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Inhibition of Na^+–H^+ exchange by cariporide reduces inflammation and heart failure in rabbits with myocardial infarction

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1 The aim of this study was to assess the effects of the Na^+–H^+ exchange inhibitor cariporide on left ventricular (LV) morphology and function as well as inflammation in rabbits with heart failure.

2 Rabbits with myocardial infarction (MI) and sham controls were randomized to receive either standard chow or chow supplemented with cariporide for 9 weeks. LV morphology was determined by echocardiography. LV systolic and diastolic function was assessed under load-dependent and -independent conditions by analysis of LV pressure–volume loops using piezo-electric crystals.

3 Plasma concentrations of C-reactive protein and aldosterone were measured.

4 Rabbits with MI developed LV dilatation that was reduced by cariporide. Systolic and diastolic LV function was impaired in rabbits with MI when compared to sham, as indicated by a decreased dP/dt_{max} (MI: 3537 ± 718 mmHg s^{-1}, sham: 5839 ± 247 mmHg s^{-1}, $P<0.05$), the load-independent preload recruitable stroke work (PRSW)(MI: 22 ± 7 mmHg, sham: 81 ± 23 mmHg, $P<0.05$) and a reduction in the time constant of relaxation tau (τ) (MI: 27 ± 1 ms, sham: 17 ± 1 ms, $P<0.05$), and significantly improved by cariporide (dP/dt_{max}: 4586 ± 374 mmHg s^{-1}, PRSW: 67 ± 18 mmHg, τ: 20 ± 2 ms; $P<0.05$ vs MI/control).

5 Induction of MI was associated with an increase in aldosterone and CRP, indicating activation of the neurohormonal and the inflammatory system that were largely reduced by cariporide.

6 Cariporide improves LV morphology and function post MI and suppresses inflammation and neurohormonal activation in congestive heart failure (CHF). Na^+–H^+ exchange inhibition may represent a new pharmaceutical approach for the treatment of CHF.
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Keywords: Myocardial infarction; rabbit; heart failure; remodeling; Na^+–H^+ exchanger; C-reactive protein; inflammation; PV loops

Abbreviations: CHF, congestive heart failure; CRP, C-reactive protein; LV, left ventricle; MI, myocardial infarction; PRSW, preload recruitable stroke work; PV loops, pressure–volume loops

Introduction

Congestive heart failure (CHF) is a common clinical syndrome characterized by abnormalities of cardiac function and morphology. Despite continued improvement in diagnosis and therapy of CHF, the prevalence and incidence remains high (Dhir & Nagueh, 2002). Myocardial infarction (MI) represents the most important cause for the development of cardiac failure, and is currently the subject of intense investigative interest (Bolognese & Cerisano, 1999). Acute MI is followed by left ventricular (LV) remodeling, which is a dynamic process characterized by morphological, functional, biochemical and molecular alterations in the myocardium, finally leading to CHF. This process includes infarct expansion as well as compensatory reactive hypertrophy and dilatation of the noninfarcted LV (McKay *et al*., 1986). It is well known that LV remodeling is accompanied by activation of the neurohormonal system, but other factors such as pro-inflammatory cytokines including TNF-α, Il-1β and IL-6 also play an important role in this process (Mann, 2002).

There is some experimental evidence suggesting that the Na–H exchanger-1 (NHE-1) is involved in the signal transduction pathway leading to the hypertrophic response in cardiomyocytes (Hori *et al*., 1990; Dostal & Baker, 1998; Hayasaki *et al*., 1999).

The NHE represents a family of transporters consisting of six family members that regulate intracellular pH by removing protons in exchange for sodium influx (Karmazyn *et al*., 1999).The NHE-1 subtype is the primary isoform in cardiac cells. There is a large body of evidence demonstrating that pharmacological inhibition of NHE-1 prevents myocardial ischemia and reperfusion injury in experimental as well as human studies (Linz *et al*., 1998; Rupprecht *et al*., 2000; Knight *et al*., 2001).

This has largely been attributed to the prevention of intracellular Ca^{2+} overload during ischemia and reperfusion by NHE-1 inhibitors. However, there is also some evidence

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suggesting that NHE-1 inhibitors have anti-inflammatory activities in experimental myocardial ischemia and reperfusion, which may contribute to the beneficial effects of these compounds (Hattori *et al*., 2001; Redlin *et al*., 2001).

Besides these beneficial effects on acute myocardial ischemia and reperfusion injury, the NHE-1 may also be involved in the (mal)adaptive hypertrophic response after MI. Indeed, the NHE-1 inhibitor cariporide reduces right and left ventricular (LV) hypertrophy, as well as improves LV function in rat models of CHF induced by MI (Karmazyn *et al*., 2001; Kusumoto *et al*., 2001; Chen *et al*., 2004).

The aim of the present study was to investigate the effect of cariporide on LV morphology and function in a rabbit model of CHF induced by MI. We applied, to our best knowledge for the first time, piezo-electric transducer crystals that allow quantification of LV systolic and diastolic function in the rabbit under both load-dependent and -independent conditions, to quantify the LV pressure–volume (*P–V*) relationship in rabbits with CHF. In addition, as there is first clinical evidence that C-reactive protein (CRP), a nonspecific marker of inflammation, is elevated in patients with CHF (Alonso Martinez *et al*., 2002), we also determined the effect of cariporide on CRP in this model.

Methods

Study design

Male New Zealand rabbits (2.5–3.5 kg) were used for all experiments. Animals were housed in separate cages in an environmentally controlled facility and were maintained on standard rabbit chow and given water *ad libitum*. All animals were treated in accordance to the guidelines of the National Institutes of Health.

Induction of MI by coronary artery ligation

Rabbits were initially anesthetized with propofol (Astra Zeneca, Wedel, Germany; 25 mg kg^{-1} i.v.,), intubated and respired with oxygen and 5% sevoflurane (Abbott, Wiesbaden, Germany) using mechanical ventilation. Animals ($n=38$) underwent a thoracotomy in the fourth intercostal space and the pericardium was opened. In 22 animals, a large branch of the LCx was ligated with a prolene suture, whereas 13 animals underwent a sham operation. Ventricular fibrillation occurred in approximately 60% of LCx-ligated animals. Defibrillation was undertaken with a 2.0 J epicardial shock. After surgery, all rabbits received oxytetracyclinhydrochlorid (Pfizer, Karlsruhe, Germany; 15 mg kg^{-1} s.c.) and metamizol (Hoechst, Unterschleissheim, Germany; 20 mg kg^{-1} i.m.) for 2 days to prevent infections. On the first day, post MI and sham animals were randomized to receive either placebo (MI: $n=10$; Sham: $n=7$) or cariporide (0.3% in chow, MI: $n=12$; Sham: $n=6$).

Echocardiographic assessment of the LV

In vivo transthoracic echocardiography of the LV with use of a 5–8 MHz linear array transducer interfaced with an HDI5000 (ATL, Solingen, Germany) was performed at baseline, 3 and 9 weeks after surgery. Measurements of LV size and function were obtained in all animals. Rabbits were lightly anesthetized with a mixture (0.5 ml kg^{-1} i.m.) of ketamine (Intervet, Unterschleissheim, Germany; 50 mg kg^{-1}) and xylazin (Bayer, Leverkusen, Germany; 10 mg kg^{-1}). M-mode echocardiograms were captured from parasternal, short-axis view. LV end-diastolic and end-systolic diameter (LVDed and LVDsys) and wall thickness of the anterior wall (AWThed) and the posterior wall (PWThed) were assessed at the midpapillary level. Two-dimensional echocardiography was performed in the parasternal long-axis and -axis view to measure the LV end-diastolic area (LVAed).

Fractional shortening (FS) was calculated as:

$$FS = (LVDed - LVDes)/LVDed \times 100$$

Pulse-Doppler was used to record the transmittal flow and LV outflow ejection time (LVET). The velocity of circumferential fiber shortening (Vcf) was calculated as:

$$Vcf = (LVDed - LVDes) \times 1000/LVDed \times LVET$$

Right ventricular (RV) outflow velocity and RV outflow tract diameter at the arch of the pulmonary artery were recorded from a parasternal long-axis view.

Cardiac output (CO) was calculated as:

$$CO = RV\,outflow\,velocity\,time - time\,integral \times [\pi \times (RV\,outflow\,diameter/2)^2] \times Heart\,rate.$$

All echocardiographic data were analyzed online and recorded on paper at 100 mm s^{-1} and on a commercially available analysis system (SonoWin®-2000, MESO).

A blood sample was taken from the auricular artery for the determination of aldosterone, CRP and the plasma concentrations of cariporide.

Open-chest determinations of LV function

At 9 weeks after surgery, rabbits were anesthetized and respired as described above. A 3F micromanometer-tipped catheter (Millar Instruments) was inserted into the LV *via* the right carotid artery. The left carotid artery was canulated for the assessment of blood pressure (BP) and heart rate (HR). A midsternotomy was performed and a flow probe (Transonic) was placed around the aortic root to measure the aortic flow (AF). In a subset of animals, four small (1.0 mm) piezo-electric transducer crystals (Sonometrics, London, Canada) were sutured on the LV to assess the long- and short-axis dimensions and to calculate the LV volume (Sham, $n=5$; Sham/cariporide, $n=4$; MI/control, $n=6$; MI/cariporide, $n=6$).

After a 15-min recovery period, a *P–V* loop was generated by simultaneously recording the left ventricular pressure (LVP) and the volume in the working heart. Left ventricular end-systolic (LVESP) and end-diastolic pressure (LVEDP), BP, HR and AF were recorded. The rate of maximum positive and negative LVP development (dP/dt_{max} and dP/dt_{min}) and tau (τ) were determined. A vena cava occlusion in the thorax was performed to determine the load-independent parameter preload recruitable stroke work (PRSW) and end-systolic *P–V* relationship (ESPVR). All parameters were digitized for 10 cardiac cycles; the averaged data are reported.

After hemodynamic measurements were taken, 5 ml of saturated potassium chloride solution was injected in the LV in order to stop the heart in the end-diastolic status. Subsequently, the heart was quickly removed and weighed.

The left (including septum) and the right ventricles were separated and weighed.

Infarct size

To confirm an equal distribution of MI sizes among the infarcted groups, infarct size was determined by planimetric measurement. The infarct area was stated as percentage of the whole LV.

Plasma concentrations of CRP, aldosterone and cariporide

The CRP levels were determined with a commercially available ELISA (American Diagnostica Inc., U.S.A.). The plasma concentrations of aldosterone were determined using a commercial available radioimmunoassay (DPC Bierheim, Bad Nauheim, Germany). To confirm stable plasma concentrations of cariporide, blood samples were taken after 3 and 8 weeks of treatment. The Plasma NHE1 activity was measured similar to the method previously described (Schwark *et al*., 1998).

Statistical analysis

Values are given as mean ± s.e.m. The statistical significance in mean values was tested by ANOVA, followed by Dunnett's test if appropriate. Calculations were carried out using a GraphPad Prism statistical program (GraphPad Software, San Diego, U.S.A.). A value of $P<0.05$ was considered statistically significant.

Materials

Cariporide (HOE 642) was synthesized by Aventis Pharma, as described previously (Weichert *et al*., 1997). The satured potassium chloride solution was obtained from Sigma Chemical Company (Sigma, Steinheim, Germany).

Results

Infarct-size, mortality, heart and body weights

In 22 rabbits, an MI was induced by ligation of a major branch of the LCx. Of the MI rabbits, 15 (68%) developed ischemia-induced ventricular fibrillation after ligation, which was successfully defibrillated (100%) with an epicardial shock. The survival rate for the entire study period of all MI rabbits was 100%. The infarct size determined by planimetric analysis of the LV showed no significant difference between MI/control and MI/cariporide (Table 1). One rabbit of each MI group was excluded from the study due to an infarct size below 30%.

Body weights were not significantly affected by MI, but tended to be smaller in the MI/cariporide group (Table 1). Heart and LV weight was significantly increased in the MI/control group but not in the MI/cariporide group when compared to sham-operated animals. However, the heart-to-body and LV-to-body ratio was significantly elevated in both infracted groups. RV weight as well as RV-to-body weight ratio was elevated in the MI/control group and this was significantly attenuated by treatment with cariporide (Table 1).

Table 1 Echocardiographic and histomorphometric data

Parameter	*Sham*	*Sham/carip.*	*MI/control*	*MI/carip.*
Histomorphometric				
Body weight (kg)	3.4±0.2	3.6±0.2	3.4±0.1	3.1±0.1
Infarct size (% of LV)	—	—	38.4±1.6	40.4±1.4
Wwt H (g)	7.6±0.5	7.7±0.4	10.2±0.3*	8.8±0.4$^{\#}$
wwt LV (g)	4.3±0.3	4.6±0.2	5.2±0.2*	4.6±0.2
wwt H/body weight	2.25±0.06	2.15±0.05	3.01±0.05*	2.8±0.09*
wwt LV/body weight	1.26±0.02	1.29±0.03	1.52±0.03*	1.46±0.05*
wwt RV (g)	1.7±0.14	1.55±0.1	2.5±0.09*	2.1±0.1*$^{\#}$
wwt RV/body weight	0.5±0.03	0.43±0.02	0.75±0.03*	0.67±0.02*$^{\#}$
Echocardiographic				
Heart rate (bpm)	183±6.9	175±3.9	163±7.2	178±6.1
LVEDA (long) (cm^2)	2.3±0.1	2.5±0.2	3.0±0.1*	2.6±0.2*$^{\#}$
LVEDA (short) (cm^2)	1.6±0.1	1.7±0.1	2.2±0.1*	2.0±0.1*$^{\#}$
AWThed (cm)	0.27±0.02	0.29±0.02	0.27±0.01	0.29±0.01
PWThed (cm)	0.25±0.02	0.26±0.01	0.26±0.01	0.28±0.02
CO ($ml\,min^{-1}$)	313±18	286±11	218±11*	281±17 $^{\#}$
Vcf ($circ\,s^{-1}$)	2.8±0.14	2.6±0.11	1.8±0.12*	2.2±0.13 *$^{\#}$
FS (%)	35.2±1.1	34.5±1.3	27.9±1.4*	30.3±1.8
Ejection fraction (%)	44±6	47.8±2.1	27±1*	32±2*$^{\#}$
Peak E DeccT (ms)	45±1.7	61±4.4	31.9±1.5*	38.7±0.9*$^{\#}$
Peak E velocity ($cm\,s^{-1}$)	56.5±3.7	52.9±2.7	58.5±1.8	53.7±2.3
Peak A velocity ($cm\,s^{-1}$)	38.9±2.7	38.1±2.3	33.8±1.8	35.6±1.9
E/*A* ratio	1.5±0.06	1.4±0.04	1.8±0.08*	1.5±0.07$^{\#}$

Echocardiographic and histomorphometric data in Sham ($n=7$), Sham/cariporide ($n=6$), MI/control ($n=9$) and MI/cariporide ($n=11$) rabbits at 9 weeks after surgery. Data are expressed as mean ± s.e.m. wwt, wet weight; H, heart; LV, left ventricle; RV, right ventricle; LVEDA, LV end-diastolic area (parasternal long- and short-axis view); AWThed, anterior wall thickness end-diastolic; PWTh, posterior wall thickness end-diastolic; CO, cardiac output; Vcf, velocity of circumferential fiber shortening; E, early; A, active/atrial and DeccT, deceleration time. *$P<0.05$ vs Sham. $^{\#}P<0.05$ vs MI/control.

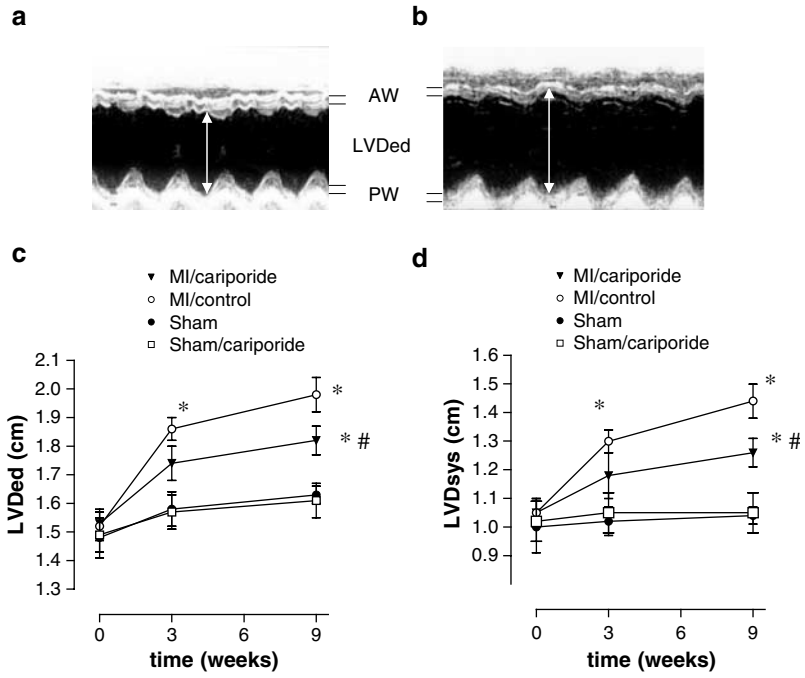

Figure 1 Representative M-mode echocardiograms obtained from MI/cariporide (a) and MI/control (b) at 9 weeks post MI. The process of cardiac enlargement, shown as LVDed (c) and LVDsys (d) of Sham (closed circles, $n=7$), Sham/cariporide (open squares, $n=6$), MI/control (open circles, $n=9$) and MI/cariporide (closed triangles, $n=11$) rabbits at baseline, 3 and 9 weeks after surgery. AW, anterior wall; PW, posterior wall. Values are expressed as mean$\pm$s.e.m. $^*P<0.05$ vs Sham. $^{\#}P<0.05$ vs MI/control.

Importantly, cariporide had no influence on heart weights in the sham-operated group.

Echocardiography

Serial echocardiographic measurements were performed at week 9 after surgery in all groups (Table 1). In the control group, MI resulted in an increase in LV end-diastolic and end-systolic diameter (LVDed and LVDsys, respectively) already at 3 weeks after the induction of MI, which further increased at 9 weeks (Figure 1a–d) when compared to sham-operated animals. LV dilation was also indicated by a significant increase in end-diastolic area (LVAed) (Table 1). In addition, cardiac output (CO), fractional shortening (FS), ejection fraction (EF), velocity of circumferential fiber shortening (Vcf), an index of contractility, as well as the deceleration time of mitral E-wave were significantly decreased in MI/control rabbits, whereas mitral inflow pattern expressed as the E/A ratio was increased, indicating the development of LV systolic and diastolic dysfunction (Table 1). Treatment of rabbits with MI with cariporide significantly attenuated LV enlargement and improved impaired systolic and diastolic function (Table 1, Figure 1). In the MI/cariporide group, there was decrease of LVDed, LVDsys, LVAed and mitral E/A ratio, as well as increase of CO, FS, EF, Vcf and decceleration time of mitral E-wave, when compared to the MI/control group (Figure 1c, d, Table 1). However, anterior and posterior wall thickness was not different between all groups. Echocardiographic measurements revealed no difference in morphology and function between the untreated and cariporide-treated sham group.

Hemodynamics

Induction of MI caused LV dilation and significantly impaired systolic and diastolic function at week 9 in the MI/control group when compared to sham-operated animals (Table 2). The LV end-systolic pressure (LVESP), dP/dt_{max}, an index of myocardial contractility, dP/dt_{min}, an index of myocardial relaxation, and aortic flow (AF) were significantly reduced, whereas the time constant of isovolumic relaxation (τ) was elevated in MI/control animals. Cariporide significantly increased LVESP, dP/dt_{max}, dP/dt_{min} and AF, and attenuated τ in the MI group, but had no effect in the sham group (Table 2). The increased left ventricular end-diastolic pressure (LVEDP) in the MI/control was not affected by treatment with cariporide (Table 2).

There was a rightward shift of the LV P–V loops in the MI/control group under steady-state conditions (Figure 2a), as well as a markedly decreased slope of the end-systolic pressure volume relationship (ESPVR) in the MI/control group compared to sham-operated animals (Figure 2b, c). In MI rabbits on cariporide, the rightward shift of the LV P–V loops was partially reversed and the slope of ESPVR was less depressed compared to MI/control (Figure 2a, c, d). The LV P–V loops showed no difference between the sham and the sham/cariporide group (data not shown). Consistent with the ESPVR data, there was a decrease in the PRSW, a parameter of systolic function that is preload independent and afterload insensitive over the physiological range, in the MI/control group, which was significantly improved by cariporide (Table 2). Finally, systolic blood pressure was significantly reduced in the MI/control (81 ± 5 mmHg) group compared to

Table 2 Hemodynamics under load-dependent and -independent conditions

Parameter	*Sham*	*Sham/carip.*	*MI/control*	*MI/carip.*
Hemodynamics				
AF (ml min^{-1})	345±16.9	307±68.8	268±18.6*	294±12.6*
LVP (mmHg)	110±5.7	106±7.8	78±3.5*	94±6 $^{\#}$
LVEDP (mmHg)	4.5±1.1	3.5±2.1	7.0±0.3*	6.3±0.5
HR (bpm)	288±15.5	273±39	294±5.5	276±5.8
Systolic function				
dP/dt_{max} (mmHg s^{-1})	5839±247	6021±596	3537±718*	4586±374*$^{\#}$
PRSW (mmHg)	80.5±22.5		21.5±6.5*	66.6±17.8$^{\#}$
Diastolic function				
dP/dt_{min} (mmHg s^{-1})	−4909±282	−4586±647	−3174±248*	−4179±376$^{\#}$
τ (ms)	17.4±0.5		26.5±1.1*	20.2±1.6$^{\#}$

Hemodynamic data obtained from Sham ($n=7$), Sham/cariporide ($n=6$), MI/Control ($n=9$) and MI/cariporide ($n=11$) rabbits 9 weeks after surgery. For the parameters PRSW and τ: Sham, $n=4$; Sham/cariporide, $n=4$; MI/control, $n=5$; MI/cariporide, $n=5$. Data are expressed as mean±s.e.m. AF, artic flow; LVP, left ventricular pressure; EDP, end-diastolic pressure; HR, heart rate; dP/dt_{max}, maximal rate of pressure development; dP/dt_{min}, maximal rate of decay of pressure; PRSW, preload recruitable stroke work; τ, monoexponential time constant of relaxation. *$P<0.05$ vs Sham. $^{\#}P<0.05$ vs MI/control.

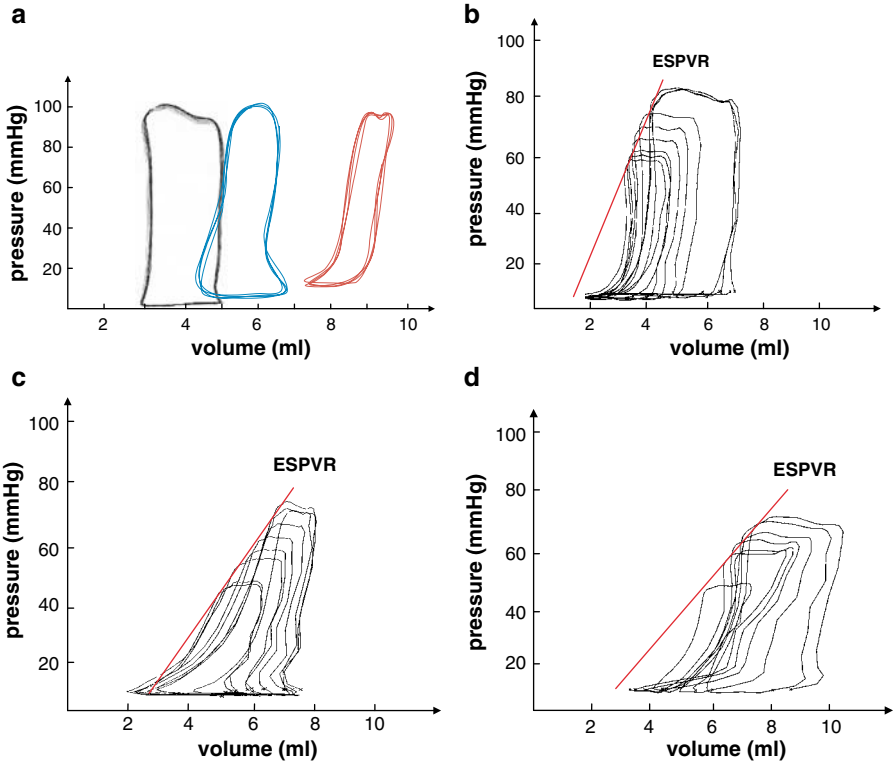

Figure 2 *P–V* loops in rabbits with heart failure induced by MI. (a) Representative, superimposed, steady-state *P–V* loops in sham-operated (black) animals, or rabbits with MI treated with (blue) or without (red) cariporide. (b–d) Representative *P–V* loops during transient reduction of cardiac preload after vena cava occlusion in rabbits at 9 weeks after sham operation (b) or MI treated with (c) or without (d) cariporide. ESPVR, end-systolic *P–V* relationship.

sham-operated (105±7 mmHg) animals, which was significantly ameliorated by cariporide treatment (95±7 mmHg).

CRP, aldosterone, cariporide levels and inhibition of NHE1 activity

Induction of MI resulted in a significant increase in the acute-phase protein, CRP, at 9 weeks when compared to sham-operated animals (MI/control: 271±52 ng ml^{-1}, sham: 130±7 ng ml^{-1}, $P<0.05$) (Figure 3a). Moreover, MI was also associated with a significant increase in aldosterone levels (MI/control: 115±24 pg ml^{-1}, sham: 53±8 pg ml^{-1}, $P<0.05$) (Figure 3b). Treatment of MI rabbits with cariporide significantly reduced both aldosterone and CRP levels (aldosterone: 145±22 pg ml^{-1}, CRP: 84±11 ng ml^{-1}, $P<0.05$), but had no effect on the neurohormonal system in the sham-operated animals (aldosterone: 55.6±9.9 pg ml^{-1}, CRP: 122±9 ng ml^{-1}) (Figure 3a, b). Supplementation of chow with

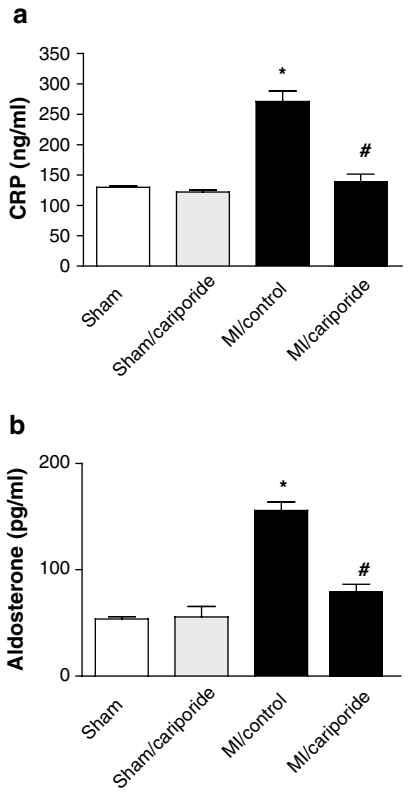

Figure 3 Aldosterone (a) and CRP (b) plasma levels in Sham ($n=11$), MI/control ($n=9$) and MI/cariporide ($n=11$) rabbits 9 weeks after surgery. Values are expressed as mean ± s.e.m. $^*P<0.05$ vs Sham. $^{\#}P<0.05$ vs MI/control.

cariporide (0.3%) resulted in an efficient plasma concentration to completely block the activity of the NHE1 (3 weeks: $2.14\pm0.1\,\mu g\,ml^{-1}$, 8 weeks: $2.73\pm0.1\,\mu g\,ml^{-1}$, $P>0.05$). These plasma concentrations completely inhibited NHE1 activity in the *in vitro* assay (data not shown).

Discussion

CHF is a severe cardiovascular disease increasing in incidence and prevalence. Despite recent advances in heart failure therapy, mortality remains high (Tendera & Ochala, 2001). Therefore, new therapeutic approaches are needed to decrease the morbidity and mortality in heart failure patients. In the present study, we investigated the effect of the Na^+/H^+ exchange inhibitor cariporide on LV remodeling after MI in rabbits. We observed that cariporide attenuated LV hypertrophy and improved LV function without decreasing the blood pressure. In addition, cariporide prevented the activation of the neurohormonal system as well as the inflammatory response, as indicated by a reduction of elevated plasma levels of aldosterone and CRP caused by MI.

Our finding that cariporide reduces left and right ventricular hypertrophy in rabbits with MI is in line with previous findings demonstrating that cariporide reduces hypertrophy of cardiomyocytes in SHR (Camilion de Hurtado *et al*., 2002), rats with heart failure induced by MI (Kusumoto *et al*., 2001) as well as in β1-adrenergic receptor transgenic mice (Engelhardt *et al*., 2002). Importantly, the effect of cariporide on cardiac hypertrophy is blood pressure- and, hence, afterload-independent (Chen *et al*., 2001; Kusumoto *et al*., 2001). Indeed, we showed that cariporide did not only reduce but also increased arterial blood pressure in rabbits with CHF. The precise mechanism explaining the involvement of NHE1 in cardiac hypertrophy or heart failure is still unclear. An increased NHE1 expression and activity has been demonstrated in myocardial samples from rats with ischemia-induced heart failure, in SHR, in β1-adrenergic receptor transgenic mice with heart failure and most importantly in patients with heart failure (Yokoyama *et al*., 2000). In addition, treatment of isolated rat cardiac myocytes with hypertrophic agonists such as α-adrenoceptor agonists (Yokoyama *et al*., 1998), endothelin-1 (Kramer *et al*., 1991), angiotensin II (Matsui *et al*., 1995) or thrombin (Yasutake *et al*., 1996) results in an increase in NHE1 activity. It has been proposed that the NHE1-dependent sodium influx is a major contributor to the hypertrophic response by these agonists, and involves sodium-induced activation of PKC (Hayasaki *et al*., 1999). Sodium load has been shown to exert hypertrophic effects in isolated cardiac myocytes. Interestingly, inhibitors of PKC reduced the hypertrophic response to α-adrenoceptor activation, whereas the NHE1 inhibitor HOE694 decreased both the hypertrophy as well as PKC activation (Hayasaki *et al*., 1999). Besides PKC, other cell-signaling mechanisms may also be involved in the NHE1-mediated induction of cardiomyocyte hypertrophy. Indeed, stretch-induced hypertrophy in cultured cardiac myocytes was associated with activation of Raf-1 and MAP kinase, both of which were partially blocked by the NHE1 inhibitor HOE694 (Yamazaki *et al*., 1998).

To our best knowledge, this is the first study in rabbits investigating the cardiac function under load-dependent and -independent conditions, which allows a more precise evaluation of the LV performance. In the present study, MI resulted in a moderate type of heart failure with depressed systolic and diastolic function, as indicated by a decrease in Vcf, FS, EF, LVP, dP/dt_{max} and dP/dt_{min} and a moderate increase in LVEDP, which was significantly improved by cariporide without decreasing systolic BP and, hence, ruling out afterload reduction as a contributing factor. The load-independent effect of cariporide on systolic LV function is further supported by our finding that cariporide significantly increased PRSW, a modification of the Frank–Starling mechanism that is more or less not influenced by ventricular geometry, preload-independent and afterload insensitive over the physiological range, as assessed by piezo-electric transducer crystals. This is of particular importance, as currently used therapeutic agents such as angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor antagonists reduce afterload, which is the basis of their beneficial effect in heart failure. What, then, is the mechanism by which cariporide improves LV function? It is well accepted that Na^+/H^+ inhibition reduces myocardial infarct size in animal models of acute ischemia and reperfusion (Linz *et al*., 1998; Karmazyn *et al*., 1999). However, in the present study, an ischemic model of permanent occlusion without reperfusion was used, resulting in myocardial infarct sizes that were not modified by cariporide. There is evidence that Na^+/H^+ inhibition prevents cardiac fibrosis. Indeed, cariporide reduced interstitial fibrosis in β1-adrenergic receptor transgenic mice with heart failure (Engelhardt *et al*., 2002). In the present study, histological investigations of the

noninfarcted region of the LV revealed similar results (data not shown). The myocardium of the cariporide-treated animals showed less interstitial fibrosis than the untreated animals. Interestingly, cardiac fibroblasts express NHE-1 and mitogenic stimuli activate NHE-1 on transcriptional and protein levels (McSwine *et al*., 1994). In addition, cariporide improves impaired calcium handling in rats with CHF (Loennechen *et al*., 2002). Thus, it is conceivable to suggest that cariporide directly inhibits myocardial remodeling of the surviving MI.

Heart failure has for a long time been viewed as a syndrome solely driven by activation of the neurohormonal system. Indeed, we demonstrate an activation of the renin–angiotensin aldosterone system (RAAS), as indicated by an increase in plasma aldosterone levels in rabbits with heart failure. Cariporide largely reduced the increase in plasma aldosterone levels, suggesting an improvement in cardiac function in CHF (Bolger *et al*., 2002; Kinugawa *et al*., 2002). However, recent studies demonstrated that inflammatory cytokines such as TNF-α, Il-1β and IL-6 might also play an important role in the pathogenesis of heart failure. Indeed, TNF-α, Il-1β and IL-6 are elevated in animal models as well as in patients with heart failure, and their plasma levels correlate with the severity of the disease according to the New York Heart Association (NYHA) classification (Kubota *et al*., 2000; Kell *et al*., 2002). Moreover, CRP, an unspecific marker of systemic inflammation, is also elevated in patients with heart failure and higher CRP levels have been observed in patients with higher NYHA functional class (Alonso Martinez *et al*., 2002). Importantly, higher CRP levels were related to a higher rate of readmission to the hospital and mortality. We demonstrate for the first time an increase in CRP levels in an animal model of heart failure, emphasizing an ongoing inflammatory process in CHF. This increase in CRP was completely prevented by cariporide. At present, it is unclear whether the normalized CRP levels under NHE1 inhibition indicate a direct involvement of NHE1 in the inflammatory process associated with heart failure or whether it is only due to an improvement in cardiac function. However, there is some evidence that the NHE1 inhibitor cariporide may have anti-inflammatory properties. In isolated coronary microvascular endothelial cells, cariporide attenuated the increased intercellular adhesion molecule-1 (ICAM-1) protein expression induced by hypoxia and reoxygenation (Hattori *et al*., 2001). Moreover, cariporide also attenuated leukocyte adhesion and emigration in rat cremaster muscle (Redlin *et al*., 2001). Further experiments are required to elucidate the potential anti-inflammatory properties of cariporide and to what extent these effects may contribute to the efficacy of cariporide in CHF.

In conclusion, the Na^+/H^+ exchange NHE-1 is directly involved in LV remodeling after MI. In addition, we provide first evidence that NHE-1 may also be involved in inflammatory response associated with CHF. Inhibition of NHE-1 activity with cariporide may be a novel, afterload-independent therapeutic strategy in the treatment of human heart failure.

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