

RESEARCH ARTICLE

HNP-1 Reverses Hypertensive Left Ventricular Hypertrophy by Inhibiting the NF- κ B Signaling Pathway

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Abstract

Background: Human neutrophil peptide-1 (HNP-1) is a commonly investigated therapeutic agent. However, its role in hypertensive left ventricular hypertrophy (HLVH) remains unclear.

Methods: We measured HNP-1 levels in patients with hypertension and treated HLVH rat and H9c2 cell hypertrophy models with HNP-1. Cardiomyocyte hypertrophy indexes (i.e., single-cell surface area, left ventricular fibrosis area, BNP levels, and β -MHC levels) were measured with hematoxylin-eosin and Masson's trichrome staining and WB. NF- κ B signaling factors (i.e., IKK β , p-IKK β , I κ B α , p-I κ B α , p65, and p-p65) were measured with WB and qPCR. Finally, inflammatory factors (i.e., IL-6, IL-1 α , and TNF- α) were measured with ELISA.

Results: HNP-1 levels were lower in the exposure than the control groups (M (95% CI), 48.83 (45.64–52.26) vs. 59.03 (55.62–62.54), P = 0.000). Diminished HNP-1 was associated with HLVH occurrence in patients. HLVH rat and H9c2 cell hypertrophy models revealed elevated cardiomyocyte hypertrophy indexes and NF- κ B signaling and inflammatory factors. However, each HNP-1 treatment group showed lower levels of the aforementioned indices than were observed in the model groups.

Conclusion: Diminished HNP-1 is a risk factor for HLVH. HNP-1 treatment may reverse HLVH by inhibiting NF- κ B signaling pathways.

Keywords: human neutrophil peptide 1, hypertensive left ventricular hypertrophy, nuclear factor- κ B

Introduction

The prevalence of hypertension has risen annually in China [1–4], thus increasing cardiovascular disease-associated morbidity and mortality [5–9],

and posing a substantial financial and health burden [2, 10–16]. Left ventricular hypertrophy is a marker of hypertensive target-organ damage and a principal pathological process in heart failure [17–20]. Reversing hypertensive left ventricular hypertrophy (HLVH) is a primary therapeutic goal to prevent heart failure, and an effective method to decrease the risk of cardiovascular disease [21–24]. However, the rate of reversal is generally low [25, 26]. Human neutrophil peptide-1 (HNP-1, sequence: ACYCRIPACIAGERRYGYCIYQGRLWAFCC), also called alpha-defensin 1, is usually produced

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by immune cells such as neutrophils, lymphocytes, monocytes, and eosinophils [27, 28]. By regulating nuclear factor-κB (NF-κB) signaling pathways, HNP-1 plays a critical role in autoimmune disorders, anti-infection, and peripheral nerve injury repair [29–40]. However, the role of HNP-1 in cardiovascular disease is debated. On the one hand, HNP-1 levels have been associated with the severity of coronary atherosclerosis [41], and may increase the risk of thrombosis by altering fibrin shape, structure, and stability [42]. On the other hand, HNP-1 may prevent atherosclerosis progression and decrease plasma low-density lipoprotein cholesterol levels [43], decrease atherosclerosis [44], and mitigate arterial thrombosis by inhibiting the aggregation of von Willebrand factor [45]. Whether HNP-1 plays a role in HLVH remains unclear. Thus, to potentially identify therapeutic targets for ameliorating HLVH, this study was aimed at exploring the association between HNP-1 and HLVH.

Materials and Methods

Study Participants

A total of 861 patients with hypertension were recruited from the Affiliated Hospital of Guizhou Medical University between May and December of 2021 in Guiyang, China. All patients were at least 18 years old and had been diagnosed with hypertension according to the 2018 Chinese Guidelines for Prevention and Treatment of Hypertension [46]. After exclusion of patients with secondary hypertension, infectious diseases, rheumatic immune diseases, tumors, diabetes, stroke, coronary atherosclerotic heart disease, heart failure, or chronic kidney disease, 216 patients with hypertension were included in the study. Ethics approval for the study was obtained from the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (No. 2021-167, March 2, 2021).

Exposure and Control Groups

The 216 study participants were divided into exposure and control groups according to their left ventricular mass index (LVMI). The exposure group comprised 100 patients with HLVH whose LVMI was $>115 \text{ g/m}^2$ in men and $>95 \text{ g/m}^2$ in women [26]. The control group comprised 116 patients

with hypertension and without left ventricular hypertrophy, whose LVMI was $\leq 115 \text{ g/m}^2$ in men and $\leq 95 \text{ g/m}^2$ in women.

Rats

Forty male Wistar rats 5–6 weeks of age were obtained from the Guizhou Medical University Laboratory Animal Center. All rats were fed SPF-rated feed and kept under 12-hour light-dark cycles at $25 \pm 1 \text{ }^\circ\text{C}$. After 1 week of adaptive feeding, 39 healthy Wistar rats were selected for this study. The animal study protocol was approved by the Animal Care and Welfare Committee of Guizhou Medical University (No. 2201437, March 31, 2022).

Establishment of the HLVH Rat Model

To establish the norepinephrine (NE)-induced HLVH model in rats, we divided 39 healthy male rats into control, NE, and NE plus HNP-1 groups. Rats in the control group received intraperitoneal injections with 0.9% saline for 18 weeks. Rats in the NE group received intraperitoneal injections with NE (R030760, Rhawn, Shanghai, China) at 1.5 mg/kg/day [47] for 10 weeks, then with 0.9% saline for 8 weeks. Rats in the NE plus HNP-1 groups received intraperitoneal injections with NE at 1.5 mg/kg/day for 10 weeks, then with HNP-1 (HSA10038, Hsynthbio, Hefei, China) at 10, 20, 30, or $40 \text{ } \mu\text{g}$, once every 2 days for 8 weeks.

Blood Pressure Monitoring in Rats

Blood pressure (BP) was measured with a non-invasive tail-cuff method (Non-Invasive Blood Pressure System, CODA, Kent Scientific Corp., Austin, TX, USA). BP was monitored weekly during NE injection administration and once every 2 weeks during HNP-1 injection administration. Before BP testing, rats were restrained on a warming table set to $35 \text{ }^\circ\text{C}$ and allowed to stabilize for 10 minutes. Next, BP measurements were taken for 15 cycles in conscious rats not under anesthesia, and the average value was used to calculate the BP.

Myocardial Tissue Staining in Rats

Before being embedded in paraffin and sectioned into $4\text{--}5 \text{ } \mu\text{m}$ thick slices, cardiac ventricles were

fixed with 10% paraformaldehyde for 24–48 hours. Then hematoxylin-eosin and Masson's trichrome staining were performed according to the manufacturers' instructions. Left ventricular HNP-1 expression was identified through immunohistochemistry staining, which was performed with rabbit anti-rat alpha-defensin 1 mAb (NBP3-05562, Novus, 1:80, Colorado, USA). Finally, the sections were digitized with a fully automatic digital slice scanner.

Establishment of the H9c2 Cell Hypertrophy Model

H9c2 cells (BNCC353655, BNCC, Beijing, China) were cultured in Dulbecco's modified Eagle's medium (HyClone, USA) at 37 °C in a 5% CO₂ incubator (BPN-80CW, Shanghai, China). After the cell density reached 50%, the H9c2 cells were randomly divided into control, Ang II, and Ang II plus HNP-1 groups. Cells in the control group were treated with 10% fetal bovine serum (10099-141, Gibco, California, USA) for 5 days. Cells in the Ang II group were treated with 1 μ M Ang II (HY-13948, MCE, New Jersey, USA) for 48 hours [24] and subsequently with 10% fetal bovine serum for 72 hours. Cells in the Ang II plus HNP-1 groups were treated with 1 μ M Ang II for 48 hours and subsequently with a different dose of HNP-1 for 72 hours.

H9c2 Cell Viability Assays

A Cell Counting Kit-8 (CCK-8, KGA317, KeyGEN, Nanjing, China) was used to determine cell viability. After stimulation of cells with HNP-1 at 0, 5, 10, 15, or 20 μ g/mL for 24, 48, or 72 hours, 10 μ L CCK-8 reagent was added to each well at 37 °C and incubated for 2 hours. The absorbance was then measured at 450 nm.

H9c2 Cell Immunofluorescence Staining

After fixation in 4% paraformaldehyde for 15 minutes, the H9c2 cells were blocked with 5% bovine serum albumin for 30 minutes. Next, mouse anti-rat F-actin mAb (OM252288, OmnimAbs, 1:100, Shanghai, China) was added to the H9c2 cells. Finally, the H9c2 cells were observed under a fluorescence microscope (CKX53, OLYMPUS, Japan).

Western Blotting

HNP-1, BNP, and β -MHC levels were measured with rabbit anti-rat alpha-Defensin 1 mAb (NBP3-05562, Novus, 1:2000, Colorado, USA), rabbit anti-rat BNP mAb (WL02126, Wanlei, 1:1000, Shenyang, China), and rabbit anti-rat β -MHC mAb (NBP2-74079, Novus, 1:2000, Colorado, USA), respectively. IKK β , p-IKK β , I κ B α , p-I κ B α , p65, p-p65, and GAPDH levels were measured with rabbit anti-rat IKK β , p-IKK β , I κ B α , p-I κ B α , p65, p-p65 mAb (all 1:500, Affinity, USA), and GAPDH mAb (1:10000, Affinity, USA), respectively.

Real-Time Quantitative Polymerase Chain Reaction

Total RNA was extracted from H9c2 cells with TRIzol reagent (CW0580S, CWBIO, Beijing, China). Subsequently, total RNA was reverse-transcribed into first-strand cDNAs with HiScript II Q RT SuperMix (R223-01, Vazyme, Nanjing, China). Finally, real-time quantitative polymerase chain reaction (qPCR) was performed with ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme, Nanjing, China), and the fluorescence was captured by an iCycler IQ system (Chemi DocTM XRS+, Bio-Rad, CA, USA). The relative expression of mRNA was calculated according to Ct values. GAPDH was used as the reference gene. All primers are listed in Table 1.

Table 1 Sequences of Primers Used in qPCR.

Gene	Sequences (5'-3')
<i>BNP</i>	Forward: TCCTGCTTTTCCTTAATCTGTC Reverse: GCTTGAACATATGTGCCATCTTG
<i>β-MHC</i>	Forward: AGGCTCATCCTTTCAGACCG Reverse: TCACCCCTGGAGACTTCGT
<i>IKKβ</i>	Forward: CAGAAGAGCGAAGTGGACATT Reverse: TGAGATTATTGGGAAGGGT
<i>IκBα</i>	Forward: TGTCTACACTTAGCCTCTATCC Reverse: GGGCAACTCATCTTCCGT
<i>p65</i>	Forward: GCAAAGGACCTACGAGACC Reverse: CGGGAAGGCACAGCAATA
<i>GAPDH</i>	Reverse: GACAACCTTTGGCATCGTGGA Reverse: ATGCAGGGATGATGTTCTGG

BNP, brain natriuretic peptide; β -MHC, β -myosin heavy chain.

Enzyme-Linked Immunosorbent Assays

An enzyme-linked immunosorbent assay (ELISA) kit (JL20063, JIANGLA, Shanghai, China) was used to measure the concentration of serum HNP-1 in patients, according to the manufacturer's instructions. ELISA kits (MM-0193R1, MM-0193R1, and MM-0180R1, MEIMIAN, Shanghai, China) were also used to measure interleukin (IL)-1 α , IL-6, and tumor necrosis factor α (TNF- α) levels, in accordance with the manufacturer's instructions.

Statistical Analysis

Statistical analyses were conducted in SPSS (21.0, IBM, USA) and GraphPad Prism 8.4 (CA, USA). The integrated density of images was calculated in ImageJ software (Media Cybernetics, USA). Student's t-test, Wilcoxon signed rank test, and one-way ANOVA were used to assess between-group differences. Pearson or Spearman correlation analyses and regression analyses were used to compare

associations among HNP-1, BP, and left ventricular hypertrophy indexes. $P < 0.05$ was considered to indicate statistical significance.

Results

Participants' Descriptive Characteristics and HNP-1 Levels

No statistically significant differences were identified between the exposure and control groups in terms of age, sex, body mass index (BMI), and cardiovascular risk factors, including smoking, TG, TC, L-DLC, H-DLC, glucose, Hcy, and history of hypertension (Table 2). In addition, no statistically significant differences were observed in the proportion of patients taking anti-hypertensive drugs, including beta-blockers, CCBs, ACEI/ARB, and diuretics (Table 2). However, the BP and HLHV indexes were higher in the exposure than the control group (Figure 1, Table 3), whereas HNP-1 levels

Table 2 Descriptive Characteristics of Participants in the Two Groups.

Variables	Exposure group (n = 100)	Control group (n = 116)	P
Age (years)	51.66 (49.85–54.18)	48.20 (45.86–50.73)	0.076
Males [†]	42 (42%)	61 (52.59%)	0.132
Smoking [†]	39 (39%)	47 (40.52%)	0.889
Body weight (kg)	67.89 (65.23–70.27)	69.53 (66.78–72.00)	0.351
BMI (kg/m ²)*	26.38 (3.87)	25.86 (3.17)	0.287
TG (mmol/L)	1.89 (1.69–2.10)	1.98 (1.77–2.16)	0.482
TC (mmol/L)	4.41 (4.26–4.59)	4.43 (4.28–4.58)	0.664
LDL-C (mmol/L)	2.70 (2.55–2.85)	2.79 (2.63–2.92)	0.308
HDL-C (mmol/L)	1.13 (1.08–1.19)	1.13 (1.07–1.09)	0.705
Hcy (μ mol/L)	14.63 (12.99–16.81)	15.22 (13.40–17.66)	0.671
Glucose (mmol/L)*	7.80 (7.32–8.31)	7.17 (6.84–7.52)	0.151
Hypertension history (years)	8.42 (6.56–10.54)	5.78 (4.35–7.33)	0.304
CCB [†]	59 (59%)	60 (51.72%)	0.338
ACEI [†]	10 (10%)	11 (10.09%)	0.816
ARB [†]	22 (22%)	21 (15.60%)	0.500
β -blockers [†]	19 (19%)	19 (16.38%)	0.721
Diuretics [†]	9 (9%)	4 (2.75%)	0.149

*Results are given as mean (SD). [†]Results are given as number (%). All other results are given as median (95% CI).

BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Hcy, homocysteine; CCB, calcium-channel blocker; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers.

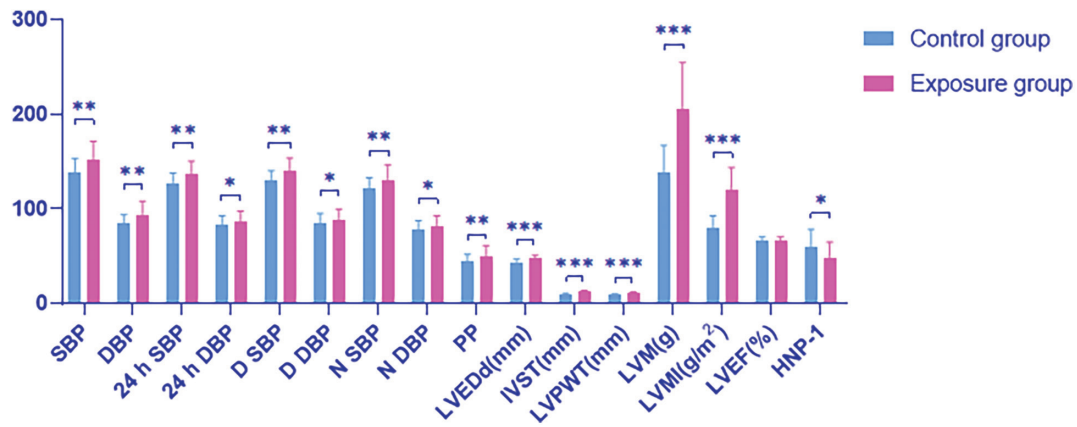


Figure 1 Blood Pressure, Left Ventricular Hypertrophy Indexes, and HNP-1 Level in the Two Groups.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. SBP, systolic blood pressure; DBP, diastolic blood pressure; 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; D SBP, daytime systolic blood pressure; D DBP, daytime diastolic blood pressure; N SBP, night systolic blood pressure; N DBP, night diastolic blood pressure; PP, pulse pressure; LVEDd, left ventricular end diastolic dimension; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass index; LVEF, left ventricle ejection fraction; HNP-1, human neutrophil peptide-1.

Table 3 Comparative Analysis of Blood Pressure and Left Ventricular Hypertrophy Indexes in the Two Groups.

Variables	Exposure group (n = 100)	Control group (n = 116)	P
SBP [†]	152.53 (148.75–156.46)	137.82 (135.08–140.99)	0.001**
DBP	94.02 ± 14.12	84.37 ± 9.68	0.001**
24 h SBP	136.62 ± 13.95	127.64 ± 10.25	0.001**
24 h DBP	86.60 ± 10.96	82.91 ± 9.65	0.03*
D SBP	139.50 ± 14.34	130.47 ± 10.23	0.001**
D DBP	88.76 ± 11.13	85.29 ± 9.81	0.045*
N SBP	130.80 ± 15.87	121.30 ± 11.56	0.001**
N DBP	81.83 ± 11.15	77.75 ± 10.08	0.020*
PP [†]	50.02 (48.05–52.05)	44.72 (43.20–46.29)	0.001**
LVEDd (cm) [†]	4.73 (4.65–4.80)	4.40 (4.35–4.46)	0.000***
IVST (cm) [†]	1.20 (1.16–1.24)	0.97 (0.95–0.99)	0.000***
LVPWT (cm) [†]	1.11 (1.08–1.14)	0.93 (0.92–0.95)	0.000***
LVM (g) [†]	205.68 (196.73–215.15)	139.79 (134.76–144.77)	0.000***
LVMI (g/m ²) [†]	120.97 (116.95–125.40)	80.48 (78.50–82.73)	0.000***
RWT [†]	0.47 (0.46–0.48)	0.42 (0.42–0.44)	0.000***
LVEF (%) [†]	65.88 (64.98–66.82)	67.21 (66.68–67.74)	0.061

[†]Results are given as median (95% CI). All other results are given as mean (SD).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

SBP, systolic blood pressure; DBP, diastolic blood pressure; 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; D SBP, daytime systolic blood pressure; D DBP, daytime diastolic blood pressure; N SBP, night systolic blood pressure; N DBP, night diastolic blood pressure; PP, pulse pressure; LVEDd, left ventricular end diastolic dimension; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass index; RWT, relative wall thickness; LVEF, left ventricle ejection fraction.

were significantly lower in the exposure than the control group (M (95% CI), 48.83 (45.64–52.26) vs. 59.03 (55.62–62.54), $P = 0.000$; Figure 1).

Furthermore, HNP-1 levels were significantly negatively correlated with BP and left ventricular hypertrophy indexes (Table 4).

Table 4 Correlations of HNP-1 Levels with Blood Pressure and Left Ventricular Hypertrophy Indexes in Patients with Hypertension.

Variables	HNP-1	
	<i>r</i>	P value
SBP (mmHg) [†]	-0.176	0.010*
DBP (mmHg) [†]	-0.196	0.004**
24 h SBP (mmHg) [†]	-0.073	0.287
24 h DBP (mmHg) [†]	-0.010	0.890
D SBP (mmHg) [†]	-0.082	0.233
D DBP (mmHg) [†]	-0.024	0.727
N SBP (mmHg) [†]	-0.052	0.447
N DBP (mmHg) [†]	0.021	0.758
PP (mmHg) [‡]	-0.161	0.018*
LVEDd (cm) [†]	-0.218	0.001**
IVST (cm) [‡]	-0.083	0.225
LVPWT (cm) [‡]	-0.122	0.074
LVM (g) [‡]	-0.186	0.006**
LVMI (g/m ²) [‡]	-0.259	0.000**
RWT [†]	-0.019	0.780
LVEF (%) [‡]	0.054	0.434

[†]Indicates Pearson correlation analysis. [‡]Indicates Spearman correlation analysis.

*indicates significant correlation at the 0.05 level (two tailed); **indicates significant correlation at the 0.01 level (two tailed).

HNP-1, human neutrophil peptide-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; D SBP, daytime systolic blood pressure; D DBP, daytime diastolic blood pressure; N SBP, night systolic blood pressure; N DBP, night diastolic blood pressure; PP, pulse pressure; LVEDd, left ventricular end diastolic dimension; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass index; RWT, relative wall thickness.

Diminished HNP-1 is a Risk Factor for HL VH in Patients

Multiple linear regression analyses revealed that diminished HNP-1 was significantly correlated with LVMI after adjustment for age, sex, SBP, DBP, 24 h SBP, 24 h DBP, D SBP, D DBP, N SBP, N DBP, and PP (Table 5). Binary logistic regression analyses indicated that diminished HNP-1 was significantly correlated with HL VH after adjustment for the aforementioned variables (Table 6).

Table 5 Multiple Linear Regression Analysis of Multiple Factors Associated with LVMI and RWT in Patients with Hypertension.

Variables	LVMI		RWT	
	<i>t</i>	P	<i>t</i>	P
Age (years)	0.100	0.921	0.158	0.874
Sex	-1.342	0.181	-1.033	0.303
HNP-1 (ng/mL)	-2.996	0.003*	-0.237	0.813
SBP (mmHg)	0.781	0.436	0.675	0.500
DBP (mmHg)	0.509	0.611	-0.367	0.714
24 h SBP (mmHg)	1.325	0.187	0.672	0.502
24 h DBP (mmHg)	-0.953	0.342	0.696	0.487
D SBP (mmHg)	1.437	0.152	0.203	0.840
D DBP (mmHg)	-0.863	0.389	0.956	0.340
N SBP (mmHg)	1.160	0.247	-1.175	0.241
N DBP (mmHg)	0.892	0.373	0.987	0.325
PP (mmHg)	1.163	0.246	0.662	0.509

*Indicates significant correlation at the 0.05 level (two tailed).

HNP-1, human neutrophil peptide-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; D SBP, daytime systolic blood pressure; D DBP, daytime diastolic blood pressure; N SBP, night systolic blood pressure; N DBP, night diastolic blood pressure; PP, pulse pressure.

HNP-1 Treatment Decreases BP in NE-Induced HL VH Rats

After injection with NE, in the fifth week, the SBP and DBP began to be significantly higher in the NE group than the control group (Figure 2A and B). In the NE group, SBP increased by 22.43 mmHg, and DBP increased by 25.96 mmHg (from baseline BP) after injection with NE for 10 weeks (Figure 2E). However, after HNP-1 injection, SBP and DBP decreased over time (Figure 2C and D). Finally, SBP and DBP were considerably lower in each NE plus HNP-1 treatment group than in the NE group (Figure 2F).

HNP-1 Treatment Increases Left Ventricular HNP-1 Expression in NE-Induced HL VH Rats

Immunohistochemistry staining indicated that HNP-1 was expressed primarily on the cardiomyocyte membranes in the left ventricle in rats.

Table 6 Binary Logistic Regression Analysis of Risk Factors for Left Ventricular Hypertrophy in Patients with Hypertension.

Variables	β	OR (95% CI)	P
Age (years)	-0.001	0.999 (0.965–1.033)	0.969
Sex	-0.881	0.952 (0.224–0.902)	0.077
HNP-1 (ng/mL)	-0.027	0.974 (0.956–0.995)	0.011*
SBP (mmHg)	0.046	1.047 (0.996–1.101)	0.072
DBP (mmHg)	0.006	1.006 (0.950–1.066)	0.839
24 h SBP (mmHg)	0.055	1.056 (0.823–1.356)	0.667
24 h DBP (mmHg)	-0.232	0.793 (0.535–1.175)	0.248
D SBP (mmHg)	0.203	1.225 (0.932–1.610)	0.146
D DBP (mmHg)	-0.109	0.896 (0.732–1.099)	0.292
N SBP (mmHg)	0.099	1.104 (0.957–1.274)	0.174
N DBP (mmHg)	-0.013	0.987 (0.858–1.136)	0.859
PP (mmHg)	-0.056	1.045 (0.730–1.113)	0.647

*Indicates significant correlation at the 0.05 level (two tailed).

HNP-1, human neutrophil peptide-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; D SBP, daytime systolic blood pressure; D DBP, daytime diastolic blood pressure; N SBP, night systolic blood pressure; N DBP, night diastolic blood pressure; PP, pulse pressure.

Furthermore, left ventricular HNP-1 expression decreased in the NE group than the control group. Left ventricular HNP-1 expression was greater in the NE plus HNP-1 groups than the NE group, and the effect was dose-dependent (Figure 3).

HNP-1 Treatment Ameliorates Ventricular Hypertrophy and Fibrosis in NE-Induced HL VH Rats

The HW/BW and HW/TL ratios were significantly higher in the NE group than the control group, and significantly lower in each NE plus HNP-1 treatment group than the NE group (Figure 4A). Hematoxylin-eosin staining revealed that left ventricular single cardiomyocyte surface area was greater in the NE group than the control group. Furthermore, the left ventricular single cardiomyocyte surface area was smaller in each NE plus HNP-1 treatment group than the NE group (Figure 4B). In addition, Masson's trichrome staining indicated that the left ventricular fibrosis area was greater in the NE group than the control group. However, compared with that in the NE group, the left ventricular fibrosis area was lower, to varying degrees, in each NE plus HNP-1 treatment group (Figure 4B).

HNP-1 Treatment Decreases the Expression of BNP, β -MHC, and NF- κ B in NE-Induced HL VH Rats

Western blotting (WB) revealed that the left ventricular expression of BNP, β -MHC, and NF- κ B signaling factors (including p-IKK β /IKK β , p-I κ B α /I κ B α , and p-p65/p65) was higher in the NE group than the control group. However, compared with that in the NE group, the expression of the aforementioned proteins was lower, to varying degrees, in the NE plus HNP-1 treatment groups (Figure 5).

HNP-1 Treatment Promotes H9c2 Cell Growth Activity and Reverses Ang II-Induced H9c2 Cell Hypertrophy

CCK8 assays indicated that HNP-1 promoted H9c2 cell growth viability. Indeed, H9c2 cell growth activity was higher after stimulation with HNP-1 at 10 and 15 μ g/mL for 72 hours than after stimulation with HNP-1 at 5 and 20 μ g/mL for 24 and 48 hours, respectively (Figure 6A). Immunofluorescence analysis revealed that the single-cell surface area was greater in the Ang II group than the control group. However, compared with that in the Ang II group, the single cell surface area was smaller, to varying degrees, in each Ang II plus HNP-1 group (Figure 6B).

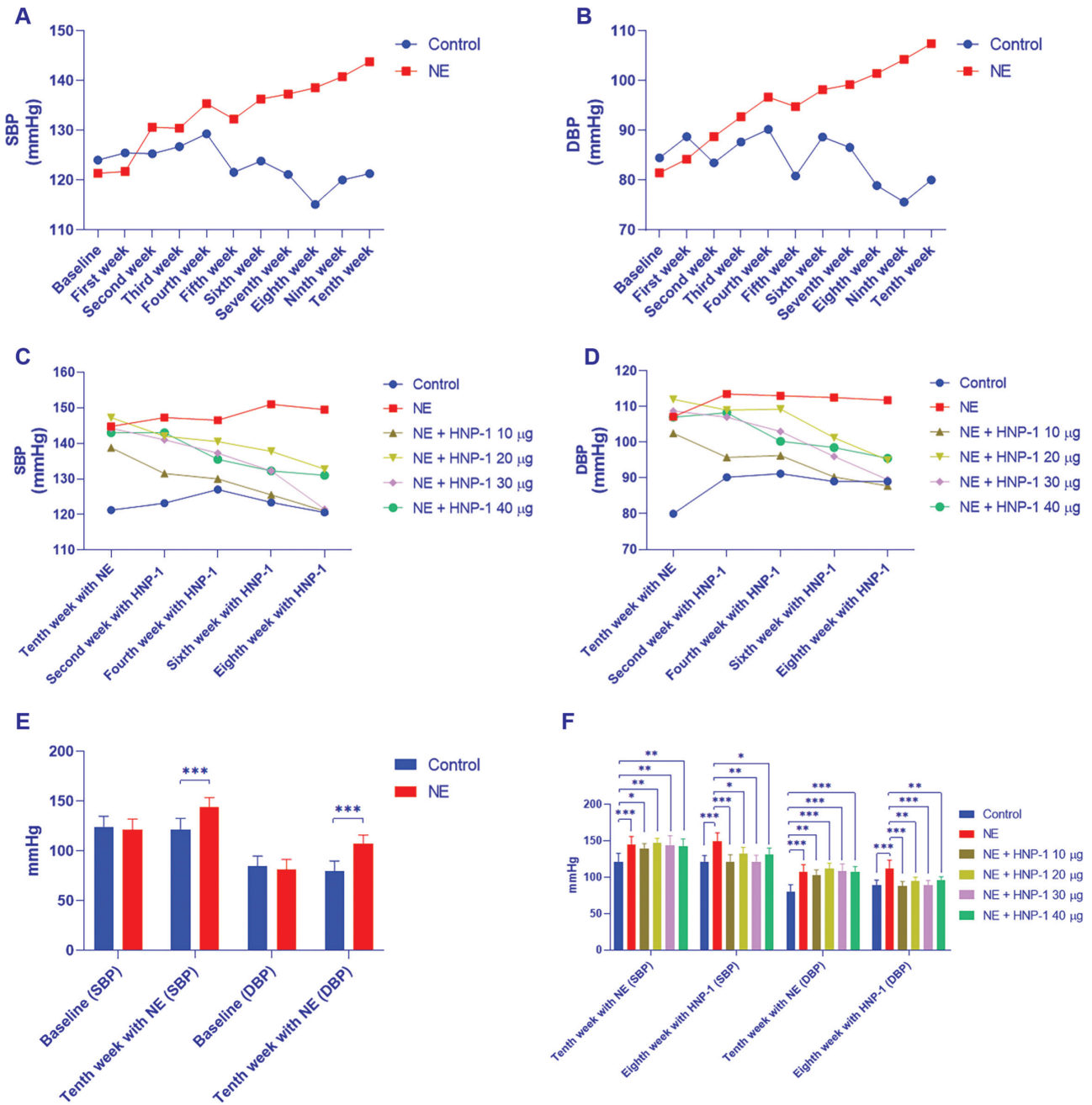


Figure 2 Levels of SBP and DBP.

A and B, SBP and DBP levels during injection with NE; **C and D**, SBP and DBP levels during injection with HNP-1; **E**, SBP and DBP levels between baseline and the tenth week of NE injection; **F**, SBP and DBP levels between the tenth week of NE injection and eighth week of HNP-1 injection. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. SBP, systolic blood pressure; DBP, diastolic blood pressure.

HNP-1 Treatment Decreases the Expression of BNP, β-MHC, NF-κB, and Inflammatory Factors in Ang II-Induced H9c2 Cell Hypertrophy

WB analysis indicated that HNP-1 levels decreased in the Ang II group compared with the control group, but it increased in the Ang II plus HNP-1 treatment

groups compared with the Ang II group (Figure 7A). WB and qPCR also revealed that BNP, β-MHC, p65, and p-p65/p65 expression increased in the Ang II group compared with the control group, but those indexes decreased in each Ang II plus HNP-1 group to varying degrees compared with the Ang II group (Figure 7A and B). Additionally, the IL-1α, IL-6, and TNF-α levels were lower in the Ang II

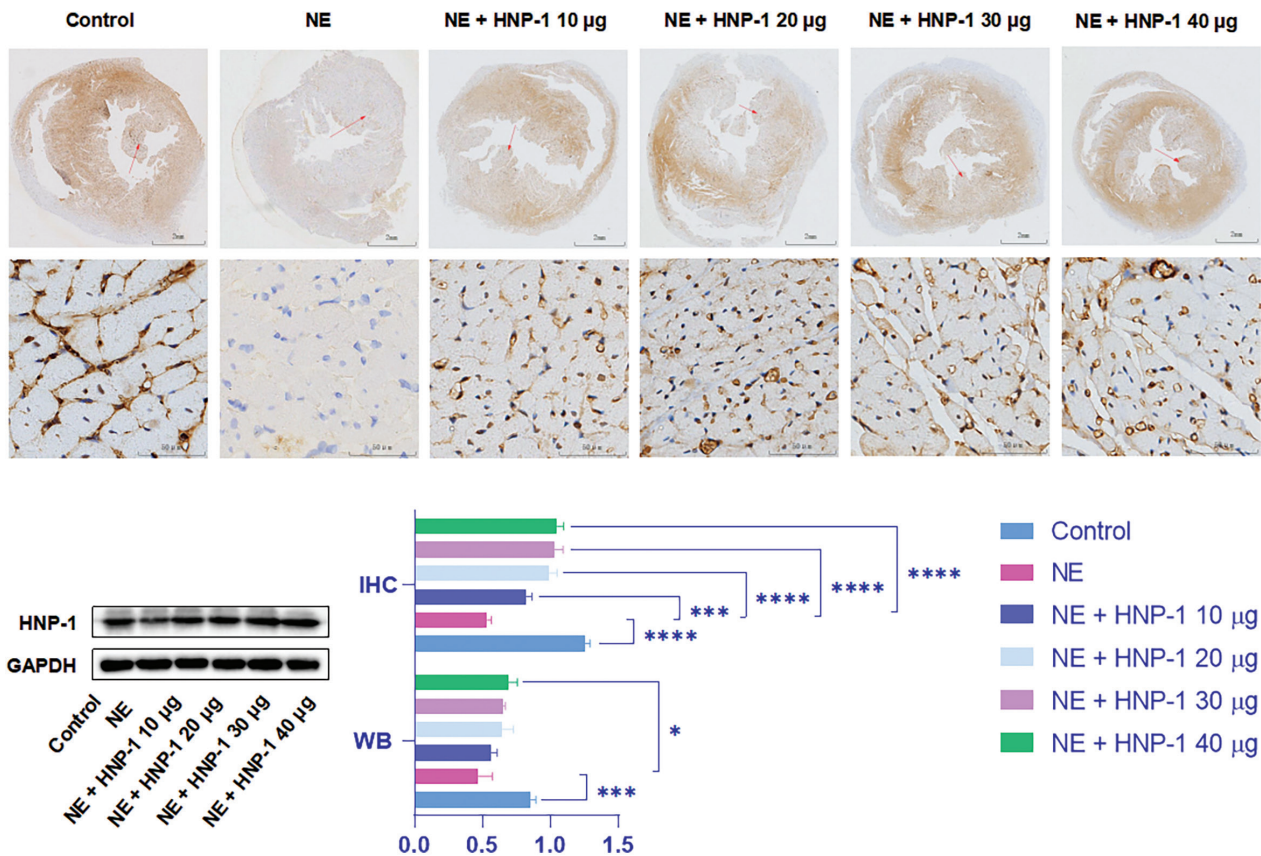


Figure 3 HNP-1 Protein Relative Expression.

Representative images were the quantitative analysis of IF and WB for the HNP-1 expression in left ventricle. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

plus HNP-1 groups than the Ang II group, particularly after treatment with HNP-1 at 15 $\mu\text{g}/\text{mL}$ and 20 $\mu\text{g}/\text{mL}$ (Figure 7C).

Discussion

This study demonstrated that HNP-1 levels were diminished in patients with HLVH and were a risk factor for HLVH. In the experimental HLVH model, HNP-1 treatment decreased NF- κ B and other heart hypertrophy markers. These findings indicated that HNP-1 reverses HLVH and may be a potential therapeutic target for cardiovascular disease. HNP-1 is the first cell-synthesized peptide demonstrated to directly enter a target cell, and subsequently affect translation and regulate protein expression [48]. Furthermore, HNP-1 is widely found in various tissues and organs but is expressed primarily in human neutrophil lineage cells [29, 49–54]. The HNP-1 gene is located on chromosome 8p23.1 [54–57]. In neutrophil lineage cells, HNP-1 is translated into a

94-amino-acid precursor form in promyelocytes and is sequentially cleaved into a 30-amino-acid mature HNP-1 in the Golgi apparatus [58, 59]. The mature HNP-1 is stored in the blue granules, and can be released by cell degranulation or secretion [54].

Previous studies have reported diminished HNP-1 levels in older people, smokers, people who are toothless or have tooth decay, and people with lipid metabolism disorders or vasodilation responses [60–62]. Decreased HNP-1 is associated with aging, decreased immune resistance, lipid metabolism disorders, and a diminished vasodilation response [63, 64]. Similarly to previous studies, this study indicated that HNP-1 levels were diminished in patients with HLVH and were a risk factor for HLVH.

This current study also demonstrated that HNP-1 treatment may decrease BP in HLVH rats, although the BP did not change in a dose-dependent manner. This finding has three possible explanations. First, HNP-1's BP-lowering effect might potentially be associated with baseline BP levels. Second, HNP-1 may have a "bipolar effect," such that inflammatory

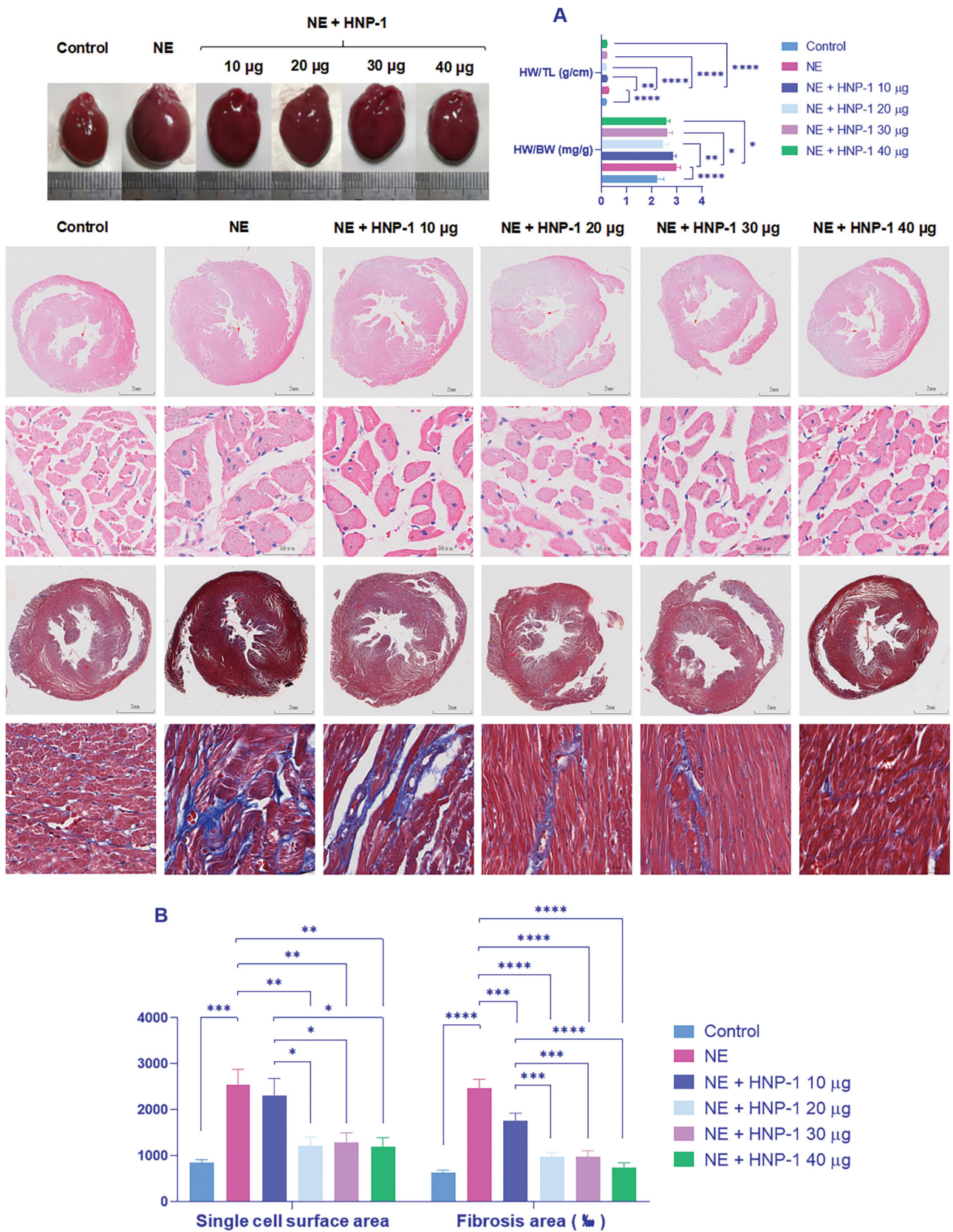


Figure 4 Left Ventricular Myocardial Cell Surface Area and Fibrosis Area. **A**, Quantitative analysis of HW/BW and HW/TL; **B**, left ventricle single cell surface area and fibrosis area. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. HW/BW, heart weight to body weight ratios; HW/TL, heart weight to tibia length ratios.

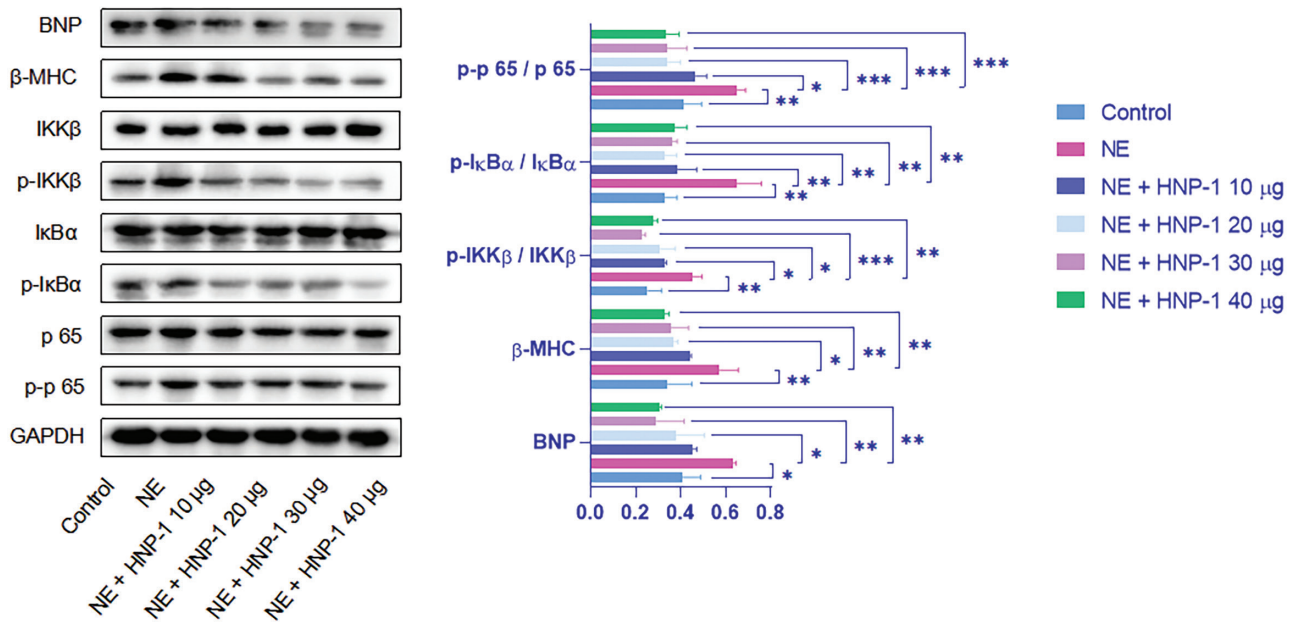


Figure 5 Protein Levels of BNP, β -MHC, and NF- κ B Signaling Factors in the Left Ventricle. Representative Images of WB and Quantitative Analysis for the Protein Expression of BNP, β -MHC, and NF- κ B in the Left Ventricle. * P < 0.05, ** P < 0.01, *** P < 0.001. BNP, brain natriuretic peptide, β -MHC, beta-myosin heavy chain.

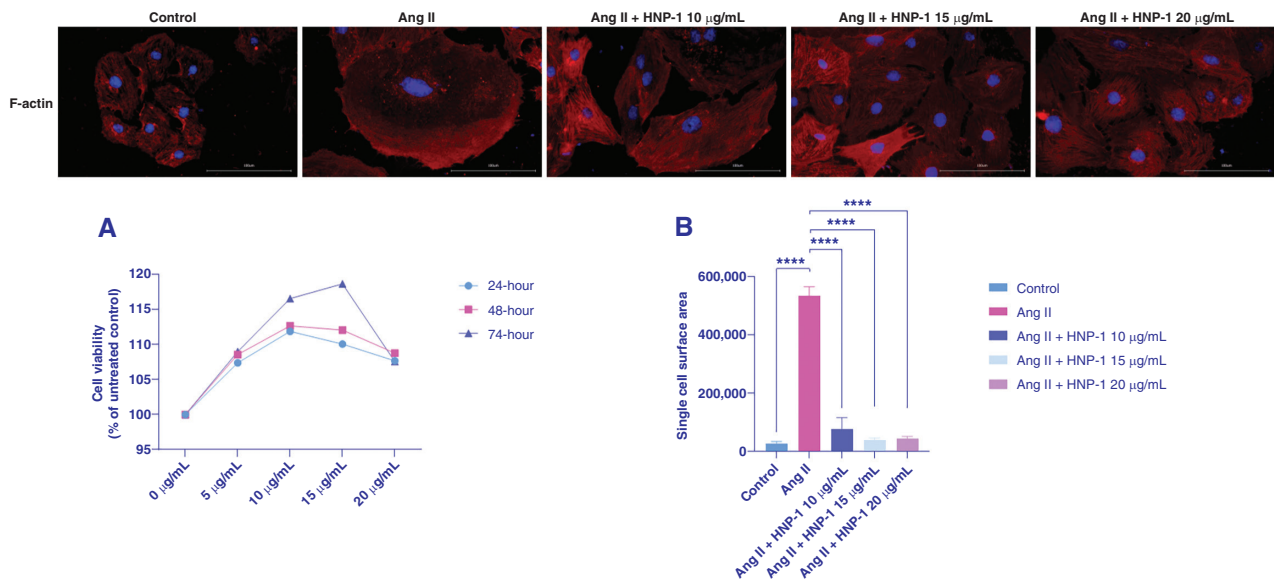


Figure 6 Cell Viability and Single Cell Surface Area. **A**, Cell growth viability measured with CCK8 assays after treatment with HNP-1 for 24, 48, or 72 hours. **B**, Quantitative analysis of single cell surfaces. Cells were stained with an anti-F-actin antibody (red), and nuclei were stained with DAPI (blue). **** P < 0.0001.

factor expression may be enhanced by a high dose of HNP-1 (100 μ g/day) and inhibited by a healthy dose of HNP-1 (5 μ g/day or 10 μ g/mL) [65]. Third, studies have reported that regulation of the inflammatory response effectively controls BP and ameliorates HLVH [65–71]. However, NF- κ B is a key regulatory signaling pathway in inflammatory

immune responses [72–75], and NF- κ B inhibition may ameliorate hypertension and HLVH [76–86]. We determined that HNP-1 may inhibit NF- κ B activation, but this inhibitory effect may not be dose-dependent.

According to our study, HNP-1 treatment increased HNP-1 expression in a HLVH rat model. In contrast,

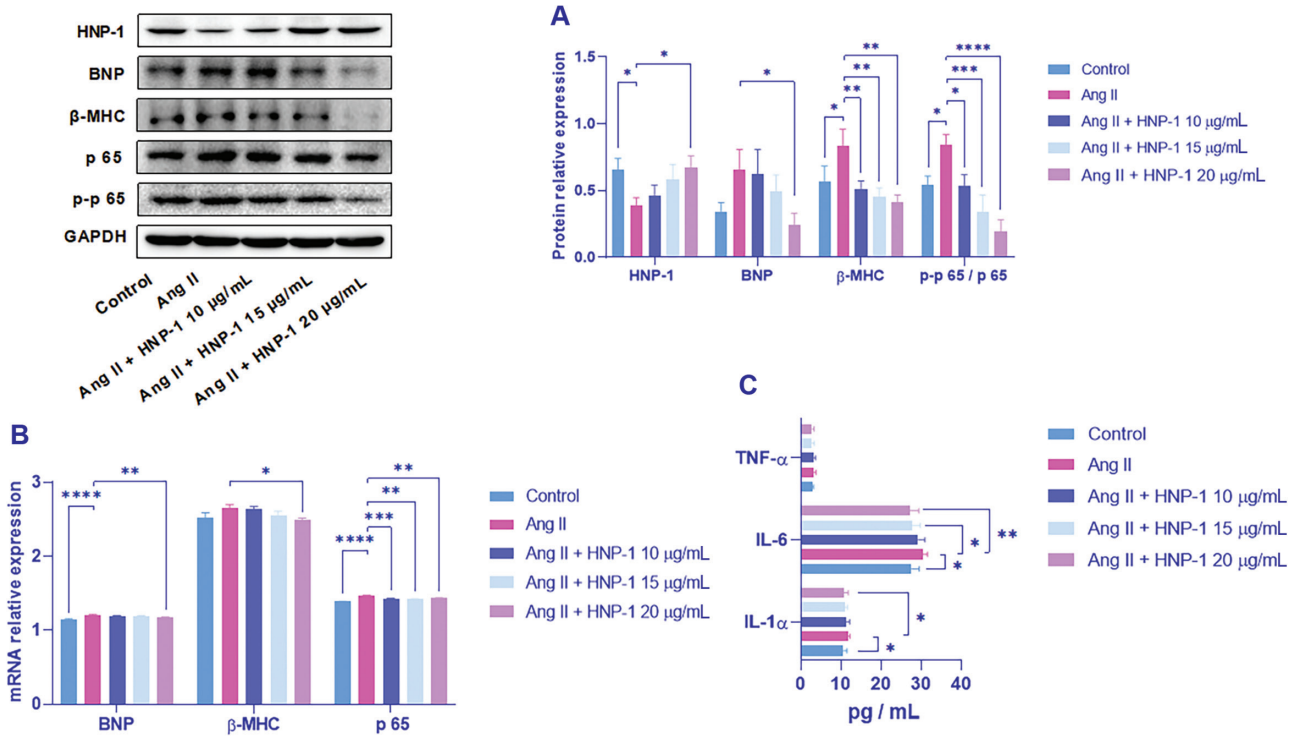


Figure 7 Expression of HNP-1, BNP, β-MHC, p65, and Inflammatory Factors in H9c2 cells. **A**, WB and quantitative analysis of the protein expression of HNP-1, BNP, β-MHC, and p-p65/p65 in H9c2 cells. **B**, qPCR and quantitative analysis of the mRNA expression of BNP, β-MHC, and p65 in H9c2 cells. **C**, ELISA and quantitative analysis of IL-1α, IL-6, and TNF-α levels in H9c2 cells. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Kou et al. [34] have reported that HNP-1 treatment does not increase HNP-1 expression in RSC 96 cells. Two possible explanations may be responsible for this difference in findings. The first is associated with differences between experimental models. Specifically, Kou et al. [34] studied RSC 96 cells in the nervous system, whereas our study focused on rat left ventricular tissue and H9c2 cells. Second, the HNP-1 treatment dose and duration differed between studies. Kou et al. [34] treated RSC 96 cells with HNP-1 at 4 or 8 μg/mL for 36 hours. In contrast, we treated HLHV rats with HNP-1 at 10, 20, 30, or 40 μg for 8 weeks, and H9c2 cells with HNP-1 at 10, 15, or 20 μg/mL for 72 hours. Furthermore, HNP-1 expression was notably associated with the treatment dose of HNP-1. HNP-1 expression was weak when the HNP-1 treatment dose was 10 μg in rats and 10 μg/mL in H9c2 cells, and was enhanced as the treatment dose of HNP-1 increased. Future studies are warranted to explore whether this effect might be due to increased endogenous HNP-1 or exogenous HNP-1.

This current study indicated that HNP-1 treatment inhibited the activation of NF-κB in an HLHV model. However, other studies have reported

differing effects of HNP-1 on NF-κB signaling pathways. For example, Kou et al. and Wang et al. [29, 34] have found that p65 expression increases in Schwann cells and CAL-1 cells after treatment with HNP-1. However, mRNA expression of AKT in Schwann cells shows lower activity when treated with 8 rather than 4 μg/mL of HNP-1. Other studies have reported that A549 cells and CD4 lymphocytes show no differences in p65 expression after treatment with HNP-1 [33, 43]. Finally, studies have indicated that HNP-1 may enhance [87] or inhibit [88] the activation of complement classical pathways. Therefore, we speculate that HNP-1 might have different regulatory effects on NF-κB signaling pathways in various tissues and cells under different experimental conditions. This study identified HNP-1's protective role in HLHV; however, the specific underlying mechanisms warrant further study.

Conclusion

HNP-1 decline is a risk factor for HLHV in patients. HNP-1 treatment may reverse HLHV and H9c2

cell hypertrophy by inhibiting NF- κ B signaling pathways.

Data Availability Statement

Experimental data are available through the corresponding author.

Author Contributions

Xiaorong Duan performed the main experiments and drafted the manuscript. Zhenqiu Yu directed the experimental research and manuscript revision. Experimental data are available through the corresponding author. Ethics Statement was provided in the part of materials and methods.

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Conflict of Interest

We declare that none of the work in this manuscript has been published in any language or is currently under consideration at any other journal. There are no conflicts of interest to declare. All authors have read and approved this submitted manuscript in its current form. Our manuscript has also been edited by a native English-speaking expert to ensure its English is sufficient for publication.

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