Redox Biology 16 (2018) 189–198

Contents lists available at ScienceDirect

Redox Biology

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

Research paper

Protective effect of dioscin against doxorubicin-induced cardiotoxicity via adjusting microRNA-140-5p-mediated myocardial oxidative stress

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ARTICLE INFO

Keywords:
Cardiotoxicity
Dioscin
Doxorubicin
MiR-140-5p
Oxidative stress

ABSTRACT

Clinical application of doxorubicin (DOX) is limited because of its cardiotoxicity. Thus, exploration of effective lead compounds against DOX-induced cardiotoxicity is necessary. The aim of the present study was to investigate the effects and possible mechanisms of dioscin against DOX-induced cardiotoxicity. The *in vitro* model of DOX-treated H9C2 cells and the *in vivo* models of DOX-treated rats and mice were used in this study. The results showed that discoin markedly increased H9C2 cell viability, decreased the levels of CK, LDH, and improved histopathological and electrocardio- gram changes in rats and mice to protect DOX-induced cardiotoxicity. Furthermore, dioscin significantly inhibited myocardial oxidative insult through adjusting the levels of intracellular ROS, MDA, SOD, GSH and GSH-Px *in vitro* and *in vivo*. Our data also indicated that dioscin activated Nrf2 and Sirt2 signaling pathways, and thereby affected the expression levels of HO-1, NQO1, Gst, GCLM, Keap1 and FOXO3a through decreasing miR-140-5p expression level. In addition, the level of intracellular ROS was significantly increased in H9C2 cells treated by DOX after miR-140-5p mimic transfection, as well as the down-regulated expression levels of Nrf2 and Sirt2, which were markedly reversed by dioscin. In conclusion, our data suggested that dioscin alleviated DOX-induced cardiotoxicity through modulating miR-140-5p-mediated myocardial oxidative stress. This natural product should be developed as a new candidate to alleviate cardiotoxicity caused by DOX in the future.

1. Introduction

Doxorubicin (DOX), an efficient chemotherapeutic drug, is widely used in clinical for cancer treatment [1,2]. Unfortunately, this drug can cause acute and chronic cardiotoxicity including tachycardia, arrhythmia, hypotension, transient depression of left ventricular function, and even refractory late-onset cardiomyopathy [3–5]. Moreover, DOX-induced cardiotoxicity will be aggravated in patients with the increased dose of DOX [6]. The incidence of heart failure will increase to 48% when the accumulation dose of DOX climbs to 700 mg/m^2 [7]. Because of the side effects, the application of DOX is limited despite its potent and effective functions.

In recent years, a large number of researches have indicated that DOX-induced myocardial injury involves in multiple biological processes including oxidative stress, lipid peroxidation, DNA damage, mitochondrial injury, apoptosis and autophagy [8,9]. Among them, oxidative stress is a key process in DOX-induced myocardial damage. Briefly, DOX produces massive superoxide anion free radicals (·O2) and reactive oxygen species (ROS), and then induces mitochondrial dysfunction and cell injury [10,11]. Therefore, inhibiting oxidative stress may be an effective prevention and treatment method against DOX-induced cardiotoxicity.

MicroRNA (miRNA) is a single-stranded non-coding RNA, which can be used as potential drug targets to treat human diseases [12–15]. Previous studies showed that miR-140-5p has the biological activities including regulating adipocyte differentiation, inhibiting cancer growth and modulating Alzheimer's disease [16–18]. Furthermore, our previous work has also proved that miR-140-5p plays an important role in DOX- induced cardiotoxicity by inducing myocardial oxidative stress via targeting nuclear erythroid factor 2-related factor 2 (Nrf2) and

Abbreviations: AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; CK, creatine kinase; DAPI, 4',6'-Diamidino-2-phenylindole; DOX, doxorubicin; ECG, electrocardiograms; FOXO3a, Forkhead box O3; FXR, farnesoid X receptor; GCLM, glutamatecysteine ligase modifier subunit; GSH, glutathione; GSH-Px, glutathione peroxidase; Gst, glutathione-S-transferase; H&E, hematoxylin-eosin; HO-1, heme oxygenase-1; Keap1, kelch like ECH- associated protein 1; LDH, lactate dehydrogenase; MDA, malondialdehyde; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5 -diphenyl tetrazolium bromide; NQO1, NAD(P)H Quinone Dehydrogenase 1; Nrf2, nuclear erythroid factor 2-related factor 2; ROS, reactive oxygen species; RT-PCR, reverse transcription polymerase chain reaction; Sirt2, silent information regulator factor 2-related enzyme 2; SOD, superoxide dismutase

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https://doi.org/10.1016/j.redox.2018.02.026
Received 31 January 2018; Received in revised form 24 February 2018; Accepted 26 February 2018
Available online 06 March 2018

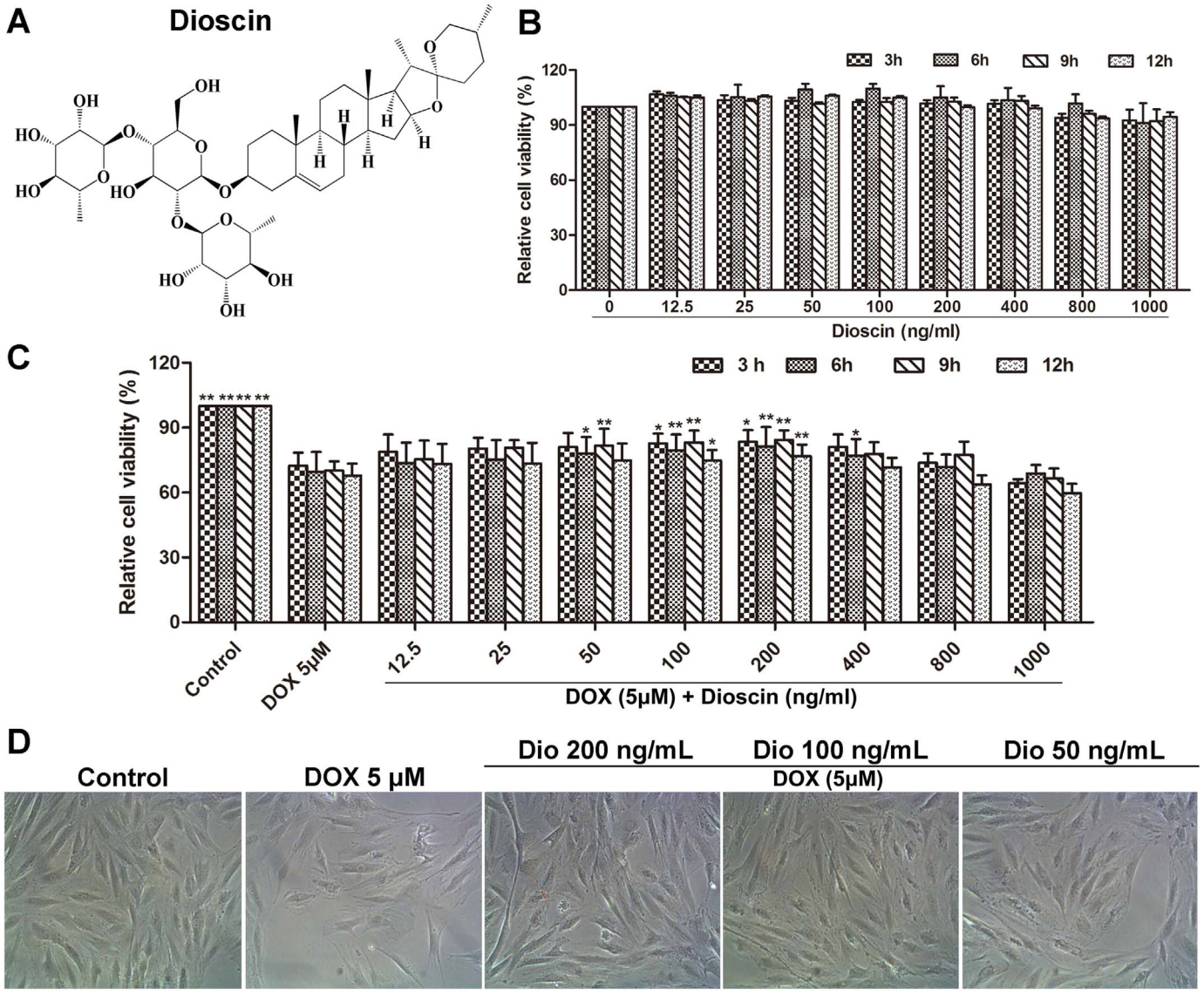

Fig. 1. Dioscin protected H9C2 cells against DOX-induced injury (A) Chemical structure of dioscin. (B) Cytotoxicity of dioscin on H9C2 cells. (C) Effects of dioscin on the cell viability of H9C2 cells induced by DOX. (D) Effects of dioscin (50, 100 and 200 ng/mL) for 12 h pretreatment on the cellular morphology of H9C2 cells by bright image (×100 magnification). Data are presented as the mean ± SD (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with DOX group.

silent information regulator factor 2-related enzyme 2 (Sirt2) [19]. In brief, Nrf2 is anchored in the cytoplasm where it binds to kelch like ECH-associated protein 1 (Keap1) under normal circumstances [20]. However, Nrf2 translocates into the nucleus and then activates its target genes through an antioxidant-response element (ARE) when Sirt proteins trigger the separation of Nrf2 and Keap1 [21]. Nrf2 can negatively regulate the dissociation and polymerization with Keap1, therefore adjust the expression levels of some anti- oxidative genes and enzymes against oxidative stress [22]. Sirt2 can regulate oxidative stress through increasing forkhead box O3 (FOXO3a) and superoxide dismutase (SOD), and then decrease ROS levels [23]. Thus, miR-140-5p inhibitor may be a potential candidate for the treatment of DOX-induced cardiotoxicity.

Dioscin (shown in Fig. 1A), a natural steroid saponin, is isolated from various herbs [24]. Pharmacological investigations have shown that dioscin has anti-tumor, anti-fungal and anti-hyperlipidemic activities [25–27]. A large number of previous studies have suggested that dioscin has potent effects against liver fibrosis, renal injury and cerebral ischemia/reperfusion injury [28–32]. Importantly, previous study has also indicated that dioscin can prevent mitochondrial apoptosis and attenuate oxidative stress in cardiac H9c2 cells [33]. However, no studies have reported the effects and molecular mechanisms of dioscin against DOX-induced cardiotoxicity in our best knowledge.

Therefore, the aim of the present work was to investigate the protective effect of dioscin against DOX-induced cardiotoxicity, and test whether this action was through adjusting miRNA-140-5p-mediated myocardial oxidative stress

2. Materials and methods

2.1. Chemicals and materials

DOX was obtained from Sigma (Santa Clara, CA, USA). Dioscin was obtained from Shanghai Tauto Biochemical Technology Co., Ltd. (Shanghai, China), which was dissolved in 0.5% carboxymethylcellulose sodium (CMC-Na) for *in vivo* experiments and in 0.1% dimethylsulfoxide (DMSO) for *in vitro* tests. Tissue Protein Extraction kit, and Nuclear and Cytoplasmic Protein Extraction kit were all purchased from KEYGEN Biotech. Co., Ltd. (Nanjing, China). ROS assay kit, bicinchoninic acid (BCA) protein assay kit and cell lysis buffer kit were