Journal of Cardiology 62 (2013) 110–116

Contents lists available at SciVerse ScienceDirect

Journal of Cardiology

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

Original article

The beneficial effects of tadalafil on left ventricular dysfunction in doxorubicin-induced cardiomyopathy

Zhe Jin (MD)[a,1], Jian Zhang (MD)[a,1], Huilan Zhi (MD)[b], Bingzhe Hong (MD)[c], Shuying Zhang (MD)[a], Huishu Guo (MD)[d], Longhu Li (MD)[a,*]

[a] Department of Cardiology, The Affiliated Zhongshan Hospital of Dalian University, Dalian, China
[b] Department of Pathology, Youyi Hospital of Dalian, Dalian, China
[c] Department of Cardiology, The Affiliated Xinhua Hospital of Dalian University, Dalian, China
[d] Centralab, The First Affiliated Hospital of Dalian Medical University, Dalian, China

ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 14 March 2013
Accepted 21 March 2013
Available online 31 May 2013

Keywords:
Doxorubicin
Cardiomyopathy
Tadalafil
Cardiomyocyte atrophy
Myocardial fibrosis

ABSTRACT

Background: It is not clear yet how tadalafil affects nonischemic cardiomyopathy, although its beneficial effects on acute myocardial infarction are well-known. We investigated tadalafil's beneficial effects on nonischemic cardiomyopathy and the specific mechanisms of its effects.
Methods: Cardiomyopathy was induced in mice by a single intraperitoneal injection of doxorubicin (15 mg/kg). In some cases, tadalafil (4 mg/kg/day, p.o., 14 days) was started simultaneously. After two weeks, cardiac function was evaluated by echocardiography and cardiac catheterization, then all of the mice were killed and cardiac specimens were subjected for hemotoxylin and eosin staining, Masson's trichrome staining, terminal deoxynucleotidyltransferase dUTP nick-end labeling assay, enzyme-linked immunosorbent assay, and Western blot.
Results: Two weeks later, left ventricular dilatation and dysfunction were apparent in mice given doxorubicin but were significantly attenuated by tadalafil treatment. Tadalafil also protected hearts against doxorubicin-induced cardiomyocyte atrophy/degeneration and myocardial fibrosis. No doxorubicin-induced apoptotic effects were seen between groups. Cardiac cGMP level was lower in the doxorubicin-treated group, however it was significantly increased with tadalafil treatment. Compared to the control group, the myocardial expression of 3 sarcomeric proteins, myosin heavy chain, troponin I, and desmin were significantly decreased in the doxorubicin-treated group, which were restored by the tadalafil treatment.
Conclusions: The present study indicates a protective effect of tadalafil mainly through cGMP signaling pathway against doxorubicin-induced nonischemic cardiomyopathy.

Introduction

The antineoplastic drug doxorubicin (DOX) is widely used in the therapy of various malignant tumors including leukemia, lymphomas, and solid tumors such as ovarian, breast, lung, cervical, and uterine cancers [1]. However, its clinical use is limited by its adverse side effects: irreversible degenerative cardiomyopathy and congestive heart failure [2,3]. The DOX-induced acute cardiotoxicity is characterized by hypotension, arrhythmia, and tachycardia while the chronic effects are manifested as cardiac dysfunction eventually leading to congestive heart failure [4,5]. The development of cumulative dose-dependent cardiomyopathy may occur many years after the cessation of DOX treatment [6,7]. Despite various therapeutic interventions adopted to protect the heart against DOX-induced cardiotoxicity, the deterioration in cardiac functions is often accompanied by high mortality rates. So far, the ability of these treatments to protect the heart from DOX-induced damage has been limited. Hence, there is an ongoing need to further investigate and develop efficient therapeutic agents to combat DOX-induced cardiac damage.

Tadalafil (Tad) is a potent long-acting selective inhibitor of cGMP-specific phosphodiesterase-5 (PDE5), which hydrolyzes and eliminates cGMP in cells [8]. A previous study has shown that the long-acting PDE5 inhibitor, Tad, induces sustained cardioprotection against lethal ischemic injury [9]. Several studies suggest that PDE5 inhibitors induce powerful beneficial effects for pulmonary arterial hypertension and ischemia/reperfusion injury [10,11]. Our most recent study suggests that prolonged inhibition of PDE5 through short hairpin RNA interference would relieve cardiac remodeling

* Corresponding author at: Department of Cardiology, The Affiliated Zhongshan Hospital of Dalian University, 6 Jiefang Road, Zhongshan, Dalian 116001, China. Tel.: +86 411 62893078; fax: +86 411 62893555.
E-mail address: lilonghu75@hotmail.com (L. Li).
[1] These authors contributed equally to this work.

0914-5087/$ – see front matter © 2013 Japanese College of Cardiology. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jjcc.2013.03.018

and dysfunction following myocardial infarction [12]. There has been only one study reporting benefits of Tad on nonischemic cardiomyopathy [13], thus little is known on the mechanisms for Tad to exert such effects. It would be worth confirming the beneficial effects of Tad on DOX-induced cardiomyopathy. In the present study, we examined the effects of Tad on DOX-induced cardiomyopathy and investigated the specific mechanisms of its effects.

Materials and methods

This study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society. Cardiomyopathy was induced in male 10-week-old C57BL/6J mice (Dalian Medical University, Dalian, China) by a single intraperitoneal injection of DOX (Sigma, St. Louis, MO, USA) at a dose of 15 mg/kg, which has been reported to be cardiotoxic [14]. The presence of DOX-induced cardiomyopathy was confirmed both functionally and histologically in all mice that were given no therapeutic intervention by the observation of decreased left ventricular (LV) function (by echocardiography and cardiac catheterization). In sham-treated mice, the same volume of saline was injected in a similar manner. Tad (Lilly, Indianapolis, IN, USA) tablets were crushed in water and given to animals orally via gauge needle at a dose of 4 mg/kg/day for successive 14 days. This dose was chosen based on the interspecies dose extrapolation scaling to result in plasma concentrations equivalent to a human dose of 20 mg/day [15]. Untreated control groups were given the same volume of saline.

Mice were randomly assigned to receive (a) saline alone (Con group, $n = 10$); (b) DOX alone (DOX group, $n = 16$); (c) DOX plus Tad (DOX + Tad group, $n = 16$), or (d) Tad alone (Tad group, $n = 10$). Two weeks later, all surviving mice were killed with an overdose of pentobarbital after physiological examination. The hearts were excised and weighed, cardiac specimens were then subjected to histological, immunohistochemical, and molecular biological analyses.

Physiological studies

Animals were anesthetized via intraperitoneal injection with pentobarbital. Echocardiograms were then recorded with an echocardiographic system (Aloka) equipped with a 7.5-MHz imaging transducer as reported previously [16]. LV end-systolic diameter (LVDs) and end-diastolic diameter (LVDd) were measured from at least three consecutive cardiac cycles. After cardiac echocardiography, the right carotid artery was cannulated with a micromanometer-tipped catheter (SPR 407; Millar Instruments, Houston, TX, USA) and advanced into the aorta and then into the LV to record pressure and $\pm dP/dt$.

Histological analysis

After the echocardiography, each heart was removed and cut into 2 transverse slices. One was fixed in 10% buffered formalin and embedded in paraffin, after which 4-μm-thick sections were stained with hematoxylin–eosin (HE) or Masson. Quantitative assessments, including cell size and fibrotic area, were performed with a multipurpose color image processor (Image Pro Plus) with 20 randomly chosen high-power fields in each heart.

Enzyme-linked immunoassay

Levels of cGMP in the myocardium were assayed with an enzyme-linked immunosorbent assay (ELISA) (CycLex, Nagano, Japan). Three hearts from each group were used for this assay.

In situ nick end-labeling

Terminal dUTP nick end-labeling (TUNEL) assays were performed in sections with an ApopTag kit (Intergene, Purchase, NY, USA) mainly according to the instructions of the supplier. Mouse mammary tissue served as a positive control.

Western blotting

Lysates/proteins from heart tissues were used for Western blot analysis. Proteins were separated and transferred to membranes by standard protocols, after which they were probed with antibodies against myosin heavy chain (MHC), troponin I (both from Santa Cruz, Dallas, TX, USA), desmin (Sigma). Three to five hearts from each group were subjected to the blotting. The blots were visualized by means of chemiluminescence (ECL, Thermo, Rockford, IL, USA), and the signals were quantified by densitometry. a-Tubulin (analyzed with an antibody from Thermo) served as the loading control.

Statistical analysis

Statistical analysis was performed by using software of SPSS17.0 (Cary, NC, USA). Values are shown as mean ± SEM. The significance of differences between groups was evaluated with 1-way ANOVA followed by the Newman–Keuls multiple comparison test. Values of $p < 0.05$ were considered significant.

Results

Survival rates and heart-to-body weight ratios

Two weeks later, we evaluated survival rates and heart-to-body weight ratios. Three of DOX and two of DOX + Tad mice were dead, no mouse was dead in the Con or Tad groups. Survival rates of the group treated with DOX + Tad (87.5%) exhibited increasing tendency compared with the DOX group (81.25%) during the 14-day experimental protocol, although this change was insignificant. The heart-to-body weight ratio was significantly increased in the DOX group, which was reversed by Tad (Table 1).

Physiological studies

The results of our physiological studies are summarized in Fig. 1. Echocardiography and cardiac catheterization performed 2 weeks after DOX administration showed that mice receiving DOX alone had significant cardiac functional deterioration characterized by enlargement of the LV cavity and signs of decreased cardiac function, i.e. increased LV diameter and end-diastolic pressure and decreased LV ejection fraction and $\pm dP/dt$, compared with sham animals. Treatment with Tad significantly mitigated the DOX-induced impairment of cardiac function. Administration of Tad to sham animals had no effect on cardiac function.

Table 1
Heart-to-body weight ratios among the groups.

Groups	*n*	Heart weights (g)	Body weights (g)	Heart-to-body weight ratios (%)
Con	10	0.098 ± 0.08	26.42 ± 0.91	0.37 ± 0.019
DOX	13	0.089 ± 0.13	21.26 ± 1.15	0.42 ± 0.037*
DOX + Tad	14	0.092 ± 0.13	25.33 ± 1.02	0.36 ± 0.046#
Tad	10	0.092 ± 0.11	26.69 ± 1.16	0.34 ± 0.038

* $p < 0.05$ versus Con group.
\# $p < 0.05$ versus DOX group.
Con, control; DOX, doxorubicin; Tad, tadalafil.