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Downregulation of *Kv4.2* and *Kv4.3* channel gene expression in right ventricular hypertrophy induced by monocrotaline in rat [1]

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ABSTRACT

AIM: To investigate the differences in gene expression of transient outward potassium ion channel between the free wall of right ventricle, free wall of left ventricle, and the septum in monocrotaline (MCT)-induced right ventricular hypertrophy of rat. **METHODS**: Twenty rats were randomly divided into two groups: a single injection of monocrotaline (MCT) 60 mg/kg (model) or saline (control). Four weeks later, hemodynamic parameters were measured and the gene expression of I_{to} channels were detected by semi-quantitative RT-PCR. **RESULTS:** After 28 d, the right ventricular systolic pressure and central venous pressure were remarkably elevated by 128 % and 533 % in the MCT-treated group, accompanied by an overt right ventricle (RV) remodeling. The difference of mRNA expression of *Kv1.4* was not significant in free wall of RV, left ventricle (LV), and septum in MCT group compared with control group. In contrast, mRNA of *Kv4.2* and *Kv4.3* in the free wall of RV in MCT-induced rat was dramatically decreased by 45.2 % and 51.1 % *vs* control, however, in free wall of LV and septum, no difference was found. In addition, mRNA expression level of *Kv4.2* in control rat was significantly lower in septum than that in free wall of RV and LV. **CONCLUSION:** Expression of *Kv1.4, Kv4.2*, and *Kv4.3* differs between regions in normal rat hearts. The down-regulation of *Kv4* family gene expression of I_{to} contributed to the pathophysiological changes in ventricular hypertrophy and pulmonary hypertension induced by MCT.

INTRODUCTION

The calcium-independent transient outward potassium current (I_{to}) is activated by depolarization and plays a key role in controlling the amplitudes and cardiac action potential duration (APD). I_{to} contributes to the phase 1 repolarization of action potential in myocytes, the prolongation of APD is the result of decrease in I_{to} density[1]. I_{to} which is encoded by genes of *Kv1.4*, *Kv4.2,* and *Kv4.3*[2], based on differences in kinetics, as well as recovery from inactivation, has been divided into two types, fast ($I_{to,f}$) and slow ($I_{to,s}$)[3]. It is believed that *Kv4.2* and *Kv4.3* contribute to the rapid component $I_{to,f}$ and *Kv1.4* underlies the slow component $I_{to,s}$[4]. Recent studies reported that changes of mRNA expression of *Kv4.2* and *Kv4.3* were correlated with changes of density of transient outward current in diseased heart[2,5].

Clinical studies have suggested that a diseased heart with heart failure and ventricular hypertrophy will evoke arrhythmia and is associated with a greater risk of cardiac sudden death[6]. Abnormal repolarization underly-

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ing the ventricular arrhythmia is attributed to multiple channelopathies[7]. I_{to} is a key contributor in waveform and early phase of repolarization of cardiac ventricular action potential. Cellular electrophysiological studies have shown that changes in early repolarization provoke the prolongation of action potential duration[8]. So I_{to} is an important target for therapy of heart diseases.

In the present study, to get more insights of pulmonary hypertension, we investigated the change in gene expression (*Kv1.4, Kv4.2,* and *Kv4.3*) of I_{to} in the right ventricle, left ventricle, and septum in hypertrophied rat hearts induced by monocrotaline (MCT).

MATERIALS AND METHODS

Animals The experiments were performed on male Sprague-Dawley rats, weighing between 200-220 g at the age of 8 weeks (from Laboratory Animal Center of Nanjing Medical University, Certificate No SCXK Jiangsu 2002-0015, China). The animals were randomly divided into two groups. In model group, rats (n=10) were injected subcutaneously (SC) a single dose of MCT (Sigma) 60 mg/kg. MCT was dissolved in 1 mol/L HCl neutralized with 0.5 mol/L NaOH and diluted with sterile distilled water to obtain a 2 % solution. Control group rats (n=10) were treated with saline, sc. The rats were allowed to access to food and water *ad libitum*, in an air-conditioned room, at 25 ºC.

Measurement of hemodynamics and right ventricular hypertrophy Four weeks after MCT injection, the animals were anesthetized with urethane (1 g/kg, ip), and surgical performance was conducted, then a polyethylene catheter (PE 50, ID: 0.58 mm; OD: 0.965 mm) connected to a pressure transducer (MPA-V, Second Medical University of PLA, Shanghai China) was inserted into the right carotid artery to measure systolic blood pressure (SBP). Subsequently, a polyethylene catheter with a curved tip was inserted into the right jugular vein to reach the level of the vena cava superior and measure the central venous pressure (CVP),then, forward further into the right ventricle for measurement of the right ventricular systolic pressure (RVSP)[9], which reflects well the pulmonary artery pressure.

After hemodynamic measurements, the heart and lung were removed and weighed quickly. Heart was divided into right ventricle (RV), left ventricle (LV), and septum (SEP), each portion was separately weighed and rapidly frozen in liquid nitrogen, then stored at -70 ºC.

RNA extraction Total RNA from the RV, LV, and SEP were isolated[10] as follows. About 100 mg tissue was homogenized in 1 mL TRIzol reagent (Sangon Gene Ltd, Shanghai, China) with a tissue homogenizer. After chloroform 0.2 mL was added, tubes was shaken vigorously for 15 s and equilibrated at room temperature for 2-3 min, then the homogenate was centrifuged at 12 000×g for 15 min at 4 ºC. The aqueous phase was transferred into a clean tube, and the total RNA was precipitated by 0.5 mL isopropyl alcohol. The mixture was incubated at room temperature for 10 min and centrifuged at 12 000×g at 4 ºC for 10 min. The supernatant was removed, and the total RNA was washed with 75 % ethanol 1 mL, finally the sample was vortexed and centrifuged at 7 500×g at 4 ºC for 5 min. The sample was dried by air, and 30 µL of diethylpyrocarbonate (DEPC) water was added. The total RNA concentration and purity were determined by ultraviolet spectrophotometer respectively. The RNA was stored at -80 ºC before use.

Reverse transcription Total RNA (2 µg) with 0.5 µg oligo d(T)18 was incubated at 70 ºC for 5 min and then chilled on ice for 5 min. AMV RT 5 µL (5×buffer),2.5 µL dNTP (20 mmol/L), 40 U RNase inhibitor(Sangon Gene Ltd, Shanghai, China), and 10 U AMV reverse transcriptase (Promega Co, USA) were added into the above tube and the final volume was added to 25 µL DEPC-water. The reaction was carried out at 42 ºC for 1 h and then inactivated at 94 ºC for 5 min. The cDNA was obtained and stored at -20 ºC.

Polymerase chain reaction Each 25 µL PCR reaction contained 1 µL cDNA, 2 mmol/L $MgCl_2$, 200 mol/L each dNTP, 0.2 nmol/L each primer, 2 U of *Taq* DNA polymerase and accompanied buffer (Promega Co, USA). The gene-specific primers were designed from coding regions of *Kv1.4* (GeneBank accession No X16002), *Kv4.2* (GeneBank accession No NM_031730), *Kv4.3* (GeneBank accession No AF334791) and β-actin (GeneBank accession No AF421789). The nucleotide sequences of primers were as follows: *Kv1.4*: sense 5'-TGGAAGGCACTGGAGGTTCTG-3', antisense 5'-AGTAGGTCAGTGTAGGAACAC-3' (643-964nt), 322 bp; *Kv4.2*: sense 5'-GTGAGTGGCACCCGTTTC-3', antisense 5'-GGCGGTATCCAAGCAGAAT-3' (371-937 nt), 567 bp; *Kv4.3*: sense 5'-CCAGCCGACAAGAACA-AG-3', antisense 5-CGAGACCGCAATGAAGAA-3' (91-588 nt), 498 bp; β-actin: sense 5'-GAAGATCCTGACC-GAGCGTG-3',antisense 5'-CGTATTCCTGCTTGCTG-ATCC-3' (632-1149 nt), 518 bp. The cDNA samples

were amplified in a DNA thermal cycler (Mastercycler personal, Eppendorff, German) under the following conditions: the mixture was initially denatured at 94 ºC for 5 min, in the cycling, denatured at 94 ºC for 40 s, extended at 72 ºC for 1 min. Annealing temperature and cyclic number of *Kv1.4, Kv4.2, Kv4.3,* and β-actin were 55 ºC for 40 s, 30 cycles; 58 ºC for 40 s, 30 cycles; 55 ºC for 40 s, 30 cycles, and 60 ºC for 40 s, 28 cycles, respectively. It was followed by a final extension at 72 ºC for 10 min to ensure complete product extension.

Quantification of PCR products The amplified products were electrophoresed on 1.2 % agarose gel, stained with ethidium bromide, visualized by ultraviolet transilluminator and photographed. The photographs were scanned by digital scanner (Syngene, England). β-Actin was used as an internal control. The optical density (*OD*) values for each band of *Kv1.4, Kv4.2, Kv4.3*, and β-actin on the gel were assayed by gel imaging analysis system (GeneGenius, Syngene, England). The *OD* values of K^+-channel signals were normalized to the *OD* values of the β-actin signals, and were expressed in arbitrary units (the ratio of K^+- channel mRNA levels to the β-actin mRNA levels) for semi-quantitative assay[11].

Statistic analysis The data was expressed as mean±SD, and performed with unpaired Student's *t*-test. Differences were considered to be statistic significant at the level of $P<0.05$.

RESULTS

Changes in hemodynamics The RVSP, an indicator for pulmonary hypertension (PH), was remarkably elevated in the MCT-treated group compared with the control group. Four weeks later in the MCT group, RVSP was increased by 128 % (18.9 mmHg±2.7 mmHg in control *vs* 43 mmHg±7 mmHg in MCT, $P<0.01$), CVP was elevated by 533 % (0.9 mmHg±0.3 mmHg in control *vs* 5.7 mmHg±2.8 mmHg in MCT, $P<0.01$). No significant difference in SBP was observed between control group and MCT group (Tab 1).

Tab 1. Changes of hemodynamics in MCT-induced right hypertrophy. Mean±SD. $^cP<0.01$ *vs* control.

Group	*n*	SBP/mmHg	CVP/mmHg	RVSP/mmHg
Control	10	142±21	0.9±0.3	18.9±2.7
MCT	6	128±14	5.7±2.8^c	43±7^c

n, number of rats; MCT, monocrotaline; SBP, systolic blood pressure; CVP, central venous pressure; RVSP, right ventricular systolic pressure.

Cardiac remodeling by MCT The weight of body, heart, and lung were weighed on d 28 of MCT, respectively. In MCT-treated rats, the ratios of RVW/BW, RVW/(LVW+SW), and LW/BW were all increased significantly by 141.5 %, 96.0 %, and 91.3 % compared with these in the control group, respectively. The BW of MCT-treated rats were decreased by 24.1 % compared with that of control on d 28 (Tab 2).

mRNA Expression of *Kv1.4, Kv4.2,* and *Kv4.3* in normal myocardium The distribution of *Kv1.4* and *Kv4* family was found to be uneven in normal myocardium. The expression of *Kv1.4* and *Kv4.3* had no difference between the three different regions, however, expression of *Kv4.2* gene was lower in septum compared with RV and LV myocardial wall (Fig 1).

mRNA Expression of *Kv1.4, Kv4.2,* and *Kv4.3* in diseased myocardium induced by MCT There was no significant difference in *Kv1.4* expression level between the RV,LV, and septum in MCT group compared with control group. For *Kv4.2* and *Kv4.3*, the mRNA expression level in the RV were dramatically decreased by 45.2 % (control *vs* MCT: 0.64±0.12 *vs* 0.35±0.11, $n=6$, $P<0.01$) and 51.1 % (control *vs* MCT: 0.96±0.09 *vs* 0.47±0.08, $n=6$, $P<0.01$) compared with that in

Tab 2. Changes in weight of lung, body, left and right ventricle, and septum in MCT rats. Mean±SD. $^cP<0.01$ *vs* control.

Group	*n*	BW/g	10^{-3}×RV/BW	RVW/(LVW+SW)	10^{-3}×(LVW+SW)/BW	10^{-3}×Lung/BW
Control	10	299±21	0.53±0.01	0.25±0.02	2.30±0.16	5.8±0.4
MCT	6	227±18^c	1.28±0.12^c	0.49±0.06^c	2.56±0.13	11.0±1.3^c

BW, body weight; RV, right ventricle weight; LVW+SW, left ventricle weight+septum weight.

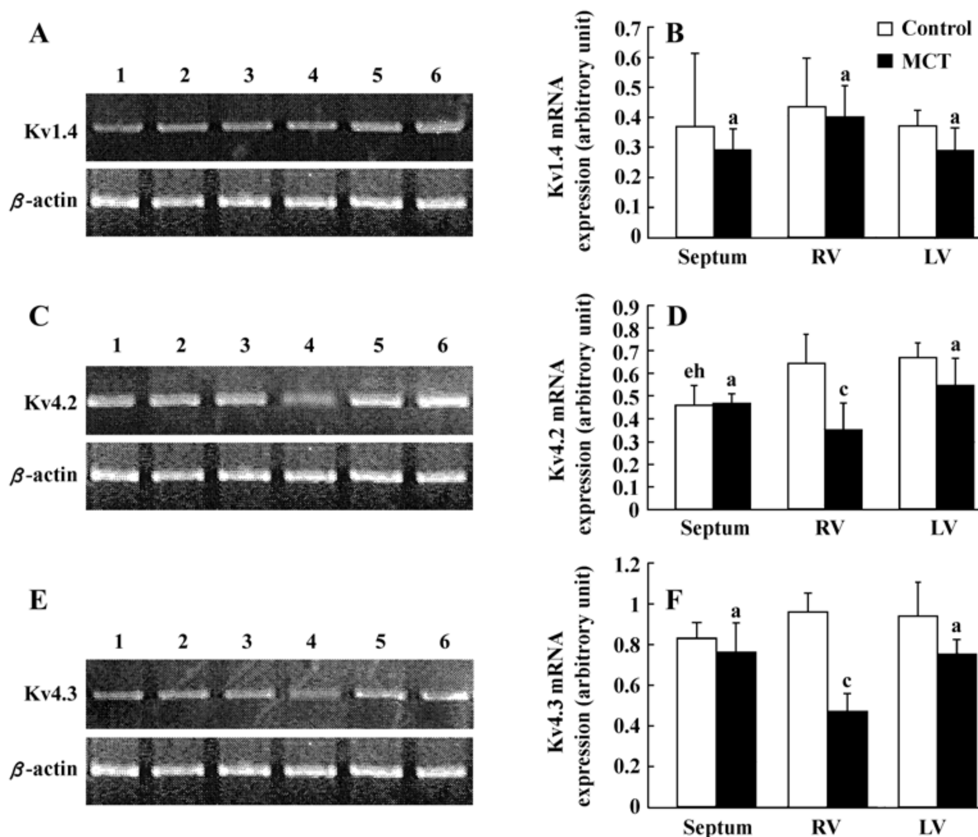

Fig 1. Calcium-independent potassium channel mRNA expression in right ventricular, left ventricular, and septum tissue of control and MCT-induced right hypertrophied rats. A, C, and E show the mRNA expression of *Kv1.4, Kv4.2,* and *Kv4.3*, respectively. β-Actin mRNA as an internal standard. Each lane stands for as following: lane 1, control septum; lane 2, MCT-induced septum; lane 3, control RV; lane 4, MCT-induced RV; lane 5, control LV; lane 6, MCT-treated LV. B,D, and F show the average of semi-quantitative amounts of *Kv1.4,Kv4.2*, and *Kv4.3*, respectively, mRNA is presented as ratio to internal standard (n=6). Mean±SD. $^{a}P>0.05$, $^{c}P<0.01$ *vs* control; $^{e}P<0.05$ *vs* RV in control; $^{h}P<0.05$ *vs* LV of control.

control rats. In the LV and septum, however, no significant difference was found (Fig 1).

DISCUSSION

Distribution of I_{to} is different among the free wall of left and right ventricle and septum in rats. The mRNA expression of *Kv4.2* is uneven in the normal myocardium, lower in the septum than that in RV and LV, which is in agreement with the finding that I_{to} is higher in the epicardium than in the endocardium[12]. So the variation in distribution of I_{to} in the rat ventricle is a matter of physiological pattern, which may not contribute to the dispersion of repolarization. Reduction in I_{to} produced by down-regulation of *Kv4.2* and *Kv4.3* gene expression is closely related to ventricular hypertrophy and action potential duration prolongation, which are likely stimulated by calcineurin in cultured neonatal rat ventricular myocytes[13].

A number of different pathological conditions can alter the functional state of I_{to} such as heart failure and ventricular hypertrophy. Therefore, I_{to} is an important target for anti-arrhythmic drugs to improve the dispersion of repolarization in a diseased heart. Consequently, a complete understanding of molecular basis and function of I_{to} under both normal and pathological conditions is needed for new drug discovery. In our previous study, the mRNA expression of voltage-gated potassium channel is down-regulated in a remodeled rat ventricle[14] which is induced by over dose of *L*-thyroxin for 10 d. MCT, a pyrrolizidine alkaloid, induced the development of pulmonary hypertension to right ventricular hypertrophy by injuring pulmonary vascular endothelial cells and inducing proliferation of pulmonary vascular smooth muscle cells[15]. In the present study the RVSP and CVP were dramatically elevated in MCT group. An elevation in CVP reflects the right heart failure that resulted from sustained elevation of pulmo-

nary arterial pressure. The mRNA level of *Kv4.2* and *Kv4.3* was dramatically reduced in RV of MCT-treated rats, which is in accord with the fact that in hypertrophied myocardium, reduction of I_{to} is a common phenomenon[16]. Two factors may be involved: first, remodeling of ion channel, caused a relative or absolute decrease in density of ion channel; second, alteration of trans-membrane signaling[17]. So we concluded that the down-regulation of mRNA expression of I_{to} was correlated to the myocardial remodeling induced by MCT, especially for the *Kv4* family such as *Kv4.2* and *Kv4.3* gene encoding channels, which might be the pharmacological target for treating cardiac remodeling in heart failure, pulmonary hypertension, and cardiac arrhythmias.

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