Reproductive Sciences
18(10) 941-947

DOI: 10.1177/1933719111400635
http://rs.sagepub.com

Remission of Endometriosis by Hyperbaric Oxygen Treatment in Rats

Yavuz Aydin[1], Alev Atis[2], Seyfettin Uludag[3], Irem Tezer[4], Damlanur Sakiz[5], Hicran Acar[2] and Akin Toklu[4]

Abstract

We designed this prospective, randomized controlled animal study to determine the effects of hyperbaric oxygen (HBO) on experimentally induced endometriosis in a rat model. Surgical induction of endometriosis was performed in 40, nonpregnant, female, Wistar-Albino rats at the Experimental Medicine Research Center of Istanbul University (DETAE). Four weeks later, the first and second laparotomies for volume measurement and peritoneal fluid (PF) collection were performed, and the rats were divided randomly into the study and control groups. The study group was exposed to HBO treatment for 6 weeks. Then, a third laparotomy was performed on all of the rats. The volume, histopathologic scores, Ki-67 labeling of the endometriotic implants, and the levels of tumor necrosis factor-α (TNF-α) in the PF were measured. The mean volume of the endometriotic implants in the study group was significantly lower than that of the control group at the end of the study (57.4 ± 12.5 vs 94.6 ± 17.2 mm^3). The mean histopathological scores (1.60 ± 0.50 vs 2.42 ± 0.51), Ki-67 immunohistochemical scores (1.50 ± 0.51 vs 2.37 ± 0.49) of the endometriotic implants, and the TNF-α levels (5.33 ± 1.02 vs 8.16 ± 1.76 pg/mL) were significantly lower in the study group than in the control group. Hyperbaric oxygen treatment for 2 hours a day for 6 weeks resulted in significant remission of endometriosis in rats.

Keywords

hyperbaric oxygen, endometriosis, rat, TNF-α, Ki-67

Introduction

Hyperbaric oxygen (HBO) is 100% oxygen administered at an elevated atmospheric pressure to patients as a therapeutic intervention. Hyperbaric oxygen can be applied to treat a variety of diseases, including traumatic injuries and acute or chronic infections. The presence of proinflammatory cytokines regulates adhesion molecules and other factors, which play a significant role in HBO effects.[1,2] Specifically, HBO impairs macrophage function.[3,4] Monocytes and macrophages isolated from HBO-exposed rodents produced fewer proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), than cells from unexposed controls.[5,6] Exposure to HBO (100% at 2.3 atm) for 1 hour a day ameliorated indomethacin-induced enteropathy in rats by decreasing TNF-α and interleukin 1β (IL-1β) production.[7]

Higher levels of several cytokines, including IL-1, 6, 8, and 10, TNF-α, and vascular endothelial growth factor (VEGF), were reported in the peritoneal fluid (PF) of women with endometriosis. These cytokines may be involved in macrophage activation, inflammatory change, and enhanced angiogenesis. They reflect impaired T- and natural killer (NK)-cell functions. Endometriotic implants produce factors, such as matrix metalloproteinases (MMPs) and Bcl-2, which affect their capacity to implant into the peritoneum.[8] Recently, the activation of transcription factors, such as nuclear factor-κ B (NF-κB), was reported to be involved in regulating these inflammatory molecules.[9] In unstimulated cells, NF-κB is found in the cytoplasm and is bound to the NF-κB inhibitor IκBα, which prevents it from entering the nuclei. When the cells are stimulated, specific kinases phosphorylate IκBα, causing its rapid degradation by proteasomes. The release of NF-κB from IκBα results in the translocation of NF-κB into the nucleus, where it binds to specific sequences in the promoter regions of target genes that

[1] Department of Obstetrics and Gynecology of Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey
[2] Department of Obstetrics and Gynecology, Sisli Etfal Training and Research Hospital, Istanbul, Turkey
[3] Obstetrics and Gynecology Department, Cerrahpasa Medical Faculty of Istanbul University, Istanbul, Turkey
[4] Department of Underwater and Hyperbaric Medicine, Istanbul University Istanbul, Turkey
[5] Hystopathology Department, Sisli Etfal Training and Research Hospital, Istanbul, Turkey

Corresponding Author:
Yavuz Aydin, Department of Obstetrics and Gynecology of Cerrahpasa Medical Faculty, Istanbul University, Atakent Mah, Soyakolimpiakent sitesi, D:12 blok No:53, 34303, Kucukcekmece/Istanbul-Turkey
Email:yavuzay@istanbul.edu.tr

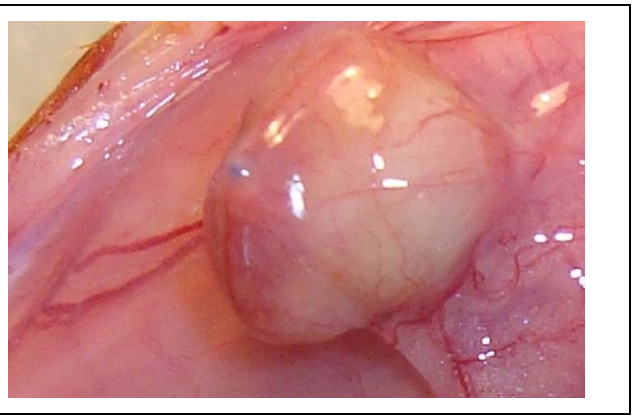

Figure 1. An endometriotic implant on peritoneal surface.

are involved in immune and inflammatory responses. The inhibition of NF-κB activation results in the decreased expression of cytokines including TNF-α.[10,11]

In many inflammatory conditions, TNF-α is present in the tissues or in the systemic circulation. While this proinflammatory cytokine contributes to the elimination of invading pathogens, inappropriate production of these molecules can be deleterious. Tumor necrosis factor-α is a well-known member of the TNF superfamily, which consists of at least 18 ligands and 29 different receptors that are involved in numerous cellular processes.[12,13] Tumor necrosis factor signals through 2 distinct receptors, TNFR1 and TNFR2, to control the expression of cytokines, immune receptors, proteases, growth factors, and cell cycle genes, which in turn regulate inflammation, survival, apoptosis, cell migration, proliferation, and differentiation.[14] Since TNF expression was discovered in the amnion and placenta, many studies have demonstrated the presence of this cytokine and its receptors in diverse human reproductive tissues.[15,16] Tumor necrosis factor has also been implicated in ovulation, corpus luteum formation, and luteolysis.[17–19]

We designed this study to test the hypothesis that relatively long-term treatment under HBO (6 weeks) would produce an immunosuppressive effect that could suppress peritoneal TNF-α and result in the remission of endometriotic lesions in a rat model.

Materials and Methods

A total of 40 mature female Wistar-Albino rats (250-300 g) were used for the experiment. All rats were provided by Experimental Medicine Research Center (DETAE) of Istanbul University and housed in the animal laboratory of same center. They were caged in a controlled environment of 22°C with 12 hours light/dark cycles and a humidity range between 40% and 60%. Standard rat feed and reverse-osmosis-purified water were provided ad libitum. All rats were allowed to have 1 week of acclimation to this environment before the experiment. This study was approved by "Animal Studies Comittee" of Istanbul University and all investigations complied with the 1996 National Academy of Science's Guide for Care and Use of Laboratory Animals.

Daily vaginal smears of the rats were taken to establish the estrous cycle of each animal. The estrous cycle was determined as follows: proestrus period (centrally nucleolated many epithelial cells), estrus period (cornified epithelial cells without nucleus), metestrus period (leukocyte, mucus, and a few cornified cells), and diestrus period (various epithelial cells, mucus, and leukocyte). Rats were observed for at least 2 successive 4-day estrous cycles. Endometriosis was induced surgically using the method described by Vernon and Wilson, during estrus.[20]

All the rats were anesthetized with an intraperitoneal (ip) administration of 50 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun, Bayer Sisli, Istanbul, Turkey).

Three 0.5 × 0.5 × 0.1 cm pieces excised by microscissors from the left uterine horn was attached onto the peritoneum; 2 pieces on right-hand side and 1 piece on left-hand side of the ventral abdominal wall close to an artery via the surgical autotransplantation technique. Of the 40 experimental rats, 1 died during the 3-week period.

Between 28 and 35 days after surgery, their daily vaginal smears were monitored and a second laparatomy was performed in their estrous phase to determine the attachment and viability of endometrial implants (Figure 1). The remaining 39 rats underwent a second exploratory laparotomy to detect endometrial implants and collect PF to measure TNF-α levels. Three dimensions of implants were measured and the volume of each was calculated by ellipsoid volume formula ($\Pi/6 \times$ length × width × height) and the total volume of implants for each rat was recorded.

After several days of recovery period, 39 rats randomly divided into 2 groups. First group consisting of 20 rats underwent HBO treatment for 6 weeks, second group is the control group consisting of 19 rats.

Hyperbaric oxygen treatment was given to study group as 2.5 atm pressure, 2 hours every day, during 6 weeks. Daily vaginal smears were monitored and at estrous cycle, a third laparotomy was performed. The 3 dimensions of the implants were measured and volumes were calculated, and the PF was collected for TNF-α levels. After endometriotic implants were totally removed and fixed with 10% formaldehyde solution for histopathological examination, animals were sacrificed. The pathologist assessing the samples was blinded to the treatment groups. The formalin-fixed endometriotic foci were embedded in paraffin blocks, sectioned at 5 mm thickness (4 sections per sample), stained with hematoxylin and eosin, and examined under a light microscope. The persistence of epithelial cells in endometrial implants was evaluated semiquantitatively as follows: 3 = well-preserved epithelial layer; 2 = moderately preserved epithelium with leukocyte infiltrate; 1 = poorly preserved epithelium (occasional epithelial cells only), and 0 = no epithelium. This evaluation was based on a previous rat endometriosis study.[21]

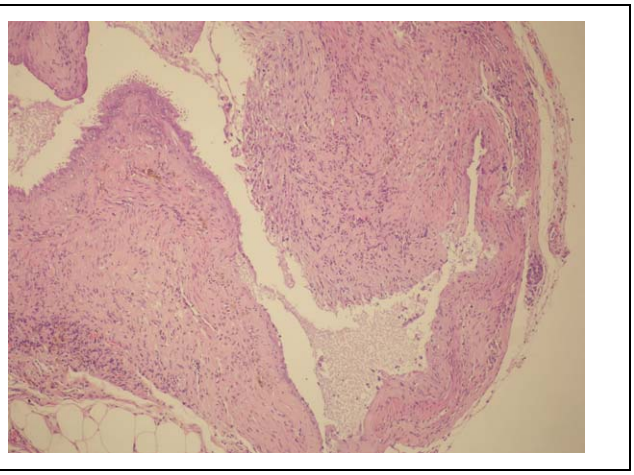

Figure 2. Histologic sections from endometriotic implant treated with hyperbaric oxygen showing poorly preserved epithelial tissue (score 1).

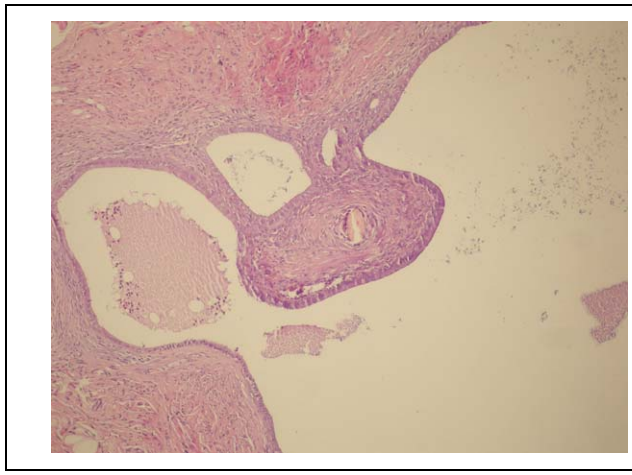

Figure 3. Histologic section from endometriotic implant of control rat indicating well-preserved viable epithelial tissue (score 3).

The sections, for Ki 67 detection, were incubated in 10 mmol/L citrate buffer (pH 6.0) at 98°C for 20 minutes and then quenched in Super Block (Abcam Laboratories, Cambridge, MA, USA) for 5 minutes at room temperature. Primary antibodies for Ki 67 (rabbit polyclonal, ready to use Abcam Laboratories) were applied to the samples and incubated at room temperature for 30 minutes. Immunodetection was performed using Ultra Tek HRP Anti-Polyvalent Lab Pack (Abcam Laboratories). Ki 67 is expressed in the cell during M, G1, S, and G2 phases of cell cycle and is absent in resting cells (G0). A semiquantitative grading system was used to score the degree of histological change of endothelial cells of implants (Figures 2 and 3). The degree of Ki 67 immunoreactivity of implant cells was evaluated as follows: 0, no Ki 67-positive cells per high-power field (×40); 1, between 1 and 10 Ki 67-positive cells; 2, between 10 and 20 Ki 67-positive cells; and 3, >20 Ki 67-positive cells.

The TNF-α level in the PF was quantitatively assessed using a commercially available enzyme-linked immunosorbent assay kit (Bio Source International, Nivelles, Belgium) according to the manufacturer's instructions. The enzyme immunoassay measures with a sensitivity of 0.5 pg/mL; it has an intra-assay variability of ±4.9% and an interassay variability of ±8.5%.

Statistical Analysis

All statistical analyses were performed on a computer with SPSS version 13.0 (SPSS, Inc, Chicago, Illinois). The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. The variables did not show a normal distribution, and hence the continuous variables were compared with the Mann-Whitney *U* and Wilcoxon Rank Sum *W* tests. The data were presented as the mean ± standard deviation of the mean (SEM). For all comparisons, statistical significance was defined by $P < .05$ (95% confidence interval).

Results

The mean weights of the rats in the endometrioma and the control groups were similar at the beginning of the study (236.1 ± 24.4 g and 239.1 ± 27.8 g, respectively), and they were also similar at the end of the study (225.8 ± 25.5 g and 228.8 ± 21.5 g, respectively). Before treatment, there were no significant differences between the groups in terms of the total volume of experimental endometriosis per animal (Table 1). There was a statistically significant decrease in the volume of mean endometriosis in the study group compared to the control group at the end of the HBO therapy ($P = .018$). When the pre and posttreatment volumes were compared within the same group, we observed that the posttreatment volume was significantly lower in the study group ($P = .024$). In the control group, the posttreatment volumes were higher than the pretreatment volumes, but these differences were not statistically significant ($P = .1$).

Peritoneal fluid TNF-α levels of 2 groups were similar at the beginning, but significantly different at the end of the study ($P < .01$). When the pre- and posttreatment TNF-α levels were compared in the study group, there was statistically significant decrease in TNF-α levels, after the HBO treatment ($P = .016$), but no difference was present within the control group ($P = .34$). Histopathological score of the implants at the end of the treatment was significantly lower in study group compared to the control group ($P < .01$). Proliferative activity defined as Ki 67 immunostaining was significantly lower in study group ($P < .01$; Figures 4 and 5).

Discussion

Although the pathogenesis of endometriosis is still unclear, research has suggested that numerous immunological and inflammatory factors are involved in the development of the disease, including proinflammatory cytokines. Many different treatment modalities are being studied to treat endometriosis, including HBO, which has been applied to treat a large variety

Table 1. Comparison of Volume of Endometrioma, TNF-α Levels, Histopathological Scoring, and Ki 67 Immunohistochemical Scoring of the Groups at the Beginning and After HBO Treatment

	Study Group	Contol Group	*P*
Beginning volume of endometrioma, mm^3	90.8 ± 20.3	86.5 ± 18.9	NS
At the end, volume of endometrioma, mm^3	57.4 ± 12.5	94.6 ± 17.2	.018
Beginning intraperitoneal TNF-α level, pg/mL	7.56 ± 1.81	7.33 ± 1.91	NS
At the end of study, TNF-α level (pg/mL)	5.33 ± 1.02	8.16 ± 1.76	<.01
Histopathological score of the implants at the end of the treatment	1.60 ± 0.50	2.42 ± 0.51	<.01
Ki 67 immunohistochemical score	1.50 ± 0.51	2.37 ± 0.49	<.01

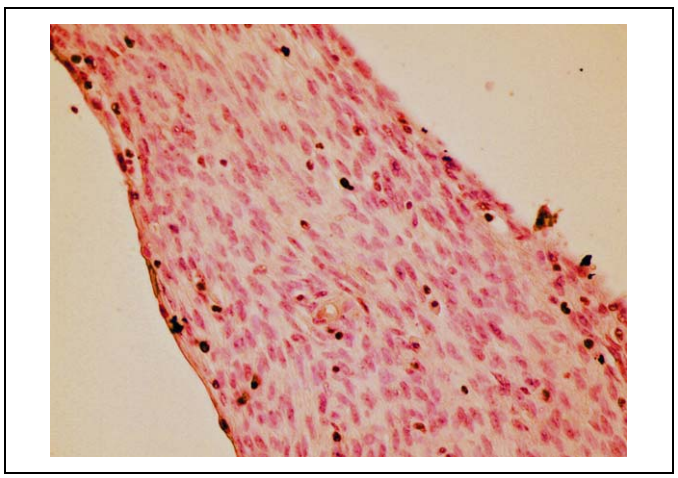

Figure 4. Ki 67 imminostaining of endometriotic implant treated with hyperbaric oxygen showing decreased proliferation.

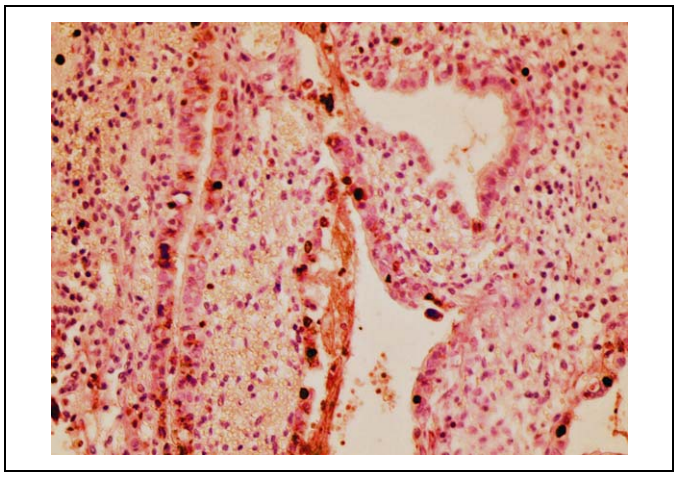

Figure 5. Ki 67 imminostaining of endometriotic implant of control rat, representing increased proliferation 203 × 152 mm.

of diseases, such as traumatic injuries and acute or chronic infections. Different application modalities of HBO using different durations and different atmospheric pressures have been reported in the literature. These studies have examined the application of HBO from a few days to a few months, from 30 minutes to a few hours per day, and from normobaric to 3 atm. The preferred HBO therapy[22] for chronic illnesses is application for 2 hours/d for several weeks under 2.5 atm.

The presence of proinflammatory cytokines regulates adhesion molecules and other factors, which play a significant role in HBO effects. Fildissis et al studied the effects of HBO on proinflammatory cytokines like TNF-α and concluded that application of HBO at 2.4 atm for 90 minutes on healthy individuals did not change the TNF-α levels in the peripheral blood.[2] They observed that if lipopolysaccharide (LPS) stimulation was performed before HBO application, then there was an increase in the production of cytokines, including TNF-α.[2] A similar increase in TNF-α was observed in a murine model when LPS stimulation preceded HBO exposure.[23] On the other hand, Benson et al performed an in vitro study, which showed that the effect of HBO on cytokine production was time dependent. Increased atmospheric pressure alone without hyperoxia had no effect upon monocyte–macrophage proinflammatory cytokine production, but exposure to hyperoxia (95% O_2) at 2.4 atm (HBO) suppressed cytokine production. Stimulation with LPS before a 90-minute exposure of HBO increased TNF-α production by 27%. The immunosuppressive effect of HBO was no longer evident in monocyte–macrophages exposed to HBO for more than 3 hours.[1] After exposing the rats to 100% O_2 for 90 minutes at 2.8 ATA, Lahat et al isolated peripheral blood monocytes and macrophages and then cultured the cells with LPS. The monocytes and macrophages from HBO-exposed rats secreted less TNF-α than cells from rats exposed to normal air.[6] In a murine zymosan-induced shock model, the plasma concentrations of TNF-α were significantly reduced in animals exposed to 100% O_2 for 60 minutes at 2 ATA compared to unexposed controls.[24] Following massive hemorrhage, the hepatic TNF-α messenger RNA (mRNA) and circulating TNF-α levels were lower in rats exposed to 3 ATA for 60 minutes than in unexposed controls.[25] Exposing rats to 100% O_2 to 2.8 ATA for 60 minutes inhibited intestinal ischemia–reperfusion, which induced the circulation of TNF-α.[26] The results in our experimental endometriosis rat model are consistent with the above in vitro and in vivo studies that examined transient HBO treatments. We observed that the application of HBO for 1 hour, 2 times a day resulted in a 29.5% decrease in the TNF-α levels, and in parallel, there was a 36.8% decrease in endometrioma volume. Our study is the first to directly apply HBO to treat endometriosis, and we demonstrate that long-term HBO treatment produces remission of endometriosis. We chose to study HBO treatment for 6 weeks

based on the previous HBO treatments for other chronic illnesses.

The reduction in proinflammatory cytokine production after HBO treatment may result from inhibition of NF-κB. Recently, elevated inflammatory cytokine levels, such as TNF-α, were shown to be positively correlated with changes in NF-κB activation in patients with acute pancreatitis. These results indicated that NF-κB activation was a key factor in the regulation of proinflammatory mediator production, angiogenesis, and the inhibition of apoptosis.[27,28] Moreover, HBO was shown to reduce the inflammatory response directly by inhibiting NF-κB activation.[29] In women with endometriosis, NF-κB is highly expressed in both eutopic and ectopic tissues compared with the normal endometrium.[30] Progestins, danazol, and other nonhormonal therapeutic agents, such as nonsteroidal anti-inflammatory drugs, all target NF-κB and have been used in the treatment of endometriosis.[31,32]

A number of studies have reported increased levels of cytokines in endometriosis. For instance, Chen et al reported 3 peaks in TNF-α levels 15 days after the injection of endometriotic tissue fragments.[33–36] The levels of both TNF-α receptors (TNFR1 and TNFR2) were shown to be increased in the PF of experimental endometriosis models.[37] In this study, we could not directly evaluate whether TNF-α increases in endometriosis or not, since both the control and the study groups had endometriosis at the beginning of the study. We observed that after the HBO treatment the TNF-α levels decreased in parallel to the decrease in the volume of the endometriotic lesions. Therefore, from these indirect observations, we conclude that peritoneal TNF-α levels increase in endometriosis and that decreases in TNF-α may be an indicator of healing processes.

We performed Ki-67 labeling to evaluate the proliferative activity of the endometriotic lesions because previous studies indicated that Ki-67 expression was high in glandular epithelium, especially during stages I and II.[38] The expression of Ki-67 protein was significantly higher in the endometrium of patients with endometriosis than in patients without endometriosis.[39] These histopathological results showed there was increased proliferative activity and increased cell proliferation markers in patients with endometriosis. Ki-67 is also used as a marker of endometriosis remission after applying treatment modalities.[40] In our study, the reduction in the Ki-67 immunohistochemical staining score after HBO treatment was consistent with the reduction in the volume of the endometriotic lesions.

One might expect that since HBO has immunosuppressive effects, patients treated with this therapy may experience a risk of infection. However, HBO acts as a bactericidal/bacteriostatic agent against anaerobic bacteria by increasing the formation of free oxygen radicals. Hyperbaric oxygen restores the bacterial-killing capacity of leukocytes in hypoxic wounds by increasing tissue oxygen tensions. In addition, HBO acts synergistically with a number of antibiotics.[41] Thus, HBO treatment is often used as either a primary or an adjunctive treatment in the management of infections, such as gas gangrene, necrotizing fasciitis, diabetic foot infections, refractory osteomyelitis, neurosurgical infections, and fungal infections.

Middle-ear barotrauma is one of the most common side effects of HBO therapy. This complication occurred in approximately 13.6% of patients treated with HBO. The incidence of middle-ear barotrauma has been shown to vary between treatment centers and patients. The risk factors for middle-ear barotrauma are repetitive treatments and difficulties with pressure equalization.[42] There are other very rare complications of HBO treatment, such as tension pneumocephalus, pulmonary barotrauma, and cerebral arterial gas embolism.[43,44]

This study warrants clinical studies to further elucidate the potential role of hyperbaric oxygen, which may represent a novel therapeutic strategy for the treatment of endometriosis. Different atmospheric pressures of HBO therapy with shorter durations, on endometriosis must be experimented.

Authors' Note

YA, AA, and HA performed study design and statistical analysis; OC, SH, and NEA collected data; YA, AA, and SU performed data interpretation and manuscript preparation; YA, SU, and IT, collected the literature; DS and NP preformed histological analysis.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Benson RM, Minter LM, Osborne BA, Granowitz EV. Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. *Clin Exp Immunol*. 2003;134(1):57-62.
2. Fildissis G, Venetsanou K, Myrianthefs P, Karatzas S, Zidianakis V, Baltopoulos G. Whole blood pro-inflammatory cytokines and adhesion molecules post-lipopolysaccharides exposure in hyperbaric conditions. *Eur Cytokine Netw*. 2004;15(3):217-221.
3. Brenner I, Shepard RJ, Shek PN. Immune function in hyperbaric environments, diving, and decompression. *Undersea Hyper Med*. 1999;26(1):27-39.
4. Murphy SA, Hyams JS, Fisher AB, Root RK. Effects of oxygen exposure on in vitro function of pulmonary alveolar macrophages. *J Clin Invest*. 1975;56(2):503-511.
5. Inamoto Y, Okuno F, Saito K, et al. Effect of hyperbaric oxygenation on macrophage function in mice. *Biochem Biophys Res Comm*. 1991;179(2):886-891.
6. Lahat N, Bitterman H, Yaniv N, Kinarty A, Bitterman N. Exposure to hyperbaric oxygen induces tumour necrosis factor-alpha (TNF-a) secretion from rat macrophages. *Clin Exp Immunol*. 1995;102(3):655-659.
7. Yang Z, Nandi J, Wang J, et al. Hyperbaric oxygenation ameliorates indomethacin-induced enteropathy in rats by modulating TNF-alpha and IL-1beta production. *Dig Dis Sci*. 2006;51(8):1426-1433.

8. Wu MY, Ho HN. The role of cytokines in endometriosis. *Am J Reprod Immunol*. 2003;49(3):285-296.
9. Ethridge RT, Hashimoto K, Chung DH, Ehlers RA, Rajaraman S, Evers BM. Selective inhibition of NF-κB attenuates the severity of cerulein-induced acute pancreatitis. *J Am Coll Surg*. 2002; 195(4):497-505.
10. Gukovsky I, Gukovskaya AS, Blinman TA, Zaninovic V, Pandol SJ. Early NF kappa B activation is associated with hormone-induced pancreatitis. *Am J Physiol*. 1998;275(6 pt 1):1402-1414.
11. Kim H, Seo JY, Roh KH, Lim JW, Kim KH. Suppression of NF-κB activation and cytokine production by N-acetylcysteine in pancreatic acinar cells. *Free Rad Biol Med*. 2000;29(7):674-683.
12. Carswell EA, Old LJ, Kassel RL, Gren S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A*. 1975;72(9):3666-3670.
13. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature*. 1984; 312(5996):724-729.
14. Locksley RM, Killen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*. 2001; 104(4):487-501.
15. Chen HL, Yang YP, Hu XL, Yelavarthi KK, Fishback JL, Hunt JC. Tumor necrosis factor alpha mRNA and protein are present in human placental and uterine cells at early and late stages of gestation. *Am J Pathol*. 1991;139(2):327-335.
16. Jokhi PP, King A, Loke YW. Cytokine production and cytokine receptor expression by cells of the human first trimester placental-uterine interface. *Cytokine*. 1997;9(2):126-137.
17. Bornstein SR, Rutkowski H, Vrezas I. Cytokines and steroidogenesis. *Mol Cell Endocrinol*. 2004;215(1-2):135-141.
18. Wuttke W, Jarry H, Pitzel L, Knoke I, Spiess S. Luteotrophic and luteolytic actions of ovarian peptides. *Hum Reprod*. 1993;8(suppl 2):141-146.
19. Terranova PF, Rice VM. Review: cytokine involvement in ovarian processes. *Am J Reprod Immunol*. 1997;37(1):50-63.
20. Vernon MW, Wilson EA. Studies on the surgical induction of endometriosis in the rat. *Fertil Steril*. 1985;44(5):684-694.
21. Keenan JA, Williams-Boyce PK, Massey PJ, Chen TT, Caudle MR, Bukovsky A regression of endometrial explants in a rat model of endometriosis treated with the immune modulators loxoribine and levamisole. *Fertil Steril*. 1999;72(1):135-141.
22. Hunter S, Langemo DK, Anderson J, Hanson D, Thompson P. Hyperbaric oxygen therapy for chronic wounds. *Adv Skin Wound Care*. 2010;23(3):116-1169.
23. Van den Blink B, van der Kleij AJ, Versteeg HH, Peppelenbosch MP. Immunomodulatory effect of oxygen and pressure. *Comp Biochem Physiol Physiol*. 2002;132(1):193-197.
24. Luongo C, Imperatore F, Cuzzocrea S, et al. Effects of hyperbaric oxygen exposure on a zymosan-induced shock model. *Crit Care Med*. 1998;26(12):1972-1976.
25. Yamashita M, Yamashita M. Hyperbaric oxygen treatment attenuates cytokine induction after massive hemorrhage. *Am J Physiol Endocrinol Metab*. 2000;278(5):811-816.
26. Yang Z, Bosco G, Montante A, Ou X, Camporesi EM. Hyperbaric O2 reduces intestinal schemia-reperfusion-induced TNF-a production and lung neutrophil sequestration. *Eur J Appl Physiol*. 2001;85(1-2):96-103.
27. Yamauchi N, Harada T, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor-alpha induced the release of interleukin-6 from endometriotic stromal cells by the nuclear factor-kappaB and mitogen-activated protein kinase pathways. *Fertil Steril*. 2004;82(suppl 3):1023-1028.
28. Yu X, Li YG, He XW, et al. Hyperbaric oxygen reduces inflammatory response in acute pancreatitis by inhibiting NF- kappaB activation. *Eur Surg Res*. 2009;42(2):130-135.
29. Yu X, Zhang SD, Hang TQ. The change of pancreatic acinar cell function in murine acute necrotizing pancreatitis. *Chin J Gen Surg*. 2000;15(5):280-282.
30. Wang F, He YL, Peng DX, Liu MB. Expressions of nuclear factor-kappaB and intercellular adhesion molecule-1 in endometriosis. *Di Yi Jun Yi Da Xue Xue Bao*. 2005;25(6):703-705.
31. Horie S, Harada T, Mitsunari M, Taniguchi F, Iwabe T, Terakawa N. Progesterone and progestational compounds attenuate tumor necrosis factor alpha-induced interleukin-8 production via nuclear factor kappa B inactivation in endometriotic stromal cells. *Fertil Steril*. 2005;83(5):1530-1535.
32. Wieser F, Vigne JL, Ryan I, Hornung D, Djalali S, Taylor RN. Sulindac suppresses nuclear factor-kappaB activation and RANTES gene and protein expression in endometrial stromal cells from women with endometriosis. *J Clin Endocrinol Metabol*. 2005;90(12):6441-6447.
33. Kuroda K, Kitade M, Kikuchi I, et al. Peritoneal vascular density assessment using narrow-band imaging and vascular analysis software, and cytokine analysis in women with and without endometriosis. *J Minim Invasive Gynecol*. 2009;3(1):704-710.
34. Kyama CM, Overbergh L, Mihalyi A, et al. Endometrial and peritoneal expression of aromatase, cytokines, and adhesion factors in women with endometriosis. *Fertil Steril*. 2008; 89(2):301-310.
35. Chen Q, Zhou W, Pu D, Li Z, Huang Q, Chen Q. The inhibitory effect of 15-R-LXA4 on experimental endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2009;145(2):200-204.
36. Chen QH, Zhou WD, Su ZY, Huang QS, Jiang JN, Chen QX. Change of proinflammatory cytokines follows certain patterns after induction of endometriosis in a mouse model. *Fertil Steril*. 2009;31(5):217-224.
37. Gogacz M, Bogusiewicz M, Putowski L, et al. Expression of tumor necrosis factor-alpha (TNF-alpha) on peritoneal fluid mononuclear cells in women with endometriosis. *Ginekol Pol*. 2008;79(1):31-35.
38. Kim HO, Yang KM, Kang IS, et al. Expression of CD44s, vascular endothelial growth factor, matrix metalloproteinase-2 and Ki-67 in peritoneal, rectovaginal and ovarian endometriosis. *J Reprod Med*. 2007;52(3):207-213.
39. Park JS, Lee JH, Kim M, Chang HJ, Hwang KJ, Chang KH. Endometrium from women with endometriosis shows increased proliferation activity. *Fertil Steril*. 2009;92(4):1246-1249.
40. Celik O, Hascalik S, Elter K, Tagluk ME, Gurates B, Aydin NE. Combating endometriosis by blocking proteasome and nuclear factor-kappaB pathways. *Hum Reprod*. 2008;23(11): 2458-2465.

41. Cimşit M, Uzun G, Yildiz S. Hyperbaric oxygen therapy as an anti-infective agent. *Expert Rev Anti Infect Ther*. 2009;7(8): 1015-1026.
42. Bessereau J, Tabah A, Genotelle N, Français A, Coulange M, Annane D. Middle-ear barotrauma after hyperbaric oxygen therapy. *Undersea Hyperb Med*. 2010;37(4):203-208.
43. Lee CH, Chen WC, Wu CI, Hsia TC. Tension pneumocephalus: a rare complication after hyperbaric oxygen therapy. *Am J Emerg Med*. 2009;27(2):251-257.
44. Rivalland G, Mitchell SJ, van Schalkwyk JM. Pulmonary barotrauma and cerebral arterial gas embolism during hyperbaric oxygen therapy. *Aviat Space Environ Med*. 2010;81(9):888-890.