DOI: 10.4274/forbes.galenos.2022.75046
Forbes J Med 2022;3(2):160-164

Original Article / Orijinal Araştırma

Effect of Hyperbaric Oxygen on Hypoxic-ischemic Damage in Cold Preserved Tissues

Hiperbarik Oksijenin Soğuk Saklamadaki Dokularda Hipoksik-iskemik Hasar Üzerine Etkisi

İbrahim ÖNCEL[1]*, Selman KESİCİ[2], Şeref Selçuk KILIÇ[3#], Saniye EKİNCİ[3], Beril TALİM[4], Benan BAYRAKCI[2]

[1]Hacettepe University Faculty of Medicine, Department of Pediatrics, Ankara, Turkey
[2]Hacettepe University Faculty of Medicine, Life Support Centre, Department of Pediatric Intensive Care, Ankara, Turkey
[3]Hacettepe University Faculty of Medicine, Department of Pediatric Surgery, Ankara, Turkey
[4]Hacettepe University Faculty of Medicine, Department of Pediatrics, Clinic of Pediatric Pathology, Ankara, Turkey

Cite as: Öncel İ, Kesici S, Kılıç ŞS, Ekinci S, Talim B, Bayrakcı B. Effect of Hyperbaric Oxygen on Hypoxic-ischemic Damage in Cold Preserved Tissues. Forbes J Med 2022;3(2):160-164

ABSTRACT

Objective: To assess the possible effect of hyperbaric oxygen (HBO) in preventing hypoxic-ischemic damage in cold-preserved organs.

Methods: Visceral organs of white male New Zealand rabbits (n=6) were removed and examined in two groups: right kidneys (n=6), right lungs (n=6), right lobe of livers (n=6) and the hearts of three animals constituted the HBO group and left kidneys (n=6), left lungs (n=6), left lobe of livers (n=6) and the hearts of the remaining animals (n=3) formed the control group. After excision, organs in the HBO group were immediately placed in 100% oxygen at 2.5 atmosphere absolute for 2 h while those in the control group were kept in room air during this period. All tissues were kept in University of Wisconsin (UW) solution, 4 °C, at the end of the experiment. Tissue sections were obtained at 2 h after removal (T1, early stage) and at 125% of the maximal acceptable ischemic time determined for each tissue (T2, late stage). Histopathological evaluation was made by blinded pathologists using semiquantitative scoring systems and scores were compared between the HBO and control groups.

Results: Tissue injury in the lungs and kidneys of the HBO group was milder compared to controls at both the early and late stages, but the difference was not statistically significant. Heart tissue in both the HBO and control groups demonstrated signs of ischemic injury at T2, while liver tissue did not change significantly from T1 to T2.

Conclusion: These preliminary results suggest that further studies with larger sample sizes, particularly involving lung and kidney tissue and conditions applicable to clinical settings, are needed to determine any effect of HBO during cold storage.

Keywords: Hyperbaric oxygen therapy, cold preservation, solid organ, transplantation, transportation

ÖZ

Amaç: Bu çalışmada soğuk saklamadaki organlarda hipoksik-iskemik hasarı önlemede hiperbarik oksijenin (HBO) etkisini değerlendirmek amaçlanmıştır.

Yöntem: Beyaz erkek Yeni Zelanda tavşanlarının (n=6) visseral organları çıkarıldıktan sonra sağ böbrek (n=6), sağ akciğer (n=6), karaciğer sağ lobu (n=6) ve 3 hayvanın kalpleri HBO grubunda yer alırken sol böbrek (n=6), sol akciğer (n=6), karaciğer sol lobu (n=6) ve geri kalan hayvanların kalpleri (n=3) kontrol grubunda yer aldı. Eksizyon sonrası HBO grubundaki organlar 2 saat boyunca 2,5 ATA'da %100 oksijende bekletildi. Kontrol grubundakiler ise bu süre zarfında oda havasında tutuldu. Tüm dokular, deneyin sonuna kadar Wisconsin Üniversitesi (UW) çözeltisinde, 4 °C'de tutuldu. Doku kesitleri, çıkarıldıktan 2 saat sonra (T1, erken evre) ve her doku için belirlenmiş maksimum kabul edilebilir iskemik sürenin

Received/Geliş: 08.04.2022
Accepted/Kabul: 21.04.2022

Corresponding Author/ Sorumlu Yazar:
İbrahim ÖNCEL MD,
Hacettepe University Faculty of Medicine, Department of Pediatrics, Ankara, Turkey
Phone: +90 532 608 16 38
dribrahimoncel@gmail.com
ORCID: 0000-0002-0710-7818

***Current address:** Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Ankara, Turkey

#Current address: Çukurova University Faculty of Medicine, Department of Pediatric Surgery, Adana, Turkey

Presented in: A summary of the results of this study was presented at the 7th World Congress on Pediatric Critical Care in 4-7 May 2014 in İstanbul, Turkey.

%125'inde (T2, geç evre) alındı. Histopatolojik değerlendirme semikantitatif skorlama sistemleri kullanılarak kör biçimde yapıldı ve skorlar HBO ve kontrol grupları arasında karşılaştırıldı.

Bulgular: HBO grubunda akciğer ve böbreklerde doku hasarı hem erken hem de geç dönemlerde kontrollere kıyasla daha hafifti, ancak aradaki fark istatistiksel olarak anlamlı değildi. Hem HBO hem de kontrol gruplarındaki kalp dokuları, T2'de iskemik hasar belirtileri gösterirken, karaciğer dokuları T1'den T2'ye belirgin değişim göstermedi.

Sonuç: Bu çalışmada edinilen sonuçlar, HBO'nun soğuk saklama sırasında özellikle akciğer ve böbrek dokuları üzerine etkisini belirlemek için daha büyük örneklemlerle ve klinik ortamlara uygulanabilir koşulları içeren ileri çalışmalara ihtiyaç olduğunu göstermektedir.

Anahtar Kelimeler: Hiperbarik oksijen, soğuk saklama, solid organ, transplantasyon, organ taşınması

INTRODUCTION

Solid organ transplantation represents a definitive treatment for many patients with end-stage organ failure. However, increasing demand and shortage of donor organs significantly restrict this possibility. Therefore, the preservation of any available organs and in particular, the prevention of ischemia after surgical removal from donors become crucial issues. Cold storage represents a simple and effective way to preserve and transport organs and is currently is currently a standard measure. It can reduce metabolic requirements, attenuate the effect of ischemia and preserve the viability of organs although complete prevention of cold ischemic injury is usually not achievable.[1]

Hyperbaric oxygen (HBO) therapy involves the application of 100% oxygen under pressure greater than one atmosphere absolute (ATA). It has been successfully used for treating carbon monoxide (CO) poisoning, decompression sickness, arterial gas embolism, and various diseases accompanied by tissue ischemia.[2] Its mechanisms of action and effects on various organs and tissues have been studied experimentally. However, methodological differences between those studies and the frequent involvement of prolonged and repetitive HBO exposures limit their application to clinical conditions and particularly to the transportation of organs. We intended to evaluate the effect of HBO on organs in cold preservation under achievable physiological conditions and during a transportation process.

METHODS

Six male white New Zealand rabbits weighing between 2800 and 3200 g were obtained from the standard care unit of the Refik Saydam Institute, Ankara, Turkey. Rabbits were kept in a room at a constant optimal temperature (20-24 °C) under 12 h of darkness and 12 h of sunlight.

Procedure

The rabbits were anesthetized with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg). The surgery was performed by two pediatric surgeons. After a midline incision, the abdominal aorta and inferior vena cava were cannulated immediately. Organs were flushed with refrigerated University of Wisconsin (UW) solution (200 mL/kg) *in situ* and recovered in the order of right and left kidneys, liver, right and left lungs, and heart. Organs were divided into two groups: the HBO group consisted of right kidneys (n=6), right lungs (n=6), right lobe of livers (n=6) and hearts of three randomly selected animals. The control group included left kidneys (n=6), left lungs (n=6), left lobe of livers (n=6) and remaining hearts (n=3). Organs in the HBO group were placed in a small hyperbaric chamber pressurized to 2.5 ATA with 100% oxygen for 2 h, and then kept in UW solution in room air at 4 °C. The control group was kept to room air and UW solution at 4 °C during the same period.

Tissue sampling was performed at 2 time points: T1 (early stage) at 2 h after removal, and T2 (late stage) at 125% of the predefined acceptable ischemic time (AIT) for each organ. T2 was 30 h for the kidney (AIT: 24 h); 15 h for the liver (AIT: 12 h); 7.5 h for the lung (AIT: 6 h); and 5 h for the heart (AIT: 4 h).[3]

Histopathological Examination

The specimens were fixed in 10% formaldehyde and embedded in paraffin. 3 µm-thick sections were stained with hematoxylin and eosin. Additionally, kidney samples were stained with Periodic Acid Schiff and heart samples were stained with masson trichrome. At least two different sections of each specimen were examined and scored by two examiners who were blinded to the study.

Scoring of kidneys was modified from Goujon et al.'s[4] technique, which grades the typical morphological patterns of proximal tubular injury including vacuolization of apical cytoplasm, tubular necrosis, tubular dilatation, cell detachment, brush border integrity, intracellular edema, denuded basement membrane on a 5-point scale as follows: 1, no abnormality; 2, mild lesion affecting 10% or less of the field of view; 3, lesions affecting 10-25% of the field of view; 4, lesions affecting 25-50% of the field of view; 5, lesions affecting 50% or more of the field of view. A total injury score was obtained by summing the scores of each morphological pattern.

Cardiac tissues were graded for the severity of myocardial damage according to a previously established grading system;[5] grade 0: normal appearance of myocytes without hydropic changes, interstitial edema, or cell disruption;

grade 1: weakly injured myocardium characterized by hydropic cardiomyocytes, low-grade interstitial edema, few contraction bands in single fields of view; grade 2: moderately injured myocardium characterized by significant interstitial and cellular edema, regular presence of contraction bands in the field of view; and grade 3: severely injured myocardium characterized by prominent contraction bands and severe interstitial and cellular edema.

A liver tissue grading system was modified from Suzuki et al.[6] which measures sinusoidal congestion, vacuolization of hepatocyte cytoplasm or ballooning, and parenchymal necrosis. Additionally, neutrophilic inflammation, sinusoidal dilatation and hydropic changes of the hepatocytes were evaluated and scored from 0 to 4 as follows: 0, no abnormality; 1, mild lesion affecting 5% or less of the field of view; 2, lesions affecting 6-20% of the field of view; 3, lesions affecting 21-50% of the field of view; 4, lesions affecting 50% or more of the field of view. The sum of the six scores formed the total injury score.

Lung tissue were scored on the basis of the prominent histopathological findings of congestion, interstitial edema, alveolar damage, intraalveolar hemorrhage and inflammation defined in the literature as follows:[7] 0, no changes; 1, focal mild changes; 2, multifocal mild changes; 3, multifocal prominent changes; 4, extensive prominent changes. A total injury score was obtained by summing the scores of each morphological pattern.

Statistical Analysis

Means of the total injury scores were used for the kidney, liver and lung, and actual scores were used for heart tissue. Statistical assessment was done with Mann-Whitney U and Wilcoxon tests, and significance was set at $p<0.05$ (Statistical Package for the Social Sciences version 15.0).

RESULTS

Histopathological examination results of various tissues are shown in Figure 1. In the lungs, the most frequent finding was congestion followed by alveolar damage and inflammation at both T1 and T2. In the kidneys, vacuolization, cell detachment and edema was noticeable at T1, and tubular dilatation and necrosis at T2. Liver sections mainly showed vacuolization at both T1 and T2, and did not show any worsening at T2 in either group (Figure 1).

The mean injury scores of the lung, kidney and liver tissue are given in Table 1. Late stage (T2) scores were higher than early (T1) scores in lung and kidney tissue of both HBO and control groups: although the scores of the control group were higher than those of the HBO group, the difference was not significant (Table 1).

The injury progression in the heart tissue was found to be slightly better in the HBO group compared to the control group (Figure 2).

DISCUSSION

This study examined whether a 2-hour exposure to HBO could prevent or reduce ischemic injury, thereby

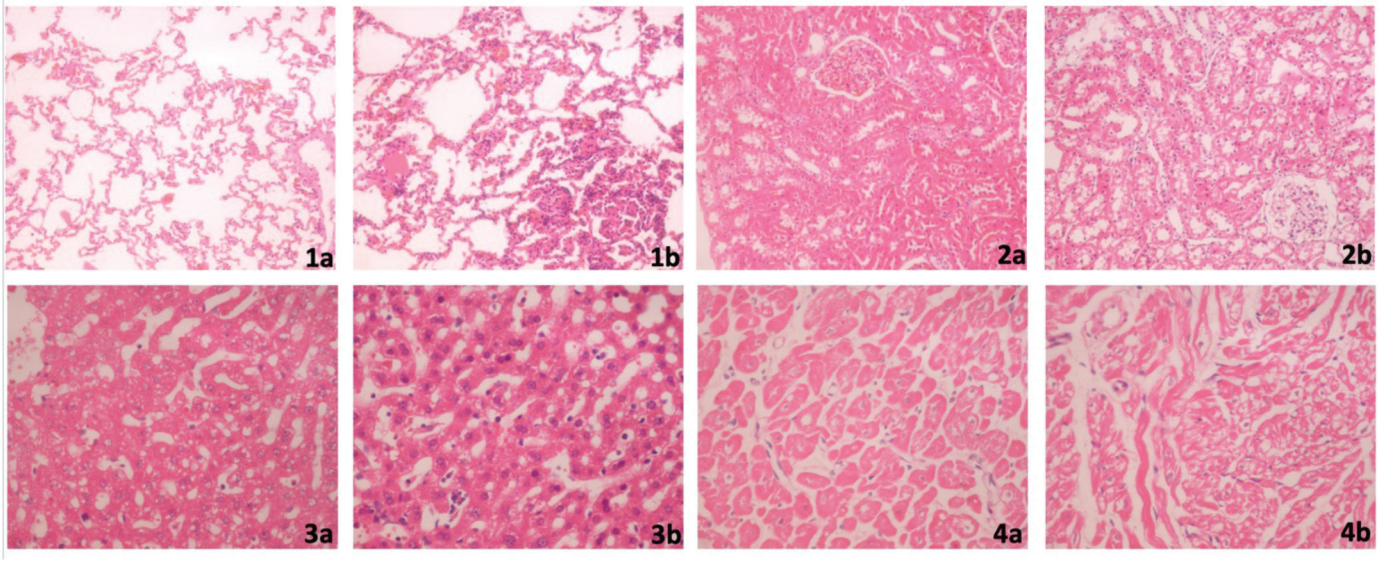

Figure 1. Histopathological findings. Histopathology of the lung (20x) showed alveolar damage and congestion at early (1a) and late (1b) stages. Kidney tissue (20x) showed mild edema, vacuolization and cell detachment at early stage (2a) and remarkable tubular dilatation at late stage (2b). The prominent histopathological finding in liver tissue (40x) was hepatocyte vacuolization at both early (3a) and late stages (3b). Cellular and interstitial edema were shown in moderately (4a) and severely (4b) injured cardiac tissues (40x) (H&E)

Table 1. Injury scores of lung, kidney and liver at early and late stages						
Organs	T1, early stage (2 hours)			T2, late stage (125% AIT)		
	Mean score		p value	Mean score		p value
	HBO	Control		HBO	Control	
Lung	8.2	8.7	0.81	9.2	10.2	0.69
Kidney	10.8	11	0.81	15.8	16.2	0.69
Liver	5.8	5.6	0.93	5.6	5.8	0.81
AIT: Acceptable ischemic time, HBO: Hyperbaric oxygen						

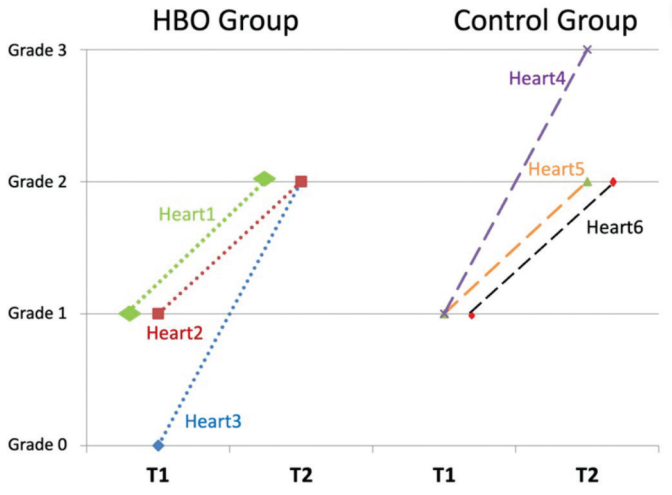

Figure 2. Grades of early (T1) and late (T2) stage injury in heart tissues

HBO: Hyperbaric oxygen

contributing to the optimal transportation of harvested organs. The effect of HBO in organs and tissues has been examined in numerous studies: in the majority, experimental conditions included extended periods and frequent, repetitive sessions of HBO.[8-11] However, few hours' exposure to HBO is more applicable during the transportation of organs and patients, as in mountain sickness. Indeed, such a device successfully used to transfer patients from high altitudes has been made commercially available.[12]

Several studies have shown certain beneficial effects of long-term HBO application during cold storage.[10,11] In earlier experiments, HBO pressures of 3 to 18 ATA were given for long periods (12 to 48 h). More recently, the optimal pressure for tissue oxygenation was determined as 2.5 ATA, where enough oxygen can be dissolved in plasma to meet physiological requirements of the organs, theoretically even without the need of hemoglobin.[13] Some of the previous studies were designed to evaluate the effect of HBO on cold ischemia, whereas some others investigated reperfusion injury in transplantation models.[14]

In a recent study, histological changes in the rat liver after HBO given at phases of ischemia, reperfusion, or both were compared: histological injury was found to be significantly lower in the organs exposed to HBO in the early ischemic phase, while HBO given in late periods of reperfusion was associated with more severe liver damage, possibly through oxidative stress.[15] Indeed, HBO treatment, especially in long and repetitive applications, increases oxidative stress markers like malondialdehyde, carbonylated protein levels and superoxide dismutase activity.[16] Such adverse effects of HBO would be of significant concern in its clinical use for organ transplantation, myocardial infarction, traumatic injury, and wound healing. Such toxicity may be avoided by shorter applications.[17] In our study, mean injury scores of the lungs and kidneys were slightly lower in the HBO group at both early and late stages compared to controls. However, the difference was not statistically significant.

Our study was inspired by the clinical observation of two siblings who received HBO (4 h in 2 sessions under 2.5 ATA) for CO intoxication but progressed into brain death after 72 h and became organ donors.[18] Despite having suffered multiple cardiac arrest episodes, these patients' harvested livers showed normal histology without any evidence of necrosis or inflammation on frozen biopsy and functioned normally after transplantation. This particular observation suggested a possible positive effect of the HBO given for CO intoxication in preventing tissue damage. In order to mimic these patients' conditions, the experimental organs in our study underwent hypoxia-ischemia followed by HBO for 2 h. We scored several parameters in the histopathological evaluation of liver and lung tissue including inflammation. HBO could have reduced or prevented inflammation through the regulation of proinflammatory cytokines and other mediators.[19,20] However, we did not observe a prominent difference in inflammation between the HBO and control groups.

Study Limitations

The main limitation of this study was the relatively small sample size reducing the statistical power. We investigated the effect of HBO on cold ischemic injury. However, when considering the clinical settings for transplantation,

evaluating solely the ischemic injury appears to be a limitation of this study instead of evaluating both ischemic and reperfusion injury through a transplantation model.

CONCLUSION

In conclusion, two hours of HBO application showed a mild beneficial effect on short-term organ preservation. As a matter of fact, our findings promote further studies with larger sample sizes including biochemical markers (i.e., oxidative stress and apoptosis markers), immunohistochemistry and electron microscopical examinations to confirm this hypothesis.

Acknowledgments

The authors thank Dr. Banu Anlar for her contributions to the writing of the article.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Animal Care Guidelines of Hacettepe University and approved by Hacettepe University Animal Care and Use Ethics Committee (approval number: 2011/22).

Informed Consent: It is an animal experiment.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.S.K., S.E., Concept: B.B., Design: B.B., Data Collection or Processing: İ.Ö., S.K., Analysis or Interpretation: İ.Ö., B.T., Literature Search: İ.Ö., Writing: İ.Ö.

Conflict of interest: No conflicts of interest are declared by the authors.

Financial Disclosure: This study was supported by Hacettepe University Scientific Research Projects Coordination Unit (project no: 011 D04 101 015).

REFERENCES

1. Shaver CM, Ware LB. Primary graft dysfunction: pathophysiology to guide new preventive therapies. Expert Rev Respir Med. 2017;11:119-28.
2. Shah J. Hyperbaric oxygen therapy. J Am Col Certif Wound Spec. 2010;2:9-13.
3. Institute of Medicine (US) Committee on Organ Procurement and Transplantation Policy. Organ Procurement and Transplantation: Assessing Current Policies and the Potential Impact of the DHHS Final Rule. Washington (DC): National Academies Press (US); 1999.
4. Goujon JM, Hauet T, Menet E, Levillain P, Babin P, Carretier M. Histological evaluation of proximal tubule cell injury in isolated perfused pig kidneys exposed to cold ischemia. J Surg Res. 1999;82:228-33.
5. Miller S, Schick F, Scheule AM, et al. Conventional high resolution versus fast T(2)-weighted MR imaging of the heart: assessment of reperfusion induced myocardial injury in an animal model. Magn Reson Imaging. 2000;18:1069-77.
6. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. Transplantation. 1993;55:1265-72.
7. Tassiopoulos AK, Carlin RE, Gao Y, et al. Role of nitric oxide and tumor necrosis factor on lung injury caused by ischemia/reperfusion of the lower extremities. J Vasc Surg. 1997;26:647-56.
8. Ladaga LG, Nabseth DC, Besznyak I, Hendry WF, McLeod G, Deterling RA Jr. Preservation of canine kidneys by hypothermia and hyperbaric oxygen: long-term survival of autografts following 24-hour storage. Ann Surg. 1966;163:553-8.
9. Largiader F, Manax WG, Lyons GW, Lillehei RC. In vitro preservation of canine heart and lung. Arch Surg. 1965;91:801-4.
10. Guimarães FA, Taha MO, Simões MJ, et al. Use of hyperbaric oxygenation in small bowel preservation for transplant. Transplant Proc. 2006;38:1796-9.
11. Ijichi H, Taketomi A, Soejima Y, et al. Effect of hyperbaric oxygen on cold storage of the liver in rats. Liver Int. 2006;26:248-53.
12. Zafren K. Gamow bag for high-altitude cerebral oedema. Lancet. 1998;352:325-6.
13. Edwards ML. Hyperbaric oxygen therapy. Part 1: history and principles. J Vet Emerg Crit Care (San Antonio). 2010;20:284-8.
14. Giannone FA, Treré D, Domenicali M, et al. An innovative hyperbaric hypothermic machine perfusion protects the liver from experimental preservation injury. ScientificWorldJournal. 2012;2012:573410.
15. Chaves JC, Neto FS, Ikejiri AT, et al. Period of Hyperbaric Oxygen Delivery Leads to Different Degrees of Hepatic Ischemia/Reperfusion Injury in Rats. Transplant Proc. 2016;48:516-20.
16. Simsek K, Ay H, Topal T, et al. Long-term exposure to repetitive hyperbaric oxygen results in cumulative oxidative stress in rat lung tissue. Inhal Toxicol. 2011;23:166-72.
17. Uluyol S, Demir L, Kilicaslan S. The Effects of Short- and Long-Term Exposure to Hyperbaric Oxygen on Nasal Mucociliary Clearance. J Craniofac Surg. 2017;28:331-3.
18. Bayrakci B. Preservation of organs from brain dead donors with hyperbaric oxygen. Pediatr Transplant. 2008;12:506-9.
19. Oyaizu T, Enomoto M, Yamamoto N, et al. Hyperbaric oxygen reduces inflammation, oxygenates injured muscle, and regenerates skeletal muscle via macrophage and satellite cell activation. Sci Rep. 2018;8:1288.
20. Kjellberg A, De Maio A, Lindholm P. Can hyperbaric oxygen safely serve as an anti-inflammatory treatment for COVID-19? Med Hypotheses. 2020;144:110224.