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The Potential Ameliorating Role of Spironolactone in Trastuzumab Mediated Cardiotoxicity: A Narrative Review

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Abstract

Background: Around 20% of breast cancers (BC) overexpress human epidermal growth factor receptor 2 (HER-2). HER-2 upregulation has been linked to increased tumor aggressiveness and poor prognosis. Trastuzumab, an anti-HER2 monoclonal antibody, has improved overall survival in early-stage and metastatic BC but at the expense of developing cardiac morbidity. In the current review, we aimed to discuss the pathogenesis of trastuzumab-induced cardiac toxicity and the potential preventive properties of spironolactone in this regard.

Methods: We thoroughly searched PubMed, Embase, Scopus, and Web of

Science according to the following search terms: trastuzumab, cardiotoxicity, heart failure,

Spironolactone.

Results: Trastuzumab deteriorates mitochondrial function and subsequently leads to the accumulation of reactive oxygen species (ROS) in cardiomyocytes. Published clinical studies have offered conflicting results regarding the efficacy of angiotensin-converting enzyme inhibitors (ACEI) and beta-blockers (BB) in respect of trastuzumab-induced cardiotoxicity.

On the other hand, spironolactone has been found to exert both antioxidant and anti-inflammatory properties. Recent in vivo studies have supported the cardioprotective

effect of spironolactone through maintaining mitochondrial ultrastructure and reducing ROS production.

Conclusions: Although spironolactone mitigates oxidative stress and mitochondrial dysfunction, there is a lack of clinical evidence to support the effectiveness of spironolactone in trastuzumab-induced cardiotoxicity. Design and implementation of clinical trials are of the essence to determine the potential beneficial impacts of spironolactone upon trastuzumab-induced cardiotoxicity.

Keywords: Spironolactone; trastuzumab; cardiotoxicity; heart failure; HER-2; cardio-oncology; chemotherapy; mitochondria; oxidative stress

Introduction

Breast cancer (BC) is the most common malignant neoplasm among women all over the world(1). Nowadays, with significant advances in early diagnosis and management of breast cancer, patients' survival has markedly improved. However, the increased life span and the use of cardiotoxic chemotherapeutic agents have led to increased incidence of cardiovascular disorders and subsequent cardiac morbidity and mortality(2). Human epidermal growth factor receptor 2 (HER-2), previously termed ErbB2, is a transmembrane proto-oncogene receptor and serves as a signal-transducing tyrosine kinase(3). HER-2 activation exerts modifying impact upon cell growth, proliferation, and survival via actuating the phosphatidylinositol-3-kinase (PI3K) and mitogen-activated phosphor kinase (MAPK) signaling pathways(4). Noteworthy, the overexpression of HER-2 receptor has been observed in approximately one-fifth of BC patients and is associated with a significantly worse prognosis(5). However, HER-2 glycoprotein upregulation is not merely confined to BC, and it could also occur in different epithelium-derived tumors(6, 7).

Of course, it goes without saying that the development of HER-2- inhibitor agents has changed the landscape of treatment for patients suffering HER-2 positive tumors(8). Adding HER-2- directed agents to the standard chemotherapeutic regimen has demonstrated survival benefits in several malignant tumors, including BC, gastric cancer, and gastroesophageal junction adenocarcinoma(9-11).

Trastuzumab is a prototype of humanized anti-HER-2 monoclonal therapeutic antibodies that selectively target the extracellular domain of the HER-2 receptor(12). Trastuzumab is believed to mediate antibody-dependent cellular cytotoxicity and trigger natural killer (NK)cells activation against tumor cells(13, 14). Besides, the amount of HER-2 receptors tends to diminish after trastuzumab exposure(15). As previously mentioned, trastuzumab can also hinder the proliferation-promoting signaling pathways(16). In a meta-analysis study that included 1497 patients with HER-2 positive metastatic BC, trastuzumab administration promoted overall survival (hazard ratio (HR)=0.79, 95% confidence interval (CI): 0.67- 0.94), but at the expense of elevating the risk of heart failure occurrence (relative risk (RR)= 3.49, 90% CI: 1.88 - 6.47)(17). Similar findings were represented in the setting of adjuvant therapy(18, 19). Accordingly, the clinical benefits of trastuzumab in BC treatment have been overshadowed by the emerging cardiovascular sequels(20-22). The present review aims to discuss the pathogenesis of trastuzumab-induced cardiac toxicity and the potential role of spironolactone in ameliorating cardiac injury.

Methods

A broad search was carried out through reliable databases, including PubMed, Embase, Scopus, and Web of Science, using the following search terms either individually or in combination: trastuzumab, cardiotoxicity, heart failure, and spironolactone. We did not limit the publication date. Relevant published articles in English were scrutinized.

Results

Trastuzumab-induced cardiotoxicity

It has been proposed that trastuzumab-induced cardiotoxicity affects 15-20% of patients(23, 24). Despite long-running endeavors, the precise underlying molecular mechanisms implicated in trastuzumab-induced cardiac toxicity have not yet been fully explicated(25, 26). HER-2 downstream signaling is the sine qua non of embryonic cardio-genesis and has a pivotal role in the regulation of cardiac homeostasis(27); ventricular-limited deletion of HER-2 in a mice model has given rise to the characteristics of dilated cardiomyopathy, including diminished contractility(28). In spite of anthracycline-induced cardiomyocyte injury and according to the biopsy specimen, there is no apparent ultrastructural remodeling attributable to trastuzumab(29). Moreover, cardiac dysfunction triggered by trastuzumab is not dose-dependent and appears to be predominantly reversible upon holding off the agent(16). However, a few studies have recommended that trastuzumab exposure might give rise to irreversible changes in cardiac tissue(30, 31).

Currently, undermining mitochondrial components via reactive oxygen species (ROS) generation and impairing metabolic pathways have garnered increasing attention in the pathogenesis of trastuzumab-induced cardiotoxicity(32, 33). Trastuzumab was found to interfere with the expression of several myocardial genes involved in mitochondrial function, metabolic pathways, global cardiac function, and contractility(34).

Trastuzumab is deemed to block neuregulin-1 (NRG-1), an epidermal growth factor that promotes HER-2/HER4 heterodimerization(35). The NRG-1/ErbB signaling pathway is essential for cardiomyocyte development and mitochondrial activity(36, 37). Animal studies have divulged that NRG-1 knockout during embryogenesis is incompatible with

life(38). Another in vivo study has found that NRG-1 injection in rats alleviates oxidative stress and preserves mitochondrial function(39). Besides, NRG-1 seems to exert an anti-apoptotic feature and facilitates the cardiomyocyte repairing process(40). Notably, trastuzumab therapy upregulates pro-apoptotic molecules such as Bcl-xS while downregulating anti-apoptotic proteins like Bcl-xL(41, 42).

It is well-established that inhibition of the HER-2 signaling is a remarkable contributor to the intensification of oxidative stress, which appears to be a rational explanation for an elevated risk of cardiac dysfunction after concomitant use of trastuzumab and anthracyclines(43). Additionally, the extraordinary ROS levels lead to the activation of mitochondrial permeability transition pore (mPTP) located in the mitochondrial inner membrane and cause significant mitochondrial osmotic damage by increasing permeability(33, 44). Interestingly enough, a recent experimental study on rabbit cardiac tissue has demonstrated that mitochondrial cristae tend to become disorganized due to edema, and mitochondrial membranes integrity could be disrupted in response to trastuzumab therapy(45). Another recent study of human-induced pluripotent stem cell-derived cardiomyocytes has shed new light on the pathogenesis of trastuzumab-induced contractile dysfunction in cardiomyocytes. Overall, this study found that trastuzumab therapy did not affect cell viability, which precludes the possibility of irreversible cardiac injury. Besides, trastuzumab led to a significant diminution in cellular ATP content, ATP-linked respiration, proteins involved in oxidative phosphorylation, and mitochondrial DNA levels per cell(46).

The clinical presentation of trastuzumab-associated cardiac dysfunction ranges from asymptomatic reduction in left ventricular ejection fraction (LVEF) to advanced heart failure(47).

Cardioprotective agents in trastuzumab induced cardiotoxicity

There is no clinically effective therapeutic approach to prevent trastuzumab-induced cardiotoxicity as of yet(48). More importantly, the cardioprotective efficacy of angiotensin-converting-enzyme inhibitors (ACEI) and beta blockers in trastuzumab-induced cardiotoxicity has been called into question, with the studies indicating conflicting results(49). A randomized, double-blind, placebo-controlled trial that included 94 patients with HER-2 positive BC receiving adjuvant trastuzumab has shown that perindopril and bisoprolol cannot prevent the remodeling associated with trastuzumab(50). In another similar study conducted on 210 patients, candesartan failed to preserve LVEF during trastuzumab adjuvant therapy(51). On the other hand, in a cohort study of 6542 patients with BC who received trastuzumab/anthracyclines, it became evident that ACEI or beta blocker exposure is connected with a statistically significantly decreased risk of all-cause mortality, that is, 21% (HR=0.79; 95% CI, 0.70-0.90)(52). A randomized, double-blind, multi-central trial conducted on 468 patients with HER-2 positive BC receiving trastuzumab and had a history of anthracycline exposure revealed that both lisinopril (HR=0.49, CI:0.27-0.89) and carvedilol (HR=0.53, CI= 0.30-0.94) could improve cardiotoxicity-free survival(53). Further, studies are limited regarding the cardioprotective effect of statins in BC patients. In a retrospective case-control study about the efficacy of statins on trastuzumab-related cardiac toxicity, patients with statin exposure had a lower risk of cardiac dysfunction (odds ratio (OR) = 0.32, p=0.049)(54). In another recent retrospective cohort of patients with BC who received trastuzumab, the statin receiving group (2.7%) had a statistically non-significant lower 5-year cumulative incidence of symptomatic heart failure in comparison with the statin-naive group (3.7%)(pv=0.09)(55).

The potential role of spironolactone in trastuzumab-induced cardiac dysfunction

Spironolactone, a potassium-sparing diuretic, competitively antagonizes mineralocorticoid receptors and exhibits antioxidant and anti-inflammatory properties(56-58). Spironolactone mitigates the deleterious effects of the last component of the renin-angiotensin-aldosterone system (RAAS) on cardiomyocytes(59, 60). It should be pointed out that aldosterone is inclined to promote inflammatory response, fibrosis, and ROS formation in cardiac tissue, particularly in the vulnerable myocardium(61, 62). Furthermore, spironolactone barricades the androgen receptors and interferes with steroidogenesis(63). Due to its diverse functions, it has been in broad clinical use for many years(64). Spironolactone has been shown to be effective in the management of resistant hypertension and reduced ejection fraction heart failure(65). Additionally, it has been recently supposed that the anti-inflammatory role of spironolactone might occur due to the suppression of NF-κB signaling. NF-κB is considered to be one of the crucial promotors of proinflammatory cytokines. (66). Spironolactone treatment puts up roadblock on apoptosis in endothelial cells by decreasing caspase 3 activity, a major executioner caspase, and cytochrome c leakage(67).

In a study conducted on the vascular tissue of Ren2 rat models, NADPH oxidase activity, a crucial source of oxidative stress, and lipid peroxidation were significantly decreased after spironolactone administration. More importantly, spironolactone upregulated the anti-apoptotic Bcl-xL protein and suppressed the activation of caspase 3, which executes apoptosis(68). It should be noted that caspase 3/7 activation can be induced by trastuzumab administration(69).

Connexin 43(Cx43) is a transmembrane gap junctional protein which involves in cardiomyocytes' cell-cell communication(70). On the other hand, Cx43 can be expressed on the mitochondrial membrane and is thought to have a vital role in controlling mitochondrial respiration(71). The overexpression of mitochondrial Cx43 has been reported as a measure to counteract the detrimental impacts of trastuzumab on mitochondrial function(72). In addition, available evidence suggests that spironolactone has a positive influence on the expression of Cx43(73, 74).

In an experimental study carried out on the streptozotocin-mediated diabetic rats, being treated with spironolactone was associated with the increments in the cardiac levels of vitamin E and decrements in nitrite production(75). It is well understood that vitamin E acts as a potent antioxidant, which can reduce superoxide and hydrogen peroxide formation within the mitochondria(76, 77).

Liu et al. recently explored the cardioprotective effect of spironolactone in rats with diabetic cardiomyopathy. Twenty-four rats were classified into three groups: non-diabetic control, diabetic treated by spironolactone, and saline receiving diabetic rats. They detected that treatment with spironolactone gives rise to a significant decline in NADPH Oxidase 4 (NOX4) expression while increasing nuclear respiratory factor 1 (NRF-1) expression. It is worth mentioning that loss of NRF-1 creates a significant elevation in ROS levels. Moreover, NOX4 serves as ROS producing enzyme. Spironolactone also promoted the expression of crucial proteins within the electron transport chain, namely, cytochrome c oxidase subunit 5B (COX5b) and ATP synthase 5a (ATP5α1)(78). In other words, spironolactone therapy maintains both ultrastructure of mitochondria and metabolic pathways.

In an experimental study involving 80 female rats, the efficacy of spironolactone in cardiotoxicity induced by trastuzumab and radiotherapy was evaluated. In a group of rats that received trastuzumab with concomitantly applied radiotherapy, spironolactone mitigated the expression of TGF-β and cardiac fibrosis score. However, it had no significant impact when trastuzumab was administered alone. Nevertheless, this study's limitation was employing a light microscope, which poses an important obstacle to evaluating ultrastructural changes(79). The role of spironolactone in doxorubicin-induced heart failure has been brought to light by several studies(80). In a meta-analysis that included 12 studies about cardioprotective approaches in doxorubicin-induced

cardiac dysfunction, spironolactone turned out to be the first appropriate therapeutic opportunity to prevent cardiac dysfunction based on LVEF changes(81).

Nonetheless, there is a shortage of clinical studies to evaluate the efficacy of spironolactone to this end. Taken together, it is evident that the deleterious impact of trastuzumab treatment on the function of cardiomyocytes is mediated through mitochondrial dysfunction and ROS generation. Current experimental studies have illustrated that spironolactone can substantially attenuate mitochondrial morphologic changes, oxidative stress, and inflammation in cardiomyocytes. Therefore, spironolactone seems to be a good candidate for further clinical investigations with the aim of ameliorating trastuzumab induced-cardiotoxicity.

Conclusion

The treatment of HER-2-amplified BC with trastuzumab might impair mitochondrial function and metabolic pathways within the cardiomyocytes. Of note, overproduction of ROS appears to have a critical role in trastuzumab-induced cardiotoxicity. Recent experimental studies have suggested that spironolactone could attenuate mitochondrial dysfunction and ameliorate oxidative stress. Nonetheless, there is a lack of clinical evidence to support the effectiveness of spironolactone in this regard. Last but not least, further clinical studies are warranted to determine the efficacy of spironolactone in the protection against trastuzumab-induced cardiotoxicity.

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Conflict of interest

The authors have no conflict of interest to declare.

References

1.   Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021;71(3):209-49.

2.   Moo T-A, Sanford R, Dang C, Morrow M. Overview of Breast Cancer Therapy. PET Clin. 2018;13(3):339-54.

3.   Nowsheen S, Viscuse PV, O'Sullivan CC, Sandhu NP, Haddad TC, Blaes A, et al. Incidence, Diagnosis, and Treatment of Cardiac Toxicity from Trastuzumab in Patients with Breast Cancer. Curr Breast Cancer Rep. 2017;9(3):173-82.

4.   Nemeth BT, Varga ZV, Wu WJ, Pacher P. Trastuzumab cardiotoxicity: from clinical trials to experimental studies. Br J Pharmacol. 2017;174(21):3727-48.

5.   Zohrian F, Alizadehasl A, Zahedi L, Ghaderian H, Anbiaee R, Azade P. Assessment of Cardiac Function by Advanced Echocardiography in Breast Cancer Patients (HER2 Positive vs HER2 Negative). Multidiscip Cardio Annal. 2020;11(2):e103004.

6.   Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. Cancer Metastasis Rev. 2015;34(1):157-64.

7.   Azin A, Zohreh R. Relationship between Breast Cancer and Cardiac Myxoma. International Journal of Hematology-Oncology and Stem Cell Research. 2021;15(2).

8.   Alizadehasl A, Maleki M, Noohi F, Adimi S, Emami A, Mousavi A, et al. The Cardiac Effects of Radiotherapy on Women with Breast Cancer Based on Laterality. International Cardiovascular Research Journal. 2021.

9.   Franchi M, Tritto R, Torroni L, Reno C, La Vecchia C, Corrao G. Effectiveness and Healthcare Cost of Adding Trastuzumab to Standard Chemotherapy for First-Line Treatment of Metastatic Gastric Cancer: A Population-Based Cohort Study. Cancers. 2020;12(6):1691.

10.   Dokmanovic M, King KE, Mohan N, Endo Y, Wu WJ. Cardiotoxicity of ErbB2-targeted therapies and its impact on drug development, a spotlight on trastuzumab. Expert Opin Drug Metab Toxicol. 2017;13(7):755-66.

11.   Lin D, Khan U, Goetze TO, Reizine N, Goodman KA, Shah MA, et al. Gastroesophageal Junction Adenocarcinoma: Is There an Optimal Management? American Society of Clinical Oncology Educational Book. 2019(39):e88-e95.

12.   Nicolazzi MA, Carnicelli A, Fuorlo M, Scaldaferri A, Masetti R, Landolfi R, et al. Anthracycline and trastuzumab-induced cardiotoxicity in breast cancer. Eur Rev Med Pharmacol Sci. 2018;22(7):2175-85.

13.   Tsao L-C, Crosby EJ, Trotter TN, Agarwal P, Hwang B-J, Acharya C, et al. CD47 blockade augmentation of trastuzumab antitumor efficacy dependent on antibody-dependent cellular phagocytosis. JCI Insight. 2019;4(24):e131882.

14.   Wang W, Erbe AK, Hank JA, Morris ZS, Sondel PM. NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity in Cancer Immunotherapy. Front Immunol. 2015;6:368-
.

15.   Gajria D, Chandarlapaty S. HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Rev Anticancer Ther. 2011;11(2):263-75.

16.   Barish R, Gates E, Barac A. Trastuzumab-Induced Cardiomyopathy. Cardiol Clin. 2019;37(4):407-18.

17.   Balduzzi S, Mantarro S, Guarneri V, Tagliabue L, Pistotti V, Moja L, et al. Trastuzumab-containing regimens for metastatic breast cancer. Cochrane Database Syst Rev. 2014;2014(6):Cd006242.

18.   Piotrowski G, Gawor R, Stasiak A, Gawor Z, Potemski P, Banach M. Cardiac complications associated with trastuzumab in the setting of adjuvant chemotherapy for

breast cancer overexpressing human epidermal growth factor receptor type 2 - a prospective study. Arch Med Sci. 2012;8(2):227-35.

19.   de Azambuja E, Ponde N, Procter M, Rastogi P, Cecchini RS, Lambertini M, et al. A pooled analysis of the cardiac events in the trastuzumab adjuvant trials. Breast Cancer Res Treat. 2020;179(1):161-71.

20.   Alizadehasl A, Amin A, Maleki M, Noohi F, Ghavamzadeh A, Farrashi M. Cardio-oncology discipline: focus on the necessities in developing countries. ESC Heart Failure. 2020;7(5):2175-83.

21.   De Keulenaer GW, Doggen K, Lemmens K. The vulnerability of the heart as a pluricellular paracrine organ: lessons from unexpected triggers of heart failure in targeted ErbB2 anticancer therapy. Circ Res. 2010;106(1):35-46.

22.   Cardinale D, Biasillo G, Cipolla CM. Curing Cancer, Saving the Heart: A Challenge That Cardioncology Should Not Miss. Curr Cardiol Rep. 2016;18(6):51.

23.   Alizadehasl A, Maleki M, Noohi F, Peighambari MM, Moradian M. Cardiomyopathies. In: Moradian M, Alizadehasl A, editors. Atlas of Echocardiography in Pediatrics and Congenital Heart Diseases. Berlin, Heidelberg: Springer Berlin Heidelberg; 2021. p. 129-37.

24.   Herrmann J. Adverse cardiac effects of cancer therapies: cardiotoxicity and arrhythmia. Nature Reviews Cardiology. 2020;17(8):474-502.

25.   Mohan N, Jiang J, Dokmanovic M, Wu WJ. Trastuzumab-mediated cardiotoxicity: current understanding, challenges, and frontiers. Antib Ther. 2018;1(1):13-7.

26.   Leemasawat K, Phrommintikul A, Chattipakorn SC, Chattipakorn N. Mechanisms and potential interventions associated with the cardiotoxicity of ErbB2-targeted drugs: Insights from in vitro, in vivo, and clinical studies in breast cancer patients. Cell Mol Life Sci. 2020;77(8):1571-89.

27.   Brown SA, Ray JC, Herrmann J. Precision Cardio-Oncology: a Systems-Based Perspective on Cardiotoxicity of Tyrosine Kinase Inhibitors and Immune Checkpoint Inhibitors. J Cardiovasc Transl Res. 2020;13(3):402-16.

28.   Crone SA, Zhao YY, Fan L, Gu Y, Minamisawa S, Liu Y, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med. 2002;8(5):459-65.

29.   Bhagat A, Kleinerman ES. Anthracycline-Induced Cardiotoxicity: Causes, Mechanisms, and Prevention. Adv Exp Med Biol. 2020;1257:181-92.

30.   Tanaka S, Ikari A, Nitta T, Horiuchi T. Long-term irreversible trastuzumab-induced cardiotoxicity for metastatic breast cancer in a patient without cardiac risk factors. Oxf Med Case Reports. 2017;2017(7):omx038-omx.

31.   Bordin P, Volpe C, Adami G, Moretti V, Ermacora P. Irreversible Cardiotoxicity after Adjuvant Treatment with Trastuzumab in a Case of Breast Cancer. Tumori Journal. 2008;94(5):777-8.

32.   Gordon LI, Burke MA, Singh ATK, Prachand S, Lieberman ED, Sun L, et al. Blockade of the erbB2 receptor induces cardiomyocyte death through mitochondrial and reactive oxygen species-dependent pathways. J Biol Chem. 2009;284(4):2080-7.

33.   Gorini S, De Angelis A, Berrino L, Malara N, Rosano G, Ferraro E. Chemotherapeutic Drugs and Mitochondrial Dysfunction: Focus on Doxorubicin, Trastuzumab, and Sunitinib. Oxidative Medicine and Cellular Longevity. 2018;2018:7582730.

34.   ElZarrad MK, Mukhopadhyay P, Mohan N, Hao E, Dokmanovic M, Hirsch DS, et al. Trastuzumab alters the expression of genes essential for cardiac function and induces ultrastructural changes of cardiomyocytes in mice. PLoS One. 2013;8(11):e79543-e.

35.   Kuramochi Y, Guo X, Sawyer DB. Neuregulin activates erbB2-dependent src/FAK signaling and cytoskeletal remodeling in isolated adult rat cardiac myocytes. J Mol Cell Cardiol. 2006;41(2):228-35.

36.   Zeglinski M, Ludke A, Jassal DS, Singal PK. Trastuzumab-induced cardiac dysfunction: A 'dual-hit'. Exp Clin Cardiol. 2011;16(3):70-4.

37.   Rupert CE, Coulombe KL. The roles of neuregulin-1 in cardiac development, homeostasis, and disease. Biomark Insights. 2015;10(Suppl 1):1-9.

38.   Pentassuglia L, Sawyer DB. The role of Neuregulin-1beta/ErbB signaling in the heart. Exp Cell Res. 2009;315(4):627-37.

39.   Li B, Zheng Z, Wei Y, Wang M, Peng J, Kang T, et al. Therapeutic effects of neuregulin-1 in diabetic cardiomyopathy rats. Cardiovascular Diabetology. 2011;10(1):69.

40.   Fock V, Plessl K, Draxler P, Otti GR, Fiala C, Knöfler M, et al. Neuregulin-1-mediated ErbB2-ErbB3 signalling protects human trophoblasts against apoptosis to preserve differentiation. J Cell Sci. 2015;128(23):4306-16.

41.   Zoeller JJ, Vagodny A, Taneja K, Tan BY, O'Brien N, Slamon DJ, et al. Neutralization of BCL-2/X(L) Enhances the Cytotoxicity of T-DM1 In Vivo. Mol Cancer Ther. 2019;18(6):1115-26.

42.   Guglin M, Cutro R, Mishkin JD. Trastuzumab-Induced Cardiomyopathy. Journal of Cardiac Failure. 2008;14(5):437-44.

43.   An J, Sheikh MS. Toxicology of Trastuzumab: An Insight into Mechanisms of Cardiotoxicity. Curr Cancer Drug Targets. 2019;19(5):400-7.

44.   Stansfield WE, Ranek M, Pendse A, Schisler JC, Wang S, Pulinilkunnil T, et al. Chapter 4 - The Pathophysiology of Cardiac Hypertrophy and Heart Failure. In: Willis MS, Homeister JW, Stone JR, editors. Cellular and Molecular Pathobiology of Cardiovascular Disease. San Diego: Academic Press; 2014. p. 51-78.

45.   Laird-Fick HS, Tokala H, Kandola S, Kehdi M, Pelosi A, Wang L, et al. Early morphological changes in cardiac mitochondria after subcutaneous administration of

trastuzumab in rabbits: possible prevention with oral selenium supplementation. Cardiovascular Pathology. 2020;44:107159.

46.   Kitani T, Ong S-G, Lam CK, Rhee J-W, Zhang JZ, Oikonomopoulos A, et al. Human-Induced Pluripotent Stem Cell Model of Trastuzumab-Induced Cardiac Dysfunction in Patients With Breast Cancer. Circulation. 2019;139(21):2451-65.

47.   Almuwaqqat Z, Meisel JL, Barac A, Parashar S. Breast Cancer and Heart Failure. Heart Fail Clin. 2019;15(1):65-75.

48.   Caron J, Nohria A. Cardiac Toxicity from Breast Cancer Treatment: Can We Avoid This? Curr Oncol Rep. 2018;20(8):61.

49.   Ohtani K, Ide T, Hiasa KI, Sakamoto I, Yamashita N, Kubo M, et al. Cardioprotective effect of renin-angiotensin inhibitors and β-blockers in trastuzumab-related cardiotoxicity. Clin Res Cardiol. 2019;108(10):1128-39.

50.   Pituskin E, Mackey JR, Koshman S, Jassal D, Pitz M, Haykowsky MJ, et al. Multidisciplinary Approach to Novel Therapies in Cardio-Oncology Research (MANTICORE 101-Breast): A Randomized Trial for the Prevention of Trastuzumab-Associated Cardiotoxicity. J Clin Oncol. 2017;35(8):870-7.

51.   Boekhout AH, Gietema JA, Milojkovic Kerklaan B, van Werkhoven ED, Altena R, Honkoop A, et al. Angiotensin II-Receptor Inhibition With Candesartan to Prevent Trastuzumab-Related Cardiotoxic Effects in Patients With Early Breast Cancer: A Randomized Clinical Trial. JAMA Oncol. 2016;2(8):1030-7.

52.   Wittayanukorn S, Qian J, Westrick SC, Billor N, Johnson B, Hansen RA. Prevention of Trastuzumab and Anthracycline-induced Cardiotoxicity Using Angiotensin-converting Enzyme Inhibitors or β-blockers in Older Adults With Breast Cancer. Am J Clin Oncol. 2018;41(9):909-18.

53.   Guglin M, Krischer J, Tamura R, Fink A, Bello-Matricaria L, McCaskill-Stevens W, et al. Randomized Trial of Lisinopril Versus Carvedilol to Prevent Trastuzumab Cardiotoxicity in Patients With Breast Cancer. J Am Coll Cardiol. 2019;73(22):2859-68.

54.   Calvillo-Argüelles O, Abdel-Qadir H, Michalowska M, Billia F, Suntheralingam S, Amir E, et al. Cardioprotective Effect of Statins in Patients With HER2-Positive Breast Cancer Receiving Trastuzumab Therapy. Can J Cardiol. 2019;35(2):153-9.

55.   Abdel-Qadir H, Bobrowski D, Zhou L, Austin PC, Calvillo-Argüelles O, Amir E, et al. Statin Exposure and Risk of Heart Failure After Anthracycline‐ or Trastuzumab‐Based Chemotherapy for Early Breast Cancer: A Propensity Score‒Matched Cohort Study. Journal of the American Heart Association. 2021;10(2):e018393.

56.   Ceron CS, Castro MM, Rizzi E, Montenegro MF, Fontana V, Salgado MCO, et al. Spironolactone and hydrochlorothiazide exert antioxidant effects and reduce vascular matrix metalloproteinase-2 activity and expression in a model of renovascular hypertension. Br J Pharmacol. 2010;160(1):77-87.

57.   Yavas G, Elsurer R, Yavas C, Elsurer C, Ata O. Does spironolactone ameliorate trastuzumab-induced cardiac toxicity? Med Hypotheses. 2013;81(2):231-4.

58.   Silva MAB, Bruder-Nascimento T, Cau SBA, Lopes RAM, Mestriner FLAC, Fais RS, et al. Spironolactone treatment attenuates vascular dysfunction in type 2 diabetic mice by decreasing oxidative stress and restoring NO/GC signaling. Frontiers in Physiology. 2015;6(269).

59.   Lu G, Li J, Zhai Y, Li Q, Xie D, Zhang J, et al. Spironolactone suppresses aldosterone-induced Kv1.5 expression by attenuating mineralocorticoid receptor-Nox1/2/4-

mediated ROS generation in neonatal rat atrial myocytes. Biochem Biophys Res Commun. 2019;520(2):379-84.

60.   Sadeghpour A, Alizadehasl A. The right ventricle: a comprehensive review from anatomy, physiology, and mechanics to hemodynamic, functional, and imaging evaluation. Arch Cardiovasc Imaging. 2015;3(4):e35717.

61.   Guitart-Mampel M, Urquiza P, Borges JI, Lymperopoulos A, Solesio ME. Impact of Aldosterone on the Failing Myocardium: Insights from Mitochondria and Adrenergic Receptors Signaling and Function. Cells. 2021;10(6):1552.

62.   Shahsavarinia K, Ghojazadeh M, Ghabousian A, Hatefnia F, Soleimanpour M, Soleimanpour H. An Umbrella Review of Clinical Efficacy and Adverse Cardiac Events Associated with Hydroxychloroquine or Chloroquine with or Without Azithromycin in Patients with COVID-19. Anesth Pain Med. 2021;In Press(In Press):e115827.

63.   Rozner RN, Freites-Martinez A, Shapiro J, Geer EB, Goldfarb S, Lacouture ME. Safety of 5α-reductase inhibitors and spironolactone in breast cancer patients receiving endocrine therapies. Breast Cancer Res Treat. 2019;174(1):15-26.

64.   Safari S, Khasraghi ZS, Chegeni MA, Ghabousian A, Amini A. The ability of Canadian Syncope risk score in differentiating cardiogenic and non-cardiogenic syncope; a cross-sectional study. The American Journal of Emergency Medicine. 2021.

65.   Kosmas CE, Silverio D, Sourlas A, Montan PD, Guzman E. Role of spironolactone in the treatment of heart failure with preserved ejection fraction. Ann Transl Med. 2018;6(23):461-.

66.   Verma I, Krishan P, Syngle A. Therapeutic potential of spironolactone in ankylosing spondylitis patients: Relation to disease activity and functional status. The Egyptian Rheumatologist. 2021;43(2):173-8.

67.	Williams TA, Verhovez A, Milan A, Veglio F, Mulatero P. Protective effect of spironolactone on endothelial cell apoptosis. Endocrinology. 2006;147(5):2496-505.

68.	Wei Y, Whaley-Connell AT, Habibi J, Rehmer J, Rehmer N, Patel K, et al. Mineralocorticoid receptor antagonism attenuates vascular apoptosis and injury via rescuing protein kinase B activation. Hypertension. 2009;53(2):158-65.

69.	Liu P, Fan J, Wang Z, Zai W, Song P, Li Y, et al. The role of autophagy in the cytotoxicity induced by trastuzumab emtansine (T-DM1) in HER2-positive breast cancer cells. AMB Express. 2020;10(1):107-.

70.	Pecoraro M, Pala B, Di Marcantonio MC, Muraro R, Marzocco S, Pinto A, et al. Doxorubicin-induced oxidative and nitrosative stress: Mitochondrial connexin 43 is at the crossroads. Int J Mol Med. 2020;46(3):1197-209.

71.	Martins-Marques T, Ribeiro-Rodrigues T, Batista-Almeida D, Aasen T, Kwak BR, Girao H. Biological Functions of Connexin43 Beyond Intercellular Communication. Trends Cell Biol. 2019;29(10):835-47.

72.	Pecoraro M, Pinto A, Popolo A. Trastuzumab-induced cardiotoxicity and role of mitochondrial connexin43 in the adaptive response. Toxicol In Vitro. 2020;67:104926.

73.	Qu J, Volpicelli FM, Garcia LI, Sandeep N, Zhang J, Márquez-Rosado L, et al. Gap junction remodeling and spironolactone-dependent reverse remodeling in the hypertrophied heart. Circ Res. 2009;104(3):365-71.

74.	De Mello WC. Aldosterone Disrupts the Intercellular Flow of Glucose in Cardiac Muscle. Front Endocrinol (Lausanne). 2015;6:185-.

75.	Mayyas F, Alzoubi KH, Bonyan R. The role of spironolactone on myocardial oxidative stress in rat model of streptozotocin-induced diabetes. Cardiovasc Ther. 2017;35(2).

76.   Chow CK. Vitamin E regulation of mitochondrial superoxide generation. Biol Signals Recept. 2001;10(1-2):112-24.

77.   Panth N, Paudel KR, Parajuli K. Reactive Oxygen Species: A Key Hallmark of Cardiovascular Disease. Adv Med. 2016;2016:9152732-.

78.   Liu W, Gong W, He M, Liu Y, Yang Y, Wang M, et al. Spironolactone Protects against Diabetic Cardiomyopathy in Streptozotocin-Induced Diabetic Rats. J Diabetes Res. 2018;2018:9232065-.

79.   Yavas G, Celik E, Yavas C, Elsurer C, Afsar RE. Spironolactone ameliorates the cardiovascular toxicity induced by concomitant trastuzumab and thoracic radiotherapy. Reports of Practical Oncology & Radiotherapy. 2017;22(4):295-302.

80.   Akpek M, Ozdogru I, Sahin O, Inanc M, Dogan A, Yazici C, et al. Protective effects of spironolactone against anthracycline-induced cardiomyopathy. Eur J Heart Fail. 2015;17(1):81-9.

81.   Alizadehasl A, Ghadimi N, Kaveh S, Maleki M, Ghavamzadeh A, Noohi F, et al. Prevention of anthracycline-induced cardiotoxicity: a systematic review and network meta-analysis. International Journal of Clinical Pharmacy. 2021;43(1):25-34.