ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Comparison of the Effects of Hyperbaric Oxygen and Medical Ozone Therapy on Wound Healing in the Experimental Burn Wound Model

Deneysel Yanık Modelinde Hiperbarik Oksijen ve Medikal Ozon Tedavisinin Yara İyileşmesi Üzerine Etkilerinin Karşılaştırılması

Zeki YAŞAR[a], Perçin KARAKOL CAŞKAN[b], Emin KAPI[c], Mehmet BOZKURT[b]

[a]Private Physician, İstanbul, TURKEY
[b]Department of Plastic, Reconstructive and Aesthetic Surgery, Health and Sciences University Bağcılar Training and Research Hospital, İstanbul, TURKEY
[c]Department of Plastic, Reconstructive and Aesthetic Surgery, Health and Sciences University Adana City Training and Research Hospital, Adana, TURKEY

This study was presented as an oral presentation at International Society for Burn Injuries (ISBI), 9-13 September 2012, Edinburgh, Scotland.

ABSTRACT Objective: Burn injury is a type of injury which is the disruptin of skin and organs by the effects of heat, electrical current, chemical caustic or burning agents. The purposes of daily burn care are removing of necrotic tissue, prevention of bacterial contamination, control of travma, stimulation of epithelization and protection of systemic complications. In this study, the effects of hyperbaric oxygen and ozone therapy were investigated, and used as adjuvant therapy in burn management, on burned areas utilizing experimental burn model. **Material and Methods:** In this study, 28 Wistar albino rats weighing 200-220 gr were used and divided into four groups. 10% burn defect areas were occured in all experiments. In the first group, rats were treated with hyperbaric oxygen. Medical ozone therapy was applied to the second group. In the third group, only debridement was used as treatment. Any treatment options were not used to rats in the fourth group. **Results:** The analysis revealed no statistically significant difference of burns surface areas between Groups 1 and 2, while a significant reduction were noted in Groups 1 and 2 when compared to the other groups. On histopathologic examination of the Groups 1 and 2 we observed a significant decrease in the inflammation and fibrosis rates when compared with other groups. **Conclusion:** As a result, we observed the affirmative effects of hyperbaric oxygen and ozone therapy on burn management. We suggest that these treatment modalities can be added to standart treatment options as adjuvant therapy. We also think that large researches are needed to support this issue.

Keywords: Hyperbaric oxygen; medical ozone; burns

ÖZET Amaç: Yanık hasarı; derinin veya organların ısı, elektrik akımı, kimyasal veya yakıcı bir ajan etkisi ile tahrip olması şeklinde gerçekleşen bir yaralanma şeklidir. Günlük yanık bakımının hedefleri nekrotik dokuları kaldırmak, bakteriyel kontaminasyonu önlemek, travmayı kontrol etmek, epitelizasyonu uyarmak ve sistemik komplikasyonlardan korunmaktır. Bu çalışmada; deneysel yanık modelinde, adjuvan tedavi olarak kullanılan hiperbarik oksijen ve medikal ozon tedavilerinin etkileri incelenmiştir.**Gereç ve Yöntemler:** Çalışmaya, ağırlıkları 200-220 gr. ağırlığında 28 adet Wistar albino sıçan dahil edildi ve 4 deney grubu oluşturuldu. Tüm deneklerde %10 yanık yüzey alanı meydana getirildi. Birinci grup hiperbarik oksijen tedavisi aldı. İkinci gruba medikal ozon tedavisi uygulandı. Üçüncü gruba sadece debridman uygulandı. Dördüncü gruptaki sıçanlara ise herhangi bir tedavi uygulanmadı. **Bulgular:** Grup 1 ve 2 arasında yanık yüzey alanı açısından istatistiksel açıdan anlamlı bir farklılık gözlenmez iken, Grup 1 ve 2 ile diğer gruplar arasında anlamlı derecede azalma gözlendi. Histopatolojik incelemede Grup 1 ve 2'de, diğer gruplara kıyasla inflamasyon ve fibrosis oranlarında belirgin azalma gözlendi. **Sonuç:** Sonuç olarak, yanık tedavisinde hiperbarik oksijen ve medikal ozon tedavisinin yararlı etkileri olduğunu gözlemledik. Bu tedavilerin standart yanık tedavisine ek olarak uygulanabileceğini düşünmekteyiz. Ancak, bu konuda geniş çaplı araştırmaların yapılması gerektiği kanaatindeyiz.

Anahtar Kelimeler: Hiperbarik oksijen; medikal ozon; yanık

Burns are a type of injury that impair the integrity of the skin, and that are likely to result in ischemia and necrosis in the affected tissue.[1,2] There are many different modalities for the treatment of burn injuries. Hyperbaric oxygen (HBO) and medical ozone (MO) therapy are among the current and

Correspondence: Emin KAPI
Department of Plastic, Reconstructive and Aesthetic Surgery, Health and Sciences University Adana Medical Faculty, Training and Research Hospital, Adana, TURKEY/TÜRKİYE
E-mail: eminkapi@gmail.com

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novel treatment methods for the treatment of burns.[3,4]

HBO therapy involves the intermittent administration of 100% oxygen ventilation under pressure greater than 1 atmosphere (1 ATA= 760 mmHg) in a closed pressure chamber.[3] The treatment improves fibroplasia, angiogenesis and re-epithelialization in the burnt skin by preventing microvascular damage, reducing edema development and providing sufficient oxygen for cellular metabolism. There have been several studies to date demonstrating reduced mortality and morbidity in burn patients who were treated with HBO.[5-8]

MO therapy is based on the administration of an oxygen/ozone mixture into the body cavities or the circulatory system. Ozone therapy has a very wide spectrum of indications for use due to its fast and effective wound healing features, in addition to its potent antibacterial, antiviral, antifungal and immunomodulator effects, and its positive effects on the transport and release of oxygen in the tissue. Ozone is described as "active oxygen" in medicine, given its very high oxidation capacity.[4,9]

A literature review uncovered very few studies comparing the efficacy of HBO and MO therapies in burn patients. Aiming to fill this gap in literature, the present experimental study makes a comparison of the effects of HBO and MO therapies on wound healing in a scald burn model.

MATERIALS AND METHOD

Approval of the Dicle University Faculty of Medicine Local Ethics Committee (DÜHADEK) was obtained prior to the study (Date: 2011.03.02/Consent no: 1), involving rats acquired from the Dicle University Prof. Dr. Selahattin Payzın Experimental Research Center (DÜSAM). The study was performed regarding of the "Guide for the Care and use of Laboratory Animals". The study was included 9-month-old, isogeneic (inbred) female 28 Wistar albino rats weighing 200-220 g. The rats were kept collectively in cages and fed with standard pellet (TAVAS Inc, Adana, Turkey), while water needs were met through standard methods. The temperature was kept stable at around 21°C; and laboratory lighting was provided in a 12-hour day and 12-hour night cycle. The room humidity level was kept stable at 45±10%. All procedures were performed by a single surgeon.

All groups were administered a mixture of ketamine sodium (Ketalar® vial; Pfizer Ltd. Şti, İstanbul, Turkey), 90 mg/kg and xylazine hydrochloride (Rompun® vial, Bayer Inc, Germany) 10 mg/kg via the intraperitoneal route for general anesthesia. The back of the rats was shaved and disinfected using 10% povidone iodine (Batticon®, Adeka İlaç Ltd. Şti, Samsun, Turkey). One hour prior to the operation, cefazolin sodium 0,25 mg/kg (Sefazol® vial, Mustafa Nevzat İlaç Sanayii, İstanbul, Turkey) was administered intramuscularly.

INDUCTION OF A BURN MODEL

In order to create a burn area amounting to 10% of the total body surface area (TBSA) in the subjects, a polyethylene plate with a 6x5 cm hole at the center was used. The back of the rats was fixed in such a way that it corresponded to the center of the polyethylene plate. The rats were held upside down a container of 95°C hot water for 20 seconds so that only a 30 cm^2 surface area of their backs was in contact with the water, as the burn area (Figure 1).

All rats with the induced burn were subcutaneously administered 20 mg/kg Pethidine HCl (Aldolan® ampoule, Gerot Pharmazeutika, Vienna, Austria) for analgesia in the post-burn early period. All of the rats were then intraperitoneally administered 2 ml of physiological saline solution in order to avoid fluid leak. After the procedure, the rats were placed in individual cages.

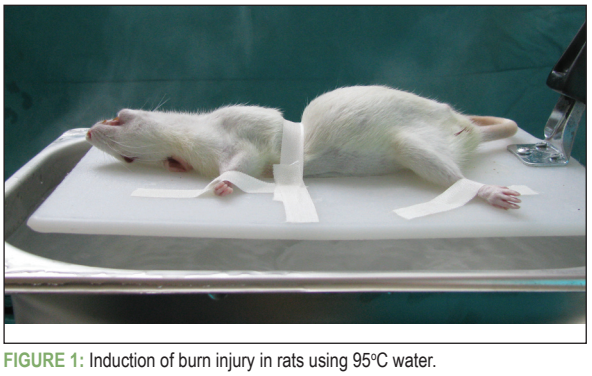


FIGURE 1: Induction of burn injury in rats using 95°C water.

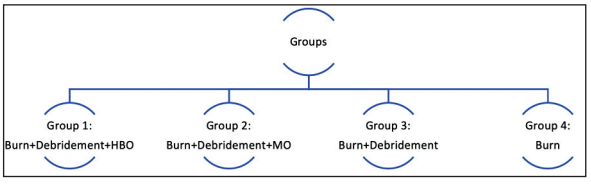


FIGURE 2: Schematic view of experimental groups.

EXPERIMENTAL GROUPS

The rats were selected based on a computer-generated randomization list, with four groups created, each with seven rats. All of the subjects were marked and numbered according to the coding system. Group 1 was planned for debridement and HBO therapy, while Group 2 was planned for debridement and MO therapy after the induction of the burn defect. Group 3 was planned for debridement alone after the induction of the burn injury, and Group 4 was planned for no procedure after the induction of the burn injury (Figure 2). Debridement was applied after 24 hours following the induced burn through the mechanical removal of the necrotic tissue in the burn area as much as needed until the end of the experiment. For all subjects, wound care was provided using octenidine hydrochloride (Octenisept®, Schülke&Mayr, GmbH, Vienna, Austria).

ADMINISTRATION OF HBO THERAPY

In Group 1, treatment was initiated using the experimental HBO device (Barotech Hyperbaric Chamber DB2, Kartal, Istanbul, Turkey) at post-burn hour 24, and continued until day 20 (Figure 3).

The HBO treatment protocol was performed in 3 stages;

1) Diving stage: Reaching the level of 1.5 bar in 15 minutes

2) Treatment stage: 90-minute ventilation at 1.5 bar

3) Exit stage: Reaching normal atmospheric pressure from 1.5 bar within 30 minutes (Figure 4).

ADMINISTRATION OF MO THERAPY

In Group 2, the rats were intraperitoneally administered 0.7 cc/kg ozone in 2 ml isotonic solution for 7 days using an ozone generator (Longevity EXT-120T, Longevity Resources Inc., Canada) at post-burn hour 24 (Figure 5).

MACROSCOPIC ASSESSMENT

At day 30 of the experiment, 1:1 macroscopic images of the burn areas were taken using a digital camera (Nikon^{Ò} D7000, SLR 50 mm) (Figure 6). The digital images were transferred to an electronic environment. An Autocad (Autocad 2008, Autodesk, Inc. USA) program was used to calculate the burn defect surface areas.

HISTOPATHOLOGICAL ASSESSMENT

Biopsies of 2 mm^2 were taken from the burn area and the normal skin meeting sites of the subjects on days 1 and 30. The tissue biopsy specimens taken for pathological assessment were kept in a 10% formaldehyde solution for 72 hours, and then subjected to routine histopathological control. The specimens were then stained with hemotoxylin&eosine (H&E) and Masson's trichrome stains, and evaluated by a specialist pathologist.

The histopathologically prepared sections were used to assess epithelization, vascularization, inflammation and fibrosis at 40x, 100x and 200x magnification. The values measured microscopically at five

FIGURE 3: View of HBO device during application (a: outer view, b: view after the subjects were placed into the device).

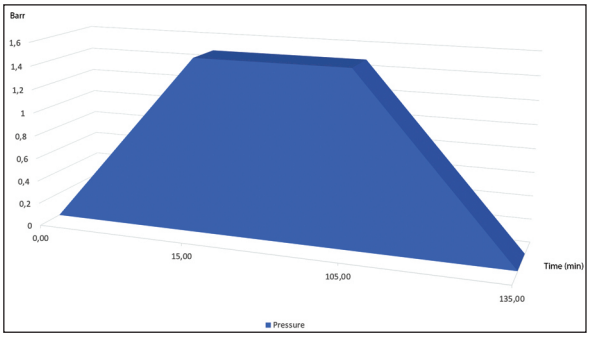


FIGURE 4: HBO therapy stages.

FIGURE 5: View of Longevity EXT- 120T ozone generator.

different locations were averaged. The findings were scored and categorized as none (0), mild (1) moderate (2) or Severe (3).

STATISTICAL ASSESSMENT

Normality control of continuous variables was perfomed with Shapiro Wilk test. Since the variables are not suitable for normal distribution, the Kruskal-Wallis test was used in comparison the median values by groups. Additionally, the binary comparison was used for the results that were found significant. Descriptive statistics are expressed with a standard deviation (SD), median and quarter values. Data analysis was performed in SPSS 21.0 (SPSS Inc., Chicago, IL, USA) package program. Two-sided p-values were considered statistically significant at $p<0.05$.

RESULTS

The researchers assessed experimental groups as double-blind while interpreting the findings. During follow-up, one rat each from Groups 1, 2 and 3, and two rats from Group 4 were excluded from the study due to exitus. All other rats healed normally and without any problems.

The mean burns surface area measurements showed a statistically significant difference among the groups ($p<0,001$) (Table 1, Table 2) (Graphic 1) while a significant difference was noted in Group 1 when compared to the Groups 3 and 4 ($p<0,05$). Similarly, there was a significant difference was noted in Group 2 when compared to the Groups 3 and 4 ($p<0,05$).

The histopathological assessment revealed:

The median values of epithelization and vascularization were found to be no significantly among groups before and after the treatment ($p>0,05$) (Figure 7).

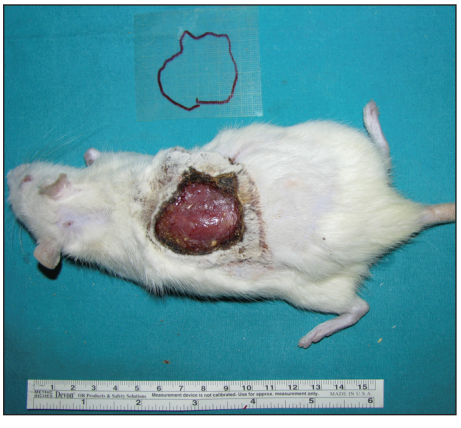


FIGURE 6: Recording of necrotic and live burn surface areas in the subject with induced burn injury.

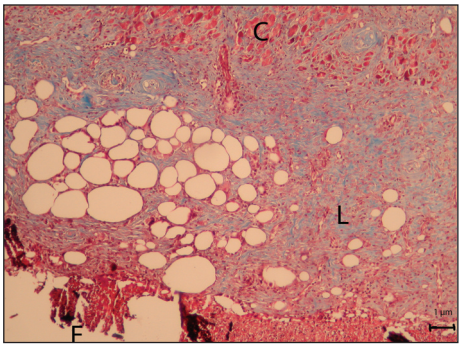


FIGURE 7: View of epithelization, lymphovascular and collagen tissues in Group 1 (H&E x 100) (E: epithelization, L: lymphovascularization, C: collagen).

TABLE 1: Inter-group comparison of burn surface areas.

Groups	Experiments 1	2	3	4	5	6	7
				cm^2			
1	0.75	0.36	3.07	exitus	0.63	3.27	2.73
2	0.96	0.79	2.31	4.62	1.01	exitus	3.04
3	4.14	exitus	11.34	12.03	11.83	16.45	11.32
4	22.74	19.21	exitus	14.35	24.36	27.98	exitus

TABLE 2: Results of the inter-group comparison of flap surface defect areas, based on a Kruskal-Wallis test.

	Groups	N	Mean	SD	Median [Q1-Q3]	p	Pairwise comparisons
Measurements	Group 1	6	1.80	1.355	1.74 [0.56-3.12]	<0.001	1-3 (p=0.014)
	Group 2	6	2.12	1.515	1.66 [0.92-3.44]		1-4 (p<0.001)
	Group 3	6	11.19	3.963	11.59 [9.53-13.14]		2-3 (p=0.037)
	Group 4	5	21.73	5.192	22.74 [16.78-26.17]		2-4 (p=0.001)

P: Kruskal-Wallis test; SD: Standard deviation.

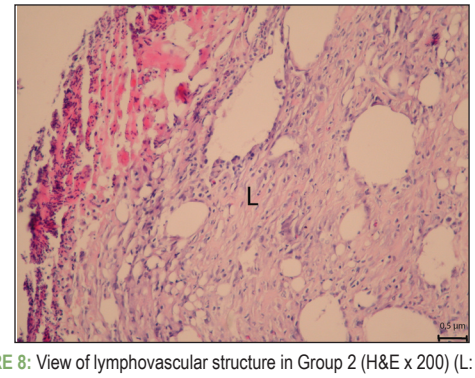


FIGURE 8: View of lymphovascular structure in Group 2 (H&E x 200) (L: lymphovascularization).

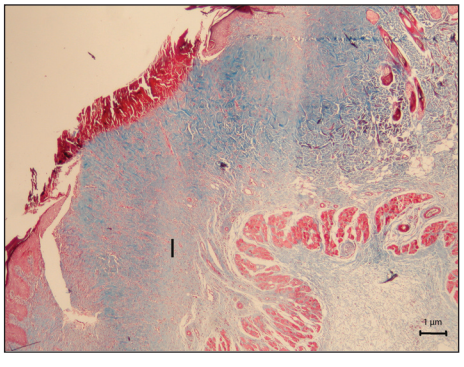


FIGURE 9: View of the intense inflammation in Group 3 (Masson's trichrome x100) (I: inflammation).

In Groups 1 and 2, the fibrosis rates were lower than the other groups (p=0,001) (Figure 8).

In Groups 3 (Figure 9) and 4 (Figure 10), inflammation and fibrosis were distinctly high (p<0,05).

While the pre- treatment median values of fibrosis parameter were not significant among to the groups, there was a significant difference after treatment (p=0,002). These differences were among Groups 1-3 (p=0,012), 1-4 (p=0,008), 2-3 (p=0,005) and 2-4 (p=0,003) (Table 3).

While the pre- treatment median values of inflammation parameter was not significant among the groups, there was a significant difference after treatment (p=0,038). These differences were among Groups 1-3 (p=0,014), 1-4 (p=0,049), and 2-3 (p=0,039) (Graphic 2).

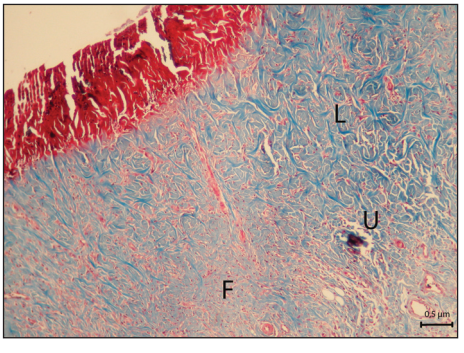
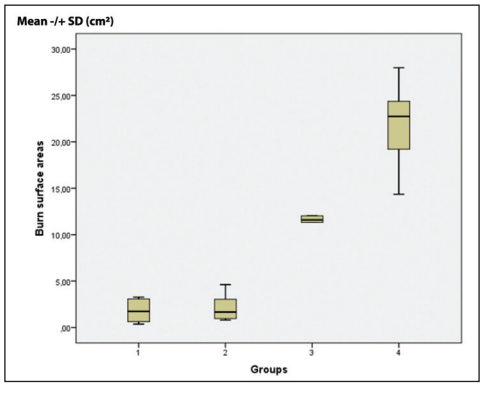


FIGURE 10: View of the low epithelization rate, collagen irregularity, intense ulceration, fibrosis and lymphovascular structure in Group 4 (Masson's trichrome x200) (U: ulceration, F: fibrosis, L: lymphovascularization).

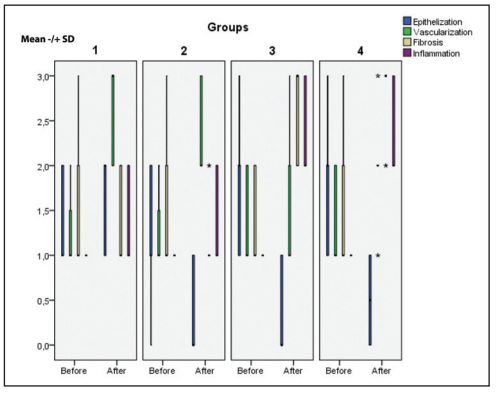
TABLE 3: Inter-group comparisons of pre- and post-operative values of parameters.

Parameters / Groups	Preoperative			Postoperative		
	Mean±SD	Median [Q1-Q3]	p	Mean±SD	Median [Q1-Q3]	p
Epithelization						
Group 1	1.29±0.76	1 [1-2]	0.887	1.33±0.82	1.5 [0.75-2]	0.160
Group 2	1.43±0.53	1 [1-2]		0.33±0.52	0 [0-1]	
Group 3	1.6±0.55	2 [1-2]		0.5±0.58	0.5 [0-1]	
Group 4	1.57±0.79	1 [1-2]		0.67±0.82	0.5 [0-1.25]	
Vascularization						
Group 1	1.29±0.49	1 [1-2]	0.971	2.5±0.55	2.5 [2-3]	0.262
Group 2	1.29±0.49	1 [1-2]		2.33±0.52	2 [2-3]	
Group 3	1.4±0.55	1 [1-2]		1.5±1	1 [1-2.5]	
Group 4	1.29±0.49	1 [1-2]		2.17±0.75	2 [1.75-3]	
Fibrosis						
Group 1	1.57±0.79	1 [1-2]	0.761	1.33±0.52	1 [1-2]	0.002
Group 2	1.43±0.53	1 [1-2]		1.17±0.41	1 [1-1.25]	1-3 (p=0.012)
Group 3	1.4±0.55	1 [1-2]		2.75±0.5	3 [2.25-3]	1-4 (p=0.008)
Group 4	1.86±0.9	2 [1-3]		2.67±0.52	3 [2-3]	2-3 (p=0.005)
						2-4 (p=0.003)
Inflammation						
Group 1	1±0	1 [1-1]	1.00	1.33±0.52	1 [1-2]	0.038
Group 2	1±0	1 [1-1]		1.5±0.55	1.5 [1-2]	1-3 (p=0.014)
Group 3	1±0	1 [1-1]		2.5±0.58	2.5 [2-3]	1-4 (p=0.049)
Group 4	1±0	1 [1-1]		2.17±0.75	2 [1.75-3]	2-3 (p=0.039)

p: Kruskal-Wallis test; SD: Standard deviation.



GRAPHIC 1: Comparison of the results of the flap surface defect areas among groups.



GRAPHIC 2: Among-groups comparison of pre-and post-operative values of parameters.

DISCUSSION

HBO therapy has been used for around 40 years for the treatment of a large variety of acute or chronic diseases, either as the primary treatment or supporting other treatment methods.[10-16] Its areas of use include, but are not limited to, such pathologies such as diabetic angiopathy, peripheral vascular impairment-related ulcers, thermal burns, healing stages of skin grafts and flaps, purpura fulminans, osteomyelitis and osteoradionecrosis.[17] The procedure is performed in an isolated pressure chamber, and involves the patient breathing 100% oxygen under pressure 2–3 times more than the atmospheric pressure at sea level (1 ATA [atmosphere absolute] = 760 mmHg).[18-22]

HBO therapy increases the amount of dissolved oxygen in blood and tissue, with a positive effect on wound healing. HBO therapy is known to contribute to the neovascularization of blood vessels in cells with reduced vascularization, with a mechanism that is based on decreased edema as a result of hyperoxic vasoconstriction, increased collagen production and the elimination of bacteria through phagocytic activity.[7,23,24] The up to 10-fold increase in tissue levels of hydroxyproline, ATP and phosphocreatine with HBO provide the fibroblast-collagen matrix support required for neovascularization, while also providing the optimum conditions for wound healing by increasing the bactericidal activities of leukocytes.[25]

Over the past 20 years, methods such as ventilatory support, proper topical and parenteral antibiotics, early debridement, enteral and parenteral nutrition have brought significant developments to the treatment of burns.[26] There is still a lack of consensus on the benefits of HBO application when used together with the standard treatment methods.[27] A review of literature reveals studies that identified no significant difference in mortality or length of hospital stay between burn patient groups who received and who did not receive HBO therapy.[28] The present study identified a macroscopically and histopathologically significant difference in the experimental animal group that underwent HBO therapy when compared to the other groups. These findings of our study suggest that HBO and MO therapies can be used adjuvant where required in clinical practice, and may reduce morbidity and mortality in burn cases, although large-scale broad studies are needed in this regard.

Ozone is a gaseous molecule composed of three oxygen atoms, and is formed through electronic discharges from oxygen. Despite the steady state of the oxygen molecule (O_2), ozone (O_3) is an unstable molecule. MO, in turn, is a mixture of 0.05% O_3-99.95% O_2 or 5% O_3-95% O_2 derived from 100% pure oxygen.[29,30]

The effect of ozone on the metabolism varies depending on the concentration and dose. Ozone is known to have bactericidal, virucidal and fungicidal effects; as well as a systemic hemostasis-restorative effect; and is known to restore the oxygen transport function of the blood; to optimize pro-and anti-oxidant systems; to restore microcirculation and peripheral circulation; to reduce blood coagulation; to stimulate hematopoiesis; to optimize the metabolisms of biological substrates such as carbohydrates, proteins and lipids (bioenergetic, biosynthetic effect); and to activate the production of biologically active substances, and the immunomodulator (immunostimulation at low doses; immunosuppression at high doses), analgesic, detoxification effects, etc.[31]

This molecule is currently used in the treatment of 350 different diseases to improve the efficacy of other treatment methods administered alone or additionally. The therapeutic effect of ozone therapy is particularly remarkable in physiopathological conditions in which there is an intense inflammatory process and when the immune system is particularly important. MO therapy has been used for wound healing, and for the treatment of ischemic and infectious diseases, dental and oral infections, burns, wound care, decubitus ulcers, poorly healing wounds, fungal egzema, herpes simplex and zoster, acne, alopecia, otitis media and externa, inflammatory genital diseases, and Hepatitis B and C. MO therapy can be administered through intravenous, intramuscular, intraarticular, intrapleural, intrarectal, intradiscal or topical routes.[4,32]

The similar mechanisms of action of HBO and MO therapies are utilized for the treatment of burns, with both applications having such effects as decreasing free radical cell injury, increasing nitric oxide (NO) release and the associated vasodilatation, preventing leukocyte adhesion to endothelial cells and decreasing superoxide radicals.[33] These factors enhance the supply of blood to skin grafts used for the treatment of burns.[34]

The literature review revealed a limited number of studies investigating the effects of HBO and MO therapies in burn cases.[35,36] The study by Al-Dalain et al. evaluated the efficacy of MO therapy in patients with diabetic foot, and found accelerated wound healing, shorter length of hospital stays, better-managed levels of glycaemia and increased antioxidant enzyme levels in patients undergoing MO therapy than in a group not undergoing MO therapy.[37] The experimental acute necrotizing pancreatitis model of Uysal

et al. compared the effects of HBO and MO therapies, and established that HBO and MO therapies significantly reduced the severity and mortality of acute necrotizing pancreatitis, with the effect being greater with MO therapy than with HBO therapy.[38] Altinel et al. evaluated the effects of HBO vs MO therapy in an experimental rat distal colitis model. The researchers found that MO therapy had a greater therapeutic effect than HBO therapy, and there were lower levels of inflammation, edema and oxidative stress in the MO therapy group.[39] Verrazzo et al. compared the effects of HBO and MO therapies on blood rheological parameters in cases with peripheral occlusive disease, and established a significant decrease in blood viscosity in the MO therapy group.[40] Villanueva et al. conducted a meta-analysis of studies of HBO therapy for the treatment of thermal burns, but could not obtain sufficient evidence to require HBO therapy in the algorithm in thermal burn cases, concluding that further studies were needed in this regard.[8]

In light of the findings from the present study, HBO and MO therapies can be stated to have positive effects on burn wound healing, and to increase epithelial regeneration and vascularization while decreasing inflammation and fibrosis. Nonetheless, we were unable to establish any statistically significant difference between the two procedures. Based on these findings and those of previous studies, we believe that the addition of HBO and MO therapies to the treatment algorithm of burn cases, especially in the presence of a wound healing problem of any kind, and an insufficient supply of blood to the graft bed, would positively impact wound healing. It can be ascertained from the literature that MO has greater efficacy than HBO therapy.[38-40] Furthermore, both the lower cost and less equipment needed for MO than with HBO therapy may bring MO therapy to the fore as a more advantageous approach.

Experimental studies have demonstrated the effects of such therapies as HBO and MO, electrical current, laser beam and ultrasound on tissue injury restoration. These treatment methods will find further areas of use for wound healing with technological developments, increased infrastructure opportunities and decreased costs. There are ongoing studies in this field being conducted to bring this field to the optimal level.[4,5,9]

CONCLUSION

We believe that HBO and MO therapies can be used as adjuvant in standard burn treatment, especially in cases with burn wound bed healing problems, although broader studies are needed in this regard.

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Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Mehmet Bozkurt;* ***Design:*** *Zeki Yaşar;* ***Control/Supervision:*** *Mehmet Bozkurt;* ***Data Collection and/or Processing:*** *Zeki Yaşar,* ***Analysis and/or Interpretation:*** *Zeki Yaşar, Emin Kapı;* ***Literature Review:*** *Zeki Yaşar, Perçin Karakol Caşkan,Emin Kapı,* ***Writing the Article:*** *Zeki Yaşar, Emin Kapı;* ***Critical Review:*** *Mehmet Bozkurt, Emin Kapı,Perçin Karakol Caşkan.*

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