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Effect of long-term B-type natriuretic peptide treatment on left ventricular remodeling and function after myocardial infarction in rats

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ABSTRACT

Although short-term B-type natriuretic peptide (BNP) treatment has been shown to be effective for decompensated congestive heart failure, little is known about the effects of long-term BNP treatment in ventricular remodeling and heart failure in response to myocardial infarction. The aim of the present study was to investigate the effects of long-term BNP treament on ventricular remodeling and heart failure after myocardial infarction in rats. Myocardial infarction was induced by ligating the left anterior descending coronary artery. The surviving rats were randomly divided into four groups: 1) vehicle-treated myocardial infarction group ('vehicle-treated group'), 2) rats treated with low-dose BNP ('low BNP group'), 3) rats treated with high-dose BNP ('high BNP group'), 4) sham-operated group. Eight weeks after the operation, rats were sacrificed. Compared with the sham-operated group, the vehicle-treated group had significantly higher collagen deposition and angiotensin II levels ($P<0.01$) and a significantly lower cardiac function ($P<0.05$). Both BNP-treated groups had significant improvement of these indexes compared with the vehicle-treated group ($P<0.01$). The high BNP group had significantly less collagen deposition and better cardiac function than the untreated and low BNP groups. Moreover, the mRNA and protein expression of TGFβ1 and Smad2 in the vehicle-treated group was significantly higher than in the sham-operated group ($P<0.01$). Both BNP-treated groups had a suppression of TGFβ1 and Smad2 expression ($P<0.01$). In conclusion, long-term treatment with BNP prevents ventricular remodeling and deterioration of cardiac function in a dose-dependent fashion, a process that may be associated with the inhibition of TGFβ1/ Smad2 signaling.

1. Introduction

The improvement of treatments for acute myocardial infarction has led to an increase in post myocardial infarction survival. However, as a consequence of this, the incidence of left ventricular remodeling and congestive heart failure associated with myocardial infarction has increased (Holmes et al., 2005; Jessup and Brozena, 2003; Mann and Bristow, 2005). Left ventricular remodeling is a complex pathological process of progressive left ventricular dilatation, leading to dysfunction and heart failure after myocardial infarction. Although it is initially adaptive, this adaptive remodeling becomes deleterious over time (Sutton and Sharpe, 2000; Tiyyagura and Pinney, 2006). Therefore, preventing the remodeling process is an important approach to prevent heart failure after myocardial infarction.

B-type natriuretic peptide (BNP) is a cardiac hormone produced primarily by ventricular myocytes. BNP not only possesses potent natriuretic, diuretic, vasorelaxant, and lusitropic properties, but also counteracts angiotensin II actions, inhibits renin and aldosterone release, and has direct and indirect antifibrotic properties (Bettencourt, 2002; Kapoun et al., 2004; Kawakami et al., 2004; Tamura et al., 2000). Plasma concentrations of BNP are markedly elevated in patients with congestive heart failure and acute myocardial infarction and are used to aid diagnosis of heart failure (Morita et al., 1993). Recent clinical and experimental studies have indicated that exogenous administration of recombinant human BNP improves hemodynamic parameters in acutely decompensated hearts. Intravenous recombinant human BNP is effective for the short-term (3 or less than 3 days) treatment of decompensated congestive heart failure (Bettencourt, 2002; Colucci et al., 2000). Moreover, Hillock et al. (2008) demonstrated that early short-term (60 h) post-infarct BNP infusions had beneficial effects on later ventricular remodeling and other cardiovascular outcomes. However, little is known about the potential effects of long-term (continue more than 4 weeks) treatment

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with BNP on cardiac remodeling and heart failure following myocardial infarction.

Numerous studies have emphasized the important role of transforming growth factor (TGF)-β1/Smad2 signaling in ventricular remodeling following myocardial infarction (Bujak and Frangogiannis, 2007; Hao et al., 1999; Khan and Sheppard, 2006; Seo and Hare, 2007). TGFβ1 is a cytokine with a broad range of regulatory effects on inflammation and cell proliferation, and it modulates these processes via signaling pathway proteins called smad (Seo and Hare, 2007). TGFβ1 signaling is activated when TGFβ1 binds to its receptors. TGFβ1 receptors 1 and 2 form a dimerized complex. Upon the binding of $TGF\beta_1$ ligand, the type II receptor kinase activates the type I receptor kinase, which initiates the intracellular signal through the phosphorylation of receptor-regulated Smads (R-Smads: Smad2 and Smad3). Once phosphorylated, R-Smads dissociate from the receptor, bind to Co-Smad (Smad4), and enter the nucleus where the complex can directly or indirectly regulate specific gene transcription by interacting with other transcription cofactors (Khan and Sheppard, 2006).

In the present study, we used a rat model of myocardial infarction to determine whether long-term BNP treatment contributes to left ventricular remodeling and cardiac function after myocardial infarction, and whether TGF-β1/Smad2 signaling are involved in BNP-mediated effects.

2. Materials and methods

The investigation was carried out according to the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

2.1. Animals and experimental designs

Male Sprague–Dawley rats (8-week old; 180–200 g) were obtained from the Experimental Animal Facility of Sun Yat-sen University, China. All animals were housed in individual cages on a 12 h light–dark cycle in a room with temperature (24±2 °C) and humidity control, and with ad libitum access to tap water and standard rodent chow. The rats were anesthetized by injection of pentobarbital sodium (40 mg/kg, i.p.) and a left thoracotomy was performed under volume-controlled mechanical ventilation (tidal-volume: 3.0 mL; respiratory rate: 70 cycles/min). Ligatures were placed around the left anterior descending coronary arteries. Similar surgery was performed in sham-operated rats without coronary artery ligation. Twenty-four hours after ligation, the surviving rats were randomly divided into three groups (n=15 per group): 1) vehicle-treated myocardial infarction group ('vehicle-treated group'), 2) rats treated with low-dose BNP ('low BNP group'), 3) rats treated with high-dose BNP ('high BNP group'). Venous access was established in each rat by introducing a 4F catheter into the isolated left extra-jugular vein. The other end of the catheter was then threaded to the back of the rat via a subcutaneous tunnel, was capped with a three-way joint, and was blocked with heparin. Heparin–saline was used to flush and then blocked it twice daily. The catheter was replaced if it was found not to be patent. Treatment started 24 h after operations and continued for 8 weeks. BNP (nesiritide, American Peptide Company, Sunnyvale, CA) was administered via the jugular vein tube by injection once a day at the dose of 5 μg/kg/day (low BNP group) or 15 μg/kg/day (high BNP group) while isovolumic sodium chloride was administrated in the same manner for the sham-operated and vehicle-treated groups.

2.2. Doppler echocardiography and hemodynamic measurements

Eight weeks after myocardial infarction, each rat was weighed. Then, transthoracic echocardiography was performed on each rat. After anesthetizing rats by intraperitoneal administration of pentobarbital (40 mg/kg body weight), left ventricular end-diastolic diameter, left ventricular posterior wall thickness, ejection fraction and fractional shortening were measured or calculated using an echocardiographic system (ATL-HDI5000) equipped with a 10-MHz imaging transducer. All measurements were averaged for 10 consecutive cardiac cycles and performed by an experienced technician who was blinded to study groups.

Then, a catheter was introduced through the right carotid artery into the left ventricle in all animals. Tracings of the left ventricular pressure were digitized with a commercially available analog-to-digital converter and BIOPAC acknowledge analysis software (Goleta, CA). The digitalized left ventricular pressure recordings were used to calculate the maximal rate of pressure rise (dP/d$t$max), the maximal rate of pressure fall (dP/d$t$min), left ventricular systolic pressure, and left ventricular end-diastolic pressure.

2.3. Cardiac morphological parameters and cardiac total collagen

Each heart was arrested in diastole by intraperitoneal injection of KCl and then excised and washed with PBS, and dried with filter paper. The left ventricle was separated and weighed. Tissue samples were rapidly obtained from the non-infarcted region (inferior half of the left ventricle and interventricular septum), frozen in liquid nitrogen, and then stored at a −70 °C freezer. These tissue samples were used for RT–PCR and Western blot. Left ventricular samples (superior half of the left ventricle) were fixed in 10% formalin, imbedded in paraffin, cut into 4 μm sections and stained with Masson's trichrome. The percentage of the area occupied by collagen was quantitated by Masson's trichrome staining which colors the collagen in blue and the myocardium in red. Eight non-vascular areas were randomly chosen in each slide under a microscope. The average value was determined by computer image analysis. The percentage of the collagen area was calculated as the sum of all collagen-positive areas divided by the sum of the muscle and collagen areas.

2.4. Measurement of angiotensinII levels

We used a radioimmunnoassay method to detect the levels of angiotension II in plasma and tissue of each rat 8 weeks after myocardial infarction. Before each rat was sacrificed, 5 ml blood was collected from the right carotid artery in a test tube containing 25 μl of 0.32 M dimercaprol, 50 μl of 0.30 M EDTA-Na_2 and 50 μl of 0.34 M 8-hydroxyquinoline. After they were mixed in ice water bath, the blood samples were centrifuged at 1000 r/min for 5 min at 4 °C. The plasma was collected immediately, and stored at −30 °C for assayed. Tissue samples consisting of the inferior half of each left ventricle, excluding the interventricular septum were used to measure the tissue concentration of angiotension II. After being weighed and minced, each sample (containing 100 mg of myocardial tissue) was added to 1 ml of normal saline containing 25 μl of 0.32 M dimercaprol, 50 μl of 0.30 M EDTA-Na_2 and 50 μl of 0.34 M 8-hydroxyquinoline, and was then homogenized individually on ice. The extracted homogenates were centrifuged at 12000 r/min for 15 min at 4 °C. The supernatant was collected and stored at −30 °C for assayed. The levels of angiotension II in plasma and tissue were measured by radioimmunoassay (Northern Biot Co, Bejing, China) according to the manufacturer's instructions. The intra-assay variation was <5%, and the inter-assay variation was <10%.

2.5. RT–PCR

To evaluate the transcriptional levels of TGF-β1 and Smad2, semiquantitative RT–PCR was performed. In brief, total RNA was extracted with Trizol Reagent (Invitrogen, Carlsbad, Calif) and quantified by

Table 1
Cardiac morphometric parameters and collagen content

Parameters	Sham (n=12)	Vehicle-treated group (n=10)	Low BNP group (n=11)	High BNP group (n=11)
Wt (g)	336.50±16.77	323.10±17.95	329.55±14.26	333.25±15.27
LVW (g)	0.78±0.07	0.81±0.09[a]	0.79±0.08[b]	0.74±0.08[b]
LVMI (mg/g)	1.76±0.11	2.30±0.15[a]	2.01±0.25[b]	1.98±0.16[b]
Collagen (%)	5.13±0.59	9.67±0.71[a]	6.77±0.37[b]	6.00±0.42[b,c]
Plasma Ang II (ng/L)	671±42	2420±290[a]	1186±103[b]	874±59[b,c]
myocardium Ang II (pg/L)	313±23	551±35[a]	433±21[b]	423±28[b]

[a]P<0.01 vs sham, [b]P<0.01 vs vehicle-treated group, [c]P<0.01 vs low BNP group. Wt: body weight, LVW: left ventricular weight, LVMI: left ventricular weight/body weight ratio, Ang II: angiotensin II.

Table 2
Echocardiography and hemodynamic measurements performed 8 weeks after myocardial infarction

Parameters	Sham (n=12)	Vehicle-treated group (n=10)	Low BNP group (n=11)	High BNP group (n=12)
HR (beats/min)	486±53	487±44	500±50	472±45
LVSP (mmHg)	133.18±3.55	120.94±4.35[a]	127.62±1.51[b]	130.11±6.15[b]
LVEDP (mmHg)	20.41±0.97	28.66±1.72[a]	20.30±1.10[b]	12.06±1.79[b,d]
dP/dt_{max} (mmHg/ms)	6.26±0.27	2.98±0.34[a]	4.78±0.41[b]	3.67±0.55[b,d]
dP/dt_{min} (mmHg/ms)	6.16±0.33	2.88±0.31[a]	4.45±0.59[b]	3.48±0.49[c,d]
EF (%)	71.02±7.11	37.93±4.92[a]	45.58±2.94[b]	60.70±3.60[b,d]
FS (%)	38.85±3.36	18.34±3.06[a]	23.70±3.22[b]	31.78±4.27[b,d]
LVDd (mm)	4.30±0.40	6.51±0.40[a]	5.63±0.53[b]	5.11±0.58[b]
LVPWd (mm)	2.14±0.25	1.43±0.10[a]	1.73±0.17[b]	1.90±0.12[b]

[a]P<0.01 vs sham-operated group, [b]P<0.01 vs vehicle-treated group, [c]P<0.05 vs vehicle-treated group, [d]P<0.01 vs low BNP group. HR: heart rate, LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, dP/dtmax: the maximal rate of pressure rise, dP/dtmin: the maximal rate of pressure fall, EF: ejection fraction; FS: fractional shortening, LVDd: left ventricular end-diastolic diameter, LVPWd: left ventricular posterior wall thickness.

spectrophotometry at 260 nm. A one-step RT–PCR kit (Toyobo, Co., Ltd., Osaka, Japan) was used to amplify the target sequences. The primers sequences were, for TGF-β1: 5′-GAAGCCATCCGTGGCCAGAT-3′ and 5′-CCAGTGACGTCAAAAGACAG-3′; for Smad2: 5′-ACTATACCCACTCCATTCCA-3′ and 5′-CACTATCACTTAGGCACTCG-3′; for β-actin: 5′-CCTTCCTGGGTATGGAATCCT-3′ and 5′-GGAGCAATGATCTTGATCTT-3′ β-actin amplification was conducted simultaneously as an endogeneous reference. Cycling conditions for cDNA production were as follows: at 38 °C for 20 min; for PCR, cycling conditions for TGF-β1 were as follows: hold at 95 °C for 5 min, followed by32 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s; cycling conditions for Smad2 and β-actin were as follows: hold at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s. The RT–PCR products were then analyzed by electrophoresis on 1.5% agarose gel with ethidium bromide staining. The relative amount of each mRNA was determined by densitometric analysis and normalized to the control (β-actin).

2.6. Western blot

Cardiac tissues were homogenized in liquid nitrogen and then in SDS sample buffer to extract total protein. Total protein concentration was determined using the BCA Protein Assay Kit. The crude protein extracts 30 μg were then loaded to a 10% SDS–polyacrylamide gel and transferred to a PVDF membrane. The membrane was blocked for 2 h at room temperature in blocking solutions and incubated with anti-TGF-β1 (1:500 dilution, Santa Cruz Biotechnology, Santa Cruz, CA), anti-Smad2 (1:1000 dilution, Cell Signal Inc., Beverly, MA.), and anti-β-actin (1:1000 dilution, Boshide Inc., Wuhan, China) antibodies diluted in TBS-T. After washing, membranes were then incubated for 1 h with rabbit anti-mouse or mouse anti-goat secondary antibodies diluted 1:5000 in TBS-T. Bands were visualized using the ECL kit according to the manufacturer's instruction, and β-actin was used as control. Results were analyzed using the gel image analysis system.

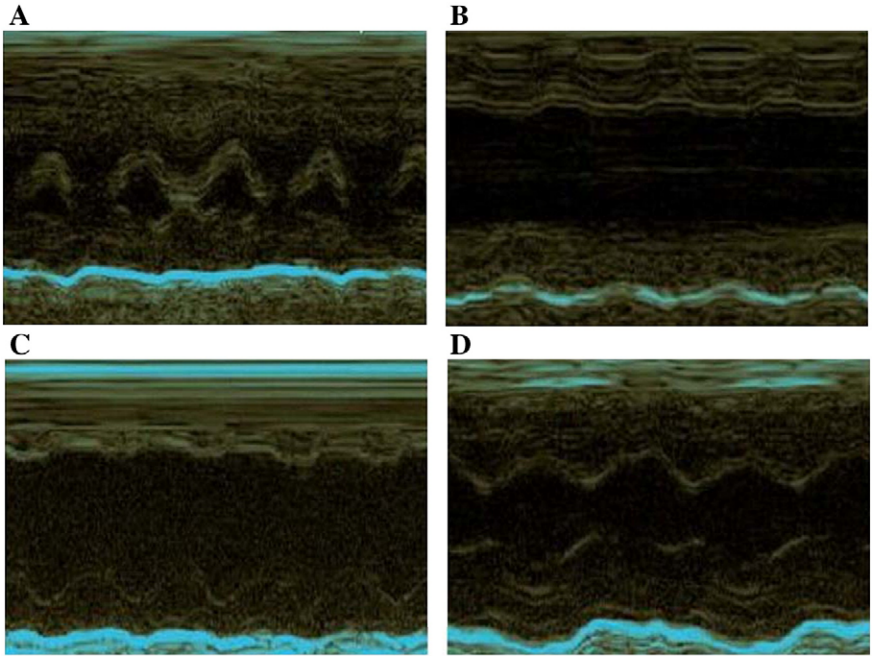

Fig. 1. Echocardiographic assessment 8 weeks after myocardial infarction. A: sham-operated group (n=12), B: vehicle-treated group (n=10), C: low BNP group (n=11), D: high BNP group (n=12).

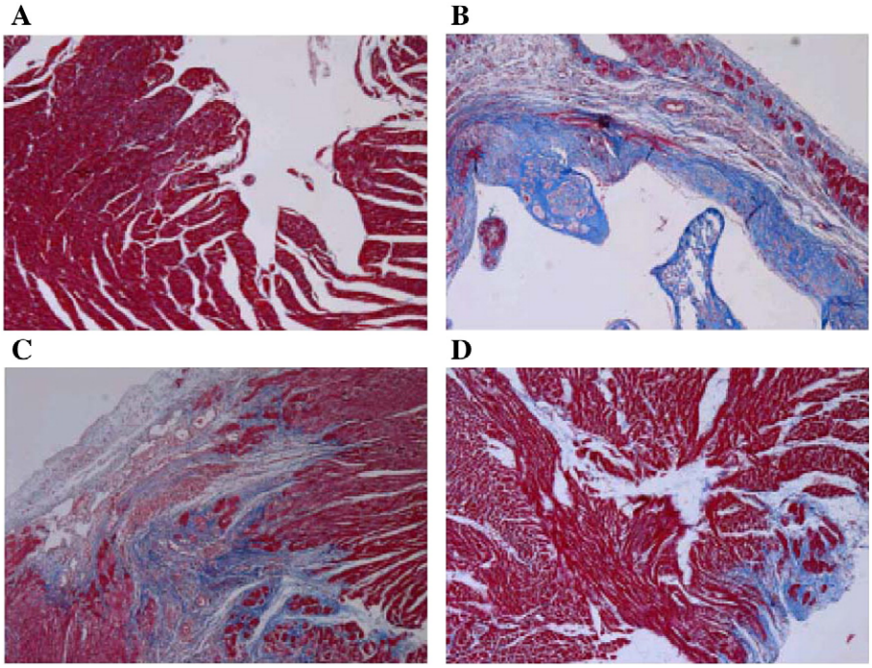

Fig. 2. Collagen deposition in 8 weeks after myocardial infarction. Blue staining shows collagen deposition while red staining shows the myocardium (Mallory's trichrome staining, original magnification×100). A: sham-operated group (n=12), B: vehicle-treated group (n=10), C: low BNP group (n=11), D: high BNP group (n=12). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

2.7. Statistical analysis

All values were expressed as mean±standard error (SE). The statistical analysis on differences among the groups was done by one-way ANOVA. It was followed by Newnam Keul's for multiple comparisons. Differences were judged to be significant when $P<0.05$. Statistical analyses were performed using SPSS 10.0 statistics software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Cardiac morphometric parameters, total collagen and angiotensin II

In our study, the rat survival rate 24 h post myocardial infarction was 70%. Eight weeks after operation, forty-five rats survived: 12 rats in the sham-operated group, 10 rats in the vehicle-treated group, 11 rats in the low BNP group, and 12 rats in the high BNP group. As shown in Table 1 and Fig. 1, left ventricular weight, left ventricular weight /body weight ratio, collagen volume fraction, plasma and myocardium angiotensin II levels were significantly higher in the vehicle-treated group than in the sham-operated group ($P<0.01$). BNP treatment prevented the increase in these parameters when comparing with the vehicle-treated group ($P<0.01$). Hearts in the high BNP group had a smaller collagen area than the low BNP group ($P<0.01$).

3.2. Echocardiographic indexes

As shown in Table 2 and Fig. 2, left ventricular posterior wall thickness, ejection fraction and fractional shortening were significantly lower in the vehicle-treated group compared with the sham-operated group ($P<0.01$). In both BNP-treated groups, left ventricular posterior wall thickness, ejection fraction, and fractional shortening were significantly improved compared with the vehicle-treated group ($P<0.01$). In addition, left ventricular end-diastolic diameter was higher in the vehicle-treated group compared with the sham-operated group ($P<0.01$), but this was attenuated in both BNP-treated groups ($P<0.01$). Effects observed in the high BNP group were more pronounced compared with the low BNP group ($P<0.01$).

3.3. Hemodynamic measurements

As shown in Table 2, left ventricular end-diastolic pressure was significantly lower and left ventricular systolic pressure, dP/d$t$max, and dP/d$t$min were higher in the vehicle-treated group compared with the sham-operated group ($P<0.05$ and $P<0.01$). Both low and high doses of BNP greatly prevented the increase in left ventricular end-diastolic pressure, dP/d$t$max, and dP/d$t$min and the decrease in left ventricular systolic pressure ($P<0.01$) when compared with the vehicle-treated group. The effects observed were more pronounced in the high BNP group compared with the low BNP group.

3.4. Expression of TGFβ1 and Smad2 mRNA

As shown in Fig. 3, TGFβ1 and Smad2 mRNA expression was significantly higher in the vehicle-treated group compared with the

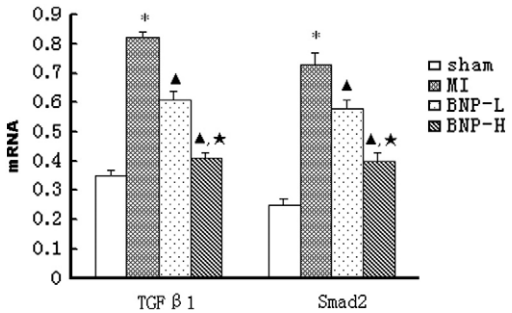

Fig. 3. Expression of TGFβ1 and Smad2 mRNA. Sham: sham-operated group (n=12), MI: vehicle-treated group (n=10), BNP-L: low BNP group (n=11), BNP-H: high BNP group (n=12). *: $P<0.01$ vs sham-operated group, ▲: $P<0.01$ vs vehicle-treated group, ★: $P<0.01$ vs low BNP group.

sham-operated group ($P<0.01$). BNP treatment attenuated this increase in TGFβ1 and Smad2 mRNA expression. Attenuation of TGFβ1 and Smad2 mRNA expression was more pronounced in the high BNP group compared with the low BNP group.

3.5. Expression of TGFβ1 and Smad 2 protein

As shown in Fig. 4, TGFβ1 and Smad2 protein expression was significantly higher in the vehicle-treated group compared with the sham-operated group ($P<0.01$). BNP treatment attenuated this increase in TGFβ1 and Smad2 protein expression. Attenuation of TGFβ1 and Smad2 protein expression was more pronounced in the high BNP group compared with the low BNP group.

4. Discussion

In the present study, we used a rat model of myocardial infarction to determine the effects of BNP treatment on ventricle remodeling and function. The rat model mimicked myocardial infarction, with 1) significant ventricular remodeling as indicated by increases in left ventricular weight, left ventricular weight /body weight ratio, collagen volume fraction, left ventricular end-diastolic diameter, and decreases in Left ventricular posterior wall thickness; and 2) decreased cardiac function as indicated by elevated left ventricular end-diastolic pressure, and decreased left ventricular systolic pressure, d*P*/d*t*max, d*P*/d*t*min, ejection fraction, and fractional shortening. Using this model, we showed that long-term treatment of BNP (starting 24 h after myocardial infarction, continue 8 weeks) significantly improved cardiac function, decreased collagen accumulation, and attenuated left ventricular remodeling in post-infarct rats. Moreover, high-dose BNP proved to be more pronounced than low-dose BNP. These results suggest that long-term treatment with exogenous BNP prevents post-infarction ventricular remodeling and decrease in cardiac function in a dose-dependent fashion.

As reported previously, BNP is synthesized and secreted in greatest abundance by cardiomyocytes, activates the natriuretic peptide receptor guanylyl cyclase-A (GC-A) that is expressed in a wide variety of tissues, including kidneys, blood vessels, adrenal glands, and heart. Activating GC-A mediates its biological actions via 3′,5′-cyclic guanosine monophosphate (cGMP) (Chen et al., 2000; Tamura et al., 2000). The BNP/cGMP system may antagonize cardiac fibroblasts proliferation and extracellular matrix production through the inhibition of renin–angiotensin and endothelin systems (Tamura et al., 2000). Nakanishi et al. (2005) found that disrupting GC-A in mice resulted in a high incidence of acute heart failure leading to increased mortality and left ventricular remodeling. Thus BNP appears to exert beneficial effects after myocardial infarction. Moreover, Hillock et al. (2008) showed that short-term treatment of BNP given soon after myocardial infarction induced incremental improvements in plasma cGMP and had beneficial effects on later ventricular remodeling. However, the effects of long-term treatment with BNP on ventricular remodeling in response to myocardial infarction are unclear. The use of peptides as chronic therapies has been limited by enzymatic degradation (BNP half-life is 22 min after IV injection). Chen et al. (2000) demonstrated that chronic subcutaneous administration of BNP was beneficial in early progressive heart failure. Plasma BNP and cGMP rapidly increase and peak 30 min after each subcutaneous BNP administration during a 10-day regimen of repeated BNP administration. The peak of plasma BNP persisted for 1 h, whereas the peak of plasma cGMP persisted for 2 h. Hobbs et al. (1996) showed that a single bolus injection of BNP at doses of 3 to 15 μg/kg produced significant beneficial hemodynamic effects in heart failure patients. The biologic effects of BNP persisted considerably longer than may be anticipated by its half-life, probably owing to receptor-mediated hemodynamic effects. In the present study, we chose to inject BNP once a day (continue 30 min every time, total 8 weeks) at a dose of 5 μg/ kg/day (low BNP group) or 15 μg/kg/day (high BNP group). We show that BNP treatment prevented the increases in above parameters. Beneficial changes included improvement in left ventricular weight, left ventricular weight /body weight ratio, collagen volume fraction, left ventricular end-diastolic diameter, Left ventricular posterior wall thickness, left ventricular end-diastolic pressure, left ventricular systolic pressure, d*P*/d*t*max, d*P*/d*t*min, ejection fraction and fractional shortening. The improvement in collagen volume fraction was more pronounced in the high BNP group compared with the low BNP group. We suggest that long-term exogenous BNP treatment prevents post-infarct ventricular remodeling and improves cardiac function in a dose-dependent fashion. These results support a potential role for BNP as a new chronic therapeutic strategy for treatment of myocardial infarction, which is consistent with the previous study (Cataliotti et al., 2007).

How does BNP affect left ventricular remodeling and cardiac function after myocardial infarction? We showed that expression of TGFβ1 and Smad2 in non-infarcted areas was significantly higher in the vehicle-treated group compared with the sham-operated group. Our results therefore suggest that TGFβ1/Smad2 signaling is associated with left ventricular remodeling and cardiac dysfunction after myocardial infarction. Previous studies indicated that TGFβ1 plays a crucial role in the process of myocardial remodeling. Several studies have demonstrated that increased myocardial TGFβ1 expression is associated with cardiac hypertrophy and fibrosis. Moreover, Smad2 protein has been identified as a mediator of TGFβ1-induced cardiac hypertrophy as well as cardiac fibrosis (Bujak and Frangogiannis, 2007; Seo and Hare, 2007). Hao et al. (1999) reported that TGF-β1 mRNA and protein levels were significantly increased in the infarct scar, while Smad 2, 3, and 4 proteins were greatly increased in the scar and scar marginal tissues. Our study is consistent with these observations.

Furthermore, our results showed that TGFβ1 and Smad2 mRNA and protein expression levels in non-infarcted area were significantly lower in BNP-treated group compared with vehicle-treated group. Moreover, the down-regulation of TGFβ1 and Smad2, the decrease in collagen deposition, and the improvement in cardiac function mediated by BNP were all dose-dependent, suggesting that exogenous BNP prevents post-infarct ventricular remodeling and improves

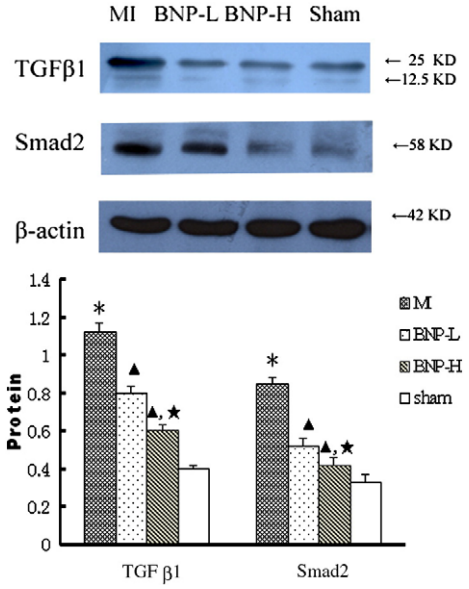

Fig. 4. Protein expression of TGFβ1 and Smad2 protein. MI: vehicle-treated group ($n=10$), BNP-L: low BNP group ($n=11$), BNP-H: high BNP group ($n=12$), sham: sham-operated group ($n=12$). *: $P<0.01$ vs sham-operated group, ▲: $P<0.01$ vs vehicle-treated group, ★: $P<0.01$ vs low BNP group.

cardiac function by modulating the expression of TGFβ1 and Smad2 in the non-infarcted area. BNP is known to act as a negative regulator of the renin–angiotensin–aldosterone system (Colucci et al., 2000; Cataliotti et al., 2007). Previous studies have shown that the renin–angiotensin–aldosterone system is activated after myocardial infarction: renin levels are markedly increased, and angiotensin II signaling is significantly enhanced (Sutton and Sharpe, 2000; Tiyyagura and Pinney, 2006). In addition, angiotensin II induces the expression of TGF-β1, a process which is mediated by the angiotensin II type1 (AT_1) receptor. Moveover, angiotensin II is associated with TGF-β1 expression in infarcted cardiac tissues, suggesting that angiotensin II stimulates fibrous tissue formation by promoting TGF-β1 synthesis via angiotensin AT_1 receptor binding (Tomita et al., 1998; Redondo et al., 2007; Gray et al., 1998). Our results showed that infarction significantly increased the levels of plasma and myocardial angiotensin II, and that BNP treatment significantly attenuated the increase in angiotensin II, which is consistent with that shown in the previous study (Schreiner and Protter, 2002). Hence we demonstrated that long-term BNP treatment contributes to the attenuation of left ventricular remodeling. BNP beneficial effect may be, at least in part, associated with direct and/or indirect inhibition of TGFβ1/Smad2 signaling, including the down-regulation of the renin–angiotensin–aldosterone system.

In summary, our results suggest that sustained upregulation of TGFβ1/ Smad2 signaling contributes to left ventricular remodeling and cardiac dysfunction after myocardial infarction. Long-term treatment with BNP prevents ventricular remodeling and cardiac dysfunction in a dose-dependent fashion, a process that may be associated with the inhibition of TGFβ1/Smad2 signaling.

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References

Bettencourt, P., 2002. Brain natriuretic peptide (nesiritide) in the treatment of heart failure. Cardiovasc. Drug Rev. 20, 27–36.

Bujak, M., Frangogiannis, N.G., 2007. The role of TGF-beta signaling in myocardial infarction and cardiac remodeling. Cardiovasc. Res. 74, 184–195.

Cataliotti, A., Chen, H.H., James, K.D., Burnett, J.C., 2007. Oral brain natriuretic peptide: a novel strategy for chronic protein therapy for cardiovascular disease. Trends Cardiovasc. Med. 17, 10–14.

Chen, H.H., Granthama, J.A., Schirger, J.A., Jougasaki, M., Redfield, M.M., Burnett, J.C., 2000. Subcutaneous administration of brain natriuretic peptide in experimental heart failure. J. Am. Coll. Cardiol. 36, 1706–1712.

Colucci, W.S., Elkayam, U., Horton, D.P., Abraham, W.T., Bourge, R.C., Johnson, A.D., Wagoner, L.E., Givertz, M.M., Liang, C.S., Neibaur, M., Haught, W.H., LeJemtel, T.H., 2000. Intravenous nesiritide, a natriuretic peptide, in the treatment of decompensated congestive heart failure. Nesiritide Study Group. N. Engl. J. Med. 343, 246–253.

Gray, M.O., Long, C.S., Kalinyak, J.E., Li, H.T., Karliner, J.S., 1998. Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-beta 1 and endothelin-1 from fibroblasts. Cardiovasc. Res. 40, 352–363.

Hao, J., Ju, H., Zhao, S., Junaid, A., Scammell-La Fleur, T., Dixon, M., 1999. Elevation of expression of Smads 2, 3, and 4, decorin and TGF-beta in the chronic phase of myocardial infarct scar healing. J. Mol. Cell. Cardiol. 31, 667–678.

Hillock, R.J., Frampton, C.M., Yandle, T.G., Troughton, R.W., Lainchbury, J.G., Richards, A.M., 2008. B-type natriuretic peptide infusions in acute myocardial infarction. Heart 94, 617–622.

Hobbs, R.E., Miller, L.W., Bott-Silverman, C., James, K.B., Rincon, G., Grossbard, E.B., 1996. Hemodynamic effects of a single intravenous injection of synthetic human brain natriuretic peptide in patients with heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am. J. Cardiol. 78, 896–901.

Holmes, J.W., Borg, T.K., Covell, J.W., 2005. Structure and mechanics of healing myocardial infarcts. Annu. Rev. Biomed. Eng. 7, 223–253.

Jessup, M., Brozena, S., 2003. Heart failure. N. Engl. J. Med. 348, 2007–2018.

Kapoun, A.M., Liang, F., O'Young, G., Damm, D.L., Quon, D., White, R.T., Munson, K., Lam, V., Schreiner, G.F., Protter, A.A., 2004. B-type natriuretic peptide exerts broad functional opposition to transforming growth factor-beta in primary human cardiac fibroblasts: fibrosis, myofibroblast conversion, proliferation, and inflammation. Circ. Res. 94, 453–461.

Kawakami, R., Saito, Y., Kishimoto, I., Harada, M., Kuwahara, K., Takahashi, N., Nakagawa, Y., Nakanishi, M., Tanimoto, K., Usami, S., Yasuno, S., Kinoshita, H., Chusho, H., Tamura, N., Ogawa, Y., Nakao, K., 2004. Overexpression of brain natriuretic peptide facilitates neutrophil infiltration and cardiac matrix metalloproteinase-9 expression after acute myocardial infarction. Circulation 110, 3306–3312.

Khan, R., Sheppard, R., 2006. Fibrosis in heart disease: understanding the role of transforming growth factor-beta in cardiomyopathy, valvular disease and arrhythmia. Immunology 118, 10–24.

Mann, D.L., Bristow, M.R., 2005. Mechanisms and models in heart failure: the biomechanical model and beyond. Circulation 111, 2837–2849.

Morita, E., Yoshimura, M., Ogawa, H., Jougasaki, M., Matsumura, T., Mukoyama, M., Nakao, K., 1993. Increased plasma levels of brain natriuretic peptide in patients with acute myocardial infarction. Circulation 88, 82–91.

Nakanishi, M., Saito, Y., Kishimoto, I., Harada, M., Kuwahara, K., Takahashi, N., Kawakami, R., Nakagawa, Y., Tanimoto, K., Yasuno, S., Usami, S., Li, Y., Adachi, Y., Fukamizu, A., David, L., Garbers, D.Y., Nakao, K., 2005. Role of natriuretic peptide receptor guanylyl cyclase-A in myocardial infarction evaluated using genetically engineered mice. Hypertension 46, 441–447.

Redondo, S., Ruiz, E., Padilla, E., Gordillo-Moscoso, A., Tejerina, T., 2007. Role of TGF-beta1 in vascular smooth muscle cell apoptosis induced by angiotensin II. Eur. J. Pharmacol. 556, 36–44.

Schreiner, G.F., Protter, A.A., 2002. B-type natriuretic peptide for the treatment of congestive heart failure. Curr. Opin. Pharmacol. 2, 142–147.

Seo, D., Hare, J.M., 2007. The transforming growth factor-beta/Smad3 pathway: coming of age as a key participant in cardiac remodeling. Circulation 116, 2096–2098.

Sutton, M.G., Sharpe, N., 2000. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation 101, 2981–2988.

Tamura, N., Ogawa, Y., Chusho, H., Nakamura, K., Nakao, K., Suda, M., Kasahara, M., Hashimoto, R., Katsuura, G., Mukoyama, M., Itoh, H., Saito, Y., Tanaka, I., Otani, H., Katsuki, M., 2000. Cardiac fibrosis in mice lacking brain natriuretic peptide. Proc. Natl. Acad. Sci. USA 97, 4239–4244.

Tiyyagura, S.R., Pinney, S.P., 2006. Left ventricular remodeling after myocardial infarction: past, present, and future. Mt. Sinai J. Med. 73, 840–851.

Tomita, H., Egashira, K., Ohara, Y., Takemoto, M., Koyanagi, M., Katoh, M., Yamamoto, H., Tamaki, K., Shimokawa, H., Takeshita, A., 1998. Early induction of transforming growth factor-beta via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. Hypertension 32, 273–279.