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Hydrogen protects against chronic intermittent hypoxia induced renal dysfunction by promoting autophagy and alleviating apoptosis

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ABSTRACT

Aims: Hydrogen gas (H_2) has a diversity of effects such as anti-apoptotic, anti-inflammatory and anti-oxidative properties. However, molecular mechanism underlying the potential effect of H_2 on chronic intermittent hypoxia (CIH) induced renal injury remains obscure.

Materials and methods: In the present study, adult male Sprague-Dawley rats were randomly allocated into four groups: control (CON) group, CIH group, CIH with H_2 treatment (CIH + H_2) group, and control with H_2 treatment (CON + H_2) group. Oxidative stress, autophagy and endoplasmic reticulum (ER) stress were detected to determine how H_2 affected the renal function of CIH exposed rats.

Key findings: We demonstrated that rats who inhale hydrogen gas showed improved renal function, alleviated pathological damage, oxidative stress and apoptosis in CIH rats. Meanwhile, CIH-induced endoplasmic reticulum stress was decreased by H_2 as the expressions of CHOP, caspase-12, and GRP78 were down-regulated. Furthermore, relative higher levels of LC3-II/I ratio and Beclin-1, with decreased expression of p62, were found after H_2 administrated. Inhibition of mTOR may be involved in the upregulation of autophagy by H_2. Finally, increased phosphorylation of p38 and JNK was involved in the CIH-induced pathological process. H_2 could inhibit the activation of p38 and JNK, suggesting H_2 played an active part in resisting renal injury via MAPK.

Significance: Taken together, our study reveals that H_2 can ameliorate CIH-induced kidney injury by decreasing endoplasmic reticulum stress and activating autophagy through inhibiting oxidative stress-dependent p38 and JNK MAPK activation.

1. Introduction

Obstructive sleep apnea (OSA) has been recognized as one of the important causes of renal damage [1]. There are several reasons to explain this excess renal risk among individuals with OSA, such as hypertension, sympathetic nervous system and renin-angiotensin-aldosterone system overactivation, endothelial dysfunction, increased oxidative stress and so on [2]. Recent studies have also demonstrated that autophagy level was increased in kidney tissue of a CIH mouse model [3]. Autophagy is an adaptive cellular response to “stress” in which long-lived proteins, damaged organelles, and cytoplasmic contents are eliminated to maintain body homeostasis [4]. A basal level of autophagy can reduce sources of stress and is thought to be beneficial to normal renal function [5]. Nevertheless, excessive autophagic activity is implicated in the progression of renal diseases, such as ischemia-reperfusion-induced kidney injury, acute kidney injury, diabetic nephropathy, and glomerular diseases [6]. Meanwhile, autophagy dysfunction can result in impaired mitochondrial function, reactive oxygen species (ROS) accumulation and oxidative stress [7]. ROS are known to deplete glutathione and result in the misfolding protein load in the endoplasmic reticulum (ER) [8]. In response to accumulation and aggregation of misfolded proteins, GRP78 was titrated away from the luminal domains of ER transmembrane signaling proteins, such as inositol-requiring enzyme 1 (IRE1), protein kinase-like ER kinase (PERK) and activating transcription factor-6 (ATF6). IRE1, PERK, and ATF6 signaling pathways, termed the unfolded protein response (UPR), act as a system to regulate the ER functions [9]. C/EBP homologous protein (CHOP) is markedly increased via UPR-dependent transcriptional induction. Studies have demonstrated that when the ER function is severely impaired, the organelle elicits apoptotic signals via CHOP [10].

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Thus, both autophagy and ER stress have been shown to be the major contributors of renal injury in the CIH-exposed rats [3,8,11].

It has been shown that molecular hydrogen (H_2) can rapidly diffuse into tissues and cells, thus function with a potent systemic antioxidant, anti-inflammatory and antiapoptotic activity [12]. Interestingly, H_2 has no known side effects as it neither interferes with metabolic redox reactions nor affects signaling reactive oxygen species (ROS) [13].

Our recent study showed that treatment of the CIH exposed rats with inhaled hydrogen could help reduce hypertension by reducing oxidative stress in rats [14].

However, whether H_2 exerts role in improving CIH-induced renal injury remains unknown. Thus, in this study, we for the first time reveal the functions and mechanisms of H_2 on CIH-induced renal injury. We investigated whether H_2 could improve CIH-induced autophagy dysfunction and ER stress by the MAPK pathway.

2. Materials and methods

2.1. Animal experiments

All animal-handling procedures were approved by the Hebei University of Chinese Medicine Animal Care and Use Committee. Adult male Sprague-Dawley rats (weight 200 ± 10 g) were kept under 12-h light/dark cycles. All efforts were made to reduce the number of animals used and their suffering.

24 rats were randomly allocated into four groups: CON group, CIH group, CIH + H_2 group, and CON + H_2 group. Exposure of animals to CIH is based on the protocol described by Guo et al. [15]. Briefly, unrestrained, freely moving rats were housed in hypoxic chambers with OxyCycler gas delivery system (BioSpherix Ltd., USA). Pure nitrogen and compressed air were distributed into each chamber through timed solenoid valves. The repeated exposure to O_2 includes a 90 s declining from 21% to 9%, 9% for 15 s, and then an increasing to 21% for 90 s period. Rats were exposed to CIH for 8 h/day for 8 h/day for 5 weeks. For the CON and H_2 group, air was forced into the chamber. The H_2 treatment groups inhaled H_2 gas for 2 h daily after CIH exposure through a ventilator circuit. The H_2 was produced using a hydrogen nebulizer machine (Shanghai Asclepius Meditec, China). At the completion of the exposure period, animals were individually housed in clear acrylic chambers.

2.2. Serum chemistry

Blood samples were collected from all animals in each group to measure the changes in serum blood urine nitrogen (BUN) and creatinine levels. Serum BUN level was measured by a urease method using urea assay kit (Nanjing Jiancheng Bioengineering Institute, China). Serum creatinine was measured by a picric acid colorimetric method using a creatinine assay kit (Nanjing Jiancheng Bioengineering Institute, China). Both serum parameters were recorded on a microplate reader (Thermo Fisher, Waltham, MA, USA).

2.3. Histopathological examination

The kidneys were carefully excised, fixed in 4% paraformaldehyde for 2 days at 4 °C. The paraffin-tissues were sliced for 4 μm and mounted onto slides. After being deparaffinized using xylene and ethanol dilutions and rehydration, the sections were stained by hematoxylin and eosin (HE) for general histological structure. As periodic acid-Schiff stain (PAS) stain can show the basement membranes of the renal tubules and the apical brush border of the proximal convoluted tubules, this method was used in this study to evaluate the fine structure.

2.4. TUNEL assay

TdT-mediated dUTP nick end labeling (TUNEL) assay was used to detect apoptosis in situ utilizing TUNEL kit (Roche Diagnostics GmbH, Mannheim, Germany). Briefly, the fixed renal tissues on the slides were treated with proteinase K for 30 min at 37 °C, and the endogenous peroxidase activity was blocked by 3% H_2O_2 for 5 min. Specimens were incubated in 50 μl of TUNEL reaction mixture for 1 h at 37 °C in the dark and humidified atmosphere. And then, the slides were rinsed with phosphate-buffered saline (PBS). Nuclei were stained with DAPI. After being washed with PBS, the sections were analyzed under light microscopy (Leica Microsystems GmbH, Wetzlar, Germany) for TUNEL-positive nuclei.

2.5. Measurement of oxidative parameters in the renal tissue

Lipid peroxidation was investigated in kidney homogenates by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Malondialdehyde (MDA) and superoxide dismutase (SOD) activities were measured as described previously [16,17]. Glutathione peroxidase (GSH-Px) activity, glutathione (GSH) levels and oxidized glutathione (GSSG) levels were assayed as described previously [18].

2.6. Western blot analyses

The expression of Bax, Bcl-2, GRP78, CHOP, caspase 12, p-p38, p-JNK, LC3, Beclin1 and p62 was detected by Western blotting analysis. Shortly, kidney samples were frozen in liquid nitrogen and homogenized with 1.5 mM EDTA in PBS supplemented with PMSF and protease inhibitors. Total proteins were extracted from the homogenate by centrifuging at 4 °C. Protein concentration was analyzed by a BCA assay kit (Kangwei Century Biotechnology, Beijing, China). SDS-PAGE was performed and then blotted to Polyviny-lidene fluoride (PVDF) membrane. Membranes were blocked in 5% skimmed milk in TBST buffer for 1 h at room temperature and then incubated overnight at 4 °C with the primary antibodies. Primary antibodies used in present study were from following sources: anti-Bcl-2 from Immunoway, anti-Bax, anti-LC3 I/II, anti-p62, and anti-beclin-1 from Servicebio; anti-GRP78, anti-Caspase 12 and anti-JNK from Arigo; anti-CHOP from GeneTex; anti-p38, anti-p-p38 and anti-p-JNK from Cell Signaling Technology. All primary antibodies were diluted as recommended by the manufacturer. Subsequently, the membranes were incubated in horseradish peroxidase (HRP)-conjugated secondary antibody and ECL detection (Vilber Fusion FX5 Spectra, Paris, France).

2.7. Immunohistochemistry

Immunohistochemistry was performed on 4 μm thick paraformaldehyde-fixed and paraffin-embedded sections. Briefly, sections were deparaffinized and rehydrated. Sections were then placed in citrate buffer and microwaved twice for antigen retrieval. Subsequently, sections were blocked by methanol containing 3% H_2O_2 to block endogenous peroxidase activity. Then, the sections were incubated overnight at 4 °C with rabbit polyclonal anti-phospho-mTOR (Ser2448) (1:1000, Santa Cruz Biotechnology). Appropriate HRP-labeled secondary antibodies (Zhongshan Biotechnology) and 3,3′ diaminobenzidine (Dako) were used to detect positive immunocytochemical reaction. The stained sections were quantified by using Image-Pro Plus software (Image-ProPlus 6.0, Media Cybernetics).

2.8. Statistical analysis

All experiments were repeated at least three times independently unless indicated otherwise. The averaged values are shown as mean ± SEM. All data were Statistically analyzed by using GraphPad Prism 5 software. One-way analysis of variance (ANOVA) was performed to analyze the data as indicated. Multiple comparison testing was made via Bonferroni's test. $p < 0.05$ was considered significant.

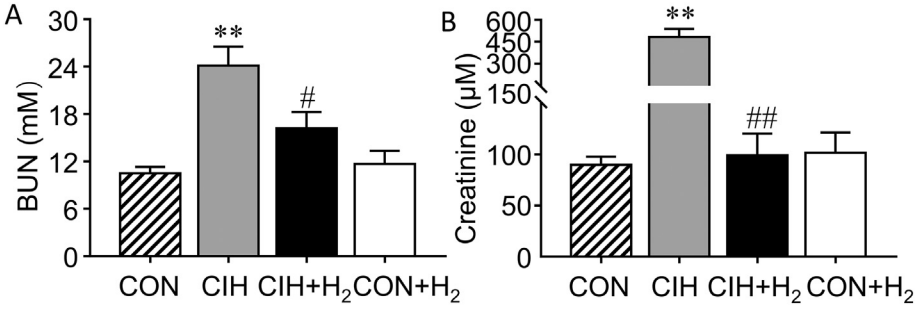

Fig. 1. (a) Blood urea nitrogen (BUN) and (b) creatinine levels in serum samples of CON, CIH, CIH + H_2 or H_2 groups. $n = 6$. $^{**}p < 0.01$ vs. CON group; $^{\#\#}p < 0.01$ vs. CIH group.

3. Results

3.1. Effects of H_2 on CIH-induced BUN and creatinine production

Serum BUN and creatinine levels were measured to evaluate the effects of H_2 on kidney function. The results showed that the levels of serum BUN and creatinine increased significantly in CIH group compared with the CON group (Fig. 1). However, treatment with H_2 significantly ($p < 0.01$) inhibited the increase in BUN and creatinine levels. In rats that underwent H_2 alone, there were no obvious changes of BUN and creatinine levels compared to controls.

3.2. Morphological and histological investigation

Micrographs of the CON group exhibited no obvious histopathological changes in the kidneys. In CIH group, we found that CIH induced remarkable renal structural damage in rats, such as glomerular and renal cysts and renal tubular epithelial cell swelling, tubular atrophy, and thickening of the glomerular basement membrane. However, these alterations were notably alleviated by H_2 (Fig. 2A, B). There were no obvious morphological changes in H_2 alone treated group compared to the CON group.

3.3. H_2 inhibited apoptosis in the kidneys

To explore the effect of H_2 treatment on protecting kidney against injury of CIH rat, the renal apoptosis cells were detected by the TUNEL assay (Fig. 3A). Compared with the CON group, an obvious increase in the number of apoptotic cells was seen in CIH group. Under H_2 treatment, the apoptosis of kidney cells were significantly up-regulated (Fig. 3B). However, renal cell apoptosis was significantly suppressed with H_2 inhalation ($p < 0.01$). Interestingly, the signal H_2 treated rats had no significant difference with CON group.

In the meantime, we used Western blot approach to detect the ratio of pro-apoptotic (Bax) to anti-apoptotic (Bcl-2) members (Fig. 3C, D). CIH induced increased Bax/Bcl-2 ratios relative to that of the CON group. Moreover, the Bax/Bcl-2 ratios were reduced by H_2 compared to CIH group. Otherwise, treatment of H_2 alone showed no apparent change in the Bax/Bcl-2 ratios.

3.4. H_2 reduces CIH-induced oxidative stress

To examine whether the increased level of oxidative stress was responsible for CIH induced cell apoptosis, MDA, SOD, GSH-Px levels and GSH/GSSG ratio were determined. Compared to the CON group, the renal MDA level was significantly higher in the CIH group. As shown in Fig. 4A, CIH-induced up-regulation of MDA was significantly attenuated in the presence of H_2. In addition, CIH significantly attenuated the SOD activity, whereas H_2 partially reversed the effects of CIH (Fig. 4B). GSH-Px is a major enzyme for removal of H_2O_2 using reduced GSH, which consequently produces GSSG. GSH-Px activity was reduced in the CIH group but significantly increased by H_2 (Fig. 4C). Furthermore, the GSH/GSSG ratio in CIH-treated rat kidneys was significantly decreased and that decrease was significantly reversed after H_2 administration (Fig. 4D).

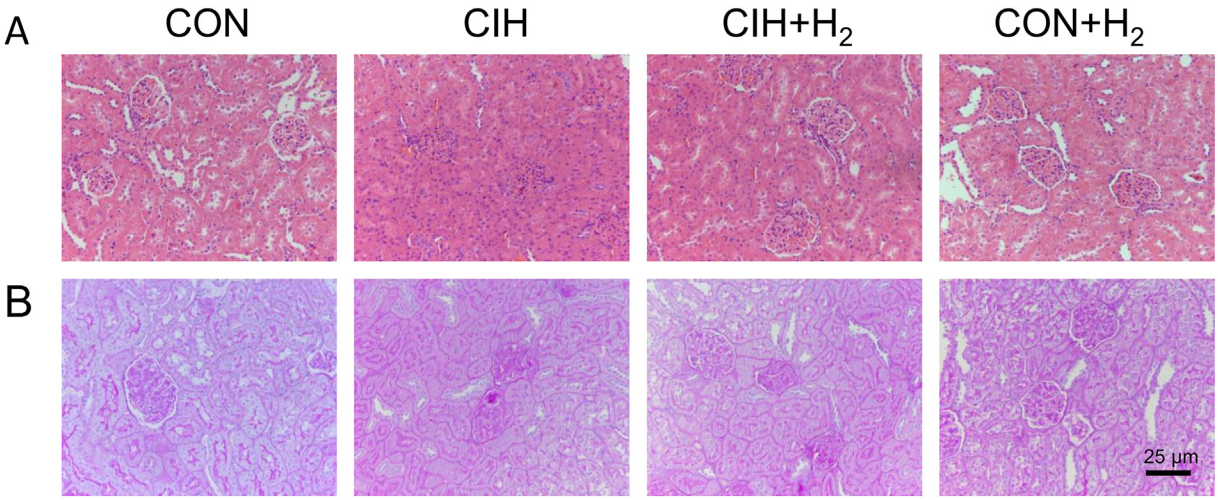

Fig. 2. Histopathological appearance in H&E and PAS-stained rat kidney sections of all groups. A. Representative H&E stained images. B. Representative PAS stained images. CIH induced remarkable renal structure damage, such as glomerular and renal cysts and renal tubular epithelial cell swelling. These alterations were notably alleviated by H_2.

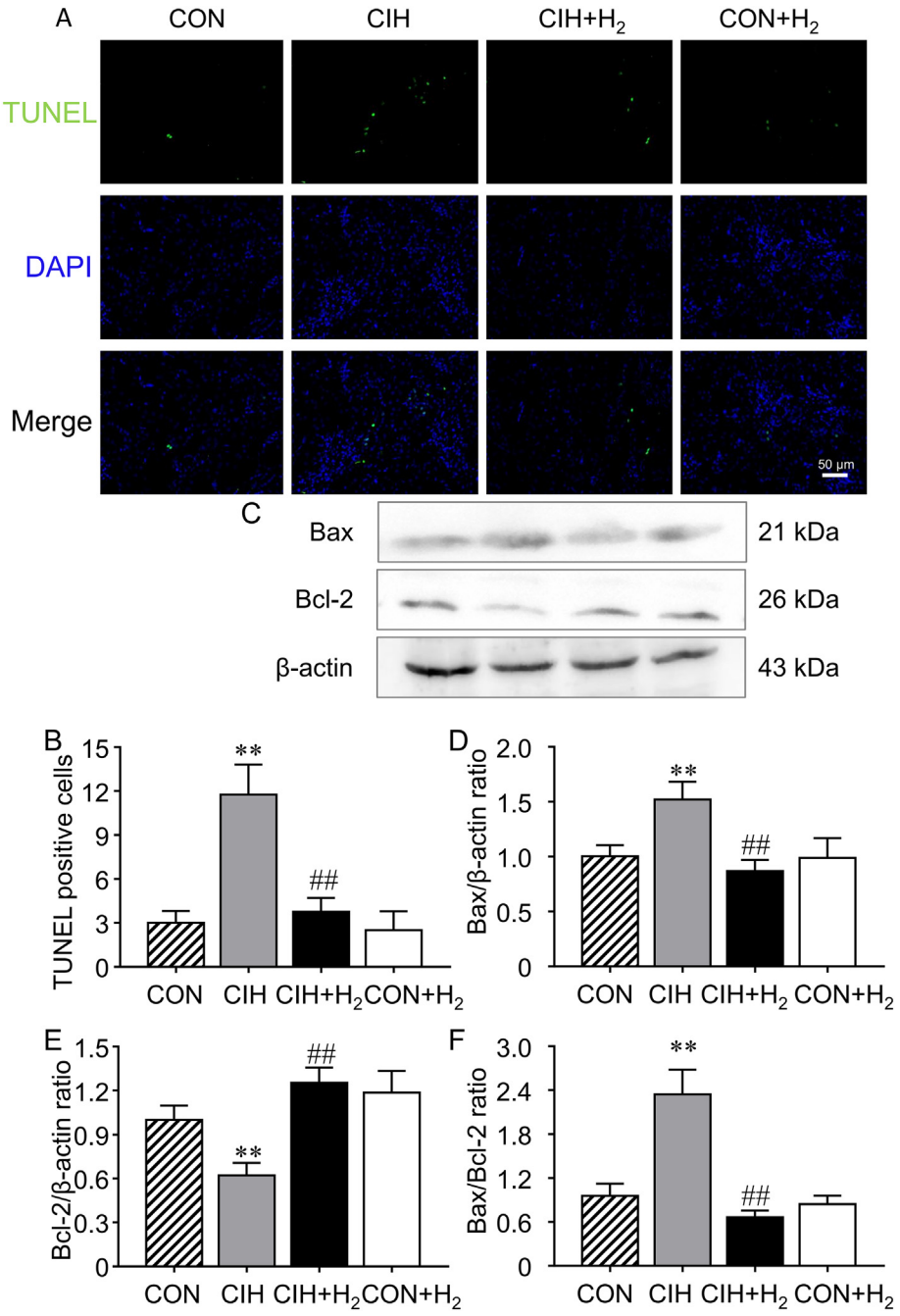

Fig. 3. Cell apoptosis in kidney tissues. A. Representative images of TUNEL staining sections. B. TUNEL staining sections was analyzed. C. Bax and bcl-2 expression levels determined by Western blot. D. Renal Bax expression. E. Renal bcl-2 expression. F. Bax/bcl-2 ratio was analyzed. $n = 6$. $^{**}p < 0.01$ vs. CON group; $^{\#\#}p < 0.01$ vs. CIH group.

3.5. H_2 alleviates CIH-induced ER stress in the kidney

To examine whether ER stress is involved or not, we detected the expression of two UPR molecules, GRP78 and CHOP, which are typical markers of ER stress. As Fig. 5 shown, CIH induced increases in both GRP78 and CHOP levels in the kidney tissue, while H_2 efficiently inhibited the increases. To investigate whether ER stress participates in apoptosis in rat kidney cells, we also detected the activation of caspase 12. We noticed that protein expression of caspase 12 increased significantly in the kidneys of the CIH group than the CON group. Expression level of caspase 12 was markedly decreased in H_2 treated rats as compared with the ones treated with CIH only.

3.6. H_2 increases autophagy in the kidney

Under cell stress, autophagy may be protective. To determine whether CIH and H_2 induce autophagy, Western blots were performed to quantify the levels of LC3, beclin1 and p62 (Fig. 6A). Both the LC3II expression and LC3II/LC3I ratio have been regarded as reliable markers of autophagy. The results showed CIH elevated the ratio of LC3-II/LC3-I but statistically nonsignificant. H_2 further increased the CIH-induced upregulation of LC3-II/LC3-I, confirming autophagic flux was enhanced by H_2 (Fig. 6B). The expression of p62 is elevated in rat model of CIH, however, p62 level decreased significantly in the kidney from the CIH + H_2 groups compared to CIH (Fig. 6C). CIH did not affect the beclin-1 level, and H_2 induced abundantly expression of beclin-1

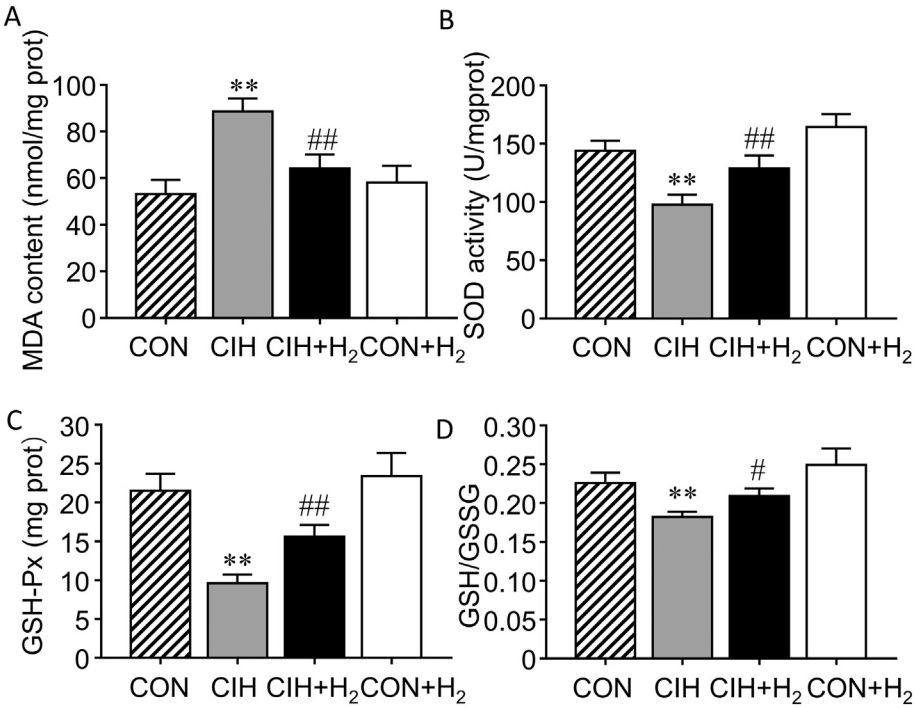

Fig. 4. Oxidative stress in kidney tissues. A. MDA content. B. SOD activity. C. GSH-Px content D. GSH/GSSG ratio. $n = 6$. $^{**}p < 0.01$ vs. CON group; $^{\#\#}p < 0.01$ vs. CIH group.

(Fig. 6D).

3.7. H_2 inhibits mTOR activation in kidney

As shown in Fig. 6E, CIH caused an increase in p-mTOR. As expected, a decreased phospho-mTOR immunohistochemical expression was found in kidney tissues of H_2 administrated CIH exposed rats compared with CIH group. H_2 alone did not alter the mTOR phosphorylation.

3.8. H_2 inhibits p38 and JNK activation in kidney

To determine whether MAPK activation contributed to the apoptosis increase in CIH-treated kidney, we assessed the phosphorylated forms of p38 and JNK. The phosphorylation of p38 and JNK markedly increased in rat renal tissues after CIH administration, compared with the CON group. However, the addition of H_2 significantly prevented the increase in p38 and JNK phosphorylations (Fig. 7).

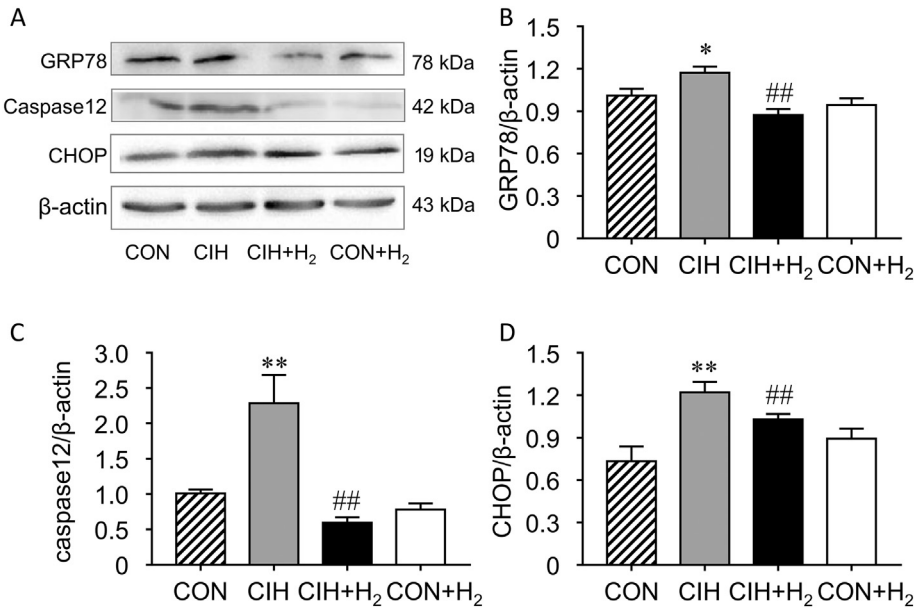

Fig. 5. The expressions of GRP78, Caspase12 and CHOP were analyzed by Western Blot. A. Representative Western blot photographs. B. Renal GRP78 expression. C. Renal caspase12 expression. D. Renal CHOP expression. $n = 6$. $^{**}p < 0.01$ vs. CON group; $^{\#\#}p < 0.01$ vs. CIH group.

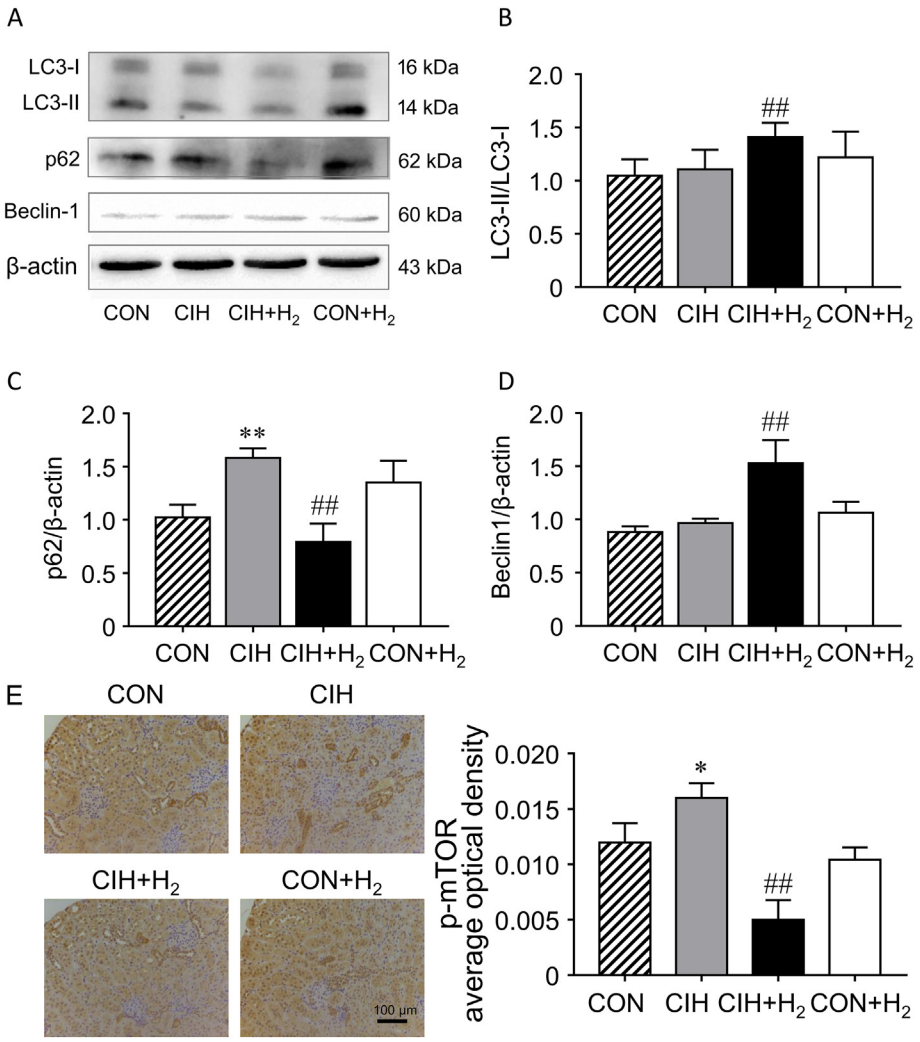

Fig. 6. The expressions of LC3 I, LC3 II, p62, beclin-1 and mTOR. A. Representative Western blot photographs. B. Renal LC3 II/LC3 I ratio. C. Renal p62 expression. D. Renal beclin-1 expression. E. p-mTOR expression. $n = 6$. $^{*}p < 0.05$ vs. CON group; $^{**}p < 0.01$ vs. CON group; $^{\#\#}p < 0.01$ vs. CIH group.

4. Discussion

Previous studies have demonstrated that OSA-associated chronic kidney disease is mainly caused by CIH triggered renal damage. For example, OSA can contribute to endothelial dysfunction and artery stiffness [19], renal tubular dysfunction-related nocturnal natriuresis [20] and activation of the systemic and renal renin-angiotensin system [21], all of which are implicated in the etiology of chronic kidney disease. Lin et al. reported more evidences in a 12-year follow up that high rate of kidney dysfunction occurred in patients who have OSA [22]. Treatment of patients with OSAS with the therapy of continuous positive airway pressure leads to a decrease in glomerular hyperfiltration [20]. This serves as further evidence that the OSA alone can alter renal function in a harmful manner directly.

The presence of basal levels of H_2 suggests that it plays a physiological role. Cardinal et al. confirmed that hydrogen gas can rapidly diffuse across the cell membrane and prevent damage from the detrimental effect of hydroxyl radicals without compromising ROS involved in cell signaling [23]. Several studies have reported that hydrogen or hydrogen-rich saline protect against renal injury with decreased levels of inflammation and lipid peroxides [24–27]. Given the increasing beneficial reports of H_2, a growing number of H_2 signaling pathways may be found. In this study, we successfully established a rat model mimicking chronic intermittent hypoxia process in OSA patients. Measurement of serum BUN and creatinine levels have been used to determine renal function in many experimental studies. The present study demonstrated that BUN and serum creatinine were increased in CIH exposed rats. Inhalation of H_2 decreased serum levels of BUN and creatinine. H_2 is therefore a powerful new tool for the treatment of CIH-induced renal injury. Another important finding in the present study is that the CIH induced histopathological renal injury as well as apoptosis, but was significantly reduced by H_2 therapy.

Oxidative stress induced-apoptosis is well-documented and manifests in renal injury progression [28]. Inhibition of oxidative stress has proved to be effective for ameliorating renal injury and facilitating recovery [29]. We disclosed that H_2 alleviated CIH-induced oxidative damage by upregulating the SOD activity, GSH-Px activity, GSH/GSSG ratio with the reduction in MDA content.

Accumulating evidence suggests that endoplasmic reticulum stress (ER stress) is activated in response to acute and chronic kidney [30]. ER stress arises after the accumulation of misfolded proteins in the ER. H_2 prevented the CIH-induced increase in oxidative stress, perhaps thereby protecting kidney against endoplasmic reticulum stress. ER stress arises after the process of protein folding is disturbed and misfolded proteins accumulate in the ER. As protein folding is highly redox-dependent, the redox status can affect protein folding mechanism and enhance further ER stress [31]. Xu et al. demonstrated that CIH in OSA patients can increase ROS generation, thus reduces the production of functional proteins and even leads to apoptosis [32]. Our results revealed that the levels of ER stress marker proteins GRP78, CHOP, and caspase12 were

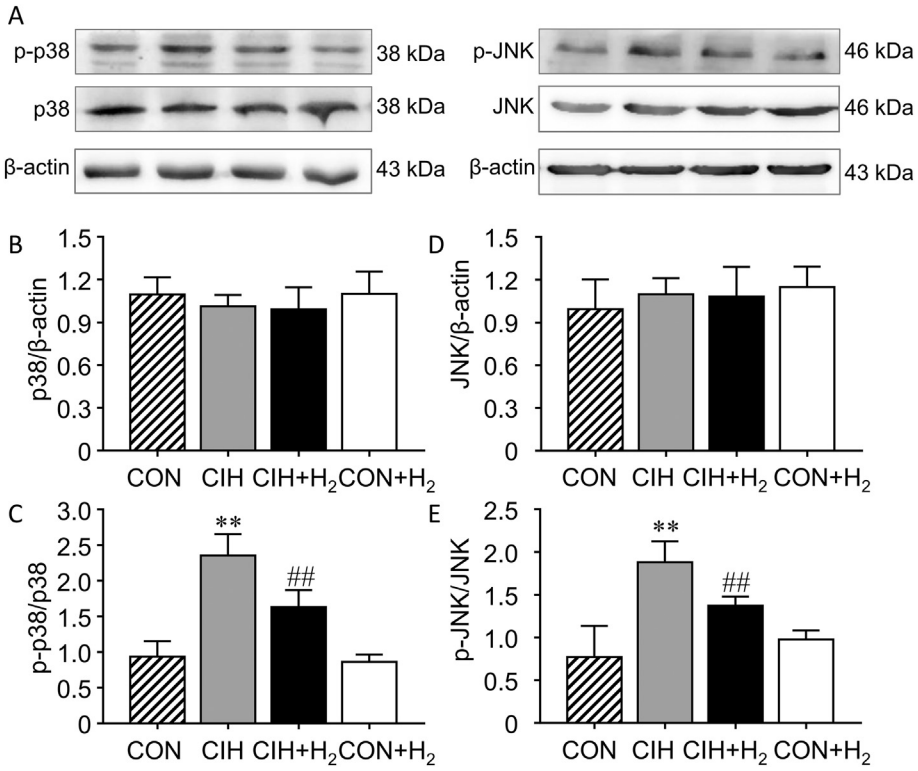

Fig. 7. p38 and JNK signaling were analyzed by Western Blot. A. Representative Western blot photographs. B. Renal p38 expression. C. Renal p38/p-p38 ratio. D. Renal JNK expression. E. Renal p-JNK/JNK ratio. $n = 6$. $^{**}p < 0.01$ vs. CON group; $^{\#\#}p < 0.01$ vs. CIH group.

dramatically increased when exposing to CIH. We also found H_2 can inhibit apoptosis, perhaps by lowering caspase12 and p38 activities. Similar anti-ER stress effects were reported for H_2 in a myocardial ischemic/reperfusion model using Wistar albino rats. Gao et al. described a protective effect of hydrogen against myocardial ischemic/reperfusion injury through inhibiting endoplasmic reticulum stress compared with ischemic postconditioning treatment [33].

Autophagy is a self-degradative process that delivers cytoplasmic components into lysosomes for degradation and reuse. On the one hand autophagy can eliminate damaged organelles and maintain the balance of protein synthesis [34], but on the other it has also been reported that uncontrolled autophagy would lead to irreversible demise of cells [35]. In healthy, selective autophagy is effective to maintain cellular homeostasis and regulating viability of renal cells [36]. However, it remains a matter of controversy, whether CIH promotes or prevents autophagy depends on experimental conditions and on the cellular context. Zhang et al. reported the upregulation of renal apoptosis and autophagy in the CIH exposed rats [3]. Their 12-week CIH exposure time is over twice as much as ours. In the present study, we demonstrated that CIH had no obvious effects on LC3-II/LC3-I ratio and beclin-1 level in comparison to controls. The p62 expression was increased in CIH-treated renal tissues when compared to control tissues. These data imply that autophagy flux was inhibited by CIH, which is consistent with a previous study that autophagy plays a renoprotective role in both ischemic and nephrotoxic acute kidney injury [37]. Jiang and colleagues reported that down-regulation of autophagy by chloroquine or conditional Atg7 ablation from proximal tubules is associated with severe acute kidney injury, whereas activation of autophagy by rapamycin protects against this injury [37]. In line with our results that H_2 in CIH-treated rats largely serve to protect kidney via activating autophagy, most of studies highlighting autophagy as a novel and very promising target for renal diseases therapy [38,39]. Beyond ER stress, mTOR may be involved in autophagy regulation in this study. mTOR can be divided into two functionally and biochemically distinct complexes, mTORC1 and mTORC2. Studies have shown that mTORC1 inhibition increases autophagy, whereas mTORC2 indirectly suppresses autophagy through the activation of mTORC1 [40]. We proved that CIH exposure ameliorates autophagy level in renal tissue. As activated JNK by ROS or ER stress induced by CIH can increase autophagy by triggering LC3 and Beclin-1 induced autophagy [41]. Thus, we speculated that activated mTOR may be responsible for lowered autophagy with CIH exposure. Blocked autophagy was reversed by H_2, suggested that the gas may play an important role in regulating mTOR signaling. Further studies are needed to assess the role for H_2 in mTOR signaling regulation is direct or not.

MAP kinases have been implicated in the regulation of oxidative stress, ER stress and autophagy [42,43]. Thus, we investigated whether the p38 and JNK pathways are involved in the protect effects of H_2 and found that H_2 significantly attenuated CIH-induced phosphorylation of p38 and JNK, suggesting that p38 and JNK were involved in the protective effects of H_2 against CIH-induced ER stress accompanied with the inhibited autophagy. Potential mechanism for the regulation of autophagy via p38 MAPK signaling might ascribe to declined expression of Unc-51-like kinase 1 [44] or increased binding of p62 to activated p38 MAPK [45]. CIH-induced ROS formation increased ER stress accompanied with the inhibited autophagy in kidney and this led to over activation of JNK signaling and subsequent renal injury. The activated JNK can promote Bax translocation from the cytoplasm into mitochondria, leads to cell apoptosis [46]. Moreover, there is a reciprocal relationship between ROS and activated p38 and JNK as ROS can activate p38 and JNK and activation of p38 or JNK is responsible for elevating ROS levels [47]. Our results indicated H_2 inhibited the activation of p38 and JNK, suggesting H_2 played an active part in resisting renal injury via MAPK.

Taken together, our work demonstrated that H_2 efficiently attenuated CIH-induced renal injury via inhibiting oxidative stress and apoptosis in rats. And the protective effects were mediated through inhibiting ER stress and increase autophagy, which was at least

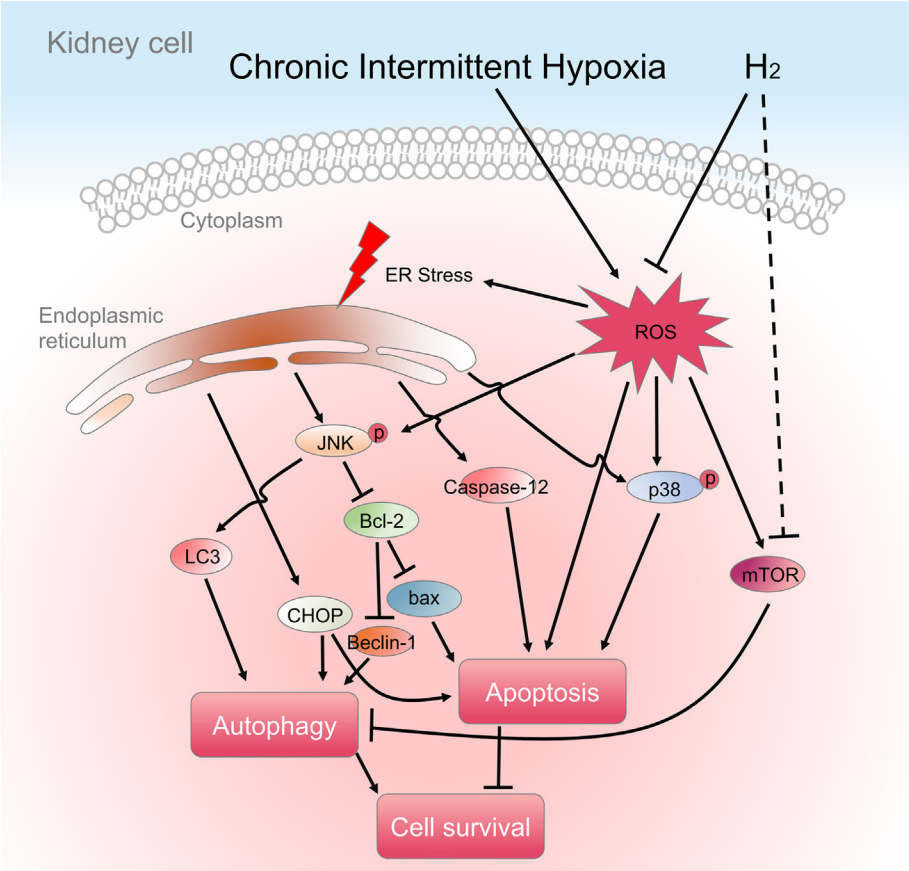

Fig. 8. Proposed signaling pathways involved in the protective effect of H_2 in CIH-induced renal injury. The renal injury, apoptosis, oxidative stress and endoplasmic reticulum stress induced by CIH were strikingly attenuated by H_2. Mechanistically, hydrogen gas attenuated CIH-induced renal injury through inhibiting ER stress and increase autophagy, which was at least partially dependent on the inactivation of p38 and JNK. What's more, autophagy may be a potential therapeutic target via mTOR to alleviate ER stress associated renal injury.

partially dependent on the inactivation of mTOR, p38 and JNK (Fig. 8). Our current knowledge will provide a theoretical foundation for further studying the effects of H_2 on renal injury.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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