Effect of Hyperbaric Oxygen on Tissue Damage and Expression of Adhesion Molecules and C3 in a Rat Model of Renal Ischemia-Reperfusion Injury After Kidney Transplantation

**Background:**

The aim of this study was to investigate the protective effect and mechanism of hyperbaric oxygen (HBO) in a rat model of renal ischemia-reperfusion injury following kidney transplantation.

**Material/Methods:**

Sprague Dawley rats were randomly divided into 3 groups (n=18): sham group, kidney transplantation group, and HBO treatment group. Six rats in each group were sacrificed at 1, 3, and 5 hours after reperfusion, and serum and renal tissue were then collected. The serum creatinine levels and histopathological changes of the renal tissue were detected. ICAM-1, VCAM-1, and C3 expression levels were also detected by immunohistochemical staining or real-time polymerase chain reaction.

**Results:**

Renal function was damaged in the kidney transplantation group and the HBO treatment group compared with sham group (P<0.05). Renal histopathological changes, including tubular cell swelling, tubular dilatation, and hyaline casts, were remarkably reduced in the HBO treatment group compared to the kidney transplantation group. In the immunohistochemical examination, the expression levels of ICAM-1, VCAM-1, and C3 expression levels were also detected by immunohistochemical staining or real-time polymerase chain reaction.

**Conclusions:**

HBO treatment exerted a protective effect on renal function through inhibition of adhesion molecule overexpression and complement system activation in a rat model of renal ischemia-reperfusion injury after kidney transplantation.

**MeSH Keywords:**

Adhesion Molecule • Complement • Hyperbaric Oxygen • Ischemia Reperfusion Injury • Kidney Transplantation • Rat

**Corresponding Authors:**

Zhihui Xie, e-mail: xiezhihui71@126.com; Guobiao Liang, e-mail: lgb1111@126.com

This study was funded by the National Natural Science Foundation of China, Grant No. 81460135; The Science and Technology Fund Project of Guizhou Province, Grant No. SY (2012) 3149; The Major Research Project of The Innovation Group of The Department of Education of Guizhou Province, Grant No. KY (2017) 045
Background

Ischemia-reperfusion injury (IRI) is a common physiopathological process in the clinic. It is also responsible for acute kidney injury that can result in acute renal failure [1]. In kidney transplantation, renal IRI is an important factor causing chronic allograft nephropathy and loss of kidney transplant function, which has a great influence on the long-term survival of the transplanted kidney. During the IRI process, the cells in the renal tissue produce inflammatory factors (such as interleukin [IL]-6 and tumor necrosis factor [TNF]) and reactive oxygen species, leading to increased expression of intercellular adhesion molecules-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The combined effect of inflammatory factors and adhesion molecules activates neutrophilic granulocytes and mononuclear macrophages, which aggregate at the injured site, leading to an inflammatory response and aggravation of the injury. Also, IRI can induce the activation of the complement system to promote an inflammatory response, leading to apoptosis of renal tubular epithelial cells and further aggravating renal IRI. The inhibition of the activation of the complement system has been found to alleviate the immunologic injury mediated by the complement system activation in renal IRI [2,3].

Hyperbaric oxygenation (HBO) treatment has been reported to have protective effect on renal IRI [4]. HBO treatment reduces the over-oxidation of oxygen radicals on the lipid membrane before ischemia and exerts a protective effect on the kidney in renal IRI. HBO treatment also protects the kidney after ischemia in renal IRI. HBO improves the functions of the kidney and increases the concentrations of Na+ and K+ in the renal tubules [4,5]. However, whether HBO protects renal function by regulating the expression of adhesion molecules and activation of the complement system in renal IRI is still unclear. Therefore, the present study aimed to evaluate the protective effect of HBO treatment and the molecular mechanisms underlying the therapeutic effect of HBO in a rat model of IRI after kidney transplantation, with a focus on the changes in the renal expression of adhesion molecules ICAM-1, VCAM-1, and C3.

Material and Methods

Reagents

Rabbit anti-rat ICAM-1 primary antibody (1: 100, BA2189), rabbit anti-rat VCAM-1 primary antibody (1: 100, BA3840), rabbit anti-rat C3 primary antibody (1: 50, BA2298-1), DAB (3,3N-diaminobenzidine tetrahydrochloride) developing kit (AR1022), and ready-to-use normal goat serum (AR0009) were all purchased from Wuhan Boster Biological Technology, Ltd. (Wuhan, China). Immunohistochemistry kit (GK500705) was purchased from Gene Technology Co. (Shanghai, China). PrimeScript Reverse Transcription Reagent Kit (RR037A) and SYBR Premix Ex Taq II (RR820A) were obtained from Takara Bio Inc. (Nojihigashi, Japan), and rat ICAM-1, C3, and β-actin primers were obtained from Generay Biotech Co. (Shanghai, China).

Animals

Sprague Dawley (SD) rats, aged 6–8 weeks, weighing 200–220 g, and specific-pathogen-free, were provided by the Experimental Animal Center, Daping Hospital, Third Military Medical University (license number: SCXK-(Ye)2012-0006). The animal study was approved by the Ethics Committee of The Affiliated Hospital of Zunyi Medical University (approval ZMUER2014-2-030).

Kidney transplantation

SD rats of 6 to 8 weeks were used for renal ischemia reperfusion surgery as described previously [6]. SD rats were fed separately and acclimated for 2 weeks. The rats were randomly divided into 3 groups: the sham group, the kidney transplantation group and the HBO treatment group. Each group was further divided into 3 subgroups (n=6): reperfusion for 1 hour, 3 hours, or 5 hours. For the sham group, the right kidneys were excised without kidney transplantation. For the kidney transplantation group, the right kidneys were excised, followed by kidney transplantation. For the HBO treatment group, the right kidneys were excised, followed by kidney transplantation and HBO treatment.

The rats were anesthetized by an intraperitoneal injection of 2% sodium pentobarbital (40 mg/kg) and placed in a supine position on the operating table. An incise was made along the middle abdomen, and the abdominal wall was cut open after iodophor sterilization.

Donor kidney operation

The donor kidney operation included the following steps. 1) The left kidney, artery and vein, aorta abdominals, and post cava were exposed, and then the left ureter was disassociated from the bladder wall. 2) For the left kidney perfusion, the abdominal aortic puncture was performed to connect a F6 trocar. The aorta abdominals in the right and left renal arteries was occluded using a vascular clamp, and an incision was made in the post cava. Then, 5 mL of renal protection fluid in a syringe was slowly pushed into the left kidney to perform perfusion until the kidney turned pale or took a granophyric shape. 3) For incision of the left kidney and ureter, the left renal artery and vein along with aorta abdominals (1.0 cm) and the whole post cava were excised, and then the whole length of the ureter and bladder flap (diameter 0.5 cm) was excised. All the excised kidneys were kept at 4°C with heparin sodium chloride as anticoagulant.

Animals

Sprague Dawley (SD) rats, aged 6–8 weeks, weighing 200–220 g, and specific-pathogen-free, were provided by the Experimental Animal Center, Daping Hospital, Third Military Medical University (license number: SCXK-(Ye)2012-0006). The animal study was approved by the Ethics Committee of The Affiliated Hospital of Zunyi Medical University (approval ZMUER2014-2-030).

Kidney transplantation

SD rats of 6 to 8 weeks were used for renal ischemia reperfusion surgery as described previously [6]. SD rats were fed separately and acclimated for 2 weeks. The rats were randomly divided into 3 groups: the sham group, the kidney transplantation group and the HBO treatment group. Each group was further divided into 3 subgroups (n=6): reperfusion for 1 hour, 3 hours, or 5 hours. For the sham group, the right kidneys were excised without kidney transplantation. For the kidney transplantation group, the right kidneys were excised, followed by kidney transplantation. For the HBO treatment group, the right kidneys were excised, followed by kidney transplantation and HBO treatment.

The rats were anesthetized by an intraperitoneal injection of 2% sodium pentobarbital (40 mg/kg) and placed in a supine position on the operating table. An incise was made along the middle abdomen, and the abdominal wall was cut open after iodophor sterilization.

Donor kidney operation

The donor kidney operation included the following steps. 1) The left kidney, artery and vein, aorta abdominals, and post cava were exposed, and then the left ureter was disassociated from the bladder wall. 2) For the left kidney perfusion, the abdominal aortic puncture was performed to connect a F6 trocar. The aorta abdominals in the right and left renal arteries was occluded using a vascular clamp, and an incision was made in the post cava. Then, 5 mL of renal protection fluid in a syringe was slowly pushed into the left kidney to perform perfusion until the kidney turned pale or took a granophyric shape. 3) For incision of the left kidney and ureter, the left renal artery and vein along with aorta abdominals (1.0 cm) and the whole post cava were excised, and then the whole length of the ureter and bladder flap (diameter 0.5 cm) was excised. All the excised kidneys were kept at 4°C with heparin sodium chloride as anticoagulant.
Recipient kidney transplantation
The recipient kidney transplantation included the following steps. 1) The aorta abdominalis and post cava were exposed and cut after being locally occluded. The vessel lumen was washed with heparin saline. 2) The end-to-lateral anastomosis was performed in the left renal artery and vein with the receptor's aorta and post cava using a 10/0 nylon suture, and in the bladder flap of ureter end with the receptor's top of bladder using a 7/0 nylon suture. The blood flow was released after surgery. 3) After the transplanted kidney turned red in color, the bilateral kidneys of the receptor were excised, and 20 U penicillin was administered to prevent infection. The excision in the abdomen was sutured.

HBO treatment
For the HBO treatment, the rats undergoing kidney transplantation were placed in a hyperbaric oxygen chamber 0, 2, and 4 hours after reperfusion. The chamber was connected to pure oxygen with increased pressure to 0.02 MPa (1.2 ATA) for 5 minutes. The pressure in the chamber was further increased to 0.1 MPa (2.0 ATA) for 5 minutes, followed by steady pressure and oxygen input for 40 minutes and decompression for 10 minutes. The oxygen concentration in the chamber was maintained more than 95%. (Note: The HBO treatment method was the same as described by Bao et al. [6]).

Sample collection
All the rats were anesthetized via intraperitoneal injection 1, 3, and 5 hours after reperfusion, and 1.5–2.0 mL of blood was collected from the post cava. The serum samples were harvested after centrifugation and stored at −20°C for later measurement of serum creatinine levels. The left kidneys from different groups were removed and divided into 2 parts. One part was frozen in liquid nitrogen and then stored at −80°C until the homogenate was completely lysed, placed at room temperature. The tissue sections were completely immersed in citric acid working solution (pH 6.0) and microwaved for 15 minutes, then cooled down to room temperature. The sections were blocked with normal goat serum for 10 minutes, and then the serum was discarded. The sections were incubated with diluted primary antibodies (ICAM-1 and VCAM-1, 1: 100; C3, 1: 50) at 4°C overnight, with PBS to replace primary antibodies as negative controls. The sections were further incubated at 37°C for 45 minutes. After washing with PBS, the sections were incubated with a secondary antibody at 37°C for 30 minutes, developed using DAB solution, followed by hematoxylin counter stain for 5 minutes.

Histologic evaluation
The formalin-fixed and paraffin-embedded kidney sections (5-μm thick) were stained with H&E, and examined under a microscope for histopathological changes.

Immunohistochemistry analyses
Paraffin-embedded sections with 5-μm thickness were dewaxed in xylene I and xylene II for 10 minutes each, rehydrated using gradient alcohol for 5 minutes, and washed with phosphate-buffered saline (PBS) and 3% H₂O₂ for 10 minutes at room temperature. The tissue sections were completely immersed in citric acid working solution (pH 6.0) and microwaved for 15 minutes, then cooled down to room temperature. The sections were blocked with normal goat serum for 10 minutes, and then the serum was discarded. The sections were incubated with a secondary antibody at 37°C for 30 minutes, developed using DAB solution, followed by hematoxylin counter stain for 5 minutes.

The kidney sections were examined with a Leica QWin image processing system (Leica, Germany). Six high power fields (400×) were randomly selected in each section, and the images were collected and analyzed using Image-Pro Plus 6.0 to measure integral optical density (IOD) for quantitative analysis.

RT-PCR for ICAM-1 and C3 mRNA expression

Total RNA extraction
TrisQuick total RNA extraction reagent and total RNA rapid extraction kit were used to obtain total RNA from renal tissue. The frozen renal tissue samples (50–100 mg) were placed into centrifugation tubes processed under high temperature and pressure. TrisQuick total RNA extraction reagent (1 mL) was added until the homogenate was completely lysed, placed at room temperature for 5 minutes, and centrifuged at 12 000 rpm for 10 minutes at 4°C. The supernatant was mixed with 0.2 mL of chloroform for 30 seconds, placed at room temperature for 2–3 minutes, and centrifuged at 12 000 rpm for 10 minutes at 4°C. The upper water phase containing total RNA was transferred into a new centrifugation tube (about 0.5–0.6 mL), and 0.5 mL of isopropanol was added per mL of TrisQuick total extraction reagent. After mixing well, the solution was placed at room temperature to precipitate for 10 minutes and centrifuged at 12 000 rpm for 10 minutes at 4°C. The supernatant was discarded, and 1 mL of 75% ethanol was added into TrisQuick total RNA extraction reagent per mL. The solution was
mixed well, and centrifuged at 12 000 rpm for 2 minutes at 4°C. The liquid was discarded, and the precipitate was dried inversely at room temperature for 5–10 minutes. An appropriate volume of DEPC (diethyl pyrocarbonate) was added to dissolve the RNA precipitate. The optical density value was read to obtain the quality and concentration of total RNA concentration. The total RNA was stored at –80°C.

cDNA synthesized by reverse transcription

RNA was reversely transcribed following the manufacturer’s protocol (reverse transcription apparatus, C1000, Bio-Rad, USA). The reagents and amounts of RT-PCR were as follows: 5× PrimeScript Buffer 2.0 µL, PrimeScript RT Enzyme Mix 0.5 µL, Oligo dT Primer (50 µM) 0.5 µL, Random 6mers (100 µM) 0.5 µL, Total RNA 500 ng/RNA concentration, Rnase Free dH2O up to 10.0 µL. The volume of the reverse transcription system was 20.0 µL, and the reaction condition was 37°C×15 minutes, 85°C×5 seconds and stored at 4°C.

PCR amplification

CFX96 Touch fluorescent quantitative PCR system (Bio-Rad) was used for PCR amplification. SYBR Premix Ex Taq II (10.0 µL) was added into the PCR reaction tube using cDNA solution as a template and β-actin as an internal reference. The volume of upstream and downstream primers was 0.8 µL, the primer concentration was 10 µmol/L, the cDNA template was 2.0 µL, and the total volume was 20.0 µL. ICAM-1 upstream primer sequence: 5’-GCTCTGCCCACCATACCTGTGA-3’; downstream primer sequence: 5’-ATAGGTTCTTGCCACCTG-3’; C3 upstream primer sequence: 5’-GTCAGAGATGAGATAAGAAGA-3’, downstream primer sequence: 5’-ACAAACACCATGAGGTCGAAAG-3’; β-actin upstream primer sequence: 5’-CTGAACCTAAGGGCCAACCG-3’, downstream primer sequence: 5’-GACCAGGCGCATACACGGGACAA-3’. Amplification conditions were as follows: pre-denaturation at 95°C for 30 seconds, PCR reaction at 95°C for 5 seconds, annealing at 60.3°C for 30 seconds (60.0°C for ICAM-1), 40 cycles.

Analysis of ICAM-1 and C3 mRNA expression

Ct values of β-actin, ICAM-1, and C3 gene amplification in each sample were detected in triplicates. The Ct value was used as a statistical parameter as follows: Ct average=(Ct1+Ct2+Ct3)/3 (triplicates), ΔΔCt=(average Ct of the target gene of sample to be tested–average Ct of the internal reference gene of sample to be tested)–(average Ct of the target gene of control sample–average Ct of the internal reference gene of control sample); relative expression level of target gene=2^(-ΔΔCt).

Statistical analysis

Measurement data were expressed as mean±standard deviation (SD). SPSS 19.0 software was used to analyze the data. One-way analysis of variance was used to analyze comparison among groups. Least significant difference method (equal variance) and Tamhane’s T2 (heterogeneity of variance) were used for comparison between groups. The Pearson correlation analysis was used to analyze the correlation among indices. A P value <0.05 indicated statistical significance.

Results

Renal function in each group (serum creatinine)

Serum creatinine values in the kidney transplantation group and the HBO treatment group significantly increased at 1, 3, and 5 hours after reperfusion compared with the sham group (P<0.05). No significant difference in the values was noted after 1 and 3 hours but the serum creatinine values significantly decreased after 5 hours in the HBO treatment group compared with the kidney transplantation group (P<0.05). The serum creatinine value significantly increased with time in the kidney transplantation group and the HBO treatment group (P<0.05). The serum creatinine value showed no statistically significant difference in the sham group for the 3 time periods (P>0.05) (Table 1). (Note: This study is the same subject as a previously published study by Bao et al. [6] with different research points in the same research direction. Therefore, the serum creatinine value of this paper shares the serum creatinine value of the Bao et al. study [6].)

Table 1. Serum creatinine value in each group (μmol/L, mean±SD, n=6).

| Group                        | 1-h reperfusion | 3-h reperfusion | 5-h reperfusion |
|------------------------------|-----------------|-----------------|-----------------|
| Sham group                   | 37.00±4.27      | 43.13±5.87      | 42.28±1.97      |
| Kidney transplant group      | 56.07±10.64*    | 115.40±10.26*   | 89.38±5.04***# |
| HBO treatment group          | 54.32±9.31*     | 83.07±11.46*#   | 86.70±8.20*     |

* P<0.05, compared with the sham group; ** P<0.05, compared with the kidney transplantation group; † P<0.05, compared with 1 hour of reperfusion; ** P<0.05, compared with 3 hours of reperfusion. SD, standard deviations.
Pathological changes in renal tissue (H&E staining)

The result of H&E staining indicated that the structure of renal tissue in the sham group at each time point was normal. Slight hyperplasia of mesangial cells, slight angiectasis and hyperemia of renal interstitium vessels, slight increase in the volume of the glomerulus, slight dilatation of the glomerulus cavity, and slight swelling of epithelial cells were observed at 1 hour after the kidney transplantation. The swelling of renal tubule epithelial cells and granular degeneration was noted after 3 hours. Lumen expansion of renal tubules, protein-like cast and red blood cell cast, obvious swelling of epithelial cells (showing granular degeneration), an increase in glomerulus volume, and some neutrophil infiltration was observed after 5 hours. No significant difference in the structure was observed after 1 and 5 hours in the HBO treatment group compared with the kidney transplantation group. The volume of the glomerulus increased after 5 hours, but the condition was not severer than the swelling of renal tubular epithelial cells and lumen expansion in the kidney transplantation group. The protein-like cast and red blood cell cast decreased, only showing limited neutrophil infiltration (Figure 1).

Figure 1. Pathological changes in the renal tissue. Histologic analysis of rat renal tissue samples taken from the sham, kidney transplantation, and HBO treatment groups at 1, 3, and 5 hours of reperfusion (n=6 for each time point for all 3 groups). Notice that the histopathological changes, including tubular epithelial degeneration, protein-like cast, and leukocyte infiltration were less severe in the HBO treatment group than in kidney transplantation group 5 hours after reperfusion (hematoxylin and eosin, 400×).
Expression of ICAM-1, VCAM-1, and C3 detected using immunohistochemistry

ICAM-1 is mainly expressed in the nuclei of renal tubular epithelial cells and interstitial cells. VCAM-1 and C3 are mainly expressed in the cytoplasm of renal tubular epithelial cells, whereas VCAM-1 is less expressed in the cytoplasm of glomerular cells. The relative quantitative results demonstrated that the expression levels of ICAM-1, VCAM-1, and C3 at 1, 3, and 5 hours after reperfusion was significantly higher in the kidney transplantation group than in the sham transplantation group at 5 hours of reperfusion (immunohistochemistry 400×).

The expression levels of each protein significantly increased with time in the kidney transplantation group and HBO treatment group (P<0.05). The expression level at each time point showed no statistically significant difference in the sham group (P>0.05) (Figures 2–5).

Changes in the expression of ICAM-1 and C3 mRNA in renal tissue

The changes in the expression of ICAM-1 and C3 mRNA in renal tissue samples are shown in Tables 2, 3, and Figure 6.
Correlation analysis between serum creatinine and immunohistochemical IOD

The analysis indicated that the\( R \) values of serum creatinine with ICAM-1, VCAM-1, and C3 expression were 0.689, 0.867, and 0.917, respectively (\( P<0.05 \)), showing a positive correlation (\( P=0.000 \)).

Discussion

Renal IRI is a common physiopathological process in patients with kidney transplants, and is associated with delayed graft function, graft rejection, chronic rejection, and chronic graft dysfunction. Renal IRI is also present in patients receiving partial nephrectomy, nephrolithotomy, extracorporeal shock wave lithotripsy, hemorrhagic shock, and cardiopulmonary bypass surgery, often associated with poor prognosis and high fatality rate [7]. Therefore, renal IRI has gained the immense attention of clinical physicians and scientists.
Renal IRI triggers inflammatory response, including increased expression of adhesion molecules and complement activation. Adhesion molecules are members of the immunoglobulin superfamily, a class of glycoproteins that mediate cell-extracellular matrix and cell-cell adhesion. ICAM-1 and VCAM-1 are important members, which play important roles in the inflammatory response, immune response, lymphocyte homing, and graft rejection. During the IRI process, the expression of ICAM-1 and VCAM-1 is enhanced under the action of tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, IL-18 and other inflammatory factors, and reactive oxygen species produced by the kidney. They work together with cytokines and chemokines to activate neutrophils and gather them at the site of injury to produce inflammatory responses. Neutrophils further produce cytokines and reactive oxygen species, aggravating tissue damage. The inhibition of adhesion molecule expression could significantly alleviate renal IRI [8,9].

Our current study showed that the expression of ICAM-1 and VCAM-1 in renal tissue significantly increased after kidney transplantation and continued to increase with reperfusion time. After HBO treatment, the expression levels significantly decreased, indicating HBO treatment can inhibit the expression of ICAM-1 and VCAM-1, reduce inflammatory response,
Table 2. Expression level of ICAM-1 mRNA in each group (2^(-DDCt), mean±SD, n=6).

| Group                  | 1-h reperfusion | 3-h reperfusion | 5-h reperfusion |
|------------------------|-----------------|-----------------|-----------------|
| Sham group             | 96.30±2.03      | 96.42±14.03     | 106.51±18.08    |
| Kidney transplant group| 122.46±17.45    | 153.33±5.46*    | 277.61±20.57*  |
| HBO treatment group    | 109.90±7.92     | 113.14±25.04*   | 166.41±7.98*   |

* P<0.05, compared with the sham group; # P<0.05, compared with 1 hour of reperfusion; ## P<0.05, compared with 3 hours of reperfusion. SD – standard deviation.

Table 3. Expression level of C3 mRNA in each group (2^(-DDCt), mean±SD, n=6).

| Group                  | 1-h reperfusion | 3-h reperfusion | 5-h reperfusion |
|------------------------|-----------------|-----------------|-----------------|
| Sham group             | 114.08±1.63     | 120.21±2.10     | 122.55±5.70     |
| Kidney transplant group| 140.81±1.34*    | 212.01±1.92**   | 313.10±11.34**  |
| HBO treatment group    | 117.73±1.56*    | 156.69±6.50**   | 200.90±4.70**   |

* P<0.05, compared with the sham group; # P<0.05, compared with 1 hour of reperfusion; ** P<0.05, compared with 3 hours of reperfusion. SD – standard deviation.

Figure 5. Expression of ICAM-1, VCAM-1, and C3 in each group. Quantitative measurement of ICAM-1, VCAM-1, and C3 immunohistochemical staining in the kidney sections. Renal tissue samples collected at 1, 3, and 5 hours of reperfusion was stained with anti-ICAM-1, VCAM-1, and C3 antibodies. Microscopic images were quantitatively analyzed using Image-Pro Plus 6.0 system. * P<0.05, compared with the sham group; # P<0.05, compared with the HBO treatment group.

and protect renal function, as indicated by decreased serum creatinine levels.

The complement system activation is crucial in IRI development. The complement system is activated in the early stage of IRI, which can not only directly cause cell damage, but also affect the expression of reactive oxygen species, neutrophils, and endothelial cell products. For example, the effector molecules produced by the activation of the complement system can not only act directly on neutrophils, but also upregulate the expression of ICAM-1 and VCAM-1 in endothelial cells, which can mediate neutrophils activation and mononuclear macrophage infiltration, thus aggravating the inflammatory response. C3a and C5a produced by the complement system can directly activate T lymphocytes and antigen presenting cells in the body, and the activated T lymphocytes can directly play the role of immune injury. During activation, various complement components are activated by proteolysis, forming a cascade reaction, which eventually forms a membrane attack complex (MAC), leading to cell disintegration and death [10–12]. C3 is the component with the highest content in the complement system and plays a center of the complement cascade reaction.
Investigators have shown that C3 was highly expressed in the renal tubular epithelial cells after kidney transplantation, and the C3 expression level is further increased with reperfusion time, accompanied by deteriorating renal function [3].

The present study reveals increased expression of C3 in the renal tissue after kidney transplantation, while HBO treatment significantly reduces the expression of C3 in renal tissue. This novel study indicated that HBO treatment can protect the renal function and structures by inhibiting the expression of inflammatory mediator C3, suggesting the use of HBO in preventing and treating renal IRI following kidney transplantation.

HBO treatment has been shown to have protective effects on renal IRI by inhibiting apoptosis in renal tissue, reducing plasma TNF-α levels, and upregulating the expression of hypoxia-inducible factor-1α mRNA [13,14].

The present study investigated the changes in the expression of adhesion molecules and C3 in renal tissue after kidney transplantation. The results indicate that adhesion molecules and C3 played important roles in the development of renal IRI and the damage of renal function. HBO treatment protect the kidney by inhibiting the overexpression of adhesion molecules in renal tissue and activation of complement system in renal tissue.

Conclusions

In conclusion, HBO treatment has protective effect on renal function and histopathological changes in IRI injury following kidney transplantation by inhibiting the overexpression of adhesion molecules and activation of the complement system. Therefore, HBO provided a theoretical basis for the treatment of IRI after kidney transplantation. More mechanisms of action and protection of HBO need to be further studied.

Acknowledgment

The authors thank the staffs of the Medical Experimental Center and Department of Pathology, Zunyi Medical University, for their help and guidance.

References:

1. Parajuli N, MacMillan-Crow LA: Role of reduced manganese superoxide dismutase in ischemia-reperfusion injury. A possible trigger for autophagy and mitochondrial biogenesis? Am J Physiol Renal Physiol, 2013; 304(3): F257–67
2. Pushpakumar SB, Perez-Abadia G, Soni C et al: Enhancing complement control on endothelial barrier reduces renal post-ischemia dysfunction. J Surg Res, 2011; 170(2): e263–70
3. Kotimaa JP, van Werkhoven MB, O’Flynn J et al: Functional assessment of mouse complement pathway activities and quantification of C3b/C3c/iC3b in an experimental model of mouse renal ischaemia/reperfusion injury. J Immunological Methods, 2015; 419: 25–34
4. Ramalho RJ, de Oliveira PS, Cavaglieri RC et al: Hyperbaric oxygen therapy induces kidney protection in an ischemia/reperfusion model in rats. Transplant Proc, 2012; 44(8): 2333–36
5. Gurer A, Ozdogan M, Gomceli I et al: Hyperbaric oxygenation attenuates renal ischemia-reperfusion injury in rats. Transplant Proc, 2006; 38(10): 3337–40
6. Bao DS, Wu YK, Fu SJ et al: Hyperbaric oxygenation protects against ischemia-reperfusion injury in transplanted rat kidneys by triggering autophagy and inhibiting inflammatory response. Ann Transplant, 2017; 22: 75–82
7. Medeiros PI, VillarimNeto A, Lima FP et al: Effect of sildenafil in renal ischemia/reperfusion injury in rats. Acta Cir Bras, 2010; 25(6): 490–95
8. Haller H, Dragun D, Miethke A et al: Antisense oligonucleotides for ICAM-1 attenuate reperfusion injury and renal failure in the rat. Kidney Int, 1996; 50(2): 473–80
9. Beiras-Fernandez A, Chappell D, Hammer C et al: Impact of polyclonal anti-thymocyte globulins on the expression of adhesion and inflammation molecules after ischemia-reperfusion injury. Transpl Immunol, 2009; 20(4): 224–28
10. Zhou W, Farrar CA, Abe K et al: Predominant role for C5b-9 in renal ischemia/reperfusion injury. J Clin Invest, 2000; 105(10): 1363–71
11. Tan B, Zhang DC, Wang ZQ: Role and mechanism of complement in ischemia reperfusion injury. J Trauma Surg, 2007; 9(4): 374–76
12. Thurman JM, Ljubanovic D, Edelstein CL et al: Lack of a functional alternative complement pathway ameliorates ischemic acute renal failure in mice. J Immunol, 2003; 170(3): 1517–23
13. Xie ZH, Chen ZP, Cao R et al: [The effect of hyperbaric oxygen on the expression of hypoxia inducible factor-1alpha mRNA in renal tissue after renal ischemia-reperfusion injury.] Chin J Phys Med Rehabil, 2010; 32(3): 182–85 [in Chinese]
14. Xie ZH, Cao R, Chen ZP et al: [The effect of hyperbaric oxygen on renal ischemia-reperfusion injury in rats.] Chin J Phys Med Rehabil, 2010; 32(2): 98–101 [in Chinese]