Int J Clin Exp Pathol 2016;9(2):1626-1634
www.ijcep.com /ISSN:1936-2625/IJCEP0018434

Original Article

Association between collagen cross-linking and cardiac function and remodeling in rats with furazolidone-induced dilated cardiomyopathy

Zhu Li[1,2], Qinfu Wang[3], Lili Meng[1,4], Weiyi Fang[5], Ning Zhu[6], Rongmei Na[1], Baiting Liu[1], Libo Chen[1,2], Wencheng Tu[1,2], Qin Yu[1]

[1]Department of Cardiology, Affiliated Zhongshan Hospital of Dalian University, Dalian, China; [2]Zunyi Medical College, Zunyi, China; [3]Life Engineering College, Dalian University, Dalian, China; [4]Emergency Internal Medicine, Jining No. 1 People' Hospital, Jining, China; [5]Department of Cardiology, Shanghai Chest Hospital, Shanghai, China; [6]Department of Cardiology, The Second Affiliated Hospital of Dalian Medical University, Dalian, China

Received October 24, 2015; Accepted January 1, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: Objective: To explore the association between collagen cross-linking (CCL) and cardiac function in rats with furazolidone induced dilated cardiomyopathy (DCM). Methods and results: Forty 2-week-old Sprague-Dawlay (SD) rats were used, 30 rats were treated with furazolidone (0.15 mg/body weight (g)/day per gavage for 8 weeks, FZ group), 10 rats were treated by equal volume water daily per gavage for 8 weeks (Control group, CON). After 8 weeks, survival rate was 100% in CON group and 73.33% in FZ group (22/30, $P<0.05$). Echocardiography revealed that both left ventricular ejection fraction (LVEF) and left ventricular fraction shortening (LVFS) were significantly reduced, while left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) were significantly increased ($P<0.001$) in FZ rats compared to CON rats. Body weight was significantly decreased while heart weight/body weight ratio was significantly increased in FZ group (both $P<0.05$). Collagen volume fraction (16.39%±2.06% versus 2.78±1.10%, $P<0.001$) and the degree of CCL (6.13±0.96 versus 5.18±0.12, $P<0.05$) were significantly increased in FZ group than in CON group. mRNA expression of myocardial collagen I, collagen III, N2B and LOX were significantly upregulated, while mRNA expression of titin and N2BA were significantly downregulated in FZ group compared with CON group (all $P<0.05$). Moreover, CCL was negatively correlated with LVEF and LVFS while positively correlated with LVEDD and LVESD in FZ group. Conclusion: Increased collagen cross-linking is linked with reduced left ventricular dysfunction and aggravated left ventricular remodeling in this FZ-induced DCM rat model.

Keywords: Dilated cardiomyopathy, furazolidone, collagen remodeling, animal model, rat

Introduction

Non-ischemic dilated cardiomyopathy (DCM), a severe and progressive cardiomyopathy, is characterized by myocardial cell necrosis, interstitial fibrosis, collagen proliferation as the main pathological features, cavity expansion, arrhythmias and congestive heart failure or even sudden death are the main clinical manifestations [1, 2]. Despite modern therapy strategies, the general outcome of DCM patients remains poor and the 5 year mortality remains as high as 20% [3]. Animal models of dilated cardiomyopathy for translational research. Animal models of cardiovascular disease have proved critically important for the discovery of pathophysiological mechanisms and for the advancement of diagnosis and therapy. They offer a number of advantages; principally the availability of adequate healthy controls and the absence of confounding factors such as marked differences in age, concomitant pathologies and pharmacological treatments. Over the past thirty years, investigators have developed numerous small and large animal models to study this very complex syndrome [4]. DCM could be induced in rats either by immunization with porcine cardiac myosin [5, 6] or by oral treatment with doxorubicin [7, 8]. Recently, several reports described the enhanced distribution of calreticulin at cardiomyocyte mitochondria [9, 10], and found that calreticulin-STAT3 signaling pathway modulates mitochondrial function in a rat model of furazolidone-induced

DCM [11], studies from our group demonstrated the beneficial effects of intramyocardial mesenchymal stem cells and VEGF165 plasmid injection in this furazolidone-induced DCM rat model [12]. Recent clinical study demonstrated that myocardial fibrosis (detected by late gadolinium enhancement cardiovascular magnetic resonance [LGE-CMR] imaging) is an independent and incremental predictor of mortality and sudden cardiac death (SCD) in nonischemic dilated cardiomyopathy [13]. Previous study also demonstrated that collagen cross-linking (CCL) is associated with elevated filling pressures in hypertensive patients with stage C heart failure, moreover, CCL is positively correlated with insoluble stiff collagen and myocardial expression of lysyl oxidase (LOX) and negatively correlated with ejection fraction (EF), suggesting that LOX-mediated excessive CCL plays an important role in the pathogenesis of heart failure [14]. Present study thus evaluated the association between the quality of collagen (CCL) and cardiac function in the furazolidone-induced DCM rat model.

Materials and methods

Reagents

Furazolidone (C8H7N3O5, MW 225.16, 99%) was purchased from Mongxin pharmaceutical of Chifeng Co, Ltd. (Chifeng city, Inner Mongolia Autonomous Region, China).

Animal model and study protocol

Forty of Sprague-Dawley rats (2 weeks old and 20~30 g), provided by Experimental Animal Center of Dalian Medical University, were randomly divided into two groups. Thirty rats were treated with FZ [43 mg/ml solution, 0.15 mg/BW (g) by gavage] for 8 weeks. Ten rats received equal volume water daily per gavage for 8 weeks (control group, CON). After 8 weeks, survived rats underwent echocardiography examination under light anesthesia (intramuscular ketamine hydrochloride injection, 22 mg/kg). All animal study protocols were approved by the Institutional Animal Research and Ethics Committee of Dalian University.

Echocardiography

Left parasternal long-axis echocardiographic images of anaesthetized rats lying in a supine position were obtained with an ultrasound system equipped with a 12.0 MHz transducer (GE LOGIQ P6 ultrasound, General Electric Company, USA). To optimize the image, a transmission gel was used. All measurements were performed by the same observer and values were obtained based on the average of three consecutive cardiac cycles. LV dimensions were obtained from a parasternal long-axis view at the level of the papillary muscles. Left ventricular fractional shortening (LVFS) was calculated as (LVEDD-LVESD)/LVEDD×100%, where LVEDD is LV end-diastolic diameter and LVESD is LV end-systolic diameter. Left ventricular ejection fraction (LVEF) was calculated according to the Teichholz formula [16].

Histological examination and immunohistochemistry

After final echocardiographic examination, rats were sacrificed under deep anesthesia (intramuscular ketamine hydrochloride injection, 100 mg/kg), hearts were excised and weighed. Atria and right ventricle were separated from left ventricle, left ventricle was cut into three sections (each 3 mm in thickness) along long axis and processed for histological (middle section), immunohistological (basal section), biochemical analysis and soluble collagen determination (apical section). For biochemical tests, sample was quick-frozen and for histological analysis the sample was fixed in 4% paraformaldehyde, embedded in paraffin. Basic myocardial histology was evaluated with hematoxylin and eosin (H&E) staining, Masson staining and Sirus Red staining, For histomorphometric examinations, single slide was taken and the total field area for ten randomly selected fields were analyzed in a blinded fashion using Image Analysis System (Media Cybernetics, Inc. (Rockville, MD USA). The collagen volume fraction (CVF = area of the collagen/area of field of vision ×100%) was measured and ten separate areas of high power fields (100×) in each section were visualized under light microscope. Ten sections from each rat were observed and the results were averaged.

Real-time PCR analysis

Total RNA was isolated from 100 mg left ventricular tissue using the High Pure RNA Isolation Kit according to the manufacturer's instructions. (Takara Bio Inc., Dalian City, China). All

Table 1. Body and heart weight and echocardiographic measurements

	CON group	FZ group
BW (g)	228.60±30.78	161.81±17.65*
HW (mg)	906.00±150.27	756.36±83.93*
HW/BW (mg/g)	3.98±0.58	4.71±0.63*
LVEDD (cm)	0.50±0.03	0.59±0.08†
LVESD (cm)	0.23±0.03	0.47±0.09†
LVEF (%)	88.51±3.91	65.69±8.45†
LVFS (%)	53.51±5.92	32.09±5.68†

BW, body weight; HW, heart weight; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LVFS, left ventricular fraction shortening. *P<0.05 vs. CON group, †P<0.001 vs. CON group.

Table 2. Histomorphological parameters

	CON group	FZ group
CVF (%)	2.78±1.10	16.39±2.06*
Insoluble collagen (μg/mg)	1.28±0.10	2.32±0.26*
Soluble collagen (μg/mg)	0.26±0.07	0.38±0.03*
CCL	5.19±0.12	6.13±0.96†

CVF, collagen volume fraction; CCL, collagen cross-linking. *P<0.001 vs. CON group, †P<0.05 vs. CON group.

the primer sequence designed and synthesized by (Beijing AuGCT Biotechnology Co. Ltd. Beijing City, China) as follow, primers specific for rat collagen type I (5'-TGC CGT GAC CTC AAG ATG TG-3' and 5'-CAC AAG CGT GCT GTA GGT GA-3'), collagen type III (5'-CTGGAC CAA AAG GTG ATG CTG-3' and 5'-TGC CAG GGA ATC CTC GATGTC-3'), Titin (5'-AAA CCA GAA GAG CCA CCA CC-3' and 5'-TTT GGC TTT GGT TCA GGT CC-3'), N2BA (5'-GTC GTT GAA GAG AAG CCA GT-3' and 5'-TCT TCC TC TTC TAC AAC GGG-3'), N2B (5'-GAA GTG GAC AAA GAG CAA AG-3' and 5'-GGG TTT TGC CTT CTT CTA TG-3'), LOX (5'-ACT CCG ACG ACA ACC CCT AT-3' and 5'-CGT GGA TGC CTG GAT GTA GT-3'), and GAPDH (5'-TCC GCC CCT TCCGCT GAT G-3' and 5'-CAC GGA AGG CCA TGC CAG TGA-3). Reverse transcription was performed with PrimeScript™ RT reagent Kitwith gDNA Eraser (Takara Bio Inc, Dalian city, China) according to the manufacturer's instructions. Real-time PCR was carried out in a 20 μl reaction system containing 10 μl SYBR® Premix Ex Taq™II (Tli RNaseH Plus), 1.6 μl each primer, 0.4 μl ROX Reference Dye II, 2 μl cDNA, and 6 μl dH2O. Real-time PCR was performed with the following protocol: an initial predenaturation step at 95°C for 30 s, 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 34 s. All samples were subjected to Real-time PCR for housekeeping gene GAPDH as a positive control and as an internal standard. The same procedure repeated 3 times. ΔΔCt was calculated. ΔΔCt = FZ group (target genes Ct value-made in Ct)-the control group (target genes Ct value-made in Ct value). Different groups of the genes on the internal change ratio = $2^{-\Delta\Delta Ct}$.

Collagen I and III immunostaining analyses

Myocardial expression of collagen I and III was examined with Instant Immunohistochemistry Kit I (Sangon Biotech, Shanghai, China). LV (basal section) were fixed with formalin, embedded with paraffin and cut into 5 μm sections. After incubation the sections for 5 min with ddH_2O_2, collagen I and III primary antibodies (GeneTex, USA; Santa Cruz, USA) were added and incubated in a humidified chamber at 4°C overnight, the slides were treated with monohydrated citrate buffer (pH 6.0) in a water bath for 3 min and cooled at room temperature for 30 min for the antigen retrieval, then incubated with inactivated enzyme reagent for 15 min at room temperature, sealing fluid in a humidified chamber at room temperature for 30 min. Each slice was dried at room temperature for 40 min, and then incubated with HRP secondary antibody at room temperature for 30 min, washed three times; brown chromogenic granules were visualized with DAB. Finally, sections were counterstained with hematoxylin for 5 min followed by dehydration and coverslip mounting. Ten separate areas of high power fields (×200) in each section were visualized under light microscope, the average optical density value of positive zone was determined.

Insoluble collagen and soluble collage analyses

The degree of CCL was calculated as the ratio between the insoluble collagen (insCol, insCol = total collage-soluble collage) and soluble collage. Briefly, LV tissue (middle section) was embedded paraffin and cut into 5 μm sections. Then the total collagen was detected with Sirius Red/Fast Green Collagen Staining Kit (Chondrex, Inc, NE Redmond, WA) according to the manufacturer's instructions. Then, the eluted dye solution was collected and 200 μl of which was injected into 96 orifice plate, and OD val-

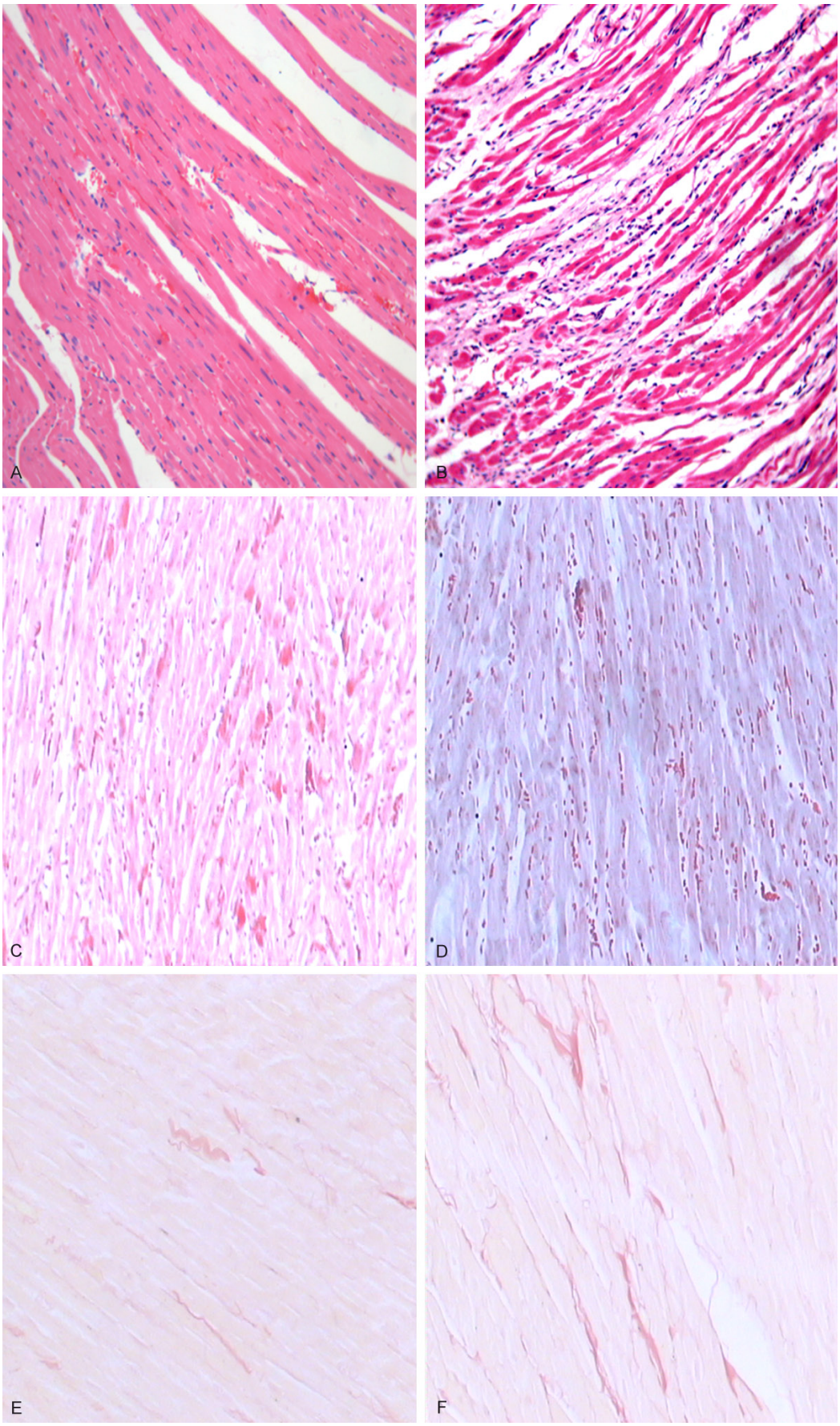
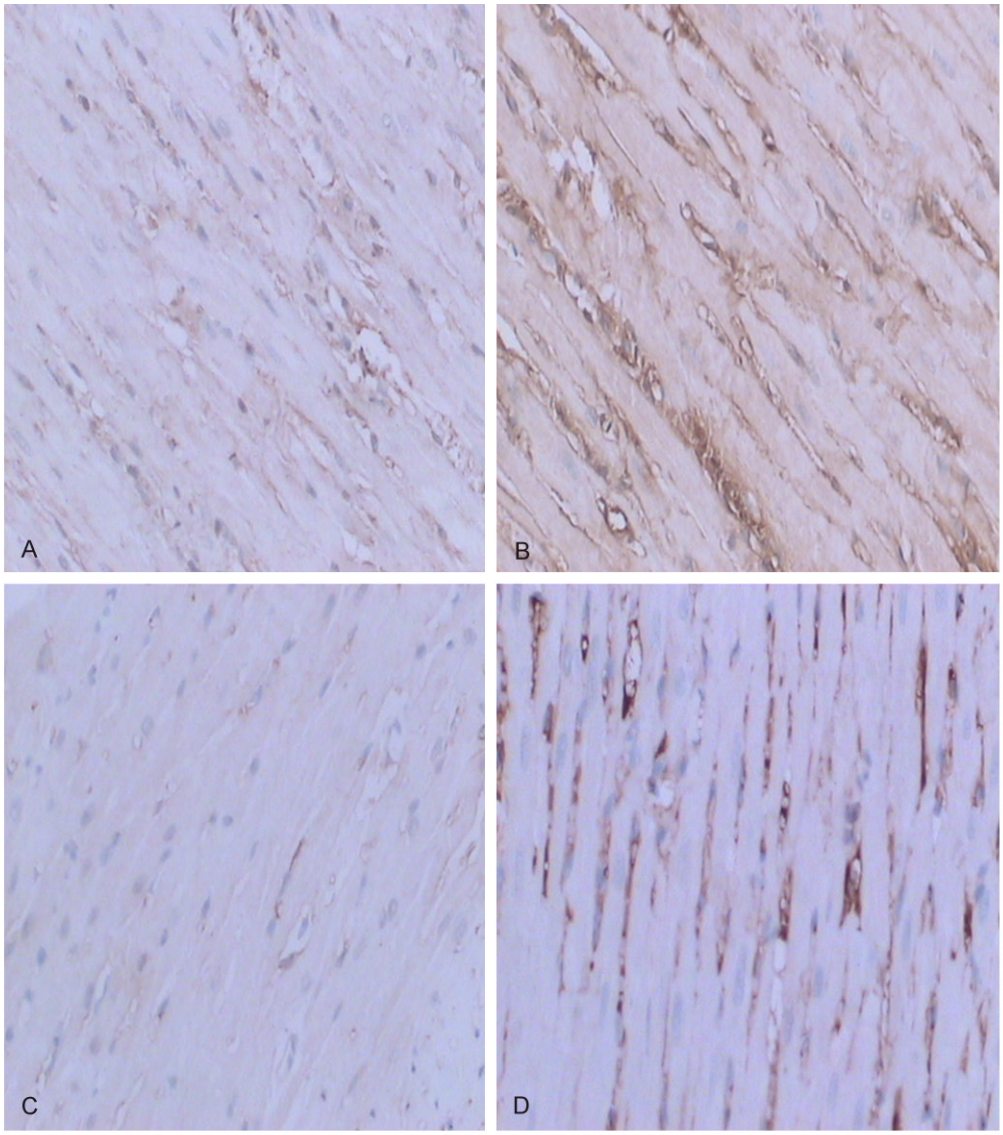

A
B
C
D
E
F

Figure 1. Representative examples of HE staining (magnification ×100) in CON group (A), FZ group (B). Representative examples of Masson staining (magnification ×100) in CON group (C), FZ group (D). Representative examples of Sirius red staining (magnification ×100) in CON group (E), FZ group (F).



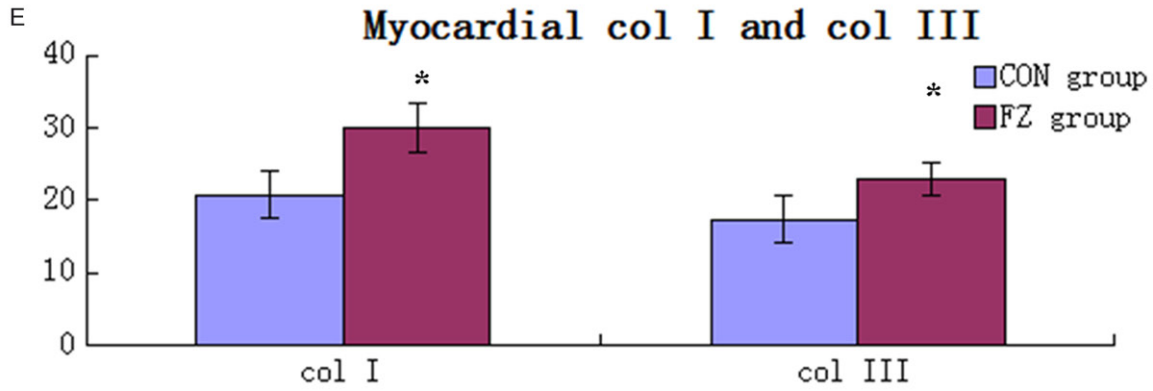


Figure 2. Representative examples of I collagen protein expression (magnification ×100) in CON group (A), FZ group (B). Representative examples of III collagen protein expression (magnification ×100) in CON group (C), FZ group

(D). Collagen I (B) and Collagen III expression (D) tended to be higher in FZ group compared to Collagen I (A) and Collagen III (C) expression in CON group. (E) Bar graph of col I (collagen I) and col III (collagen III). *P<0.05 vs. CON group. Myocardial mRNA expressions.

Table 3. Myocardial mRNA expressions

	Col I	Col III	Titin	N2B	N2BA	LOX
CON group	1.01±0.17	1.01±0.16	1.01±0.17	1.02±0.24	1.03±0.32	1.00±0.06
FZ group	2.57±0.63*	1.45±0.34*	0.50±0.13*	2.56±0.57*	0.41±0.05*	1.78±0.79*

Col I, collagen I; Col III, collagen III; LOX, lysyl oxidase. *P<0.05 vs. CON group.

Table 4. N2BA/N2B isoform rate

	N2B	N2BA	N2BA/N2B
CON group	1.02±0.24	1.03±0.32	0.99±0.02
FZ group	2.56±0.57*	0.41±0.05*	0.16±0.03*

*P<0.05 vs. CON group.

ues at 540 nm and 605 nm was obtained with a spectrophotometer. Total collagen (μg/section) = [OD 540 value - (OD 605 value × 0.291)]/0.0378. Soluble collagen was detected with Sircol Soluble Collagen Assay (Biocolor Ltd, Northern Ireland, U.K.) according to the manufacturer's instructions. For tissue samples, the Acid-Pepsin (0.1 mg Pepsin: 1 ml 0.5M Acid) Extraction procedure was performed and samples were incubated overnight at 4°C. 200 μl sample was transferred to individual wells of a 96 micro well plate, and microplate reader was set to 555 nm, the sircol soluble collagen value was calculated according to standard curve.

Statistical analysis

Data (mean ± SD) were analyzed by one-way or two-way ANOVA followed by Bonferroni's post-hoc comparisons with the SPSS statistical program (Statistical Product and Service Solutions); IBM (International Business Machines Corporation), (Armonk, NY, USA). A p value < 0.05 was considered as statistically significant.

Results

Survival rate and echocardiographic results on survived rats

Twenty-two out of thirty rats (73.3%) survived at 8 weeks in FZ group, and 10 rats survived (100%) at 8 weeks in CON group.

Table 1 shown body weight, heart weight and echocardiographic parameters assessed after 8 weeks of the 2 groups. LVEDD and LVESD were significantly higher while LVEF and LVFS were significantly lower in FZ group than that in CON group. Heart weights were lower and HW/BW ratios were higher in FZ group than in CON group.

Histological analysis

As shown in **Table 2**, myocardial CVF, insolule and soluble collagen as well as CCL were all significantly increased in FZ group compared to CON group.

Figure 1 shown representative HE staining (**Figure 1A** and **1B**), Masson staining (**Figure 1C** and **1D**) and Sirius red staining (**Figure 1E** and **1F**) results in CON group and FZ group. HE staining showed that myocardial fibers were dyed uniform and neat. The nucleus structure of cardiomyocytes was clear, size was uniform, only a small amount of collagen fibers in heart muscle fibers or around the blood vessels was detected in CON group (**Figure 1A**). In FZ group, the nucleus of cardiomyocytes was enlarged, and myocardial collagen fiber was increased and presented in disordered form (**Figure 1B**). Masson staining demonstrated that there were visible neat rows of dyed red myocardial fibers and a small amount of collagen fibers around the blood vessels in CON group (**Figure 1C**), while a large number of collagen fibers could be detected in disordered form in FZ group (**Figure 1D**). Sirius red staining showed that the arrangement of myocardial fibers was neatly, a small amount of scarlet collagen fibers was found around myocardial interstitial or blood vessels in CON group (**Figure 1E**). The content of collagen fiber increased, which was widely distributed in the myocardial interstitial in disordered arrangement in FZ group (**Figure 1F**).

Figure 2 showed myocardial Collagen I, Collagen III expression in myocardial tissue of rats

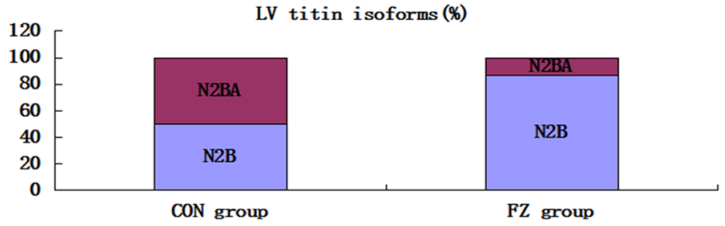


Figure 3. LV titin isoforms in CON group and FZ group. **Figure 3** shown N2BA/N2B isoform rate was reduced in FZ group. *P=0.046. *P<0.05 vs. CON group.

Table 5. Correlations of CCL and myocardial mRNA expressions echocardiographic parameters in FZ group

	CCL		InsCol		Col I		Col III	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
LVEF	-0.76	<0.05	-0.43	NS	-0.88	<0.05	-0.88	<0.05
LVEDD	0.66	<0.05	0.18	NS	0.51	<0.05	0.66	<0.05
LVESD	0.74	<0.05	0.29	NS	0.66	<0.05	0.78	<0.05
LVFS	-0.77	<0.05	-0.43	NS	-0.86	<0.05	-0.86	<0.05
	N2B		N2BA		Titin		LOX	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
LVEF	-0.74	<0.05	0.71	<0.05	0.74	<0.05	-0.83	<0.05
LVEDD	0.52	<0.05	-0.70	<0.05	-0.62	<0.05	0.56	<0.05
LVESD	0.64	<0.05	-0.75	<0.05	-0.70	<0.05	0.69	<0.05
LVFS	-0.74	<0.05	0.71	<0.05	0.74	<0.05	-0.80	<0.05

CCL, collagen cross-linking; InsCol, insoluble collagen; Col I, collagen I; Col III, collagen III; LOX, lysyl oxidase; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVFS, left ventricular fraction shortening. NS, not significant.

in CON group and FZ group. Few brown stained collagen I (**Figure 2A**) and collagen III (**Figure 2C**) were detected in the myocardial interstitial cells in CON group. Increased collagen I (**Figure 2B**) and collagen III (**Figure 2D**) were detected around the blood vessels and in the myocardial interstitial cells in FZ group. Both collagens I and III expressions were significantly increased in FZ group compared to CON group (**Figure 2E**).

The mRNA expression of collagen I, collagen III, Titin, N2B, N2BA and LOX was detected by Real-time PCR. $2^{-\Delta\Delta Ct}$ was applicated. Compared with CON group, the mRNA expressions of collagen I, collagen III, N2B, LOX were significantly upregulated while the mRNA expressions of Titin and N2BA were significantly downregulated in FZ group (**Table 3**). **Table 4** and **Figure 3** demonstrated reduced N2BA/N2B isoform rate in FZ group. Thus, the myocardium might be become stiffer in FZ group.

Analysis of associations

Correlations of CCL and myocardial mRNA expressions and echocardiographic parameters in FZ group are presented in **Table 5**. CCL, collagen I and collagen III, N2B and LOX mRNA expressions were negatively correlated with LVEF and LVFS and positively correlated with LVEDD and LVESD while N2BA and titin mRNA expressions were positively correlated with LVEF and LVFS and negatively correlated with LVEDD and LVESD.

Discussion

The DCM animal model in rat induced by FZ imitated the characteristic performance of DCM in human including enlarged LVEDd and LVESd, reduced LVEF and LVFS, above changes are joined by increased heart weight/body weight ratio and increased myocardial collagen expressions including increased CCL in FZ rats. Moreover, increased CCL is linked with reduced cardiac function and aggravated cardiac remodeling in this DCM model.

To our knowledge, that is the first report describing the association between CCL and cardiac function and remodeling in this model. Previous study [14] found that it is the degree of CCL and the abundance of insCol but not the amount of total or type I collagen fibers that associate with LV chamber stiffness (i.e., LV chamber stiffness constant) and FPs (i.e., PCWP) in patients with HHD and stage C HF. In line with above report, we found that the increase of CCL is negatively correlated with LVEF and LVFS, suggesting that the qualitative alterations of the myocardial collagen matrix might have a functional impact in FZ-induced DCM rats. It is shown that CCL is positively correlated with the expression of lysyl oxidase (LOX) [14], and expression of LOX is markedly increased in fibrotic tissues, including models of dermal, lung, liver, and arterial fibrosis [15]. Similarly, the myocardial LOX expression was significantly upregulated in FZ rats, supporting the hypothesis that LOX-mediated excessive CCL might

facilitate LV dysfunction and remodeling in this FZ-induced DCM model.

CCL is only one of the determinants of collagen mechanics [16]. Properties such as collagen fiber diameter and the ratio of collagen I to III all contribute to the mechanical behavior at the tissue level [17, 18]. Tissues with predominance of Col I (thick collagen fibers) are characterized by strength and stiffness, whereas tissues containing large amounts of Col III (thin collagen fibers) are characterized by greater elasticity. Because of their different physical properties, the altered Col I/Col III ratio may therefore have a major impact on the diastolic and systolic function of the heart [19]. Our results revealed increased myocardial fibrosis (increased CVF) and myocardial mRNA expressions of Col I and Col III in FZ rats. Since myocardial fibrosis is a known detrimental determinant of LV function and remodeling evolution [20, 21], increased myocardial fibrosis might be responsible for the FZ-induced LV dysfunction and aggravated remodeling in this model [21].

In 1954, Huxley and Hanson found a third type of filament in sarcomere. Since then, people recognized that the sarcomere is consists of thick filament, thin filament, and titin. Titin is a giant sarcomeric protein that extends from the Z disk to the M-line and is encoded by a single gene [22]. Its differential splicing leads in the myocardium to the expression of N2B and N2BA isoforms that differ in size [22]. A key difference is that the N2B isoform is stiffer than the N2BA isoform, so that it is not surprising that N2B tends to predominate in stiffer ventricles, whereas N2BA occurs in more compliant hearts [23]. Titin-transcript expression was studied previously in a guinea-pig model of hypertrophic cardiomyopathy, demonstrating increased titin-mRNA transcripts in compensated hypertrophy but declining mRNA-levels in the transition to decompensated CHF [24]. An increased content of the less stiff N2BA isoform has been reported in human dilated cardiomyopathy [25]. By RT-PCR, we found that the total-titin and N2BA-transcript expression were reduced in FZ hearts compared with control hearts, N2B-transcript expression was increased compared with control hearts. Previous studies found that a 47% reduction in total-titin mRNA levels in human DCM compared with control hearts, but no differences in N2B, N2BA transcripts [26]. Future studies are needed to verify the divergent finding in N2B and N2BA between human DCM and FZ-induced rat DCM model.

In conclusion, FZ-induced DCM rat model might be a useful tool to explore the pathological mechanisms and new therapeutic strategies of DCM. Myocardial collagen remodeling, especially increased CCL in this model is significantly correlated with LV dysfunction and remodeling.

Acknowledgements

This study was supported by Scientific Research Fund from Dalian Science and Technology Bureau (grant number: 2007E21SF203; 2014E14SF149) and the Specialized Research Fund of Higher Education of Liaoning Provincial Education Department (grant number: L2010023), Grant to promote Dalian's medical soft power from Dalian Municipal Government.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qin Yu, Department of Cardiology, Affiliated Zhongshan Hospital of Dalian University, 6 Jiefang Street, Zhongshan District, Dalian 116001, China. Tel: 86-411-6288-7018; Fax: 86-411-62893555; E-mail: dryuqin06-0621@sina.com; yuqin060621@126.com

References

[1] Luk A, Ahn E, Soor GS, Butany J. Dilated cardiomyopathy: A review. J Clin Pathol 2009; 62: 219-225.

[2] Jefferies JL, Towbin JA. Dilated cardiomyopathy. Lancet 2010; 375: 752-762.

[3] Felker GM, Thompson RE, Hare JM, Hruban RH, Clemetson DE, Howard DL, Baughman KL, Kasper EK. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med 2000; 342: 1077-1084.

[4] Recchia FA, Lionetti V. Animal models of dilated cardiomyopathy for translational research. Veter Res Commun 2007; 31 Suppl 1: 35-41

[5] Kodama M, Zhang S, Hanawa H, Saeki M, Inomata T, Suzuki K, Koyama S, Shibata A. Effects of 15-deoxyspergualin on experimental autoimmune giant cell myocarditis of the rat. Circulation 1995; 91: 1116-1122.

[6] Watanabe K, Ohta Y, Nakazawa M, Higuchi H, Hasegawa G, Naito M, Fuse K, Ito M, Hirono S, Tanabe N, Hanawa H, Kato K, Kodama M, Aizawa Y. Low dose carvedilol inhibits progression

of heart failure in rats with dilated cardiomyopathy. Br J Pharmacol 2000; 130: 1489-1495.
[7] Leontyev S, Schlegel F, Spath C, Schmiedel R, Nichtitz M, Boldt A, Rubsamen R, Salameh A, Kostelka M, Mohr FW, Dhein S. Transplantation of engineered heart tissue as a biological cardiac assist device for treatment of dilated cardiomyopathy. Eur J Heart Fail 2013; 15: 23-35.
[8] Yu Q, Li Q, Na R, Li X, Liu B, Meng L, Liutong H, Fang W, Zhu N, Zheng X. Impact of repeated intravenous bone marrow mesenchymal stem cells infusion on myocardial collagen network remodeling in a rat model of doxorubicin-induced dilated cardiomyopathy. Mol Cell Biochem 2014; 387: 279-285.
[9] Shan H, Wei J, Zhang M, Lin L, Yan R, Zhu Y, Zhang R. Calreticulin is localized at mitochondria of rat cardiomyocytes and affected by furazolidone. Mol Cell Biochem 2014; 397: 125-130.
[10] Zhang M, Wei J, Li Y, Shan H, Yan R, Lin L, Zhang Q, Xue J. Novel distribution of calreticulin to cardiomyocyte mitochondria and its increase in a rat model of dilated cardiomyopathy. Biochem Biophys Res Commun 2014; 449: 62-68.
[11] Zhang M, Wei J, Shan H, Wang H, Zhu Y, Xue J, Lin L, Yan R. Calreticulin-stat3 signaling pathway modulates mitochondrial function in a rat model of furazolidone-induced dilated cardiomyopathy. PLoS One 2013; 8: e66779.
[12] Yu Q, Fang W, Zhu N, Zheng X, Na R, Liu B, Meng L, Li Z, Li Q, Li X. Beneficial effects of intramyocardial mesenchymal stem cells and vegf165 plasmid injection in rats with furazolidone induced dilated cardiomyopathy. J Cell Mol Med 2015; 19: 1868-1876.
[13] Gulati A, Jabbour A, Ismail TF, Guha K, Khwaja J, Raza S, Morarji K, Brown TD, Ismail NA, Dweck MR, Di Pietro E, Roughton M, Wage R, Daryani Y, O'Hanlon R, Sheppard MN, Alpendurada F, Lyon AR, Cook SA, Cowie MR, Assomull RG, Pennell DJ, Prasad SK. Association of fibrosis with mortality and sudden cardiac death in patients with nonischemic dilated cardiomyopathy. JAMA 2013; 309: 896-908.
[14] Lopez B, Querejeta R, Gonzalez A, Larman M, Diez J. Collagen cross-linking but not collagen amount associates with elevated filling pressures in hypertensive patients with stage c heart failure: Potential role of lysyl oxidase. Hypertension 2012; 60: 677-683.
[15] Smith-Mungo LI, Kagan HM. Lysyl oxidase: Properties, regulation and multiple functions in biology. Matr Biol 1998; 16: 387-398.
[16] Voorhees AP, DeLeon-Pennell KY, Ma Y, Halade GV, Yabluchanskiy A, Iyer RP, Flynn E, Cates CA, Lindsey ML, Han HC. Building a better infarct: Modulation of collagen cross-linking to increase infarct stiffness and reduce left ventricular dilation post-myocardial infarction. J Mol Cell Cardiol 2015; 85: 229-239.
[17] Voorhees AP, Han HC. A model to determine the effect of collagen fiber alignment on heart function post myocardial infarction. Theoret Biol Med Model 2014; 11: 6.
[18] Fomovsky GM, Thomopoulos S, Holmes JW. Contribution of extracellular matrix to the mechanical properties of the heart. J Mol Cell Cardiol 2010; 48: 490-496.
[19] Pauschinger M, Knopf D, Petschauer S, Doerner A, Poller W, Schwimmbeck PL, Kuhl U, Schultheiss HP. Dilated cardiomyopathy is associated with significant changes in collagen type i/iii ratio. Circulation 1999; 99: 2750-2756.
[20] Patrianakos AP, Parthenakis FI, Nyktari E, Malliaraki N, Karakitsos DN, Vardas PE. Central aortic stiffness in patients with nonischemic dilated cardiomyopathy: Relationship with neurohumoral activation. J Card Fail 2009; 15: 665-672.
[21] Segura AM, Frazier OH, Buja LM. Fibrosis and heart failure. Heart Fail Rev 2014; 19: 173-185.
[22] Granzier HL, Labeit S. The giant protein titin: A major player in myocardial mechanics, signaling, and disease. Circulat Res 2004; 94: 284-295.
[23] Katz AM, Zile MR. New molecular mechanism in diastolic heart failure. Circulation 2006; 113: 1922-1925.
[24] Collins JF, Pawloski-Dahm C, Davis MG, Ball N, Dorn GW 2nd, Walsh RA. The role of the cytoskeleton in left ventricular pressure overload hypertrophy and failure. J Mol Cell Cardiol 1996; 28: 1435-1443.
[25] Nagueh SF, Shah G, Wu Y, Torre-Amione G, King NM, Lahmers S, Witt CC, Becker K, Labeit S, Granzier HL. Altered titin expression, myocardial stiffness, and left ventricular function in patients with dilated cardiomyopathy. Circulation 2004; 110: 155-162.
[26] Makarenko I, Opitz CA, Leake MC, Neagoe C, Kulke M, Gwathmey JK, del Monte F, Hajjar RJ, Linke WA. Passive stiffness changes caused by upregulation of compliant titin isoforms in human dilated cardiomyopathy hearts. Circulat Res 2004; 95: 708-716.