

Effects of Hyperbaric Oxygen Treatment on Renal System

Orhan Tezcan,¹ Ahmet Caliskan,¹ Sinan Demirtas,¹ Celal Yavuz,¹
Mahir Kuyumcu,² Yusuf Nergiz,³ Abdulmenap Guzel,²
Oğuz Karahan,¹ Şeyhmus Ari,⁴ Sevda Soker,³ İbrahim Yalinkilic,⁵
Kenan Ahmet Turkdogan⁶

¹Department of Cardiovascular Surgery, Medical School of Dicle University, Diyarbakir, Turkey

²Department of Anesthesiology, Medical School of Dicle University, Diyarbakir, Turkey

³Department of Histology and Embryology, Medical School of Dicle University, Diyarbakir, Turkey

⁴Department of Ophthalmology, Medical School of Dicle University, Diyarbakir, Turkey

⁵Department of Medical Biology, Medical School of Dicle University, Diyarbakir, Turkey

⁶Department of Emergency Medicine, Medical School of Adnan Menderes University, Diyarbakir, Turkey

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Introduction. Hyperbaric oxygen (HBO) treatment is steadily increasing as a therapeutic modality for various types of diseases. Although good clinical outcomes were reported with HBO treatment for various diseases, the multisystemic effects of this modality are still unclear. This study aimed to investigate the renal effects of HBO experimentally.

Materials and Methods. Fourteen New Zealand White rabbits were divided into 2 groups randomly as the control group and the study group. The study group received HBO treatment for 28 days (100% oxygen at 2.5 atmospheres for 90 minutes daily) and the control group was used to obtain normal renal tissue of the animal genus. After the intervention period, venous blood samples were obtained, and renal tissue samples were harvested for comparisons.

Results. Normal histological morphology was determined with Masson trichrome staining and periodic acid-Schiff staining in the control group. Atrophic glomerular structures, vacuolated tubule cells, and degeneration were detected in the renal samples of the study group with Masson trichrome staining. Additionally, flattening was observed on the brush borders of the proximal tubules, and tubular dilatation was visualized with periodic acid-Schiff staining. The histopathologic disruption of renal morphology was verified with detection of significantly elevated kidney function laboratory biomarkers in the study group.

Conclusions. Our findings suggests that HBO has adverse effects on renal glomerulus and proximal tubules. However, the functional effects of this alteration should be investigated with further studies.

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INTRODUCTION

Hyperbaric oxygen (HBO) treatment has gradually gained popularity and become a concomitant or single therapeutic method for various types of diseases such as carbon monoxide poisoning, anemia, wound healing, and ischemic disorders.¹ This treatment is based on 95% to 100% oxygen breathing. The procedure is applied at 1.0, 2.0, or 2.5 atmospheres absolute (ATA) for a minimum

of 90 minutes daily.^{1,2}

Although HBO treatment has been used since the 1930s, scientific data regarding its benefits and adverse effects are insufficient. However, this treatment is known to reduce hemoglobin requirement by increasing blood oxygenation, and it has antibacterial effects with bacteriostatic or bactericidal activities.^{1,3,4} In addition, HBO can lead to systemic effects. It was speculated that HBO can

induce oxidant substance formation by producing peroxynitrite from the reaction of superoxide with nitric oxide, which disrupts nitric oxide mediated vasodilatation. This can lead to peripheral vascular resistance and can trigger tachycardia with increased parasympathetic activation.⁵⁻⁷ Moreover, this vasoconstrictive action affects all systemic organs.⁶ Similar interactions may lead to renal effects as well as other systemic outcomes.

In the current study, we aimed to investigate the renal effects of repetitive HBO application in an experimental animal model.

MATERIALS AND METHODS

Animals

Healthy male New Zealand White rabbits (weighing 2.1 kg to 3.3 kg) were obtained from the laboratory production unit of Dicle University. All animals were kept in 12-hour light-dark cycle cages programmed with standard humidity ($50 \pm 5\%$) and temperature ($22 \pm 2^\circ\text{C}$). The animals were allowed free access to a standard diet and tap water until study protocols began. Ethical approval was obtained from the local animal ethics committee of the university, and the study protocol was designed in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals.

Grouping and Protocols

Fourteen animals were enrolled in this study and divided into 2 equal groups randomly. The control group animals were kept in standard cage conditions and received standard libitum. This group was created for detecting normal histopathology of the renal tissue in the rabbit genus. The remaining rabbits were separated as the study group. This group received daily HBO treatment (100% oxygen at 2.5 ATA for 90 minutes) for 28 days. The duration and pressure of HBO was determined in accordance to previous experimental reports.^{8,9} After the completion of 28 days of HBO treatment, all of the rabbits were euthanized, and the kidneys were harvested after obtaining 2 mL of blood sample from the ear veins in each group.

Laboratory Analysis

Venous blood samples were centrifuged at 5000 rpm for 10 minutes, and the obtained serums were stored in Eppendorf tubes. Thereafter, serum urea (mmol/L), creatinine ($\mu\text{mol/L}$), and potassium (mg/dL) values were measured by using an autoanalyzer (Abbott ARCHITECT C-16000, Abbott Diagnostics, Chicago, IL, USA).

Preparation of Tissue for Histopathological Examination

The kidneys were fixed in 10% formalin solution after harvest. Thereafter, tissues were embedded in paraffin blocks. Each paraffin block was sliced into 6 μm pieces for staining. Slices were examined microscopically after Masson's trichrome staining and periodic acid-Schiff staining. An ApoTome Axio Imager Z2 (Zeiss) light microscope was used to evaluation tissues and obtain micrographs. Finally, the groups were compared in accordance to laboratory and histopathological findings.

Statistical Analysis

The SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, IL, USA) was used to perform statistical analysis. The laboratory parameters were expressed as mean \pm standard deviation using an unpaired the Student *t* test, with statistical significance considered at a *P* value less than .05.

RESULTS

Laboratory kidney function tests were found to be markedly higher in HBO-treated rabbits ($P < .05$). The comparison of the two groups is summarized in the Table. The normal histopathological imaging of kidney tissue and histological morphology of basal lamina and microvillus structures in proximal tubules are shown in Figure 1. Disrupted and atrophic glomeruli structure and vacuolated tubule cells were detected in the study group (Figure 2). Moreover, histologic examination revealed flattening or loss of the brush borders of the proximal tubules and tubular dilatation

Laboratory Kidney Function Tests in Rabbit Treated With Hyperbaric Oxygen (HBO) and Controls

Parameter	Control Group	HBO Group	<i>P</i>
Blood urea, mmol/L	11.71 \pm 5.63	26.55 \pm 12.34	.005
Serum creatinine, $\mu\text{mol/L}$	84.03 \pm 11.12	102.48 \pm 19.42	< .001
Serum potassium, mg/dL	4.89 \pm 1.63	5.55 \pm 2.12	.002

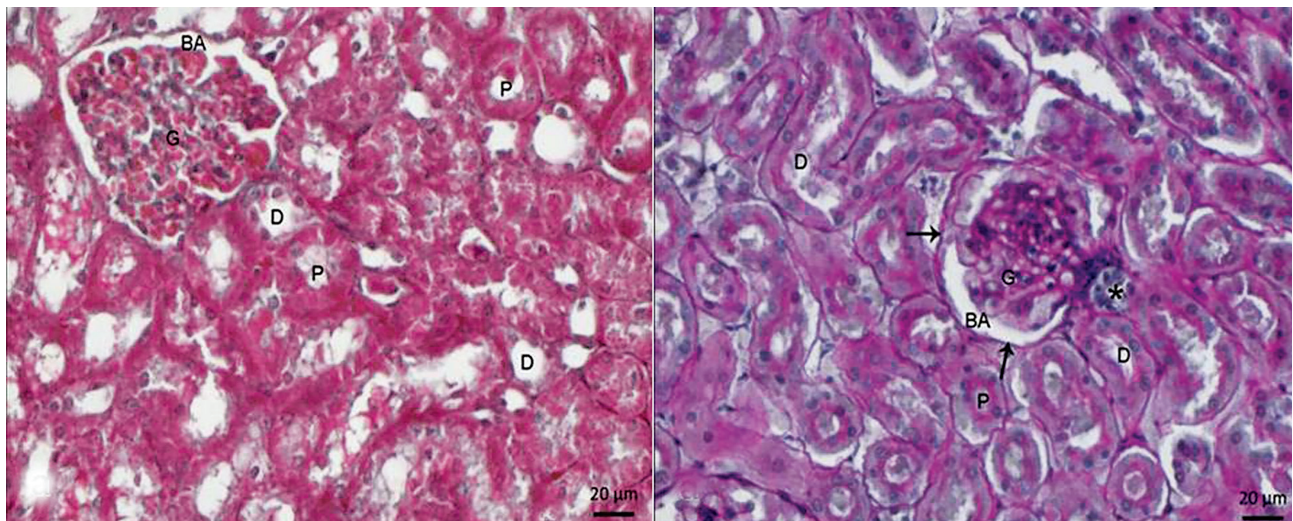


Figure 1. Left, Normal morphology of renal structures in the control group (Masson trichrome). Right, Normal histology of border brush and basal lamina of proximal tubules in the control group. Arrows show basal lamina and the asterisk show macula densa complex (periodic acid-Schiff). G indicates glomeruli, BA, Bowman space; P, proximal tubule; and D, distal tubule.

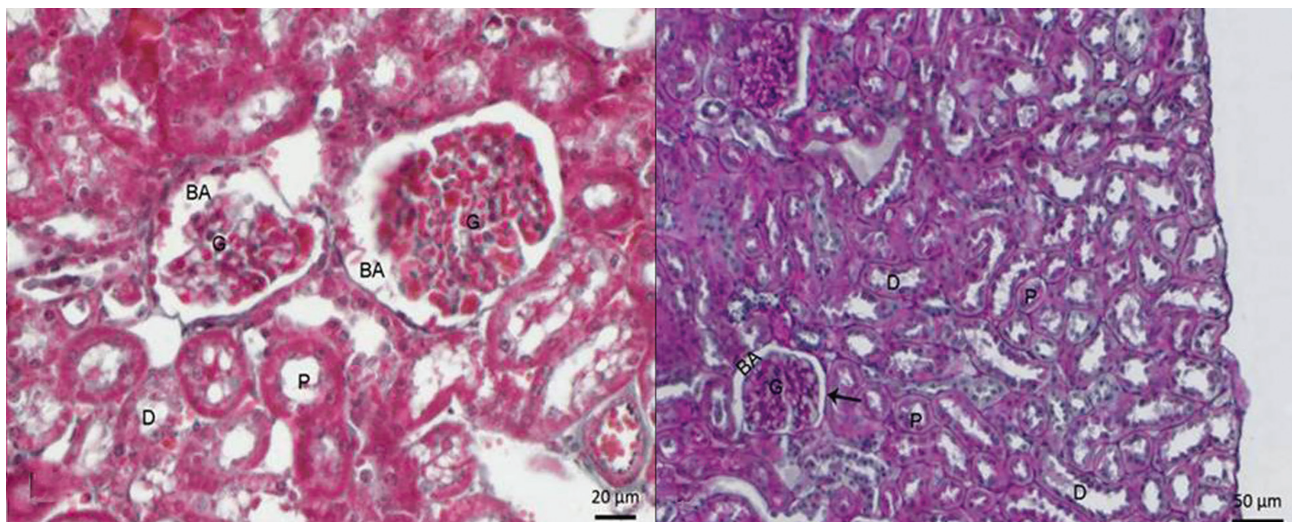


Figure 2. Left, In the histopathology of the renal structure in rabbits treated with hyperbaric oxygen, glomerular atrophy, basement membrane thickening, tubular vacuolization, and degeneration were visualized (Masson trichrome). Right, Flattening or loss of the brush borders of the proximal tubules and tubular dilatation in rabbits with hyperbaric oxygen treatment. Arrows show basal lamina (periodic acid-Schiff). G indicates glomeruli, BA, Bowman space; P, proximal tubule; and D, distal tubule.

(Figure 2) in the renal tissue samples of the study group.

DISCUSSION

Our findings demonstrated that HBO treatment for 28 days led to glomerular atrophy and degeneration, as well as vacuolated tubule cells. Even further evaluation with periodic acid-Schiff staining revealed an important disruption in essential structures for kidney functions, such as the shortening of the proximal tubular brush border and the renal tubular dilatation. Additionally,

markedly impaired laboratory kidney function markers were detected in the HBO-treated group.

The therapeutic facilities of HBO are gaining popularity for the treatment of different kinds of diseases. This treatment provides the high oxygen supply to the end organs and increases the tissue oxygenation.^{1,2} Although it may have a potential therapeutic role for ischemic or inflammatory events, the higher oxygen supply to healthy tissues can lead to undesirable cellular effects.⁵⁻⁷ Previous reports speculated some adverse effects of high oxygen concentration in end organs such as lungs

(pulmonary toxicity and resorption atelectasis), systemic redox reactions with increased production of reactive oxygen species, vasoconstrictive on vascular structures, and cellular metabolic changes.^{10,11} Despite the reported technical or patient-related risk factors for complications, most of the current reports have presented beneficial effects of oxygen treatment in normobaric and hyperbaric conditions.^{11,12} Nonetheless, the count of conflicting studies is still substantial. For instance, Efrati and colleagues reported that the ineffective increment of tissue oxygenation in nonhealing diabetic ulcers by HBO therapy leads to higher oxidative stress and reduced nitric oxide bioavailability.¹³¹

The hyperoxia can also affect the kidneys. Hinkelbein and colleagues¹⁴ studied the short-term effects of hyperoxia in rat kidneys in which 6 experimental groups were conducted: 3 groups with normobaric hyperoxia (exposure to 100% oxygen for 3 hours) and 3 groups with normobaric normoxia (room air). The study demonstrated the protein alterations in hyperoxic injury of rat renal cells. Finally, they suggested that clarifying organ-specific responses to hyperoxia is critical to identifying cellular reactions and understanding the basic mechanisms that lead to alterations in renal tissue.¹⁴ Experimental models claim that HBO therapy protects the kidneys against the oxidative stress induced by renal ischemia perfusion by inhibiting neutrophil infiltration, and it ameliorates the glomerular filtration rates against sepsis-related impairment of renal functions.^{15,16} Berkovitch and coworkers reported that HBO did not caused renal destruction in rats treated with HBO (100% oxygen at 2 ATA for 60 minutes daily) for 10 days.¹⁷ In addition, they could not find any histopathological deformation that interpreted as structural impairment, such as cellular swelling, focal tubular necrosis, proximal tubule dilatation, and flattened brush borders. In response to their findings, they concluded that HBO is safer for healthy renal tissues.¹⁷ An earlier study reported that higher atmospheric (4.8 or 6.8 ATA) HBO applications could lead to convulsions that were related to alterations in kidney function.¹⁸ Our results revealed both functional impairment of laboratory markers and histopathological destruction of renal tissue samples in rabbits treated with HBO for 28 days (100% oxygen at 2.5 ATA for 90 minutes daily). These findings conflict

with previous results. This difference might be related to the duration of therapy (Berkovitch and colleagues¹⁷ applied 100% oxygen at 2 ATA for 60 minutes daily for 10 days) or the healthy nature of renal tissue samples instead of septic or ischemic exposure (Ilhan and coworkers¹⁵ and Edremitlioglu and colleagues¹⁶ reported improved kidney function with HBO treatment in ischemia reperfusion or septic exposure kidneys).

CONCLUSIONS

Our findings suggest that HBO treatment can lead to destruction of renal tissue and disruption of the kidney function in healthy rabbits. This is speculative because monitoring of kidney function is required during HBO. However, to obtain definitive conclusions, future efforts should be focused on the effects of HBO treatment in clinical studies that include human subjects.

CONFLICT OF INTEREST

None declared.

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Correspondence to:

Orhan Tezcan, MD

Department of Cardiovascular Surgery, Medical School of Dicle University, Diyarbakir, Turkey

Tel: +90 412 248 8001

Fax: +90 412 248 8523

E-mail: dr.orhantezcan@gmail.com

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