Protective effect of lyophilized recombinant human brain natriuretic peptide on renal ischemia/reperfusion injury in mice

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ABSTRACT. Brain natriuretic peptide (BNP) has a protective effect on acute injury of the heart, brain, and lung. However, its role in acute kidney injury (AKI) remains unclear. The aim of this study was to investigate the effect of lyophilized recombinant human BNP (lrh-BNP) on AKI and the underlying molecular mechanisms. An experimental model for AKI was established using an ischemia/reperfusion (I/R) procedure. Healthy adult BALB/c mice were randomized to the sham, I/R, and lrh-BNP-treated post-I/R (BNP + I/R) groups. Post-operatively, the BNP + I/R group was subcutaneously injected with lrh-BNP (0.03 µg·kg^{-1}·min^{-1}), whereas the other groups received saline at the same dose. Serum creatinine (Scr) and blood urea nitrogen levels were examined; tissue staining was performed to evaluate the degree of I/R injury (IRI). Ki67 positive staining of renal tubular epithelial cells was observed using immunofluorescence confocal laser scanning to assess the effect of BNP on cell proliferation after IRI. Inflammatory factor expression levels were detected to evaluate the effect of BNP on renal inflammation. Compared with the sham group, the I/R

group showed increased Scr levels, severe tubular injury of the renal outer medulla, increased Kim-1 mRNA expression, an increased number of infiltrative macrophages in the renal interstitium, and increased TNF-α, IL-1β, IL-6, MCP-1, and HIF-1α mRNA expression. BNP delivery significantly reduced all pathological changes in the I/R group. The protective role of BNP in murine renal IRI may be associated with its inhibition of renal interstitial inflammation and hypoxia and its promotion of renal tubule repair.

Key words: Renal ischemia/reperfusion injury; Inflammation; Hypoxia; Brain natriuretic peptide

INTRODUCTION

The kidney is one of the most susceptible organs to ischemia/reperfusion injury (IRI), which results in a relatively high rate of morbidity and mortality (Devarajan, 2006; Bonventre and Yang, 2011; Basile et al., 2012). The mechanisms underlying IRI are complex and include oxyradical-induced lipid peroxidation injury, imbalance of relaxation and contraction factors produced by renal vascular endothelial cells, and inflammation (Devarajan, 2006; Bonventre and Yang, 2011; Basile et al., 2012). Renal IRI is the major cause of ischemic acute renal failure.

Renal interstitial inflammation plays an important role in the progression of renal IRI. The associated mechanisms include intracellular calcium overload, mitochondrial dysfunction, activation of multiple enzyme systems, and secretion of diverse pro-inflammatory cytokines (Devarajan, 2006). During renal IRI progression, the numbers of infiltrative neutrophil granulocytes and macrophages increase in the renal interstitium (Decleves et al., 1999) Large amounts of inflammatory cytokines are produced by these interstitial renal cells, which are themselves targets of these cytokines. This process aggravates the inflammation and accelerates tissue damage (Awad et al., 2009). Therefore, reducing inflammation can alleviate the severity of renal IRI (Ranganathan et al., 2013).

During ischemic acute kidney injury (AKI), peritubular capillary (PTC) loss occurs, which is associated with renal tubular damage, hypoxia, and inflammatory cell infiltration (Lien et al., 2003; Ishii et al., 2005). Furthermore, PTC loss occurring during renal ischemic injury is positively correlated with renal failure and renal interstitial fibrosis (Li et al., 2010; Lin et al., 2010). Promoting reparative regeneration of the renal vascular system can reverse these pathological changes (Li et al., 2006; Li et al., 2010). Apoptosis and necrosis of renal tubular epithelial cells are the main pathological features of AKI, which is closely related to the degree of IRI, and appeared at different time point post injury (Gobe et al., 1999). Accordingly, recovery from renal IRI requires reparative regeneration of the renal tubular epithelial cells (Esson and Schrier, 2002). Therefore, proliferation of these cells is important in the repair of damaged kidneys.

In the last few years, research on ischemic kidney disease and injury has mainly focused on the physiological action of atrial natriuretic peptide (ANP), a functionally similar subtype of brain (or B-type) natriuretic peptide (BNP). Studies have shown that ANP can not only protect the myocardium from damage caused by IRI (Kousholt, 2012) but can also have a protective effect on the lungs and liver. The mechanism of the protective effect of ANP is related to reduction in oxidative stress, inhibition of inflammation and inflammatory cell infiltration, and protection of endothelial function (Dodd-o et al., 2008; Aoyama et al., 2009; Yamada et al., 2013). A recent study on renal IRI reported that ANP also plays a protective role in ischemic renal injury (Koga et al., 2012). It has been established that the physiological effect of BNP is somewhat similar to that of ANP.

BNP was first extracted from pig brain tissue by a Japanese group (Sudoh et al., 1988). BNP belongs to the natriuretic peptide family and is synthesized and secreted mainly by cardiac myocytes, where it is used as a hemodynamic parameter that can reflect left ventricular dysfunction and decompensated heart failure (Jaffe et al., 2006). Many studies have demonstrated that BNP functions as an anti-inflammatory, antioxidant and anti-apoptotic factor that can protect the heart from IRI (Hu et al., 2014). However, whether BNP can also protect the kidney from damage is unclear. There are a few reports on the effects of BNP on AKI but the results are ambiguous. Kim et al. (2010) observed that delivery of BNP in mice with AKI did not result in an improvement in the filtration function of the kidneys. In contrast, Song et al. (2013) examined the effect of BNP in a canine model of AKI and found that BNP had a mild protective effect. Therefore, the effect of BNP on AKI needs to be further examined and clarified.

Based on the aforementioned evidence, we investigated the effect of BNP on renal IRI and examined the possible mechanisms underlying its effect. The results of this study may be useful for future clinical applications of BNP in the treatment of IRI-induced AKI.

MATERIAL AND METHODS

Animals

A total of 36 healthy adult BALB/c male mice (aged 8-10 weeks; weighing approximately 20-25 g) were included in this study. The mice were randomly divided into three groups (N = 12): sham operation group, renal ischemia/reperfusion (I/R) group, and lyophilized recombinant human BNP (lrh-BNP) treatment (BNP + I/R) group. The surgical procedures were approved by the Institute of Animal Ethics of the Fourth Affiliated Hospital of Harbin Medical University (China).

Model establishment

The mice were fasted for 12 h before the operation was performed. Pre-operatively, 5% chloral hydrate solution was injected intraperitoneally at 7 mL/kg for anesthesia at room temperature (25-28°C). For the procedure, incisions were made along both sides of the lateral dorsal abdomen. Skin and muscles were cut and separated layer by layer. After the renal hilum was exposed on both sides, the renal pedicles were carefully separated. After stabilization for 5 min, the renal pedicles were clamped with artery clamps. Renal ischemia was achieved when the color of the kidneys switched from pink to purple-black, and the artery clamps were removed after 30 min of ischemia. Reperfusion was considered successful when the color of the kidneys switched from purple-black back to pink. In the sham operation group, the kidneys were exposed but clamping of the renal artery was not performed. The remaining operative procedures were identical in all groups.

Treatment

In the BNP + I/R group, immediately following reperfusion, lrh-BNP ($0.03\ \mu g \cdot kg^{-1} \cdot min^{-1}$; Chengdu Nuodikang Biology Pharmacy Limited Company, China) was continuously delivered subcutaneously using osmotic pressure pumps (Alzet, CA, USA) buried under the shoulder blades of the mice. Saline was injected in the sham and I/R groups at the same rate described above.

Sample collection and processing

After the procedure, 12 mice in each group were divided into 2 sub-groups (N = 6) according to the reperfusion time (12 h or 24 h). Before sacrifice, the mice were again anesthetized and blood was collected from the orbital venous plexus of the mice. Both kidneys of each mouse were removed after saline perfusion and half of each kidney was preserved in liquid nitrogen. A quarter of each kidney was fixed with 10% neutral formaldehyde and paraffin-embedded. The last quarter of each kidney was fixed in periodate-lysine-paraformaldehyde (PLP) for 2 h, dehydrated using 18% sucrose overnight, opti-mum cutting temperature compound (OCT)-embedded, and then stored at -80°C.

Biochemical analysis

Serum creatinine (Scr) and blood urea nitrogen (BUN) levels were obtained by following routine procedures using a fully automated biochemical analyzer (Beckman Coulter AU5800, CA, US).

Renal histopathology

The paraffin-embedded tissue samples were sectioned serially into 3-5-µm-thick slices, dewaxed, and dehydrated; then, they were stained with hematoxylin-eosin (HE). The renal morphological damage was observed under a light microscope (Leica Microsystems, Germany) after I/R. All parameters were evaluated and scored on a scale of 0 to 5 points according to severity (0 for normal, 1 for ≤ 10%, 2 for 11-25%, 3 for 26-45%, 4 for 46-75%, and 5 for >75% damage). Twenty visual fields of the renal cortices were selected at high magnification (400X). The degree of renal tubular damage was observed and evaluated according to the proportion of impaired renal tubules to total renal tubules.

Quantitative polymerase chain reaction (qPCR)

The mRNA levels of the renal tissue-associated factors hypoxia-inducible factor 1α (HIF-1α) and kidney injury molecule-1 (Kim-1) were detected using qPCR. Total RNA was extracted using TRIzol reagent (Invitrogen, Life Technologies, CA, USA). cDNA was synthesized according to manufacturer instructions (Applied Biosystems, USA). Real-time PCR was performed according to the manufacturer protocol of the reverse transcription-PCR (RT-PCR) kit (Promega, Madison, USA). The total reaction volume was 25 µL and contained 2 µL of 100 ng cDNA, 1 µL of 10 µML upstream primer, 1 µL of 10 µML downstream primer, 12.5 µL 2X PCR mix and the appropriate amount of H_2O. The following reaction conditions were used: pre-denaturation at 95°C for 30 s, annealing at 49°C for 30 s and extension at 72°C for 60 s. The PCR reaction continued for another 35 cycles of the following: 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C. The final extension step was 72°C for 7 min. PCR products were subjected to 2% agarose gel electrophoresis and β-actin was used as the internal reference. The mRNA expression of the target was represented by the ratio between its optical density (OD) and that of the internal reference. Sequences for all primers used are summarized in Table 1.

Table 1. Primers used for qPCR.

Gene (mouse)	Primer Sequence (5'→3')
TNF-α	F-GCCACCACGCTCTTCTGTCT
	R-TTGACGGCAGAGAGGAGGTT
IL-1β	F-CTTTCCCGTGGACCTTCCA
	R-GGGTGTGCCGTCTTTCATTAC
IL-6	F-GAGTTGTGCAATGGCAATTCTG
	R-CACTCCTTCTGTGACTCCAGCTT
MCP-1	F-AAAACCTGGATCGGAACCAAA
	R-TGCTTGAGGTGGTTGTGGAA
Kim-1	F-GTTAAACCAGAGATTCCCACACG
	R-TCTCATGGGGACAAAATGTAGTG
HIF-1α	F-GATGACGGVGACATGGTTTAC
	R-CTCACTGGGCCATTTCTGTGT
β-actin	F-GGAAATCGTGCGTGACATTA
	R-AGGAAGGAAGGCTGGAAGAG

TNF-α = tumor necrosis factor α; IL-1β/6 = interleukin-1β/6; MCP-1 = monocyte chemoattractant protein-1; Kim-1 = kidney injury molecule-1; HIF-1α = hypoxia-inducible factor 1α; F = forward primer; R = reverse primer.

Immunofluorescence confocal laser scanning

The frozen sections (2-3 µm) prepared were blocked at room temperature for 30 min with serum working solution and then incubated with primary antibody at 4°C overnight. The sections were washed with 0.01 mM PBS (pH 7.2-7.4) and then incubated with secondary antibody at room temperature for 1 h. The nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI) for 10 min. After washing with PBS, the sections were mounted using anti-fluorescence quenching mounting medium. The following primary antibodies were used to detect targeted cellular antigens: rat anti-mouse F4/80 (1:200, clone BM8; eBioscience, CA, USA), rabbit anti-mouse CD31 (1:50; Abcam, Hong Kong, China), rabbit anti-mouse Ki-67 (1:200, clone SP6; Thermo Scientific, CA, USA). The secondary antibodies used in this study included Alexa Fluor 488-conjugated goat anti-rat and Alexa Fluor 594-conjugated goat anti-rabbit antibodies (1:400; Jackson ImmunoResearch Laboratories, PA, USA). Images were observed and captured using fluorescence microscopy (Nikon, Tokyo, Japan). Positive staining was identified as green fluorescence detected in the cytoplasm of the renal tubule epithelial cells, and negative staining was identified as no fluorescence signal detected. Ten non-overlapping visual fields on the renal cortex and outer medulla of each sample were randomly selected, and average data were calculated. Targeted parameters were calculated by a self-equipped analysis system of the laser scanning confocal microscope. The number of F4/80 positively stained macrophages was calculated as a detection value for each mouse at high magnification (400X). The number of Ki67 positively stained renal tubular epithelial cells was calculated at high magnification (400X). A visual field at 200X magnification was divided into 225 grids. The number of grids without CD31+ staining was counted and then divided by the total number of grids. The percentage of this ratio was the renal PTC loss frequency.

Statistical analysis

All the data are presented as the mean ± standard deviation. The software package SPSS 17.0 was used for one-way ANOVA analysis. The least significant difference method was used for differences between multiple samples for variance homogeneity. The nonparametric test was used

for variance non-homogeneity. Differences were considered statistically significant at d = 0.05, P < 0.05.

RESULTS

BNP improves the renal function of mice with IRI

There was no weight difference in the mice between all groups before the procedure (Figure 1A). The Scr and BUN levels of the mice in the I/R and BNP + I/R groups were significantly higher than those of the sham group (P < 0.05, Figures 1B and C, respectively). Compared with the I/R group, the BNP + I/R group showed significantly decreased Scr and BUN levels (P < 0.05, Figures 1B and C, respectively).
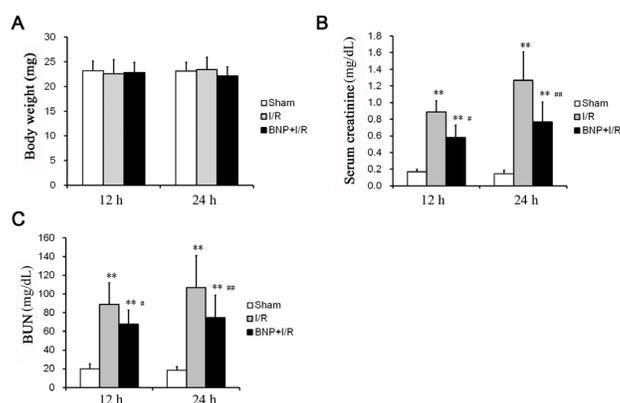
Figure 1. Effect of BNP on renal function in IRI mice. **A.** Body weight of the mice before the procedure. **B.** Serum creatinine (Scr) levels in the mice 12 or 24 h after reperfusion. **C.** Blood urea nitrogen (BUN) levels in the mice 12 or 24 h after reperfusion. The three groups are as follows: Sham (control), I/R (ischemia-reperfusion), and BNP + I/R (BNP treatment following I/R). Data are shown as the mean ± SD: #P < 0.05, ##P < 0.01 *vs* Sham group; and *P < 0.05 **P < 0.01 *vs* I/R group.
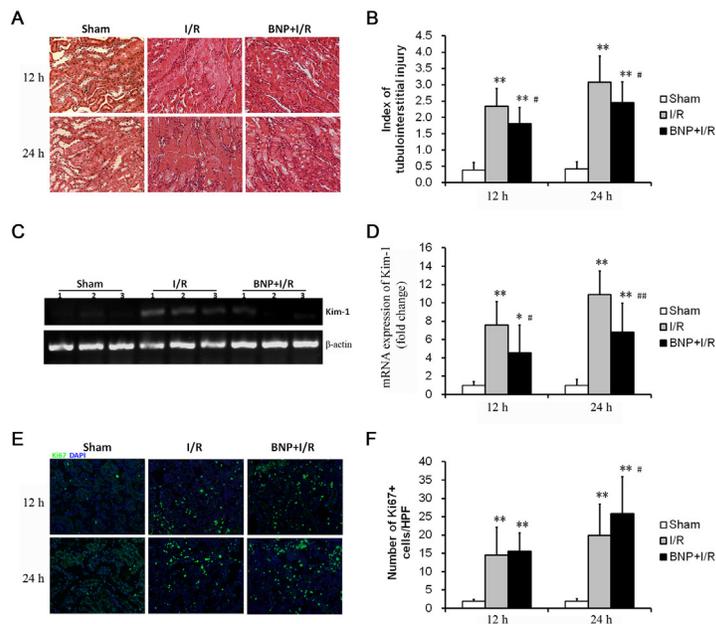
BNP alleviates renal tubular damage and promotes proliferative repair of the renal tubular epithelia of mice with IRI

Renal histopathology in each group was observed following HE staining. In the sham group, no obvious changes were apparent in the renal tissue: the morphology of the renal cells was normal, the staining was well distributed, and no necrotic or scaled cells appeared in the renal tubular lumen. In the I/R group, the following was observed: most renal tubular epithelial cells were swollen and demonstrated vacuolar degeneration in the renal outer medulla; some renal tubular epithelial cells were coagulative necrotic and shedding; the brush border was absent; the renal tubular lumen was narrowed; a substantial number of necrotic and scaled cells were apparent in the lumen; the basement membrane collapsed; cellular cast and protein cast were observed in some renal tubules; and some pathological changes were noticed in the renal tubules located in the cortex, but no changes in the glomerulus were observed. After BNP intervention following I/R, the pathological changes of the kidney were significantly alleviated compared to those in the I/R group: only slightly swollen renal tubular epithelial cells, a mostly intact basement membrane, and

limited cast in the renal tubular lumen were observed (Figure 2A).

The semi-quantitative results of the renal tubular damage as evaluated by H_E staining revealed the following: 1) the most severe renal tubular damage occurred in the I/R group; and 2) the renal tubular damage was substantially alleviated after BNP intervention compared to that in the I/R group but was still more severe than that in the sham group (P < 0.05, Figure 2B). Furthermore, the mRNA levels of Kim-1 significantly increased in renal tissue during I/R and then significantly decreased after BNP treatment (P < 0.05, Figures 2C and D).

To further investigate whether BNP can promote proliferative repair of the renal tubular epithelia, we counted the number of proliferative renal tubular epithelial cells using Ki67 as a proliferative cell marker (Figure 2E). We found that although IRI can promote self-proliferative repair of the epithelial cells, BNP intervention can amplify the repair. The quantitative statistical data analysis verified this amplification function (P < 0.05, Figure 2F).


Figure 2. BNP alleviates renal tubular damage and promotes proliferative repair of renal tubular epithelia in IRI mice. (**A, B**) H_E staining and quantification of the outer medulla of the renal tissue sections 12 or 24 h after reperfusion (200X magnification). (**C**) Agarose gel electrophoresis and Kim-1 mRNA in mouse renal tissue 24 h after IRI (normalized to β-actin; *Lines 1-3* represent the three samples in each group, respectively). (**D**) Quantitative Kim-1 mRNA expression 12 and 24 h after IRI. (E, F) Immunofluorescence and quantification (by cell counting: cells/HPF (high-power field)) of Ki67 staining of mouse renal tissue sections 12 or 24 h after reperfusion (200X magnification). The three groups are as follows: sham (control), I/R (ischemia-reperfusion), and BNP + I/R (BNP treatment following I/R). Data are shown as the mean ± SD: #P < 0.05, ##P < 0.01 *vs* Sham group; and *P < 0.05 **P < 0.01 *vs* I/R group.

BNP inhibits renal interstitial inflammation caused by IRI

To observe the influence of BNP on renal tissue inflammation caused by IRI, we first used F4/80 as a macrophage marker to detect the renal interstitial infiltrative macrophages by immunofluorescence staining. Compared with the sham group, the number of renal interstitial

infiltrative macrophages increased in the I/R group; however, after BNP intervention, the number of infiltrative macrophages decreased (Figure 3A). The cell counting result also supported this observation (P < 0.05, Figure 3B). We also evaluated the mRNA levels of several renal tissue inflammatory cellular factors using RT-PCR. These results showed that the expression levels of renal tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and monocyte chemoattractant protein-1 (MCP-1) were significantly higher in the I/R group than in the sham group (P < 0.05), and the expression of these inflammatory factors decreased significantly in the BNP + I/R group compared to the I/R group (P < 0.05, Figures 3C and D).
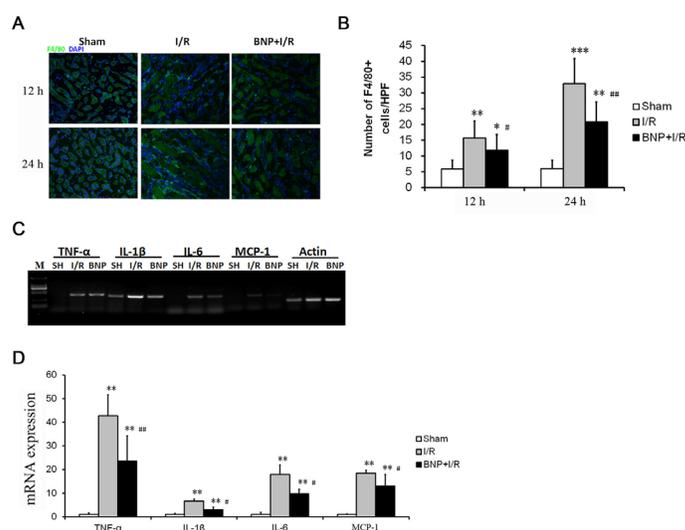
Figure 3. BNP inhibits renal interstitial inflammation induced by IRI. (**A, B**) Immunofluorescence and quantification (by cell counting: cells/HPF (high-power field)) of F4/80+ cells in the outer renal medulla 12 or 24 h after reperfusion (200X magnification). (**C, D**) Agarose gel (M is molecular marker) and quantification of inflammatory factor (TNF-α, IL-1β, IL-6 and MCP-1) mRNA expression in mouse renal tissue 24 h after IRI. The three groups are as follows: Sham (SH; control), I/R (ischemia-reperfusion), and BNP + I/R (BNP; BNP treatment following I/R). Data are shown as the mean ± SD: #P < 0.05, ##P < 0.01 *vs* Sham group; and *P < 0.05 **P < 0.01 *vs* I/R group.

BNP protects renal PTC and alleviates renal hypoxia

To quantitate the loss of renal PTC, we used CD31 to mark endothelial cells. The immunofluorescence results showed that the density of renal PTC decreased in the mice in the I/R group compared with the sham group; however, after BNP treatment, the renal PTC density increased, indicating recovery of PTC loss (Figure 4A). Densitometric analysis of this data supports this conclusion (Figure 4B). HIF-1α is a protein that is primarily expressed in the renal tubular cells when the kidney experiences hypoxia-ischemia damage. HIF-1α can trigger the expression of downstream target genes, which protect the kidney by adapting to the hypoxia-ischemia condition (Leonard et al., 2003). Therefore, the expression level of HIF-1α can represent the degree of tissue hypoxia. We found that HIF-1α mRNA expression in the renal tissues of mice in the I/R group was significantly higher than that in the sham group but HIF-1α mRNA expression decreased after BNP intervention compared to that in the I/R group at the same time point (P < 0.05, Figures 4C and D).
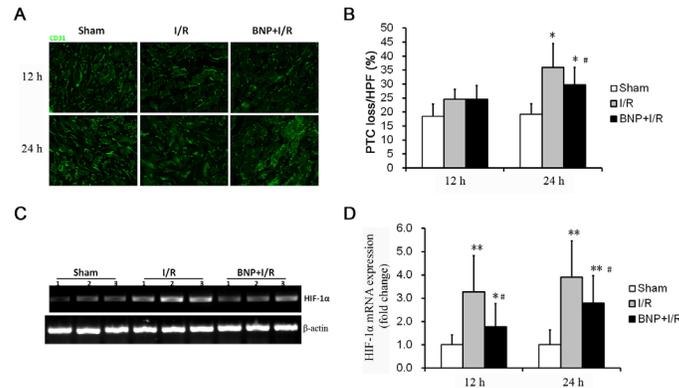
Figure 4. BNP protects renal PTCs and alleviates renal hypoxia. (**A, B**) Immunofluorescence and quantification of PTC loss by CD31+ staining for endothelial cells (by cell counting: cells/HPF (high-power field)) in the outer renal medulla 12 or 24 h after reperfusion (200X magnification). (**C**) Agarose gel electrophoresis HIF-lα mRNA expression in mouse renal tissue 24 h after IRI (lines 1-3 represent the three samples in each group, respectively). (**D**) Quantitative HIF-1α mRNA expression 12 and 24 h after IRI The three groups are as follows: Sham (SH; control), I/R (ischemia-reperfusion), and BNP + I/R (BNP; BNP treatment following I/R). Data are shown as the mean ± SD: [#]P < 0.05, [##]P < 0.01 *vs* Sham group; and *P < 0.05 **P < 0.01 *vs* I/R group.

DISCUSSION

IRI is the primary pathogenic cause of ischemic acute renal failure and delayed graft function after renal transplantation. BNP is a member of the natriuretic peptide family and recent evidence has established that it has a protective effect on acute heart (Liu et al., 2015), cerebral (Taub et al., 2011), and lung injury (Ruocco et al., 2015). However, the effect of BNP on renal damage caused by IRI remains to be explored. Therefore, in this study, we investigated the effect of lrh-BNP on IRI-induced AKI and explored the molecular mechanisms involved in I/R and the beneficial outcome of BNP treatment.

We first explored the protective effect of BNP on AKI from the morphological perspective. In the I/R group, noticeable pathological changes were observed, including swollen renal tubular epithelial cells and an absent brush border. After BNP treatment, the number of renal tubular epithelial cells increased by approximately 5%. This result is consistent with the finding that BNP is able to stimulate cardiac progenitor cell proliferation and differentiation in murine hearts after birth (Bielmann et al., 2015), which indicates that BNP can also promote the proliferation of renal tubular epithelial cells. Furthermore, our results showed that serum Scr and BUN levels significantly decreased in the BNP + I/R group compared with the I/R group. BNP has long been used as a marker of renal function; however, the direct effect of BNP on the filtration function of the kidneys has not been previously reported. Our results indicate that BNP can improve renal filtration function, although the underlying mechanism needs to be further explored. As a marker of renal tubular epithelial damage, the expression level of Kim-1 increases upon epithelial damage (Vaidya et al., 2010; Hu and Moe, 2012; Sohotnik et al., 2013). Our data indicate that the mRNA expression of Kim-1 significantly increases in renal tissue during I/R and then significantly decreases after BNP treatment (Figures 2C and D). This process indicates the favorable function of BNP on renal tubular damage of mice with IRI.

In the early stages of IRI, infiltration of macrophages manifests a pro-inflammatory phenotype by secreting pro-inflammatory factors and preventing renal pathological damage. In the

later stages of IRI, the role of macrophages transforms into an anti-inflammatory one, as they repair renal damage (Duffield, 2011; Huen and Cantley, 2015). It has been previously shown that BNP can inhibit cardiac inflammation induced by IRI (Hu et al., 2014). We also observed macrophage infiltration at an early stage of IRI (24 h after reperfusion) and noticed a pro-inflammatory phenotype of the macrophages (Kinsey, 2014; Huen and Cantley, 2015). However, the number of macrophages declined after BNP intervention. Some factors expressed in the acute stage of IRI, such as TNF-α, IL-1β, IL-6 and MCP-1, are associated with inflammatory regulation and immune response. These factors are important pro-inflammatory factors during renal IRI (Bomsztyk et al., 2013) and can have multiple functions in protecting the kidney after I/R damage. They can generate neutrophil granulocyte activity, increased inflammation, glomerular fibrosis deposition, vessel contraction, decreased glomerular filterability, and neutralization of pro-inflammatory factors. In our study, the expression levels of TNF-α, IL-1β, IL-6 and MCP-1 increased after I/R but decreased after treatment with BNP (compared to those in the I/R group). Due to dual promotion of pro-inflammatory and inflammatory factors, an amplification cascade of inflammation occurs. Therefore, we hypothesize that BNP may inhibit macrophage infiltration by restraining the secretion of pro-inflammatory factors. Moreover, the decline in inflammatory macrophages further decreased the secretion of pro-inflammatory factors.

Our results demonstrate that BNP has a protective effect on IKI. This finding contradicts the results reported by Kim et al. (2010) but is in accordance with those reported by Song et al. (2013). Presumably, this contradiction is a result of the difference in observational time points. In the study conducted by Kim et al. (2010), the effect of BNP was observed within 5 h of delivery. Our study shows that BNP exerts its protective effect by promoting cell proliferation and inhibiting excessive inflammatory reactions. If this mechanism holds true, the effect of BNP would not be observed within such a short time span. In our study, we observed the effect of BNP at 12 and 24 h after treatment, which is in accordance with the observational time points used in the study by Song et al. (2013). While these and our studies demonstrate that BNP has a protective effect in IRI, it is still not known if BNP exerts more long-term effects, as all observational times were under 24 h.

Recent studies have shown that PTC plexus stability is the decisive factor for maintaining renal function (Ivanyi et al., 2011; Wang et al., 2012). If renal ischemia leads to renal insufficiency, the pathological damage appears earliest in PTC endothelial cells (Bonventre and Yang, 2011). Clinical renal biopsies revealed that PTC loss was associated with renal tubular damage, hypoxia, and inflammation (Lien et al., 2003; Ishii et al., 2005). Vascular endothelial damage and dysfunction can aggravate ischemic injury, cause vascular congestion and swelling, and further worsen inflammatory infiltration (Lien et al., 2003). During acute renal ischemic injury, PTC loss was positively correlated with renal failure and renal interstitial fibrosis (Li et al., 2010; Lin et al., 2010). Promoting reparative regeneration of renal interstitial fibrosis can reverse these pathological changes (Li et al., 2006; Li et al., 2010). It has been reported that ANP improves capillary permeability through vascular endothelial receptor A (NPR-A) to protect capillary integrity (Dodd-o et al., 2008). Therefore, we investigated the protective effect of BNP on the renal capillary system during IRI. We found that the renal PTC density of the mice in the I/R group significantly decreased compared to the sham group; however, after BNP treatment, the renal PTC density increased, indicating that renal PTC loss can be recovered. Hypoxia is an important factor that mediates acute renal ischemic injury (Amran-Cohen et al., 2003). During renal ischemic injury, HIF-1α is primarily expressed in renal tubular cells. HIF-1α can promote glycolysis, improve oxygen utilization efficiency, raise erythropoiesis, and increase glucose transport (Conde et al., 2012). HIF-1α expression can be induced by ischemic hypoxia, which can trigger the transcription of

downstream genes and protect renal function (Leonard et al., 2003; Conde et al., 2012). Our results show that HIF-1α expression determined the degree of hypoxia after IRI despite the lack of high HIF-1α mRNA levels after BNP treatment. This could be explained by the effect of BNP on improving PTC integrity, in which oxygen delivery capacity is increased and hypoxia is alleviated.

There are a few limitations to this study. The first is the use of mice for model establishment. Considering the physiological differences among species, these experiments need to be repeated in higher-level organisms in order to confirm the results obtained in this study. Second, we observed the effect of BNP on IRI 12 and 24 h after administration. Whether BNP aids in later-stage restoration after IRI needs to be examined.

In conclusion, our results demonstrate that BNP has a protective effect on murine IRI. This effect may be associated with its inhibition of renal interstitial inflammation and hypoxia and its promotion of renal tubule repair. As a commonly used medicine in clinical practice, BNP has been proven safe and reliable based on drug toxicity and metabolism tests. The results of this study suggest that it may be used as an alternative medicine for the treatment of renal IRI in clinical practice.

Conflicts of Interest

The authors declare no conflict of interest.

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