Toxicology 333 (2015) 76–88

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Gene expression profiling identifies the novel role of immunoproteasome in doxorubicin-induced cardiotoxicity

Wen-Jie Zhao [a,b,c], Sheng-Nan Wei [a,b,c], Xiang-Jun Zeng [a,**], Yun-Long Xia [d], Jie Du [a,b,c], Hui-Hua Li [a,b,c,*]

[a] Department of Physiology and Pathophysiology, Beijing Anzhen Hospital the Key Laboratory of Remodeling-Related Cardiovascular Diseases, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China
[b] Department of Cardiology, Chaoyang Hospital, Capital Medical University, Beijing 100069, China
[c] Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing 100029, China
[d] Department of Cardiology, Institute of Cardiovascular Diseases, First Affiliated Hospital of Dalian Medical University, Dalian 116011, China

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 13 April 2015
Accepted 14 April 2015
Available online 17 April 2015

Keywords:
Cardiotoxicity
Doxorubicin
Apoptosis
Ubiquitin-proteasome system
Immunoproteasome
Microarray

ABSTRACT

The most well-known cause of chemotherapy-induced cardiotoxicity is doxorubicin (DOX). The ubiquitin-proteasome system (UPS) is the main cellular machinery for protein degradation in eukaryotic cells. However, the expression pattern of the UPS in DOX-induced cardiotoxicity remains unclear. C57BL/6 mice were intraperitoneally injected with a single dose of DOX (15 mg/kg). After 1, 3 and 5 days, cardiac function and apoptosis were detected with echocardiography and TUNEL assay. Microarray assay and qPCR analysis were also performed at day 5. We found that DOX caused a significant decrease in cardiac function at day 5 and increase in cardiomyocyte apoptosis at days 3 and 5. Microarray data revealed that totally 1185 genes were significantly regulated in DOX-treated heart, and genes involved in apoptosis and the UPS were mostly altered. Among them, the expression of 3 immunoproteasome catalytic subunits (β1i, β2i and β5i) was markedly down-regulated. Moreover, DOX significantly decreased proteasome activities and enhanced polyubiquitinated proteins in the heart. Importantly, overexpression of immunoproteasome catalytic subunits (β1i, β2i or β5i) significantly attenuated DOX-induced cardiomyocyte apoptosis and other UPS gene expression while knockdown of them significantly increased DOX-induced cardiomyocyte apoptosis. These effects were partially associated with increased degradation of multiple pro-apoptotic proteins. In conclusion, our studies suggest that immunoproteasome plays an important role in DOX-induced cardiomyocyte apoptosis, and may be a novel therapeutic target for prevention of DOX-induced cardiotoxicity.

1. Introduction

DOX is one of the most effective anticancer agents of a variety of solid tumors but its use is limited primarily due to cardiotoxicity (Carvalho et al., 2014; Chatterjee et al., 2010; Octavia et al., 2012; Ranek and Wang, 2009). DOX-induced cardiotoxicity may be acute or chronic. The incidence of acute cardiotoxicity is much higher than chronic cardiotoxicity, which are approximately 11% and 1.7%, respectively. They develop dilated cardiomyopathy and congestive heart failure eventually (Carvalho et al., 2014; Chatterjee et al., 2010). Emerging evidence demonstrates multiple mechanisms for cardiotoxic effects of DOX which include the generation of ROS, membrane lipid peroxidation, mitochondrial dysfunction, decreased activity of Na^+–K^+ ATPase, intracellular calcium dysregulation and these cellular changes eventually lead to myocardial apoptosis and cardiac dysfunction (Carvalho et al., 2014; Chatterjee et al., 2010; Octavia et al., 2012). Recently, increased UPS activity has been reported to be associated with DOX-induced cardiotoxicity (Ranek and Wang, 2009; Shi et al., 2011). While Sishi et al. demonstrated that DOX decreased chymotrypsin-like activity and increased protein ubiquitination in the heart (Sishi et al., 2013).

Abbreviations: DOX, doxorubicin; ROS, reactive oxygen species; UPS, ubiquitin-proteasome system; E1s, ubiquitin activating enzymes; E2s, ubiquitin conjugating enzymes; E3s, ubiquitin ligases; DUBs, deubiquitinases.

* Corresponding author at: Department of Physiology and Pathophysiology, Capital Medical University, No 10 Xitoutiao, You An Men, Beijing 100069, China. Tel.: +861083950091; fax:+861083950091
** Corresponding author at: Department of Physiology and Pathophysiology, Beijing Anzhen Hospital the Key Laboratory of Remodeling-Related Cardiovascular Diseases, Department of Cardiology, Beijing Chaoyang Hospital, Capital Medical University, No.10 Xitoutiao, You An Men, Beijing 100069, China. Tel.:+861083950090; fax: +86 10 83950091.
E-mail addresses: megan_zeng@163.com (X.-J. Zeng), hhli1995@yahoo.com (H.-H. Li).

http://dx.doi.org/10.1016/j.tox.2015.04.009
0300-483X/

The UPS plays a key role in mediating the degradation of intracellular proteins (Wang and Robbins, 2014). UPS-mediated proteolysis includes 2 major steps: ubiquitination and proteasome-mediated degradation. Ubiquitination is a series of enzymatic reactions involving the E1s, E2s and E3s (Powell et al., 2012; Wang and Robbins, 2014). DUBs hydrolyze ubiquitin chains to make ubiquitin molecular to proceed to next ubiquitination process (Wang and Robbins, 2014). Finally, the polyubiquitinated proteins are rapidly degraded by the 26S proteasome. Dysfunction of the UPS has been implicated in numerous cardiovascular diseases, such as atherosclerosis, myocardial ischemia, hypertrophy and heart failure (Li and Wang, 2011; Powell et al., 2012). And it has been revealed that UPS is correlated with cardiomyocyte apoptosis by degradation of pro-survival transcriptional factors (Ranek and Wang, 2009).

The 26S proteasome consists of a 20S core particle and two regulatory particles (Tian et al., 2012). The 20S core harbors the three constitutive catalytic subunits β1, β2 and β5 in the inner β-rings (Angeles et al., 2012; Tian et al., 2012). However, in response to inflammatory cytokines such as interferon-γ (IFN-γ), the constitutively expressed catalytic β-subunits (β1, β2 and β5) are replaced by inducible β-counterparts known as immunosubunits (Angeles et al., 2012; Ferrington and Gregerson, 2012). These immunosubunits, including β1i (also known as LMP2 or PSMB9), β2i (also known as MECL-1 or PSMB10) and β5i (also known as LMP7 or PSMB8), are preferentially incorporated during proteasome assemble to form the immunoproteasome (Angeles et al., 2012; Ferrington and Gregerson, 2012). Now it is known that immunoproteasome-mediated proteolysis has emerged as a critical molecular mechanism for regulating both the innate and adaptive immune responses, protecting against oxidative stress and maintaining cellular protein homeostasis (Angeles et al., 2012). But it is still unclear which components of UPS play a key role in DOX-induced cardiotoxicity.

Therefore, the present study aimed to search for the altered components of the UPS and to determine the role of immunoproteasome expression in DOX-induced cardiotoxocity.

2. Materials and methods

2.1. Antibodies and reagents

Anti-ubiquitin antibody was purchased from Millipore (Temecula, CA, USA). Anti-β1, β5, α-actinin and β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-β2, β1i, β5i and PA28α antibodies were from Abcam (Cambridge, UK). Anti-β2i antibody was purchased from Enzo Life Science (Farmingdale, NY, USA). Anti-Bcl-2 and Bax Horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG secondary antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). Apoptosis detection kit (DeadEnd™ Fluorometric TUNEL System) was purchased from Promega (Madison, WI, USA). Fluorescent substrates Suc-LLVY-AMC, Z-LLE-AMC and Ac-RLR-AMC, proteasome inhibitor epoxomicin and MG132 were purchased from Boston Biochem (Cambridge, MA, USA). Ac-DEVD-AFC was acquired from Biomol (Plymouth Meeting, PA, USA). The DMEM/F12 culture medium and fetal bovine serum were obtained from Hyclone (Thermo; USA). Doxorubicin and other reagents were purchased from Sigma–Aldrich (Louis, MO, USA).

2.2. Animals and treatment

Male 8-week-old C57BL/6 mice were administered intraperitoneally with a single dose of 15 mg/kg body weight of DOX. In control mice, the same volume of saline was injected (Li et al., 2006; Zhu et al., 2011). All mice underwent echocardiography measurements at days 1, 3 and 5. Then the mice were anesthetized by an overdose of pentobarbital (100 mg/kg, i.p.) and heart tissues were quickly harvested and prepared for further histological and molecular analysis. This study was approved by the Institutional Animal Care and Use Committee of Capital Medical University and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.3. Cardiac echocardiography detection

The hair of left chest wall was removed by depilatory cream. The mouse was placed in the mouse anesthesia induction chamber to induce anesthesia (the oxygen level: 1 L/min; the concentration of isoflurane: 2.5%). And then masked and reset the concentration of isoflurane to 1–1.5% to maintain the anesthesia. The mouse paws were placed on the ECG electrode contact pads with dermatological tape. Echocardiograms were obtained by transthoracic echocardiograpy using metal ECG connector. We measured the left ventricular anterior wall thickness (LVAW), the left ventricular posterior wall thickness (LVPW) and the left ventricular internal diameter in systole (s) and diastole (d), respectively. And then we calculated ejection fraction (EF) and fractional shortening (FS) as the following formula: FS% = (LVID;d-LVID;s)/LVID;d × 100%; LV Vol;d = [7.0/(2.4 + LVID;d)] × LVID;d^3 × 1000; LV Vol;s = [7.0/(2.4 + LVID;s)] × LVID;s^3 × 1000; EF% = (LV Vol;d-LV Vol;s)/LV vol;d × 100%.

2.4. Primary cardiomyocyte culture and adenovirus infection

Recombinant adenovirus expressing GFP alone (Ad-GFP), β1i and GFP (Ad-β1i), β2i and GFP (Ad-β2i), β5i and GFP (Ad-β5i) were constructed using the AdMax adenoviral expression system by Genechem. The RNA interference negative control adenovirus (Ad-shC) expressed scrambled shRNA: TTCTCCGAACGTGTCACGT. The RNA interference shRNA sequence of β1i, β2i, β5i was respectively ACCATCATGGCTGTGGAAT (Ad-shβ1i), GGCTTCTCTTTCGAGAACT (Ad-shβ2i) and GGAATGCAGGCTATACTAT (Ad-shβ5i). Recombinant adenovirus expressing shRNA was generated using the AdMax adenoviral expression system. Neonatal rat cardiomyocytes (NRCMs) were isolated by enzymatic disassociating hearts of 1–3-day-old Sprague-Dawley (SD) rats as described (Liu et al., 2014). NRCMs were infected with overexpression adenovirus or RNA interference adenovirus 24 h and then stimulated by DOX (0.5, 1, 5 μM) for additional 24 h (Zhang et al., 2011). NRCMs were subjected to TUNEL assay and lysed by western blot analysis after DOX treatment.

2.5. TUNEL assay

TUNEL assay was performed with the DeadEnd™ Fluorometric TUNEL System according to manufacturer's instructions. The samples were counterstained with DAPI as described (Liu et al., 2014; Wang et al., 2013). The apoptotic nuclei and the total nuclei were counted at a magnification of 100× (Olympus BX-63, Japan). The number of TUNEL-positive nuclei was calculated using Image J software.

2.6. Caspase-3 activity assay

Caspase-3 activity was measured as described (Wang et al., 2013). Briefly, cells were homogenized in ice-cold lysis buffer. Samples were then incubated with assay buffer containing Ac-DEVD-AFC (50 μM) at 37 °C for 1.5 h. Activity of caspase-3 was determined using a PerkinElmer 2030 Multilabel Microplate Reader at 405 nm.