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ORIGINAL PAPER

Hyperbaric Oxygen Therapy Is as Effective as Dexamethasone in the Treatment of TNBS-E-Induced Experimental Colitis

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Abstract *Introduction* Hyperbaric oxygen (HBO) has been demonstrated to be useful as an adjunctive therapy for Crohn's disease. In the present study, HBO was tested as a treatment for trinitrobenzenesulfonic acid–ethanol (TNBS-E)-induced distal colitis, and its effects were compared with dexamethasone therapy. *Methods* A total of 48 Sprague-Dawley rats were separated into six groups: the control, and those treated with vehicle, TNBS-E, HBO, dexamethasone, or combined HBO + dexamethasone. The HBO treatment group was exposed to 100% HBO at 2 ATM for 75 min twice daily at 6-h intervals in a HBO chamber, both on the day of colitis induction and 3 days thereafter. Treatment with intraperitoneal dexamethasone twice daily was started 1 h before the induction of colitis and was continued for 7 days in the dexamethasone group. The rats were decapitated 8 days after the induction of colitis, and the colonic tissue wet weight, macroscopic and microscopic lesion score, and tissue myeloperoxidase (MPO) activity were determined. *Results* HBO therapy decreased the activity of experimental colitis measured by the tissue wet weight, macroscopic score, microscopic score, and MPO activity. The dexamethasone treatment significantly reduced the colitis activity as determined by the tissue MPO activity and wet weight. There were also decreases in the macroscopic and microscopic activity scores with the dexamethasone therapy; however, these changes were not statistically significant. The combined therapy with HBO and dexamethasone provided no additional benefit over HBO therapy alone. *Conclusion* HBO therapy can be a valuable therapeutic option in treatment of patients with inflammatory bowel disease. HBO therapy in the refractory patients deserves further, larger clinical studies.

Keywords Hyperbaric oxygen therapy · Dexamethasone · TNBS-E · Experimental colitis · Inflammatory bowel disease

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Introduction

Evolving therapies for inflammatory bowel disease (IBD) have held promise in the last decade, but some cases still necessitate alternative drugs and supportive therapies. This constant search for new and more effective treatment modalities has generated some promising approaches, such as hyperbaric oxygen (HBO) therapy as reported by Rachmilewitz et al. in an experimental model of colitis [1]. Acceleration of colonic wound healing using HBO therapy in patients with colon anastomoses was also presented [2].

HBO is defined as inhalation of 100% oxygen under a pressure more than 1 atmosphere absolute. This amount of oxygen results in an almost 20-fold increase of plasma oxygen level [3, 4]. Because of these properties, HBO therapy is mostly recommended for the treatment of carbon monoxide poisoning and decompression sickness. Oxygen is also crucial for immune reactions and has been shown to have bactericidal effects in clostridial myonecrosis,

refractory osteomyelitis, and chronic wounds, which are other indications of HBO therapy [5–10].

The aims of the present study were to investigate whether HBO is effective in ameliorating experimental colitis and to compare its effects with dexamethasone treatment.

Materials and methods

Animals

Sprague-Dawley rats of either sex (200–250 g) were kept in a room at a constant temperature of 22 ± 1°C, with a 12-h/12-h light/ dark cycle, and fed standard pellet chow and water ad libitum. There were six groups and each consisted of eight rats. The study was approved by Marmara University, School of Medicine, Animal Care and Use committee.

Induction of colitis

After overnight fasting, inflammation was induced in the colon, under light ether anesthesia, by means of intrarectal administration of 1 ml of a 30-mg/ml trinitrobenzenesulfonic acid–ethanol (TNBS-E) solution dissolved in 50% ethanol in saline using an 8-cm-long cannula (Colitis group) [5]. Comparisons were carried out with rats administered an equal volume (1 ml) of either saline solution (control group) or solvent (50% ethanol in saline) (vehicle group). In addition, treatment with dexamethasone (0.1 ml/100 g of body weight/i.p./twice daily) was started 1 h before the induction of colitis and was continued for 7 days (Dexamethasone group). Rats in the HBO treatment group were exposed to 100% HBO at 2 ATM for 75 min twice daily at 6-h intervals in a HBO chamber on the day of colitis induction and 3 days thereafter (HBO group). Another group of rats received both dexamethasone for 7 days and HBO therapy for 3 days (HBO + Dexamethasone group).

The rats were decapitated 8 days after the induction of colitis, the last 8 cm of the colon was excised, opened longitudinally and rinsed with saline solution. Then, the distal colon was weighed and the mucosal lesions were examined macroscopically. The samples were taken for microscopic evaluation of the lesions and for determination of myeloperoxidase (MPO) activity.

Determination of colonic damage

Criteria outlined in Table 1 [11] were used to assess the macroscopic damage score. The distal part of the colon was excised into two parts, one of which was stained with Hematoxylin–Eosin; the criteria outlined in Table 2 [12] were used to assess the histological changes. The second sample was stored at –70°C for subsequent measurement of MPO activity. An observer unaware of the study groups performed macroscopic and microscopic scoring of tissue samples.

Table 1 Macroscopic colonic damage score

Score	Appearance
0	No damage
1	Localized hyperemia, no ulcers
2	Ulceration without hyperemia or bowel thickening
3	Ulceration with inflammation at one site
4	Two or more sites of ulceration/inflammation
5	Major sites of damage extending more than 1 cm along the length of colon
6–10	If damage extends more than 2 cm along the length of the colon, the score is increased by one for each additional 1 cm

Determination of tissue myeloperoxidase activity

Tissue MPO activity was determined in 250- to 500-mg samples. The tissue samples were homogenized first in 10

Table 2 Microscopic colonic damage score

Score	Description
Submucosal edema	
0	None
1	Mild
2	Moderate
3	Severe
Damage/necrosis	
0	None
1	Mild
2	Moderate
3	Severe
Inflammatory cell infiltration	
0	None
1	Mild
2	Moderate
3	Severe
Vasculitis	
0	None
1	Mild
2	Moderate
3	Severe
Perforation	
0	–
1	+
Maximum score: 13	

vol of cold-potassium buffer (20 mmol/l K_2HPO_4, pH 7.4). Then the homogenate was centrifuged at 500 *g* for 10 min at 4°C. The pellet was re-homogenized with an equivalent volume of 50 mmol/l K_2HPO_4 containing 0.5% (w/v) hexadecyltrimethyl-ammonium hydroxide. MPO activity was assessed by measuring the H_2O_2-dependent oxidation of *o*-dianisidine 2HCl. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance of 1.0/min at 460 nm and 37°C [13].

Statistical analysis

Data were expressed as mean ± SE. Groups of data were analyzed using analysis of variance (ANOVA) followed by Tukey–Kramer test. Values of $P < 0.05$ were accepted as significant.

Results

The macroscopic score, wet weight, microscopic score and tissue MPO activities were significantly higher in TNBS-E-treated rats than in the control and vehicle groups.

Macroscopic lesion score

Macroscopic lesion score of the colitis group was higher than that of the control and vehicle-treated groups. HBO treatment reduced the colonic damage relative to that in the untreated colitis group. Dexamethasone treatment showed no significant reduction when compared with the untreated colitis group. HBO therapy was found to be more effective than dexamethasone treatment in ameliorating the macroscopic lesion score. The difference between the dexamethasone-treated group and the HBO + dexamethasone group was not statistically significant (Fig. 1).

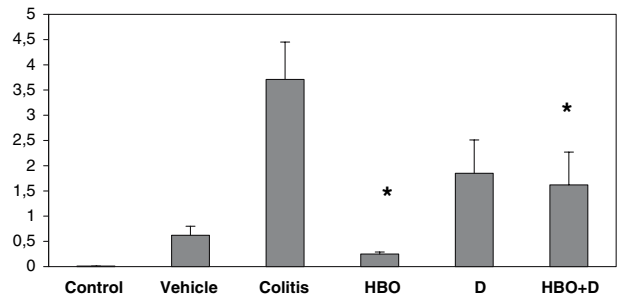

Fig. 1 The macroscopic colonic damage scores. *HBO*hyperbaric oxygen therapy group, *D* dexamethasone group, *HBO + D* hyperbaric oxygen and dexamethasone combined therapy group. $*P < 0.001$ versus the colitis group

Microscopic lesion score

Both HBO therapy and combined therapy of HBO with dexamethasone reduced microscopic lesion scores relative to those in the colitis-group, significantly. However, treatment with dexamethasone alone did not affect this score (Fig. 2).

Tissue wet weight

The tissue wet weight was found to be significantly reduced in the HBO and combined HBO + dexamethasone-treated rats when compared with the colitis group. The dexamethasone therapy alone also decreased the tissue wet weight, although not significantly. There were no significant changes in the tissue wet weight scores among the HBO, dexamethasone, and combined HBO + dexamethasone groups (Fig. 3).

Tissue MPO activity

The differences in tissue MPO activity between the HBO and colitis groups and the dexamethasone and colitis

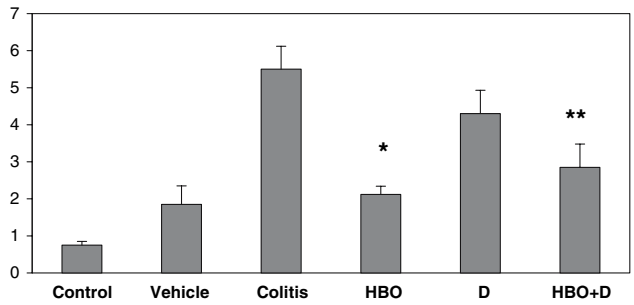

Fig. 2 Microscopic colonic damage scores. *HBO* the hyperbaric oxygen therapy group, *D* the dexamethasone group, *HBO + D* the hyperbaric oxygen and dexamethasone combined therapy group. $*P < 0.001$ and $**P < 0.05$ versus the colitis group

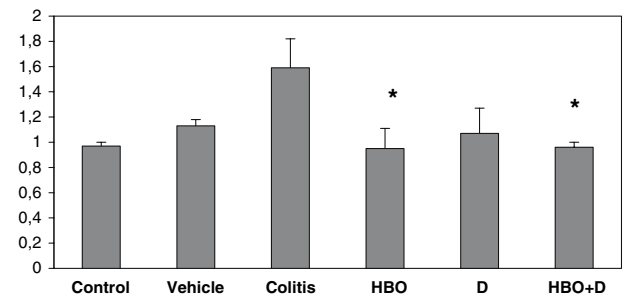

Fig. 3 The tissue wet weight (g). *HBO* the hyperbaric oxygen therapy group, *D* the dexamethasone group, *HBO + D* the hyperbaric oxygen and dexamethasone combined therapy group. $*P < 0.05$ versus the colitis group

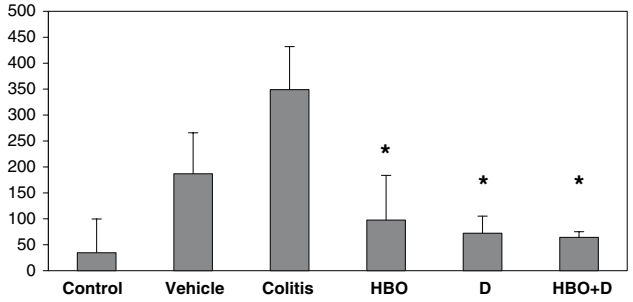

Fig. 4 The tissue myeloperoxidase activity (Units). *HBO* the hyperbaric oxygen therapy group, *D* the dexamethasone group, *HBO + D* the hyperbaric oxygen and dexamethasone combined therapy group. *$P < 0.001$ versus the colitis group

groups were statistically significant, but there was no difference between the HBO + dexamethasone groups. The combined HBO + dexamethasone therapy was not more effective than the HBO or the dexamethasone therapy alone (Fig. 4).

Discussion

In this study, we demonstrated that HBO therapy effectively decreased the activity of experimental colitis as measured by the tissue wet weight, macroscopic score, microscopic score, and tissue MPO activity. The dexamethasone treatment significantly reduced the colitis activity determined by the tissue wet weight and MPO activity. There were also decreases in macroscopic and microscopic activity scores with the dexamethasone therapy, although this was not statistically significant. The combined therapy with HBO and dexamethasone provided no additional benefit over HBO therapy alone.

A routine HBO therapy consists of intermittent inhalation of 100% oxygen at pressures greater than that at sea level. Oxygen inhaled at pressures greater than room air pressure dissolves in plasma. Approximately 6.8% vol. of oxygen is sufficient to tissue oxygenization in the absence of hemoglobin [4, 14]. Besides improving tissue hypoxia, HBO increases the resistance of the tissues against infections. HBO is bactericidal for certain anaerobe bacteria such as Clostridia and Escherichia. It also improves postischemic injuries and accelerates wound healing [15].

Lavy et al. reported that HBO treatment was safe and effective in a small series of patients with refractory perianal Crohn's disease [16]. In an experimental study, Rachmilewitz et al. found HBO therapy to effectively decrease the injury in both TNBS-E- and acetic-acid-induced models of colitis [1]. Results of our study supported Rachmilewitz et al. Additionally our study demonstrated that HBO therapy is as effective as dexamethasone therapy, which is known as one of the most important therapeutic agents in IBD management.

Rothfuss et al. pointed to a controversial topic that HBO itself may increase reactive oxygen species and may cause DNA damage in human subjects. This concept could have blocked the further HBO investigations in IBD therapy. However, interestingly, in their human study, DNA damage was detected only after the first treatment and not after further treatments under the same conditions. Their results indicated that HBO induces an increase in antioxidant defense system as an adaptive response that protects tissues against oxidative stress. In their human study, they pointed out that HBO-induced protection lasts for at least 1 week, although with some degree of interindividual variation, and is a cellular effect [17].

Gulec et al. tested HBO for acetic-acid-induced colitis and evaluated antioxidant systems to clarify its possible effects. HBO decreased malondialdehyde levels in erythrocyte, plasma, and intestinal tissue; and it increased the levels of glutathione peroxidase and superoxide dismutase activities. The activation of these antioxidant systems was supposed toinvolve the mode of HBO action [18]. In another study, HBO treatment significantly ameliorated trinitrobenzenesulfonic acid-induced chronic colitis via decreasing the MPO activity [19].

Gorgulu et al. revealed that HBO therapy is effective in reducing the extent of colitis induced by acetic acid, although it is not as potent as 5-aminosalicylic acid. The combination of HBO and 5-aminosalicylic acid was found to be much more potent in the reduction of the severity of colitis [20].

HBO therapy suppresses free oxygen radicals which have been reported to be increased in IBD [21]. Microvascular thrombosis has also been claimed as a pathogenic factor, especially in Crohn's disease. Prothrombotic state and ischemia have been proposed to cause necrosis, inflammation, and ulceration seen in IBD. HBO may also ameliorate the activity of colitis by increasing tissue O_2 diffusion [22].

It is accepted that in a genetically susceptible host, commensal bacteria and their products cause a constant antigenic stimulus which results in an aggressive immune response in IBD [23]. Antibiotics such as metronidazole and ciprofloxacin have been proven to be useful in management of Crohn's disease [24–27]. HBO may enhance bactericidal activity and alter the function of immune system. Weisz et al. showed that HBO therapy decreases interleukin-1, interleukin-6 and tumor necrosis factor-α from circulating monocytes [28].

Some adverse effects such as reversible myopia and cataract formation may occur. In addition, rupture of the middle ear or damage to cranial sinuses, teeth, and lungs due to barotraumas have been reported. However, these

complications are very rare and when standard protocols are used HBO therapy is quite safe [29]. HBO might be useful, especially in the treatment of refractory IBD patients, as a therapeutic option and deserves further and larger clinical studies.

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