Biological effects of hyperbaric oxygen on human severe periodontitis

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Chen T, Zhou Y, Liu J, Xu B, Wu Z, and Li D. Biological effects of hyperbaric oxygen on severe human periodontitis. Undersea Hyperb Med 2002; 29(3):159-166 - This paper reports the effects of Hyperbaric Oxygen (HBO\textsubscript{2}) in a controlled study of periodontitis in 24 patients. The patients received either HBO\textsubscript{2} or no HBO\textsubscript{2}, and study teeth were divided into 4 groups based on treatment: 1- HBO\textsubscript{2} therapy, 2- HBO\textsubscript{2} + scaling, 3-scaling, 4-control. We measured indices of periodontal disease and gingival blood flow (GBF). The microorganisms in a periodontal pocket were stained and the percentage of straight rods (Rods), curved rods (Cur), fusiforms (Fusi) and spirochetes (Spiro) were observed. The numbers of anaerobic organisms were measured by routine anaerobic culture. Highly significant differences in Gingival Indices (GI), Sulcus Bleeding Indices (SBI), Probing Depth (PD), Attachment Loss (AL), Plaque Index (PLI), and GBF were seen in the HBO\textsubscript{2}, the HBO\textsubscript{2} + Scaling and the Scaling Groups compared to the Control Group (P<0.01). The number of subgingival anaerobes as well as the number of Rods, Cur, Fusi, and Spiro were reduced markedly in these three treatment groups. Statistically greater differences in clinical indices, GBF, subgingival anaerobe number and number of Rods, Cur, Fusi and Spiro were found by comparison of HBO\textsubscript{2} + Scaling and HBO\textsubscript{2} Groups, as well as between the HBO\textsubscript{2} + Scaling and Scaling Groups, but no significant differences were observed in GI, SBI, PD, or AL between the HBO\textsubscript{2} and Scaling Groups. In conclusion, HBO\textsubscript{2} had beneficial therapeutic effects on severe periodontitis. HBO\textsubscript{2} therapy combined with scaling and root planing was the most beneficial in the treatment of periodontitis. Clinical follow-up suggests that this treatment effect could last more than 1 year.

Hyperbaric oxygen, periodontitis, microcirculation, spirochetes, subgingival anaerobes

INTRODUCTION

Due to the prevalence and complex pathophysiology, there is at present no effective treatment for periodontitis. Experimental work, however, indicates that hyperbaric oxygen (HBO\textsubscript{2}) can inhibit the growth of anaerobes in periodontal pockets\textsuperscript{[1]} and increase gingival blood flow (GBF)\textsuperscript{[2-3]}. In recent years, some clinical case reports of HBO\textsubscript{2} therapy of periodontitis have been reported in Chinese medical journals\textsuperscript{[1,4]}. Myers et al also reported that HBO\textsubscript{2} increases the number of open blood vessels and blood flow of irradiated tissue\textsuperscript{[5]}. But to date, no research reports have been published on treatment regimen or mechanism of action of HBO\textsubscript{2} treatment of periodontitis. In
this study, outpatients with severe periodontitis were treated with HBO$_2$ in a controlled trial. Close observations were made on treatment results among different treatment groups and controls. Observations were compared on changes in clinical data, gingival blood flow (GBF), and both anaerobic and aerobic microorganisms in periodontal pockets pre and post treatment. Our experimental results suggest value in the use of HBO$_2$ for the treatment of periodontitis.

**MATERIALS AND METHODS**

**Research subjects.** Twenty-four patients (16 males, 8 females) with periodontitis (17 chronic generalized deep periodontitis, 7 aggressive generalized deep periodontitis) were instructed on oral hygiene, referred to the Periodontal Department of Stomatological Research Center and chosen for our study. Their ages ranged from 18 to 65 years (mean age 45.5±5.5). Patients were selected on the basis of generalized severe periodontitis, where pockets could be probed to 4 mm or more and where approximately one quarter or more of the original alveolar bone height had been lost. All patients had at least 20 remaining teeth and were willing to participate in the study. They had not received any irradiation and other periodontal therapy in the past 3 years, nor did they have any history of allergy, diabetes, blood dyscrasias, or chronic liver or kidney disease. None of the patients were heavy smokers or alcohol drinkers. None of the women were pregnant. They were randomly divided into 2 groups, HBO$_2$ therapy and non-HBO$_2$ therapy. For HBO$_2$ therapy, the patients were exposed to a pressure of 2.5ATA (0.25MPa) breathing pure oxygen once a day for 90 minutes with an air break for 10 minutes in the middle of the session (exposure schedule: pure oxygen 45 minutes, air 10 minutes and pure oxygen 45 minutes). The subjects received a total of 10 treatment sessions [1]. The left-sided teeth of all patients were treated with scaling and root planing with the same techniques, namely, ultrasonic combined with manual mechanical scaling. The right-sided teeth remained untreated. The initial 2 groups of patients were each divided into 2 additional sub-groups, based on different treatments. The HBO$_2$ group was divided into the HBO$_2$ Group (G1) and the HBO$_2$ + Scaling Group (G2). The Non-HBO$_2$ Group was divided into the Scaling Group (G3) and the Control Group (G4).

**Clinical Assessment.** Clinical data were assessed as follows, Gingival Indices (GI) were measured in accordance with the Loe Standard [6] during the first clinical visit, immediately after the last HBO$_2$, and 1 year after HBO$_2$ therapy. Sulcus bleeding indices (SBI) were assessed in accordance with the Muhlemann Standard, [7] and plaque deposits were recorded using the plaque index (PLI, Silness)[8]. Probing depth (PD) and attachment loss (AL) was recorded to the nearest mm by a single investigator (Ramfjord) [9]. Clinical data of six test teeth of each group were measured, i.e. the three maxillary and three mandible teeth of each group were chosen at random to measure the clinical indices. Two teeth of six teeth in each group were selected for measurement of gingival blood flow and assessment of anaerobes, and four of the six teeth in each group were chosen to measure subgingival bacteria. The measurement of gingival blood flow, anaerobes and subgingival bacteria for all experimental teeth before HBO$_2$ therapy, immediately after the last HBO$_2$ therapy and 1 year after HBO$_2$ therapy were made at the same time as the clinical assessment.
**Measurement of Gingival Blood Flow.** The GBF of subjects were measured with a Laser Doppler Flow-III instrument for all experimental teeth pre and post therapy. The following parameters were typically used: time constant (0.22s), frequency (12kHz) and gain ×3 for measurement. The signal was fed into a computer, and analyzed with JI-200 laser microcirculatory dynamic analysis software. The probes were placed vertically 3mm away from the gingival margin and then touched to the margin gently\(^{10}\).

**Assessment of Anaerobes.** Bacterial samples were obtained as follows: when no detectable supra-gingival microbial accumulations were present, a clean and standard curette was introduced through the pocket orifice as far as possible and the contents removed. The process was repeated to obtain enough material. If the crown was covered with substantial accumulation of bacterial debris, the latter was first scraped off from the tooth surface coronal to the pocket orifice prior to sampling. The plaque from the base of each periodontal pocket was placed in 1.0ml of sterile thioglycolate salt solution and then ultrasonicated for 30 seconds. Immediately afterwards, bacterial suspensions of 10 fold dilution were prepared and 0.1ml of the diluents were spread on 5% blood agar plates, one with and one without 1.0ml of streptomycin and 300ug of clindamycin phosphate per ml. All plates were cultured under anaerobic conditions for 4 days. Colonies of anaerobes were confirmed by Gram-stain, colony morphology and biochemical tests. The number of anaerobes was calculated, and the total sum of anaerobes in the diluted specimen was assessed\(^{11}\).

**Subgingival Plaque Stain.** The plaque microorganisms in each periodontal pocket base were stained with 2% Congo red and pure hydrochloric acid and a proper field of vision was selected for measurement. The slide was examined by light microscopy at a magnification of ×1200. The percentages of coccoid cells (Cocci), straight rods (Rods), curved rods (Cur), fusiforms (Fusi) and spirochetes (Spiro) were calculated under the light microscope\(^{12}\).

**Statistical Analysis.** Differences between before HBO\(_2\) therapy and after the last HBO\(_2\) therapy, and between immediately after the last HBO\(_2\) therapy and 1 year after HBO\(_2\) therapy values for each parameter were analyzed using the Student \(t\)-test for paired observations. Differences between groups were analyzed by means of Student \(t\)-test for unpaired observations. The level of statistical significance was taken at \(p=0.05\).

**RESULTS**

**Effects of HBO\(_2\) on GI, SBI, PD, AL and PLI.** Assessment of Gingival Indices, Sulcus Bleeding Indices, Probing Depth, Attachment Loss, and Plaque Indices indicated that highly statistically significant differences were seen in the HBO\(_2\) Group, the HBO\(_2\) + Scaling Group and the Scaling Group compared with the Control Group. Significant differences could also be seen by comparison of the HBO\(_2\) + Scaling Group and the HBO\(_2\) Group, as well as between the HBO\(_2\) + Scaling Group and the Scaling Group (\(p<0.01\), see Table1). Very significant differences could also be seen in certain clinical data, e.g.Gingival Indices, Sulcus Bleeding Indices, Probing Depth, Attachment Loss, and Plaque Indices between the pre and post treatment groups in the HBO\(_2\) Group, the HBO\(_2\) + Scaling Group and the Scaling Group (\(p<0.01\)). However, no significant differences...
were observed in the Gingival Indices, Sulcus Bleeding Indices, Probing Depth, and Attachment Loss between the HBO₂ Group and the Scaling Group.

Table 1. Comparison of GI, SBI, PD, AL and PLI between groups after HBO₂ therapy ( x ±s ) *

<table>
<thead>
<tr>
<th>Group</th>
<th>Tooth number</th>
<th>GI</th>
<th>SBI</th>
<th>PD</th>
<th>AL</th>
<th>PLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group (G4)</td>
<td>72</td>
<td>2.1±0.5</td>
<td>2.3±0.4</td>
<td>4.8±0.5</td>
<td>5.2±0.5</td>
<td>2.8±0.5</td>
</tr>
<tr>
<td>Scaling Group (G3)</td>
<td>72</td>
<td>1.2±0.5*</td>
<td>1.4±0.3*</td>
<td>4.2±0.5*</td>
<td>4.6±0.5*</td>
<td>1.2±0.5*</td>
</tr>
<tr>
<td>HBO₂ Group (G1)</td>
<td>72</td>
<td>1.0±0.5*</td>
<td>1.4±0.4*</td>
<td>4.1±0.5*</td>
<td>4.5±0.6*</td>
<td>2.0±0.4*##</td>
</tr>
<tr>
<td>HBO₂+ Scaling Group (G2)</td>
<td>72</td>
<td>0.6±0.5*##</td>
<td>1.0±0.5*##</td>
<td>3.7±0.5*##</td>
<td>3.7±0.6*##</td>
<td>0.7±0.3*##</td>
</tr>
</tbody>
</table>

* Significant differences were noted between three treatment groups and G4, p<0.01.
# Significant differences were noted between G2 and G3, and also between G1 and G3, p<0.01.
† Significant differences were noted between G2 and G1, p<0.01.
✿ Numbers are mean ± standard error of the mean.

Effects of HBO₂ on Gingival Blood Flow. Statistically significant differences in Gingival Blood Flow could be seen between the pre and post treatment groups in the HBO₂ Group, the HBO₂ + Scaling Group and the Scaling Group. Assessment of Gingival Blood Flow indicated that highly statistically significant differences were seen in the HBO₂ Group, the HBO₂ + Scaling Group and the Scaling Group compared with the Control Group. Very significant differences in Gingival Blood Flow could also be seen when comparisons were made between the HBO₂ + Scaling Group and the HBO₂ Group, as well as between the HBO₂ + Scaling Group and the Scaling Group (p<0.01, see Table 2).

Table 2 Comparisons of GBF between groups (MV, x ±s ) *

<table>
<thead>
<tr>
<th>Group</th>
<th>Tooth number</th>
<th>GBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4</td>
<td>24</td>
<td>139.8±31.5</td>
</tr>
<tr>
<td>G3</td>
<td>24</td>
<td>210.2 ±3.5*</td>
</tr>
<tr>
<td>G1</td>
<td>24</td>
<td>255.7±18.5##</td>
</tr>
<tr>
<td>G2</td>
<td>24</td>
<td>485.8±33.4*##</td>
</tr>
</tbody>
</table>

* Significant differences were noted between three treatment groups and G4, p<0.01.
# Significant differences were noted between G2 and G3, and also between G1 and G3, p<0.01.
† Significant differences were noted between G2 and G1, p<0.01.
✿ Numbers are mean ± standard error of the mean.
Effects of HBO2 on Subgingival Anaerobes. The number of subgingival anaerobes recovered from patients with periodontitis included in the three treatment groups decreased significantly when compared with baseline figures. Very significant differences in subgingival anaerobes were seen in the three treatment groups and the control group. More significant differences were also seen between the HBO2 + Scaling Group and the HBO2 Group, and also between the HBO2 + Scaling Group and the Scaling Group (p<0.01, Table 3).

Table 3. Comparisons of Subgingival anaerobe number between groups (x ±s, CFU/ml) *

<table>
<thead>
<tr>
<th>Group</th>
<th>Tooth number</th>
<th>Subgingival anaerobe number (×10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4</td>
<td>24</td>
<td>29584.3±35.5</td>
</tr>
<tr>
<td>G3</td>
<td>24</td>
<td>20118.1±16.8*</td>
</tr>
<tr>
<td>G1</td>
<td>24</td>
<td>361.3±12.5*#</td>
</tr>
<tr>
<td>G2</td>
<td>24</td>
<td>12.9±1.6*#†</td>
</tr>
</tbody>
</table>

* Significant differences were noted between three treatment groups and G4, p<0.01.
# Significant differences were noted between G2 and G3, and also between G1 and G3, p<0.01.
† Significant differences were noted between G2 and G1, p<0.01.

Effects of HBO2 on Subgingival Plaque Morphology. Assessment of subgingival plaque morphology for the three treatment groups indicated that numbers of Straight Rod, Curved Rod, Fusiform, and Spirochetes all decreased compared to baseline (p<0.01), but the number of coccoid cells increased. Statistically significant differences in number of bacteria could be seen in the HBO2 Group, the HBO2 + Scaling Group and the Scaling Group compared with the Control Group. Very significant differences of the bacteria were also seen between the HBO2 + Scaling Group and the HBO2 Group, and between the HBO2 + Scaling Group and the Scaling Group (Table 4).

Table 4. Comparison of subgingival plaque morphology between groups (%. x±s) *

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Cocci</th>
<th>Rods</th>
<th>Cur</th>
<th>Fusi</th>
<th>Spiro</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4</td>
<td>48</td>
<td>24.1±3.4</td>
<td>14.4±1.0</td>
<td>10.3±2.1</td>
<td>7.1±0.8</td>
</tr>
<tr>
<td>G3</td>
<td>48</td>
<td>43.9±3.9*</td>
<td>9.8±1.2*</td>
<td>7.9±0.5*</td>
<td>4.5±0.5*</td>
</tr>
<tr>
<td>G1</td>
<td>48</td>
<td>49.2±5.3*#</td>
<td>9.1±2.1*</td>
<td>7.2±0.6*</td>
<td>4.3±0.5*</td>
</tr>
<tr>
<td>G2</td>
<td>48</td>
<td>65.0±2.5*#†</td>
<td>5.6±0.9*#†</td>
<td>4.0±0.7*#†</td>
<td>2.5±0.6*#†</td>
</tr>
</tbody>
</table>

* Significant differences were noted between three treatment groups and G4, p<0.01.
# Significant differences were noted between G2 and G3, and also between G1 and G3, p<0.01.
† Significant differences were noted between G2 and G1, p<0.01.
*x each number is mean ± standard error of the mean.
Follow up data on the effects of HBO on periodontitis. Assessment of gingival indices, sulcus bleeding indices, probing depth, attachment loss, and plaque indices one year after treatment indicated statistically significant differences in the HBO\textsuperscript{2} Group, the HBO\textsuperscript{2} + Scaling Group and the Scaling Group compared with the Control Group. Identical results could be seen when comparisons were made between the HBO\textsuperscript{2} + Scaling Group and the HBO\textsuperscript{2} Group, and also between the HBO\textsuperscript{2} + Scaling Group and the Scaling Group (p<0.01, see Table 5). Significant differences were also observed in clinical data such as gingival indices and sulcus bleeding indices measured after termination of HBO\textsuperscript{2} therapy and 1 year after HBO\textsuperscript{2} therapy in the HBO\textsuperscript{2} Group, the HBO\textsuperscript{2} + Scaling Group and the Scaling Group (p<0.05). However, no significant differences were found in the probing depth, attachment loss, and plaque indices assessed at the same time.

Table 5. Comparison of GI, SBI, PD, AL and PLI between groups one year after HBO\textsuperscript{2} therapy (x±s) *

<table>
<thead>
<tr>
<th>Group</th>
<th>Tooth number</th>
<th>GI</th>
<th>SBI</th>
<th>PD</th>
<th>AL</th>
<th>PLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group(G4)</td>
<td>72</td>
<td>2.9±0.2</td>
<td>3.8±0.3</td>
<td>5.1±0.6</td>
<td>5.4±0.3</td>
<td>2.9±0.5</td>
</tr>
<tr>
<td>Scaling Group(G3)</td>
<td>72</td>
<td>1.6±0.7*</td>
<td>1.8±0.6*</td>
<td>4.3±0.6*</td>
<td>4.7±0.6*</td>
<td>1.4±0.4*</td>
</tr>
<tr>
<td>HBO Therapy Group (G1)</td>
<td>72</td>
<td>1.4±0.6*</td>
<td>1.7±0.5*</td>
<td>4.1±0.4*</td>
<td>4.5±0.5*</td>
<td>2.3±0.5*#</td>
</tr>
<tr>
<td>HBO + Scaling Group (G2)</td>
<td>72</td>
<td>0.9±0.4*#†</td>
<td>1.3±0.3*#†</td>
<td>3.7±0.3*#†</td>
<td>3.7±0.3*#†</td>
<td>0.9±0.3*#†</td>
</tr>
</tbody>
</table>

* Significant differences were noted between three treatment groups and G4, p<0.01.
# Significant differences were noted between G2 and G3, and also between G1 and G3, p<0.01.
† Significant differences were noted between G2 and G1, p<0.01.

DISCUSSION

The present study was designed to investigate the effects of HBO\textsuperscript{2} on the gingival inflammatory response of patients with periodontitis. Anaerobes are culprits of periodontitis; HBO\textsuperscript{2} kills or inhibits anaerobes and can be applied in the treatment of periodontitis\textsuperscript{[1,4]}. Our controlled study clearly showed that plaque index, sulcus bleeding index, gingival index, probing depth, and attachment loss of all three treatment groups decreased, and those of the HBO\textsuperscript{2} and Scaling Group decreased markedly, indicating that HBO\textsuperscript{2} had significant effects on periodontitis, and also indicating that HBO\textsuperscript{2} combined with scaling and root planing is most beneficial for treating periodontitis. The treatment effects can last at least one year.

Constantino et al (1995) reported that radiotherapy coupled with HBO\textsuperscript{2} treatment significantly reduced the occurrence of osteoradionecrosis and the amount of bone loss, indicating that HBO\textsuperscript{2} therapy could effectively inhibit alveolar resorption\textsuperscript{[13]}. Yoblon\textsuperscript{[14]} reported that rats with a fracture exposed to 0.3MPa pressure two sessions a day, 1 hour each, had abundant new subperiosteal bones formed at 40 days, indicating that HBO\textsuperscript{2} could enhance new bone formation.
Jiang (1995) reported that the oxygen pressure in periodontal pockets was lowered when periodontitis was active\[15\]. As can be seen from our experiment and other literature, HBO\(_2\) exposure can increase local oxygen delivery, which will raise oxygen concentration in periodontal pocket bottoms, on the one hand inhibiting the growth of anaerobes, and on the other enabling ischemic tissues to get enough oxygen supply to facilitate early recovery of tissue metabolism\[16\].

It has been reported that bacteria can secrete different enzymes that can destroy collagen and growth factors. When the oxygen concentration in gingival tissue is low, the number of bacteria in the periodontal pockets is increased. HBO\(_2\) can effectively decrease the number of bacteria and at the same time inhibit secretion of collagenase. A study by Rabkin showed that oxygen at 0.20MPa could inhibit the growth of certain pathogens related to periodontitis\[17\]. Manheim\[18\] reported that HBO\(_2\) kills or inhibits actinomyces, bacteroides and streptococcus, which contribute to the development of periodontitis. Our results show that the number of anaerobes in the HBO\(_2\) Group obviously decreased, and there were significant differences between the HBO\(_2\) Group and the Control groups. Scaling and root planing may decrease markedly the number of microorganisms in periodontal pockets, so HBO\(_2\) therapy combined with scaling and root planing will be most advantageous for treating periodontitis.

**CONCLUSIONS**

Our study provides good evidence that HBO\(_2\) inhibits the growth of subgingival bacteria, improves microcirculation, and increase gingival blood flow, thus promoting healing of peridontium, which will be of help in the treatment of periodontitis. HBO\(_2\) therapy combined with scaling and root planing appears to be even better for treating periodontitis. This treatment effect can last more than one year. The mechanism of the treatment of HBO\(_2\) on periodontitis is unknown. The following might be involved in the mechanism of action of HBO\(_2\): an increase in GBF, inhibition of growth and reproduction of subgingival plaque and spirochetes, and in particular the growth of anaerobes at the base of pockets. Collectively, these effects indicate a therapeutic benefit of HBO\(_2\) for treatment of severe periodontitis.

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**REFERENCES**


