Doxorubicin and Trastuzumab Regimen Induces Biventricular Failure in Mice

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Background: An increased risk for cardiac dysfunction is reported when the anti–epidermal growth factor receptor type 2 (ErbB2) antibody trastuzumab (Trz) is combined with doxorubicin (Dox) as adjuvant chemotherapy for patients with ErbB2-positive breast cancer. The aim of this study was to develop and characterize a novel mouse model of cardiotoxicity that recapitulates the clinical therapeutic protocols of consecutive cycles of Dox followed by Trz therapy.

Methods: Chronic cardiotoxicity was induced in mice by administering six intraperitoneal injections of Dox weekly over a 2-week period (n = 38; cumulative dose, 24 mg/kg), Trz alone (n = 15; cumulative dose, 10 mg/kg), Trz administered 1 week after Dox treatment (n = 35), or an equivalent volume of saline (n = 24).

Results: Echocardiography and pressure-volume analysis indicated that Dox administration was responsible for both left ventricular (LV) and right ventricular (RV) systolic dysfunction and dilatation, further exacerbated by subsequent Trz treatment. Trz alone induced a short down-regulation of LV ErbB2/4 expression associated with reversible LV dysfunction but did not affect receptor expression and RV performance. Dox and Trz in combination decreased the ratio of LV weight to tibia length as well as LV and RV wall thickness compared with Dox treatment. Plasma cardiac troponin I levels and myocardial oxidative stress were higher in mice treated with Dox and Trz than in those treated with Dox alone, while a similar increase of interstitial collagen I deposition was observed in both groups. Trz alone did not affect LV and RV remodeling.

Conclusions: These findings suggest that a combined Dox and Trz regimen provokes a detrimental synergistic global cardiac injury extending to both the LV and RV chambers. (J Am Soc Echocardiogr 2014;27:568-79.)

Keywords: Cardiotoxicity, Mouse model, Echocardiography, Hemodynamics

The addition of trastuzumab (Trz) to adjuvant doxorubicin (Dox) chemotherapy has reduced the risk for breast cancer recurrence by 50% and mortality by 30% in early epidermal growth factor receptor type 2 (ErbB2)–positive women.[1,2] However, the major limitation of this therapeutic regimen is the onset of serious cardiac side effects.[2] Dox, one of the most effective antitumor anthracyclines used in cancer therapy, is well known to exert a dose-dependent cardiotoxic action, due primarily to the generation of reactive oxygen species, myofibrillar disarray, cardiac cell death, and subsequent myocardial remodeling.[3,4] Trz, a humanized monoclonal antibody against human ErbB2, marketed as Herceptin (Roche, Basel, Switzerland), may induce cardiotoxicity by deregulating the ErbB2–phosphoinositide 3-kinase survival pathway, which in turn is responsible for cardiomyocyte integrity in response to stress.[2,3,5,6]

The association of combined Dox and Trz therapy provokes a synergic detrimental cardiotoxic effect. The risk for developing cardiomyopathy or symptomatic heart failure (HF) is relatively low, although it is increased in patients treated with Dox and Trz compared with anthracycline alone. Cardiomyopathy or symptomatic HF induced by combined Dox and Trz therapy is reported to range between 2% and 4% in controlled clinical trials,[7-9] with peaks to 30% in population-based observational studies.[10] The reversibility rate upon Trz discontinuation varies from 50% to 85%.[7-9] To minimize the risk for cardiomyopathy or symptomatic HF, Dox-Trz therapy has been limited to subjects meeting well-defined cardiovascular eligibility criteria.[8,11] As a consequence, a significant proportion of patients are currently not eligible for Dox-Trz therapy.

A better understanding of the functional and molecular mechanisms responsible for the synergistic Trz-induced cardiac injury after exposure to anthracycline treatment may be important for novel

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Abbreviations

cTnI = Cardiac troponin I

Dox = Doxorubicin

ErbB2 = Epidermal growth factor receptor type 2

HF = Heart failure

LV = Left ventricular

LVEDV = Left ventricular end-diastolic volume

LVEF = Left ventricular ejection fraction

LVESV = Left ventricular end-systolic volume

PA = Pulmonary artery

PBS = Phosphate-buffered saline

RV = Right ventricular

3-NT = 3-Nitrotirosine

TL = Tibia length

Trz = Trastuzumab

protective or preventive therapeutic strategies. Animal studies of cardiotoxicity induced by the acute administration of a single dose of Dox, given simultaneously with Trz, have reported a synergistic increase of LV dilatation and systolic dysfunction due to myocardial apoptosis and oxidative stress.[12,13] These models, however, do not fully recapitulate the current standard clinical therapeutic protocols. Therefore, in the present study, we developed a novel mouse model of cardiotoxicity, mimicking the standard clinical protocol of Dox-Trz-associated therapy, in which multiple administrations of Dox are followed by multiple doses of Trz. To better assess the extent and the severity of cardiac toxicity, we extended the evaluation of cardiac injury at functional and molecular levels to both the left and right ventricles.

METHODS

Animals and Experimental Protocol

We generated a chronic in vivo mouse model of cardiotoxicity induced by either Dox (Sigma-Aldrich, St Louis, MO) or Trz (Herceptin) alone or Dox followed by Trz treatment. A range of cumulative doses of Dox were previously tested, starting from 18 to 33 mg/kg. Eventually, the dose of 24 mg/kg showed severe cardiotoxicity with a low mortality rate. Female C57Bl/6 wild-type mice (Charles River Laboratories, Calco, Italy) aged 8 to 10 weeks were randomly divided into five groups (Figure 1). In the first group (Dox, $n = 38$), Dox was administered in six equal intraperitoneal injections over a period of 2 weeks (4 mg/kg each; cumulative dose, 24 mg/kg). In the second group (Trz, $n = 15$), Trz was administered in six equal injections over a period of 2 weeks (1.66 mg/kg each; cumulative dose, 10 mg/kg).[13] In the third group (saline 1, $n = 14$), control mice were treated with physiologic saline in same manner as the regimens for the Dox and Trz groups. In the fourth group (Dox and Trz, $n = 35$), after 1 week of recovery following 2 weeks of Dox treatment (4 mg/kg each; cumulative dose, 24 mg/kg), mice were treated with Trz over a period of 2 weeks (1.66 mg/kg each; cumulative dose, 10 mg/kg). In the fifth group (saline 2, $n = 10$), control mice were treated with physiologic saline simulating the combined Dox and Trz regimen.

Protocols complied with national and international law and policies (4D.L. N.116, G.U., supplement 40, 18-2-1992; EEC Council Directive 86/609, OJ L 358,1,12-12-1987; The Guidelines of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals; and US National Research Council 1996).

Echocardiography

Transthoracic echocardiography was performed using the Vevo 2100 high-resolution imaging system (VisualSonics, Toronto, ON, Canada) and a 40-MHz linear transducer with simultaneous electrocardiographic recording. Analyses were performed on mice lightly anesthetized with 0.5% to 1% isoflurane (heart rate, 480–550 beats/min), 1 day before starting treatments (baseline) and on day 21 or day 42 after the first drug administration (Figure 1). Two-dimensional short-axis M-mode echocardiography was performed at the level of the midpapillary muscle to measure left ventricular (LV) end-systolic volume (LVESV) and LV end-diastolic volume (LVEDV), LV anterior wall thickness in systole, and LV ejection fraction (LVEF).

Right ventricular (RV) internal diameters in systole and in diastole were acquired from a parasternal long-axis view and measured from images acquired in M mode, using the depth interval (in millimeters) generic measurements tool.[14-16] Pulsed-wave Doppler measurements of blood flow across the proximal main pulmonary artery (PA) were obtained as a further indicator of RV function, placing the probe in a parasternal long-axis position and adjusting the angle orientation (in all cases $<10°$) to optimize visualization of the proximal main PA.[14-17] Pulsed flow Doppler imaging was then overlaid to measure the peak PA velocity-time integral, PA diameter, and heart rate. RV cardiac output was calculated according the following equation: cardiac output = (PA diameter/2)^2 $\times$ π $\times$ PA velocity-time integral $\times$ heart rate.[18] All measurements were averaged from a minimum of three cycles during diastole and systole corresponding to the electrocardiogram. Data and imaging were analyzed using the VisualSonics Cardiac Measurements Package by a blinded investigator.

Hemodynamics

LV and RV performance was analyzed using a Millar pressure-volume conductance catheter (SPR-839; Millar Instruments, Houston, TX), as previously described.[19-22] On days 21 and 42, mice were anesthetized with an intraperitoneal injection of ketamine/metador cocktail (100/10 mg/kg). The trachea was cannulated, and the animal was connected to a positive-pressure volume-controlled rodent ventilator (MiniVent; Harvard Apparatus, Holliston, MA). For LV measurements (LVESV, LVEDV, $\pm$dP/dt, and τ), the catheter was introduced through the right carotid artery into the ascending aorta and then into the LV cavity. For RV measurements (RV end-systolic volume, RV end-diastolic volume, $\pm$dP/dt, and τ), the catheter was inserted into the right jugular vein and then advanced into the RV cavity. It should be mentioned that for RV volume, in this case we considered indices valid only to represent changes rather than validated volumes.

Hematologic Analysis

Retro-orbital bleeding was performed with mice anesthetized with 1.5% isoflurane. Whole blood was collected into ethylenediaminetetraacetic acid–coated tubes (Fisher HealthCare, Houston, TX), and hematocrit was analyzed. Plasma release of cardiac troponin I (cTnI) was determined using an enzyme-linked immunosorbent assay kit (Life Diagnostics, Inc, West Chester, PA) according to the protocol provided by the manufacturer. Results are expressed in nanograms per milliliter.

Western Blotting

Hearts isolated from mice were separated into left and right ventricles and immediately frozen. Ventricles were lysed in radioimmunoprecipitation assay buffer in the presence of proteases (P8849; Sigma-Aldrich) and phosphatase inhibitors (04906837001; Roche Diagnostics GmbH, Mannheim, Germany), and total protein extracts (100 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis