Therapeutic effects of continuous infusion of brain natriuretic peptides on postmyocardial infarction ventricular remodelling in rats

Effet thérapeutique d’une perfusion continue de BNP sur le remodelage ventriculaire post-infarctus du myocarde chez le rat

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Summary

Background. — Previous studies have shown protective effects of brain natriuretic peptide (BNP) against the postmyocardial infarction (MI) remodelling process. The transcription factor NF-κB is known to play an important role after MI.

Aims. — To investigate if NF-κB is involved in the protective effects of BNP against adverse post-MI remodelling.

Methods. — Rats were randomly assigned to five groups: sham-operation; MI by coronary ligation; MI treated with chronic BNP infusion; MI treated with enalapril; MI treated with BNP + enalapril. Rats were closely monitored for survival rate analysis. Rats from each group were sacrificed on days 3, 7 and 28 postoperation.

KEYWORDS
Brain natriuretic peptide; Matrix metalloproteinase; NF-κB; Myocardial infarction; Remodelling

Abbreviations: Ang II, angiotensin II; ANOVA, analysis of variance; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; CVF, collagen volume fraction; FS, fractional shortening; IL, interleukin; LAD, left anterior descending; LSD, least significant difference; LV, left ventricle/ventricular; LVEDD, left ventricular end-diastolic diameter; LVEDP, left ventricular end-diastolic pressure; LVESD, left ventricular end-systolic diameter; LVW/BW, left ventricular weight/body weight; MAP, mean arterial pressure; MI, myocardial infarction; MMP, matrix metalloproteinase; NPRA, natriuretic peptide receptor A; p, phosphorylated; TGF, transforming growth factor; TNF, tumour necrosis factor; TIMP, tissue inhibitor of metalloproteinase.

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Introduction

BNP, as an important natriuretic peptide secreted from the ventricle, can act via the natriuretic peptide receptor A/guanylyl cyclase/cGMP signalling pathway, maintaining cardiorenal homeostasis through diuresis, natriuresis, vasodilatation and inhibition of aldosterone synthesis and renin secretion under physiological and pathological conditions [1]. Release of the stored peptides due to altered chamber loading and myocyte stretch [2] has been observed in MI [3]. Interestingly, exogenous BNP infusion can protect the heart-limiting infarct size in an ischaemia/reperfusion injury rat model, possibly through adenosine triphosphate-sensitive potassium channels and nitric oxide synthase activation [4]. In addition, natriuretic peptide receptor A/cGMP signalling activated by BNP can modulate cardiac responses to hypertrophic stimuli and hence improve cardiac remodelling via mediation of the calcineurin-nuclear factor of activated T cells pathway [5,6], indicating that BNP also plays an important role in the post-MI remodelling process.

Previous studies have shown that intravenous infusion of BNP can improve clinical condition in patients with heart failure [7,8] and MI [9]. The long-term beneficial effect of BNP therapy against ventricular remodelling has also been

Results. — The results showed that chronic continuous BNP infusion achieved similar effects to enalapril therapy, as evidenced by improved survival rate within the 28-day observation period compared with MI group rats; this effect was closely associated with preserved cardiac geometry and performance. The treatment combination did not offer extra benefits in terms of survival rate. Both BNP and enalapril therapy produced higher heart tissue concentrations of cyclic guanosine monophosphate and lower expression levels of inflammatory cytokines, including tumour necrosis factor-α, interleukin-1 and interleukin-6. These benefits were associated with lower phosphorylation levels of NF-κB subunits IκBα, IκBδ, p50 and p65. While enalapril significantly inhibited extracellular matrix remodelling via regulation of the protein expression ratio of matrix metalloproteinase/tissue inhibitor of metalloproteinase and the activity of matrix metalloproteinase, these variables were not affected by BNP, indicating that the two therapies involve different mechanisms.

Conclusion. — Chronic BNP infusion can provide beneficial effects against adverse post-MI remodelling.

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demonstrated in a rat MI model, which was associated with inhibition of TGFβ1/Smad2 signalling [10]. However, it is still not fully known how BNP infusion attenuates TGFβ expression. NF-κB is a key transcription factor that mediates inflammatory cytokine expression in response to a variety of stimuli [11], especially after MI [12]. Therefore, using a rat MI model, we aimed to investigate whether modulation of NF-κB activity (and hence pro-inflammatory cytokine expression) is involved in the improved remodelling process associated with chronic BNP infusion. The benefits of BNP infusion were compared with those of the angiotensin-converting enzyme inhibitor enalapril, and additive effects were tested when the two therapies were combined.

**Materials and methods**

Male, 8-week-old Sprague-Dawley rats (Experimental Animal Center, Fudan University, Shanghai, China) were used in this study. The animal research study protocol complied with The Guide for the Care of Use of Laboratory Animals published by the National Institute of Health (NIH Publication No. 85-23, revised 1996). All rats were housed for a 2-week acclimatization period before the study started.

**Animal preparation**

The MI model was induced as described in our previous report [13] by LAD coronary ligation. In brief, after anaesthesia with pentobarbital (50 mg/kg, intraperitoneally) all rats (weighing 320–380 g) underwent an open chest operation and the LAD coronary artery was encircled just distal to its first branch. The infarct was confirmed by a pale area below the suture or ST-T elevation on an electrocardiogram. The control group underwent sham operation with passage of a suture around the LAD without tie-down. All rats were then allowed to recover under close observation.

**Criteria for rat enrolment**

To minimize the variability of infarct size, only rats with moderate infarct size were enrolled. A pilot MI study was performed on 21 rats. Twenty-four hours after LAD ligation, blood serum was collected to measure troponin I concentration (Siemens Medical Solution Diagnostics, Tarrytown, NY, USA). The heart was harvested from each rat and the LV was cut into five parallel slices. Triphenyltetrazolium chloride staining was performed and the extent of necrosis was quantified by computerized planimetry. The total infarct size was expressed as a percentage of the total sum of the infarct area on each slice/LV corrected by weight. The correlation curve was then derived for infarct size and serum troponin I concentration (Fig. 1A) with a correlation coefficient of 0.979 ($P$ < 0.05). In panel B, Kaplan-Meier curves were created for the MI group, the MI + enalapril group, the MI + BNP group and the MI + combination therapy group. All the rats were rigorously monitored for evaluation of survival rate. Both enalapril and BNP infusion improved survival rate ($P$ < 0.05, respectively) but no further significant beneficial effects were obtained with combination therapy. *$P$ < 0.05 vs MI group. BNP: brain natriuretic peptide; CTnI: cardiac troponin I; MI: myocardial infarction.

**Experimental protocols**

**Protocol I**

To evaluate survival rate, 160 MI rats with an appropriate troponin I concentration were randomly assigned to one of the following groups ($n$ = 40 in each group): MI group; MI + enalapril group (1 day after LAD ligation, MI rats were given enalapril [Berlin-Chemie AG, Berlin, Germany] 10 mg/kg/day by gavage); MI + BNP group (1 day after LAD ligation, MI rats were given continuous intravenous infusion of rat-BNP-32 [Jingmei Biotech Co. Ltd., Shenzhen, China] 0.06 g/kg/minute through an osmotic mini-pump [Alza Corporation, Mountainview, CA, USA] [14] implanted intraperitoneally); MI + combination group (MI rats were given both the enalapril and BNP treatments). All MI rats were rigorously monitored to check the cause of sudden cardiac death. A careful autopsy was performed for each rat with reference to cardiac rupture. Survival rate was analyzed to evaluate treatment benefits.

**Protocol II**

Time-course changes in the post-MI remodelling process were evaluated. The other 64 MI rats were then randomly assigned to the four groups ($n$ = 16 to each group). Rats were
sacrificed on day 3 and day 7 after the MI (n = 8 for each time point), respectively.

Before the rats were sacrificed, echocardiography (ACUSON SEQUOIA 512 equipped with 14 MHz mini probe) was performed, using the same anaesthesia as described above. Two-dimensional and M-mode echocardiograms were obtained. LVEDD and LVESD were measured in the short-axis view at papillary muscle level. FS was also obtained. All values were averages of three consecutive cardiac cycles and were analysed by two independent blinded researchers. Invasive haemodynamic measurements were also taken by right carotid artery cannulation with a pre-heparinized fine polyethylene tube connected to a fluid-filled pressure transducer (MPA-CFS, Allocott Biotech, Shanghai, China). After euthanasia, the heart from each rat was harvested. LV weight was recorded for comparison with body weight, and at papillary muscle level, one LV cross-sectional tissue slice around 5 mm in thickness was obtained, fixed in 4% formalin and embedded in paraffin for the histological examination. Non-infarcted LV tissue was obtained from the rest of the LV, and was snap frozen in liquid nitrogen and stored at −80°C. Eight rats that had completed 28 days of rigorous observation were randomly selected from each group and underwent the same procedure as described above.

Another 24 sham-operated control rats were also sacrificed on day 3, day 7 and day 28; these rats underwent the same procedure as described above.

Quantification of collagen deposition and infarct size

Paraffin-embedded heart tissue was stained by Mallory’s method, which is collagen specific. Three sections per animal and eight fields per section were scanned and computerized with a Leica Q500MC digital image analyser at a magnification of 10. The sections from different groups were stained in one batch. CVF was obtained from the ratio of the connective tissue area divided by the total tissue area within the same microscopic field. The infarct (expressed as fibrotic area) perimeter was traced and the size of the MI was normalized to the LV area using the following equation: percentage infarct perimeter = circumference of infarct scar/[(epicardium perimeter + endocardium perimeter)/2] × 100%.

Immunohistochemistry

After dewaxing, the sections of LV tissue of 5 μm thickness were immersed in 3% hydrogen peroxide for 10 minutes at room temperature. After incubation with 5% bovine serum albumin (Boster, Wuhan, China) for 20 minutes, the tissue sections were probed with rabbit anti-rat type I and type III collagen antibodies (Boster, Wuhan, China) (1:100) at 4°C overnight. The sections were then incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2000; Boster) for 20 minutes at 37°C then reacted with streptavidin-biotin-peroxidase complex (Boster) for 20 minutes and coloured by diaminobenzaldehyde (Boster). Quantification of resulting image data from immunohistochemical staining was performed using a Leica Q500MC digital image analyser.

Enzyme-linked immunosorbent assay

A double-antibody sandwich-based enzyme-linked immunosorbent assay kit (USCN Life Science Inc., Wuhan, China) was used to detect angiotensin II (Ang II), cGMP, BNP, TNFα, IL-1 and IL-6 concentrations in non-infarcted cardiac tissue, according to the manufacturer’s instructions. The detection limits of each assay were as follows: Ang II, 1.56 ng/mL; cGMP, 1.56 pmol/mL; BNP, 156 pg/mL; TNFα, 3.9 pg/mL; IL-1, 39 pg/mL; IL-6, 7.8 pg/mL. The intra- and intervariability for each kit were < 8%, respectively.

Western blotting

The detailed method of western blotting has been described previously using the rat MI model [15]. The primary antibodies against different proteins of MMP, including MMP-2 (R&D Systems, Minneapolis, MN, USA), MMP-9, TIMP-1 (Abcam, Cambridge, MA, USA), phosphorylated (p)-NF-κB-p50, NF-κB-p50 (Abcam, USA), p-IκB-α (Ser32), IκB-α, p-NF-κB-p65 and NF-κB-p65 (Cell Signaling Technology, Danvers, MA, USA), were used to probe the target protein. An antibody against β-actin (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was also used for protein loading. The bands for different proteins were quantified by densitometry using Image J software (version 1.41, National Institutes of Health, Bethesda, MD, USA) and normalized to the expression of β-actin.

Zymography

Gelatine zymography was used to determine MMP protein activity as described. Approximately 10 mg of myocardial tissue were extracted from the non-infarcted area and equal volumes (10 μL) of samples normalized for protein concentration were subjected to electrophoresis (without boiling or reduction) through a 10% polyacrylamide gel co-polymerized with gelatine (0.5 mg/mL), at 4°C. After electrophoresis, the gel was incubated for 1 hour at 25°C in a 2.5% Triton X-100 solution and then incubated overnight at 37°C in a 0.05 M Tris-HCl buffer, pH 8.0, containing 5 mM CaCl2. The gels were fixed with 20% methanol and 7% acetic acid. The gels were stained with 0.25% Coomassie Brilliant Blue R250 and then destained with 10% methanol and 7% acetic acid. The gels were scanned using the EagleEye II imaging system for relative lytic activity. A mixture of human MMP-2 and MMP-9 (Chemicon, Hofheim, Germany) served as a zymography standard.

Statistical analysis

All data are expressed as means ± standard deviations. Kaplan-Meier survival curves were analysed to check the differences between MI rats with and without treatments. Time-course changes in the haemodynamic heart tissue expression of MMP, cGMP, Ang II and BNP, and the extent of fibrosis were compared within groups by using repeated ANOVA measures, and between groups by using two-way ANOVA followed by LSD-corrected multiple comparisons. One-way ANOVA followed by LSD correction was used for comparing the differences in all other variables between groups. Non-parametric data were analysed using Fisher’s exact method. A P-value < 0.05 was considered as statistically significant. Statistical analyses were performed.
using SPSS 13.0 statistics software (SPSS Inc., Chicago, IL, USA).

Results

Survival rate

Troponin I values were similar in the four groups of rats with MI (MI group, 35.8 ± 6.0 g/L; MI + enalapril group, 33.6 ± 4.7 g/L; MI + BNP group, 36.8 ± 5.8 g/L; MI + combination group, 37.0 ± 5.4 g/L), with no significant differences (P > 0.05, respectively). However, during the 28-day observation period, survival rates improved in rats treated with BNP or enalapril, with no significant difference between these two groups (P = 1.000; Fig. 1B). The combination of BNP and enalapril did not provide additional benefits in terms of survival rate during this period. Rigorous monitoring combined with autopsy showed that cardiac ruptures occurred within 7 days after MI (two in the MI + enalapril group, two in the MI + BNP group, two in the MI + combination group and three in the MI group) with no differences between the four groups (P = 1.000, respectively).

Tissue weight and percentage infarct perimeter

There were no significant differences in infarct perimeter between the four MI groups at any time point post MI. Neither enalapril nor BNP therapy exerted any protective effects on infarct size (Table 1) and their combination did not produce additive effects in terms of limiting infarct size.

The ratio of left ventricular weight/body weight (LVW/BW) was markedly increased in the MI group compared with the sham control group on day 28 post MI (P < 0.001), and this increase in LVW/BW ratio was attenuated by both enalapril (P = 0.024, vs. MI group) and BNP administration (P = 0.002, vs. MI group). The combination of enalapril and BNP showed a non-significant trend towards producing a further decrease in LVW/BW (P = 0.890, vs. MI + BNP group and MI + Enalapril group, respectively).

Echocardiographic examination

At each time point, MI group rats had increased LVEDD compared with sham controls (day 3, P = 0.002; day 7, P < 0.001 and day 28, P < 0.001), and within the MI group, the LV was further dilated on day 28 compared with day 3 (P < 0.001). Compared with the MI group, the increase in LVEDD on day 28 was attenuated by both enalapril (P = 0.033) and BNP (P = 0.044) (Table 1).

Similarly, at each time point, MI group rats had lower FS than sham controls (P < 0.001, respectively); within the MI group, FS was further decreased on day 28 compared with on day 3 (P = 0.004) and day 7 (P = 0.028). The beneficial effects of enalapril and BNP were only seen on day 28, indicated by an increase in FS compared with the MI group on day 28 (MI + enalapril group, P = 0.036; MI + BNP group, P = 0.045; Table 1).

However, the combination of enalapril and BNP only showed a non-significant trend towards further improve-ment in LV geometry and FS (P = 1.000, vs. MI + BNP group and MI + Enalapril group, respectively; Table 1).

Haemodynamic variables

The heart rates of MI group rats were similar at each time point and were not different from those of the sham control rats. Treatment with enalapril, BNP or combination therapy did not produce any changes in heart rate (data not shown).

At each time point, MI group rats only showed a non-significant trend towards a decrease in MAP compared with sham controls. Both enalapril and combination therapy produced significant decreases in MAP compared with sham rats (day 3: enalapril group, P = 0.005 and combination group, P < 0.001; day 7: P < 0.001, respectively and day 28: enalapril group, P = 0.006 and combination group, P = 0.008). However, BNP produced a decrease in MAP only on day 3 (P < 0.001, vs. sham controls) (Table 1).

Impaired LV function was also evidenced by an increase in LVEDP at each time point in MI group rats, with a further increase within the MI group on day 28 (P < 0.001, vs. day 3 and day 7; respectively). Again, enalapril, BNP and combination therapy resulted in improved LVEDP only on day 28 compared with the MI group (P < 0.001, respectively). Combination therapy resulted in a further decrease in LVEDP compared with the enalapril group alone (P = 0.017; Table 1).

Ang II, cGMP and BNP concentrations

The concentration of Ang II was increased in MI group rats compared with sham controls on day 3 (P < 0.001) and day 7 (P = 0.001) but had returned to a level similar to that in sham controls on day 28. Enalapril treatment resulted in decreases in Ang II concentration compared with the MI group on day 3 (P = 0.006) and day 7 (P = 0.020); however, BNP infusion only showed a non-significant trend towards a reduction in Ang II (P = 0.115, vs. MI group) on day 3. Combination therapy achieved similar Ang II levels as enalapril (P = 1.000, at each time point; respectively) (Fig. 2A).

MI group rats had similar cGMP concentrations to sham controls at each time point (P = 1.000, respectively). A higher concentration of cardiac cGMP was achieved by both enalapril and BNP at each time point (P < 0.001 vs sham controls, respectively), with no significant differences between these two treatment groups at any time point. A further increase was achieved when enalapril and BNP were combined, but only on day 7 (P = 0.018, vs. MI + Enalapril group) (Fig. 2B).

BNP concentration was increased in MI group rats on day 3 compared with sham controls (P < 0.001), but decreased gradually to the level of sham controls on day 7 and day 28. Enalapril treatment did not affect BNP concentration significantly compared with MI group rats. By contrast, a higher concentration of BNP was achieved by chronic BNP infusion throughout the whole observation period compared with MI group rats. The combination therapy exerted an effect on BNP concentration that was similar to that achieved with BNP treatment alone (Fig. 2C).
Table 1  Infarct perimeter, cardiac geometry, function and haemodynamic data for each group.

<table>
<thead>
<tr>
<th>Observation period</th>
<th>Groups</th>
<th>IP (%)</th>
<th>MAP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LVW/BW (mg/g)</th>
<th>LVEDD (mm)</th>
<th>FS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>Sham</td>
<td>—</td>
<td>114 ± 12</td>
<td>3.4 ± 1.0</td>
<td>1.76 ± 0.11</td>
<td>5.33 ± 0.33</td>
<td>61.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>38.35 ± 4.93</td>
<td>103 ± 10</td>
<td>8.3 ± 1.3(^a)</td>
<td>1.89 ± 0.09</td>
<td>6.93 ± 0.92(^a)</td>
<td>34.7 ± 9.3(^a)</td>
</tr>
<tr>
<td></td>
<td>MI + enalapril</td>
<td>37.00 ± 3.45</td>
<td>95 ± 6(^a)</td>
<td>8.9 ± 1.5(^a)</td>
<td>1.91 ± 0.08</td>
<td>6.97 ± 0.37(^a)</td>
<td>33.7 ± 12.2(^a)</td>
</tr>
<tr>
<td></td>
<td>MI + BNP</td>
<td>39.13 ± 4.56</td>
<td>92 ± 5(^a)</td>
<td>7.2 ± 0.9(^a)</td>
<td>1.87 ± 0.06</td>
<td>7.20 ± 0.54(^a)</td>
<td>37.2 ± 8.3(^a)</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>38.27 ± 6.21</td>
<td>88 ± 5(^a)</td>
<td>6.9 ± 1.0(^a)</td>
<td>1.91 ± 0.07</td>
<td>7.10 ± 0.82(^a)</td>
<td>38.6 ± 6.9(^a)</td>
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<tr>
<td>7 days</td>
<td>Sham</td>
<td>—</td>
<td>114 ± 6</td>
<td>2.9 ± 0.8</td>
<td>1.76 ± 0.08</td>
<td>5.32 ± 0.338</td>
<td>60.3 ± 4.5</td>
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<td></td>
<td>MI</td>
<td>42.10 ± 5.78</td>
<td>104 ± 7</td>
<td>10.2 ± 1.1(^a)</td>
<td>1.93 ± 0.09</td>
<td>7.85 ± 0.58(^a)</td>
<td>30.9 ± 6.6(^a)</td>
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<tr>
<td></td>
<td>MI + enalapril</td>
<td>41.11 ± 7.33</td>
<td>93 ± 5(^a)</td>
<td>8.4 ± 1.3(^a)</td>
<td>1.86 ± 0.14</td>
<td>7.00 ± 0.53(^a)</td>
<td>33.3 ± 6.1(^a)</td>
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<tr>
<td></td>
<td>MI + BNP</td>
<td>42.13 ± 6.23</td>
<td>103 ± 8</td>
<td>7.2 ± 1.5(^a)</td>
<td>1.89 ± 0.08</td>
<td>7.13 ± 0.63(^a)</td>
<td>31.9 ± 7.0(^a)</td>
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<tr>
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<td>Combination</td>
<td>41.35 ± 3.46</td>
<td>92 ± 7(^a)</td>
<td>6.5 ± 1.2(^a)</td>
<td>1.85 ± 0.08</td>
<td>6.77 ± 0.54(^a)</td>
<td>36.3 ± 4.4(^a)</td>
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<tr>
<td>28 days</td>
<td>Sham</td>
<td>—</td>
<td>116 ± 12</td>
<td>2.8 ± 1.0</td>
<td>1.71 ± 0.13</td>
<td>5.68 ± 0.62</td>
<td>62.5 ± 4.2</td>
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<td>MI</td>
<td>41.18 ± 5.92</td>
<td>106 ± 8</td>
<td>14.2 ± 1.7(^a)</td>
<td>2.19 ± 0.12(^a)</td>
<td>8.83 ± 0.61(^b)</td>
<td>19.2 ± 2.6(^a)</td>
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<td>MI + enalapril</td>
<td>41.14 ± 6.25</td>
<td>96 ± 5(^a)</td>
<td>9.5 ± 1.0(^a)</td>
<td>1.90 ± 0.25(^b)</td>
<td>7.47 ± 0.73(^b)</td>
<td>27.7 ± 5.6(^b)</td>
</tr>
<tr>
<td></td>
<td>MI + BNP</td>
<td>42.05 ± 6.08</td>
<td>104 ± 9</td>
<td>7.7 ± 1.2(^a)</td>
<td>1.82 ± 0.11(^b)</td>
<td>7.52 ± 0.97(^b)</td>
<td>27.5 ± 3.9(^b)</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>42.63 ± 7.31</td>
<td>97 ± 7(^a)</td>
<td>6.8 ± 1.5(^a)</td>
<td>1.82 ± 0.07(^b)</td>
<td>6.92 ± 0.65(^b)</td>
<td>31.4 ± 5.9(^b)</td>
</tr>
</tbody>
</table>

All data are expressed as means ± standard deviations. BNP: brain natriuretic peptide; FS: fractional shortening; IP: infarct perimeter; LVEDD: left ventricular end-diastolic diameter; LVEDP: left ventricular end-diastolic pressure; LVW/BW: left ventricular weight/body weight; MAP: mean arterial pressures; MI: myocardial infarction.

\(^a\) P < 0.05 vs sham control group at the same time point.
\(^b\) P < 0.05 vs MI group at the same time point.
\(^c\) P < 0.05 vs MI + enalapril group at the same time point.
\(^d\) P < 0.05 vs MI group on day 3.
\(^e\) P < 0.05 vs MI group on day 7.
Activation of nuclear factor NF-κB and cytokine levels

Phosphorylation levels of the NF-κB subunits IκBα, p50, and p65 were enhanced in the non-infarcted LV of MI group rats on day 28 post-MI \( (P < 0.001, \) vs. sham controls; respectively). However, both BNP and enalapril treatment attenuated phosphorylation levels of IκBα, p50, and p65 compared with the MI group \( (P < 0.001, \) respectively) (Fig. 3).

The concentrations of TNFα, IL-1 and IL-6 were increased in the non-infarcted LV of MI rats on day 28 post-MI \( (P < 0.001, \) vs. sham controls; respectively), these were inhibited by both enalapril and BNP treatment \( (P < 0.001, \) vs. MI group; respectively).

Combination therapy did not further suppress either the phosphorylation levels of NF-κB subunits \( (IκBα, P = 1.000; \) p50, \( P = 1.000 \) and p65, \( P = 0.853, \) vs. MI + Enalapril group, respectively) or the expression levels of TNFα and IL-6 \( (P = 1.000 \) and \( P = 0.107, \) vs. MI + Enalapril group; respectively). However, combination therapy did further suppress the expression levels of IL-1β \( (P = 0.002 \) vs MI + enalapril group; \( P = 0.022 \) vs MI + BNP group).

MMP-2/TIMP-1 and MMP-9/TIMP-1 protein expression ratios

On day 7, compared with MI group rats, there were significant decreases in both MMP-2/TIMP-1 and MMP-9/TIMP-1 in enalapril-treated rats \( (P = 0.016 \) for MMP-2/TIMP-1; \( P = 0.001 \) for MMP-9/TIMP-1) but not in BNP-treated rats \( (P = 0.631 \) for MMP-2/TIMP-1; \( P = 1.000 \) for MMP-9/TIMP-1). Combination therapy only resulted in a non-significant trend towards further decreases in MMP-2/TIMP-1 and MMP-9/TIMP-1 \( (P = 0.672 \) and \( P = 1.000, \) vs. MI + Enalapril group; respectively) (Fig. 4).

MMP gelatinolytic activity

Two lytic bands were observed for MMP-2, one corresponding to pro-MMP-2 (72 kDa) and the other to active-MMP-2 (66 kDa). Another lytic band represented pro-MMP-9 (92 kDa) activity (Fig. 5).

Compared with sham controls, pro-MMP-2 gelatinolytic activity increased two-fold on day 3 and 2.7-fold on day 7 in the non-infarcted area of MI group rats. Enalapril resulted in decreases in pro-MMP-2 gelatinolytic activity on day 3 \( (P = 0.015, \) vs. MI group) and day 7 \( (P < 0.001, \) vs. MI group), whereas BNP treatment alone had no effect on MMP-2 gelatinolytic activity. Combination therapy had similar effects to enalapril on pro-MMP-2 gelatinolytic activity on day 3 and day 7. On day 28, pro-MMP-2 gelatinolytic activity was similar in all five groups \( (P > 0.05, \) respectively) (Fig. 5).

On day 3, the levels of pro-MMP-9 gelatinolytic activity were quite low in sham controls. In contrast, MI group rats had a significant increase in pro-MMP-9 activity (Fig. 5). Both enalapril and combination therapy resulted in similar decreases in pro-MMP-9 gelatinolytic activity in non-infarcted LV tissue compared with the MI group (enalapril group, \( P = 0.032 \); combination group, \( P = 0.020 \)). Again, BNP infusion alone had no effects on pro-MMP-9 gelatinolytic activity \( (P = 1.000, \) vs. MI group). MMP-9 activity was not detectable.
on either day 7 or day 28 in non-infarcted LV tissue obtained from rats from any of the groups (Fig. 5).

Collagen quantification

The infarct area showed no changes in CVF induced by MI compared with sham controls; CVF was not affected by enalapril, BNP or combination therapy at any time point ($P > 0.05$, respectively) (Fig. 6A).

In non-infarcted LV tissue, MI group rats showed a gradual increase in CVF (day 3 vs day 7, $P = 0.047$; day 7 vs day 28, $P = 0.028$). Both enalapril and BNP treatment improved CVF on day 28 ($P = 0.001$, respectively) compared with MI group rats. However, no further decrease in CVF was achieved with combination therapy ($P = 0.740$, vs. MI + Enalapril group) (Fig. 6B).

MI group rats also exhibited a time-course increase in collagen type I expression in non-infarcted LV tissue (day 3 vs day 7, $P = 0.015$; day 7 vs day 28, $P = 0.003$) compared with sham controls. Both enalapril and BNP treatment resulted in lower collagen type I expression on day 28 ($P < 0.001$, respectively) compared with MI group rats, and a further decrease in collagen type I expression was observed only on day 28 when the two therapies were combined ($P = 0.041$, vs. MI + Enalapril group) (Fig. 6C).

Within the MI group, compared with on day 3, a similar increase in collagen type III expression was present in non-infarcted LV tissue on day 7 ($P = 0.013$) and day 28 ($P = 0.017$). However, collagen type III expression was not affected by enalapril, BNP or combination treatment (Fig. 6D).

Discussion

Our data demonstrate that chronic continuous BNP administration via an osmotic mini-pump after acute MI maintains a satisfactory BNP concentration in cardiac tissue, which, in turn, increases the concentration of cGMP in heart tissue. Both BNP infusion and enalapril administration were closely associated with an attenuated phosphorylation level of the
NF-κB subunits IκBα, p50 and p65, and hence lower expression of the cytokines TNF, IL-1 and IL-6 in non-infarcted heart tissue. Improved collagen deposition was also observed in non-infarcted areas. All these benefits resulted in improved cardiac geometry and performance, leading to improved survival rates during the 28-day observation period.

It is well established that inflammation is an important pathophysiological process during the remodelling process. Although appropriate inflammation is necessary for the healing process early after MI, overt and sustained inflammatory responses induced by combined volume and pressure overload in the setting of significant loss of myocytes can eventually result in left ventricular dilation and dysfunction [16]. NF-κB, as a transcription factor, plays a key role in mediating the inflammatory responses. A variety of stimuli, including ischaemia, oxidative stress and cytokines, can activate NF-κB, which, in turn, can induce further expression of pro-inflammatory cytokines [11]. It has been shown that attenuation of NF-κB activities can protect the heart against cardiac hypertrophy and improve cardiac performance in various pathological processes [17,18], especially in the setting of MI [19–21]. However, the role of NF-κB in mediating the inflammatory reaction has been questioned by a recent study in which p50 knock-out mice exhibited a worse post-MI remodelling process [22], and the protective role of NF-κB was further demonstrated in another study at the cellular level [23]. Our study has shown that the improved cardiac remodelling process provided by BNP infusion and enalapril therapy was, in fact, associated with less activation of the three NF-κB subunits (IκBα, p50 and p65), thus further supporting the idea that suppressed NF-κB signalling can protect the heart against the post-MI remodelling process. The apparently different role for subunit p50 in the anti-inflammatory process indicated in other studies could be due to the different models or pharmacological agents used.

Intriguingly, in our study, although NF-κB signalling activation was attenuated with BNP infusion, MMP-2 expression and MMP-9 expression were not decreased. In contrast, enalapril administration resulted in a significant decrease in MMP-2/TIMP-1 and MMP-9/TIMP-1 [24]. This is in striking contrast to previous data that have shown that MMP-9 is regulated by NF-κB, as there is a binding site in the promoter of the MMP-9 gene [25], indicating that a different mechanism is involved in regulating MMP expression. It has also been shown that transgenic mice overexpressing BNP can induce MMP-9 expression [26]. Enhanced MMP expression and/or activity could potentially cause sudden death due to cardiac rupture [26,27]. Although MMP-2 may exert a dual role in the post-MI remodelling process [28], in these studies, MMP-2 expression could only be induced at a very high BNP concentration in vitro (∼10^{-7} – 10^{-6} mol/L) [27], or at a plasma BNP concentration of 3 × 10^{-9} mol/L, as seen in BNP overexpression transgenic mice [26]. This concentration is much higher than the clinical therapeutic concentration (8 × 10^{-11} mol/L) [9] and the myocardial

Figure 4. The representative bands for MMP-2, MMP-9 and TIMP-1 are shown in panel A for day 3, panel B for day 7 and panel C for day 28. The expression level was quantified by densitometry and normalized to that of β-actin. The expression ratios were then calculated and shown in a bar graph for both MMP-2/TIMP-1 (panel D) and MMP-9/TIMP-1 (panel E) for day 7. All data are expressed as means ± standard deviations. *P < 0.05 vs sham controls; §P < 0.05 vs MI group; ¶, P < 0.05 vs MI + enalapril group; ∆P < 0.05 vs MI + BNP group. ACEI: angiotensin-converting enzyme inhibitor; BNP: brain natriuretic peptide; MI: myocardial infarction; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase.
tissue BNP concentration in our present study. Therefore, this side effect might only be seen at an extremely high dose of BNP in the clinical setting. In fact, cardiac rupture was not reported in either of the earlier clinical trials or in animal experiments in the setting of MI, although BNP was only given for 60 hours to patients with MI [9] or was delivered once-a-day in a rat MI model [10]. In our present study, BNP was continuously delivered via an osmotic mini-pump implanted intraperitoneally throughout the whole observation period. Therefore, the cardiac BNP concentration was maintained at a stable level. Neither expression nor activity of MMP-2 and MMP-9 was significantly increased by BNP infusion, and no increased incidence of cardiac rupture was observed with our rigorous monitoring protocol. Therefore, our data confirmed that continuous BNP infusion does not induce a significant increase in MMP-9 and MMP-2 expression, and does not result in cardiac rupture.

In addition, it has been shown that activation of the TGFβ1/Smad2 signalling pathway can be activated by Ang II, and results in collagen deposition and fibrosis [29,30]. In cell cultures of human fibroblasts, BNP administration can increase the cGMP concentration and hence inhibit TGFβ1/Smad2 signalling, while activating the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway [31]. This protective effect was also observed in an in vivo rat MI model, by the inhibition of TGFβ1/Smad2 signalling [10]. Our data show that BNP infusion can attenuate the proinflammatory responses via inactivation of NF-κB, which, in turn, can suppress the expression level of proinflammatory cytokines and hence inhibit TGFβ1/Smad2 signalling. However, it is still not understood why the Ang II expression level was not affected by BNP infusion, indicating that a different pathway is involved in regulating Ang II expression; further study is therefore certainly warranted.

Limitations

There are conflicting data in our study regarding the mechanisms via which BNP therapy improved the remodelling process. First, Ang II expression was not affected by BNP infusion, which contradicts the fact that NF-κB activation is attenuated. Second, BNP concentration was not affected by enalapril therapy although cardiac function was significantly improved. Third, MMP expression and/or activities in the non-infarcted area were also differentially modulated by BNP and enalapril. In the present study, we are not able to elucidate the underlying mechanisms by which BNP might act differently from enalapril. We reason that these data indicate that different mechanisms are involved in the two treatments in the setting of MI. In addition, the moderate...
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Figure 6. The effects of BNP and enalapril on CVF were quantified in the infarct area (panel A) and in non-infarcted left ventricular tissue (panel B). In non-infarcted left ventricular tissue, both collagen type I (panel C) and collagen type III (panel D) content were also quantified. For comparison between groups at the same time point: *P<0.05 vs sham controls; §P<0.05 vs MI group; ¶P<0.05 vs MI + enalapril group. For comparison within the MI group: #P<0.05 on day 3; ΔP<0.05 on day 7. BNP: brain natriuretic peptide; CVF: collagen volume fraction; MI: myocardial infarction.

Infarct size we selected could potentially mask the predictive role regarding prognosis when enalapril was given. In addition, we cannot fully exclude that the improved remodelling process is due to improved haemodynamic status, such as vasodilatation effects of BNP infusion. However, the long-term beneficial effect on the remodelling process (independent of the infarct size sparing effects) of vasodilating drugs such as nitrates is still not fully established [32]; they could eventually be found to exert a protective effect via anti-inflammatory effects, as shown in the present study.

Conclusion

BNP infusion can increase the cGMP concentration in cardiac tissue and protect against adverse LV remodelling, resulting in similar effects as angiotensin-converting enzyme inhibitor treatment. The improved remodelling process is closely associated with attenuation of NF-κB activation. Further study is warranted to elucidate the underlying mechanisms by which Ang II expression is differentially regulated by BNP infusion and angiotensin-converting enzyme inhibitors, especially the link between inhibition of NFκB and the TGFβ1/Smad2 pathways during the post-MI remodelling process.

Conflict of interest statement

None.

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References


