MANAGEMENT OF ROOT CARIES USING A NOVEL OZONE DELIVERY SYSTEM \textit{IN-VIVO}

A. BAYSAN$^{1,2}$, E. LYNCH$^1$, 

$^1$Restorative Dentistry and Gerodontology, Queen's University Belfast, Belfast, Northern Ireland and $^2$St. Bartholomew’s and the Royal London School of Medicine and Dentistry, Department of Adult Oral Health, UK

Author for correspondence:
Aylin Baysan BDS MSc
St. Bartholomew’s and the Royal London School of Medicine and Dentistry, Department of Adult Oral Health, Turner Street Whitechapel, E1 2AD
Tel: +44 207 377 7000/2186
Fax: +44 207 377 7375
E-mail: abaysan@mds.qmw.ac.uk

Key words: Ozone, root caries, anti-microbial effect
Abstract

The aim of this study was to assess the antimicrobial effect of ozone using a novel ozone delivery system on primary root carious lesions (PRCLs). 26 patients with 70 PRCLs requiring restorations were entered. Each PRCL was classified in terms of colour, texture, cavitation, size, hardness, distance from the gingival margin and severity. Overlying plaque was then removed and each tooth was dried. Half of each PRCL was then removed using a sterile excavator. Subsequently, the remaining lesions were exposed to ozone gas for a period of either 10 s (n: 35) or 20 s (n: 35) of ozone at room temperature (23°C) and a further biopsy was taken. Using a Paired Student t test, a significant ($p < 0.00001$) difference (mean ± SE) was observed in the ozone-treated samples with either a 10 s ($\log_{10} 4.35 \pm 0.49$) or 20 s ($\log_{10} 0.46 \pm 0.25$) ozone application compared with the control samples ($\log_{10} 7.00 \pm 0.24$) and ($\log_{10} 6.00 \pm 0.20$) respectively. Using Pearson's correlation tests, there were significant correlations for the reduction in total micro-organisms after 10 s of ozone application with cavitation, size, distance from gingival margin and severity of PRCLs ($p < 0.05$). In conclusion, this novel treatment regime using ozone may therefore be considered to be potentially an effective alternative to conventional "drilling and filling" for the management of PRCLs.
Introduction

Most elderly people may encounter significant oral problems that can ultimately have a profound impact on the quality of their lives. The most common causes of tooth loss are attributed to root caries and periodontal diseases, and this phenomenon can diminish function and contribute to loss of self-esteem in elderly populations.

It is now clear that the problem is widespread and can be quite serious in certain older population. Lynch (1996) reported that primary root carious lesions (PRCLs) containing > $10^3$ total micro-organisms were more likely to be deemed to require operative intervention. However, treatment of PRCLs poses many problems. Reversal of PRCLs is associated with remineralisation and a corresponding reduction in acidogenic and aciduric micro-organisms (Schüpbach et al., 1996; Beighton et al., 1993). An anti-microbial method to manage PRCLs can therefore be considered (Lynch, 1996). Compared to enamel caries, there has been relatively limited research into the pharmaceutical management of root caries, and many of these studies have been carried out in-vitro, with limited numbers of clinical trials.

As an alternative management strategy for root caries, ozone can be considered as a useful therapeutic agent. Ozone (a pale blue-coloured gas) plays an important role as a natural constituent in the higher layer of the Earth’s atmosphere. Ozone acts as a disinfectant, and has the unique feature of decomposing to a harmless, non-toxic and environmentally safe molecule (oxygen). Recently Baysan et al., (2000) reported that ozone application either for 10 or 20 s was effective to kill the great majority of micro-organisms in PRCLs in-vitro and this application for a period of 10 s was also capable of reducing the numbers of S. mutans and S. sobrinus in vitro.

The aim of this study was to assess the anti-microbial effect of ozone using a novel ozone delivery system on PRCLs in-vivo.
Materials and Method

All participants were recruited from the patients of St. Bart's and the Royal London School of Medicine and Dentistry who were attending its Dental Institute for routine oral health care. Each subject had been given their informed consents for both dental examinations and ozone treatment to be undertaken.

The data with which this whole work is concerned have been obtained from a total of 70 PRCLs in 26 patients. A total of 15 (58%) male and 11 (42%) female, with at least one PRCL were selected. The mean ± standard error age of the subjects at baseline was (65.88 ± 2.34) years with a minimum of 37 and maximum of 86 years.

The ozone delivery system.

In this study, a novel ozone delivery system (HealOzone, CurOzone USA) was employed (Figure 1). The ozone delivery system is a portable apparatus for the treatment of root caries. This system delivers at a concentration of 2,100 ppm ± 10% ozone. The vacuum pump suctions air through the generator at a rate of 615 cc/minute to supply ozone to the lesion. The same vacuum pump is used to purge the system of ozone after the treatment. It includes a source of oxidising gas and a dental handpiece with a cup for delivering the gas to the lesion. A removable silicon cup, which is attached to the handpiece is provided for receiving the gas and exposing a selected area of the tooth to the gas. The tightly fitting cup includes a resilient edge for sealing the edge of the cup against the selected area on the tooth to prevent escape of ozone (Figure 2).
**Figure 1.** Ozone delivery system

**Figure 2.** Handpiece with a cup
Study design

All lesions were examined using a visual/tactile method. Each PRCL was classified subjectively in terms of colour, texture, cavitation, size, hardness, distance from the gingival margin and severity (Lynch and Beighton 1993; Baysan et al., 2001). Leathery lesions with severity index 1 or 2 were selected.

A total of 70 root carious lesions were randomly divided into two groups to test the anti-microbial effect on PRCLs from exposure to ozone for a period of either 10 or 20 s. Overlying plaque was then removed using a hand held standard fine nylon fibre sterile toothbrush with water as a lubricant. Each tooth was dried using dry sterile cotton wool rolls and a dental 3 in 1-air syringe. The excavator blade was used to traverse the lesion in line with long axis of the tooth across the maximum gingival/occlusal dimension. Half of each lesion was removed using a sterile excavator for the control group. Subsequently, the remaining lesion was exposed to the ozone gas for a period of either 10 seconds or 20 seconds at room temperature (23°C) and a further sample was taken. After the ozone application, each subject was followed up between 3 and 5.5 months to assess any adverse events related to the ozone treatment and severity of PRCLs.

Microbiological analysis

Each sample was put into a preweighed sterile vial and weighed. 1 ml of fastidious anaerobe broth (FAB, Lab M Ltd., Bury, Lancs, UK) with sterile glass beads (3.5 - 4.5 mm in diameter, BDH, Poole, Dorset, UK) was added to these vials and vortexted for 30 s to facilitate the extraction of any microorganisms from carious dentine and disperse any aggregates. Dilutions were performed by transferring 1 ml of the resulting suspensions into 9 ml of FAB. This process was repeated in 10-fold dilution to $10^4$. After decimal dilution with FAB, 100 μl aliquots (for both test and control groups) were spread on fastidious anaerobe agar (FAA, Lab M, Bury, Lancs, UK) supplemented with 5% (v/v) horse blood and placed in an anaerobic chamber at 37°C for four days. The total number of
colony forming units (cfus) was calculated.

**Statistical analyses**

Microbiological counts from test and control groups for each study were transformed as $\log_{10}(\text{colony forming units} + 1)$ prior to statistical analyses in order to normalise their distributions. Statistical analyses of the data were obtained by paired Student t-tests to determine differences between test and control groups, with the threshold of significance chosen at 0.05. Means and standard errors were also recorded. Pearson's correlation tests were also performed to correlate the reduction in total micro-organisms and clinical assessments with the threshold of significance chosen at 0.05.
Results

At baseline 26 patients (70 lesions) were recruited for the ozone treatment. Of these two subjects failed to attend for their follow-up examinations. There were no adverse events observed during and after the ozone treatment. Only subjects attending to the re-call appointments were eligible for inclusion in the final re-call analyses. Out of the 63 PRCLs reviewed, 38 lesions had become hard, 18 lesions reversed to severity index 1 from severity index 2, and 7 lesions remained the same.

Reduction in total micro-organisms

Using the Paired Student t tests, a significant ($p < 0.00001$) reduction (mean ± SE) was observed in the ozone-treated samples with either a 10 or 20 s ozone application compared with the control samples respectively (Table 1).

Table 1. Mean ± SE $\log_{10}$ cfu + 1 before and after ozone application for either 10 or 20 s

<table>
<thead>
<tr>
<th>Groups</th>
<th>10 s $\log_{10}$ (cfu +1)</th>
<th>20 s $\log_{10}$ (cfu +1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Samples</td>
<td>7.00 ± 0.24</td>
<td>6.00 ± 0.21</td>
</tr>
<tr>
<td>Ozonated Samples</td>
<td>4.35 ± 0.49</td>
<td>0.46 ± 0.26</td>
</tr>
</tbody>
</table>

Colour

Colour of PRCLs failed to correlate with the reduction of micro-organisms after the ozone application for a period of either 10 or 20 s.

Cavitation

The depth of lesion cavitation estimated the loss of tooth structure between the lesion surface and the original contour of the tooth. Using Pearson's correlation test, there was significant ($p < 0.05$) correlation for the reduction in total micro-organisms after the ozone application for a period of 10 s
(r= 0.39) (Figure 3). Reduction in total micro-organisms after ozone application for a period of 20 s failed to correlate with cavitation (r= -0.22).

**Figure 3. Correlation of reduction in total micro-organisms after ozone application for a period of 10 s and cavitation of PRCLs**

![Box & Whisker Plot](image)

**Size**

The size of each lesion was estimated by multiplying the height of the lesion by its width. Using Pearson's correlation test, reduction in total micro-organisms after ozone application for a period of 10 s correlated with size of PRCLs (r = 0.44) (Figure 4). Small lesions presented greater reduction in total micro-organisms than large lesions following ozone application. Reduction in total micro-organisms after ozone application for a period of 20 s failed to correlate with size (r= -0.05).
**Figure 4. Correlation of reduction in total micro-organisms after ozone application for a period of 10 s and size of PRCLs**

Using Pearson’s correlation test, reduction in total micro-organisms after ozone application for a period of 10 s correlated with distance of PRCLs from the gingival margin ($r = 0.36$). Lesions which were far away from the gingival margin presented greater reduction in total micro-organisms than lesions close to the gingival margin following ozone application. Reduction in total micro-organisms after ozone application for a period of 20 s failed to correlate with distance of PRCLs from the gingival margin ($r = -0.24$).

**Distance from the gingival margin**

Using Pearson’s correlation test, reduction in total micro-organisms after ozone application for a period of 10 s correlated with distance of PRCLs from the gingival margin ($r = 0.36$). Lesions which were far away from the gingival margin presented greater reduction in total micro-organisms than lesions close to the gingival margin following ozone application. Reduction in total micro-organisms after ozone application for a period of 20 s failed to correlate with distance of PRCLs from the gingival margin ($r = -0.24$).

**Severity index.**

Using Duncan’s multiple range tests, reduction of total micro-organisms after treating test samples with ozone either for a period of 10 or 20 s showed significant differences between severity indices.
1 and 2 (p < 0.05) (Figures 5 and 6). Total micro-organisms in clinical severity index 1 showed significantly greater reduction compared to the total micro-organisms in severity index 2. Using Pearson’s correlation test, there was also a clear relationship between the reduction of total micro-organisms and clinical severity indices 1 and 2 for PRCLs (r = -0.48).

**Figure 5. Log\(_{10}\) (cfu + 1) reduction in total micro-organisms related to the severity indices of PRCLs after ozone application for a period of 10 s**
Figure 6. Log_{10} (cfu + 1) reduction in total micro-organisms related to the severity indices of PRCLs after ozone application for a period of 20 s
Discussion

Methodology

In this study, sampling method employed involved full depth of the lesion. It can therefore be speculated that ozone penetrates the lesion and immediately kills micro-organisms in the carious lesion. The rapid inactivation of micro-organisms is one of ozone’s outstanding characteristics. Presumably, ozone dissipates quickly in water (Bocci et al., 1993) and kills micro-organisms via a mechanism involving the rupture of their membranes in the lesions. In this study, different species from PRCLs were not investigated. Total counts of micro-organisms were counted before and after the ozone applications by the employment of microbiological analyses. However, ozone has the potential to inactivate all organisms (Watkins 1997) and reduce the numbers of micro-organisms present in root caries. Yamayoshi and Tatsumi (1993) also demonstrated that ozone was a strong oxidiser to cell walls and cytoplasmic membranes of micro-organisms.

Clinical assessments

Clinical criteria used to detect PRCLs (i.e., size, cavitation, distance from gingival margin and severity of the lesions) were correlated with the reduction in total micro-organisms after the application of ozone for a period of 10 s. Total micro-organisms in small, non cavitated and less severe lesions showed greater reduction after the application of ozone. This related to the findings of Lynch (1996), who reported that non-cavitated, small lesions had less cariogenic micro-organisms than cavitated and large lesions. However, clinical assessments failed to correlate with the reduction in total micro-organisms after the ozone application for a period of 20 s. In this group, 86% of ozonated carious lesions had no micro-organisms (i.e., zero micro-organisms). It can be speculated that ozone killed all micro-organisms in PRCLs. Therefore, all the categories of the lesions had substantial microbial reduction after the ozone application for a period of 20 s. Hence, total micro-organisms in PRCLs were dramatically reduced regardless their size, cavitation and severity of lesions when the ozone application doubled.
Follow-up appointments and safety of ozone treatment

The main clinical problem with the pharmaceutical approach for the management of root caries is the difficulty in suppressing or eliminating micro-organisms for extended periods. After a treatment with the pharmaceutical approach, these organisms may proliferate and re-colonise in PRCLs. In this study, root carious lesions for each patient were reviewed in terms of their severity status following the application of ozone. Interestingly, 60% of PRCLs had become hard either after 10 or 20 s of ozone application. Arends (1990) found that the use of an oxidant on root dentine carious lesions improved their potential to remineralise. This may partly account for the dramatic remineralisation results shown after ozone application in this study. Emilson (1981) also reported that after a short-term intensive treatment of the dentition with chlorhexidine, \textit{S mutans} is suppressed \textit{in-vivo} for a significant length of time (for 2 to 6 months). Re-colonised time also differed among the subjects. In addition, in subjects in whom mutans streptococci had been reduced to 99% after the treatment, micro-organisms returned more slowly than in subjects with less microbial reduction.

The importance of ozone treatment in elderly population

The percentage of elderly people is increasing rapidly in industrialised countries. A survey conducted in 1988 presented for the first time information regarding root caries, the highest prevalence being found in the 55-64 year old group with a mean of 0.7 teeth having root caries, and 1.2 having had restorations placed in their roots (Downer 1991). Root caries prevalence was reported to be over 80% in elderly institutionalised people (Banting et al., 1980). A conventional approach to treat root caries is difficult in view of the difficulties of visibility, moisture control, access to carious lesions, proximity of the pulp, proximity to the gingival margin and the high organic content of the dentine (Titus 1991). Restorative management of PRCLs has therefore become challenging especially for the high percentage of the elderly population in special care units. There
is a major requirement for the best management strategy for root caries inhibition. Barmes (2000) suggested that the acceptance of broad-based preventive behaviours to preserve oral health in old age is essential as a practical strategy. Emilson et al., (1993) reported that it was evident to convert active root caries to inactive lesions by an intensive prophylactic programme. The pharmaceutical approach for the management of root caries in elderly people can therefore be considered.

In conclusion, ozone application either for 10 or 20 s dramatically reduced most of the micro-organisms in PRCLs without any side effects recorded at recall intervals between 3 and 6 months. Clinical criteria used to detect PRCLs showed significant correlation with the reduction in total micro-organisms following 10 s ozone application. This novel treatment regime using ozone may therefore be considered to be potentially an effective alternative to conventional “drilling and filling” for the management of PRCLs. Based on these findings, a longitudinal study is in process to investigate the application of ozone for a period of 10 s on root carious lesions.
References

Arends -------To add

Beighton D, Lynch E:
Relationships between yeasts and primary root-caries lesions.

Lynch E:
Relationships between clinical criteria and microflora of primary root caries.