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Alteration of endothelin system and calcium handling protein in left ventricles following drug treatment in dilated cardiomyopathy rats¹

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KEY WORDS congestive cardiomyopathy; ventricular remodeling; adrenergic receptors; calcium; endothelin receptors

ABSTRACT

AIM: To investigate the changes of cardiac calcium handling proteins and endothelin system in dilated cardiomyopathy (DCM) rats and the effects of perindopril and bisoprolol on the remodeling ventricles. **METHODS:** DCM rats were employed using a 2-kidney, 1-clip hypertensive and diabetic model. Some of the DCM rats were treated with perindopril and bisoprolol for 3 months, respectively. The ratio of left ventricular weight to body weight (LVW/BW), mRNA expressions of calcium handling proteins and endothelin receptors were determined. The alterations of maximum binding capacity (B_{max}) and equilibrium dissociation constant (K_D) values of cardiac endothelin receptors (ETR) and its subtypes were detected. **RESULTS:** Compared with those of normal control, blood pressure, and LVW/BW in the DCM rats were elevated. Sarcoplasmic reticulum calcium pump (SERCA) mRNA expression and SERCA activity decreased in the left ventricle. The ETR B_{max} decreased, especially the endothelin receptor A. Endothelin converting enzyme activity and expression were elevated, and mRNA expressions of β_1 -adrenoreceptor and inositol-3-phosphate receptor in some hearts increased as well. The administration of perindopril and bisoprolol could reverse myocardial hypertrophy and restore the imbalance of calcium handling proteins and endothelin system. **CONCLUSION:** The disorder of calcium handling proteins and endothelin system existed in the hearts of DCM rats. Treatment of perindopril and bisoprolol could reverse myocardial hypertrophy and changes in DCM rats.

INTRODUCTION

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by progressive depression of myocardial contractile function and by ventricular

dilation. The pathogenic mechanism of DCM is still not clear. In the present study, we measured the changes of cardiac calcium handling proteins and the endothelin system in left ventricular remodeling from DCM rats, and investigated the influences of β -blockers and inhibitor of angiotensin converting enzyme (ACE-I) on the regulation of intracellular calcium and endothelin system.

MATERIALS AND METHODS

DCM model and protocol The DCM model

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adopted was the 2-kidney and 1-clip combined with diabetes rat (SD rats were obtained from Shanghai SIPPR/BK Limited Company, male, 160-180 g), which was a well established and widely used model^[1] with a tail BP over 170 mmHg and blood glucose over 17.78 mmol/L. The DCM rats were divided into a DCM model group (D), a perindopril-treated DCM group (A, ACE-I 1 mg·kg⁻¹·d⁻¹) and a bisoprolol-treated DCM group (B, β_1 antagonist 0.83 mg·kg⁻¹·d⁻¹). Normal SD rats were housed as a control group (S). All rats were sacrificed after treatment for 3 months. The hearts and ventricles were immediately separated and weighted to determine the ratio of left ventricular weight to body weight (LVW/BW). The left ventricles were put into liquid nitrogen immediately, then stored at -75 °C for further study.

mRNA level The mRNA levels of β_1 -adrenoreceptor (β_1 AR), L-Ca²⁺ channel, sarcoplasmic reticulum calcium pump (SERCA), inositol-3-phosphate receptor type I (IP₃-1R), angiotensin II receptor (AT₁R), ryanodine receptor (RyR), endothelin receptor A (ETAR), endothelin receptor B (ETBR) and endothelin converting enzyme (ECE) in the left ventricles from the rats were determined by RT-PCR.

SERCA and ECE activities The activities of SERCA and ECE were determined following the methods of Larsen^[2] and Ahn^[3] respectively, with some modification.

Maximum binding capacity (B_{max}) and equilibrium dissociation constant (K_D) of the endothelin receptor The alterations of B_{max} and K_D of cardiac endothelin receptors (ETR) and its subtypes were detected by radio-ligand binding assay^[4,5]. ET-1 (MERCA) was labeled by National Science Nuclear Institute (Beijing, China).

Statistics Data were expressed as mean±SD. *n* refers to the number of rats. Statistical analysis was performed using analysis of variance or Student's *t*-test.

A value of $P < 0.05$ was considered to be significant.

RESULTS

Systolic blood pressure (SBP) and LV/BW The SBP and LV/BW of the DCM model group were significantly higher than those of the control group ($P < 0.05$). Compared with those of DCM model group, the SBP and LVW/BW of perindopril-treated group decreased very significantly ($P < 0.05$), the SBP of bisoprolol-treated group decreased as well (Tab 1).

Tab 1. Data of dilated cardiomyopathy rats. Mean±SD. ^b $P < 0.05$ vs control group. ^c $P < 0.05$ vs model group.

Groups	<i>n</i>	SBP (mmHg)	LVW/BW (mg·g ⁻¹)
Control group	9	134±6	1.92±0.15
Model group	9	181±11 ^b	2.31±0.28 ^b
Perindopril-treated	6	133±19 ^c	1.98±0.21 ^c
Bisoprolol-treated	6	132±25 ^c	2.25±0.14 ^b

mRNA expression of membrane receptors and calcium handling proteins The mRNA expression of β_1 AR and IP₃-1R in model group was higher than that of control group ($P < 0.05$). No significant differences in mRNA expressions of AT₁R, RyR₂ and L-Ca²⁺ were found between control and DCM model group. Compared with those of DCM model group, the expression of β_1 AR and IP₃-1R of perindopril-treated group was significantly lower ($P < 0.05$). Meanwhile, in perindopril-treated group, the expressions of AT₁R and RyR reduced significantly ($P < 0.05$), SERCA and L-Ca²⁺ channel unchanged. Compared with those of model group, the expressions of IP₃-1R, AT₁R, RyR₂ and L-Ca²⁺ of bisoprolol-treated group decreased, the β_1 AR and

Tab 2. The mRNA expressions of β_1 -AR, AT₁-R and calcium-handling proteins in DCM rats. Mean±SD. ^b $P < 0.05$ vs control group. ^c $P < 0.05$ vs model group.

	Control (<i>n</i> =12)	Model (<i>n</i> =8)	Perindopril-treated (<i>n</i> =8)	Bisoprolol-treated (<i>n</i> =8)
β_1 -AR	0.049±0.023	0.18±0.16 ^b	0.10±0.09 ^c	0.15±0.10
AT ₁ -R	1.0±0.7	1.1±0.5	0.6±0.4 ^c	0.6±0.4 ^c
SERCA ₂	0.46±0.21	0.40±0.16	0.38±0.18	0.34±0.14
IP ₃ -1R	0.16±0.07	0.23±0.14	0.14±0.09 ^c	0.15±0.06
RyR	1.2±0.4	1.00±0.42	0.77±0.19 ^b	0.64±0.27 ^{bc}
L-Ca ²⁺	0.51±0.26	0.44±0.20	0.41±0.17	0.26±0.15 ^{bc}

SERCA unchanged (Tab 2). The SERCA activity of model group was lower than that of control group significantly ($2.656 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ protein vs $1.652 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ protein, $P<0.01$).

B_{max} and K_{D} values of cardiac ETR, $\text{ET}_{\text{A}}\text{R}$ and $\text{ET}_{\text{B}}\text{R}$ The ETR density (B_{max}) of the DCM model group decreased by 29 % compared with the S group, and $\text{ET}_{\text{A}}\text{R}$ was reduced by 33 %. A slight tendency of $\text{ET}_{\text{B}}\text{R}$ reduction was also observed in the same group. High ETR densities were observed in the perindopril-treated and bisoprolol-treated groups, which increased by 62 % and 39 % respectively compared with the DCM model group, $P<0.05$. The subtype of $\text{ET}_{\text{A}}\text{R}$ showed a remarkable increase in the perindopril-treated and bisoprolol-treated groups. No significant differences were observed in $\text{ET}_{\text{B}}\text{R}$ density when the perindopril-treated and bisoprolol-treated groups were compared with the model group (Tab 3).

$\text{ET}_{\text{A}}\text{R}$ and $\text{ET}_{\text{B}}\text{R}$ mRNA expressions The $\text{ET}_{\text{A}}\text{R}$ mRNA level of left ventricles was markedly lower in the DCM model group than in the control group [(0.108 ± 0.025) vs (0.20 ± 0.06) , $P<0.05$]. The $\text{ET}_{\text{B}}\text{R}$ mRNA level was slightly attenuated, but not significantly [(0.296 ± 0.013) vs (0.38 ± 0.05) , $P>0.05$]. The expressions of $\text{ET}_{\text{A}}\text{R}$ and $\text{ET}_{\text{B}}\text{R}$ mRNA in the perindopril-treated and bisoprolol-treated groups were both significantly increased compared with those of the DCM model group [$\text{ET}_{\text{A}}\text{R}$: (0.41 ± 0.06) and (0.45 ± 0.10) , $P<0.01$; $\text{ET}_{\text{B}}\text{R}$: (0.55 ± 0.04) and (0.79 ± 0.26) , $P<0.05$].

mRNA expression and activity of ECE Compared with that of the S group, the ECE mRNA expression of the DCM model group increased by (1.2 ± 0.4) vs (0.7 ± 0.3) , $P<0.01$. The ECE expressions in the perindopril-treated and bisoprolol-treated groups were 0.98 ± 0.02 and 0.73 ± 0.24 , respectively, which were remarkably lower than that of DCM model group, especially the bisoprolol-treated group ($P<0.05$). The

ECE activities of the control, DCM model, perindopril-treated, and bisoprolol-treated groups were (42 ± 10) , (58 ± 9) , (22 ± 6) , and (60 ± 12) pmol/L, respectively. The ECE activity of the DCM model group was markedly higher than those of control and bisoprolol-treated group ($P<0.05$), and was not different to that of the perindopril-treated group.

DISCUSSION

The hypertensive combined diabetic rat as DCM model has been used widely since the pathologic change in the heart is similar to that of human DCM^[6]. Our data demonstrated that LVW/BW increased in the model group than that in the control group ($P<0.05$). The data revealed that left ventricular remodeling was occurring at 4 months, and regulations of calcium handling proteins and membrane receptors were injured in the myocardial cells. The activity and mRNA expression of SERCA decreased and $\text{IP}_3\text{-1R}$ expression increased (by 8 % and 44 %, respectively), which would result in calcium accumulation in myocardial cells, since calcium pump density was decreased and the release channel density was increased. Chronic calcium overload in the myocardial cell has been well known to play an important role for tissue growth through stimulating protein kinase C pathway^[7]. The mRNA expression of $\beta_1\text{-AR}$ in the left ventricles of DCM rats increased by 3.6-fold compared to the control group. Previous data from our laboratory showed that B_{max} of $\beta\text{-AR}$ increased about 40 % with no change of the K_{D} value in left ventricles at the early stage of DCM^[8]. Through stimulation of the cyclic adenosine monophosphate and kinase upregulation, $\beta\text{-AR}$ would at least partially promote the hypertrophic process in the ventricle. Both the gene expression and the density of ETR were markedly attenuated in left ventricular of DCM rats. At the same

Tab 3. The B_{max} and K_{D} values of ETR and subtypes in the left ventricle of DCM rats. Mean \pm SD. ^b $P<0.05$ vs control group. ^c $P<0.05$ vs model group. B_{max} [nmol/g (protein)], K_{D} (nmol/L).

	Control (n=7)	Model (n=7)	Perindopril-treated (n=6)	Bisoprolol-treated(n=6)
ETR B_{max}	92 \pm 34	66 \pm 17 ^b	106 \pm 24 ^c	91 \pm 24 ^c
ETR K_{D}	0.044 \pm 0.007	0.053 \pm 0.014	0.059 \pm 0.011 ^b	0.053 \pm 0.012
$\text{ET}_{\text{A}}\text{R}$ B_{max}	64 \pm 23	43 \pm 5 ^b	89 \pm 20 ^c	64 \pm 16 ^c
$\text{ET}_{\text{A}}\text{R}$ K_{D}	0.040 \pm 0.012	0.035 \pm 0.010	0.061 \pm 0.012 ^{bc}	0.044 \pm 0.010
$\text{ET}_{\text{B}}\text{R}$ B_{max}	24 \pm 14	19 \pm 5	18 \pm 8	21 \pm 12

time both ECE expression and activity were elevated in DCM rats. The administration of perindopril and bisoprolol could at least partially reverse myocardial hypertrophy in DCM rats by lowering the mRNA expression of β_1 -AR, AT₁R, IP₃R, RyR, and L-Ca²⁺ channel in the left ventricular and restoring the balance of calcium handling proteins and endothelin system. These changes in the calcium handling proteins and endothelin system indicated the existence of cross-talk among endothelial system, renin-angiotensin system, and beta-adrenergic system^[9,10]. The effects of each system could be strengthened by interacting with each other.

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