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Original Research Article

Effects of Hyperbaric Oxygen Therapy for *Clostridioides difficile*-associated Colitis: A Retrospective Study

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Abstract

Objectives: *Clostridioides difficile* (CD) is an anaerobic spore-forming Gram-positive rod that is a major cause of antibiotic-associated diarrhea. Hyperbaric oxygen therapy (HBO) is a well-established treatment for Clostridium perfringens, but there are no reports that have examined the efficacy of HBO against CD, which is also an anaerobic bacterium.

Methods: In this study, we retrospectively examined whether HBO therapy affects the prognosis following CD infections (CDI). This study included 92 inpatients diagnosed with CDI at our hospital between January 2013 and December 2022. Of these, 16 patients received HBO therapy. The indications for HBO therapy were stroke in five patients, ileus in four patients, cancer in two patients, acute peripheral circulatory disturbance in two patients, and others in three patients. The mean observation period was 5.4 years.

Results: In the univariate analysis, there was no significant difference in severity, mortality, hospitalization, or overall survival between patients who did and did not receive HBO therapy. However, the HBO group had a significantly lower recurrence rate (0% vs. 22.4%, p=0.0363) and a shorter symptomatic period (6.2 vs. 13.6 days, p=0.0217).

Conclusions: HBO may have beneficial effect on CDI by shortening the symptomatic period and preventing recurrence.

Keywords

Clostridioides difficile infection, hyperbaric oxygen therapy

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Introduction

Clostridioides difficile (CD) is an anaerobic, spore-forming, Gram-positive rod-shaped bacterium that is a major cause of antimicrobial-associated diarrhea [1,2]. Toxins A and B, which are produced by CD that proliferate due to imbalances in the intestinal flora caused by antimicrobial agents, can be pathogenic and cause CD infections (CDI) [3,4]. Clostridial gas gangrene is characterized by rapid myonecrosis due to Clostridium perfringens. Hyperbaric oxygen therapy (HBO) is a highly effective treatment option that provides high concentrations of dissolved oxygen to hemodynamically impaired tissues, activates the oxygen-dependent oxidative killing of leukocytes, and exerts bactericidal effects against anaerobic and other bacteria. HBO also effectively increases intracellular antibiotic transport and

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promotes oxygen-free radical synthesis [5,6], making it an important and valuable treatment option for patients with Clostridial gas gangrene.

There is currently no consensus regarding the inhibitory effects of HBO on CDI, particularly its potential toxicity. Recently, however, the anti-inflammatory effects of HBO have been acknowledged in other forms of intestinal inflammation, including inflammatory bowel disease. Inflammatory and anti-inflammatory cytokines play a role in the pathogenesis of CDI and contribute significantly to its manifestations by influencing the immune system [7]. Various cytokines, such as interleukin (IL)-1 and IL-6, are upregulated in CDI, while HBO downregulates their expression [8,9]. Furthermore, secondary bile acids inhibit CDI progression at different stages, and the loss of mucus-adherent taxa resulting from HBO therapy reduces microbial diversity and increases the proportion of secondary bile acids [10]. It was anticipated that HBO would exhibit inhibitory effects against CDI by suppressing inflammatory immune responses primarily through the reduction of these cytokines and by inhibiting CD development through the augmentation of secondary bile acids. In this study, we investigated whether HBO provided clinical benefits to patients with CDI.

Methods

This clinical trial was approved by the Ethics Committee of Tobata Kyoritsu Hospital and complied with the Declaration of Helsinki. The option to opt out was clearly defined and was always available to patients by presenting this information on the website. This retrospective study was conducted at Tobata Kyoritsu Hospital (Fukuoka, Japan) from January 1, 2013 to December 31, 2022. And the consent has been obtained from all patients and relevant persons (such as the parent or legal guardian) to publish the information, including photographs. A confirmed diagnosis of CDI was based on a positive toxin test using a rapid membrane enzyme immunoassay performed with the commercially available C. DIFF WUIK CHEK COMPLETE kit (Abbott, Chicago, IL, USA) as well as a clinical diagnosis. Patients with positive stool cultures or endoscopically proven pseudomembranes were eligible for recruitment. Patients aged <20 years and outpatients were excluded from the study. CDI recurrence was defined as a positive result on a toxin test or treatment with antibiotics specifically targeting *C. difficile* (orally administered vancomycin, metronidazole, or both) within 8 weeks of enrollment [11]. Clinical data were obtained on the first day of antibiotic treatment specifically targeting *C. difficile*. In cases wherein clinical data were unavailable on the first day of treatment, laboratory data measured the day before and obtained within the period from the onset of CDI to the third day after treatment initiation were used. The primary endpoint of this study was the short-term prognosis of patients diagnosed with CDI, including the duration of symptomatic illness and hospitalization. Additionally, the recurrence rate was assessed as an important outcome measure. Moreover, the long-term prognosis of patients was evaluated by examining their overall survival rates.

The Mikamo-Nakamura (MN) criteria were proposed as the first Japanese CDI severity scoring system in 2017 [6] and were based on nine categorical variables: age, abdominal pain/distension, body temperature, episodes of diarrhea, hematochezia, white blood cell (WBC) count, estimated glomerular filtration rate, serum albumin (Alb) level, and imaging findings. Each variable is scored on a 3-point scale. Asaoka et al. [12] reported the accuracy of the MN criteria in determining CDI severity and conclusively demonstrated that Alb levels and WBC counts are critical in accurately determine CDI severity.

The duration of HBO therapy was determined based on the underlying pathology. The indications for HBO were stroke in five patients, ileus in four patients, cancer in two patients, acute peripheral circulatory disturbance in two patients, and others in three patients. The treatment protocol consisted of HBO administration daily, comprising of 100% oxygen at 2.5 atmosphere absolute for 60 minutes.

All statistical analyses were performed using JMP software (version 16.0; SAS Institute, Cary, NC, USA). Clinicopathological variables were compared using the Chi-squared test (or Fisher's exact test). Logistic regression was used to identify potentially relevant variables based on univariate analyses ($P < 0.05$). Survival rates were calculated using the Kaplan-Meier method and compared using the generalized Wilcoxon test.

Results

The median follow-up period was 5.4 years. Overall, 195 patients were included in this study (Figure 1). Nineteen patients were excluded because they received HBO therapy outside the treatment period for CDI. In the remaining 176 cases, severity was scored based on the MN criteria. The 92 patients with available MN data were classified into four groups according to the severity of CDI: mild (n=9), moderate (n = 60), severe (n=18), and fulminant (n=5). Additionally, patients were divided into the HBO (16 patients, 17.4%) and non-HBO groups (76 patients, 82.6%). The average age of patients in the HBO and non-HBO groups was 78.6 years (standard deviation [SD]: ± 10.3) and 82.6 years (SD: ± 10.5), respectively. There were no significant differences in sex, hospitalization before current admission, or category of admission between the two groups; however, the body mass index was higher in the HBO group (p=0.0212).

Concerning risk factors such as comorbidities, only the incidence of liver disease was higher in the HBO group than

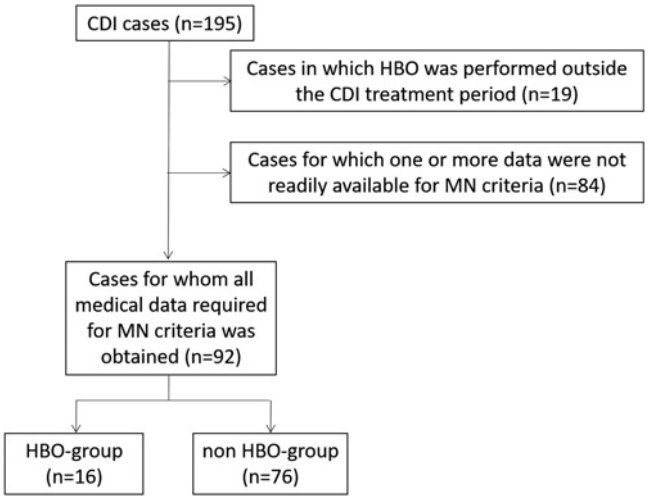

Figure 1. Flow chart of cases included in this study.

in the non-HBO group (p=0.0097). There were no significant differences in the proportion of patients with comorbidities, medications, or symptoms.

There were no significant differences between the two groups in terms of symptoms such as fever, abdominal pain, abdominal fullness, or diarrhea. However, the WBC count was significantly higher in the non-HBO group than in the HBO group. Meanwhile, there were no differences in the other laboratory parameters.

Recurrent CDI was usually defined as onset within 8 weeks of a previous episode (A). Relapsed CDI is known to be refractory and relapse with high frequency after treatment, placing a significant burden on healthcare (A). Of the 92 patients with CDI, 3 cases (3.2%) had recurrent CDI. All of these patients were in the non-HBO group, but there was no statistically significant difference between the two groups.

There was no significant difference in severity scoring (MN criteria) between the HBO and non-HBO groups. As for clinical events, the length of ICU stays and complementary surgical procedures were not significantly different between the two groups (Table 1).

In the HBO group, vancomycin and metronidazole were administered to eight and seven patients, respectively. In the non-HBO group, vancomycin was administered to 35 patients, while metronidazole was administered to 25 patients. Additionally, both antibiotics were administered to 13 patients. No statistically significant differences were found in the use of antibiotics.

The short-term outcomes are presented in Table 2. In the HBO group, the short-term prognosis was better than that in the non-HBO group, as evidenced by the shorter duration of symptoms (abdominal pain, distention, and diarrhea) (p=0.0217) and absence of recurrence (p=0.0388). However, there were no significant differences in the length of hospital stay and hospital mortality between the two groups. Next, we compared the long-term outcomes between the HBO and non-HBO groups. The overall survival rate did not differ significantly between the two groups (Figure 2a). On the other hand, the recurrence rate was significantly lower in the HBO group. In addition, short-term and long-term prognoses were additionally examined by the severity of CDI. There was no significant difference in overall survival between the HBO and non-HBO groups by severity of disease (Figure 2b). In a study of all CDI patients, the recurrence rate was lower in the HBO group (Figure 3a). Examination of recurrence rates by severity with and without HBO showed no statistically significant differences (Figure 3b). The recurrence rate was significantly lower in the HBO group for patients with less severe disease, except for fulminant cases (Figure 3c). Nevertheless, it should be noted, that there were no fulminant cases in the HBO group. In the severity-based examination of symptom duration, a reduction in symptom duration in HBO was observed, especially in the group of patients with moderate disease (Figure 4).

Discussion

In healthy individuals, CD is a nonpathogenic colonizer of the colon. For CD to cause disease, several conditions must be met, including disruption of the normal diversity of the gut microbiome that is often triggered by antibiotic treatment or cancer chemotherapy. Additionally, the host must be colonized with a toxigenic strain of CD that can produce cellular toxins. Colonization may occur before or after microbiome disruption. Furthermore, the transformation of CD bacteria into a vegetative state is required for the activation of CD genes that produce cytotoxic proteins, which damage the enteric mucosa and cause local inflammation, ultimately leading to disease [13]. The heightened frequency and severity of CDI have resulted in a major economic burden on healthcare systems owing to the costs associated with treatment and extended hospital stays [2,3]. Oxygen therapy is considered a potential treatment for anaerobic infections, including CDI [14]. In 1960, Brummelkamp et al. reported the successful use of HBO therapy in four cases of gas gangrene [15]. Thereafter, HBO has become an indispensable treatment method that can increase survival rates and preserve affected limbs. In cases of intestinal infections, successful treatment of toxic megacolonization with HBO therapy was reported by Kuroki [16]. Furthermore, there are case reports demonstrating favorable outcomes following enteral oxygenation for CDI [17].

However, the current scientific validation of HBO for CDI is inadequate. Similar to the research conducted on *Clostridium perfringens (C. perfringens)*, there is a need for basic and clinical studies on *C. difficile* to ascertain the effectiveness of HBO therapy in treating CDI. In clinical studies, it

Table 1. Comparison of the Clinical Characteristics of Patients with CDI.

Factor Background characteristics	HBO (n=16)	Non-HBO (n=76)	P-value
Age	78.6±10.3	82.6±10.5	0.1269
Sex (male/female)	10/6	35/41	0.2316
BMI	20.5±4.0	18.1±3.0	0.0212
Hospitalization before the current admission			
Never or >12 months before	10 (62.5%)	36 (47.4%)	0.5183
2-12 months before	2 (12.5%)	13 (17.1%)	0.6504
<2 months before	4 (25%)	27 (35.5%)	0.4181
Category of admission			0.5832
Medicine	11 (68.8%)	60 (79.0%)	
Emergency surgery	4 (25%)	11 (14.5%)	
Scheduled surgery	1 (6.2%)	5 (6.5%)	
Comorbidities			
Hepatic	3 (18.8%)	2 (2.63%)	0.0097
Cardiovascular	5 (31.3%)	22 (29.0%)	0.8541
Pulmonary	6 (37.5%)	33 (43.4%)	0.6631
Renal	4 (25%)	22 (29.0%)	0.7499
Immunosuppression	0	2 (2.63%)	0.5118
Inflammatory bowel disease	0	1 (1.32%)	0.6446
Diabetes mellitus	6 (37.5%)	24 (31.6%)	0.6461
Malignancy	5 (31.3%)	14 (18.4%)	0.2493
Medication use			
Antibiotic	14 (87.5%)	61 (80.3%)	0.4978
Proton pump inhibitor	7 (43.8%)	27 (35.5%)	0.5357
H2 antagonist	0	8 (10.5%)	0.1744
Chemotherapy	2 (12.5%)	1 (1.32%)	0.0221
Glucocorticoid	1 (6.3%)	2 (2.6%)	0.4589
NSAID	3 (18.8%)	7 (9.2%)	0.2652
Symptom			
Body temperature (ºC)	37.8±0.6	37.9±0.7	0.8729
Abdominal pain or distention	5 (31.3%)	12 (15.8%)	0.1476
Diarrhea (Bristol scale≥5)	6.1±3.9	5.2±3.4	0.5965
Laboratory			
White blood cell count (/μL)	8238±2902	12923±7324	0.0058
Platelet count ($\times 10^4/mm^3$)	28.3±16.3	27.4±10.2	0.9180
CRP (mg/dL)	10.8±6.9	8.0±7.2	0.0580
Albumin (g/dL)	2.4±0.5	2.5±0.5	0.5477
CRE (mg/dL)	1.2±1.0	1.5±1.7	0.9918
eGFR (mL/min/1.73 m^2)	64.7±36.5	58.2±36.1	0.5433
MN criteria (Mild/Moderate/Severe/Fulminant)	2/12/2/0	7/48/16/5	0.5772
Recurrent CDI	0	3 (3.9%)	
ICU stay	0	4 (5.3%)	0.3481
Surgery for CDI	0	0	

CDI, Clostridium difficile infection; HBO, Hyperbaric oxygen therapy: BMI, Body mass index; H2, Histamine type 2; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs; CRP, C-reactive protein: Cre, Creatinine: eGFR, estimated Glomerular Filtration Rate

is necessary to measure the levels of *Clostridioides* bacteria and toxin activity in stool before and after HBO therapy and to compare the HBO group with the pure oxygen inhalation group under normal atmospheric pressure [18]. To explore the potential use of HBO as a treatment for CDI, we selected patients who had received HBO for other conditions at the onset of CDI and compared them with a control group that had not undergone HBO. In this study, although there was no significant difference in the long-term prognosis between the two groups, the HBO group exhibited a

Table 2. Comparison of Prognosis of Patients with CDI.

	HBO (n=16)	Non-HBO (n=76)	P-value
Short-term prognosis			
Duration of symptoms (days)	6.2±3.5	13.6±15.5	0.0217
Duration of hospital stay (days)	46.4±24	39.6±24	0.2703
Death during hospital stay	0	4 (5.26%)	0.3481
Recurrence	0	17 (22.4)	0.0361
Long-term prognosis			
Overall survival (months)	23.7±27.6	19.2±26.4	0.1839

HBO, Hyperbaric oxygen therapy; CDI, Clostridium difficile infection

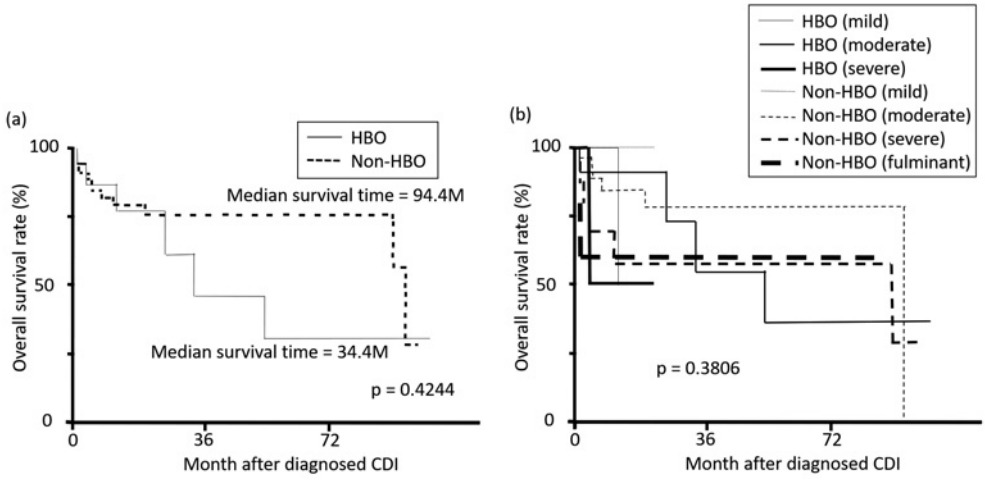

Figure 2. (a) Overall survival rate after treatment for CDI in patients with or without HBO. (b) Overall survival rate of CDI patients by severity of illness.

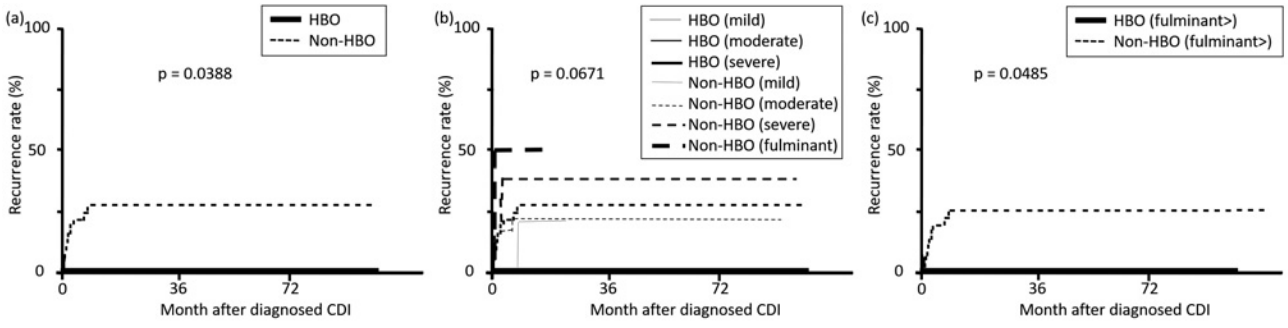

Figure 3. (a) Recurrence rate after treatment for CDI in patients with or without HBO. (b) Recurrence rate after treatment for CDI in patients by severity of illness. (c) Recurrence rate after treatment for CDI with and without HBO excluding fulminant cases.

shorter symptomatic period and lower recurrence rate than the non-HBO group. The recurrence rate was significantly lower in the HBO group for patients with less severe disease, except for fulminant cases. Nevertheless, it should be noted, that there were no fulminant cases in the HBO group. In the severity-based examination of symptom duration, a reduction in symptom duration in HBO was observed, especially in the group of patients with moderate disease. These results suggest that HBO may contribute to improved prognosis, at least in patients with less severe disease, especially in patients with moderate CDI. However, since the number of cases in which HBO was performed in patients with fulminant or severe CDI was small, the possibility of good results even in severe cases should be considered, depending on future case accumulation and research.

There are multiple possible mechanisms by which HBO

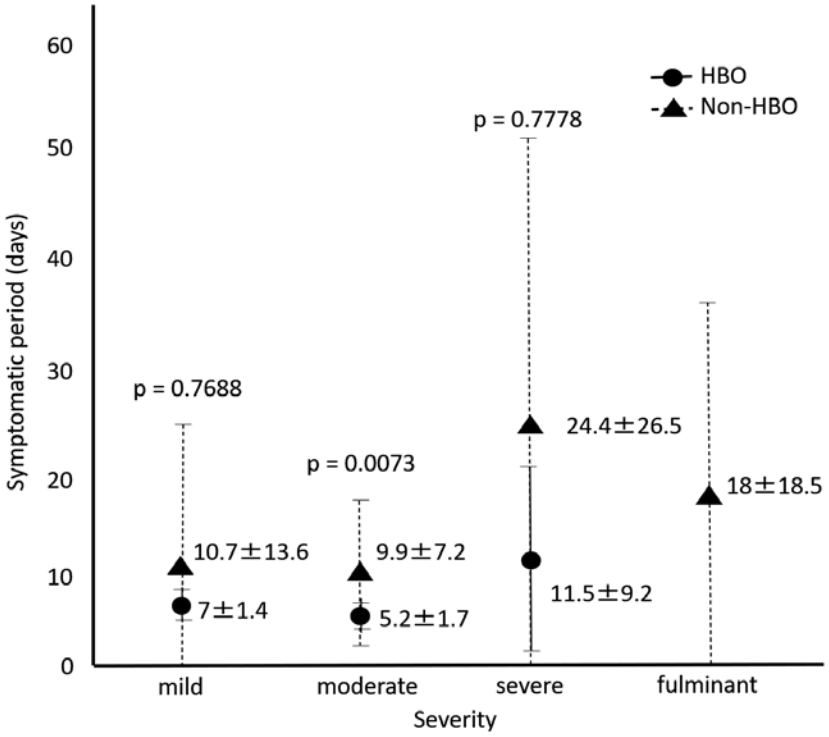

Figure 4. Comparison of symptomatic duration of CDI by severity and with and without HBO.

affects CDI. Oxygen is toxic to anaerobic bacteria, either through the production of free radical superoxides or by increasing the redox potential [19]. HBO has been shown to kill anaerobes, suppress toxin production by *C. perfringens*, and inhibit the growth of *Escherichia coli* and other aerobes [20]. Based on several case reports, oxygen therapy may suppress CD [21,22].

Recently, the role of inflammation and cytokines in the severity of CDI has gained significant attention. HBO not only improves hypoxia but also affects inflammation and immune mediators [21]. CDI is characterized by a robust inflammatory response [22]. Toxins produced by CD directly damage the intestinal epithelium, triggering the release of cytokines and causing significant neutrophil activation and recruitment, leading to further intestinal injury [23]. Once an inflammatory response is initiated, the effectiveness of antibiotics in protecting against the disease is limited. Recent findings indicate that persistent diarrhea in patients with CDI undergoing appropriate antibiotic therapy correlates with intestinal inflammation rather than bacterial burden [24]. Inhibition of the acute inflammatory response can potentially reduce intestinal injury [23]. Notably, experimental studies have demonstrated that anti-inflammatory agents can attenuate intestinal injury in patients with CDI [24,25], suggesting that modulating the host inflammatory response could be an effective strategy when combined with antibiotic or probiotic approaches for CDI therapy and prevention. Hence, gaining insight into the molecular drivers of acute inflammation is crucial for developing innovative host-centered therapies. Moreover, the influx of neutrophils into the epithelium and submucosa is a characteristic feature of severe CDI [26]. Therefore, cytokines induced by immune cells in response to CDI may be associated with disease severity and, if confirmed, could serve as therapeutic targets. Several cytokines have emerged as biologically relevant markers of the disease and disease severity in patients with CDI. Elevated levels of IL-6, IL-8, and IL-1β have been consistently observed in CDI, indicating their crucial involvement in its pathogenesis [7,27,28]. In human studies and model systems, HBO suppresses neutrophil adhesion and attenuates the production of inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor-α [8,9]. IL-1 is a multifunctional cytokine that affects several cell types and frequently act synergistically with other cytokines and small molecules. While IL-1 is primarily a highly proinflammatory cytokine, it also offers clinical advantages such as promoting lymphocyte- and colony-stimulating growth factors. However, the therapeutic window for IL-1 in humans is narrow, with potential toxicity concerns [29]. IL-6, which is a member of the inflammatory cytokine family, triggers the expression of proteins contributing to acute inflammation. IL-6 trans-signaling plays a role in the induction of ICAM-1 expression in response to heat and facilitates the migration of lymphocytes into inflamed tissues [30]. In patients with

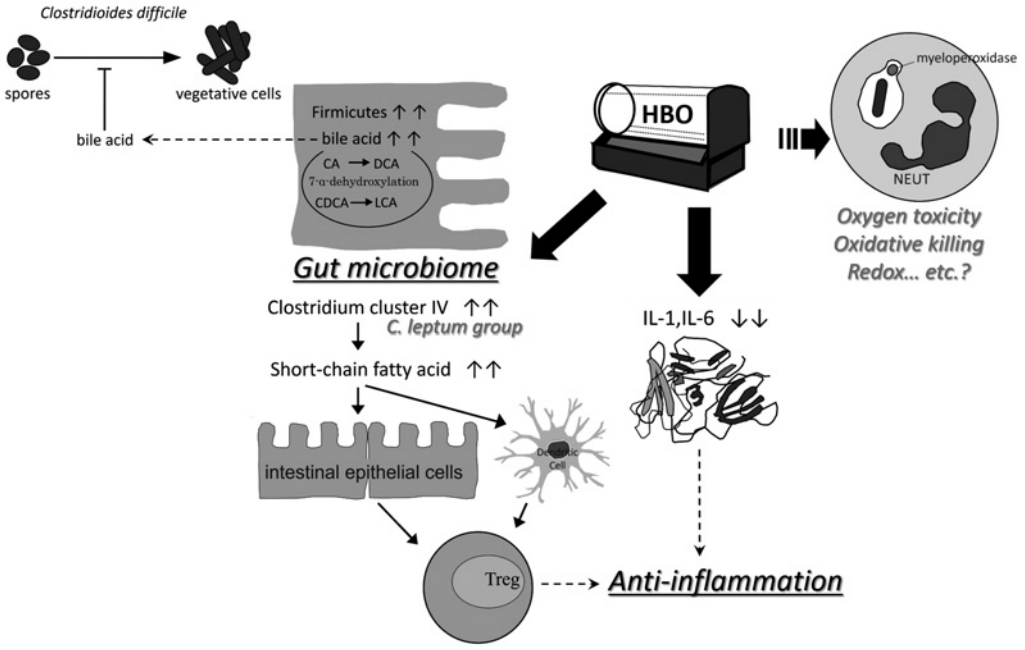

Figure 5. Relationship between gut microbiome, bile acids, Clostridium difficile and Interaction between immune system and HBO.

CDI, HBO therapy may alleviate intestinal inflammation and associated injury by suppressing IL-1 and IL-6.

Additionally, several studies have explored the connection between intestinal microbiota and CD, and it is plausible that HBO inhibits CDI by influencing the intestinal microbiota. The gut microbiota is essential for the host's nutritional metabolism, defense mechanisms, and immune system [31]. The breakdown of indigestible carbohydrates into short-chain fatty acids, synthesis of vitamins and amino acids, and metabolism of lipids as well as primary and secondary bile acids are necessary for host energy acquisition and maintenance of homeostasis in the digestive tract [32-34], Ursodeoxycholic acid has protective effects on colon cells by preventing apoptosis and oxidative damage [35]. Additionally, *C. difficile* is susceptible to the bactericidal effects of secondary bile acids. The secondary bile acids deoxycholic acid and lithocholic acid (LCA) inhibit CD in vitro, ex vivo, and in vivo [36]. Secondary bile acids are believed to play three potential roles for gut bacteria: acting as terminal electron acceptors for energy production; facilitating the formation of less hydrophobic bile acids that are less damaging to cell membranes; and influencing pathogen virulence [37,38]. In the case of CD, secondary bile acids modulate pathogenicity by inhibiting various stages of its life cycle [38-43]. These examples highlight the diverse and potentially multifaceted functions of bile acids in host and microbial physiology [36]. HBO significantly influences the microbial composition and function of the colon in animal studies [44]. Gonzalez et al. [10] investigated the role of HBO-induced microbial changes in mediating the effects of HBO on hosts. Following HBO, notable acute shedding of bacterial subpopulations was observed, accompanied by an increase in *Firmicutes* and LCA levels [44]. Animal models have shown that LCA supplementation exerts protective effects against colitis [45]. Furthermore, HBO increases levels of bile acids in healthy bacteria, particularly *Firmicutes*, which are involved in bile acid production [44,46]. Other possible mechanisms include Clostridium cluster IV and Clostridium subcluster XIVa are thought to activate intestinal epithelial cells and dendritic cells by producing short-chain fatty acids, a mechanism that induces regulatory T cells and activates intestinal immunity. These clusters have important anti-inflammatory properties and may improve glucose metabolism in the human body [46]. The genus Bacteroides has been reported to be involved in the induction of regulatory T cells; OYA et al. [46] studied the fecal microbiota of healthy volunteers who exposed hyperbaric conditions and reported that the frequency of distribution of Bacteroides. and subcluster XIVa was altered and cluster IV was increased. This suggests that the increase in Clostridium cluster IV due to hyperbaric conditions may have positive effects on intestinal immunity, such as anti-inflammatory effects. These findings suggest that HBO may alter the bacterial flora, leading to an elevation in protective secondary bile acids against colitis, including CDI (Figure 5).

Although regarded as safe, HBO has complications, with ear barotrauma being the most frequently encountered that occasionally hinders the treatment process [47,48]. Additionally, claustrophobia can affect up to 12% of patients, particularly those undergoing treatment in monoplace chambers,

and may lead to treatment interruption [49]. Nonetheless, in our study, no adverse effects related to HBO were observed.

The present study has several limitations. First, the study was retrospective, and the sample size was small. Second, ribotypes were not tested and could not be included in the study. Third, the recommended genetic testing (nucleic acid amplification test; NAAT) for glutamate dehydrogenase-positive and -negative cases in Japanese clinical practice was not performed in this study because of the lack of insurance coverage until 2019 [50]. Our study also did not include patients requiring mechanical ventilation. Furthermore, fidaxomicin administration and fecal transplantation were not performed in the present study.

Conclusion

HBO is a potential treatment method to reduce the recurrence rate and shorten the symptomatic period in patients with CDI.

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Conflicts of Interest

There are no conflicts of interest.

Author Contributions

Daisuke Muroya contributed to the concept and design and data acquisition and analysis and drafted and revised the manuscript; Shinya Nadayoshi, Koito Yamada, Yutaro Kai, Naoki Masuda, Takamichi Nishida, Masayuki Shimokobe, and Toru Hisaka contributed to data acquisition, revised the manuscript, and approved the final version.

Approval by Institutional Review Board (IRB)

This research was approved by the institutional review board of Tobata Kyoritsu Hospital (approval code: 22-05)

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