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Doxorubicin toxicity changes myocardial energy metabolism in rats

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ABSTRACT

Background: Doxorubicin (DOX) is an antitumor antibiotics used against malignancies. But its toxicity limits the therapy of DOX.
Objective: The purpose of this study was to evaluate DOX toxicity and the alteration of energy metabolism after short term and long term treatment.
Methods: Male Sprague–Dawley rats were randomly assigned to four groups: Short term control group, short term DOX treatment group, long term control group and long term DOX treatment group. In short term treated group, rats were injected with DOX i.p. at a dose of 2.5 mg/kg every 48 h for six equal injections. In long term, treated group, rats were tail-intravenously injected with DOX at a dose of 3 mg/kg once a week for four weeks. At the end of the experiment, histopathological changes, general blood biomarkers, endogenous antioxidant enzymes, cardiac energy metabolism and related mRNA expression of AMPK signal pathway were determined.
Results: DOX induced prominent oxidative stress, a higher mortality rate, histological and ECG changes, obvious cardiac hypertrophy, acute cardiac damage and cardiac energy impairment in short term treatment rats. In long term treatment rats, DOX caused serious nephropathy and systolic dysfunction, terrible cardiac energy impairment, clear alteration of substrate utilization and AMPK signal pathway.
Conclusion: DOX treatment can induce different damages after short term and long term treatment. In short term treatment group, rats experienced a terrible mortality rate about 40%, the acute cardiac damage, cardiac energy impairment and an early heart failure which are potential connected with reduction of glucose utilization. In the long term treatment group, serious nephropathy and obvious changes of mRNA expressions of AMPK signal pathway were observed. Meanwhile, the serious cardiac energy impairment and substrate utilization alteration denote an obviously heart failure. This study could be helpful to develop therapy strategies of DOX complications for clinical application.

1. Introduction

Doxorubicin (DOX), a drug of the anthracycline group, is one of the most extensively used chemotherapeutic drugs for different types of human tumors [1,2]. However, the DOX therapy has been limited by its complications, such as hypersensitivity reactions, liver and kidney dysfunction, severe cardiotoxicity [3–5]. Cardiotoxicity, involving acute and chronic, is the most dangerous complication of DOX. This cumulative cardiotoxicity will eventually lead to irreversible heart failure. The mechanisms of action of DOX cardiotoxicity include oxidative stress and nonoxidative damages (myofibrillar deterioration, intracellular calcium dysregulation, myocardial energy impairment and DNA degradation). It can even occur decades after therapy with a mortality rate about 20% [6]. To

Abbreviations: DOX, Doxorubicin; OS, oxidative stress; AMPK, AMP-activated protein kinase; BW, body weight; HWI, heart weight index; LVWI, left ventricular weight index; TG, triglyceride; TP, total protein; BUN, blood urea nitrogen; CRE, creatinine; CHO, cholesterol; ALB, albumin; GLO, globulin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; BNP, brain natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; ATPase, adenosine triphosphatase; PCr, Phosphocreatine; ATP, adenosine-triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; TAN, total adenine nucleotides; AMPKα2, AMP-activated protein kinase catalytic subunit alpha-2; PPARα, peroxisome proliferator-activated receptor alpha; PCG-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

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investigate cardiotoxicity of DOX, various animal models have been used, for example, with rats [7], dogs [8], monkeys [9], rabbits [10] and mice [11]. These studies demonstrate that toxic effect of DOX is a multifactorial process, which is mainly including oxidative stress (OS) and impairment of energy metabolism [12].

Myocardial energy metabolism relates to mitochondrial respiratory chain, oxidative phosphorylation, myocardial substrate utilization, high-energy phosphate storage and transfer and energy signal pathway [12,13]. Heart consumes large amounts of adenosine triphosphate (ATP) to sustain cardiac systolic function. Hence, energy metabolism is vital to cardiac function. Several studies show that DOX can impair most processes of myocardial energy metabolism, including oxidative phosphorylation, mitochondrial respiratory chain and AMP-activated protein kinase (AMPK) signal pathway [7,12–14]. Nicolay et al. have evaluated the alterations of myocardial energy metabolism in DOX-treated rats for one week [7]. But, the differences of the alterations in rats between a shorter term and a longer term DOX-treatment are still not quite clear.

In this paper, we chose a short term treated rat model (three weeks) and a long term treated rat model (up to six weeks) to evaluate DOX toxicity and the alterations of myocardial energy metabolism. Measurements of cardiac weight indexes, histological changes, general blood biomarkers, brain natriuretic peptide (BNP), endogenous antioxidant enzymes, adenosine triphosphatase (ATPase), adenine nucleotides and related mRNA expressions of AMPK signal pathway were performed. This study may be helpful in investigating the adverse events and its molecular bases during DOX therapy in different duration and providing basises for animal experimental studies (DOX cardiomyopathy models).

2. Materials and methods

2.1. Materials

Doxorubicin Hydrochloride was purchased from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China).

2.2. Animals and experimental design

Male Sprague–Dawley rats (roughly 200 g, age: 4 weeks) were randomly assigned to four groups. Each short term group contains 15 animals, each short term group contains 11 animals.

1. Short term control group (S-cont): rats were intraperitoneally (i.p.) injected with normal saline (the solvent for DOX).
2. Short term DOX treatment group (S-DOX): rats were injected with DOX i.p. at a dose of 2.5 mg/kg every 48 h for six equal injections to achieve an accumulative dose of 15 mg/kg. This total dose is according to Xiao et al. [15].
3. Long term control group (L-cont): rats were tail-intravenously injected with normal saline (the solvent for DOX).
4. Long term DOX treatment group (L-DOX): rats received a tail-intravenous injection of DOX (3 mg/kg) once a week for four weeks to achieve an accumulative dose of 12 mg/kg. It is similar to the research of Chang et al. [16].

All rats used in this study were handled in compliance with the guideline for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Animal Care and Use Committee of the Shanghai University of Traditional Chinese Medicine. The accumulative doses of DOX (15 mg/kg and 12 mg/kg; 487 mg/m^2 and 389 mg/m^2) are above the threshold dose of clinical DOX cardiomyopathy (630 mg for a 70 kg human; 370 mg/m^2) [15]. Rats in short term and long term treatment groups were euthanized 21 days and 42 days after the first injection respectively. Mortality of rats was recorded. Blood samples of short term treated rats were collected 24 h after the last injection and centrifuged (4 °C, 2325 g, 10 min) to recover the serum for measuring general blood biomarkers. At the end of the experiment, rats were sacrificed under anesthesia (urethane, 1 g/kg), and serum and heart tissues were collected after recording the body weight (BW), elctrocardiography (ECG) and blood pressure. The heart was excised and the left ventricle was separated. Heart weight (HW) and left ventricle weight (LVW) were measured. The upper part of left ventricular was fixed in 10% buffered neutral formalin solution, and the lower part and serum were stored at −80 °C.

2.3. Assessment of general blood biomarkers

The general biomarkers of serum of short term treated rats and long term treated rats were measured respectively by the Hitachi 7080 biochemical analyzer (Tokyo, Japan), including triglyceride (TG), total protein (TP), blood urea nitrogen (BUN), creatinine (CRE), cholesterol (CHO), albumin (ALB), globulin (GLO), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH). These kits were purchased from Shino-Test Co., Ltd. (Tokyo, Japan).

2.4. ECG and blood pressure record

At the end of the experiment, blood pressure and ECG (position II) of rats were recorded after anesthesia with multi-channel biological signal analysis system (RM6240C, Chengdu Instrument factory). Heart Rate, Sα–T segment and QRS complex segment were calculated for evaluating DOX cardiotoxicity [17].

2.5. Histopathological analysis and cardiac weight indexes

The fixed part of left ventricle was dehydrated in ethanol (70–100%), then cleared in xylene, and embedded in paraffin. Five-mm thick slices were prepared and stained with hematoxylin-eosin (H&E). Heart weight index (HWI, mg/g) and left ventricular weight index (LVWI, mg/g) were calculated by the ratios of HW to BW and LVW to BW respectively.

2.6. Measurement of serum BNP

The serum BNP (μg/L) content was measured with ELISA method. The kit was purchased from Shanghai Jianglai Bioengineering Technology Co., Ltd. (Shanghai, China).

2.7. Estimation of malondialdehyde (MDA) level and the activities of antioxidant enzymes in serum

Serum MDA content, activities of superoxide dismutase (SOD, U/ml) and glutathione peroxidase (GSH-Px, U) were determined using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.8. Measurement of the activities of myocardial mitochondrial ATPases

Mitochondria of cardiac tissues were recovered with differential centrifugation [18]. Activities of mitochondrial ATPases (Na^+K^+-ATPase and $Ca^{++}Mg^{++}$-ATPase) were assayed with kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).