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LONG-TERM INHIBITION OF Na^+/H^+ EXCHANGE ATTENUATES CARDIAC REMODELING AFTER MYOCARDIAL INFARCTION IN RATS

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Abstract: Objective: In addition to pH regulation, Na^+/H^+ exchanger (NHE) has been shown to facilitate cell growth and proliferation. However, the effects of long-term inhibition of Na^+/H^+ exchange on cardiac structural and functional remodeling post myocardial infarction (MI) are still controversial. The present study was therefore carried out to further investigate the effects of long-term treatment with cariporide, a specific inhibitor of NHE-1, on cardiac remodeling after MI in rats; Methods: Male Wistar rats that underwent coronary ligation were randomly selected for cariporide treatment starting 6 h after induction of MI or no treatment. Treatment was continued up to 6 weeks post MI, after which, the arterial, venous and left ventricular catheters were chronically implanted. Twenty-four h later, after hemodynamic signals were recorded in conscious rats, they were sacrificed and hearts were taken out for morphological examinations; Results: Cariporide treatment decreased the heart weight and heart weight to body weight ratio (both $P < 0.05$), decreased left ventricular end-diastolic pressure ($P < 0.001$), improved myocardial contractility (dP/dt_{max}) ($P < 0.05$) and tended to increase the survival of treated rats compared to that of untreated infarct rats; Conclusion: The results of the present study indicate that the long-term inhibition of NHE with cariporide can attenuate cardiac structural remodeling and improve left ventricular dysfunction in infarcted rats, and suggest that Na^+/H^+ exchange inhibition could be an effective therapeutic strategy for myocardial infarction-induced heart failure.

Key words: myocardial infarction, Na^+/H^+ exchange inhibitor, remodeling, ventricular function
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INTRODUCTION

The Na^+/H^+ exchanger (NHE) is a pH-regulatory protein present in the plasma membrance of cardiomyocytes and other cell types. Although several isoforms of NHE have been decribed, the predominant isoform in the heart is the ubiquitous NHE-1, which under centain physiological conditions, removes one intracellular H^+ in exchange for an extracellular Na^+ (H^+ out, Na^+ in) and thus is involved in the regulation of intracellular pH and in the control of cell volume and of intracellular Na^+ concentration (Fliegel, 1999). The NHE is activated primarily by a reduction in intracellular pH. Recent evidence suggests that a variety of extracellular signals (e.g., catecholamines, angiotensin Ⅱ, thrombin, endothelin, and oxidant stress) also modulate NHE activity by altering its sensitivity to intracellular H^+. Activation of NHE leads to an increase in intracellular Na^+ and subsequent intracellular Ca^{2+} overload through Na^+/Ca^{2+} exchange, which is thought to play an important role in myocardial tissue injury following ischemia and reperfusion. In addition, NHE facilitates the induction of cell growth and proliferation in response to numerous growth factors (Cingolani, 1999). Therefore, the activation of NHE has been recognized as a contributor to various types of cardiac pathologies. Administration of NHE inhibitors in different animal models of ischemia and reperfusion injury have been shown to improve myocardial function (Karmazyn, 1988), decrease incidence of arrhythmias (Yasutake et al., 1994), attenuate calcium uptake (Duan et al., 1992), reduce release of intracellular enzymes (Scholz et al., 1995), diminishcell death and limit infarct size (Gumina et

al., 1998; Klein et al., 1995). However, the effects of long-term inhibition of NHE on cardiac structural and functional remodeling post myocardial infarction (MI) are still controversial. Hasegawa et al. (1995) reported that orally admi nistration of a non-specific NHE inhibitor (amiloride) for 4 weeks attenuated cardiac hypertrophy, reduced diameter of myocardial fiber and left ventricular cavity dimension in infarcted rats. In contrast, Ruzicka et al. (1999) reported that 4 weeks-monotherapy with amiloride starting 3 days before coronary ligation did not significantly attenuate cardiac hypertrophy and improve left ventricular function in rats. The present study was therefore carried out to further investigate the effects of long-term treatment with cariporide, a recently synthesised, specific inhibitor of NHE-1, on cardiac remodeling after MI in rats.

METHODS

1. Animals and model of Myocardial Infarction

Male normotensive Wistar rats weighing 250 – 280 g were obtained from Charles River Inc. (Sulzfeld, Germany). Before operation, the rats were housed in our animal facility for at least 5 days under controlled condition of constant temperature and humidity and exposed to a 12-h light/dark cycle. The rats had free access to a standard rat chow (Ssniff Spezialditen GmbH, Soest, Germany) and drinking water. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85 – 23, revised 1996). The ligation of the left descending coronary artery and sham operations were performed as previously described (Xia et al., 1999). Briefly, animals were anesthetized with ether and an i.v. injection of methohexital-sodium (initially 10 mg/kg), and artificially ventilated. After left thoracotomy, the heart was exposed, and the left descending coronary artery was then ligated with a sterile 6.0 silk suture (Ethibond, Ethicon, Norderstedt, Germany) under a stereomicroscope. Sham-operated rats were subjected to the same protocol, except that the ligation was placed beside the coronary artery. The muscle and skin were sutured and the thorax was closed in layers.

2. Experimental protocol

Rats were divided randomly into three groups and treated according to the following protocol:

Group 1 (sham-operated group, $n = 11$): no treatment.

Group 2 (MI, untreated group, $n = 13$): no treatment.

Group 3 (MI, cariporide treated group, $n = 11$): cariporide treatment was started 6 h after MI.

The compound cariporide was provided in the rat chow pelleted at 3000×10^{-6} (Aventis Pharma Deutschland GmbH, Frankfurt/Main, Germany). Cariporide treatment was started 6 h after surgery and continued up to 6 weeks after induction of MI. Because the food intake was markedly decreased after surgery, cariporide 50 mg/kg · day was additionally administrated by subcutaneous injection in day 1 – 3 after coronary ligation. At the end of the treatment period, arterial, venous and left ventricular catheters were chronically implanted. twenty-four h later, hemodynamic signals were recorded in conscious rats. After the hemodynamic recording, the rats were sacrificed and hearts were taken out for morphological examinations.

3. Hemodynamic studies

Under chloral hydrate (400 mg/kg, i.p.) anaesthesia, polypropylene tubes (Portex, London, UK) were inserted into the right femoral artery and vein and a specially constructed pigtail catheter via the right carotid artery was introduced in the left ventricle. Then the catheters were exteriorized and anchored at the posterior neck region.

Blood pressure, heart rate and left ventricular pressure in conscious rats were recorded Twenty-four h after catheterization. Mean arterial blood pressure, heart rate, left ventricular end-diastolic pressure and dP/dt_{max} were analysed by a computer-based recording and analysing system (MEGA) (Stauss et al., 1990).

4. Cardiac morphological examinations

After recording of the hemodynamic signals, the rats were anaesthetized with ether and the hearts were arrested in diastole by an intravenous injection of KCl solution. The hearts were excised and the atria and large vessels removed. The hearts were cleaned and weighed, then

placed in 4% phosphate-buffered formalin for at least 24 h, and cut transversely into five sections of approximately identical thickness from the apex to the base. These sections were transferred into 10% phosphate-buffered formalin and were kept overnight. After dehydration, the sections were embedded in paraffin, and cut in serial 4-μm-thick slices. The slices were mounted onto glass slides and were stained with haematoxylin-eosin or Goldner, respectively. Morphological parameters were measured by a computerised morphometric system (Quantimet 570, Leica, Cambridge, UK).

5. Measurement of interstitial collagen

After deparaffinization, the sections were stained for 90 min with 0.1% Sirius red F3BA (C.I 35780, Polysciences, Warrington PA, USA) in saturated aqueous picric acid. They were then dehydrated and mounted with coverslips (Junqueira et al., 1979). The collagen content was determined under a microscope coupled to a computerized morphometric system (Quantimet 570, Leica, Cambridge, UK), and was expressed as the percentage of the sum of positive Sirius red areas to the sum of measured areas in the non-infarcted left ventricular free wall and the interventricular septum. Measurements were restricted to the interstitial collagen; perivascular and endocardial collagen were excluded from the measurements.

6. Statistical analysis

All values are expressed as mean ± SD. Statistical analysis of obtained morphological and hemodynamic data was performed using one-way ANOVA. If the ANOVA revealed significance, differences between individual groups were evaluated using Bonferroni test. Survival rates of the treated and untreated groups were comparad using Kaplan-Meier analysis followed by Log-rank test. Differences were considered significant at the level of $P < 0.05$.

RESULTS

1. Infarct size

Infarct size of all animals was measured at the end of the experiment. None of the sham-operated rats had evidence of myocardial infarction, while the coronary ligation animals had microscopic evidence of transmural infarction. Two animals with infarct size of less than 25% were excluded from the study. Compared to the untreated infarct group, cariporide did not significantly decrease the infarct size when treatment started 6 h after induction of MI.

2. Heart mass

After induction of myocardial infarction, food and water intake was reduced in all animals and a decrease of body weight (23.9 ± 8.6) g was observed. One week after coronary ligation the food and water intake was normalized and was not different from sham-operated animals (data not shown). At the end of the study, the body weights were similar in the three groups, although infarcted rats had a small, insignificant decrease in body weight.

Heart mass was evaluated by determining the heart weight (HW) and heart weight to body weight ratio (HW/BW). Although 40.2% the of left ventricular myocardium was replaced by paper-thin scar tissue, HW and HW/BW still were significantly increased in the untreated infarct group compared to sham-operated animals ($P < 0.01$ and 0.001), indicating reactive myocardial hypertrophy has been occurred in remaining myocardium. Cariporide treatment significantly decreased HW and HW/BW compared to untreated MI group (both $P < 0.05$), suggesting NHE inhibitor, cariporide, prevented cardiac hypertrophy.

3. Interstitial collagen content

The interstitial collagen content (ICC) was markedly increased in untreated infarct rats compared to sham-operated animals ($P < 0.001$), and ICC was slightly decreased by cariporide treatment compared to untreated infarct group, but the difference did not reach statistical significance compared with sham-operated rats.

4. Left ventricular size

The left ventricular inner diameter (LVD) and left ventricular circumference (LVC) were used to determine the left ventricular size and dilation. In untreated infarct rats, LVD and LVC were significantly increased compared to sham-operated rats (both $P < 0.001$), cariporide treatment practically did not modify LVD and LVC compared to untreated infarct rats.

Myocardial infarction resulted in a decrease

of septal thickness when compared to the sham-operated group, cariporide treatment slightly but not significantly increased the septal thickness.

5. Left ventricular function

Six weeks after induction of myocardial infarction, mean arterial blood pressure (MAP) was slightly but not significantly decreased in untreated infarct animals compared to sham-operated rats. Cariporide treatment did not influence MAP compared to untreated infarct rats.

Heart rate showed little change in either untreated infarct animals or cariporide-treated infarct rats, nor was there a significant difference between groups.

Left ventricular end-diastolic pressure (LVEDP) was significantly elevated in untreated infarct rats when compared to sham-operated rats ($P < 0.001$). In cariporide-treated animals, LVEDP was markedly decreased compared to the untreated infarct group ($P < 0.001$).

Myocardial contractility (dP/dtmax) was seriously impaired in untreated infarct rats when compared to sham-operated rats ($P < 0.001$). Cariporide treatment improved dP/dtmax when compared to untreated infarct rats ($P < 0.05$).

6. Survival

No animal in the sham-operated group died during the 6 weeks study. At the end of the study, 29 (out of 42, 69%) in the untreated infarct group, 11 (out of 22, 50%) in the cariporide treated group died respectively, which indicated Na^+/H^+ exchange inhibitor cariporide treatment tended to improve survival, although the difference did not reach statistical significance ($P > 0.05$)(Table 1).

Table 1 Effect of cariporide treatment on morphological and hemodynamic parameters

	Sham-operated rats ($n = 11$)	Infarcted rats treated with	
		no treatment ($n = 13$)	cariporide ($n = 11$)
Heart weight(g)	1.20 ± 0.07	1.36 ± 0.11[a]	1.24 ± 0.10[c]
Heart weight/body weight	2.63 ± 0.17	3.05 ± 0.29[b]	2.75 ± 0.23[c]
Infarct size(%)	0	40.2 ± 8.3	38.5 ± 9.6
LV inner diameter (mm)	5.3 ± 0.7	7.7 ± 1.1[b]	7.6 ± 1.0[d]
LV circumference(mm)	22.1 ± 2.0	27.2 ± 2.5[b]	27.3 ± 2.0[d]
Interstitial coolagen content(%)	2.15 ± 0.20	5.22 ± 0.43[b]	4.89 ± 0.30[b]
Septal thickness(mm)	2.02 ± 0.36	1.78 ± 0.25	1.85 ± 0.37
Mean arterial pressure (mmHg)	108 ± 16	98 ± 11	97 ± 10
Heart rate (beats/min)	378 ± 36	388 ± 36	381 ± 39
LVEDP(mmHg)	8.3 ± 3.6	22.4 ± 5.4[b]	12.8 ± 5.6[d]
dP/dt_{max}(1000 mmHg/s)	10.32 ± 2.18	5.81 ± 2.52[b]	8.50 ± 1.59 \ + c

Values represent the mean ± SD. LV, left ventricular; LVEDP, left ventrcular end-diastolic pressure; dP/dt_{max}, the maximum rate of rise of the left ventricular systolic pressure;

[a] $P < 0.01$, [b] $P < 0.001$ as compared to sham-operated group; [c] $P < 0.05$, [d] $P < 0.0001$ as compared to no treatment infarct group.

DISCUSSION

The hypothesis that scarcolemmal Na^+/H^+ exchanger activity may contribute to myocardial injury during ischamia and reperfusion was raised by Lasdunski et al in 1985 (Ladzdunski et al., 1985). Initial pharmacological evidence in support of the Na^+/H^+ exchange hypothesis was first provided by Karmazyn (1988), who showed that Na^+/H^+ exchange inhibitor, amiloride, enhanced the postischamic recovery of the contractility function and reduced the release of creatine kinase in rat hearts subjected to global ischamia and reperfusion. Since then, a number of experimental studies have demonstrated that the Na^+/H^+ exchange inhibitors, such as HOE 694, HOE 642 (cariporide) were cardioprotective in a variety of animal models of ischamia and reperfusion. However, the information is limited and conflicted regarding the effects of long-term inhibition of Na^+/H^+ exchange on cardiac structural and functional remodeling in myocardial infarction-induced heart failure. Accordingly, the present study was designed to further investigate

the effects of long-term treatment with cariporide on cardiac morphological remodeling and left ventricular dysfunction in a rat model of MI-induced heart failure. Our results showed that long-term treatment with cariporide attenuated cardiac hypertrophy and improved left ventricular function after MI in rats.

1. The effects of Na^+/H^+ exchanger inhibitor cariporide on cardiac structural remodeling

The present study showed that chronic administration with cariporide prevented increase in heart weight and heart weight to body weight ratio, but did not attenuate interstitial collagen deposition and left ventricular dilation, indicating the scarcolemmal NHE – 1 could play an important role in MI-induced cardiac hypertrophy. Our results are in agreement with a recent study of Yoshida et al. (2000), in which the pretreatment with cariporide for 1 week prevented the increase in heart weight, myocyte lengths and area in infarcted rats.

The mechanisms by which cariporide prevented cardiac hypertrophy are not completely understood. Acute myocardial infarction has been demonstrated to activate neurohormonal systems including the renin-angiotensin system, the sympathetic nervous system, and endothelin system. Angiotensin II, cardiac sympathetic activity and endothelin have been implicated in the cardiac remodeling after MI (Sigurdsson et al., 1996). In addition, some evidence suggests that angiotensin II, endothelin and catecholamines can activate NHE (Iwakura et al., 1990; Wallert et al., 1992; Grace et al., 1996; Kramer et al., 1991). In addition, Hori et al. (Hori et al., 1990) reported that Na^+/H^+ exchange inhibitor, amiloride, significantly attenuated the norepinephrine mediated protein synthesis in neonatal rat cardiomyocytes. Accordingly, we speculate that the activation of neurohormonal systems stimulate NHE and might be one of important mechanisms in MI-induced cardiac hypertrophy. However, it is not known whether the elevated NHE activity is a common final pathway for these growth stimuli or one of the parallel mediating mechanisms for MI-induced cardiac hypertrophy. Thus, further studies will be necessary to determine the role of Na^+/H^+ exchanger in MI-induced cardiac hypertrophy.

2. The effects of Na^+/H^+ exchanger inhibitor cariporide on functional remodeling of left ventricle

Six weeks after induction of MI, LVEDP was markedly elevated, whereas myocardial contractility ($dP/dt\max$) was seriously impaired in untreated infarct rats compared to sham-operated animals; cariporide treatment significantly improved left ventricular function. These functional effects of cariporide are in line with data reported previously by others (Hasegawa et al., 1995). Although Na^+/H^+ exchange inhibition is cardioprotective in ischemic myocardium, the mechanisms of action remain speculative. Because intracellular Ca^{2+} overload is one of major factors for reperfusion injury. It has been demonstrated that the Na^+/Ca^{2+} exchanger is mainly responsible for the intracellular Ca^{2+} overload, which occurs secondary to intracellular Na^+ overload. In ischemic myocardium, the Na^+/H^+ exchanger is activated, and results in intracellular Na^+ overload and subsequent Ca^{2+} overload through Na^+/Ca^{2+} exchange. In addition, intracellular acidosis can protect the myocardium against reperfusion injury. Accordingly, it was hypothesized that the inhibition of Na^+/H^+ exchange by attenuation of intracellular Ca^{2+} overload and prolongation of acidosis might be cardioprotective. This hypothesis has been confirmed by recent studies (Hartmann et al., 1999; Stromer et al., 2000). However, whether the cardioprotection of cariporide on MI-induced left ventricular dysfunction is through similar mechanisms need to be further studied.

CONCLUSIONS

This study demonstrated that chronic treatment with a Na^+/H^+ exchanger-1 inhibitor cariporide can attenuate cardiac hypertrophic remodeling and improve left ventricular function and survival after myocardial infarction in rats. Our findings suggest that Na^+/H^+ exchange inhibition could be a effective therapeutic strategy for myocardial infarction-induced heart failure. Further studies will be required to reveal the action mechanisms of Na^+/H^+ exchange inhibitors in myocardial infarction-induced cardiac remodeling.

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