

Original Article

Sevoflurane pretreatment enhance HIF-2 α expression in mice after renal ischemia/reperfusion injury

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Abstract: Ischemia/reperfusion (I/R) injury often occurs, which is one of the major causes of acute kidney injury, thus increasing in-hospital mortality. HIF-2 α has a protective role against ischemia of the kidney. Renal ischemia/reperfusion under sevoflurane anesthesia resulted in drastic improvements in renal function. We hypothesized that underlying mechanism responsible for renal protection from sevoflurane pretreatment involves the upregulation of HIF-2 α . Sevoflurane pretreatment were performed on WT and HIF-2 α knockout mice before renal ischemia/reperfusion. Levels of blood urea nitrogen (BUN) and serum creatinine (Cr) were determined with a standard clinical automatic analyzer. The left kidneys were taken for morphological examination. Expression of HIF-2 α in kidney tissue was examined by western blotting. In WT mice, group I/R injury had significantly higher BUN and Cr levels than group control, whereas group I/R + Sev had significantly lower BUN and Cr levels than group I/R injury. Renal HIF-2 α expression levels were significantly higher in WT mice of group I/R + Sev than group control and group I/R. In HIF-2 α ^{-/-} mice, group I/R + Sev showed much higher BUN and Cr levels and severer histological damage than group I/R and group control. Renal HIF-2 α expression levels were significantly higher in WT mice of group I/R + Sev than group control and group I/R. Our findings suggested that HIF-2 α might contribute to the beneficial effect of sevoflurane in renal ischemia/reperfusion injury.

Keywords: Sevoflurane, renal ischemia/reperfusion injury, HIF-2 α

Introduction

In renal transplantation, ischemia/reperfusion (I/R) injury often occurs, which is one of the major causes of acute kidney injury, thus increasing in-hospital mortality [1, 2]. The kidney is particularly susceptible to hypoxic injury because of its high specialized vascular anatomy and the relatively low tissue pO₂ levels [3, 4]. Cellular responses to oxygen play an essential role in the pathophysiology of acute ischemic injuries [5]. Central mediator to cellular responses is the hypoxia-inducible factor (HIF), which is composed of α and β subunits. HIF-1 α is induced mainly in tubular and glomerular epithelia cells in response to hypoxia, whereas HIF-2 α is localized in glomerular cells, peritubular endothelia cells, and fibroblasts [6, 7]. Some studies indicated that endothelial HIF-2 α had a protective role against ischemia of the kidney and represented a potential therapeutic target for renoprotection and prevention of fibrosis following acute ischemic injury [5, 8, 9].

Sevoflurane has anti-inflammation properties. Several studies showed that renal ischemia/reperfusion (I/R) under sevoflurane anesthesia, which was given both during and after renal ischemia, resulted in drastic improvements in renal function [10-12]. Recently, Zhou et al. demonstrated that sevoflurane pretreatment could also induce an ischemic tolerance against ischemic injury to provide a renal protection [12]. Sun et al. reported that preconditioning of mesenchymal stem cells by sevoflurane could improve their therapeutic potential to produce beneficial effects on ischemia-reperfusion injury by upregulating HIF-2 α [13]. Thus, we hypothesized that underlying mechanism responsible for renal protection from sevoflurane pretreatment involved the upregulation of HIF-2 α .

In the present study, we subjected HIF-2 α knockout mice to sevoflurane pretreatment followed by renal ischemia/reperfusion (I/R) injury to assess the role of HIF-2 α in protection of renal function.

Materials and methods

Ethic statements and mice

All the procedures were conducted with the approval of the Animal Use and Care Committee of Shanghai Jiaotong University School of Medicine in accordance with the NIH Guide for the Care and Use of the Laboratory Animals.

The HIF-2 α ^{-/-} mice and wild-type (WT) mice were provided from another team in our hospital [8]. A total of 27 WT mice were included in our study. They were randomly assigned to one of three groups (N=9 per group): group control: sham operation; group I/R: renal I/R injury; group I/R + Sev: sevoflurane pretreatment + renal I/R injury. A total of 27 HIF-2 α ^{-/-} mice were included in our study as well. HIF-2 α ^{-/-} mice were also assigned to one of three groups: group control; group I/R; group I/R + Sev.

Sevoflurane pretreatment

Before undergoing renal I/R injury, mice in group I/R + Sev were put into a closed box. An animal anesthesia machine (RM-AS-I; Hairui Man Information Technology Co., Ltd. Shanghai, China) was connected to the closed box on one side. And an anesthetic gas monitor (Datex-Ultima, Helsinki, Finland) was connected to the box on the other side. Sodalime was placed in the box. Temperature was maintained at \pm 36°C. Sevoflurane was administered at rate of 1L/min. Mice in group I/R + Sev were put into the closed box for 60 min, when sevoflurane concentration reached 2%. Then the mice were removed from the box and maintained in the ambient environment for 15 min. 24 h later, renal I/R injury was conducted.

Renal ischemia/reperfusion model (I/R)

Mice in group I/R and in group I/R + Sev were placed on a temperature-controlled pad to maintain rectal temperature 36°C and were anesthetized by sodium pentobarbital (60 mg/kg body weight). A warm renal I/R model was used as described [12, 14]. A midline abdominal incision was made after sterilization. The pedicle of the left kidney was exposed and clamped. The right kidney was removed. After inspection of ischemia (kidney became gray in color), mice were covered with sterile surgical dressing to maintain the body temperature. After 25 min, reperfusion was performed by

releasing the clamps. Successful reperfusion was defined by the kidney becoming red in color. Then, incisions were closed. Mice were given food and water. Mice in group control had same procedures but without vascular occlusion. All of the mice were killed 24 h after reperfusion by exsanguination followed by blood and renal samples for analysis.

Biochemical analysis and renal histology

Blood samples, including blood urea nitrogen (BUN) and serum creatinine (Cr), were collected from the mice 24 h after reperfusion and processed within 2 h after collection. Biochemical analyses were measured with a standard clinical automatic analyzer (Siemens Dade behring dimension xpand).

The left kidney was harvested. Kidney tissues were fixed in 10% neutral formalin solution for 24 h, dehydrated, embedded in paraffin and sectioned at 5 μ m. Sections were stained with Periodic Acid-Schiff (PAS). A nephro-pathologist evaluated the stained sections for tubular cell necrosis, tubular dilation, intratubular cell detachment and cast formation (original magnification \times 200). At least three fields were randomly selected and analyzed.

Western blotting

At 24 h after reperfusion, tissues from kidneys were homogenized with Cell & Tissue Protein Extraction Reagent (1% Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail, and Phenyl Methylsulfonyl Fluoride Proteomics Grade were added to the protein extraction reagent before use; Kangchen Bio-Tech, Shanghai, China). Then, the supernatant was obtained by centrifugation at 11,000 \times g for 10 min at 4°C. Protein concentration was determined with a BCA Protein Assay Kit (Tiangen, Beijing, China). A total 30 μ g of protein from each sample was fractionated by 10% SDS-PAGE and transferred onto a PVDF membrane. The membranes were incubated in TBS-Tween solution containing 5% nonfat dry milk for 4 h at 25°C. The blots were then incubated for 2 h at 25°C with primary antibodies for HIF-2 α (1:1000, Abcam, USA), followed by incubation with HRP-conjugated anti-rabbit secondary antibody (Sigma-Aldrich) for 1 h at room temperature. Protein signals were detected by using the Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software from Bio-

Sevoflurane pretreatment on renal HIF-2 α expression

Table 1. Physiological characteristics and BUN and Cr levels

Variable	WT mice			HIF-2 α ^{-/-} mice			P
	control	I/R	I/R + Sev	control	I/R	I/R + Sev	
Weight (g)	219 \pm 5.8	222 \pm 6.1	220 \pm 5.9	221 \pm 6.4	223 \pm 5.6	224 \pm 6.3	0.235
BUN (mmol/L)	8.3 \pm 0.5	58.4 \pm 7.2*	28.6 \pm 10.8	5.9 \pm 0.5	66.8 \pm 5.3*	59.6 \pm 5.9* [#]	<0.001
Cr (μ mol/L)	8.3 \pm 0.9	130.2 \pm 32.2*	63.2 \pm 30.4	7.1 \pm 2.8	151.7 \pm 11.1*	132.7 \pm 22.4* [#]	<0.001

Group control: sham operation; group I/R: renal I/R injury; group I/R + Sev: sevoflurane pretreatment + renal I/R injury. N = 9 per group. BUN: blood urea nitrogen; Cr: serum creatinine. $P < 0.05$ indicates a significant difference among six groups.

*Significant difference compared with group control. #Significant difference in group I/R + Sev of HIF-2 α ^{-/-} mice compared with WT mice.

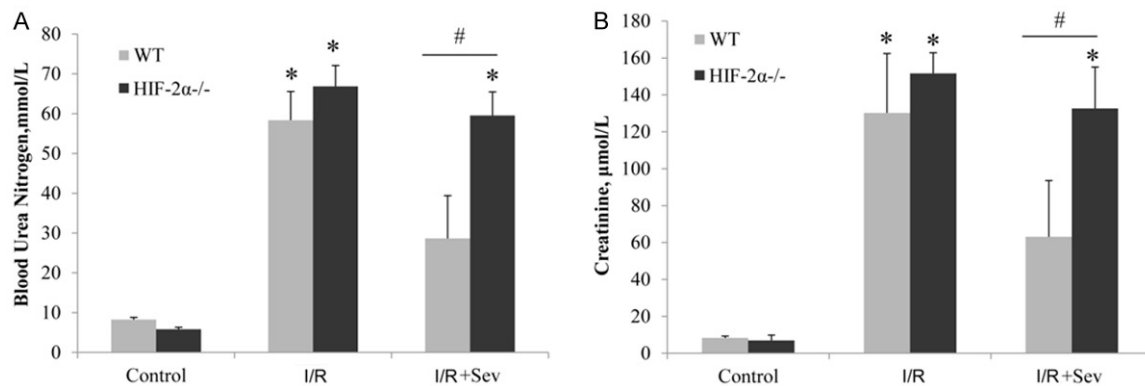


Figure 1. Blood urea nitrogen (BUN) and creatinine (Cr) concentrations. group control: sham operation; group I/R: renal I/R injury; group I/R + Sev: sevoflurane pretreatment + I/R injury. $P < 0.05$ indicates a significant difference among six groups. *Significant difference compared with group control. #Significant difference in HIF-2 α ^{-/-} mice of group I/R + Sev compared with WT mice.

Rad. Experiments were repeated at least three times and the relative expression of the target protein was normalized to the level of β -actin in the same sample.

Statistical analysis

All data were presented as means \pm SD. Statistical analysis was performed with analysis of variance (ANOVA). Analyses were performed using Excel 2000 (Microsoft, Redmond, WA, USA) and SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined at $P < 0.05$ (two-sided).

Results

Physiological characteristics of the mice groups

There was no significant difference in body weight among the groups (Table 1). There was no significant difference in group control between WT mice and HIF-2 α ^{-/-} mice in respect of blood urea nitrogen (BUN) and serum creati-

nine (Cr) concentrations before renal I/R injury (Table 1).

Effect of sevoflurane pretreatment on BUN and Cr levels after I/R injury

Renal I/R injury had a significant effect on blood urea nitrogen (BUN) and serum creatinine (Cr) levels. Whether in WT mice or in HIF-2 α ^{-/-} mice, BUN and Cr levels were significantly higher in group I/R than those in group control ($P < 0.001$) (Table 1).

In WT mice, BUN and Cr concentrations decreased more significantly in group I/R + Sev than in group I/R, while there were no obvious changes in group I/R + Sev of HIF-2 α ^{-/-} mice (Figure 1). BUN and Cr levels were significantly higher in group I/R + Sev of HIF-2 α ^{-/-} mice compared with WT mice (Figure 1).

Effect of sevoflurane pretreatment on histopathology after I/R injury

After renal I/R injury, the similar histopathological acute kidney injury was found in WT mice

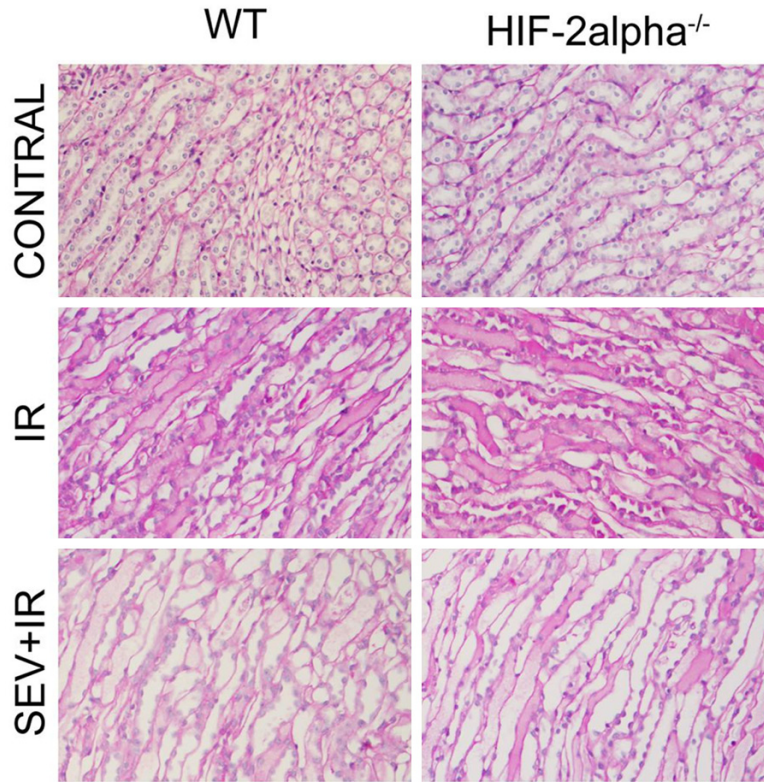


Figure 2. Representative renal PAS-stained sections from WT mice and HIF-2 α ^{-/-} mice in group I/R and group I/R + Sev (original magnification $\times 200$). In group I/R, the similar histopathological acute kidney injury was found: tubular cell necrosis, tubular dilation, neutrophil recruitment and intratubular cell detachment. Group I/R + Sev in WT mice manifested much mitigated tubular injury, compared with group I/R. Group I/R + Sev in HIF-2 α ^{-/-} mice had much severer renal damage compared with group I/R.

and HIF-2 α ^{-/-} mice: tubular cell necrosis, tubular dilation, neutrophil recruitment and intratubular cell detachment (**Figure 2**). WT mice in group I/R + Sev manifested much mitigated tubular injury, compared with group I/R injury renal sections. In contrast, HIF-2 α ^{-/-} mice had much severer renal damage in group I/R + Sev (**Figure 2**).

Sevoflurane pretreatment caused accumulation of HIF-2 α

Figure 3 shows the renal HIF-2 α expression in WT mice. Compared with group control, the renal HIF-2 α expression was significantly increased in group I/R. And there was a significantly further increase of the renal HIF-2 α expression in group I/R + Sev (**Figure 3**).

Discussion

In the present study, we indicated that sevoflurane pretreatment provided some protection

against renal I/R injury in mice, which was mediated by increasing renal expression of HIF-2 α , at least in part.

Renal ischemia and reperfusion (I/R) injury is one of the main causes of acute renal failure in renal transplantation, which is associated with a high mortality rate in humans. Attenuating renal I/R injury has important clinical significance. Many studies demonstrated that sevoflurane preconditioning or post-conditioning could protect against reperfusion injury in various organs such as heart [15], pulmonary [16] or kidneys [10, 12]. It was reported that rats in sevoflurane anesthesia group had significantly lower serum creatinine 24 h after reperfusion, significantly lower plasma TNF- α and IL-6 concentrations 2 h after reperfusion [11]. Recently, Zhou et al. [12] demonstrated that sevoflurane pretreatment could induce an ischemic tolerance against ischemic injury to provide a

renal protection in diabetic rats by altering none-receptor tyrosine kinases steroid receptor coactivator (Src) and focal adhesion kinase (FAK) expression (Src and FAK are mediators of cellular apoptosis and necrosis). Sun et al. [13] found that sevoflurane pretreatment could produce effects on survival and migration of induce mesenchymal stem cells against ischemia-reperfusion injury, by the elevation of HIF-2 α expression. These protective mechanism mainly involved of inflammatory and oxidative damage, but the specific cells and the underlying molecular mechanisms were not clear.

According to the above studies associated with oxidative damage, we focused on examining changes in the expression of HIFs. HIFs are primary regulators of oxygen homeostasis. Once activated, they can stimulate numerous genes important for energy metabolism, vasomotor regulation, angiogenic growth and erythropoie-

Sevoflurane pretreatment on renal HIF-2 α expression

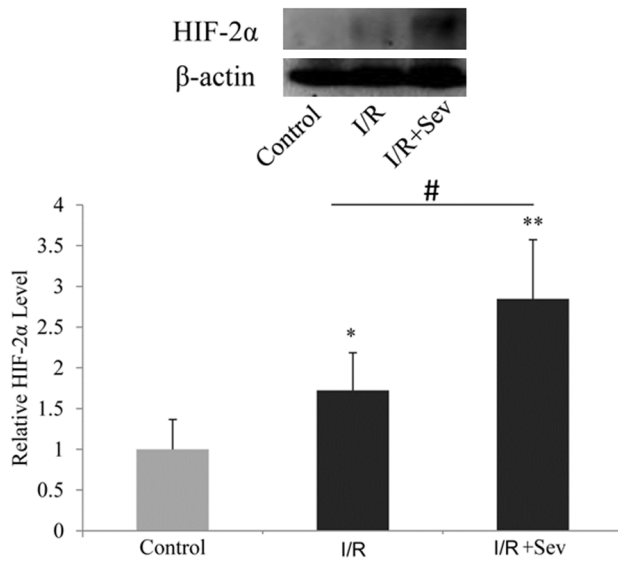


Figure 3. Expression of HIF-2 α protein in WT mice kidney. * $P < 0.05$ versus group control, ** $P < 0.01$ versus control group, # $P < 0.05$ versus group I/R. Compared with control group, the renal HIF-2 α expression was significantly increased in group I/R and in group I/R + Sev. There was a significantly further increase of the renal HIF-2 α expression in group I/R + Sev than in group I/R.

sis [5, 6, 8]. Some studies used *in vivo* gene transfer of dominant negative HIF to demonstrate involvement of HIFs in ischemia and reperfusion (I/R) injury of the kidney [17]. The two main subunits, HIF-1 α and HIF-2 α , are both involved. HIF-1 α , known as a key regulator in a wide range of cellular adaptation responses to hypoxia, is induced mainly in tubular and glomerular epithelia cells in response to hypoxia, whereas HIF-2 α is localized in glomerular cells, peritubular endothelia cells, and fibroblasts [6, 7]. Some studies tried to explore the role of the two hypoxia-inducible factors, HIF-1 α and HIF-2 α , in the renoprotective effects of renal I/R injury. Kojima et al. first clarified direct evidence of a protective role of HIF-2 α against an exogenous insult *in vivo*. It was testified that reduction in HIF-2 α made the kidney more susceptible to renal I/R injury [5]. Then, other studies also indicated that endothelial HIF-2 α has a protective role against ischemia of the kidney and represents a potential therapeutic target for renoprotection and prevention of fibrosis following acute ischemic injury [8, 9]. Based on the above results, we created HIF-2 α knockout mice and subject them to sevoflurane pretreatment following by renal I/R injury to explore the role of HIF-2 α in the renoprotective effects of sevoflurane pretreatment.

In our study, we found that one day after renal I/R injury, serum Cr and BUN levels were markedly increased in group I/R compared with group control (sham-operated) both in WT mice and in HIF-2 α ^{-/-} mice. This suggested that renal I/R injury may severely impair renal function. In WT mice, sevoflurane pretreatment significantly reduced serum CR and BUN levels after renal I/R injury. The histological manifestations reinforced the results of BUN and Cr levels: sevoflurane pretreatment following renal I/R injury could prevent the tubular loss and preserved the integrity of neurons. It suggested that sevoflurane pretreatment had some degree protective effect on renal I/R injury in WT mice. We performed experiment to check the changes of HIF-2 α and we found the obviously elevated HIF-2 α expression under the circumstance of sevoflurane pretreatment. No differences could be observed between group I/R and group I/R + Sev in HIF-2 α knockout mice in serum Cr and BUN levels. In histological observations, group I/R + Sev in HIF-2 α knockout mice even had severer renal damage. Therefore, up-regulation of HIF-2 α might be responsible for the protective mechanism of sevofluranepretreatment.

In conclusion, the present study illustrated that sevoflurane pretreatment could improve renal function after renal ischemia and reperfusion injury. This beneficial effect was mediated in part by upregulation of HIF-2 α .

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Disclosure of conflict of interest

None.

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