Turk Thorac J 2018; 19: 61-5 DOI: 10.5152/TurkThoracJ.2018.17060

Original Article

The Role of Duration of Hyperbaric Oxygen Therapy on Lung Injury: An Experimental Study Lung Injury and Hyperbaric Oxygen Therapy

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Cite this article as: Oruç M, Esen B, Taylan M, et al. The role of duration of hyperbaric oxygen therapy on lung injury: An experimental study Lung injury and hyperbaric oxygen therapy. Turk Thorac J 2018: 19; 61-5.

Abstract

OBJECTIVES: We aim to histopathologically analyze the effect of hyperbaric oxygen (HBO) therapy in the lung tissue.

MATERIAL AND METHODS: A total of 21 rabbits were divided into three groups, with each containing seven rabbits. Group 1 was the control group. Group 2 underwent HBO of 3 atmosphere absolute (ATA) for 90 min/day for 7 days. In group 3, HBO at 3 ATA was administered 90 min/day for 28 days. Oxygen saturation (SpO_2) was determined by pulse oximetry before and after administration of HBO. Rabbits were sacrificed, and the apex of the right lung was excised.

RESULTS: SpO_2 was 98-100% in all rabbits before HBO administration. After the procedure, the mean SpO_2 was 92% and 83% in groups 2 and 3, respectively. As expected, histopathologic examination in group 1 was normal. In group 2, congestion in the lung vessels, mononuclear cell infiltration in the bronchial mucosa, interstitial edema, and alveolar dilation were evident. Histopathologic examination in group 3 indicated diffuse alveolar edema, peribronchial mononuclear cell infiltration, thickening of the alveolar and vessel wall, and intraalveolar hemorrhage.

CONCLUSION: There is a strict relationship between duration of HBO administration and severity of lung injury.

KEYWORDS: Hyperbaric oxygen therapy, lung injury, blood oxygen saturation

Received: *21.08.2017* ***Accepted:*** *19.11.2017*

INTRODUCTION

Hyperbaric oxygen (HBO) therapy is a procedure to administer 100% oxygen in a chamber with a higher pressure than sea level. HBO has positive therapeutic effects on immunity, oxygen transport, and hemodynamics on the injured tissues and maintains tissue viability in microvascular complications of wounds, burns, necrotizing soft tissue infections, osteomyelitis, intracranial abscess, and carbon monoxide poisoning [1,2]. In addition to the beneficial effect of HBO, several studies pointed out its adverse effects in the liver, central nervous system, and lung by production of reactive oxygen species (ROS). Standard dose of HBO is 2 or 3 atmosphere absolute (ATA) (1 ATA: 760 mm Hg) [1]. The conflict between recovery and duration of the procedure continues regarding its benefits and disadvantages. There are limited studies relating to lung tissue injuries in animals [3-5]. In these studies, the same duration was compared with different pressures. However, there is a lack of study that examines the same pressure within different durations.

In the present study, our aim is to experimentally examine the effect of HBO at different durations but at the same pressure in the lung tissue in rabbits.

MATERIAL AND METHODS

The ethics committee of experimental animal studies of Dicle University approved the study. Twenty-one New Zealand rabbits between 2.5 and 3 kg in weight (mean weight 2.7 kg) were equally divided into three groups, kept in appropriate heat and humidity, and received daylight for 12 h/day as described by Jamieson [4]. Before the procedure, sedative anesthesia was administered intraperitoneally with ketamine hydrochloride (Ketalar 5 mg/kg; Pfizer) plus xylazine (Rompun 2 mg/kg; Bayer). Feathers on the anterior and posterior sides of the ears were removed, and vessels became visible by alcohol administration. Oxygen saturation (SpO_2) was non-invasively analyzed by using ear probes.

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Group 1 consists of control rabbits with no intervention instituted. Rabbits in groups 2 and 3 received HBO at 3 ATA for 90 min/day for 7 and 28 days, respectively. Rabbits were one by one placed in a cylinder containing 100% oxygen for 90 min. These oxygen cages have 90 cm internal diameter and 1 m length. Consequently, atmospheric pressure was gradually increased to 3 ATA and gradually decreased to 1 ATA. Rabbits are free to move in the cylinder. None of rabbits were intubated or received extra respiratory support except 100% oxygen. Similarly, no other medication or chemical agent was administered. After the procedure, rabbits were again transferred to their shelter. Lastly, SpO_2 of rabbits was measured and recorded. High dose Pentothal was used for sacrifice, and thoracotomy was subsequently performed for excisional biopsy. Lung tissue specimens were fixed with 10% neutral formalin and embedded into paraffin blocks. Specimens with 5 µm thickness were stained with hematoxylin and eosin. Histopathologic examination was performed by using light microscopy (Zeiss Imager 2). Alveolar hemorrhage was evaluated as Grade 0, Grade 1, Grade 2, and Grade 3 as shown in Table 1 [6]. Histological findings were evaluated as normal, very mild, moderate, and severe lesions in Table 2 [7].

Table 1. Evaluation of alveolar hemorrhage

Grading	Alveolar hemorrhage in light microscope
Grade 0	No hemorrhage
Grade 1	Few erythrocytes in alveoli
Grade 2	Clusters of erythrocytes that do not fully fill alveoli
Grade 3	Erythrocyte populations completely filling alveoli

Table 2. Pathological examination

Grading	Pathological lesions
Normal	No pathological lesions
Mild	Focal inflammation
Moderate	Perivascular, peribronchial edema, vascular congestion, and inflammation
Severe	Intrapulmonary, interstitial edema, severe vascular congestion, and thrombosis

Statistical Analysis

Statistical Package for Social Sciences version 18.0 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. Since the normal distribution was not appropriate, Mann-Whitney U test

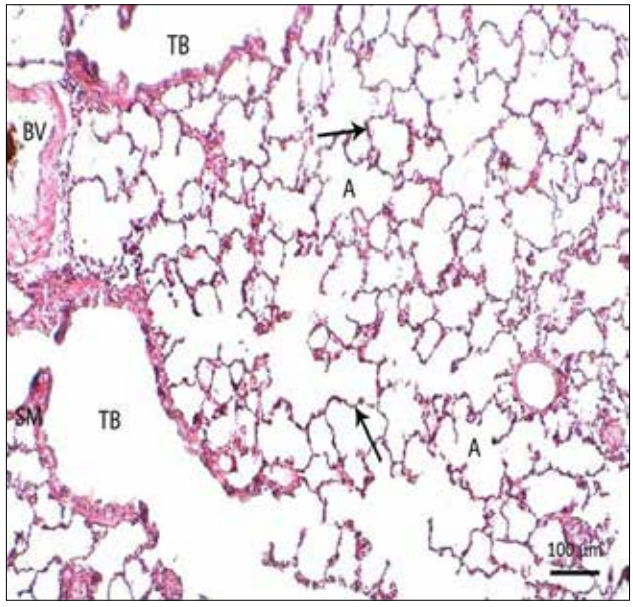

Figure 1. Histologic features of larger terminal bronchioles and alveoli in the control group. TB: terminal bronchioles; A, alveoli. Arrow: Intraalveolar septum
BV: blood vessel; SM: smooth muscle (H&E, bar: 100 µm)

Table 3. Pre- and post-HBO saturation levels (%) of rabbit groups

	Pulse SpO_2 level				
N	G1 (control)	G2 (pre-HBO)	G2 (post-HBO)	G3 (pre-HBO)	G3 (post-HBO)
1	100	99	87	99	91
2	98	99	88	100	90
3	99	100	91	100	80
4	100	100	93	99	79
5	100	100	95	97	82
6	99	100	94	100	85
7	100	99	96	100	77
Min-max	98-100	99-100	87-96	97-100	77-100
Median	100	100	94	100	94
Mean±SD	99.4±0.7	99.5±0.5	92.1±3.5	99.2±1.1	91.6±7.5
	p between groups				
	Group	p	Group	p	
	G1-G2 pre	0.827	G1-G2 post	0.002	
	G1-G3 pre	0.943	G1-G3 post	0.002	
	G2-G3 pre	0.827	G2-G3 post	0.01	

HBO: hyperbaric oxygen; SpO_2: oxygen saturation

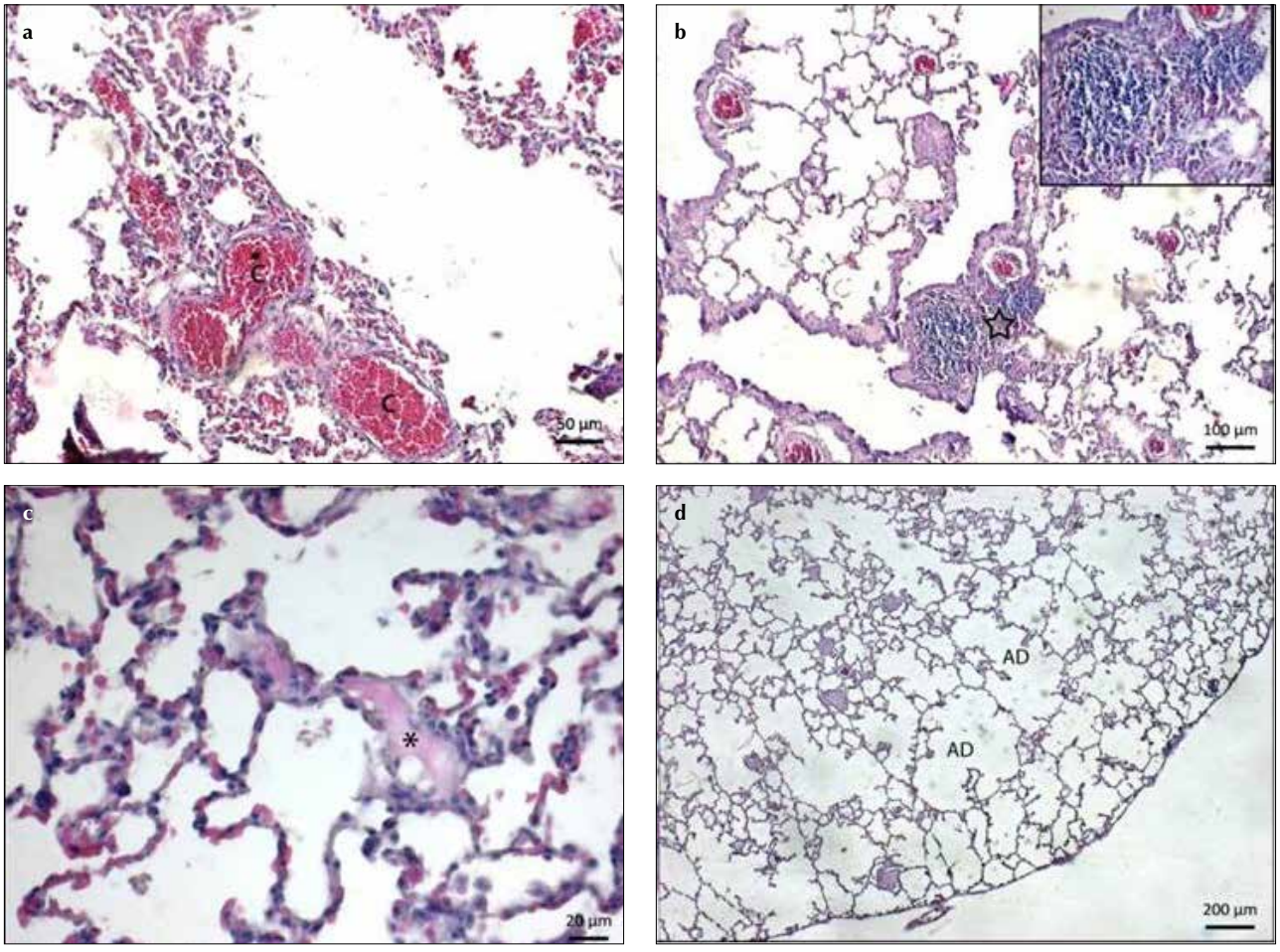

Figure 2. a-d. Histopathologic examination of rats that received hyperbaric oxygen for 7 days. (a) Congestion in the vessels, (b) Mononuclear cell infiltration in the peribronchial and perivascular area. Inset: A higher magnification of the rectangle in the upper right corner reveals mononuclear cell infiltration, (c) Minimal interstitial edema, (d) A lower magnification of the lung reveals dilatation of the alveoli (emphysematous changes).
C: congestion; ¶ : mononuclear cell infiltration; *: edema; AD: alveolar dilatation (H&E, a. bar: 50 µm, b. bar: 100 µm (inset, bar: 20 µm), c. bar: 100 µm, d. bar: 200 µm)

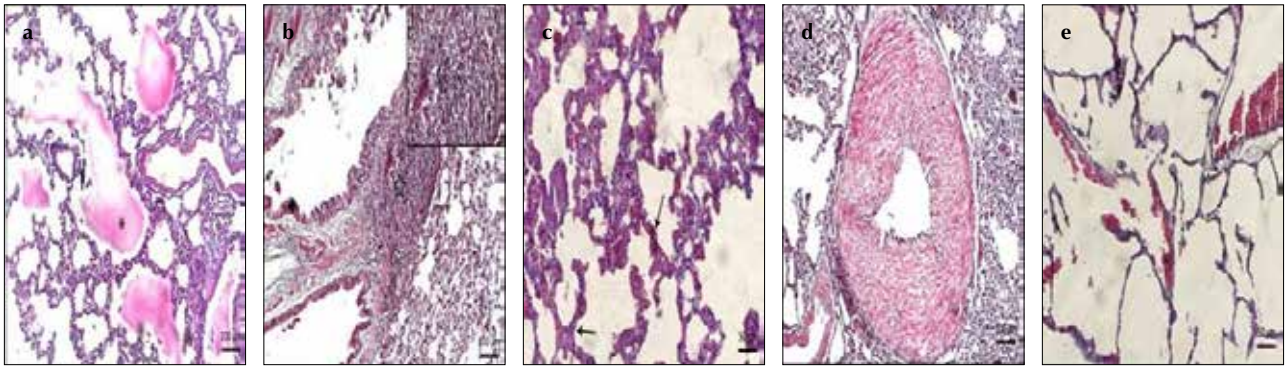

Figure 3. a-e. Histopathologic examination of rats that received hyperbaric oxygen for 28 days. (a) Diffuse intraalveolar edema and alveolar wall thickening, (b) Peribronchial mononuclear cell infiltration. Inset: A higher magnification of the rectangle in the upper right corner reveals mononuclear cell infiltration, (c) Apparent alveolar wall thickening, (d) Thickening of the vessel wall, (e) Intraalveolar hemorrhage.
*: edema; ☆: mononuclear cell infiltration; arrow: alveolar wall thickening; A: alveolus; H: intraalveolar hemorrhage. a. H&E, bar: 100 µm. b. Trichrome Masson, bar: 100 µm (inset, bar: 20 µm). c. H&E, bar: 50 µm. d. Trichrome Masson, bar: 100 µm. e. Trichrome Masson, bar: 50 µm

was used to compare the continuous variables of each of the two groups. A p value of <0.05 was considered significant.

RESULTS

Every rabbit survived until sacrificed, and none exhibited symptoms such as nausea, vomiting, or weight loss. At baseline evaluation, the mean SpO_2 of rabbits in three groups was 98%-100%. After the procedure, control rabbits kept SpO_2 level at98%-100%. The mean SpO_2 of rabbits in groups 2 and 3 was 92.28% (87-96) and 83.42% (77-91), respectively (Table 3).

Figure 1 shows the normal histopathologic examination of rabbits in group 1. Histopathologic examination of rabbits in group 2 that were exposed to 3 ATA for 7 days indicated congestion in the lung vessels, mononuclear cell infiltration in

Table 4. Evaluation of specimens in terms of alveolar hemorrhage and pathological lesions

Groups	Specimen	Alveolar hemorrhage*	Pathological lesions**
Group 1			
	1	0	-
	2	0	-
	3	0	-
	4	1	+
	5	0	
	6	0	-
	7	0	-
Group 2			
	1	1	+
	2	1	+
	3	2	++
	4	2	++
	5	2	++
	6	1	+
	7	1	+
Group 3			
	1	3	+++
	2	3	+++
	3	2	++
	4	3	+++
	5	3	+++
	6	3	+++
	7	2	++

* 0: Grade 0 ** (-) No pathological lesions

1: Grade 1 (+) Mild pathological lesions

2: Grade 2 (++) Moderate pathological lesions

3: Grade 3 (+++) Severe pathological lesions

the bronchial mucosa, interstitial edema, alveolar dilatation, and moderate pathological lesions (Figure 2). In group 3, diffuse alveolar edema, peribronchial mononuclear cell infiltration, thickening of the alveolar and vessel wall, intraalveolar hemorrhage, and severe pathological lesions were observed (Figure 3). When the specimens were graded on the light microscope for hemorrhage, 14% of the rabbit's lungs in group 1 had Grade 1 and the remaining 86% had Grade 0; 42% in group 2 had Grade 2 and the remaining 58% had Grade 1; and 28% in group 3 had Grade 2 and the remaining 72% had Grade 3 (Table 4).

At the initial evaluation, there was no difference in SpO_2 in three groups. After HBO exposure, the saturation level of group 2 was lower than that of the control group (p=0.002). As the same, the post-HBO SpO_2 of group 3 was lower than that of the control group (p=0.002). Group 3 exposed to HBO for 28 days was found to be significantly desaturated compared with group 2 exposed to HBO for 7 days (p=0.01). We observed that the increased duration of HBO exposure is related to deeper oxygen desaturation (Table 1).

DISCUSSION

The present study indicates that the adverse effects of HBO therapy in the lung tissue increase as the duration of HBO exposure prolongs. For optimal beneficial effect, HBO exposure should be minimized and discontinued when the target of therapy is achieved.

In normal conditions, very low percent of oxygen is dissolved in blood. However, in hyperbaric situations, high percent of oxygen that is adequate for daily need is dissolved [8]. The aim of HBO therapy is to increase the percent of dissolved oxygen and improve tissue oxygenation. Additionally, HBO helps wound healing by augmentation of tissue oxygenation. HBO therapy also supports conversion of carboxyhemoglobin to oxyhemoglobin in mitochondrial cytochrome in severe carbon monoxide intoxication [9].

Excessive oxygen supply may cause harmful effect by production of ROS that have potential to cause structural changes in proteins, lipids, carbohydrates, and deoxyribonucleic acid [10,11]. Peroxidation is the major mechanism of oxygen radicals related to cell membrane injury [12]. There are similar studies with regard to toxic dose of oxygenation. Raman et al. [13] concluded that HBO at 2 ATA may cause free oxygen radicals and oxidative stress. Oxygen-derived free radicals are short-acting reactants in aerobic microorganisms, which are associated with metabolic deterioration and cell death [4,8,10]. Researchers of another study showed that 100% oxygen, even in normal pressure, may cause increased activity of ROS [14]. Oter et al. [15] emphasized that there is no absolute dose limit or duration of HBO that causes harmful effect. It would be reasonable to state that the beneficial and harmful effect of HBO administration should be considered, and therapy should be individualized depending on the severity of the disorder.

A number of pathogenetic mechanisms of lung injury are attributed to HBO therapy. HBO administration increases epithelial permeability of the lung endothelium leading to interstitial and alveolar edema and hemorrhage [16]. Similarly, we observed peribronchial and perivascular mononuclear cell infiltration, interstitial edema, and alveolar dilatation with HBO administration. As the duration of HBO therapy was quadruplicated in the same dose, we showed that diffuse intraalveolar edema, alveolar and vessel wall thickness, and intraalveolar hemorrhage increase. Lung injury was significantly associated with a decrease of SpO_2 in groups 2 and 3. Histopathologic examination determined that the severity of lung injury was well correlated with a decrease in SpO_2.

Jamieson et al. [4] emphasized that independent from the duration of the procedure, dose of HBO has the strongest association with lung injury. Jacobs et al. [17] concluded that ROS are seen at the first 48 h of HBO therapy in various degrees depending on the dose of HBO. Patel et al. [5] demonstrated that HBO at 3 ATA for 120 min was relatively safe; however, longer durations and higher pressures may cause otalgia, pneumothorax, and air embolism. Severe complications related to ruptures in pulmonary vascular bed are rarely

seen. In an experimental animal study, severe edema in the lungs and subsequent death were reported in rats subjected to HBO for 90 min at 4 ATA [18]. Oter et al. [19] concluded that for optimal beneficial effect, HBO should be administered at the lowest effective dose to minimize HBO related to oxidative stress.

Efforts are ongoing to diminish the harmful effects of HBO therapy. It would be reasonable to state that HBO therapy should be started at minimum duration and gradually increased to achieve the goal of therapy. Concomitantly, participants should be carefully monitored for possible adverse outcomes. Therapy should be discontinued when the target of therapy is achieved and maintained.

A growing number of studies recommend HBO use, but there are several studies relating to its hazards in the lung tissues. There are few studies associated with HBO use at the same pressure and longer durations. We aimed to increase oxygen pressure in blood to histopathologically define the hazards of HBO use in histological examination and pulse oximeter measurement.

The present study has several potential drawbacks. The first limitation was that we did not examine arterial blood gases and biochemical variables. The second limitation was that measurement of ROS may provide beneficial information on the toxic effects of HBO in the lung tissue.

In conclusion, as indications of HBO therapy increase and HBO use becomes widespread, side effects are more frequently observed. Augmentation of pressure has no additional beneficial effect, but toxic events may increase. Duration of treatment and quantity of pressure are critical to determine the risk of HBO toxicity. Large scale studies are warranted to reach more precise conclusion.

Ethics Committee Approval: Ethics committee approval was received for this study from Dicle University (Protocol No: 2015/35).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.O., B.E., A.Ş., Y.N.; Design - M.O., B.E.; Supervision - M.O., A.Ş.; Resource - M.O., M.T.; Materials - M.O., B.E.; Data Collection and/or Processing - M.O., Y.N., B.E.; Analysis and/or Interpretation - M.O., B.E.; Literature Search - M.O., A.Ş.; Writing - M.O., B.E.; Critical Reviews - M.O., A.Ş., B.E.

Acknowledgements: The authors would like to thank Dr. A. Engin Atay for his contribution on editing of the manuscript.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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