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The exploration of modeling of rats with diabetic cardiomyopathy

Luyan Yu[1], Yongjun Mao[2,*]

[1]Qingdao University, Qingdao, 266071, China

[2]The Affiliated Hospital of Qingdao University, Qingdao, 266003, China

Abstract: **The complication of diabetic cardiomyopathy (DCM) is one of the major causes of death in diabetes mellitus (DM) patients. Accordingly, an effective animal model is playing an important role in investigating the pathogenesis of DCM. In this study, we randomized Sprague-Dawley (SD) rats into the Control group and the DCM group. The DCM rats were induced with intraperitoneal streptozotocin (STZ) injection. Eight weeks after STZ injection, compared with the Control rats, the DCM rats exhibited a series of symptoms such as polydipsia, polyphagia, polyuria, weakness, hyperglycemia and emaciation. Echocardiography results revealed that the DCM rats had a lower left ventricular ejection fraction (LVEF), fractional shortening (FS) and E wave to A wave (E/A) ratio than the Control rats. The results of hematoxylin-eosin (HE) staining showed that the left ventricle myocytes of the DCM rats were disordered and their cytoplasm was stained unevenly and their nucleus was irregular compared with the Control rats. Subsequently, as detected by transmission electron microscopy, compared with the Control rats, the apical tissue of heart displayed disorganized myofibrils and fragmented, vacuolated and swollen mitochondria in the DCM rats. Therefore, the DCM rats model were successfully induced via injecting intraperitoneally with a single STZ (65 mg/kg).**

Keywords: Diabetic cardiomyopathy; Streptozotocin; Echocardiography

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*Corresponding Author: YongjunMao, mmc168@126.com

1. Introduction

Recently, growing evidence has suggested that DM is one of the major threats to human health and its morbidity is increasing year by year[1]. Both experimental and clinical studies have indicated that the complication of DCM is the main cause of mortality in DM patients and DM is a global health problem accounting for the diabetes of over 550 million people by 2030[2-4]. However, the research of pathogenesis, diagnosis and treatment of DCM is not perfect, and the clinical diagnosis of the disease via tissue research (especially the acquisition of myocardial tissue) is difficult at present. Therefore, an effective DCM animal model is important because it can not only help us to clarify the complex mechanism of DCM in humans more easily but also give us chance to find a cure for DCM.

Diabetes is a group of metabolic diseases, including high blood glucose levels over a prolonged period. Hyperglycemia is caused by insulin deficiency and/or resistance. DCM is a kind of cardiac disease except for other complications such as hypertension, coronary artery disease, and it is a chronic myocardial pathological change that has developed on the basis of diabetes[1]. To date, several studies have demonstrated that STZ is an important naturally occurring chemical which is toxic to insulin-producing beta cells of the pancreas, so we can use it to induce DCM animal model, and more importantly, the STZ-induced DCM animal model has a high survival rate due to its mild toxicity to the organism[5]. Here, we performed a research to explore the DCM rat model which was induced by STZ.

2. Materials and Methods

2.1. Materials

2.1.1. Animals

A total of 20 male SD rats weighing (150–200)g were obtained from Jinan Peng Yue Experimental Animal Breeding Co., Ltd. All the rats were housed with 12h light and 12h dark-cycle at 25 ±2 ℃, free to feed and drink. They were fed for one week before experiments. All studies were in compliance with the Guide for Care and Use of Laboratory Animals and approved by the institutional Animal Care and Use Committee in XBL-China.

2.1.2. Reagents and Instruments

STZ (Sigma, USA), Glucose Assay Kit (Shanghai Rongsheng bio Pharmaceutical Co., Ltd, China); Ultrasound device (Vevo 2100, Toronto, Canada), Light microscope (Leica, Germany), Advanced multi-spectrum Automatic Analysis System of the glass (Perkin Elmer, USA), Transmission electron micrograph (JEM-1200EX; JEOL, Tokyo, Japan).

2.2. Methods

2.2.1. Grouping and Modelling

All SD rats were randomly divided into two groups: Control group (n=8) and DCM group (n=12). After fasting overnight, on the basis of the method performed as described previously[6,7], the rats of DCM group were injected intraperitoneally with a single STZ (65mg/kg dissolved in citrate buffer PH=4.34), while the rats in the Control group were

injected intraperitoneally with the equivalent volume of citrate buffer. Three days later, after fasting overnight, blood samples were taken from the posterior orbital venous plexus of the rats. The rats with the fasting blood glucose levels ≥16.7mmol/L and with the symptoms of polydipsia, polyphagia, polyuria and emaciation were regard as DM rats. After one week, 11 DM rats were established. According to the method described previously[8,9], eight weeks after STZ injection, all rats were anaesthetized with intraperitoneal injection of chloral hydrate (3ml/kg). We chose M-mode echocardiography and Pulsed Doppler echocardiography to check the cardiac systolic and diastolic function indexes: LVEF, FS and E/A ratio. The results exhibited that the rats with cardiac dysfunction were regarded as DCM rats. In the end, a total of 9 DCM rats were successfully induced.

2.2.2. General conditions

We observed and recorded the general conditions of the rats every day[10].

2.2.3. Monitoring of fasting blood glucose and body weight

The fasting blood glucose and body weight were dynamically monitored in the first week, the second week, the fifth week and the eighth week, respectively. Blood glucose testing was strictly carried out according to the manufacturer's instructions.

2.2.4. HE staining

All the rats were anaesthetized with chloral hydrate (3ml/kg) by intraperitoneal injection. The left ventricular myocardium of the hearts was excised rapidly. According to the method performed and described previously[11], some of the tissues were fixed in 10% formalin for 24h. Next, the samples were embedded in paraffin and sectioned to 5μm thickness, mounted on slide, stained with HE and observed with a light microscope.

2.2.5. Transmission electron microscopy

According to the previous described method[12, 13], approximately 1mm^3 cubes of apical tissue were prepared and fixed in 2.5% glutaraldehyde in PBS (0.1mL/L, pH=7.4) overnight at 4 ℃. Before washing in 0.1M PBS, the samples were fixed in cacodylate-buffered 1% osmium tetroxide. Specimens were dehydrated in a graded ethanol series and acetone, embedded in Epon 812, and polymerized at 37 ℃, 45 ℃ and 60 ℃, respectively for 24h. Ultrathin slices (70nm) were well prepared, and stained with uranyl acetate and lead citrate. Subsequently, they were observed under transmission electron microscopy.

2.2.6. Statistical analysis

Statistical analyses were used by SPSS software version 22.0. All statistical data were expressed as mean ± SD. Comparisons between 2 groups were performed using the unpaired Student test. $P<0.05$ was considered statistically significant. All statistical tests were expressed by GraphPad Prism software version 6.0.

3. Results

3.1. General conditions

Compared with the Control rats, DCM rats exhibited dull reactivity, hair lackluster, cyanotic lips, skin ulcer, and so on (Table 1), which were accompanied by a series of symptoms such as polydipsia, polyphagia, polyuria and weakness. Eight weeks after STZ injection, there were two deaths in DCM rats but no death in Control rats.

Table 1. The general condition of the rats (Eight weeks after STZ injection)

	Reactivity	Hair				Lip	Skin	Urine
Group	Dull	Lacklustre	Crude	Sparse	Dirty	Cynotic	Ulcer	Smell
Control	0	0	0	0	0	0	0	0
DCM	+++	+++	+++	+++	+++	+++	+++	+++

Note: "0" expresses normal, "+++" expresses severe.

3.2. Fasting blood glucose and body weight

As shown in Figure 1. A and B, compared with Control rats, there was an obvious increase in fasting blood glucose ($P<0.01$) and decrease in fasting body weight in DCM rats ($P<0.01$).

3.3. Echocardiographic evaluation

As expected, the echocardiography results of DCM rats displayed significant cardiac dilatation (Figure 2. A, B), markedly decreased left LVEF, FS and E/A ratio compared with Control rats ($P<0.01$) (Figure 2. C-E).

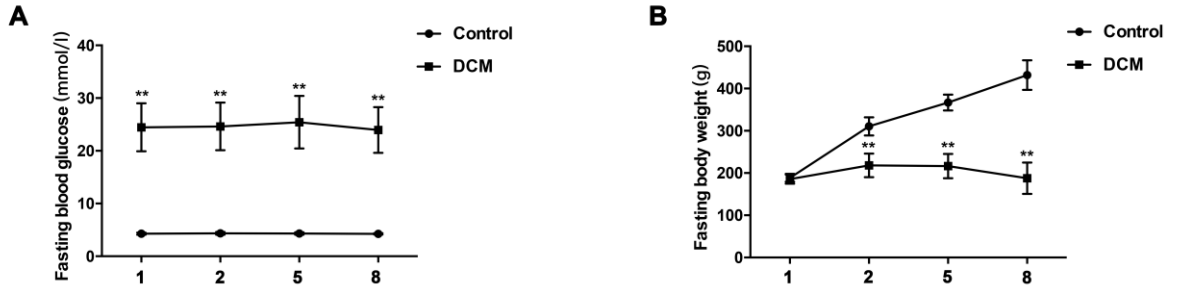

Figure 1. The results of fasting blood glucose and body weight. (A and B) The fasting blood glucose and body weight were dynamically monitored in the first week, the second week, the fifth week and the eighth week, respectively. Data are mean ± SD (n=6). **P<0.01vs Control rats.

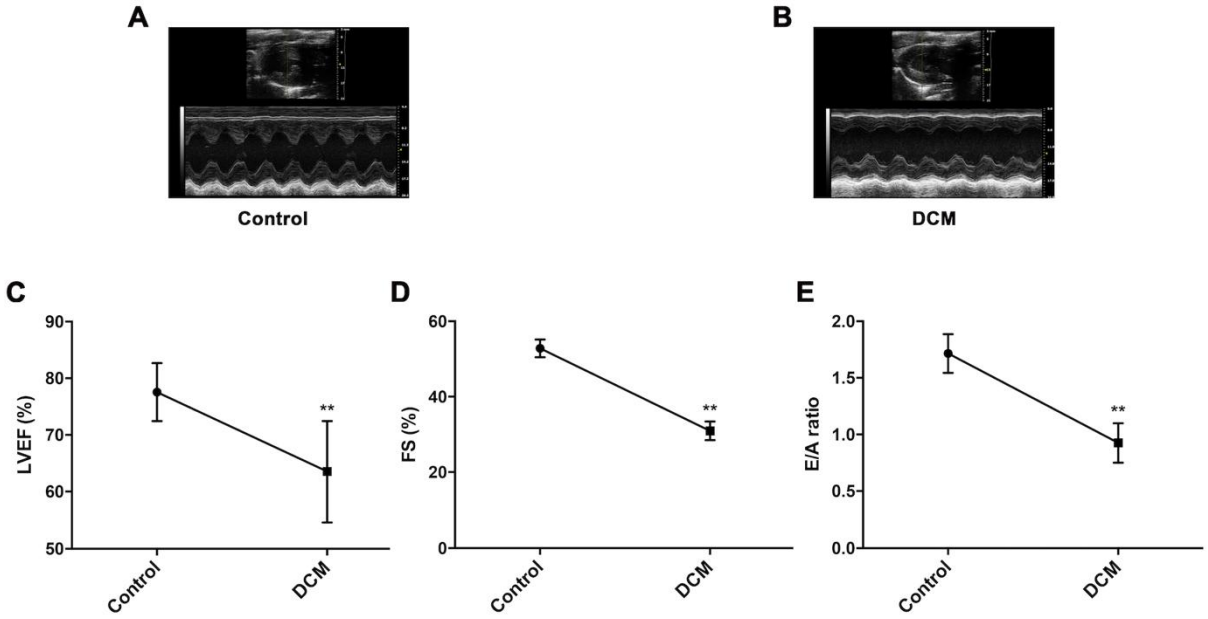

Figure 2. The results of echocardiography. (A and B) Representative echocardiography images. (C-E) The cardiac systolic and diastolic function indexes: LVEF, FS and E/A ratio. Data are mean ± SD (n=6). **P<0.01vs Control rats.

3.4. Histological analysis

According to the HE staining, compared with Control rats, the results showed that the left ventricle myocytes were disordered and their cytoplasm was stained unevenly and their nucleus was irregular in DCM rats (Figure 3. A). Ultrastructural studies of DCM rats' apical tissue of heart, as detected by transmission electron microscopy, showed disorganized myofibrils, fragmented, vacuolated and swollen mitochondria compared with Control rats (Figure 3. B).

4. Discussion

DCM, is a serious complication in DM, has attracted increasing attention, and the growing incidence and prevalence of diabetes has also brought a significant economic burden to the world, especially in the developed countries. It is estimated that 12% of the world's health care expenditure will be used for the treatment and prevention of diabetes[2]. Hence, effective DCM animal model not only plays an essential role in clarifying the complex mechanism of DCM in humans but also provided us a chance to find a cure for DCM.

In this study, the DCM rats were injected intraperitoneally with a single STZ (65 mg/kg). With the prolongation of modeling time, the DCM rats displayed a series of symptoms such as weakness, hyperglycemia, polydipsia, polyphagia, polyuria and emaciation. There were two deaths and one un-modeled rats in DCM group. Accordingly, this dose of STZ can effectively avoid the high mortality and the model instability.

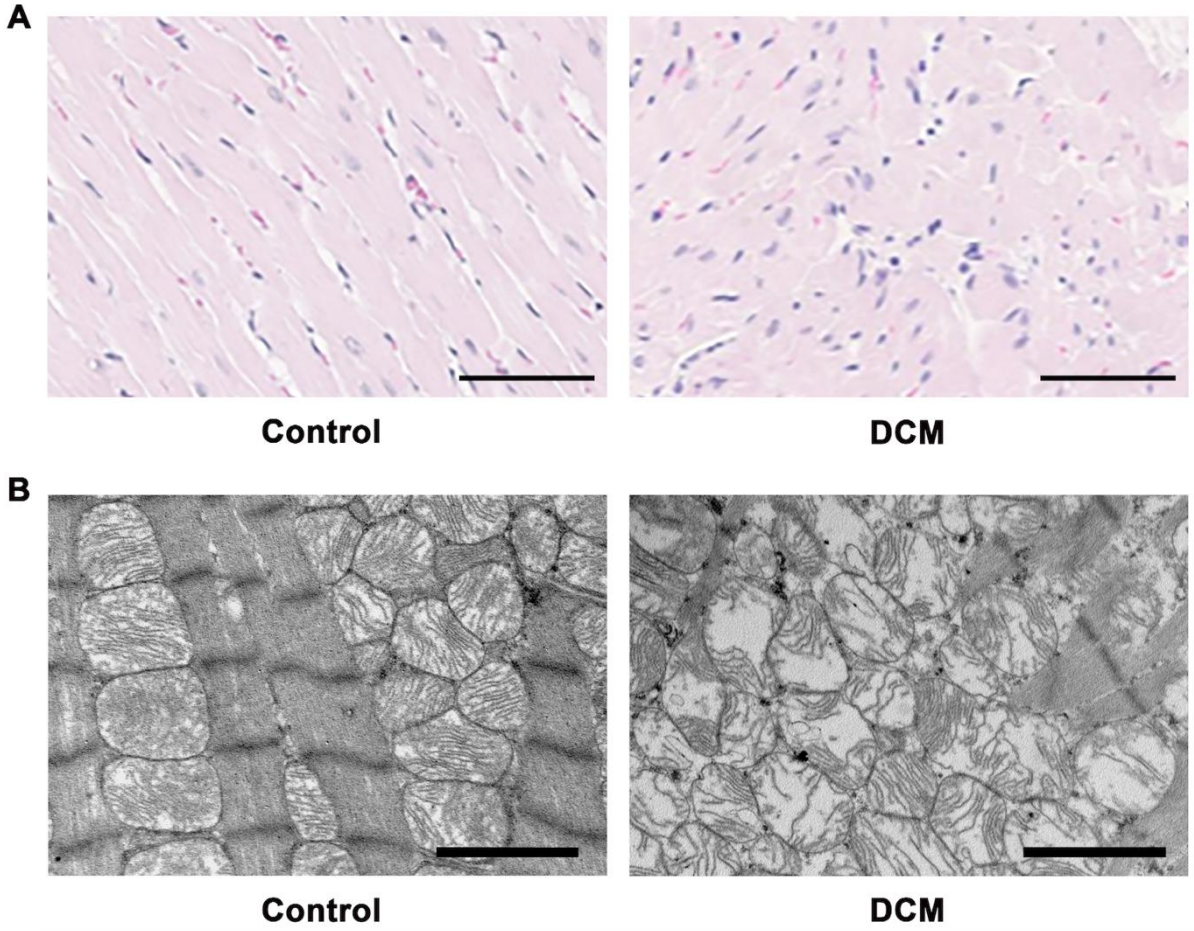

Figure 3. (A)The HE staining results of cardiac tissue: the cell nuclei stained blue and the cytoplasm stained red (Scale bar = 50μm, magnification 400×), n=6 per group. (B)Transmission electron micrograph of cardiac muscle. (Scale bar = 2μm, magnification 10,000×), n=6 per group.

The left ventricular diastolic dysfunction as one of the first symptoms of DCM often occurs before systolic dysfunction in human beings; furthermore, the morbidity of diastolic heart failure is rising year by year[1,14]. Diastolic dysfunction is associated with preserved ejection fraction or diastolic heart failure, and other injuries, such as concentric hypertrophy and vascular stiffness[15]. There also exists the phenomenon that the isolated diastolic dysfunction remains as an indicator of DCM, due to the fact that the diabetic patients are not regularly assessed for diastolic function in the early phase; in addition, the cardiac complications from diabetes are usually investigated only after symptoms become apparent[16]. A large number of evidences of diabetic cardiomyopathy suggest that left ventricular diastolic dysfunction often appears primarily in isolation or prior to left ventricular systolic dysfunction; however, the minority of evidence indicate that abnormalities in systolic function may also be obvious in clinical and experimental researches[17]. In this study, eight weeks after STZ injection, the echocardiography results of DCM rats exhibited significant cardiac dilatation and cardiac systolic and diastolic dysfunction compared with Control rats, which were consistent with the expression of DCM in human beings.

In human beings, the pathological changes are often caused by a series of complex factors including left ventricular systolic and diastolic dysfunction, myocardial interstitial fibrosis, cardiomyocyte hypertrophy, apoptosis, oxidative stress, and so on[18-20]. In this study, the DCM rats' histological results showed that the disordered left ventricle myocytes and unevenly stained cytoplasm and irregular nucleus. The ultrastructural changes of apical tissues of heart showed disorganized and decreased myofibrils, fragmented, vacuolated and swollen mitochondria in the DCM rats, which were similar to the expression of DCM in human beings. Studies have revealed that reduced myofibril results in a decline in systolic function and mitochondrial damage can lead to the decrease of myocardial compliance [21,22].

5. Conclusion

The DCM rat model was successfully established in our experiment. Although the method is only suitable for the DCM model on the basis of type 1

diabetes, the findings, which are of research and practical values, provide reliable guarantee for further studies.

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