Original Research

Protective effect of truncated Na+/K+-ATPase β on ischemia/reperfusion-induced renal injury in rats

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Abstract

Renal ischemia/reperfusion(I/R) is an important injury part of ischemic acute renal failure, and it is also the main factor that affects the early functional recovery and the long-term survival of transplanted kidney in renal transplantation. In this study, we cloned and expressed truncated Na+/K+-ATPase β(tNKAβ) and demonstrated that tNKAβ could activate NKA α subunit and induce protective effect on human kidney-2(HK-2) cells via PKCε signal pathway. The half maximum effective concentrations (EC_{50}) of tNKAβ were 0.24 μM. Furthermore, the application of EAVSLKPT (PKCε inhibitor) could abolish the protective effect of tNKAβ in HK-2 cells subjected to ischemia/reperfusion. To identify the protective effect of tNKAβ against the I/R injury in the kidney, Sprague-Dawley rats were treated with tNKAβ (75 mg/kg) for 2 h before ischemia. The tNKAβ-treated group demonstrated a significant improvement in renal function with a lower serum creatinine and blood urea nitrogen (BUN) levels on postoperative days 1–6. Renal sections obtained from rats of the I/R group showed serious renal injury which included degeneration of tubular structure, tubular dilation, swelling and necrosis, luminal congestion, and muddy brown casts formed by sloughing of severely damaged tubular epithelial cells. However, sections of rats that were administered with tNKAβ 2 h before reperfusion showed marked reduction of the histological features of renal injury compared with kidneys that were subjected to I/R only. In conclusion, the protective effects of tNKAβ against renal I/R injury have been evaluated for the first time, and these protective effects may occur via stimulation of PKCε pathways.

Keywords: I/R, AKF, HK-2 cell, tNKAβ, PKCε

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Introduction

Ischemia/reperfusion (I/R) is an important pathologic mechanism leading to organ failure in shock. Acute kidney injury following I/R injuryis a frequent clinical problem associated with high morbidity and mortality.[1] Much has been learned about the mechanism of renal I/R injury, but fewer therapeutic strategies have been shown to reduce mortality.[2]

The Na+/K+-ATPase (NKA) pump is comprised of a 100 kDa α-subunit and a 55 kDa β-subunit. It is situated on the plasma membrane of almost all animal cells, and exchanges for three sodium ions out of the cell for two potassium ions into the cell.[3] Within the recent decade, many independent laboratories have demonstrated that in addition to the classical ion transporting, this membrane protein can also relay extracellular binding signalling into the cell through regulation of protein tyrosine phosphorylation.[4] It is reported that a polyclonal antibody targets the DR region (Asp 897–Arg 911) of the NKA α-subunit and stimulates the NKA activity.[5,6] This stimulation not only increases intracellular calcium ions and leads to enhanced heart contractility through opening of L-type Ca^{2+} channels,[7] but also induces cardioprotective effect against I/R injury via stimulation of the Src/ERK1/2 pathway.[6]

The NKA β-subunit plays a fundamental role in the structural and functional maturation of the catalytic α-subunit.[8] Recently, the extracellular side of the NKAβ-subunit was found as the primary interaction site, which mediates a salt bridge with the carboxy-terminal part of the NKA α-subunit (Asp 892–Gln 910), which is almost overlapped with the DR region (Asp 897–Arg 911).[9,10] However, the protective role of the NKA β-subunit in renal I/R injury has not been investigated. In the present study, we constructed a truncated Na+/K+-ATPase β (tNKAβ), which contains most of the extracellular side of NKAβ-subunit to test whether activation of NKAα with tNKAβ can produce

protective effect on I/R injury in a rat model and expose the signaling mechanisms for its protective effects.

Materials and methods

Animals

Sprague-Dawley (SD) male rats that weighted 250–300 g were housed under pathogen-free conditions. All animal care and experimental procedures in this study were approved by the Institutional Animal Care and Use Committees of Xi'an Jiaotong University. All the procedures were approved by the Animal Ethics Committee of the University.

Clone and expression of tNKAβ

Total RNA was isolated from HK-2 cells using Trizol (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. The specific primers for tNKAβ were as follows: 5′-ctc gag atg ctg aaa ccc acg tac cag-3′ (sense) and 5′-gga tcc tca gct ctt aac ttc aat-3′ (anti-sense). Polymerase chain reaction (PCR) fragments with XhoI and BamHI restriction sites were inserted into pET-15b (Novagen, Madison, WI, USA), and recombinant plasmid with inserted sequence was verified by DNA sequencing. Plasmid was transformed into *Escherichia coli* BL21 (DE3) and gene expression was induced with 0.8 mM isopropyl b-D-thiogalactoside (IPTG). Then, the protein was purified using a Ni-NTA purification system (Invitrogen) and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. Blots were probed with anti-human NKAβ (Novus Biologicals, Littleton, CO, USA) and anti-His antibody (Serotec, Raleigh, NC, USA) at a 1:1000 dilution, followed by alkaline phosphatase-conjugated rabbit anti-mouse IgG at a 1:2500 dilution. AP detection was performed using the picoBLUE immunoscreening kit (Statagene, La Jolla, CA, USA).

Purification of NKA and measurement of NKA activity

NKA was purified from HK-2 cells as described previously, with modifications as reported in detail.[6] Briefly, cells were homogenized in a solution, containing 250 mM sucrose, 30 mM histidine, 5 mM imidazole, and 1 mM EDTA (4°C; pH 7.4), and then centrifuged at $6000 \times g$ for 15 min. The supernatant was re-spun at $15{,}000 \times g$ for 30 min at 4°C and the resultant supernatant was centrifuged at $150{,}000 \times g$ for 90 min at 4°C (Beckman OptimaL-80XP 70Ti). The pellet was suspended in a homogenizing medium, and incubated with 0.4 mg SDS per milligram protein per milliliter for 30 min at room temperature in the presence of 3 mM/L ATP, 2 mM/L EDTA, and 50 mM/L imidazole, pH 7.5. The resultant suspension was applied to discontinuous sucrose gradients, consisting of 0.32, 0.8, 1.0, and 1.2 M/L of layers buffered with 30 mM/L of histidine and 5 mM/L of imidazole (pH 7.4), and centrifuged at $150{,}000 \times g$ for 120 min. The pellet appearing at the bottom was resuspended in a homogenizing medium to a protein concentration of 2 mg/mL, and stored at −80°C.

Purified NKA (10 μg/mL) was incubated with or without different concentrations of tNKAβ or bovine serum albumin (BSA) at 37°C for 60 min. The reaction was initiated by adding Mg-ATP (3 mM) in a final volume of 0.2 mL at 37°C for 30 min and terminated by adding 0.75 mL quench solution (0.5% ammonium molybdate + 0.5 M H_2SO_4) and 0.02 mL developer (25 mg/mL of the mixture of 0.2 g 1-amino-2-naphthol- 4-sulfonic acid + 1.2 g sodium bisulfate + 1.2 g sodium sulfite). Color was developed for 30 min at room temperature and the concentration of phosphate was then determined at 700 nm using a spectrophotometer.

Flow cytometry analyses

HK-2 cells were harvested and divided into three samples, each sample containing 1×10^5 cells. Cells were incubated with tNKAβ or BSA, and then stained with the PE-conjugated anti-His antibody (BD PharmingenTM). The antibody-stained cells were analyzed using a Becton Dickinson FACS Calibur flow cytometer (BD Biosciences), and data were analyzed using Flow-Jo software (Tree Star, Inc., San Carlos, CA, USA).

Cell viability analysis

Cell viability was evaluated with the Methylthiazolyldiphenyl-tetrazolium bromide (MTT) method. In brief, 100 μL of the HK-2 cells preparation in DMEM were plated in a 96-well plate format for each of the four treatment groups. Cells were treated with tNKAβ or BSA at different concentrations for 2 h and then subjected to ischemia and reperfusion. To mimic ischemia, simulated ischemia solution (glucose-free Krebs buffer containing 5 mM sodium lactate, 20 mM 2-deoxy-D-glucose [2-DOG, an inhibitor of glycolysis], and 20 mM sodium dithionite [$Na_2S_2O_4$, an oxygen scavenger]), pH 6.6 was used. The cells were subjected to the simulated-ischemia solution for 10 min and then washed with normal culture medium for reperfusion. After the 10-min reperfusion, MTT was added to the culture media to reach a final concentration of 0.5 mg/mL and incubated at 37°C for 4 h. The culture media containing MTT were then removed. Dimethyl sulfoxide was added into each well. Optical density was read at 560 nm and background, measured at 670 nm, was subtracted.

Western blot analysis

HK-2 cells were washed with PBS twice and scraped in Radio-Immunoprecipitation Assay (RIPA) lysis buffer including protease inhibitors. The cell lysate was centrifuged at $1000 \times g$ at 4°C for 10 min for rough partition between cytosolic and membrane fractions. The supernatant was re-centrifuged at $16{,}000 \times g$ at 4°C for 15 min to remove contaminating pellet materials, and collected as the cytosolic fraction. The initial pellets were resuspended in 100 μl cell lysis buffer containing 1% Triton X-100 and shaken on ice for another 60 min, and then centrifuged at $16{,}000 \times g$ at 4°C for 15 min. The second supernatant was collected as the membrane fraction. Equal amounts of protein were loaded and electrophoresed using 8% SDS-PAGE. After being transferred to a nitrocellulose membrane,

proteins were blocked with 10% non-fat milk for 1 h, and probed first with the primary antibody against PKCε (Santa Cruz Biotechnology) at 4°C overnight. Immunoreactivity was detected using an ECL advanced Western blot detection kit (Amersham Biosciences Biotech, Piscataway, NJ, USA).

Animal model of I/R injury

Specific pathogen-free male SD rats weighing 180–220 g were used throughout the experiments. The renal I/R injury protocol was as previously reported.[11,12] The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg; Abbott Laboratories, North Chicago, IL, USA). In all rats, a midline incision was made and then a left nephrectomy was performed. In the I/R injury groups, the right pedicle was clamped with a non-crushing micro vascular clamp for 45 min. The presence of ischemia was visually confirmed by observing blanching of the kidney. tNKAβ (75 mg/kg) or saline was administered intravenously 2 h before reperfusion. After 45 min of ischemia, the clamps were removed, the wounds were closed with 3-0 silk, and the animals were returned to their cages. The rats in the sham-operation group underwent the same procedure except that clamping was not done. At specified times after reperfusion, animals were anesthetized by intraperitoneal administration of pentobarbital (50 mg/kg) and killed by exsanguination from the abdominal aorta. Blood was collected in tubes and centrifuged at $2000 \times g$ for 10 min. Serum levels of blood urea nitrogen (BUN) and creatinine were measured with standard urease assays and picric acid reactions.[13]

Histopathological studies of the kidneys

The rats' kidneys were fixed in 10% formalin, embedded in paraffin, sectioned (at 5 μm thicknesses), and stained with hematoxylin and eosin. Histological changes were evaluated by assessments of tubular necrosis, which was evaluated by determining the percentage of tubules in the cortex and medulla in which epithelial necrosis or necrotic debris was observed. Each variable was evaluated in 20 high-power fields. Samples were analyzed by a pathologist blinded to the experimental group to which the rat belonged.

Histology and histologic scoring of renal sections were used for the assessment of renal I/R injury.[14] Briefly, 100 intersections were examined for each kidney and a score from 0 to 3 was given for each tubular profile that involved an intersection: 0, normal histology; 1, tubular cell swelling, brush border loss, nuclear condensation, with up to one-third of tubular profile showing nuclear loss; 2, as for score 1 but greater than one-third and less than two-thirds of tubular profile showing nuclear loss; and 3, greater than two-thirds of tubular profile showing nuclear loss. The total score for each kidney was calculated by addition of all 100 scores with a maximum score of 300.

Statistical analysis

Data were processed using the SPSS13.0 software package for Windows (SPSS Inc., Chicago, IL, USA). All results were expressed as the mean + SD (standard deviation). One-way analysis of variance (ANOVA) was used to determine the difference between the groups. Results were considered statistically significant if $P < 0.05$.

Results

Expression of tNKAβ protein

For cloning the tNKAβ gene, total RNA was isolated from HK-2 cells, and tNKAβ gene was cloned by reverse transcription-PCR. The PCR product was analyzed and a band between 700 bp and 800 bp was confirmed (Figure 1a). The size was identical to the DNA of tNKAβ (726 bp). Recombinant tNKAβ containing the 6-His tag was purified from bacterial lysates using a Ni-NTA column, and then a distinct band of 30 kDa corresponding to tNKAβ was readily resolved by SDS-PAGE (Figure 1(b) and (c)). Western blot analysis demonstrated that the prepared human tNKAβ was recognized by anti-His antibody and anti-human NKAβ antibody (Figure 1d).

Binding of tNKAβ to NKAα sububit increases NKA activity

The binding of tNKAβ to NKA was detected by flow cytometry. Strong immunofluorescent signals were detected on HK-2 cells which were treated with tNKAβ, but not control or BSA (Figure 1e). To identify whether the binding of NKA with tNKAβ could stimulate the NKA activity, both tNKAβ and BSA were used. The activities of NKA after treatment with tNKAβ were significantly increased in the presence of 0.25, 0.5, 1.0, and 2.0 μM tNKAβ, compared with that of control and BSA (Figure 1f). The half maximum effective concentrations (EC_{50}) were 0.24 μM.

Protective effect of tNKAβ in HK-2 cells subjected to ischemia/reperfusion

To investigate if the treatment with tNKAβ confers protective effect against cellular injury caused by I/R, HK-2 cells were incubated with 0.25 μM of tNKAβ for 60, 120 or 180 min, then treated with simulated ischemic buffer for 10 min followed by reperfusion with normal culture medium for another 10 min (Figure 2a). Cell viability was determined 10 min after reperfusion. Cell viability was significantly decreased after I/R (Figure 2b). tNKAβ obviously increased cell viability when compared with that in the PBS control and BSA group. The optimal cultured time of tNKAβ for protective effect was about 2 h. This finding suggests that tNKAβ may also protect kidney against I/R-induced injury.

PKCε is involved in the protective effect of tNKAβ

PKCε is a well-known pro-survival protein kinase. We examined whether PKCε is involved in the protection offered by tNKAβ-induced protective effect. HK-2 cells were treated with EAVSLKPT (a PKCε-selective peptide

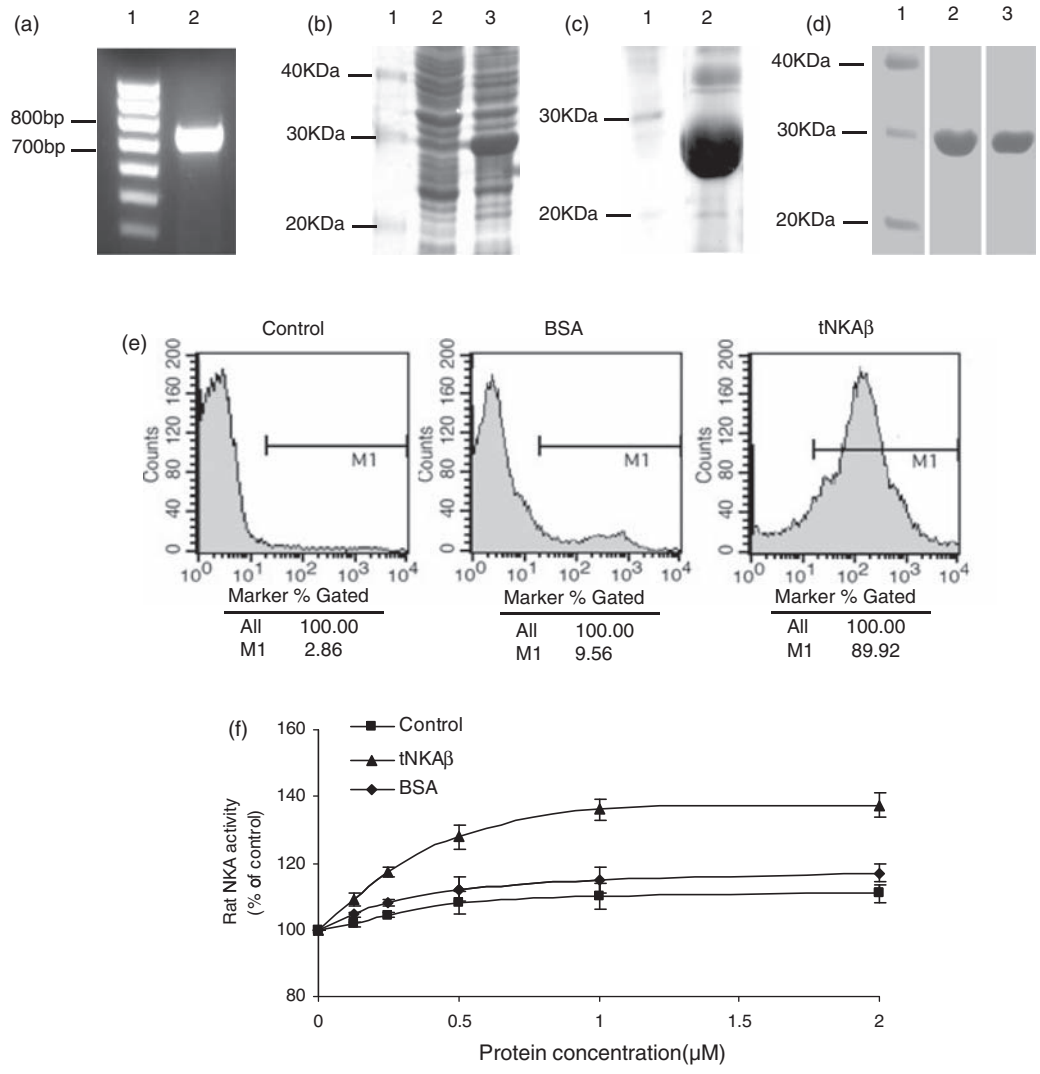

Figure 1 Expression of tNKAβ protein and binding of tNKAβ to NKAα subunit increases NKA activity. (a) The DNA band of tNKAβ (726 bp) was apparent between 700 bp and 800 bp. (1) DNA molecular marker; (2) PCR product of tNKAβ DNA. (b) tNKAβ was expressed by the pET-15b bacterial vector in *E. coli* BL21 (DE3) and a distinct band of 30 kDa corresponding to tNKAβ resolved by SDS-PAGE and evaluated by Western blotting. (1) Protein molecular marker; (2) bacteria transformed with empty pET-15b vector; (3) bacteria transformed with tNKAβ recombinant pET-15b vector. (c) Recombinant tNKAβ purified with Ni-NTA column. (1) Purified protein from bacteria transformed with empty pET-15b vector; (2) purified protein from bacteria transformed with tNKAβ recombinant pET-15b vector. (d) Western blot analysis of purified tNKAβ. (1) Protein molecular marker; (2) proteins probed with anti-human NKAβ antibody; (3) proteins probed with anti-His antibody. (e) Flow cytometry analysis of tNKAβ binding on HK-2 cells. (f) Concentration-dependent effect of tNKAβ on NKA activities, $n = 4$. $^*P < 0.05$ versus control and BSA

translocation inhibitor) 30 min before treatment with tNKAβ (Figure 2c). As shown in Figure 2(d), EAVSLKPT abolished the protective effect of tNKAβ on HK-2 cells viability. We further investigated whether PKCα and PKCδ were playing a role in tNKAβ-induced protection. Chelerythrine (a PKCα inhibitor) and Gő 6976 (a PKCδ inhibitor) failed to reverse the protection induced by tNKAβ (data not shown).

To determine the activated PKCε induced by tNKAβ, subcellular distributions of PKCε were examined with Western blotting. tNKAβ induced PKCε translocation from cytosol to membrane, and EAVSLKPT effectively inhibited the translocation (Figure 2e). These data suggest that the tNKAβ-induced protective effect is mediated by PKCε pathways.

Protective effect of tNKAβ in rat I/R injury model

SD rats were treated with either the tNKAβ ($n = 8$) or saline as control ($n = 8$) for 2 h, and then subjected to left nephrectomy followed by right renal ischemia for 45 min (Figure 3a). Sham animals underwent laparotomy, exposure of the left nephrectomy, and right renal pedicle. All control and treatment animals demonstrated a significant rise in serum creatinine and BUN levels in the first two days, and all animals showed an improvement in renal function by day 7. The tNKAβ-treated group demonstrated a significant improvement in renal function with a lower serum creatinine and BUN levels on post-operative days 1–6 (Figure 3(b) and (c)), indicating that tNKAβ could prevent the tissue damage and promote the recovery of the tissue damage.

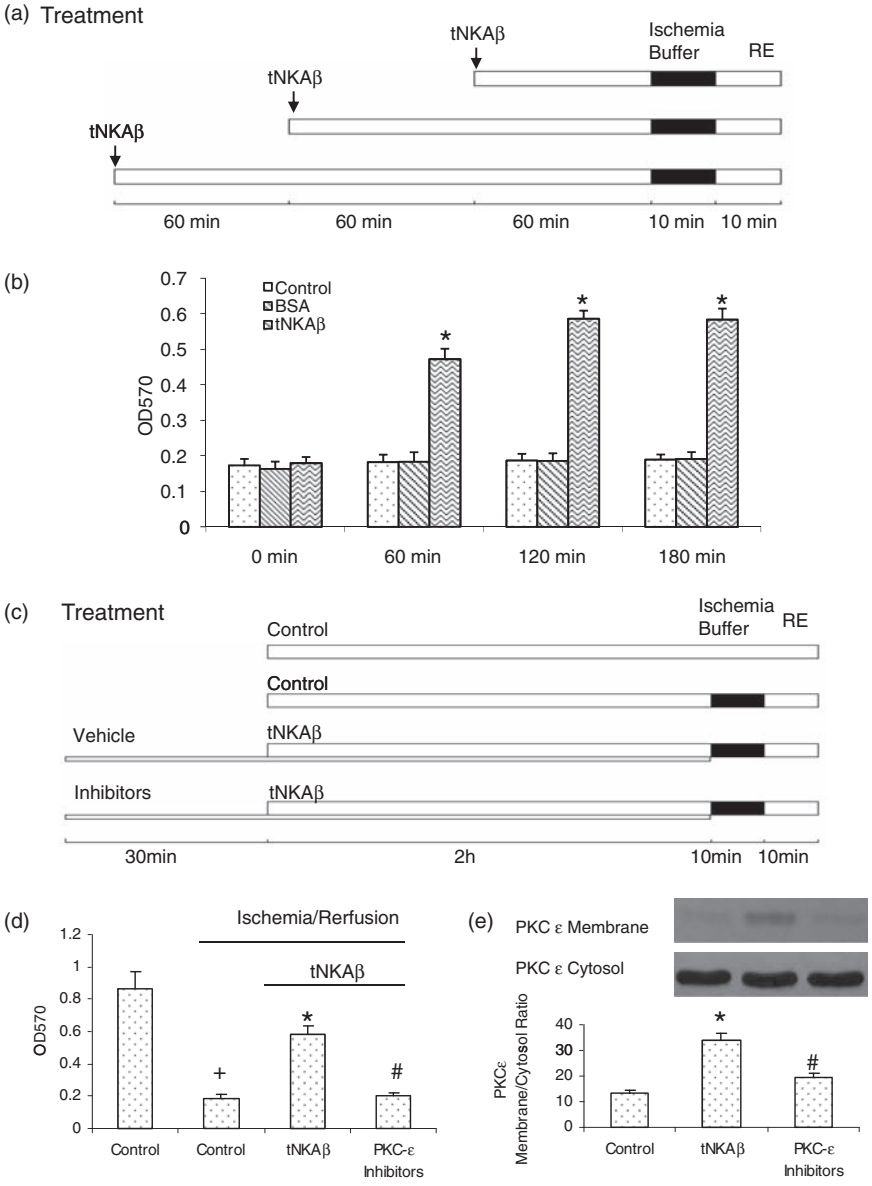

Figure 2 Protective effect of tNKAβ in HK-2 cells subjected to I/R. (a) Experimental protocols. RE, reperfusion with normal culture medium. HK-2 cells were incubated with tNKAβ for 60, 120 or 180 min, respectively, and then treated with ischemia buffer for 10 min and reperfusion with normal medium for 10 min. (b) The optimal culture time of tNKAβ for protective effect was approximately 120 min, $n = 3$. (c) Experimental protocols. Vehicle, PBS; HK-2 cells were treated with tNKAβ for 2 h. EAVSLKPT was given 30 min before the tNKAβ treatment. Cell viability was measured 10 min after reperfusion. (d) Blockade of PKCε with EAVSLKPT abolished the protection of tNKAβ, $n = 3$. (e) Western blot analysis showing tNKAβ-induced activation of PKCε, $n = 3$. Values were mean ± SEM, $+P < 0.05$ versus control (without ischemia), $^*P < 0.05$ versus control, # $P < 0.05$ versus vehicle (tNKAβ alone)

Effect of tNKAβ on histologic alterations after I/R injury in rats

In comparison with the renal histology that was taken from sham-operated rats (Figure 4a), rats that underwent renal I/R demonstrated the recognized features of renal injury (Figure 4b). But, there was no obvious structural damage to the renal corpuscles. The renal injury included degeneration of tubular structure, tubular dilation, swelling and necrosis and luminal congestion. In contrast, renal sections that were obtained from rats of tNKAβ treatment group (Figure 4c) demonstrated marked reduction of the histologic features of renal injury compared with kidneys that were subjected to I/R only (Figure 4b). In comparison with the histology score that was measured in sham-operated animals, renal I/R produced a significant increase in histology score. However, administration of tNKAβ significantly reduced the histology score when compared with scores from the rats that were subjected to renal I/R only (Figure 4d). We also found the muddy brown casts formed by sloughing of severely damaged tubular epithelial cells in histopathologic slide, especially in the I/R group (Figure 5b). The renal sections of tNKAβ treatment group showed the vacuolar degeneration of renal tubular epithelial cells and no casts were found. Taken together, our founding further confirmed that tNKAβ could induce a reduction in I/R injury.

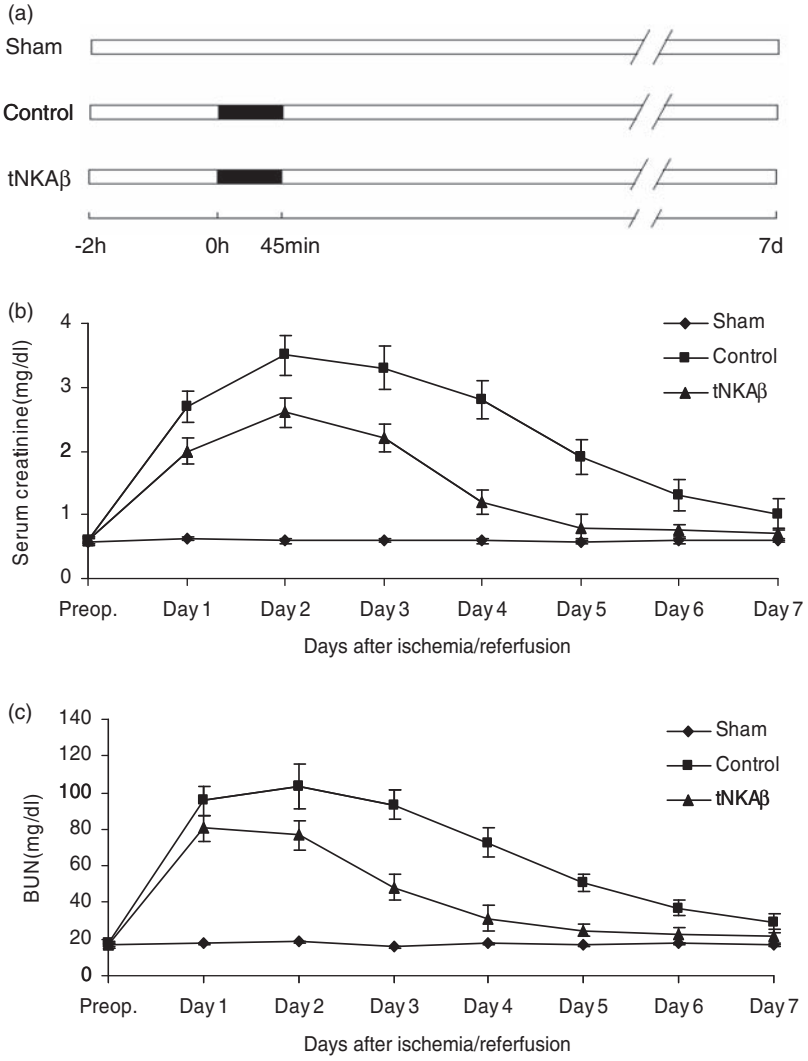

Figure 3 Effect of tNKAβ treatment on renal function in I/R rats. (a) Experiment schedule. (b and c) The tNKAβ treated group demonstrated a significant improvement in renal function with a lower serum creatinine and BUN level on postoperative days 1–6 ($P < 0.05$). Data are presented as the mean ± SE ($n = 8$)

Discussion

Ischemic acute renal failure is a syndrome that develops following a transient drop in total or regional blood flow to the kidney. Although reperfusion is essential for the survival of ischemic tissue, there is evidence that reperfusion itself causes additional cellular injury.[15,16] The mechanisms of renal I/R injury involve both vascular and tubular factors. Tubular injury contributes to reductions in the glomerular filtration rate (GFR) by producing tubular obstruction and via epithelial ''back leak'' of solutes including urea and creatinine, which leads to the increase in BUN and creatinine in serum.[17] Therefore, reducing the tubular epithelial damage and promoting epithelial recovery are key to restore I/R injury.

NKA is a member of the P-type ATPase superfamily. It was found that the native activity of NKA is markedly elevated when protein–protein interaction occurs at the extracellular DVEDSYGQQWTYEQR(DR) region in the a-subunit of the enzyme. Binding of activator SSA412 to the activation site of NKA(DR region) not only accelerates the enzyme catalytic activity,[5] but also initiates signaling property of NKA, leading to the positive regulation of L-type Ca^{2+} channels (LTCC) function through a Src/Erk1/2 signaling cascade, which phosphorylates and assembles other proteins into different signaling modules. This in turn activates multiple protein kinase cascades including mitogen-activated protein kinases and protein kinase C isozymes in a cell-specific manner.[18] The PKC family of serine/threonine kinases is a central component of the signaling pathways that play a significant role in mediating various cellular functions including apoptosis, proliferation, migration, motility, chemoresistance, and differentiation.[19–21] Numerous studies have documented a central role of PKC in the cardioprotection caused by ischaemic preconditioning.[22,23]

In our previous study, we found that antibodies against DR region significantly prolonged the survival time of isolated cardiac myocytes and protected cardiac myocytes against I/R injury by activating NKA and its subsequent signaling pathway.[6] The NKA β subunit has a crucial role in

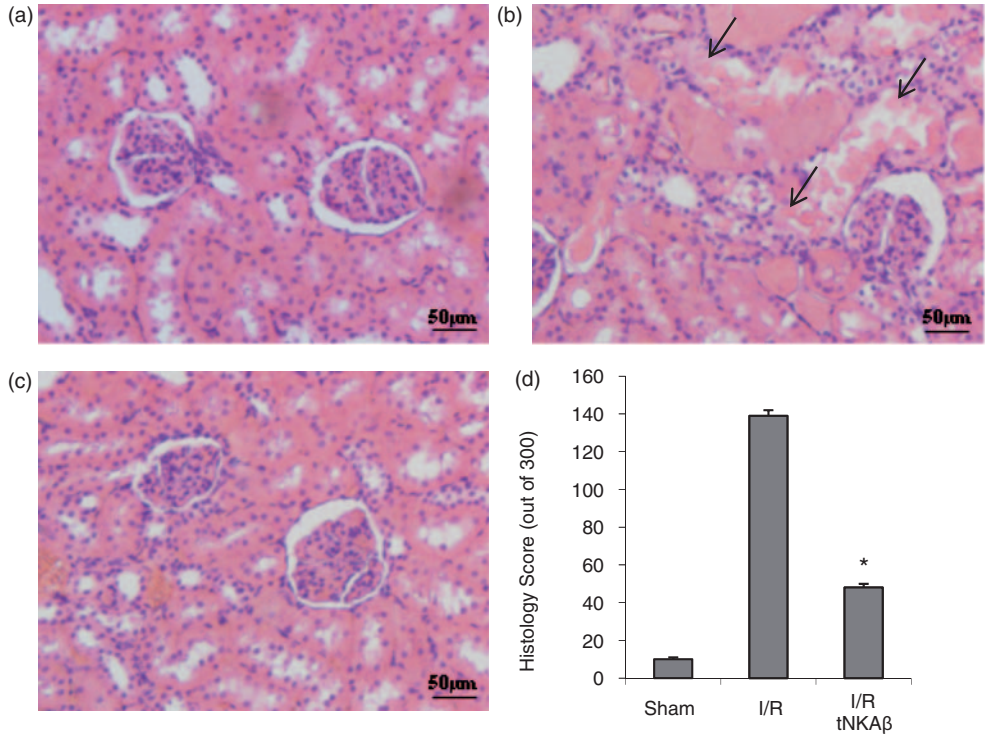

Figure 4 Effect of tNKAβ on renal cortex histologic examination. (a) Renal sections from a sham-operated rat. (b) Renal sections from a rat that was subjected to renal I/R. (c) Renal sections from a rat that was administered tNKAβ (75 mg/kg) 2 h before reperfusion and subjected to renal I/R. Figures are representative of at least three experiments that were performed on different days ($n = 8$ for all groups; hematoxylin and eosin). (d) Renal sections were scored for characteristic histologic signs of renal injury out of a total of 300 subsequent to sham operation (sham; $n = 3$) or renal I/R (I/R; $n = 3$). Data are means ± SEM. $P < 0.05$ vs I/R. Arrows in (b) show the degeneration of tubular structure, tubular dilation, swelling and necrosis, and luminal congestion. For (a–d), original magnification × 200. (A color version of this figure is available in the online journal)

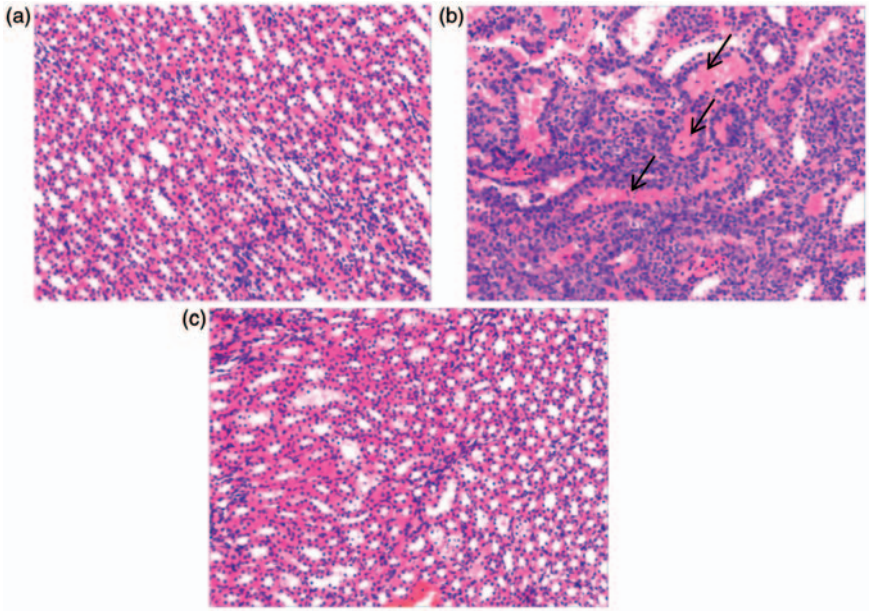

Figure 5 The observation of casts on renal medulla histologic examination. (a) Renal sections from a sham-operated rat. (b) Renal sections from a rat that was subjected to renal I/R. (c). Renal sections from a rat that was administered tNKAβ (75 mg/kg) 2 h before reperfusion and subjected to renal I/R. Figures are representative of at least three experiments that were performed on different days ($n = 8$ for all groups; hematoxylin and eosin). Arrows in (b) show the muddy brown casts formed by sloughing of severely damaged tubular epithelial cells. For (a–c), original magnification × 100. (A color version of this figure is available in the online journal)

the structural and functional maturation of NKA and modulates its transport properties. The chaperone function of the beta subunit is essential, for example, in the formation of tight junctions and cell polarity. Recent studies suggest that beta subunits also have inherent functions, which may be involved in cell–cell adhesiveness and the suppression of cell motility.[24] In addition, the extracellular side of the NKAβ-subunit has been found as the primary interaction

site which mediates a salt bridge with the carboxy-terminal part of NKA α-subunit (Asp 892–Gln 910),[10] which almost overlapps with the DR region (Asp 897–Arg 911). Therefore, we hypothesised that the extracellular side of NKAβ-subunit might have the ability to produce protective effect similar to that elicited by the activator against the DR region.

In this study, we expressed the tNKAβ, which includes most of the extracellular side of NKAβ, and confirmed that tNKA β could bind at the surface of HK-2 cells. More importantly, we found that treatment with tNKA β produced protective effects in both HK-2 cells and the kidney *in vivo*. tNKA β significantly improved the survival of HK-2 cells and protected kidney against I/R injury. Moreover, tNKA β markedly promoted the recovery of the kidney function and reduced tissue damage in kidneys subjected to I/R. Our findings indicate that tNKA β may produce protective effects on I/R injury *in vivo*.

We further examined the signaling mechanism underlying the protective effect of tNKAβ. In the present study, we tested the involvement of PKC and its isoforms in tNKAβ-induced protective effect on I/R injury in SD rats. Since PKCα and PKCε were recently reported as essential proliferative and survival molecules in different cell lines,[25,26] we examined the protection of tNKAβ in the presence of chelerythrine, a general PKC inhibitor, Gő 6976, a selective PKCa inhibitor, and EAVSLKPT, a selective PKCε peptide translocation inhibitor. The protection induced by tNKAβ was only attenuated by EAVSLKPT. These data suggest that the protection induced by tNKAβ was mediated by PKCε.

In conclusion, we provide evidence for the first time that tNKAβ expressed by *E. coli* may ellicit protective effect by activation of NKA via the PKCε pathway activation. tNKAβ also promoted the recovery of the kidney function and tissue damage subjected to I/R. This unique property may improve the early functional recovery and the long-term survival of transplanted kidney in renal transplantation.

Author contributions: HG and JS have contributed equally to this manuscript. JZ, HG, and JS designed research, performed, and wrote the paper. WX, PT, XD, HY, and YL interpreted the data. All authors read and approved the final manuscript.

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