Short communication

Low-dose carvedilol reduces transmural heterogeneity of ventricular repolarization in congestive heart failure[1]

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Key words

Abstract

Aim: To study the effects of carvedilol on the transmural heterogeneity of ventricular repolarization in rabbits with congestive heart failure (CHF). **Methods:** Rabbits were randomly divided into 3 groups: control, CHF and carvedilol treated CHF group. Monophasic action potential duration (MAPD) in the 3 myocardial layers was simultaneously recorded. **Results:** All the rabbits in the CHF group had signs of severe CHF. Compared with the control group, the mean blood pressure and cardiac output were significantly decreased, while peripheral resistance was significantly increased in the CHF group. This proved that the CHF model was successful created with adriamycin in this study. Compared to the control group, the ventricular fibrillation threshold (VFT) was remarkably decreased and all MAPD of the 3 myocardial layers were extended in rabbits with CHF. However, the extension of MAPD in the midmyocardium was more obvious. The transmural dispersion of repolarization (TDR) was significantly increased in CHF. Low-dose carvedilol (0.25 mg/kg, twice daily) had no effects on ventricular remodeling. Treatment with low-dose carvedilol significantly increased VFT. Although the MAPD of the 3 myocardial layers were further prolonged in the carvedilol treated CHF group, the prolongation of MAPD in the midmyocardium was shorter than those in the epicardium and endocardium. Treatment with low-dose carvedilol significantly decreased TDR in CHF. **Conclusion:** In the present study, the transmural heterogeneity of ventricular repolarization increased in the rabbits with CHF. Low-dose carvedilol decreased the transmural heterogeneity of ventricular repolarization in CHF, which may be related to its direct electrophysiological property rather than its effect on ventricular remodeling.

Introduction

Congestive heart failure (CHF) is the final stage of various structural cardiovascular, such as dilated cardio-myopathy, coronary heart disease, and hypertension. Patients with CHF have a high incidence of ventricular arrhythmias causing sudden cardiac death (SCD)[1,2]. However, the mechanism of ventricular arrhythmia in CHF is unclear.

On the other hand, recently some researches have proved that there are different electrophysiological characters among epicardial, midmyocardial, and endocardial cells. It is known that the midmyocardium has longer monophasic action potential duration (MAPD) than the epicardium and endocardium[3,4]. The transmural dispersion of repolarization (TDR) provides a basis for the development of malignant ventricular arrhythmia, such as Torsade de pointes[5]. After myocardial infarction in animals, an increase of TDR leads to ventricular transmural re-entrance and re-entrant arrhythmia[6,7], so the increasing TDR may be the main electrophysiological mechanism for malignant ventricular arrhythmia in CHF.

Carvedilol, a nonselective alpha- and beta-adrenergic receptor antagonist, has been reported to reduce ventricular arrhythmias in patients with CHF[8,9]. However, the electrophysiological mechanisms underlying this improvement are

not fully understood. We hypothesized that the anti-arrhythmic action of carvedilol may be related to its electrophysiological effects on the midmyocardium.

To verify our hypotheses, we recorded the monophasic action potential (MAP) of the 3 myocardial layers in rabbits with CHF and observed the effects of carvedilol on the transmural heterogeneity of ventricular repolarization. Our final aim was to study the electrophysiological mechanism of ventricular arrhythmia and reduce SCD in patients with CHF.

Materials and methods

Animal models Twenty four adults, New Zealand White rabbits of either sex, weighing 2–3 kg, were randomly divided into 3 groups: control, CHF, and carvedilol treated CHF group. The rabbit model with CHF used in this study was previously described[10]. Adriamycin (1 mg/kg, twice weekly) was intravenously administered via a marginal ear vein for 8 weeks. In the CHF group treated with carvedilol, the rabbits received low-dose carvedilol (0.25 mg/kg, twice daily) orally for 8 weeks at the same time[11]. In both of the groups, the last administration of adriamycin or carvedilol was given 48 h before the experiment. All the rabbit hearts were perfused with Tyrode's solution *in vitro*, and the monophasic action potential (MAP) in the 3 myocardial layers was recorded.

Measurement of hemodynamic parameters Aortic catheters were chronically placed into the aorta of all the animals to measure hemodynamic parameters, including mean blood pressure (MBP), cardiac output (CO), and peripheral resistance (PR) by the thermodilution technique using 5% dextrose injected into the right atrium at 25 °C. The thermistor catheter was inserted into the aorta via the left ilio-lumbar artery under anesthesia and positioned just below the renal arteries[12]. Right atrial and central ear artery thermistor catheters were inserted under local anaesthesia (2% xylocaine) at least 1 h before the study.

Heart perfusion All rabbits were anticoagulated with heparin (1.7 μkat/kg, iv) and anesthetized with urethane (1 g/kg, iv). The chest was opened via a left thoracotomy, and the heart was excised and placed in cold Tyrode's solution and stored at 4 °C. The aorta was cannulated and the heart was connected to a Longendorff perfusion system (Radnoti Glass Technology, Monrovia, CA, USA). The preparation was then placed in a small tissue bath and arterially perfused with Tyrode's solution (115.0 mmol/L NaCl, 5.4 mmol/L KCl, 1.0 mmol/L MgCl$_2$, 1.8 mmol/L CaCl$_2$, 1.0 mmol/L NaH$_2$PO$_4$, 5.0 mmol/L HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), and 10.0 mmol/L glucose buffered with 95% O$_2$ and 5% CO$_2$) at 37 °C. The perfusate was delivered by a roller pump (Cole-Parmer Instruments, Chicago, IL, USA). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Sarasota, FL, USA) and maintained between 50 and 60 mmHg by adjustment of the perfusion flow rate. The rabbit hearts were allowed to equilibrate in the tissue bath until they were electrically stable, usually for about 1 h[13,14].

Electrophysiological recordings A transmural electrocardiogram (ECG) signal was obtained with 4 silver–silver chloride electrodes and amplified with a standard ECG amplifier. The electrodes for the 3 layers of the myocardium were made from a 0.3 mm diameter silver-silver chloride electrode thread insulated with Teflon except at the tip. The epicardium electrode was placed on the anterior wall of the left ventricle[12,13]. Compared to the left ventricular wall and midmyocardial region thickness in normal rabbits and the left ventricular wall thickness in heart failure rabbits, the electrode was inserted into the midmyocardial region, with the tip of the electrode placed 2.0–2.5 mm up to the epicardial surface (2 mm in the CHF and CHF treated with carvedilol groups and 2.5 mm in the control group, Figure 1)[13]. The endocardium electrode was inserted into the left ventricular cavity and maintained tight contact with the endocardium.
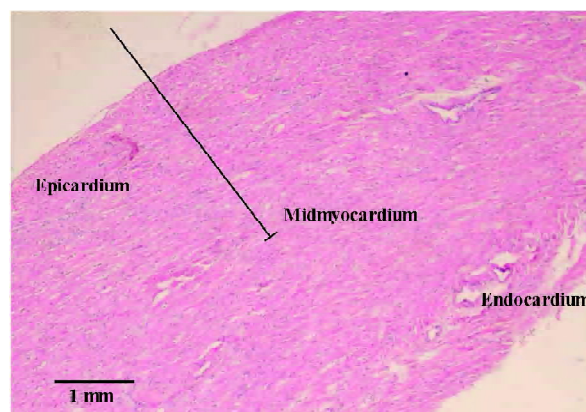

Figure 1. Histological section (×100) showing site of electrode in the midmyocardial region of the left ventricular wall, and the tip of the electrode placed 2.5 mm up to the epicardial surface.

The atrioventricular node was ablated by cautery to slow the intrinsic heart rate and allow the heart to pace at a fixed rate. Right ventricular pacing was set at a cycle length of 1 s for at least 20 min to assure steady parameters. The MAP electrodes of the epicardium, midmyocardium, and endocardium were connected to a high-input impedance amplifier, and the action potentials in the 3 myocardial layers were

simultaneously recorded. The MAPD was measured at 90% repolarization ($MAPD_{90}$). $\Delta MAPD_{90}$ was the difference of $MAPD_{90}$ fixed between the midmyocardium and endocardium, which was not related to the $MAPD_{90}$ of the epicardium. The TDR was defined as the difference between the longest and the shortest $MAPD_{90}$ among the 3 myocardial layers[14].

For the next protocol step, heart pacing was continued at a fixed cycle length of 1 s. An approximate ventricular fibrillation threshold (VFT) was measured by increasing the amplitude in 0.3 V steps from 1 V until ventricular fibrillation (VF) and sustained for at least 4 s[15] (Figure 2).
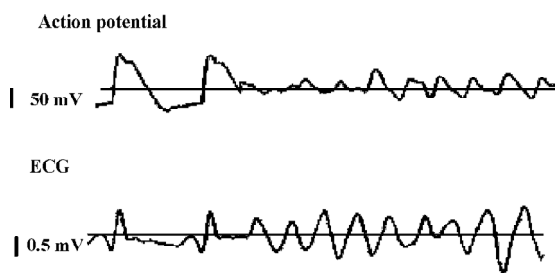
Figure 2. Examples showing VF respectively recorded on ECG and action potential to measure the VF threshold by increasing the amplitude from 1 V until VF was initiated.

Measurement of ventricles Each rabbit heart was weighed. The ventricular internal dimension (VID) and ventricular wall thickness (VWT) were measured by incising the rabbit hearts along with the lower edger of the coronary artery[16].

Statistical analysis All data were presented as mean±SD. The statistical analysis of data was performed using one-way analysis of variance (ANOVA). Significance was defined as a value of *P*<0.05.

Results

Measurements of ventricles and hemodynamic parameters As shown in Table 1, all the rabbits in the CHF and the carvedilol treated CHF groups had signs of severe CHF after being treated with adriamycin for 8 weeks. Heart weight/body weight (HW/BW) and VID were significantly increased (*P*<0.05), and VWT was significantly decreased (*P*<0.05) in the CHF group. Compared with the control group, the MBP fell from 78.2±2.4 to 66.8±2.5 mmHg (*P*<0.05) and CO decreased from 786±24 to 650±47 mL/min (*P*<0.01), while PR increased from 7865±374 to 9470±468 dyn·s·cm⁻⁵ (*P*<0.01) in the CHF group. However, there were no differences in the ventricular cavity, ventricular wall, and hemodynamic parameters between the CHF and the the carvedilol treated CHF groups

(*P*>0.05). Short-term treatment with low-dose carvedilol had no effects on ventricular remodeling.

Table 1. Parameters of ventricular structure and hemodynamics of the 3 groups. ᵇ*P*<0.05, ᶜ*P*<0.01 *vs* control.

	Control (*n*=8)	CHF (*n*=8)	HF+carvedilol (*n*=8)
HW/BW (g/kg)	2.6±0.1	3.0±0.2ᵇ	2.9±0.1
LVID (mm)	5.6±0.4	7.4±0.6ᵇ	7.1±0.6
RVID (mm)	0.9±0.1	1.6±0.2ᵇ	1.5±0.2
LVWT (mm)	5.8±0.5	5.1±0.4ᵇ	5.1±0.7
RVWT (mm)	2.6±0.2	1.8±0.2ᵇ	1.9±0.2
MBP (mmHg)	78.2±2.4	66.8±2.5ᵇ	64.3±2.5
CO (mL/min)	786±24	650±47ᵇ	638±36
PR (dyn·s·cm⁻⁵)	7865±374	9470±468ᵇ	9285±487

LVID, left ventricular internal dimension; LVWT, left ventricular wall thickness; RVID, right ventricular internal dimension; RVWT, right ventricular wall thickness.

Electrophysiological parameters in the control and the CHF group VFT was remarkably decreased (*P*<0.01) in the CHF rabbits compared with the controls. MAPD analysis showed that the midmyocardium exhibited a longer $MAPD_{90}$ than the epicardium and endocardium (*P*<0.05) in the control group. Compared to the control group, all $MAPD_{90}$ of the 3 myocardial layers in the CHF group were extended. Furthermore, compared with those in the epicardium and endocardium, the extension of MAPD in the midmyocardium was more obvious in the CHF group (*P*<0.01). TDR and $\Delta MAPD_{90}$ were significantly increased (*P*<0.05) in the CHF group (Table 2, Figure 3).

Table 2. Electrophysiological parameters among the control group, the CHF group, and the carvedilol treated CHF group. Mean±SD. *n*=8. ᵇ*P*<0.05 *vs* endocardium and epicardium. ᵉ*P*<0.05, ᶠ*P*<0.01 *vs* control; ʰ*P*>0.05, ⁱ*P*<0.05 *vs* CHF.

	Control (*n*=8)	CHF (*n*=8)	HF+carvedilol (*n*=8)
VFT (V)	13.2±2.6	6.7±1.8ᶠ	9.4±1.7ⁱ
Endo-$MAPD_{90}$ (ms)	197.5±16.8	224.6±14.2ᶠ	274.0±30.5ⁱ
Mid-$MAPD_{90}$ (ms)	231.4±20.4ᵇ	280.4±28.8ᶠ	310.2±29.3ʰ
Epi-$MAPD_{90}$ (ms)	205.8±15.1	244.7±19.8ᶠ	283.4±25.3ⁱ
$\Delta MAPD_{90}$ (ms)	20.8±3.0	28.1±4.9ᵉ	22.5±4.8ⁱ
TDR (ms)	25.6±6.4	36.6±15.3ᵉ	26.8±9.2ⁱ
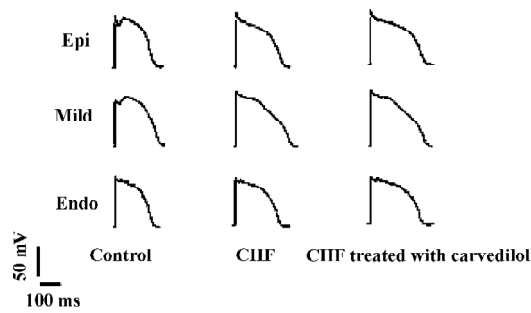
Figure 3. Comparison of MAPD$_{90}$ of the 3 myocardial layers among the control group, the CHF group, and the carvedilol treated CHF group. MAPD$_{90}$ of all myocardial layers were extended in the CHF group, and the extension in the midmyocardium was more obvious (*P* <0.05). Although the MAPD$_{90}$ of the 3 myocardial layers were further extended in the carvedilol treated CHF group, the extension of MAPD$_{90}$ in the midmyocardium was shorter than those in the epicardium and endocardium, while TDR was significantly decreased (*P*< 0.05).

Electrophysiological parameters in the CHF treated with carvedilol group Interestingly, carvedilol treatment significantly increased VFT in the carvedilol treated CHF group. Although the MAPD$_{90}$ of the 3 myocardial layers were further prolonged in the carvedilol treated CHF group, the prolongation of MAPD$_{90}$ in the midmyocardium was significant shorter than those in the epicardium and endocardium (*P*< 0.05). Therefore, TDR and ΔMAPD$_{90}$ were significantly decreased (*P*<0.05) in the carvedilol treated CHF group (Table 2, Figure 3).

Discussion

In the present study, our results showed that chronic administration of adriamycin in rabbits produced an animal model with low output heart failure. All parameters of ventricular structure and hemodynamics were consistent with the characteristics of CHF in the CHF group. Specifically, although it is reported that carvedilol can improve ventricular remodeling, there were no differences between the CHF and the carvedilol treated CHF groups in the ventricular cavity, ventricular wall, and hemodynamic parameters in our study. We temperately ascribed the results to the low-dose administration of carvedilol. This is in accordance with a previous observation that carvedilol has dose-related effects in the prevention of volume expansion and ventricular hypertrophy[17].

CHF is often accompanied with malignant ventricular arrhythmia, such as ventricular tachycardia and fibrillation[1,2]. Our result showed that VFT was significantly decreased in the CHF group, which provides evidence that VF is more

easily induced in CHF. The pathophysiological mechanisms of VF in CHF are probably due to extensive myocardial degeneration, myocardial fibrosis, and increased load, which effect the electrophysiological properties of myocardial cells and promote the occurrence of VF[18,19]. However, the electrophysiological mechanisms of VF in CHF are not well known.

More recently, some researchers have proved that there are different electrophysiological characters among epicardial, midmyocardial, and endocardial cells. MAPD$_{90}$ is significantly longer in the midmyocardium than that in the endocardium and epicardium, which leads to electrophysiological heterogeneity in the ventricular transmural wall[3,4]. Our results are in accordance with this viewpoint.

MAPD dispersion among epicardial, endocardial, and midmyocardial cells provides the electrophysiological basis of ventricular transmural reentrance. In pathophysiological conditions, such as myocardial infarction and hypoxia, ventricular transmural repolarization is increased, which induces early after depolarization and triggers activity[5,20]. Finally, it results in ventricular re-entrant arrhythmia, such as ventricular tachycardia and ventricular fibrillation. The results in our study showed that the prolongation of MAPD$_{90}$ was more marked in the midmyocardium as compared to the epicardium and endocardium. TDR and ΔMAPD$_{90}$ were significant increased in the CHF rabbits.

It is reported that ventricular myocytes of the 3 myocardial layers have different electrophysiological responses to pathological changes. The midmyocardial cells are more sensitive to pathological stimulation than epicardial and endocardial cells[21]. In addition, it is shown that gap junctions among midmyocardial cells with CHF are damaged, and coupled conduction is delayed and even off, which weakens the mutual restraint of midmyocardial cells[22]. Therefore, we speculated that midmyocardial cells with CHF manifest more prominent electrophysiological characteristics and display longer MAPD$_{90}$ than epicardial and endocardial cells.

Interestingly, previous studies have demonstrated that some anti-arrhythmic drugs including quinidine and d-sotalol can extend the 3 myocardial MAPD$_{90}$. However, the extension of MAPD in the midmyocardium is more obvious, which is bound to result in an increase of myocardial heterogeneity and induce fatal arrhythmia such as ventricular tachycardia and ventricular fibrillation[23,24]. Contrarily, carveldilol, a nonselective alpha- and beta-adrenoceptor antagonist, produces a significant reduction in SCD in CHF patients[1,2]. Further studies also show that carvedilol can prevent ventricular arrhythmia in various animal models[25,26]. However, the detailed mechanisms of the anti-arrhythmic action of carvedilol

are not fully understood.

Our study also proved the anti-arrhythmic action in the CHF rabbit model. The results showed that VFT was significantly increased in the carvedilol treated CHF group. Although Keating *et al* reported that it was an indirect result and related to the beneficial effects of carvedilol on ventricular remodeling[27], our result found that low-dose caveldilol could produce a direct beneficial electrophysiological property and reduce TDR among the 3 myocardial layers in the CHF rabbits. However, the ionic mechanisms of the effects of carvedilol on midmyocardial cells are poorly understood.

In conclusion, the current study shows that the transmural heterogeneity of ventricular repolarization increases in rabbits with CHF, which may be the one mechanism of malignant ventricular arrhythmias in CHF. Low-dose carvedilol can decrease the transmural heterogeneity of ventricular repolarization in CHF rabbits, which may be related to its direct electrophysiological property rather than its effect on ventricular remodeling.

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