Cardio Protective Effect of Neuregillin-1 against Cardio Toxicity Induced by Trastuzumab in Adult Mice

Yousuf Qays Majeed*1, Mustafa Ghazi AL-Abbassi2, Ghaith Ali Jasim Al-Zubaidy3

Department of Pharmacology and Toxicology, College of Pharmacy, Al-Mustansiri yah University

*Corresponding author’s: Yousuf Qays Majeed

Abstract

Objective: This work will be carried out to investigate the potential therapeutic effect of Neuregulin-1 against cardio toxicity induced by trastuzumab in male mice.

Materials and Methods: Forty five adult male and female mice (weighing 35-50 gm) were divided into 3 groups randomly, each group contains 15 animals, group A which received distilled water as control by using intraperitoneal injection (IP) route for 10 days group B, received Trastuzumab as 6 mg/kg/day by using intraperitoneal injection route for 10 days while group C, received Neuregulin-1 (2.5 mg/day/animal) just before the administration of Trastuzumab (6mg/kg/day) given by IP route for 10 days. The significance of difference was tested by using ANOVA followed by Tukey test. Statistical significance depends on the P<0.05.

Results: Cardiac troponin I concentration was significantly elevated in the trastuzumab group when compared with the control group; while, concentration has been significantly lower in the group C when compared with the trastuzumab group. While, results of total antioxidant capacity had significantly higher in the group B and group C in compare with control group.

Conclusion: Neuregulin-1 can be used as a cardio protective in cardiac complications of cancer treatment by its ability to attenuate the cardio toxic effect of trastuzumab by significantly lowering cardiac troponin I concentrations.

Keywords: Neuregillin-1, Cardio toxicity, Trastuzumab, Cardiac troponins.

Introduction

Cardio toxicity is the ‘toxicity that affects the heart’, which represent direct effect of the drug on the heart and an indirect effect due to enhancement of haemodynamic flow alterations or due to thrombotic events (1). The improvement in cancer management and cardiotoxicity that have a relationship which lead to improve survival and the adverse effects of treatments that have significant effect on patient outcome (2–4).

Most of anticancer drugs may cause severe cardiac dysfunctions by the dose dependent toxicity in those patients in treatment period and for health care professionals in multiple periods of antineoplastic agents (5, 6). Cardio toxic events may comprise some blood pressure changes, arrhythmias and cardiomyopathy (7). Several mechanisms of antineoplastic agents causing cardiotoxicity may include cellular damage, and production of reactive oxygen species and many immunological reactions by presenting of antigen presenting cells (APC) in the heart (8).

The intensity of side effects can be acute, subacute, or as chronic progress (9). Trastuzumab is considered as humanized monoclonal antibody against the extracellular domain of human epidermal growth factor receptor 2 (HER2) that improves survival with those patients of metastatic breast cancer and prolongs disease free survival and overall survival in patients with early-stage breast cancer (EBC) treated trastuzumab as adjuvant therapy (10–13).

Cardio toxicity that related to Trastuzumab therapy is classified as Type II which is mechanistically and clinically distinct, is not dose-dependent, reversible, and is not related to ultrastructural changes (14, 15).
Myocardial stunning may considered as mechanism trastuzumab induced cardiac dysfunction and differ from this of anthracyclines.

The HER2 signalling pathway has an important role in the cardiac development and function and some studies showed that mice models of lacking HER2 may involve multiple properties of dilated cardiomyopathy, including wall thinning, chamber dilation, and decreased contractility (16, 17).

The most acceptable mechanism of drug-induced cardiac toxicity is due to oxidative stress and the generation of reactive oxygen species (ROS) (18, 19). Anthracyclines may involve redox cycling and formation of reactive oxygen species like superoxide anion and hydrogen peroxide (H₂O₂), upon oxidative stress consequence, loss of energy in cardiomyocytes, defect in mitochondrial function, damage to cellular membrane, apoptosis, and cytotoxicity (20). Biomarkers like Cardiac troponins I (cTnI) and T (cTnT) are considered as sensitive and specific for detecting myocardial abnormality and damage, while B-type natriuretic peptide (BNP) is detecting the volume overload.

These markers are considered as potential indicators of drugs-remediared cardiotoxicity (21-23). The elevation of troponins may be associated with intensity of clinical symptoms and future prognosis. Troponin may be considered as an evaluation parameter for identification of myocardial injury in those patients with acute coronary diseases.

In cardiac diseases, the using of troponins as clinical biomarker has been taken to a wide range of pathologies characterized by cardiac injury, including left ventricular hypertrophy, congestive HF, pulmonary embolism, blunt trauma, sepsis, renal insufficiency, and diabetes mellitus (24). In spite of the effective treatment of chemotherapy, a cardiotoxicity has been occurred and for that reason many strategies required at the starting of treatment CVD risk factors, preventing cardiac side effects just before or during chemotherapy and also many protocols used for the management of cardiac side effects either sub-clinically or clinically. Thus many of cardio-protective agents are used with early identification and induction of therapy to get the best results of getting rid from cardiac side effects. Dexrazoxane is considered as a chelating agent of iron and it exerts its effects by preventing the complexes of anthracycline-iron to be formed, and thereby a reduction in the ROS production (25). Angiotensin-converting enzyme (ACE) inhibitors are considered as the best agent of systolic heart failure therapy as many studies showing a free mortality benefit (26). Several studies showed the protective role for ACE inhibitors in cancer-induced cardiotoxicity and also the significant reduction in LVEF (27).

Beta blockers have an important role in decreasing the developing risk of cancer therapy related cardiomyopathy and the important effects of this class showed with agents like carvedilol and nebivolol (28). Statins are lipid lowering drugs has the ability to reduce morbidity and mortality due to their actions as anti-inflammatory and antioxidant properties (29,30). Sildenafil, a phosphodiesterase-5 inhibitor which has the ability to limit the ischemic and myocardial injury, and provide the protection from doxorubicin-induced myocardial toxicity by its effect on nitric oxide pathway and on the opening of ATP-sensitive potassium channels in mitochondria (31,32).

Neuregulin-1 (NRG-1) is belongs to family of neuregulin growth factor, it is responsible for many processes in cell it is could be either via paracrine or juxtacrine signalling mediated by tyrosine kinase receptor called “erythoblastic leukemia viral oncogene homologs (ErbBs)” of epidermal growth factor receptors (33). So that’s mean, NRG-1 is going to reduce in the pathologic changes in those studied animal with heart failure accompanied with other cardiovascular diseases through one or of these mechanisms mention previously and thus lead to the improvement in cardiac muscle integrity and performance and also enhance the function of left ventricles and decreased mortality (34). This work will be carried out to investigate the potential therapeutic effect of neuregulin-1 against cardiotoxicity induced by trastuzumab in mice.

Materials and Methods

Animals and Study Design

Forty five adult male and female mice (weighing 35-50 gm) were have been used in the experiment after getting approval from...
The specific antibody that already existed in micro ELISA plate wells will bind to standards or samples. Then the addition of a biotinylated antibody and avidin-horseradish peroxidase (HRP) conjugate to each micro plate well and be incubated. Wash buffer will remove the unbound conjugates. TMB substrate is used to let the HRP enzymatic reaction visible. HRB will be as a catalyst for TMB to produce blue color agent that change into yellow after the addition of acidic stop solution.

The yellow color density is proportional to the amount of biomarker sample captured in plate. The optical density optical density is measured spectrophotometrically at 450nm in microplate reader, then the concentration of biomarkers can be calculated in the sample by comparing the optical density of the samples to the standard curve (35).

Statistical Analysis

Analysis of data was performed by using SPSS-16.0 software (Statistical Packages for Social Sciences- version 16) for windows (SPSS Inc, Chicago, IL). Data were presented in simple measures of mean± standard deviation. The significance of difference were tested by using ANOVA test for difference among more than two independent means followed by Tukey test. Statistical significance depends on the P value when it was less than 0.05 (36).

Results

Serum Cardiac Troponin I Level (CTNI)

The descriptive statistics, which represent as mean ± standard deviation for cardiac troponin I concentration was significantly elevated in the TRZ group has been matched with the control group (23.44 ± 6.99 vs. 15.01 ±1.11 pg/ml, respectively; P < 0.05). The cardiac troponin I concentration has been significantly lower in the NRG-1 + TRZ group were compare with the TRZ group (17.51± 3.73 pg/ml; P < 0.05).as shown in the Table and Figure (1).

In compare with control group (group A) using ANOVA test followed by Tukey, there was a significant difference (P<0.05) for TRZ group (group B) while, data recorded a non-significant difference when comparing the control group with NRG-1 (group C) P=0.572. All group when, generally, compared with

ethical committee in college of pharmacy/Al-Mustansiriyah University. Animals were divided into 3 groups randomly each group contains 15 animals, group A (control group ) which received Distilled water as control by using intraperitoneal injection route for 10 days group B (TRZ group) received Trastuzumab (TRZ) as 6 mg/kg/day by using intraperitoneal injection route for 10 days while group C (NRG-1+TRZ group) received Neuregulin-1 (2.5 mg/day/animal) just before the administration of Trastuzumab 6mg/kg/day given by IP route for 10 days.

Treatment Administration

Each day of the experiment a calculated dose of Trastuzumab and Neuregulin-1 for each mouse (in both group B and C) with equal volume of distilled water (in Group A) was drawn in an insulin syringe then delivered to the animal via intraperitoneal injection of a conscious mouse. Using adequate manual restraint, the animal was turned over so abdomen is exposed and injection should be in the lower right or left quadrant of abdomen trying to avoid hitting bladder, liver, or other internal organs. As the animal injected a gentle aspiration to check if the needle hit an internal organ or not, if no blood incoming with the aspiration that’s mean no damage had been occurred and complete the injection.

Animal Sacrifice

At the end of 10 days, blood collected from animals by cardiac puncture then mice euthanized by decapitation.

Serum Sample Preparation

Collected blood was allowed to clot for 30 min in plane tube without an anticoagulant at 25°C. Then, blood samples were centrifuged at 3,000 x g for 10 min, and serum layers were pipetted off without disturbing the white buffy layers. Serum were stored in frozen state for detection of total antioxidant capacity test (TAOC) and cardiac troponin-I.

Detection of Serum Mouse Biomarkers: Troponin I and Total Antioxidant Capacity):

All kits are based on sandwich enzyme – linked imunosorbent assay (ELISA) technology. Anti-biomarkers antibody is pre-coated onto 96-well plates.
control group (group A) using ANOVA test followed by Tukey.

### Table 1: Serum cardiac troponin I level among the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>15.0302</td>
<td>1.10512</td>
<td>.45116</td>
<td>13.8704 to 16.1899</td>
<td>14.02</td>
<td>16.04</td>
</tr>
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<td>TRZ</td>
<td>23.4372</td>
<td>6.99566</td>
<td>1.94025</td>
<td>19.2097 to 27.6646</td>
<td>16.41</td>
<td>37.60</td>
</tr>
<tr>
<td>NRG</td>
<td>17.5079</td>
<td>3.72842</td>
<td>.96267</td>
<td>15.4431 to 19.5726</td>
<td>13.31</td>
<td>25.06</td>
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<tr>
<td>Total</td>
<td>19.3377</td>
<td>5.94933</td>
<td>1.02030</td>
<td>17.2619 to 21.4135</td>
<td>13.31</td>
<td>37.60</td>
</tr>
</tbody>
</table>

Figure 1: Serum cardiac troponin I level among the studied groups

### Serum Total Antioxidant Capacity Level (TAOC)

The descriptive statistics, which represent as mean ± standard deviation for total antioxidant capacity (TAOC) concentration. Results of the present study showed that total antioxidant capacity (TAOC) concentration had significantly higher values in the TRZ group and NRG-1 + TRZ group in compare to control group (13.26 ± 1.62 & 12.8305 ± 2.38) respectively vs (9.55 ± 0.62 unit/ml; P < 0.05). Total antioxidant capacity data recorded a decrease in the NRG-1 + TRZ group compared to TRZ group with no significant results (P =0.822) as shown in the table and Figure (2). All group when, generally, compared with control group (group A) using ANOVA test followed by Tukey.

### Table 2: Serum Total antioxidant capacity (TAOC) level among the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>9.55</td>
<td>0.62</td>
<td>0.25</td>
<td>8.89 to 10.21</td>
<td>8.98</td>
<td>10.12</td>
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<td>TRZ</td>
<td>13.26</td>
<td>1.62</td>
<td>0.45</td>
<td>12.28 to 14.24</td>
<td>9.86</td>
<td>15.15</td>
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<td>NRG</td>
<td>12.83</td>
<td>2.38</td>
<td>0.61</td>
<td>11.51 to 14.15</td>
<td>8.48</td>
<td>17.97</td>
</tr>
<tr>
<td>Total</td>
<td>12.42</td>
<td>2.29</td>
<td>0.39</td>
<td>11.62 to 13.22</td>
<td>8.48</td>
<td>17.97</td>
</tr>
</tbody>
</table>

Figure 2: Serum Total antioxidant capacity (TAOC) level among the studied groups
Discussion

Drug related cardio toxicity may affect health and drug development. There are several indicators of cardiotoxicity which significantly changed only after severe heart injuries occurrence. In this study the investigation of most sensitive and reliable indicators to evaluate and predict the cardiac dysfunction. Thus, mice cardiotoxicity models had been created by Trastuzumab (6 mg/kg/day), followed by different statistical and integration tests and after selecting some of biomarkers that evaluate the occurrence of cardiotoxicity like biochemical and histopathological assessment.

Serum Cardiac Troponin I Level (CTNI)

Cardiac troponins are released due to damage of cardiomyocyte which may be induced by many of mechanisms such as ischemia, inflammation, oxidative stress, or apoptosis. It may consider one of the best biomarkers used to assess trastuzumab-induced cardiotoxicity. Many studies have shown number of patients with cTnI elevation, magnitude of the elevation, time of TRZ administration, and prognosis cardiac dysfunction. Estimation of cardiac toxicity with cardiac troponin T (cTnT) was as less accurate as compared with cardiac troponin I (cTnI). Cardiac troponin I, is considered useful as a diagnostic marker for many of heart disorders, results of the present study showed that cardiac troponin I concentration was significantly elevated in the TRZ group when compared with the control group (23.44 ± 6.99 vs. 15.01 ± 1.11 pg/ml, respectively; P < 0.05) which indicates that trastuzumab is associated with an increased risk of cardiotoxicity. The ErbB2 has an important role in normal and the overall incidence of trastuzumab-related cardiovascular development and physiology and that cardiotoxicity varies according to the definitioneffect obviously demonstrated in those mice with used and the population evaluated. In a previous knockout of cardiac ErbB2. Endothelial cells study that observed the rates of trastuzumab (ECs) in rat showed an expression of multiple related cardiotoxicity are much higher in the NRG-1 + TRZ group when compared with the TRZ group (17.51± 3.73 pg/ml; P < 0.05), as shown in the Table and figure (1). In this study, the efficiency of NRG-1 can be used as a standardized sequential chemotherapy combined with trastuzumab. In the NRG-1 + TRZ group, the use of NRG-1 was associated with lower levels of serum CTNI, which indicated that NRG-1 could relieve side effects of cardiotoxicity induced by trastuzumab. In addition, NRG-1 displayed an obviously protective effect against the cardiomyocyte apoptosis. This in vivo study on Mice demonstrated that combination with NRG-1 can be considered as standardized sequential chemotherapy with trastuzumab.

This result came in agreement with a previous study demonstrated on rats using trastuzumab with Dexrazoxane which recorded lower levels of cTnI (38). In compare with control group (group A) using ANOVA test followed by Tukey, data recorded a non-significant difference when compared with NRG-1 + TRZ group (P=0.572), which strongly aids the protective effect of NRG-1 against the cardiomyocyte apoptosis (39). NRG-1 has different types of isoforms that are expressed mainly in the endothelial cells and cardiomyocytes. The effects of NRG-18 are mainly on cardiac biology and vascular cells and could be beneficial on cardiac function. The essential role of these peptides is mainly on the development of the cardiovascular system, involving many processes such as angiogenesis and also compensation of cardiac function.

The cardiac troponin I concentration has been significantly lower in the NRG-1 + TRZ group when compared with the TRZ group (17.51± 3.73 pg/ml; P < 0.05), as shown in the Table and figure (1). In this study, the efficiency of NRG-1 can be used as a standardized sequential chemotherapy combined with trastuzumab. In the NRG-1 + TRZ group, the use of NRG-1 was associated with lower levels of serum CTNI, which indicated that NRG-1 could relieve side effects of cardiotoxicity induced by trastuzumab. In addition, NRG-1 displayed an obviously protective effect against the cardiomyocyte apoptosis. This in vivo study on Mice demonstrated that combination with NRG-1 can be considered as standardized sequential chemotherapy with trastuzumab.

The most common and serious side effect resulting from antineoplastic agents is cardiomyopathy. The cardio toxic effects may ranging from mild reduction in ejection fraction to serious heart failure. Some results show that, in mice, the treatment with TRZ induces many of effects on myocardial genes expression that involved in myocardial functions, stress adaptation, and repair of DNA. These alterations in the genes are associated with elevation in myocardial oxidative and nitrosamine stress, and may activate several pathways of apoptosis, leading to increased serum troponin-I and cardiac myosin light chain-1 (cMLC1) levels (37).
Serum Total Antioxidant Capacity Level (TAOC)

Total antioxidant capacity (TAOC) is considered as a parameter used for characterization of food products and the antioxidant status of the body. The term TAOC is not optimal since the assay methods only part of antioxidant capacity, typically excluding enzymatic activities. It has been described that different antioxidants can be produced depending on types of ROS that formed. Plasma antioxidant status can therefore vary according to which antioxidant is quantified (41). Results of the present study showed that total antioxidant capacity (TAOC) concentration had significantly higher values in the TRZ group and NRG-1 + TRZ group in compare to control group (13.26 ± 1.62 & 12.8305 ± 2.38) respectively (9.55 ± 0.62 unit/ml; P < 0.05).

Total antioxidant capacity data recorded a decrease in the NRG-1 + TRZ group compared to TRZ group with no significant results (P =0.822) as shown in the table and figure (2). As mentioned above regarding the effect of TRZ treatment on the expression of myocardial genes, and how these alterations are related to myocardial oxidative and nitrosative stress, and also the activation of apoptotic pathways (47). Oxidative stress may induce deleterious consequences to many of cellular compounds such as DNA, lipids, and proteins.

The oxidative stress can mediate the attack on endogenous molecules by the reaction with phospholipids of cellular membrane, and lead to generation of secondary reactive intermediates (42).

Trastuzumab may worsen sub-lethal anthracycline-related damage in addition to causing HER2-specific damage and inhibition of cardiomyocyte repair and also signal regulation in the case of trastuzumab-induced cardiac dysfunction potentiated by anthracycline exposure (43,44). Cardiotoxicity induced by TRZ was confirmed by cultures of cardiac cells in vitro and in vivo on animal models that reported the effect of and functionality (45-49).

Trastuzumab treatment on both functional and structural properties of the heart. It causes the alterations of the genetic expression of several cardiac functions, pressure adaptation, vasodilatation and contractility. Trastuzumab raised myocardial levels of products of oxidative and nitrative stress that stimulate the mitochondrial dysfunction and cause the damage to cardiac tissue.

Also treatment with TRZ can cause cardiomyocyte ultrastructure changes, associated with ultrastructural damages of cardiomyocytes in mice. The oxidative stress and structural changes are considered as the main pathways in anticancer related cardiac toxicity. Also many of evidences showed that apoptotic pathways in cardiomyocytes of mice treated with trastuzumab may be activated due to that therapy and that obvious through the increase in apoptotic cells and the extent of cardiac fibrosis in cardiomyocytes in mice treated with trastuzumab when compared to control group. Changes in the functional activity of heart in treated mice with TRZ were increasing left ventricular end-diastolic dimension (LVEDD).

Trastuzumab is simulating the doxorubicin in reducing radial strain (RS) before the change in fractional shortening (FS) two days post therapy. In vitro study of Trastuzumab, a reduction in cell viability of cardiomyocytes has been shown.

Trastuzumab may increase the levels of activated caspase3 and reduces the levels of Bcl-XL in cardiomyocytes, thus apoptosis induction. The occurrence of cardiotoxic effects of trastuzumab may be due to the prevention of NRG-1/ErbB2/ErbB4 complex that required for cardiomyocyte survival. The trastuzumab treatment has an important role in the inhibition of ligand-induced cell proliferation, and also the ligand-induced ErbB2/ErbB4 dimerization signaling pathway that required for cell growth.

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