Vagus Nerve Stimulation Attenuates Acute Kidney Injury Induced by Hepatic Ischemia/Reperfusion Injury by Suppressing Inflammation, Oxidative Stress, and Apoptosis in Rats

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Article

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Abstract

Hepatic ischemia reperfusion (I/R) injury, caused by limited blood supply and subsequent blood supply, is a causative factor resulting in morbidity and mortality during liver transplantation (LT) and liver resection. Hepatic I/R injury frequently contributes to remote organ injury, such as kidney, lung, and heart. It has been demonstrated that vagus nerve stimulation (VNS) is effective in remote organ injury after ischemia reperfusion injury. Here, our aim is to investigate the potential action of VNS on hepatic I/R injury-induced acute kidney injury (AKI) and explore its underlying mechanisms. To test this hypothesis, male Sprague-Dawley rats were randomly assigned into three experimental groups: Sham group (sham operation, n=6); I/R group (hepatic I/R with sham VNS, n=6); and VNS group (hepatic I/R with VNS, n=6). VNS was performed during the entire hepatic I/R process. Our results showed that throughout the hepatic I/R process, VNS significantly reduced inflammation, oxidative stress, and apoptosis, and greatly enhanced the protein expression levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and hemeoxygenase-1 (HO-1) in the kidneys. These findings suggest that VNS may ameliorate hepatic I/R injury-induced AKI by suppressing inflammation, oxidative stress, and apoptosis probably through activating the Nrf2/HO-1 signaling pathway.

1. Introduction

As a two-stage pathophysiological process, hepatic ischemia reperfusion (I/R) injury is a leading cause of acute liver failure and is a severe clinical complication that inevitably occurs in complex liver surgeries, such as liver transplantation (LT) and major liver resection [1]. In addition to acute liver injury, hepatic I/R injury often causes remote organ injury, particularly in acute kidney injury (AKI) [2]. AKI is frequently associated with increased mortality, prolonged hospital stays, and higher healthcare costs in patients suffering from LT [3,4]. In addition, during the peritransplant period of LT, 78% of patients with AKI have a five-fold increased risk of death [5].

The etiology of AKI is complex and multifactorial, mainly attributed to acute ischemia and acute hypoxia caused by hemodynamic instability [6]. However, the pathomechanisms remain incompletely characterized. Although many advances have been made in the management of hepatic I/R injury over past decades, the morbidity and mortality rates associated with hepatic I/R injury-induced AKI remain high and are of increasing concern. Hepatic I/R injury accompanied with AKI is an independent prognostic factor of AKI around a 40% rate, depending on the diagnosis and heterogeneity [7]. Few specific preventive and therapeutic options exist and there is an urgent need to seek novel effective approaches to reduce the incidence and severity of hepatic I/R injury-induced AKI.

VNS, as an FDA-approved therapeutic measure, is a well-tolerated adjunctive treatment for medically refractory partial-onset epilepsy and treatment-resistant depression [8]. Since 1989, the year of the first human VNS implantation, more than 100,000 patients have been treated with VNS worldwide. Additionally, VNS has been proven to reduce I/R injury in multiple organs, including the kidneys, brain, and

heart [9–11]. Furthermore, Lai *et al.* focused on the effect of VNS treatment on acute liver injury caused by kidney I/R injury [12]. However, to the best of our knowledge, whether VNS can also reduce kidney injury after hepatic I/R injury remains unknown. The current study aimed to investigate whether VNS can protect against AKI induced by hepatic I/R injury and to explore its potential mechanisms.

2. Results

2.1 VNS Significantly Protected Against AKI after Hepatic I/R Injury.

Kidney injury was determined via histological examination and blood detection. Upon histological examination, there were no detectable alterations in the kidneys of the sham group (see Fig. 1(a)). There were several hallmark signs of kidney damage in the I/R group, including tubular necrosis, loss of brush borders, cast formation, and tubular dilatation (see Fig. 1(a)). In contrast, compared to the I/R group, partial recovery was observed in the tubular cells of the VNS group (see Fig. 1(a)). Parallelly, compared to the sham group, higher histological scores were observed in the I/R group (see Fig. 1(b)). However, lower histological scores were observed in the VNS group than in the I/R group (see Fig. 1(b)). Consistent with the histological changes, the serum BUN and Cr levels were markedly higher in the I/R group than in the sham group (see Figs. 1(c)-1(d)). When compared with the I/R group, the VNS group exhibited a significant reduction in serum BUN and Cr levels (see Figs. 1(c)-1(d)). These data indicate that VNS may exert a protective effect on the kidneys after hepatic I/R injury.

2.2 VNS Significantly Inhibited Inflammation in the Kidneys after Hepatic I/R Injury.

The serum levels of inflammatory cytokines were measured to examine whether VNS could modulate inflammation in the kidneys after hepatic I/R injury. The serum levels of TNF-α, IL-6, and IL-1β were significantly increased in the I/R group compared to those in the sham group (see Figs. 2(a)-2(c)). In contrast, when compared with the I/R group, VNS treatment greatly lowered the serum levels of TNF-α, IL-6, and IL-1β (see Figs. 2(a)-2(c)).

MCP-1 and MIP-2 mRNA levels were evaluated by qRT-PCR. MCP-1 and MIP-2 relative mRNA expression levels were elevated in the I/R group compared to those in the sham group (see Figs. 2(d)-2(e)). However, VNS treatment lowered the relative mRNA expression levels of MCP-1 and MIP-2 compared with those in the I/R group (see Figs. 2(d)-2(e)). These data suggest that VNS significantly inhibited inflammation in the kidneys after hepatic I/R injury.

2.3 VNS Significantly Alleviated Oxidative Stress in the Kidneys after Hepatic I/R Injury.

Oxidative stress leads to overproduction of ROS, formation of MDA and MPO, and decreased expression of anti-oxidative enzymes, such as GSH and SOD. The levels of MDA and GSH, as well as the activity of MPO and SOD, were examined to evaluate oxidative stress in the kidneys. The level of MDA and the activity of MPO were significantly increased in the I/R group compared to the sham group (see Figs. 3(a)-3(b)). In contrast, when compared to the I/R group, VNS treatment greatly reversed these changes during the hepatic I/R process (see Figs. 3(a)-3(b)). Compared with the sham group, the level of GSH and the activity of SOD were noticeably decreased in the I/R group (see Figs. 3(c)-3(d)). However, this decrease was significantly reversed by VNS treatment during the hepatic I/R process when compared to the I/R group (see Figs. 3(c)-3(d)). These data suggest that VNS significantly alleviated oxidative stress in the kidneys after hepatic I/R injury.

2.4 VNS Significantly Improved Redox Status in the Kidneys after Hepatic I/R Injury.

To further assess the redox status, the levels of TOS and T-AOC in the kidneys were determined. The level of TOS was significantly higher and the level of TAS was markedly lower in the I/R group than in the sham group (see Figs. 4(a)-4(b)). However, when compared to the I/R group, these changes were greatly reversed by VNS treatment during the hepatic I/R process (see Figs. 4(a)-4(b)). In parallel, higher OSI values were observed in the I/R group than in the sham group (see Fig. 4(c)). However, lower OSI values were observed in the VNS group than in the I/R group (see Fig. 4(c)). These data indicate that VNS prominently improved the redox status in the kidneys after hepatic I/R injury.

2.5 VNS Significantly Reduced Apoptosis in the Kidneys after Hepatic I/R Injury.

To detect apoptotic cells in the kidneys among the three groups, TUNEL staining was performed. The representative micrographs of immunofluorescence staining for DAPI (blue) and TUNEL (green) in kidney cell nuclei from the three groups are shown in Fig. 5(a). Compared with the sham group, the percentage of TUNEL-positive cells was markedly increased in the I/R group (see Fig. 5(b)). However, when compared to the I/R group, the VNS group showed an obvious reduction in the percentage of TUNEL-positive cells (see Fig. 5(b)).

Western blotting was performed to further evaluate apoptosis in the kidneys. Compared with the sham group, the relative protein expression level of Bax, which induces apoptosis, was significantly increased in the I/R group and the relative protein expression level of Bcl-2, which inhibits apoptosis, was markedly decreased in the I/R group (see Figs. 6(a)-6(b)). The relative protein expression levels of cleaved caspase3 and caspase7 were greatly increased in the I/R group (see Figs. 6(c)-6(d)). VNS reversed these changes as described above, when compared to the I/R group (see Figs. 6(a)-6(d)). The representative blots and relative protein levels of Bax, Bcl-2, Cleaved caspase3, and caspase7 in kidney tissues from the

three groups are shown in Fig. 6(e). These data suggest that VNS significantly reduced apoptosis in the kidneys after hepatic I/R injury.

2.6 VNS Significantly Enhanced the Nrf2/HO-1 Pathway in the Kidneys after Hepatic I/R Injury.

Western blotting was used to analyze protein expression levels in the three groups. Compared with the sham group, hepatic I/R injury markedly increased the relative protein expression levels of Nrf2 and HO-1 in the kidneys (see Figs. 7(a)-7(d)). In contrast, when compared to the I/R group, VNS greatly augmented the increases described above (see Figs. 7(a)-7(d)). These data suggest that the Nrf2/HO-1 pathway was activated in the kidneys after hepatic I/R injury and greatly enhanced by VNS treatment during the hepatic I/R process.

3. Discussion

The essential findings of the present study are that hepatic I/R injury can induce remote kidney injury and that VNS treatment protects against AKI after hepatic I/R injury. Notably, we demonstrated for the first time that VNS significantly attenuated inflammation, oxidative stress, and apoptosis in the kidneys during the hepatic I/R process. Additionally, VNS was firstly noted to facilitate the activation of the Nrf2/HO-1 pathway in the kidneys after hepatic I/R injury.

AKI, also regarded as acute renal failure, is characterized by a sudden reduction in renal function or glomerular filtration rate, evolving from early injury to severe damage [6]. It can result in chronic kidney disease (CKD) or even kidney failure, requiring kidney replacement therapy. Population data suggests that in liver recipients who experienced postoperative acute kidney failure, the risk of developing CKD increased by 100% [13]. The kidneys are vulnerable to a series of challenges from remote hepatic I/R injury, owing to their abundant blood perfusion. Animal and clinical evidence of causality between hepatic I/R injury and AKI has been well documented. In rodents, hepatic I/R injury has been proven to cause AKI [14]. In humans, a large single-center, case-controlled study has shown the same trend in increasing mortality and morbidity of AKI during the perioperative period [5]. Previous findings support a strong relationship between hepatic I/R injury and AKI. Therefore, it is meaningful to progress an effective approach to arrest the progression of AKI.

From an anatomical point of view, except for the adrenal glands, the kidneys receive a more extensive nerve supply than any other abdominal organ [15]. The kidney arteries were sympathetically innervated because of the numerous kidney sympathetic nerves close to the lumen of the kidney arteries [16]. The pathogenesis of AKI was associated with the heightened activity of the sympathetic nervous system [17]. This is consistent with a previous study where in the initial 24 h-period following hepatic I/R injury, the occurrence of AKI was attributed to intrarenal vasoconstriction resulting from splanchnic vasodilatation following portal hypertension [18]. These observations indicate that activation of the sympathetic nervous

system negatively influences kidney function. The activation of the vagus nerve system, which naturally offsets sympathetic vasoconstrictive activity, is an optional approach to restore autonomic regulatory function. Inoue *et al*. verified that 24 h before kidney ischemia, VNS markedly attenuated AKI and inhibited systemic inflammation through α7 nicotinic acetylcholine receptor (α7nAChR)-positive splenocytes [11]. Hence, we originally proposed the hypothesis that VNS treatment might protect against AKI after hepatic I/R injury. It has already been revealed that mice subjected to severe hepatic I/R injury develop AKI characterized by immediate kidney peritubular capillary endothelial cell apoptosis with subsequent kidney proximal tubular necrosis and inflammation [18]. Therefore, we explored whether VNS could block these pathways to exert protective effects against AKI induced after hepatic I/R injury.

In the development of hepatic I/R injury-induced AKI, a substantial systematic inflammatory response always bears the brunt. It is widely known that acute liver injury following hepatic I/R injury can cause a systemic inflammatory response, resulting in remote organ injury [19]. Park *et al*. observed that hepatic I/R injury was attributed to a significant upregulation of proinflammatory cytokines in the kidneys [20]. Furthermore, Koopman *et al*. reported that the approach for VNS to mitigate inflammatory diseases was to inhibit the production of proinflammatory cytokines [21]. The VNS-mediated cholinergic anti-inflammatory pathway has been well described in the kidneys [9]. A similar finding was reported by Okusa *et al*., indicating that the previously-described pathway has the potential to limit ischemic acute kidney injury [22]. Recently, several researchers have applied vagal anti-inflammatory effects in remote organ injury after I/R injury. Lai *et al*. reported that VNS exhibited a protective effect in acute liver injury after kidney I/R injury by reducing the release of various inflammatory cytokines (e.g., TNF-α and IL-6) [12]. Consistent with these previous studies, our data suggests that VNS greatly ameliorated inflammation in the kidneys after hepatic I/R injury.

Oxidative stress is another crucial contributor to the pathogenesis of AKI. During I/R, reactive oxygen species (ROS) are massively released and various ROS subsequently initiate lipid peroxidation (LP) reactions, contributing to inflammation activation and tissue damage. MDA serves as an oxidative stress marker and has been proven to increase in kidneys after hepatic I/R injury [18]. A range of studies reveal that when AKI develops, systemic oxidative stress greatly increases, whether in humans or animals [23,24]. In addition, a subsequent study further demonstrated that hepatic I/R injury can acutely induce a remote renal cortical oxidative stress response [25]. Accumulating evidence from past studies suggested that VNS can reduce oxidative stress in multiple organs [9,26]. Our study showed that VNS increased GSH levels and SOD activity, and decreased MDA levels and MPO activity in the kidneys after hepatic I/R injury. Based on the above, our results suggest that VNS protected against hepatic I/R injury-induced AKI, partly through its antioxidative properties.

Kidney apoptosis is a hallmark of AKI [27]. Cellular apoptosis is a recognized pathological characteristic in the literature. A previous study revealed that VNS exerted protective effects on the liver after kidney I/R injury, probably by suppressing cellular apoptosis [12]. Kidney is another important organ of the liver-kidney axis, compared to the previous study, our data showed that VNS exhibited the similar protective effect in

the kidneys after hepatic I/R injury. Our study is the first to provide evidence and support the concept that VNS can alleviate apoptosis in the kidneys after hepatic I/R injury.

Crosstalk between organs affects each other's functioning via various pathways, including endocrine, neural, and direct cell-cell signaling pathways [28]. The pathophysiology of hepatic I/R injury-induced AKI is complex and multifactorial, with inflammation, oxidative stress, apoptosis, and activation of various signaling pathways. Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor, mediates cellular defense against oxidative stress in organs. Under physiological cellular conditions, Nrf2 is sequestered in the cytoplasm in association with its protein inhibitor, Kelth-like ECH-associated protein-1 (Keap-1). Under pathological cellular conditions, Nrf2 is transferred into the nucleus where it upregulates relevant cytoprotective enzymes, such as heme oxygenase-1 (HO-1) [29]. Kudoh *et al*. reported that in a rat model of hepatic I/R injury, the depletion of Nrf2 aggravated inflammation and lead to oxidative stress and apoptosis [30]. It is widely accepted that Nrf2 activation induces HO-1 transcription, which has been demonstrated to be closely involved in alleviating AKI by minimizing cellular oxidative stress in recent finding [31]. α7nAChR is the target of the cholinergic anti-inflammatory pathway. Several studies have found that α7nAChR activation can directly induce the Nrf2/HO-1 pathway [32,33]. Navarro *et al*. further demonstrated that the deletion of Nrf2 removed the protective effect of the α7nAChR agonist [33]. As noted above, the Nrf2/HO-1 pathway may be an important signaling pathway for the protection of VNS in AKI. Consistent with previous findings, our data showed that VNS strongly enhanced the expression levels of Nrf2/HO-1. It is possible to conclude that the Nrf2/HO-1 pathway may serve as an important mediator of the protective effects of VNS in the setting of AKI following hepatic I/R injury.

Liver transplantation is the only definitive long-term treatment for end-stage liver disease and hepatic malignant tumors, with implementation beginning in the 1950s [34]. AKI is a common clinical complication in liver recipients and is considered to be a lethal threat. The data from our study show that VNS may be a potential clinical treatment for hepatic I/R injury-induced AKI. Traditional VNS is always invasive and requires device implantation; therefore, the application of VNS is widely restricted. Auricular VNS, a non-invasive VNS, has been shown to obtain the similar effects as invasive VNS [35]. Considering these observations together, non-invasive VNS might be a prospective clinical treatment for hepatic I/R injury-induced AKI.

There are limitations to our study which ought to be mentioned. First, we chose pentobarbital to anesthetize all experimental animals, which may affect the autonomic nervous system function. Second, different VNS frequencies, intensities, and durations have been shown to exert different therapeutic effects. However, only one stimulation parameter was used in the present study. It is unclear if there is an enhanced effect with a different parameter. Third, this experiment was tested in only one animal model, and extrapolation from rats to humans can be difficult.

In conclusion, our data suggest that VNS can protect against AKI after hepatic I/R injury. The potential mechanisms may involve inhibiting inflammation, suppressing oxidative stress, and reducing apoptosis. Additionally, VNS greatly activated the Nrf2/HO-1 pathway in the kidneys. Taken together, VNS may

attenuate hepatic I/R injury-induced AKI by suppressing inflammation, oxidative stress, and apoptosis probably via the Nrf2/HO-1 pathway (see Fig. 8). With the development of non-invasive VNS, VNS might provide a novel clinical treatment for patients with hepatic I/R injury-induced AKI.

4. Methods

4.1 Animal Preparation and Experimental Groups.

All animal experiments complied with the ARRIVE guidelines. Healthy male Sprague-Dawley rats (each weighing 280–350 g) used in this study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85 – 23, revised 1996) and approved by the Animal Care and Use Committee of the Second Xiangya Hospital of Central South University. Rats were housed in an environment with controlled temperature (22°C ± 1°C) and relative humidity (50%±5%) on a 12:12-h light/dark cycle, with free access to sterile food and water. Eighteen rats were randomly allocated into three groups and received different treatments: sham group (sham operation, n = 6); I/R group (hepatic I/R with sham VNS, n = 6); and VNS group (hepatic I/R with VNS, n = 6). A flowchart of the experimental design is shown in Fig. 9(a), and the location of left vagus nerve is shown in Fig. 9(b). During the whole experiment, the body surface electrocardiogram of rats was recorded with a TECHMAN biological signal acquisition system (BL-420F, Chengdu City, China).

4.2 Preparation of Hepatic I/R Injury Model.

According to the method of Zhang *et al.* [10], we established an acute segmental (70%) hepatic I/R injury model in this experiment. All rats fasted overnight before surgery. On the day of the operation, all rats were weighed and anesthetized with 1% pentobarbital sodium (40 mg/kg body weight) by intraperitoneal injection. Briefly, a midline abdominal incision was made, and an atraumatic vascular clamp was used to interrupt the arterial and portal venous blood supply to the left and median liver lobes. The clamp was removed after 1 h to initiate a 6 h hepatic reperfusion period. Throughout the operation, the body temperature was monitored using a rectal probe and maintained at 37°C with a heating pad. At the end of the experiment, all rats were anesthetized by inhaling methoxyflurane and sacrificed by exsanguination.

4.3 Vagus Nerve Stimulation

In this study, we chose the left cervical vagal trunk as the stimulating target, which was carefully separated from the surrounding connective tissues and stimulated (SDZ-IIB, Hwato, Suzhou City, China) through use of a bipolar silver wire electrode. Subsequently, continuous low-frequency stimulation (HFS, 20 Hz, 0.2 ms in duration, square waves) were delivered. The stimulus intensity should achieve a 10% reduction in the sinus rate and adjust hourly [10].

4.4 Collection of Blood and Tissue Samples.

At the end of reperfusion, blood samples were drawn from the inferior caval vein and centrifuged to separate the serum (3,000 rpm, 15 min, 4°C). At the end of the experiment, kidney tissues were collected

quickly from each rat. The left kidney was coronally dissected. Half of the kidney was fixed with paraformaldehyde and the other half was frozen with liquid nitrogen. Both blood and tissue samples were stored at -80°C until the biochemical assays, molecular biological detection, and histological examination.

4.5 Assessment of Function and Determination of Inflammatory Cytokines.

Kidney function and inflammation after hepatic I/R injury were determined by measuring the serum levels of blood urea nitrogen (BUN) and creatinine (Cr), which were detected using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, China). The serum levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1-beta (IL-1β) were analyzed by ELISA (ELK Biotechnology, Wuhan City, China) as per the manufacturer's instructions.

4.6 Measurements of Oxidative Stress in Kidney Tissues after Hepatic I/R Injury.

The levels of malondialdehyde (MDA), myeloperoxidase (MPO), glutathione (GSH), and superoxide dismutase (SOD) were measured in the kidney tissues using different assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, China). To assess the cumulative antioxidant action and the oxidative stress level, the levels of total oxidant status (TOS) and total antioxidant capacity (T-AOC) were determined in the kidney tissues using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, China). The oxidative stress index (OSI), represented by the ratio of TOS to T-AOC, is an indicator of the degree of oxidative stress. The OSI value is calculated according to the formula: OSI = TOS (μmol/gprot) / T-AOC (mmol/gprot) ×100.

4.7 Histological Examination and Quantification of Kidney Injury after Hepatic I/R Injury.

Kidney tissue samples from different groups were separated and fixed with 10% paraformaldehyde for 48 h. After automated dehydration through a graded alcohol series, the kidney slices were embedded in paraffin. Sections (4-μm thick) were stained with hematoxylin and eosin (H&E) to determine morphological changes in the kidney tissues. Finally, images of the sections were captured under a light microscope (×400 magnification). Histological scoring was performed by grading tubular necrosis, loss of brush border, cast formation, and tubular dilatation as follows: 0, none; 1, < 10%; 2, 11–25%; 3, 26–45%; 4, 46–75%; and 5, > 76% [36]. Ten views from each tissue sample among the three groups were randomly screened, and the mean was regarded as the representative value of the sample. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) staining was performed to determine apoptosis. Kidney tissue sections (4-μm thick) were deparaffinized in xylene and then hydrated in graded ethanol. After treatment with protease K, the sections were rinsed with phosphate-buffered saline (PBS) and then incubated completely in a TUNEL reaction reagent for 60 min at 37°C. After washing three times with PBS, the sections were treated with 4,6-diamidino-2-phe-

nylindole (DAPI) and incubated in the dark. The stained sections were analyzed using a fluorescence microscope (Nikon DS-U3, Tokyo, Japan). Cells with nuclei containing irregular green particles were regarded as TUNEL-positive cells, and the percentage of TUNEL-positive cells was recorded and averaged in three random high-power fields (×400 magnification).

4.8 Western Blotting Analysis.

The protein expression levels of Bax, Bcl-2, cleaved caspase 3, and caspase 7 in kidney tissues, which are often regarded as indicators of apoptosis, were assessed to determine the effect of VNS on kidney apoptosis in the hepatic I/R injury model. The level of HO-1 was measured in kidney lysates, and Nrf2 levels were detected in nuclear lysates. Briefly, cells and tissues were completely homogenized in a lysis buffer. Total proteins were extracted from the supernatant and extracted into 1.5 ml EP tubes. Equal amounts of denatured protein solution were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat milk for 1 h at 25°C and then incubated with rabbit anti-glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH, 1:10000 dilution, Abcam, Cambridge, UK), rabbit anti-Histone H3 antibody (1:3000 dilution, CST, Boston, USA), rabbit anti-Bax antibody (1:2000 dilution, CST, Boston, USA), rabbit anti-Bcl-2 antibody (1:2000 dilution, Abcam, Cambridge, UK), rabbit anti-cleaved caspase3 antibody (1:500 dilution, Affbiotech, Shanghai City, China), rabbit anti-caspase7 antibody (1:500 dilution, Abcam, Cambridge, UK), rabbit anti-nuclear factor erythroid 2-related factor 2 antibody (Nrf2, 1:500 dilution, Abcam, Cambridge, UK), and rabbit anti-heme oxygenase-1 antibody (HO-1, 1:2000 dilution, CST, Boston, USA) overnight at 4°C. After washing in Tris-buffered saline containing Tween (TBST), the PVDF membranes were incubated with horseradish peroxidase (HRP)-goat anti-rabbit secondary antibodies at room temperature for 1h. Finally, the relative protein expression was standardized to GAPDH from the same sample and quantified using a AlphaEase FC image analyzer software (Alpha Innotech, California, USA).

4.9 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR).

The mRNA levels of monocyte chemotactic protein 1 (MCP-1) and macrophage inflammatory protein 2 (MIP-2) were evaluated by qRT-PCR. Total RNA was extracted from kidney tissues using TRIpure Total RNA Extraction Reagent (ELK Biotechnology, Wuhan City, China) following the manufacturer's protocol. First-strand cDNA was synthesized using the EntiLink™ 1st Strand cDNA Synthesis Kit (ELK Biotechnology, Wuhan City, China). Target gene expression levels were measured with EnTurbo™ SYBR Green PCR SuperMix (ELK Biotechnology, Wuhan City, China) using a StepOne™ RT-PCR thermocycler (Life Technologies, Massachusetts, USA). The mRNA levels of MCP-1 and MIP-2 were normalized to the β-actin mRNA level in the same sample and calculated using the Delta-Delta-CT method. The primer sequences were as follows: MCP-1, forward: 5'-GGCCTGTTGTTCACAGTTGCT-3'; reverse, 5'-GCCGACTCATTGGGATCATC-3'; MIP-2, forward: 5'-GTCAATGCCTGACGACCCTAC-3', reverse: 5'-CCTTCCCAGGTCAGTTAGCCT-3'; and β-actin, forward: 5'- CGTTGACATCCGTAAAGACCTC-3', reverse: 5'-TAGGAGCCAGGGCAGTAATCT-3'.

4.10 Statistical Analysis

All continuous variables were presented as mean ± standard deviation (SD). GraphPad Prism version 7.0 software (GraphPad Software, Inc. San Diego, CA) was used for statistical analysis and graphing. Between-group differences were analyzed by one-way analysis of variance (ANOVA) and two-tailed $p \leq 0.05$, indicating statistical significance.

Declarations

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Author contributions

SMD and YFZ contributed equally to this study. XQH conceived and designed the experiments. SMD and YFZ performed the experiments. SMD and YFZ analysed the data and wrote the manuscript. YX reviewed and edited the manuscript. All authors have approved the final version of the manuscript and are prepared to take public responsibility for the work and share accountability for the results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Competing interests statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Liu, Y. *et al.* Activation of YAP attenuates hepatic damage and fibrosis in liver ischemia-reperfusion injury. *Journal of hepatology* **71**, 719-730, doi:10.1016/j.jhep.2019.05.029 (2019).
2. Zhang, H. *et al.* Hepatic Surgical Stress Promotes Systemic Immunothrombosis That Results in Distant Organ Injury. *Frontiers in immunology* **11**, 987, doi:10.3389/fimmu.2020.00987 (2020).
3. Jagarlamudi, N. & Wong, F. Acute kidney injury: prediction, prognostication and optimisation for liver transplant. *Hepatology international* **14**, 167-179, doi:10.1007/s12072-020-10018-0 (2020).

4. Leithead, J. A., Rajoriya, N., Gunson, B. K., Muiesan, P. & Ferguson, J. W. The evolving use of higher risk grafts is associated with an increased incidence of acute kidney injury after liver transplantation. *Journal of hepatology* **60**, 1180-1186, doi:10.1016/j.jhep.2014.02.019 (2014).
5. Jochmans, I. *et al.* Hepatic ischemia/reperfusion injury associates with acute kidney injury in liver transplantation: Prospective cohort study. *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* **23**, 634-644, doi:10.1002/lt.24728 (2017).
6. Ronco, C., Bellomo, R. & Kellum, J. A. Acute kidney injury. *Lancet (London, England)* **394**, 1949-1964, doi:10.1016/s0140-6736(19)32563-2 (2019).
7. Betrosian, A. P., Agarwal, B. & Douzinas, E. E. Acute renal dysfunction in liver diseases. *World journal of gastroenterology* **13**, 5552-5559, doi:10.3748/wjg.v13.i42.5552 (2007).
8. Shuchman, M. Approving the vagus-nerve stimulator for depression. *The New England journal of medicine* **356**, 1604-1607, doi:10.1056/NEJMp078035 (2007).
9. Wang, M. *et al.* Vagus Nerve Stimulation Ameliorates Renal Ischemia-Reperfusion Injury through Inhibiting NF-κB Activation and iNOS Protein Expression. *Oxidative medicine and cellular longevity* **2020**, 7106525, doi:10.1155/2020/7106525 (2020).
10. Zhang, Q. *et al.* Vagus Nerve Stimulation Attenuates Hepatic Ischemia/Reperfusion Injury via the Nrf2/HO-1 Pathway. *Oxidative medicine and cellular longevity* **2019**, 9549506, doi:10.1155/2019/9549506 (2019).
11. Inoue, T. *et al.* Vagus nerve stimulation mediates protection from kidney ischemia-reperfusion injury through α7nAChR+ splenocytes. *The Journal of clinical investigation* **126**, 1939-1952, doi:10.1172/jci83658 (2016).
12. Lai, Y. *et al.* Vagus nerve stimulation protects against acute liver injury induced by renal ischemia reperfusion via antioxidant stress and anti-inflammation. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **117**, 109062, doi:10.1016/j.biopha.2019.109062 (2019).
13. Ojo, A. O. *et al.* Chronic renal failure after transplantation of a nonrenal organ. *The New England journal of medicine* **349**, 931-940, doi:10.1056/NEJMoa021744 (2003).
14. Cabezuelo, J. B. *et al.* Does the standard vs piggyback surgical technique affect the development of early acute renal failure after orthotopic liver transplantation? *Transplantation proceedings* **35**, 1913-1914, doi:10.1016/s0041-1345(03)00598-0 (2003).
15. Norvell, J. E. The aorticorenal ganglion and its role in renal innervation. *The Journal of comparative neurology* **133**, 101-111, doi:10.1002/cne.901330107 (1968).
16. Atherton, D. S., Deep, N. L. & Mendelsohn, F. O. Micro-anatomy of the renal sympathetic nervous system: a human postmortem histologic study. *Clinical anatomy (New York, N.Y.)* **25**, 628-633, doi:10.1002/ca.21280 (2012).
17. Grisk, O. The sympathetic nervous system in acute kidney injury. *Acta physiologica (Oxford, England)* **228**, e13404, doi:10.1111/apha.13404 (2020).

18. Lee, H. T., Park, S. W., Kim, M. & D'Agati, V. D. Acute kidney injury after hepatic ischemia and reperfusion injury in mice. *Laboratory investigation; a journal of technical methods and pathology* **89**, 196-208, doi:10.1038/labinvest.2008.124 (2009).
19. Colletti, L. M. *et al.* The production of tumor necrosis factor alpha and the development of a pulmonary capillary injury following hepatic ischemia/reperfusion. *Transplantation* **49**, 268-272, doi:10.1097/00007890-199002000-00008 (1990).
20. Park, S. W., Kim, M., Chen, S. W., D'Agati, V. D. & Lee, H. T. Sphinganine-1-phosphate attenuates both hepatic and renal injury induced by hepatic ischemia and reperfusion in mice. *Shock (Augusta, Ga.)* **33**, 31-42, doi:10.1097/SHK.0b013e3181c02c1f (2010).
21. Koopman, F. A. *et al.* Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 8284-8289, doi:10.1073/pnas.1605635113 (2016).
22. Okusa, M. D., Rosin, D. L. & Tracey, K. J. Targeting neural reflex circuits in immunity to treat kidney disease. *Nature reviews. Nephrology* **13**, 669-680, doi:10.1038/nrneph.2017.132 (2017).
23. Golab, F. *et al.* Ischemic and non-ischemic acute kidney injury cause hepatic damage. *Kidney international* **75**, 783-792, doi:10.1038/ki.2008.683 (2009).
24. Himmelfarb, J. *et al.* Oxidative stress is increased in critically ill patients with acute renal failure. *Journal of the American Society of Nephrology : JASN* **15**, 2449-2456, doi:10.1097/01.Asn.0000138232.68452.3b (2004).
25. Zager, R. A., Johnson, A. C. & Frostad, K. B. Acute hepatic ischemic-reperfusion injury induces a renal cortical "stress response," renal "cytoresistance," and an endotoxin hyperresponsive state. *American journal of physiology. Renal physiology* **307**, F856-868, doi:10.1152/ajprenal.00378.2014 (2014).
26. Zhang, Y. *et al.* Vagus Nerve Stimulation Attenuates Acute Skeletal Muscle Injury Induced by Ischemia-Reperfusion in Rats. *Oxidative medicine and cellular longevity* **2019**, 9208949, doi:10.1155/2019/9208949 (2019).
27. Arai, S. *et al.* Apoptosis inhibitor of macrophage protein enhances intraluminal debris clearance and ameliorates acute kidney injury in mice. *Nature medicine* **22**, 183-193, doi:10.1038/nm.4012 (2016).
28. Lane, K., Dixon, J. J., MacPhee, I. A. & Philips, B. J. Renohepatic crosstalk: does acute kidney injury cause liver dysfunction? *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **28**, 1634-1647, doi:10.1093/ndt/gft091 (2013).
29. Rojo de la Vega, M., Chapman, E. & Zhang, D. D. NRF2 and the Hallmarks of Cancer. *Cancer cell* **34**, 21-43, doi:10.1016/j.ccell.2018.03.022 (2018).
30. Kudoh, K., Uchinami, H., Yoshioka, M., Seki, E. & Yamamoto, Y. Nrf2 activation protects the liver from ischemia/reperfusion injury in mice. *Annals of surgery* **260**, 118-127, doi:10.1097/sla.0000000000000287 (2014).
31. Amini, N. *et al.* Protective effects of naringin and trimetazidine on remote effect of acute renal injury on oxidative stress and myocardial injury through Nrf-2 regulation. *Pharmacological reports : PR* **71**,

1059-1066, doi:10.1016/j.pharep.2019.06.007 (2019).

32. Kalkman, H. O. & Feuerbach, D. Modulatory effects of α7 nAChRs on the immune system and its relevance for CNS disorders. *Cellular and molecular life sciences : CMLS* **73**, 2511-2530, doi:10.1007/s00018-016-2175-4 (2016).
33. Navarro, E. *et al.* Heme-Oxygenase I and PCG-1α Regulate Mitochondrial Biogenesis via Microglial Activation of Alpha7 Nicotinic Acetylcholine Receptors Using PNU282987. *Antioxidants & redox signaling* **27**, 93-105, doi:10.1089/ars.2016.6698 (2017).
34. Im, G. Y., Cameron, A. M. & Lucey, M. R. Liver transplantation for alcoholic hepatitis. *Journal of hepatology* **70**, 328-334, doi:10.1016/j.jhep.2018.11.007 (2019).
35. Neuser, M. P. *et al.* Vagus nerve stimulation boosts the drive to work for rewards. *Nature communications* **11**, 3555, doi:10.1038/s41467-020-17344-9 (2020).
36. Melnikov, V. Y. *et al.* Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. *The Journal of clinical investigation* **110**, 1083-1091, doi:10.1172/jci15623 (2002).

Figures

Figure 1

VNS alleviates kidney injury. (a) Representative H&E staining images of kidney tissues in the three groups are shown. (b) Kidney histological scores are shown. Levels of serum (c) BUN and (d) Cr are presented. *p<0.05 vs. the Sham group; #p<0.05 vs. the I/R group. BUN: Blood urea nitrogen; Cr: creatinine.

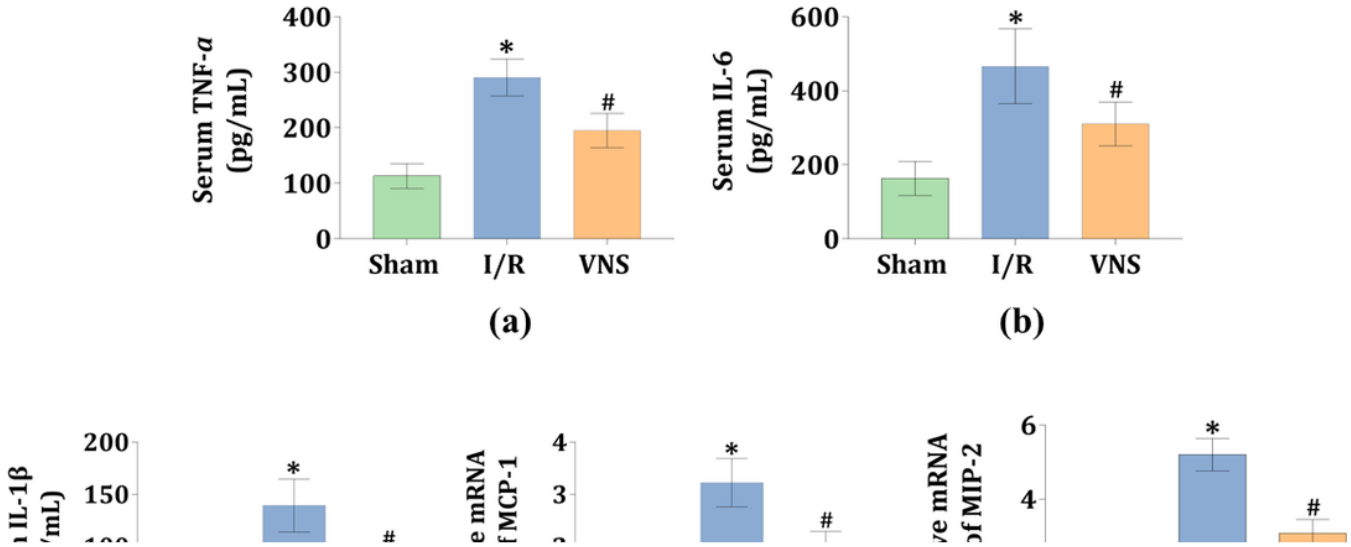

Figure 2

VNS mitigates inflammation in the kidneys after hepatic I/R injury. The effect of VNS on the serum levels of (a) TNF-α, (b) IL-6, and (c) IL-1β is shown. The relative mRNA levels of (d) MCP-1 and (e) MIP-2 in kidney tissues from the three groups are shown. Data are presented as mean ± SD. *p<0.05 versus the Sham group; #p<0.05 versus the I/R group. TNF-α: tumor necrosis factor alpha; IL-6: interleukin 6; IL-1β: interleukin1-beta; MCP-1: monocyte chemoattractive protein 1; MIP-2: macrophage inflammatory protein 2.

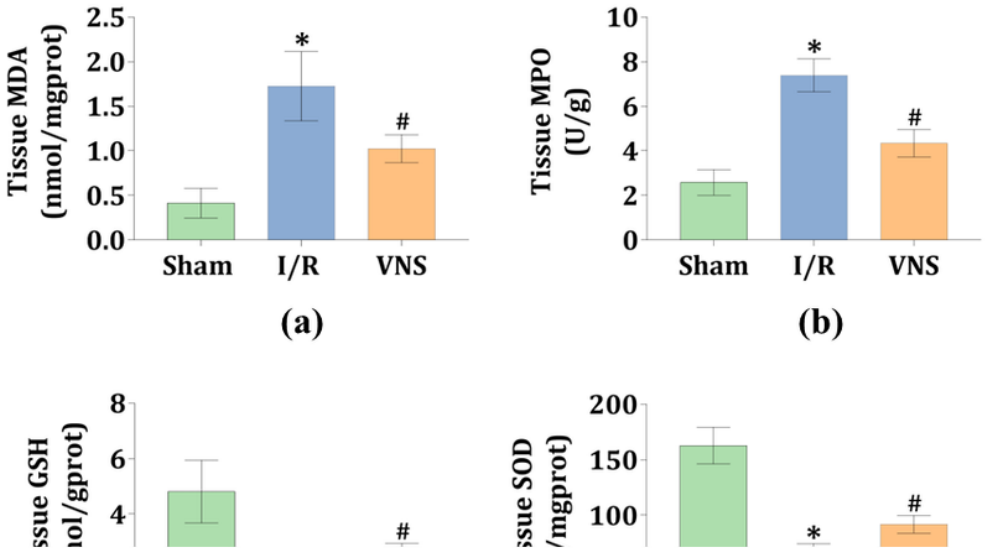

Figure 3

VNS attenuates oxidative stress in the kidneys after hepatic I/R injury. The effect of VNS on the levels of (a) MDA and (c) GSH in kidney tissues is shown. The effect of VNS on the activity of (b) MPO and (d) SOD in kidney tissues is shown. Data are expressed as mean ± SD. *p<0.05 versus the Sham group; #p<0.05 versus the I/R group. MDA: myeloperoxidase; MPO: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase.

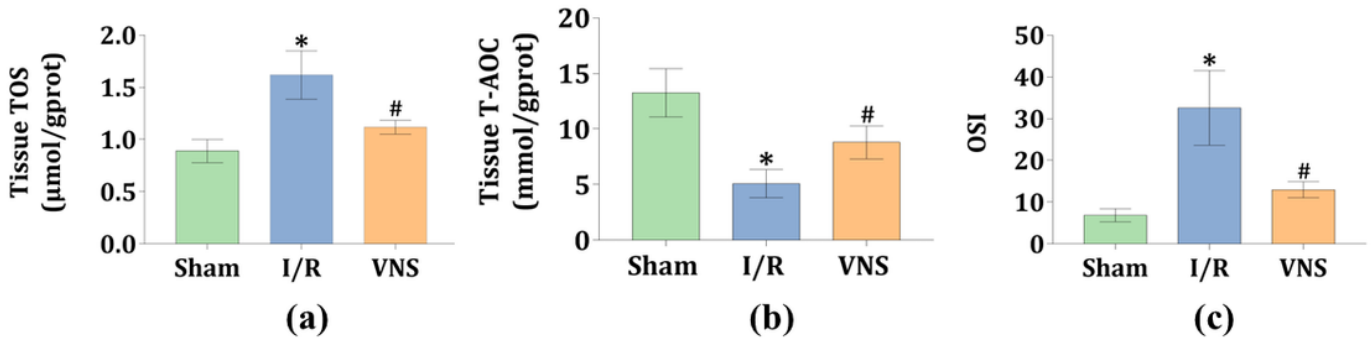

Figure 4

VNS improves redox status in the kidneys after hepatic I/R injury. The effect of VNS on the levels of (a) TOS and (b) T-AOC in kidney tissues is shown. (c) The OSI values is shown. Data are expressed as mean ± SD. *p<0.05 versus the Sham group; #p<0.05 versus the I/R group. TOS: total oxidant status; T-AOC: total antioxidant capacity; OSI: oxidative stress index.

Figure 5

VNS reduces cell apoptosis in kidneys after hepatic I/R injury. (a) Representative micrographs of immunofluorescence staining for DAPI (blue) and TUNEL (green) in kidney cell nuclei from the three groups are shown. (b) Quantification of kidney cell apoptosis from the three groups is shown. *p<0.05 vs. the Sham group; #p<0.05 vs. the I/R group.

Figure 6

VNS reduces cell apoptosis by regulating Bax, Bcl-2, Cleaved caspase3, and caspase7. (E) Representative blots and relative protein levels of (a) Bax, (b) Bcl-2, (c) Cleaved caspase3, and (d) caspase7 in kidney tissues from the three groups are shown. *p<0.05 vs. the Sham group; #p<0.05 vs. the I/R group.

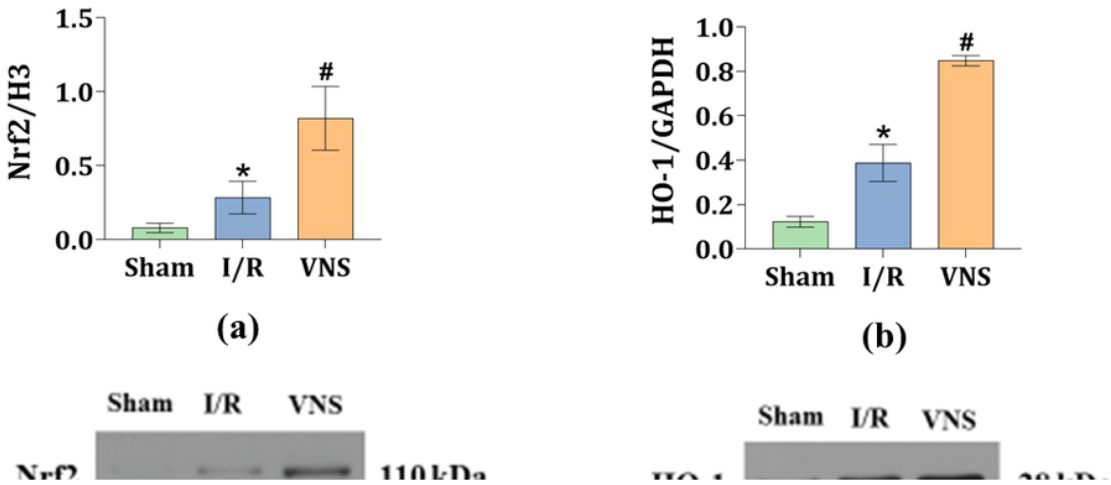

Figure 7

VNS activates the Nrf2/HO-1 pathway in the kidneys after hepatic I/R injury. (c. d) Representative blots and relative protein levels of (a) Nrf2 and (b) HO-1 in kidney tissues from the three groups are shown. *p<0.05 vs. the Sham group; #p<0.05 vs. the I/R group. Nrf2: nuclear factor erythroid 2-related factor 2; HO-1: heme oxygenase-1.

Figure 8

Schematic diagram depicting the protective effect of VNS on AKI after hepatic I/R injury and its potential mechanisms. VNS protects against AKI after hepatic I/R injury by suppressing inflammation, oxidative stress, and apoptosis probably via the Nrf2/HO-1 pathway.

Figure 9

Experimental protocol (a) and location of the left vagus nerve (b). Sham: sham operation; I/R: ischemia-reperfusion; VNS: vagus nerve stimulation.

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