Original Research

Turk J Immunol 2023;11(1):37-41
DOI: 10.4274/tji.galenos.2023.78942

Effects of Hyperbaric Oxygen on Anti-dsDNA Antibodies and IL-6 in BALB/c Mice with Pristane-Induced Lupus Nephritis

Indri Dwi Murbani[1], Titut Harnanik[2], Soetjipto[3]

[1]Master Program of Basic Medical Science, Faculty of Medicine, Airlangga University, Surabaya, Indonesia
[2]Department of Physiology, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia
[3]Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

Cite as: Murbani ID, Harnanik T, Soetjipto. Effects of Hyperbaric Oxygen on Anti-dsDNA Antibodies and IL-6 in BALB/c Mice with Pristane-Induced Lupus Nephritis. Turk J Immunol 2023;11(1):37-41

Received: 25.08.2022 **Accepted:** 05.04.2023

Corresponding Author: Soetjipto, Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, Indonesia
Phone: +6281331340518 **E-mail:** soetjipto1950@gmail.com **ORCID:** orcid.org/0000-0003-2203-5565

Abstract

Objective: Lupus nephritis (LN) has negative outcomes in patients. It is associated with enhanced oxidative stress. The aim in this study was to explore whether hyperbaric oxygen (HBO) made beneficial effects on the levels of anti-double stranded DNA (dsDNA) antibodies and interleukin-6 (IL-6) within the serum of LN mode treated with HBO compared to LN mode without HBO and negative controls.

Materials and Methods: The samples were separated into 3 groups (negative control, LN and LN + HBO groups) in 24 mice. Pristane was injected into female BALB/c mice to make the LN model. After 60 days, HBO therapy was administered to the LN model for 10 sessions. Levels of anti-dsDNA antibodies and IL-6 serum of all groups were examined by ELISA.

Results: The level of anti-dsDNA antibodies and IL-6, as the biomarker of LN activity, was essentially diminished within HBO [a statistically significant decrease ($p<0.050$) between the LN + HBO group and LN group].

Conclusion: The inflammation in LN can be reduced by decreasing anti-dsDNA antibodies and IL-6 levels with HBO through the repairment of hypoxia.

Keywords: Anti-dsDNA, IL-6, lupus nephritis, hyperbaric oxygen, pristane

Introduction

Lupus nephritis (LN) is one of the problematic systemic lupus erythematosus (SLE) manifestations (1). It is represented by impaired renal function, inflammatory cytokines secretion, and production of autoantibody (2). The immunosuppressive regimens and corticosteroids are not specific to the disease, and they have led to side effects on the renal damage (3,4). Fewer toxicity therapies are needed to find.

LN may cause proteinuria with more than 0.5 grams per day (5,6). Renal injury and tissue hypoxia are initiated by immune complex deposition and subsequent glomerular pathology (7). The secretion of anti-double stranded DNA (dsDNA) antibodies could be evaluated as diagnostic and prognostic marker (8,9). It has been accepted as a surrogate marker for disease activity of LN in everyday clinical routines (10). The increased levels of interleukin-6 (IL-6) in serum are related to anti-dsDNA antibodies titers and the activity of the disease (11).

LN can be induced by pristane, or TMPD (2,6,10,14-tetramethylpentadecane) in mice (12). Pristane injection generates autoantibodies that are comparable to LN in humans (13). Pristane is the finest example of an environmental element inducing lupus-like disease (14). This is usually utilized in the experiment setting (12).

Hyperbaric oxygen (HBO) therapy is a treatment with breathing 100% oxygen in the chamber and the pressure above sea level decreases inflammation (15). Its effecy in LN is yet to be identified. Therefore, the aim of this study was to evaluate whether HBO therapy has beneficial effects on LN in mice model by decreasing anti-dsDNA antibodies and IL-6.

ORCID: I. D. Murbani 0000-0003-4212-3298, T. Harnanik 0000-0003-1282-7614, S. Soetjipto 0000-0003-2203-5565

Materials and Methods

Subjects

Female mice with the strain BALB/c and 8 weeks of age (body weight: 25 ± 3 g) were obtained from Biosains Laboratory, Brawijaya University, Indonesia. Then, they were kept 7 days of acclimatization before the trial. All mice were given sterilized food, water ad libitum, and were housed for 12:12 h in light and dark. The room temperature was controlled at 22 ± 1°C, with positive pressure laminar flow. Mice were housed (maximum, 8 per cage). This trial was held between January and March 2022 and approved by the Ethical Committee of Medical Faculty, Airlangga University, Indonesia.

Experimental Design

A simple random sampling was used to select and divide all female mice into three groups (including 8 mice in each group). There was a control group (injection with normal saline and not exposed to HBO), LN group (a single injection of 0.5 cc or 0.783 g/mL pristane intraperitoneally and not exposed to HBO), and LN + HBO group (a single injection of 0.5 cc or 0.783 g/mL pristane intraperitoneally and exposed to HBO). LN was characterized by the occurrence of proteinuria observed two months after pristane injection.

HBO Therapy

The LN + HBO group got oxygen in the chamber for the animal within 10 days. The therapy session started with 10 minutes of breathing the air from 2.4 atmospheres absolute (ATA) and followed by 90 minutes of breathing 100% oxygen (partitioned into 3 divisions each of 30 minutes with five minutes intervals of breathing the air). After that, breathing the air for 10 minutes at 1 ATA. HBO therapy schedule is shown in Figure 1.

Determination of Anti-dsDNA Antibodies and IL-6

Thirty minutes after the group of LN + HBO completed HBO therapy, all mice were anesthetized. Serum was collected from the hearts of mice. The examinations of anti-dsDNA antibodies and IL-6 were carried out by ELISA. Anti-dsDNA antibodies and IL-6 levels were obtained by mouse anti-double stranded DNA and IL-6 antibodies from Korain Biotech Co., Ltd, Shanghai, China. Then, they were read by an ELISA reader.

Statistical Analysis

The version 24.0 from IBM SPSS was utilized for analyses. The descriptive data were shown as mean ± SD. Statistical analysis with the level of significance was appraised as p=0.050.

Results

After the 10-day administration of HBO, no animal died. The urine examination was carried out on the 60th day. Mice in the control group did not show proteinuria, while the LN group and the LN + HBO group showed proteinuria with an average of +3 (≥500 mg/dL).

The normality test performed by using the One-Sample Kolmogorov-Smirnov analysis revealed that the levels of anti-dsDNA antibodies and IL-6 had normal distribution with p>0.050. The results of anti-dsDNA antibodies and IL-6 showed homogeneous variance with the Levene's test (p>0.05). The test results of anti-dsDNA antibodies between the groups are shown in Table 1.

Two months after pristane injection, the value of mean ± SD increased in the serum anti dsDNA antibodies (77.37 ± 1.31 ng/mL) in the LN group compared to the negative control group (17.64 ± 3.46 ng/mL). Furthermore, after HBO therapy, the LN + HBO group exhibited lower levels of the serum anti dsDNA antibodies (74.28 ± 2.44 ng/mL) than the LN group.

LN group had statistically significantly higher (p=0.001) level of anti-dsDNA antibody. Anti-ds-DNA antibody level was statistically signifantly lower in LN+HBO group compared to that of LN group (p=0.025).

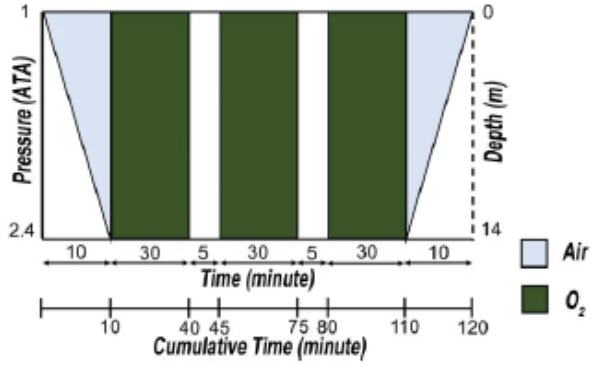

Figure 1. HBO therapy schedule. Therapy started with breathing normal air for 10 minutes from a pressure of 2.4 ATA and followed by breathing 100% O_2 for three fraction each of 30 minutes with two intervals of five minutes breathing normal air and then breathing normal air for 10 minutes at 1 ATA.

Table 1. Titres of anti-dsDNA antibodies

Group	Anti-dsDNA antibodies (ng/mL)			
	Mean ± SD	Minimum	Maximum	p-value
Control group	17.64 ± 3.46	13.47	22.70	*0.001
LN	77.37 ± 1.31	75.15	79.60	
LN + HBO	74.28 ± 2.44	70.08	77.69	*0.025
F=1379				

*Mean difference was compared to that of the LN group, significant if p<0.05

Two months after the pristane injection, IL-6 was increased (26.80 ± 4.28 pg/mL) in the LN group, in comparison with the control group (22.78 ± 2.02 pg/mL). Furthermore, after HBO therapy, the LN + HBO group exhibited lower levels of IL-6 (21.40 ± 4.13 pg/mL) than the that of LN group. IL-6 measurements are shown in Table 2.

The mean IL-6 level of the LN + HBO group was statistically significantly decreased (p=0.007) when compared to that of the LN group. IL-6 levels were not found to be statistically significantly different between LN + HBO and control groups (p=0.454) (Figure 2).

Discussion

Nowadays, despite being beneficial to suppressing the progression of the disease, common treatments for LN seldom offer remission in the long term (2). The existing drugs have a small impact on kidney complications (16). Recent studies have shown that HBO therapy diminished inflammation (17). Therefore, one may speculate that HBO therapy can reduce disease activity in LN through decreased inflammation. This hypothesis is supported by Chen et al., who described that HBO therapy reduces spontaneous immunoglobulin production, proteinuria, and anti-dsDNA antibodies in murine models of autoimmunity (18). However, the exact role of HBO therapy in the disease activity in LN remains unclear. We aimed to evaluate whether HBO therapy had beneficial effects on LN. This study limited the use of HBO therapy in changing anti-dsDNA antibodies and IL-6.

Table 2. Description of differences in IL-6 between the experimental groups

Group	IL-6 (pg/mL)			
	Mean ± SD	Minimum	Maximum	p-value
Negative control	22.78 ± 2.02	20.10	26.85	*0.038
LN	26.80 ± 4.28	21.51	33.81	
LN + HBO	21.40 ± 4.13	17.45	30.48	*0.007
F=4.777				

*Mean difference was compared to that of the LN group, significant if p<0.05

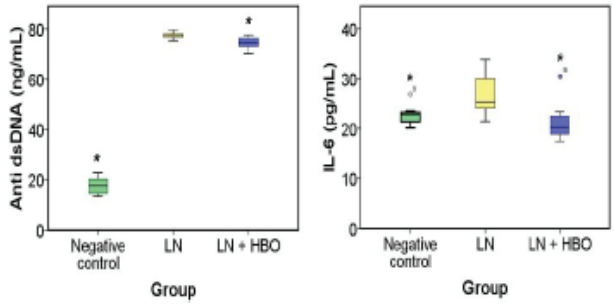

Figure 2. The comparison diagram of the average of anti-dsDNA antibodies and IL-6 in the experimental groups.

In this study, we utilized a widely accepted animal model. Our results confirmed that administration of pristane injection in experimental animals could induce LN, which is characterized by proteinuria. Proteinuria in LN model mice was detected 2 months after pristane injection. Urine analysis showed that, mice in control group showed no proteinuria, while mice in the LN group and the LN + HBO group were found have proteinuria with an average of +3 (≥500 mg/dL). This was in accordance with previous research that LN model in which. LN was induced with proteinuria (2,18-20).

The mean of anti dsDNA antibodies showed a significant increase after the mice received pristane injection. The level anti dsDNA antibodies in LN model mice that received OHB therapy in the LN + HBO group decreased when compared to the mean anti dsDNA antibodies in nephrotic models of mice that did not receive HBO therapy. These results were similar to those in the the study conducted by Lin et al. (21) on BALB/c mice injected with pristane, showing an increased in the anti-dsDNA antibodies.

A anti-dsDNA antibodies have a cut-off value of 12 ng/mL. High anti-dsDNA antibodies are characterized with the levels of more than 100 ng/mL in humans (22). Anti-dsDNA antibodies in mice injected with pristane averaged 59.78 ng/mL, whereas, it was 38.26 ng/mL in the control group (23). Anti-dsDNA antibodies are specific for LN (24). It contributes to organ damage (25). HBO therapy in this study reduced anti-dsDNA antibodies. Decreased anti-dsDNA antibodies presented improvement in LN activity. This consideration is in line with a past report by Saito et al. (15), in which it was shown that HBO particularly diminished the level of anti-DNA antibodies in patients with SLE. This suggests that HBO may have functions on immune responses.

Administration of pristane injection increased IL-6 in animal models (16). The principal finding in this study was that IL-6 of the LN + HBO group after 10 sessions of HBO therapy was significantly decreased compared to the LN group. IL-6 is a proinflammatory cytokine that facilitates adaptive T helper-2 (Th2) and Th17 responses, B cell activation, and antibody production, one of which is anti-dsDNA (26). These results indicated that administration of HBO therapy could reduce the disease activity of LN, which was accompanied by a decrease in IL-6. The results, in accordance with the study of Memar et al. in 2019, showed that HBO had the effect to suppress proinflammatory cytokines such as IL-6 (27).

Evidence is scarce regarding the impact of HBO on LN. One of the studies investigating the effects of the HBO therapy on lupus was previously carried out by Chen et al., using lupus-prone autoimmune (NZB x NZW) F1 mice with 90 minutes daily pressure using 2.5 ATA

in 2 weeks (28). The result showed that HBO therapy increased survival, diminished proteinuria, and anti-dsDNA antibodies (28). Different from the previous studies, they investigated the lupus activity using a different strain of mice through proteinuria and anti-dsDNA antibodies after HBO (28). They utilized higher pressure than that in our experiment. The length of therapy was over 2 weeks (28).

Novak et al. (29) studied HBO therapy in BALB/c mice with colitis. They showed that HBO therapy could reduce IL-6. Saito et al. (15) also demonstrated that long-term HBO therapy in another strain of autoimmune mice suppressed the development of autoimmune symptoms. Hypoxia initiated an inflammatory response, which leads to decreased secretion of proinflammatory cytokines such as IL-6 (30). HBO therapy induced changes of oxidative capabilities of immune cells and diminished the expression of the pro-inflammatory cytokines (31).

In the study on SLE in 18 female patients treated with HBO with 2.2 ATA fat a duration of 60 minutes for 10 days, anti-nuclear antibody titres were not found to be different in 10^{th} and 30^{th} day follow-up. (32). The air pressure, duration, and parameters are also different in this study.

We utilized the Treatment Table 9, the HBO table dosing protocol created by the United States Navy. The LN + HBO group was exposed to 2.4 ATA breathing the air for 10 minutes. After that, they breathed 100% oxygen for 90 minutes, partitioned by 3 × 30 minutes, and breathed the air at 2.4 ATA at two-time intervals of 5 minutes. Then, after 10 minutes of breathing the air, the pressure was decreased to 1 ATA. The treatment group received HBO treatment for 10 consecutive days.

The impact of HBO therapy in combination with drugs should further studied. It is also needed to evaluate the effectiveness of the long-term use of HBO on LN activity.

Conclusion

HBO could be effective in LN treatment. We have shown that HBO could diminish LN activity by reducing the levels of anti-dsDNA and IL-6.

Ethics

Ethics Committee Approval: The Health Research, Airlangga University Faculty of Medicine, Surabaya was approved by the Ethics Committee (approval number: 17/EC/KEPK/FKUA/2022, date: 24.01.2022).

Informed Consent: Informed consent from all patients were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: I.D.M., T.H., S., Design: I.D.M., S., Data Collection or Processing: I.D.M., Analysis or Interpretation: I.D.M., T.H., S., Literature Search: I.D.M., T.H., S., Writing: I.D.M., S.

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

Financial Disclosure: The authors declare that they have no relevant financial.

References

1. Yang F, He Y, Zhai Z, Sun E, Guan Q. Programmed Cell Death Pathways in the Pathogenesis of Systemic Lupus Erythematosus. J Immunol Res. 2019;2019:1.
2. Yan Y, Zhang Z, Chen Y, Hou B, Liu K, Qin H, et al. Coptisine Alleviates Pristane-Induced Lupus-Like Disease and Associated Kidney and Cardiovascular Complications in Mice. Front Pharmacol. 2020;11:1-2.
3. Morell M, Pérez-Cózar F, Marañón C. Immune-related urine biomarkers for the diagnosis of lupus nephritis. Int J Mol Sci. 2021;22:1-2.
4. Pan Q, Chen X, Liao S, Chen X, Zhao C, Xu YZ, et al. Updated advances of linking psychosocial factors and sex hormones with systemic lupus erythematosus susceptibility and development. PeerJ. 2019;2019:1-2.
5. Almaani S, Meara A, Rovin BH. Update on lupus nephritis. Clin J Am Soc Nephrol. 2017;12:825-35.
6. Selvaraja M, Abdullah M, Arip M, Chin VK, Shah A, Nordin SA. Elevated interleukin-25 and its association to Th2 cytokines in systemic lupus erythematosus with lupus nephritis. PLoS One. 2019;14:1-12.
7. Ping-Min Chen, Parker C. Wilson, Justin A. Shyer, Margaret Veselits, Holly R. Steach, Can Cui, Gilbert Moeckel, Marcus R. Clark JC. Kidney Tissue Hypoxia Dictates T-Cell Mediated Injury in Murine Lupus Nephritis. Sci Transl Med. 2020;12:1-2.
8. Kelley BP, Corrigan JJ, Patel SC, Griffith BD. Neuropsychiatric lupus with antibody-mediated striatal encephalitis. Am J Neuroradiol. 2018;39:2263-9.
9. Tunakan Dalgıç C, Mete Gökmen EN, Sin AZ. Comparison of Different Laboratory Methods in the Detection of Anti-dsDNA Antibodies and Their Diagnostic Utility. Turk J Immunol. 2020;8:38.
10. Caster DJ, Powell DW. Utilization of Biomarkers in Lupus Nephritis. Adv Chronic Kidney Dis. 2019;26:351-9.
11. Samotij D, Reich A. Biologics in the treatment of lupus erythematosus: A critical literature review. Biomed Res Int. 2019;2019:1-10.
12. Fu D, Senouthai S, Wang J, You Y. Vasoactive intestinal peptide ameliorates renal injury in a pristane-induced lupus mouse model by modulating Th17/Treg balance. BMC Nephrol. 2019;20:2.
13. Devarapu SK, Anders HJ. Toll-like receptors in lupus nephritis. J Biomed Sci. 2018;25:1-11.
14. Richard ML, Gilkeson G. Mouse models of lupus: What they tell us and what they don't. Lupus Sci Med. 2018;5:3.
15. Saito K, Tanaka Y, Ota T, Eto S, Yamashita U. Suppressive effect of hyperbaric oxygenation on immune responses of normal and autoimmune mice. Clin Exp Immunol. 1991;86:322-7.
16. Hikmah Z, Endaryanto A, Gede ID, Setijo A. Heliyon Nigella sativa L. as immunomodulator and preventive effect on renal tissue damage of lupus mice induced by pristane. 2022;8:1.

17. Benkő R, Miklós Z, Ágoston VA, Ihonvien K, Répás C, Csépányi-Kömi R, et al. Hyperbaric oxygen therapy dampens inflammatory cytokine production and does not worsen the cardiac function and oxidative state of diabetic rats. Antioxidants. 2019;8:2.

18. Atzeni F, Masala IF, Cirillo M, Boccassini L, Sorbara S, Alciati A. Hyperbaric oxygen therapy in fibromyalgia and the diseases involving the central nervous system. Clin Exp Rheumatol. 2020;38:S94-8.

19. Li D, Shi G, Wang J, Zhang D, Pan Y, Dou H, et al. Baicalein ameliorates pristane-induced lupus nephritis via activating Nrf2/HO-1 in myeloid-derived suppressor cells. Arthritis Res Ther. 2019;21:1-14.

20. Bonomini F, Dos Santos M, Veronese FV, Rezzani R. NLRP3 inflammasome modulation by melatonin supplementation in chronic pristane-induced lupus nephritis. Int J Mol Sci. 2019;20:1.

21. Lin Y, Yan Y, Zhang H, Chen Y, He Y, Wang S, et al. Salvianolic acid A alleviates renal injury in systemic lupus erythematosus induced by pristane in BALB/c mice. Acta Pharm Sin B 2017;7:159-66.

22. Hanaoka H, Satoh T, Yasuoka H, Kuwana M. Circulating anti-double-stranded DNA antibody-secreting cells in patients with systemic lupus erythematosus : a novel biomarker for disease activity. Lupus. 2012;21:1286.

23. Handono K, Sunarti S, Pratama MZ, Hidayat S, Solikhin MB. The Mango ' s Mistletoe Leaves Extract Ameliorates Lupus by Inhibiting the Anti-dsDNA Antibody Production, the Percentages of CD8 CD28 and CD4 CD28 T Cells. Maced J Med Sci. 2022;10:253.

24. Rekvig OP. Systemic lupus erythematosus: Definitions, contexts, conflicts, enigmas. Front Immunol. 2018;9:1-16.

25. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC. Pathogenesis of Human Systemic Lupus Erythematosus: A Cellular Perspective. Trends Mol Med. 2017;23:615-35.

26. Arriens C, Wren JD, Munroe ME, Mohan C. Systemic lupus erythematosus biomarkers: The challenging quest. Rheumatology (Oxford). 2017;56:i32-45.

27. Memar MY, Yekani M, Alidazeh N, Baghi HB. Hiperbaric oxygen therapy: antimicrobial mechanisms and clinical application for infections. Biomed Pharmacother. 2019;109:440-7.

28. Chen SY, Chen YC, Wang JK, Hsu HP, Ho PS, Chen YC, Sytwu HK. Early hyperbaric oxygen therapy attenuates disease severity in lupus-prone autoimmune (NZB × NZW) F1 mice. Clinical Immunology. 2003;108:103-10.

29. Novak S, Drenjancevic I, Vukovic R, Kellermayer Z, Cosic A, Tolusic Levak M, et al. Anti-Inflammatory Effects of Hyperbaric Oxygenation during DSS-Induced Colitis in BALB/c Mice Include Changes in Gene Expression of HIF-1 α, Proinflammatory Cytokines, and Antioxidative Enzymes. Mediators Inflamm. 2016;2016:1-17.

30. Choudhury R. Hypoxia and hyperbaric oxygen therapy: A review. Int J Gen Med. 2018;11:431-42.

31. Sureda A, Batle JM, Martorell M, Capó X, Tejada S, Tur JA, et al. Antioxidant response of chronic wounds to hyperbaric oxygen therapy. PLoS One. 2016;11:1-14.

32. Rabrenović M, Nikolić T, Rabrenović V, Bradić J, Trešnjić S, Petković A, et al. Impact of the hyperbaric oxygen therapy on the redox status in the patients with systemic lupus erythematosus. Vojnosanit Pregl. 2019;76:412-21.