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Research Article

Inhibitory Effect of Doxycycline on MMP2/9 and its Improvement in Cardiac Function and Left Ventricular Remodeling After Myocardial Infarction in Rats

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Abstract

The objective of this research was to investigate the inhibitory effect of doxycycline (DOX) on MMP2/9 and the improvement in cardiac function and left ventricular remodeling (LVR) after myocardial infarction (MI) in rats. For this purpose, thirty-six rats were randomly divided into control (group A), model (group B) and experimental (group C) groups. A MI model was established in groups B and C by ligation of the left anterior descending (LAD) of the coronary artery, while the rats in group A underwent thoracotomy without ligation only. The rats in groups A and B were injected with normal saline, and the rats in group C were injected with DOX. Two weeks after the operation, the cardiac function of the rats was evaluated by color Doppler ultrasound, myocardial infarct size by Masson staining, collagen content and the ratio of type I/III collagen by immunohistochemistry and immunoblotting respectively and activities of MMP2/9 by gelatin enzyme method. The results revealed DOX to cause reduction in the degree of ventricular enlargement and cardiac wall thinning, reduce the collagen content, increase the ratio of type I/III collagen, decrease the activities of MMP2/9, and reduce myocardial destruction and remodeling in rats after MI. However, further clinical research is recommended for the evaluation of the effectiveness of DOX.

Keywords: Doxycycline, Left ventricular remodeling, Myocardial infarction, Matrix metalloproteinases

Introduction

Ventricular remodeling after myocardial infarction (MI) is an important pathogenesis of heart failure that results from neurohumoral and other factors [1]. The mortality of patients with MI has gradually decreased, but the incidence of cardiac dysfunction caused by left ventricular remodeling (LVR) after MI has significantly increased. Heart failure after MI is gradually becoming the main cause of death in patients with MI [2]. This is due to the reason that the treatments fail to specifically address the underlying pathophysiological mechanisms [3]. The activities of many kinds of matrix metalloproteinases (MMPs), especially MMP-9, in the myocardium increase after MI [4]. Increased MMPs promote the development of LVR after MI by regulating extracellular matrix metabolism, which in turn aggravates cardiac function damage in patients and model animals after MI [5]. The inhibition of MMP activity in the myocardium after MI may reduce LVR and improve cardiac function in patients with MI [6,7].

LVR after acute MI (AMI) is a common pathophysiological process of progressive development in the clinic and begins within a few hours after AMI. Uncoordinated elongation and thinning of the necrotic myocardium and eccentric hypertrophy of the non-infarcted area lead to ventricular wall dilation, ventricular enlargement and changes in ventricular chamber geometry in the infarcted area [8]. This process increases the ventricular volume and leads to the impairment of LV function, which is considered to be the most important factor in determining the survival and prognosis of patients [9]. Doxycycline (DOX) is a tetracycline antibiotic and a broad-spectrum inhibitor of matrix metalloproteinases that can reduce the expression of MMPs by binding to the active center of MMPs and inhibiting their transcription [10]. However, it is not clear whether DOX can inhibit myocardial matrix metalloproteinases-2 and-9 and improve cardiac function and LVR in rats after MI. Therefore, the purpose of this study was to explore the effects of DOX on cardiac function and LVR after MI in rats and the possible underlying mechanism.

Materials and Methods

Ethical Statement

This study was approved by the Xi 'an Daxing Hospital committee (Approval No: 20230911).

Experimental Animals

Thirty-six male SD rats weighing 260±10 g were purchased from the Experimental Animal Center of Anhui Medical University and raised in the laboratory of the Animal Center. The temperature ranged from 20-22°C, and the humidity ranged from 45-55%.

Experimental Instruments and Reagents

Small ventilators and high-speed centrifugal freezers were purchased from the Eppendorf Company of Germany, IE33 echocardiography machines were purchased from the Shanghai Ohaus Company, and a ZD-9556 horizontal decolorizing shaker, horizontal electrophoresis instrument and inverted microscope were purchased from the Jiangsu Guosheng Experimental Instrument Factory. DOX was purchased from Haikou Kangliyuan Pharmaceutical Co., Ltd. (national drug standard: H20060405), a Masson staining kit was purchased from Beijing Biotechnology Co., Ltd. (production batch number: ZC00126), and a GENMED matrix metalloproteinases and gelatin zymography electrophoresis analysis kit was purchased from Shanghai Jiemei Genome Pharmaceutical Technology Co., Ltd.

Establishment of a Rat Model of MI

Thirty-six rats were anesthetized by intraperitoneal injection of pentobarbital sodium (PB). An electrophysiological instrument was used to monitor the electrocardiogram, endotracheal intubation and ventilation. The respiratory rate was set at 45 beats per minute, and the tidal volume was 30 mL. After the thoracotomy test, the anterior descending branch was marked, and the LAD of the coronary artery was ligated 1 mm below the root of the left atrial appendage. The rats whose electrocardiogram showed characteristic STT changes of MI after the operation that lasted for more than half an hour were judged to be successful in establishing the MI model. The successful rats were randomly divided into model (group B) (n = 12) and experimental (group C) groups (n = 12). Three days after the establishment of the MI model, group C was given 15 mg kg-1 body weight (BW) DOX with a 1 mL saline dilution and then injected intraperitoneally twice. In group B, only 0.5 mL of saline was injected intraperitoneally twice a day. In the other 12 rats, the chest was only opened to expose the heart, but the coronary artery was not ligated. In group a, 0.5 mg of normal saline was injected intraperitoneally twice a day for 5 days.

Ultrasonic Electrocardiogram Examination

Two weeks later, three groups of rats with MI were anesthetized and weighed by intraperitoneal injection of PB, and the heart was examined by HP-5500 color Doppler echocardiography. The LVAW, LVPW and LVDd were recorded by M-mode echocardiography at the LV short axis papillary muscle plane. The LVEF and LVFS were calculated by the Teichholtz method. All the data were analyzed offline after recording, and each parameter was measured for 3 cardiac cycles and averaged.

Specimen Handling and Masson Staining

After blood was collected from the right ventricle of the open chest, the heart was washed, the fat was removed with PBS solution, and the whole heart weight (HW) was measured. Compared with BW, the proportion of HW/BW was calculated. A 2 mm thick tissue layer was cut from the middle part of the rat heart along the cross section and fixed with 10% formalin, while the apical part was quickly frozen and preserved in liquid nitrogen.

After the myocardial tissue was fixed, it was embedded in paraffin and cut into 5 μm thick sections. A Masson staining kit was used to dye the slices. Images were taken under a 10x microscope and saved in TIF format. The images were analyzed with imaging software to calculate the ratio of the infarct area to the LV area.

Determination of the Total Amount of Collagen and the Ratio of Type I/III Collagen

According to the hydroxyproline digestion kit and type I/III collagen immunohistochemical detection kit, the images were analyzed and processed by a CMIAS true color medical image analysis system. By observing the ratio of collagen to the area of the heart in the visual field, the content of collagen can be estimated indirectly, and then the ratio of type I/type III collagen can be calculated.

Zymography and Immunoblotting

The myocardial tissue samples of 6 rats in the three groups were treated, ground in lysis buffer, and centrifuged at 12.000 rpm for 15 min, after which the supernatant was collected for subsequent analysis. The protein content in the supernatant was determined by a bicinchoninic acid (BCA) protein concentration assay. Samples with a protein content of 60 μg were selected, and the activities of the two target enzymes were determined according to the instructions of the MMP-2 and MMP-9 activity detection kits. The image with the target band was captured by a Bio-Rad imaging analysis system, and the grayscale value of the target band was analyzed by Quantity-One software. The gray values obtained were compared with those in group A to evaluate the degree of MI. After protein extraction, the frozen rat myocardium was extracted by immunoblotting and subjected to transmembrane staining, incubated with primary and secondary antibodies and treated with a DAB chromogenic agent.

Table 1. LVR and cardiac function in rats in each group

Group	N	LVAW (mm)	LVPW (mm)	LVDd (mm)	LVSd (mm)	LVFS (%)	LVEF (%)
A	12	0.26±0.08	2.39±0.34	4.56±0.90	3.46±0.64	40.32±9.56	74.14±9.63
B	12	1.93±0.14[a]	2.25±0.42[a]	6.90±1.08[a]	5.05±0.92[a]	27.21±4.10[a]	52.40±6.51[a]
C	12	1.43±0.17[ab]	2.42±0.43[ab]	5.82±0.92[ab]	4.26±0.83[ab]	33.53±4.72[ab]	66.73±5.22[ab]
F		481.730	0.620	17.490	11.700	11.86	27.080
P		<0.001	0.543	<0.001	<0.001	<0.001	<0.001

** Parameters' values across different groups in the same column having similar superscripts are statistically non-significant*
LVAW = Left Ventricular Anterior Wall, LVPW = Left Ventricular Posterior Wall, LVDd = Left Ventricular End-Diastolic Diameter, LVSd = Left Ventricular End-Systolic Diameter, LVFS = Left Ventricular Fractional Shortening, LVEF = Left Ventricular Ejection Fraction

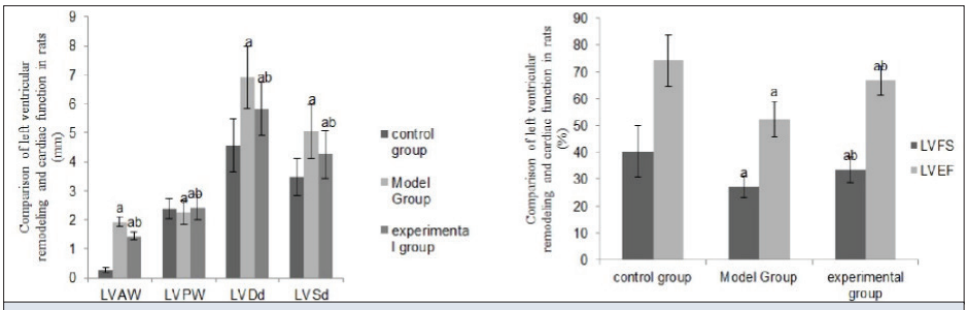

Fig 1. Comparison of LVR and cardiac function in rats

Table 2. MI size in rats

Group	N	HW/BW
A	12	3.92±0.11
B	12	5.03±0.16[a]
C	12	4.27±0.14[ab]
F		202.320
P		<0.001

** Parameters' values across different groups in the same column having similar superscripts are statistically non-significant*
HW/BW = Heart Weight Body Weight ratio

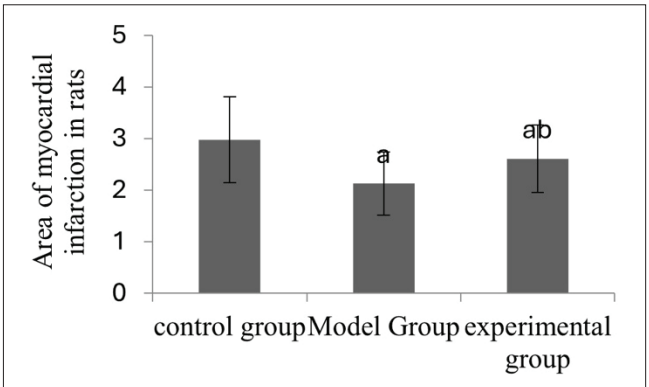

Fig 2. Comparison of MI size among the rats in each group

Statistical Analysis

All the data are represented by (`x±s), and the experimental data were analyzed by SPSS 20.0 statistical software. Compared with that of group A, the [a]P of group B was less than 0.05. Compared with that of group B, the [b]P of group C was less than 0.05.

Results

Effects of DOX on LVR and Cardiac Function After MI in Rats

The results of echocardiography showed that the LV cavity of rats after MI was enlarged, the LV anterior wall membrane became thinner, and many dilated into ventricular aneurysms. The LVAW, LVDd and LVSd in group B were greater than those in group A, while these values decreased, and the LVFS and LVEF increased in group C compared with those in group B *(Table 1, Fig. 1)*.

Analysis of Myocardial Infarct Size by Masson Staining

The results of Masson staining showed that the infarcted myocardial tissue was replaced by collagen, which was light blue, while the normal myocardial tissue was red. In group B, the ventricular wall in the infarcted area became thinner, and the LV cavity became enlarged, while the LV wall thinning and bulging in group C improved compared with those in group B. The HW/BW ratio of group B was increased group A, and the HW/BW ratio of group C decreased than group B *(Table 2, Fig. 2)*.

Comparison of the Ratio of Myocardial Collagen and I/III Collagen

The collagen content of group B was greater than that of group A, and the ratio of I/III collagen decreased, while that of group C decreased, and the ratio of I/III collagen increased compared with that of group B *(Table 3, Fig. 3)*.

Table 3. *The ratios of myocardial collagen and I/III collagen in rats*

Group	N	Collagen Content (μg/mg)	I/III Collagen
A	12	34.62±1.53	2.98±0.83
B	12	38.65±2.12[a]	2.13±0.61[a]
C	12	36.17±2.05[ab]	2.61±0.65[ab]
F		13.480	4.410
P		<0.001	0.020

** Parameters' values across different groups in the same column having similar superscripts are statistically non-significant*
HW/BW = Collagen type I and III ratio

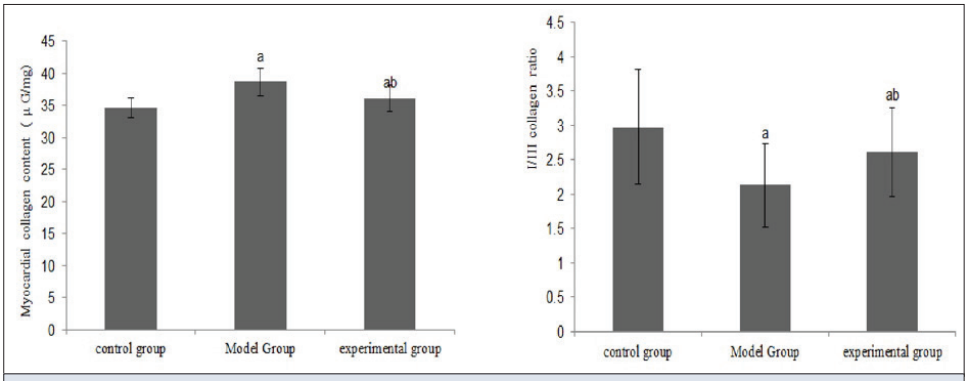

Fig 3. Comparison of the ratios of myocardial collagen and I/III collagen

Table 4. *Comparison of myocardial MMP-2 and MMP-9 activities in rats*

Group	N	MMP-2	MMP-9
A	12	1.03±0.02	1.02±0.03
B	12	1.87±0.14[a]	2.18±0.16[a]
C	12	1.52±0.12[ab]	1.34±0.14[ab]
F		186.310	28.300
P		<0.001	<0.001

** Parameters' values across different groups in the same column having similar superscripts are statistically non-significant*
MMP-2 = Matrix Metalloproteinase-2, MMP-9= Matrix Metalloproteinase-9

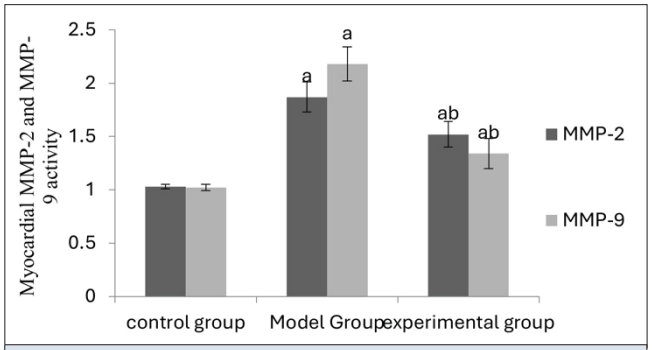

Fig 4. Comparison of myocardial MMP-2 and MMP-9 activities in rats

Comparison of Myocardial MMP-2 and MMP-9 Activities

The MMP-2 and MMP-9 in group B were greater than those in group A, while those in group C were less than group B *(Table 4, Fig. 4)*.

Discussion

MI can lead to severe myocardial injury. Gene expression changes after injury, resulting in increased expression of inflammatory factors, MMPs and growth factors; cardiomyocyte hypertrophy; necrosis; apoptosis; fibroblast proliferation; extracellular matrix degradation; and collagen accumulation, that is, fibrosis [11,12]. Exploring the mechanism of ventricular remodeling caused by MI and to identify effective therapeutic drugs is important.

The results showed that the ratio of LV weight to BW in group C was lower than group B, indicating that DOX could inhibit the development of cardiac remodeling to some extent. The results of Masson staining showed that the infarcted myocardial tissue was replaced by collagen, which was light blue, while the normal myocardial tissue was red. In group B, the ventricular wall in the infarcted

area became thinner, and the LV cavity became enlarged, while the LV wall thinning and bulging in the DOX group improved than group B. These findings suggest that DOX therapy can inhibit the development of ventricular remodeling to some extent. The ultrasound results also showed that DOX could improve LVR, reduce the thinning of the ventricular wall in the infarcted area and reduce the enlargement of the LV cavity. After MI, the process of LVR develops with a decrease in cardiac function, which may eventually lead to heart failure. The cardiac function of the rats was evaluated by color Doppler echocardiography. The LVAW, LVDd and LVSd in group C were reduced than group B. These findings suggested that DOX can inhibit the process of LVR, reduce the degree of LV dilatation and improve the cardiac function of MI rats to some extent.

LVR is the result of many factors, especially an increase in MMP activity, which promotes intercellular matrix degradation and fibrosis [13]. DOX is a tetracycline antibiotic that not only can inhibit bacterial protein synthesis but is also a broad-spectrum and efficient MMP inhibitor [14,15]. The activities of MMP-2 and MMP-9 in group C were lower than those in group B. Analysis revealed that DOX can reduce the expression of MMP-2 by reducing the half-life of mRNAs related to MMP-2 expression [10]. DOX can also inhibit the transcription of MMPs and reduce the activity and expression of MMPs in tissue by binding to the active center of MMPs [16]. Moreover, it has been shown that DOX can also reduce both the local and systemic inflammation as observed in various animal models [17,18]. This is achieved through factors such as C-reactive protein and myeloperoxide that further inhibit the progression of LVR. DOX can also inhibit cardiomyocyte apoptosis, thus inhibiting the development of ventricular fibrosis and improving LVR [19,20].

Collagen is the extracellular matrix and plays an important role in the functional unit of cardiomyocytes, especially type I and type III collagen [21]. After MI, ventricular remodeling occurs, and the early repair process is mainly characterized by an increase in the expression of type III collagen [22]. However, water tissue composed of type III collagen is not as strong as that composed mainly of type I collagen. Therefore, changes in collagen composition after MI can lead to cardiac enlargement [23]. Hydroxyproline accounts for 13.4% of collagen, very little in elastin, and does not exist in other proteins [24,25]. After treatment with DOX, the collagen content of group C was reduced than group B, and the ratio of group I/III collagen was optimized. Thus, DOX can reduce the degradation of collagen and inhibit LVR.

In summary, the activation of MMPs in myocardial tissue after MI promotes the development of LVR and heart failure. DOX can improve LVR and cardiac function after MI to some extent by inhibiting the activity of MMPs.

Declarations

Availability of Data and Materials: All data generated or analyzed during this study are available from the corresponding author (J. Li) upon reasonable request.

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Ethical Statement: This study was approved by the Xi 'an Daxing Hospital committee (Approval No: 20230911).

Competing Interests: The authors declare that they have no competing interests.

Declaration of Generative Artificial Intelligence (AI): This article, including its tables and figures, was not written or created by generative artificial intelligence or AI-assisted technologies. These technologies were used solely to enhance readability and language clarity, not for content generation.

Authors' Contribution: YC and JL designed the experiment, carried out research collected data and performed data analysis whereas KL was involved in manuscript drafting, improving the language of the manuscript and reviewing of the final draft. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work. This statement complies with the"ETHICAL PRINCIPLES AND PUBLICATION POLICY/Authorship and Authors Rights"guidelines of the journal.

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