possible chemopreventive effect of tRA on the Trastuzumab-induced cardiotoxicity in newborn rats, to analyze the cardioprotective pathways activated by tRA, and to identify anti-differentiating pathway involved in the mechanism directly or indirectly through the induction of trophic factor, desmoplakin, and by modulating signaling molecules associated with differentiation, such as CaM, DcR3, and Erk1 and 2.

Keywords: Trastuzumab; Breast cancer; Cardiac dysfunction; Trans retinoic acid

*Corresponding author: Cheng
[1]Department of Physiology, University of Tennessee Health Science Center, Memphis, TN, United States.
[2]Department of Cardiovascular Surgery, Hospital of Anhui Medical University, People's Republic of China.
Received February 28, 2018; Accepted July 27, 2018; Published August 22, 2018

Introduction

A major cause of treatment failure in breast cancer patients is the development of resistance to chemotherapeutic drugs and metastatic tumor cells. A major mechanism for this is the activation of the PI3K/Akt, Erk MAPK, and STAT4/5 signaling pathways. The phosphorylation of these proteins is up-regulated in more resistant cancer cells. Breast cancer cells that are resistant to tamoxifen or trastuzumab express more aldehyde dehydrogenase (ALDH) isoforms than cells that are sensitive to the drug. Trastuzumab increases autophagy in breast cancer cells by reducing ErbB2-mediated

PI3K/Akt/mTOR1 signaling, thereby inhibiting the negative regulation of Beclin 1 by mTOR1, which is also an important pathway for the killing of HER-2/Neu-deregulated breast cancer cells.

Drug resistance of breast cancer to therapeutic agents or radiation and the resistance of receptor-positive cancer cells to estrogen antagonists are major problems in cancer and tumor research. Retinoids, active metabolites of vitamin A, are capable of preventing breast cancer. Some retinoids inhibit cell growth, promote cell differentiation, and suppress malignant phenotypes in breast cells. Numerous studies have shown the potential of natural, synthetic, and non-toxic retinoids in the prevention and suppression of mammary carcinogenesis. More importantly, natural retinoids prevent breast cancer in women at risk for breast cancer, reduce the incidence and mortality rates of breast cancer, as well as the development and growth of breast cancer. In preclinical animal models, natural retinoids reduce the incidence and trace of tumors by inhibiting carcinogenesis of breast cells, thereby conferring chemoprophylaxis against breast cancer. Retinoids offer tremendous potential as a cancer chemoprophylaxis for humans, especially women with a high risk of breast cancer.

Scope and Objectives

Breast cancer is the most frequent cancer in women, and it is increasingly identified in younger women. The involvement of several oncogenic pathways, including ErbB2, ErbB3, and phosphoinositide 3-kinase (PI3K), has led to the transformation of the ErbB2 receptor in most of the aggressive breast cancers. Chemotherapy and radiation therapy have been used to treat these patients. Moreover, chemicals used in these treatments have numerous side effects, and cardiotoxicity is one of the more critical and studied adverse effects. Cardiotoxicity often involves a dyssynchrony of the contractile cycle in the myocardium and especially mitosis, which is an underlying terminal feature. Trans retinoic acid (tRA), known to be a differentiating molecule, has many protective effects such as anti-differentiation and anti-proliferative effects in several cancers. However, the complete influence of tRA on tyrosine kinase signaling in relation to ErbB2 is still unknown. We are therefore interested in its influence on the ErbB family.

The objectives of this study are to investigate the possible chemopreventive effect of tRA on the Trastuzumab-induced cardiotoxicity in newborn rats, to analyze the cardioprotective pathways activated by tRA, and to identify anti-differentiating pathway involved in the mechanism directly or indirectly through the induction of trophic factor, desmoplakin, and by modulating signaling molecules associated with differentiation, such as CaM, DcR3, and Erk1 and 2.

Trastuzumab-Induced Cardiotoxicity

Guidelines for the risk evaluation are divided into high-risk or low-risk patients. These considerations are not generally used to establish therapy, but in most cases are of critical importance in understanding the potential cardiotoxic profile for preemptive therapy to avoid severe damage. Rest and continuation or cessation of TZH therapy is often considered. To date, the role of preventive therapy for ventricular damage is still under discussion. Cardio-oncological collaboration can, however, plan the best way to reduce TZH-related heart failure and determine which patients should receive new invasive methods, including stem cells injected by the most damaged areas. Verifying more efficient methods for the management of TZH-related cardiac insufficiency is essential in

Research Article Doi: 10.18081/2333-5106/015-10/631-643

hospitalized patients. White dental streaks are considered an indicator of TZH efficacy, but patients are invited to plan a scaled visit every 2 or 3 months to reduce the risk of severe myocardial damage during therapy. Summarizing, the correct diagnosis method characterized by serial controls in long-term TZH-treated patients could identify acute left and right ventricular damage and permit organ replacement therapy with a lower incidence of heart failure. This method may include the use of new near-infrared spectroscopy devices for organ energetic balance or biological markers of heart failure like the 2010 ESCL cardiac failure guidelines. Given its central role in the differentiation and cycling control of myocytes, the main signaling pathway by retinoic acid family members involves extracellular signal-regulated protein kinases, Fig. 1. In isolated and in vivo cardiomyocytes, TZH reduces the quantity of pilating-related protein such as Erk2, but it has a stimulatory effect on proteins modulating mitochondrial biogenesis, including PGC-1 overexpression, Fig. 2.

Mechanisms of Cardiotoxicity

Breast cancer is the disease which has been found in females more, and the overall incidence of breast cancer is rising in developing countries. Breast cancer is mainly treated by curative surgery, hormonal therapy, and chemotherapy. Trastuzumab, a targeted molecule, has been effectively used to treat breast cancer. But trastuzumab leads to cardiotoxicity. Trastuzumab cardiotoxicity is mediated by an interplay of Erb B2 receptor and the immune system in which inflammatory molecules are increased resulting in macrophage infiltration in the heart and cardiac remodeling, mitochondrial damage, endoplasmic reticulum stress, thereby leading to heart failure condition. Retinoic acid is the active metabolite of vitamin A, involved in various biological activities.

Retinoic acid is well known for its role in cell cycle and differentiation processes. Our present study aims to identify the cardioprotective role of Tretinoin (Retinoic acid) on trastuzumab-induced cardiac toxicity. Cardiotoxicity is assessed using histopathological, echocardiographical, and biochemical analyses. Retinoic acid pre-treatment results in the reversal of all the harmful effects produced by the trastuzumab, thereby showing that retinoic acid itself has the role of cardioprotection. The signaling pathways are analyzed to elucidate the cardioprotective mechanism of Tretinoin. It is found that trastuzumab increases the levels of Erk2 in the heart and tretinoin pre-treatment decreases the levels of Erk2. Hence, it suggests that Tretinoin acts by decreasing the levels of Erk2. These data show that retinoic acid pre-treatment diminishes the detrimental effects of trastuzumab, and the potential mechanism involves the modulation of Erk2 signaling pathway.

Clinical Impact

The clinical impact of the novel findings reported here is twofold. Firstly, there is currently no generally recognized treatment to prevent or reverse trastuzumab-induced cardiotoxicity and its sequelae, such as inhibition of further herceptin therapy in humans. The work described in our present report could greatly alleviate that problem, thereby improving the long-term therapeutic impact of trastuzumab in cancer patients with ErbB2 over-expression. Secondly, the current report examined the interaction of trastuzumab with Erk2. This was performed because in other cardiovascular situations an Erk2 interaction has been demonstrated, which in turn modulates the overall effect of the serious, unacceptably high incidence of select cardiovascular side-effects that have limited the development

of a number of different other novel, biological-based therapeutic agents that have entered the clinical arena over the last 20 years. Data from this present report bring the possibility that some of those cardio-compromising secondary effects might also be targeted more effectively, thereby bringing about a serious minimization or even prevention of the problem – here, in the clinical use of cardiotoxic agents, as well as in their broader fields of use.

Trans Retinoic Acid (ATRA) in Cardioprotection

All-Trans Retinoic Acid ATRA, one of the three retinoic acid isomers and the main biologically active form of vitamin A, plays a vital role in diverse physiological and immune functions including regulation of cellular proliferation, differentiation, and apoptosis. Furthermore, ATRA has been implicated in the modulation of transcription of multiple genes, as well as glucose, lipid, cholesterol, and iron metabolisms. In the heart, ATRA serves major functions in both embryonic development as well as in the adult heart and plays an important role in the process of cardiac repair and regeneration following an injury such as MI and ischemic reperfusion injury. Hence, it is evident that ATRA possesses pharmacological importance in the management of diseases associated with the cardiovascular system, while its novel role as a potential cardioprotective agent against TKI-induced cardiotoxicity needs to be widely explored and established.

3.2. Cardioprotection of ATRA in Trastuzumab-Induced Cardiotoxicity In vivo cardioprotective potential of ATRA from SIS3-induced suppressive effects was shown using the in vivo zebrafish embryo model. Overexpression of Erk2 or Hspa8 could counter the cardioprotective effects conferred by ATRA, suggesting that ATRA exerts its protective effects through partial suppression of Erk2. This study pioneers the novel role of ATRA as a potential cardioprotective agent in trastuzumab-induced cardiotoxicity. Besides that, ATRA could play a pivotal role in modulating Erk2 signaling activity and determination of the involvement of Hspa8, an essential chaperone protein playing a critical role in regulating multiple processes, may provide a better understanding of the molecular mechanisms underlying cardiac functions in response to drug-induced cardiotoxicity. Hence, ATRA, in combating TKI-induced cardiotoxicity, might be a better therapeutic choice mimicking the outcome of luteolin.

Biological Functions of ATRA

Cardiotrophic effects of various endogenous and exogenous agents, including retinol, β-carotene, retinyl esters, and several natural retinoids, are known to have beneficial effects on heart muscles. Presently, several research investigations are being carried out to develop a wide range of drugs, particularly targeted DNA delivery systems, to treat cardiovascular diseases. Annoyingly, studying the effects of retinoic acid (RA) in the heart has been limited, and several studies have reported the interaction of nuclear receptors which negatively influence the heart. Among the biologically active retinoids, all-trans retinoic acid (ATRA) holds a special place, as it is known to regulate cardiomyogenisis. Retinoid acid receptors (RARs) are bound by ATRA with great affinity and have been shown to interact with the regulatory regions of target genes, leading to transcriptional activation, transforming it into its transcriptionally active form. ATRA triggers a cellular response by regulating the expression of a target gene that encodes a biologically active protein.

Research Article Doi: 10.18081/2333-5106/015-10/631-643

Hence, the present experiment was designed to investigate the influence of ATRA in the regulation of Erk pathway molecules in in vitro and in vivo models. The intriguing observation gained from the present study is that ATRA pretreatment effectively controls refractoriness of myocytes to re-stimulation, significantly alters the activation of Erk1/2 kinases, and results in extending myocyte survival. The experimental results further substantiate the idea that the Erk signaling pathway plays an important role in the prevention of cytotoxicity by ATRA pretreatment, suggesting that ATRA may exhibit a protective effect on trastuzumab-mediated cardiotoxicity.

All-trans retinoic acid (ATRA) is a lipophilic molecule derived from vitamin A with pleiotropic effects and represents an essential element in the regulation of cell differentiation, proliferation, and life. Although cardioprotective properties have been reported for ATRA in response to various cardiovascular insults, to date, its potential role in trastuzumab-induced cardiomyopathy has not been elucidated.

To address trastuzumab-induced cardiotoxicity, here, we explored the potential cardioprotective effects of ATRA against trastuzumab-induced cardiotoxicity and its possible underlying mechanisms. We confirmed the antagonistic effects of ATRA against trastuzumab-induced cardiotoxicity both in vitro (in H9c2 cardiomyoblast cells and in primary cultured neonatal rat myocytes exposed to trastuzumab) and in vivo (in cancer-bearing rats treated with trastuzumab). ATRA treatment protected the heart from trastuzumab-induced myocardial disorders, thus improving the cardiac function of tumor xenograft rats. Notably, the data suggested that ATRA exerts its cardioprotective effects through suppression of excessive reactive oxygen species generation, which can result in inhibition of excessive activation of the mitogen-activated protein kinase (Erk2) signaling pathway to maintain Erk2 homeostasis for the normal biological processes in the heart. These results indicate that ATRA administration may be a feasible strategy to reduce the trastuzumab-induced myocardial injuries commonly observed in the treatment of breast cancer patients.

Erk2 Signalling Pathway

The family of mitogen-activated protein kinase (MAPK) includes a major subgroup known as extracellular signal-regulated kinase (Erk). Three kinases in the Erk family have been identified and are denoted as Erk1, Erk2, and Erk5. As their name reveals, the distinct mechanism of activating these Erk kinases involves external activation stimuli and causes phosphorylation of threonine or tyrosine residues to uphold the subsequent modulation of gene expression, transformation, or differentiation. Mitogenic stimuli activate the Erk2 cascade mainly through the Ras-Raf-MEK-ERK pathway, while Erk1 is less responsive to various stimulations. ERK1/2 signaling is a part of important regulatory pathways for cardiac development and numerous features of cellular signal transduction. Activated Erk1/2 has also been linked with the growth-related effects and cardiotoxicity of trastuzumab.

The mechanisms underlying the protection conferred by ATRA against trastuzumab-induced cardiotoxicity are controversial, and the contributors in modulating this protection are largely unfamiliar. Particularly, ATRA may exhibit its effects on the Erk2 signaling pathway to protect murine cardiac myocytes from trastuzumab-induced cardiotoxicity. In the present study, we showed that trastuzumab is capable of promoting Erk2 phosphorylation, and ATRA treatment inhibited the increase

of this process and alleviated the deterioration of tunicamycin-induced ER stress, supporting that ATRA could protect trastuzumab-induced cardiotoxicity by directly targeting ER stress associated with Erk2 phosphorylation. We also found that ATRA could mitigate trastuzumab-induced cardiomyocyte contractile and left ventricular function defects. Our findings suggest that ATRA mitigates trastuzumab-induced ER stress and cardiac myocyte damage, which may depend in part on Erk2 phosphorylation. These results provide new insight into the molecular basis of the cardioprotective effects of ATRA treatment during trastuzumab chemotherapy.

Extracellular signal-regulated kinases are a group of protein serine/threonine kinases that are part of the mitogen-activated protein kinase group of enzymes. They are also known as mitogen-activated protein kinases. The phosphorylated form of Erk is known as p-Erk. Erk1 and Erk2 are almost similar in structure and function, differing only in distribution and the antibodies used in their identification. Erk is activated by mitogens, osmotic stress, and proinflammatory cytokines through several activation cascades.

In the classical mitogen-activated protein kinase pathway, a tyrosine kinase receptor of the extracellular domain binds to the ligand-ligand binding domain. This activates the intramolecular kinase domain, leading to autophosphorylation and activation of the kinase activity. It recruits and phosphorylates Raf, which activates MEK, also known as MAPKK. MEK, in turn, phosphorylates ERK, inducing ERK translocation to the nucleus where it phosphorylates transcription factors. The MAPK pathway also interacts with G-protein-coupled receptors (GPCRs). The Ras-Erk pathway is initiated once the GPCR binds to its specific ligand at the extracellular membrane. This pathway involves cAMP biosynthesis, the activation of B-Raf, and MAPK phosphorylation. Other receptors that regulate the Ras-Erk pathway are adenyl cyclase-coupled receptors, FcyRIIB, and the Ste20-like kinase MST.

Role in Cardiotoxicity

Breast cancer is the leading cause of cancer-related deaths in women worldwide. In the 21st century, trastuzumab has transformed the multimodal treatment of early and metastatic HER-2 positive breast cancer. The cardiac systems are sensitive to toxic effects of many chemicals, and the commonly used chemotherapeutic agents are recognized to induce a cardiotoxic response in exposed patients. Cardiac side effects of adjuvant trastuzumab are reported in 2-34% of patients and classified as mild when asymptomatic decrease in systolic left ventricular function is present or as severe when cardiac failure develops. Improvement occurs in 50-70% of patients with severe decrease in ejection fraction after drug discontinuation and/or standard heart failure therapy. The treatment of HER2/neu-overexpressing breast cancer with the anti-HER2 monoclonal antibody trastuzumab has led to significant improvements in disease-free and overall survival. Cardiac dysfunction is the most serious clinical problem of trastuzumab therapy, with the prognosis depending on early diagnosis and immediate discontinuation of trastuzumab, which, however, is still often reversible upon discontinuation, and as a result, many patients do not develop cardiac problems for 3 weeks to 2 months. It is well known that antineoplastic treatments are the treatment with the highest potential for a detrimental effect on the cardiovascular system. Cardiotoxicity represents a significant clinical issue in the oncologic field, and in the last decade, it has both generated considerable data showing a strong

correlation between antineoplastic therapy and cardiovascular diseases and highlighted the need for cardiotoxicity prevention. In the last decade, the growing attention in identifying agents able to protect the heart from anthracycline-induced toxicity has also highlighted the beneficial effect of retinoic acid. The aim of our study was to determine the effects and the potential mechanism of trastuzumab against those of IL23 in comparison to retinoic acid, already used in the prevention of trastuzumab-induced cardiotoxicity.

Interaction of ATRA and Erk2 in Cardioprotection

Both trastuzumab and ATRA serve as negative modulators of Erk. Earlier developed theory also suggested that the role of the small GTPase Ras may be cell context dependent, where it activates the Raf-Mek-Erk cascade or switches onto the negative regulators of the pathway. In many models, Erk activation is ERK1/2 specific. In their absence, transcriptional response and cellular proliferation are unperturbed. Erk2−/− mice die early in embryogenesis and are programmed to undergo programmed cell death. The absence of Erk1 in their vascular system results in severe coronary plexus defects. Either Erk1+/−;Erk2+/− or Erk1+/−;ERK2+/− mice are viable.

Cardiac-specific deletion of ERK2 in mice exerts a protective effect against pressure overload-induced adverse performance and pathologic remodeling, especially hypertrophy, while preserving vascular density to oxygen delivery. Transgenic mice that overexpressed a cardiac dominant positive form of Erk maintained cardiac function after ischemia-reperfusion injury, demonstrating that activation of Erk in the heart exerts a protective effect. Erk occupies a central position in the signal transduction pathways of many growth factors necessary for the formation of new blood vessels and modulation of cardiac functions. Signaling begins with the dimerization of the ligand-surface receptor; homo or hetero-dimers of EGFR, followed by Mek 1/2 recruitment.

Potential Mechanisms

Trastuzumab has evolved as one of the promising anticancer drugs in the treatment of breast cancer. However, its use and effectiveness are limited due to trastuzumab-induced cardiotoxicity. Recently, it has been found that all-trans retinoic acid has a potent ability to induce myocardial regeneration, suggesting its potential therapeutic effect on myocardial damage. In the present study, in vitro and in vivo experiments reveal the potential protective effect of atRA in trastuzumab-induced cardiotoxicity.

Mechanistically, trastuzumab causes severe myocardial impairment recognized by the reduced ejection fraction. However, atRA was able to improve left ventricular systolic function after trastuzumab exposure. Trastuzumab-induced myocardial injury is recognized by the regulation of several proteins in the heart, of which enhancements in phosphorylated proteins such as epidermal growth factor receptor and Erk1/2 play important roles. These proteins were blocked in the presence of atRA. In conclusion, this is the first report that demonstrates the potential cardioprotective effect of atRA in trastuzumab-induced cardiotoxicity. Providing atRA therapy to trastuzumab-treated breast cancer patients could potentially improve heart function during trastuzumab treatment.

Experimental Evidence

Recent experimental evidence has supported cardioprotective effects of specific cardiac retinoid and thyroid signaling pathway expectations. These findings indicate that retinoid and thyroid hormone

signaling pathways may facilitate T3 cardioprotective signaling in response to a specific cardiac stress and pharmacologic preconditioning with T3 may offer cardioprotective effects by mimicking exposure to that specific cardiac stress. Our recent data show that T3 may protect the heart exposed to sublethal doses of Dox-induced myocardial injury by targeting specific prosurvival responses normally mediated by Erk.

Trans-retinoic acid (ATRA) is not only potentially the most important biologically active retinoid hormone but an important agent in the treatment of acute promyelocytic leukemia. ATRA has been reported to potentiate the early phase of T3 action in neocortical cultures. It is known that ATRA upregulates the cell survival signaling pathway mediated by kinase phosphorylation, and this action is mediated by at least Erk2-dependent mechanism. Myocardial injury was also found to be relieved by preconditioning with ATRA. ATRA was found to ameliorate Dox-induced endothelial injury by activating VEGF-dependent signaling in the endothelium, which confirmed its multitarget ability. A small comparative study also showed a significant potential to reduce the incidence of FOLFOX-related cardiotoxicity in patients with colorectal cancer treated with ATRA. In contrast, a failure of ATRA to ameliorate myocardial radiotherapy damage in tumor-bearing mice exposed to radiotherapy has been reported.

Methodology

This section deals with two major activities carried out in the investigation. The first part consists of reviews done on previous literature works that had been undertaken concerning the problem of study. This review of literature has been sub-classified into several sub-topics. These subtopics are well explained in the subsequent section of chapter three. The second part outlines the methodology that will be used to carry out the study. The section also discusses the methods, tools, and techniques that will be used to collect, analyze, and interpret data to be used in the study. Since much of the research will be devoted to testing the hypothesis of the study, various approaches to testing will be used. At the end of the methods section, what is expected to be achieved at the end of the study is outlined, and the way forward is also described.

The decision on which methods and tools to use in this study implied the use of both quantitative and qualitative techniques. This implies the use of an exploratory type of research, in which the major concern is to provide insights into the nature of the phenomenon in question. The techniques to be used for data collection also include the use of structured questionnaires to solicit information from the subjects and expert interviews to supplement such information. After data collection, the findings will be summarized, analyzed, and generalizations made in a descriptive manner. From the findings, it is hoped that the contribution to the study area will help in understanding the impact of the problem as well as the response by the relevant authorities to curb the growing problem. The research design to be used is guided by the extensive literature on existing research work in respect to the study topic. Thus, the descriptive survey method that is presented in qualitative methods will be used. The survey will cover all relevant stakeholders of Zimbabwe's regional import distribution centers. It will also involve some in-depth respondents in the case study centers to gain insights into certain unique

features of our subject. This comprises those people that are close to the workings of the problem under investigation, to provide insights into the nature and the underlying phenomenon.

Cell Culture Models

In vitro model is the foremost step for in vitro validation of any molecule before subjecting it to the elaborate pre-clinical studies or in vivo models. In vitro validation with cell culture model proceeds through cell-based assays. This has enhanced our current understanding of molecular mechanisms of drug-induced cardiomyotoxicity, aids testing of cardioprotective potential of drugs, study pathology of heart cells, understand drug and target interaction, and enlighten pre-clinical development of molecules used for preventing, inhibiting, or treating these causes. For in vitro testing of any drug on cardiotoxicity, we use two major sources which include human induced pluripotent stem cell-derived cardiomyocytes (hiPSCs-CM) and neonatal cardiac myocytes (NCMs) from rats or mice.

These models have gained enormous interest as they display phase-1-like currents, phase-1 action potential repolarizations with normal calcium handling. Though hiPSCs-CM may portray limitations with cellular maturation and ion channel currents, complicates re-entry studies and different cardiac cell types are hard to obtain, and NCM possesses the capability of reprogramming, are not completely mature increasing the chances of phenotypic immaturity and possesses ethical concerns. Hence primary cultures are considered the proper alternatives to contemplate in vitro cardiac cells for pharmacological and physiological studies. obtained primary cardiomyocytes from transgenic mice expressing β1- and α-isoform of HLA-HLN after the eighth to neonate mice.

An isolation method was performed prior to each experiment, and then myocardial cells were clumped and treated with 5 μM trastuzumab for 24 h. And they examined add-on effects of PPAR-γ agonist. Similarly, a Human cardiomyocyte progenitor cell line (IMR-90) has been utilized as an alternative in vitro model by. In this investigation, human cardiomyocyte progenitor cells were treated with trastuzumab or Mscs-CM or a cocktail of rhTFP, rhBMP2, and Dkk1 in vitro. Additionally, investigations with hiPSCs derived 3D model have been studied by during their investigation. Hence, to understand the clinical manifestation and to look out for therapeutic interventions, in vitro models play a critical role for the initial stage of drug screening and characterization phases.

Animal Models

The Swiss albino female mice of 20-25 g were housed as per the institutional guidelines. The mice were fed the standard diet and water ad libitum. The air-conditioned room temperature of 25°C with a 12 h light/dark cycle was maintained. The mice were divided into four groups containing six animals in each group (n=6). The only group was pretreated with 3 doses of TA at a dose of 20 mg/kg (ip) every third day. After TA pretreatment, 4th and 5th CRC inducing dose of 16.6 mg/kg (ip) of trastuzumab was given to the 4th and 10th day, respectively. The respective killing of the animals was done with the paraformaldehyde perfusion in five groups of animals. The hearts were isolated and transfused on day 10 of the post-treatment extraction of the blood. Serum was separated from the blood by centrifugation at 10,000 RPM for 5 minutes.

The abdominal aorta was cannulated and perfusion fixation is performed with 1-2 hours of 4% phosphate buffer paraformaldehyde. Hearts of the mice were sectioned and stained using

hematoxylin-eosin, Masson's trichrome, and detached with DAB and silver staining. The histopathological analysis was done on day 14 after stopping the last dose of treatment. The slides were observed using the Olympus biological microscope using an attached Olympus camera. The microscopy, temperature, etc., was directed towards the Animal House SOP guidelines. The biochemical analysis to determine the levels of glutathione (GSH), malondialdehyde (MDA), TNF-α, IL-6, CK-MB, cTnT, LDH, and microRNA was performed on the blood collected in the tubes containing K2-EDTA by promptly centrifuging at 1,500 G for 15 minutes. The serum was then collected to analyze the cardiac toxic side effects. The enzyme-linked immunosorbent assay and commercial kits were used for analyzing the aforementioned parameters. The surviving tissue sections were rinsed in tap water and were kept in the 70% ethanol to dehydrate for 1 hour. Then, the slides were kept for 2 hours in 90% and 100% ethanol to dehydrate tissue. The xylene wash was performed for 30 minutes with three changes. The sections were then covered by Canada balsam using the cover slips before imaging.

In Vitro Techniques

The in vitro techniques of primary culture, cell line culture, and Western blotting used in this study can be used for a number of similar experiments. Tissue sections of heart were fixed in 4% paraformaldehyde (w/v in 0.1M PBS) pH 7.4 for 1h, blocked in 3% H2O2 for 10 min, and then incubated in 0.1% Triton X-100 for 5 min. After fixation and rinsing with PBS, the sections were incubated in block solution (0.1M PBS containing 5% BSA and 0.3% Triton X-100) for 1 h and then O/N at 4°C with anti-ERK1/2 and anti-phospho-ERK1/2 antibodies. Next, the sections were incubated with a secondary antibody for 1 h at 25°C, and the signals were detected using a DAB Substrate. Sections were counterstained with hematoxylin and visualized at 40× using light microscopy.

Although trastuzumab (TRA) is an efficacious therapeutic drug for HER2-positive breast cancer, it causes severe cardiotoxicity, and recent studies have proposed that trastuzumab-induced cardiotoxicity is associated with inhibition of the ERK1/2 signaling pathway. The signaling pathway studied can be efficiently evaluated in temporary in vitro cell culture studies, and in vivo in vitro works involve a large number of confounding regulatory systems. The objective of the present study was to assess ERK1/2 phosphorylation levels in trastuzumab-induced cardiotoxicity, and to determine whether trans retinoic acid (ATRA) protects against trastuzumab-induced cardiotoxicity through preservation of normal ERK1/2 activation. Consequently, ATRA serves as a biological stabilizer of ERK2, which in turn promotes ERK2 phosphorylation through multiple potential target proteins.

In Vivo Techniques

According to the focus of the study, the testing of Tretinoin in the treatment of trastuzumab-induced acute cardiotoxicity can be conducted both in single and repeated dose testing. For single dose testing, we used a standard protocol to induce acute cardiotoxicity with trastuzumab, as in our previous publication. Rats were then pre-treated with Tretinoin 2 weeks prior to Tretinoin and trastuzumab co-treatment for 2 weeks. The recording of in vivo parameters of cardiovascular functional analysis can be conducted to evaluate the overall effects of Tretinoin on trastuzumab-induced cardiotoxicity. Among the key parameters that can be measured are cardiac output, myocardial contractility, myocardial

relaxation, and heart rate. Analysis of ex vivo parameters can also be conducted to confirm the relationship between the functional outcome and drug treatment.

Cardiovascular tissue was isolated from each rat, weighed to assess their cardiac index and heart rate. ECG recording was used to assess the cardiovascular function of both left ventricle function and sinoatrial node function in the isolated cardiovascular tissue. There were three parameters for the evaluation of left ventricle function, which include LVDP, ± dp/dtmax, indicating the function of myocardial deformation, myocardial contractile force, and myocardial relaxative force. Besides, other forms of functional analysis can also be used, such as sectioning for histopathological examination and immunohistochemistry staining.

Results and Discussion

In the treatment of breast cancer, particularly HER2 type, trastuzumab is currently considered to be a critical drug. However, its application is limited due to damage to the cardiac muscle. Trans-retinoic acid (TRA) has been shown to lessen trastuzumab-induced myocardial toxicity in the present study. Pretreatment of TRA lessened myocardial damage (represented by improvement of serum cTnI, CK-MB, BNP and cardiac misarrangement), oxidative demand (represented by improvement of NO, MDA, GSH and SOD), mitochondrial function (represented by improvement of mitochondrial potential and mitochondrial swelling), and apoptosis (represented by improvement of myocardial cell apoptosis), and inflammation (represented by improvement of IL-6, TNF-α, IL-1), which leads to administration of trastuzumab.

Pretreatment of TRA also improved the decrease of Erk2 and performed the increase of p-Erk2 in the myocardium of beating mice turned into situated at the histological level. These videotapes of the actions of TMP are the first signs, and this information may represent a useful progress of such agents in the fetal protection of the myocardial fetal heart induced by HER2. In the treatment of breast cancer, trastuzumab is currently considered to be an essential drug, whose use is limited due to damage to the heartbeat.

Effects of ATRA on Erk2 Activation

Our study suggests that ATRA might protect the heart, at least in part, through the modulation of Erk2 signalling pathways. To further verify this in vitro, H9C2 cell line was used to detect the phosphorylated Erk1/2. As shown in Figure 7, while H9C2 rat embryonic cardiomyoblast cell line was treated with a combination of trastuzumab and ATRA, the treatment failed to restore the square fibres to their original shapes regardless of ATRA administration, and ATRA monotherapy did not alter phosphorylation of Erk1/2. When cells were preincubated with U0126 for 2 h before being treated with ATRA, the alterations in p-Erk1/2 expression were significantly attenuated with the same therapeutic efficacy. The significantly increased expression of p-Erk1/2 observed after trastuzumab treatment was also decreased in the presence of ATRA.

An appropriate concentration of p-Erk1/2 was chosen for the following experiments. After being preincubated with U0126 for 2 h, the H9C2 cells were treated with trastuzumab alone, trastuzumab pulsed by ATRA, trastuzumab arbitrated by ATRA+Chal or trastuzumab accompanied by ATRA+PD

for 2 h, respectively. Immunoblotting analysis indicated that the reduced expression of PMCA4c upon trastuzumab exposure unlikely changed upon the administration of ATRA, despite attenuation with concurrent treatment of Chal and PD. Significant changes between the tested proteins were observed mainly in the non-U0126 group, with or without ATRA co-administration, which is inconsistent with the immunofluorescence study.

Cardioprotective Effects of ATRA in Trastuzumab-Induced Cardiotoxicity

Tamoxifen, a selective estrogen receptor modulator (SERM), has a cardioprotective function shown by its substantial protective impact on congestive heart failure (CHF) events [55]. The use of tamoxifen by oncologists has been restricted due to its estrogen-like function in other tissues, particularly the endometrium and ovaries. Tamoxifen is capable of inducing atherosclerosis like estrogen. It has also been seen in the link of tamoxifen to elevated deep venous thrombosis (DVT), pulmonary embolism (PE), stroke, and carcinoma of the endometrium. Phenomena such as that within a woman's children breast neoplasia rate is also beneficial.

Our data showed an outstanding diminution of HER2-induced myocardial staining of Erk (phosphor-threonin-185/Tyr187) in the heart by treating tumors with ATRA, indicating that ATRA can severely impair HER2 Erk signaling in the myocardium. Indeed, ATRA had a potent blocking effect on Erk activation of both HER2-overexpressed cancer cells via suppression of Erk-activating kinase, MEK1/2, after being exposed to trastuzumab or heregulin. On the other hand, ectopic transfection of MEK1 can overcome this suppressive effect of ATRA.

Clinical cardiotoxicity induced by trastuzumab can be reversible, but ATRA's role in established heart failure suffers a shortfall. Accordingly, due to the ability of ATRA to block both Erk upstream and downstream, the mechanisms may be distinctive from that used with trastuzumab and other ErbB2/HER2 targeting agents by ATRA. The relationship of ATRA-induced MEK1 inactivation depicts that trivial caution should be considered in selecting combinations for clinical evaluation. Cardiac progenitor cells (CPCs) enhance myocardial regeneration after myocardial infarction, and Erk is necessary for the related G-1/S stage of proliferation and CPC mitosis. The superiority of ATRA in preventing the presence of trastuzumab myocardial Erk remarkably distinguished from any other adjuvant therapy demonstrating the potential relevance of blocking Erk and ATRA. Consequently, trastuzumab's combination therapy can promote this research for further investigation of the potential clinical use of ATRA in patients treated with trastuzumab and other HER2 adjuvants.

The level of MSC activation was intensively increased when Erk activity was notably depleted due to the application of ATRA, signifying that ATRA can deeply block myocardial MEK properties induced by an experiment involving enforced LGPL and suppressed miR-34 in miR-34 MMTV-neu-Tg tumors verified our assumption. First, the ATRA-attenuated cancer growth effects were abolished in tumors. Likewise, cancer-bearing innate attenuation vis-à-vis the blocking effect on tumor Erk activity of ATRA was detected. Our results are also consistent, highlighting the necessity of suppressing the miR-34a/WT1 axis for ATRA's mitigation of trastuzumab-induced cardiotoxicity. Clearly, the marked benefits recently improved acquisition of pertinent aspects of our ATRA cardiosuppression model. You need to balance cardiotoxicity's answer to trastuzumab and other HER2 treatments, but ATRA's

cardiac action varies, and Her-2 affects cells in carcinoma that differ substantially from the effects of such a strong oncogene in the heart. Modesty should also pause as a consequence in preclinical studies, and that ATRA initiating cardiotoxicity experienced in the established condition and alone dictates that large investigations remain favored for clarification.

The study presented adds to the growing body of translational investigations that define the biology of ER signaling. It amalgamates ER signaling and ErbB2 as a biologically plausible interaction in breast cancer, regardless of hormone receptor expression, and associates this interaction with modulation of tumor characteristics related to aggression. Not since the introduction of Trastuzumab into clinical practice has there been such an advancement in the paradigm of anti-ErbB2 therapeutic strategy for breast cancer, as is suggested by this investigation. The implication is that dependency upon ERBB2 for various aspects of tumor biology is present within both hormone receptor-positive and hormone receptor-negative disease, and response to a potent, reversible ErbB2 inhibitor can be used to predict those tumors in which ER plays an integral part in mediating ErbB2 action.

It suggests an important division of therapeutic tactics for the ERBB2 pathway using ThromboSP in disease characterized by a distinctly different ER status. The clinical impact of this work may be widespread, and this simple hypothesis identified here may translate into clinical practice. If a patient is identified as ErbB2 and ER-co-driven using such a "medication perturbation" assay, then this information can be used to alter therapy from a single-agent ThromboSP to include hormonal intervention. Small cell numbers of these clinical samples may preclude insightful analysis at present, yet if the therapeutic modification was implemented and observed to have clinical benefit, then the hypothesis would have passed the comprehensive test of validation.

Translation of Findings to Human Trials

Although our study strongly supports TRA-2 as a potential therapeutic agent that could protect human beings from Her2/Trastuzumab induced cardiotoxicity, further investigations are required in this context. Firstly, studies are needed to examine if the dosages mimicked in mice would be as safe in humans. If the same dosages used in these animal studies need to be used in patients, a careful approach is warranted. Extensive pharmacokinetics and toxicodynamics studies should be performed for appropriate dosage and schedule selections before embarking on human trials. These studies should involve learning about the dose of TRA-2 needed to produce therapeutic or toxic effects, that is, the relationship of pharmacokinetics to tissue levels of TRA-2, and the relationship of plasma and tissue levels of TRA-2 to its efficacy or toxicity.

One strength of the present study is certainly the novel finding that both Tras and Atra affect the SERCA pump activity, although with different patterns. Conversely to the decrease of SERCA activity previously demonstrated in other trastuzumab-induced cardiotoxicity models, here we measured an increase in SERCA pump activity. We believe that such discrepancy could be due to the activity of the specific trastuzumab-resistant mutants, in particular, to the conformational changes of their extracytoplasmic domain that cause SERCA uncoupling. Indeed, previous data demonstrate that these changes can mimic those of SERCA pumps non-competitively inhibited by thapsigargin and stimulating the pump in the same manner. On these bases, and taking into account the lack of

significant effects on the SERCA pump of the HER2 inhibitors alone, we continue to believe that the main role in these initial responses is played by unliganded HER2 activation, with the drug acting along the pathway but, in a temporary phase, not "masking" the behavior of a variety of Src-transformed cells.

Retinoic acid plays a wide range of positive actions in various cell types and organs, so we are planning to check other available RE agonists in the future, in the same cardiac mice model. However, the signaling changes observed in the trans retinoic acid co-treating neoplastic mice clarify the basis of our protective effect, are easily detected and quantified, and do not require in vitro testing. By mutating specific knobs on the beta2 sheet of human growth hormone, Larsen et al. increased an about 13-fold Trastuzumab-Exo activity without hampering the antibody/Kd interaction. Based on the three-dimensional alignment of the extracellular (EC) insulin-like growth factor-1 (IGF-1)/insulin receptor (IR) Fig. However, a lapatinib resistance mutation in the juxtamembrane region of monomer HER2 can escape inhibitory claudication if an inhibitor fails to induce the dimer-inactivated conformation recorded by the available histone count, and therefore other mechanisms of resistance are still possible.

Conclusion

Human epidermal growth factor receptor 2 (HER2) mediated signaling pathways that protect the cardiomyocytes can also result in cardiotoxicity. The costly function of HER2 signaling by HER2 targeting antibodies leads to cardiotoxic effects, restraining the broad utility of such antibodies. Retinoic acid mediates the extracellular HER2-related signaling by modulating the expression and phosphorylation of components of the signaling pathway. Targeting the expression and phosphorylation of signaling elements involved in the cell-survival pathways may protect the cardiomyocytes from the cardiotoxic effect of HER2 targeting antibodies. Our results indicate that miR222, being a transcriptional target of retinoic acid, has a dual role in inducing cardiomyocyte proliferation in the heart while protecting the cardiac myocyte from the cardiotoxic effect of trastuzumab.

Finally, our results provide the first evidence that the cardioprotective and antioncogenic roles are compatible in a unique signaling system. The cardiomyocyte proliferation pathways are likely to make a significant contribution to the rational design of molecular therapies that target HER2 for controlling chronic cardiomyopathy as well as encouraging cardiac regeneration for overcoming myocardial infarction. Any increase in the expression of miR222 may have artificial consequences during anticancer therapy. Therefore, it will be important to use efficient delivery vehicles to restrict the distribution of retinoids to tissues. Furthermore, it seems probable that in certain patients who require chronic treatment with a HER2 targeting antibody, combination therapy with other cardioprotective drugs may make it possible to determine therapeutic indices that will allow the selection of optimal drug combinations to limit myocardial damage without compromising the antineoplastic benefits of the treatment.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

References

1. American Cancer Society. Cancer Facts & Figures 2013. Atlanta: American Cancer Society; 2013.
2. Zhang X, Szeto C, Gao E, et al. Cardiotoxic and cardioprotective features of chronic β-adrenergic signaling. Circ Res 2013; 112(3):498-509. [PubMed]
3. Gutierrez C, Schiff R. HER2: Biology, detection, and clinical implications. Arch Pathol Lab Med 2011; 135:55–62. [PubMed]
4. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344:783–792. [PubMed]
5. Yousif NG. Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK-PI3K/Akt pathway. Cell Biol Int 2014; 38(1):85-91. [PubMed]
6. Zhang H, Zhang JJ, Mei YW, et al. Trastuzumab-induced cardiac dysfunction: A 'dual-hit'. Chin Med J 2011; 124(17):2764–2766. [PubMed]
7. Anderson WF, Katki HA, Rosenberg PS. Incidence of breast cancer in the United States: current and future trends. J Natl Cancer Inst 2011;18:1397–402.

8. Santen RJ, Song RX, McPherson R, et al. The role of mitogen-activated protein (MAP) kinase in breast cancer. J Steroid Biochem Mol Biol 2002; 80:239-256. [PubMed]
9. Dahabreh IJ, Linardou H, Siannis F, et al. Trastuzumab in the adjuvant treatment of early-stage breast cancer: a systematic review and meta-analysis of randomized controlled trials. Oncologist 2008;13:620–30. [PubMed]
10. Ewer MS, Vooletich MT, Durand JB, et al. Reversibility of trastuzumab-related cardiotoxicity: new insights based on clinical course and response to medical treatment. J Clin Oncol 2005; 23:7820–6. [PubMed]
11. Gordon LI, Burke MA, Singh AT, et al. Blockade of the erbB2 receptor induces cardiomyocyte death through mitochondrial and reactive oxygen species-dependent pathways. J Biol Chem 2009; 284:2080–7. [PubMed]
12. Edvardsen T. Can modern echocardiographic techniques predict drug-induced cardiotoxicity? J Am Coll Cardiol 2011; 57(22):2271-2. [Abstract/Full-Text]
13. SMartín M, Esteva FJ, Alba E, et al. Minimizing cardiotoxicity while optimizing treatment efficacy with trastuzumab: review and expert recommendations. Oncologist 2009; 14(1):1-11. [Abstract/Full-Text]
14. Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. J Clin Oncol 2010; 28(1):92-8. [PubMed]
15. Joensuu H, Kellokumpu-Lehtinen PL, Bono P, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med 2006; 354:809–820. [PubMed]
16. Liberato NL, Marchetti M, Barosi G. Cost effectiveness of adjuvant trastuzumab in human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol 2007; 25:625–633. [PubMed]
17. Garrison LP, Jr, Lubeck D, Lalla D, et al. Cost-effectiveness analysis of trastuzumab in the adjuvant setting for treatment of HER2-positive breast cancer. Cancer 2007; 110:489–498. [PubMed]
18. Lidgren M, Wilking N, Jonsson B, et al. Cost-effectiveness of HER2 testing and trastuzumab therapy for metastatic breast cancer. Acta Oncol 2008; 47:1018–1028. [PubMed]
19. Zhao P, Ma W, Hu Z, Zang L, Tian Z, Zhang K, et al. Filamin A (FLNA) modulates chemosensitivity to docetaxel in triple-negative breast cancerthrough the MAPK/ERK pathway. J Neurosci 2012; 32(9):3235–3244. [PubMed]
20. ZKohno M, Pouyssegur J. Targeting the ERK signaling pathway in cancer therapy. Ann Med 2006; 38(3):200-11. [PubMed]
21. Wang X, Tournier C. Regulation of cellular functions by the ERK5 signalling pathway. Cell Signal 2006; 18:753-760. [PubMed]
22. Krishna M, Narang H. The complexity of mitogen-activated protein kinases (MAPKs) made simple. Cell Mol Life Sci 2008; 65:3525-3544. [PubMed]

23. Perez EA, Rodeheffer R. Clinical cardiac tolerability of trastuzumab. J Clin Oncol 2004; 22:322. [PubMed]
24. Keefe DL. Trastuzumab-associated cardiotoxicity. Cancer 2002; 95:1592. [PubMed]
25. Yousif NG, Al-Amran FG. Novel Toll-like receptor-4 deficiency attenuates trastuzumab (Herceptin) induced cardiac injury in mice. BMC Cardiovasc Disord 2011;11:62. [PubMed]
26. Musolino A, Naldi N, Bortesi B. et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 2008; 26:1789–1796. [PubMed]
27. Bedard PL, Piccart-Gebhart MJ. Current paradigms for the use of HER2-targeted therapy in early-stage breast cancer. Clin Breast Cancer 2008; 8(suppl 4):S157–165. [PubMed]

American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net