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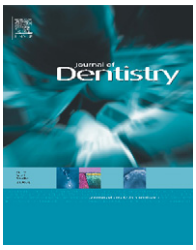

Letter to the Editor

Comment on "The application of Ozone in dentistry: A systematic review of the literature"

I read the recent article[1] on the application of ozone in dentistry (Journal of Dentistry 2008;36:104–16.). Thank you for the opportunity to comment briefly on this recent report. There were many useful parts in this paper.

Ozone cannot do everything and certainly should not be a treatment isolated from our individualized preventive oral health care. To be effective, Ozone must be prescribed in sufficient concentration, for an adequate time and must be delivered into lesions. When conflicting results were presented,[1] unfortunately, doses of delivered Ozone were not discussed. Would we expect less than 10 mg of Amoxicillin to have the same efficacy as 250 mg Amoxicillin? Of course not, and this paper did not point out the relationships between doses of Ozone delivered and the results achieved. Further optimum ozone dosage experiments are underway but if in doubt then deliver more than the minimum required as ozonated water a fully biocompatible antimicrobial.

1. Ozone in oral microbiology

For example, the paper stated that there was ''conflicting evidence about the antimicrobial efficacy of ozone but that there was some evidence that ozone is effective in removing the microorganisms from dental unit water lines, the oral cavity, and dentures''.

Ozone is one of the most powerful antimicrobial agents we could use in dentistry[2] and clearly, there are enormous advantages to kill pathogens. The paper correctly mentioned papers proving the antimicrobial effectiveness of ozone but did not discuss the limitations of the studies that showed poor results. All active ingredients need to be used in sufficient dose and with the appropriate method to be effective.

For example, the paper[1] published a section entitled ''Ozone in oral microbiology''. They identified four studies[3–6] investigating the bactericidal effect of ozone in the oral cavity. They stated ''Ozone might be useful to control oral infectious microorganisms in dental plaque. However, the evidence available is controversial: while some researchers found an incomplete efficacy of ozone (in aqueous or gaseous form) in eliminating the viable bacteria,[5,6] the others found ozonated water effective for killing gram-positive and gram-negative oral microorganisms and oral *Candida albicans*.[4] Also, a high level of biocompatibility of aqueous ozone on human oral epithelial cells, gingival fibroblast cells, and periodontal cells has been found.[3,7] This is important for decontamination of avulsed teeth before replantation.[3] Moreover, four studies were found that showed the *in vitro* antimicrobial efficacy of ozone as denture cleaners[8–10] and ozone gas was found to be more effective than ozonated water.[11] Therefore, gaseous ozone can be clinically useful for disinfection of dentures''.

The authors state that the above results were controversial as the Muller et al.[5] and the Walker et al.[6] studies had relatively poor results.

Muller et al.[5] reported less than a one log reduction of bacteria measured after using ozone gas above biofilms in culture media, which was a similar reduction to that achieved by using 0.2% chlorhexidine or photoactivated disinfection. However, the authors did not mention that ozone will react immediately with reductants in culture media and the authors did not bubble the ozone into the biofilm. It is recommended that ozone be delivered under pressure into a lesion by pressing the delivery tube onto the carious surface so that it can penetrate the lesion.[12] In vivo lesions (unlike artificial biofilms) contain many molecules (such as iron) which increase the antimicrobial effectiveness of ozone in caries.

Walker et al.[6] reported that Ozone (even at a very low dose and a short time of application), achieved a 57% reduction in biofilm coverage and a 65% reduction in viable bacteria in the biofilm in model dental unit water lines. It was not mentioned that the contact time for ozone in this study was at least 96 times less than the contact time for the other disinfectant products used. Other studies have also defined optimum dosages of ozone to achieve significant results.

2. Ozone in endodontics

Again, the authors report on Ozone in endodontics and ignore the dose of Ozone used and its relationship to the results.

The review[1] identified four studies[5,13–15] investigating the bactericidal effect of Ozone as compared to 2.5–5% sodium hypochlorite (NaOCl) as irrigation solutions in endodontics.

DOIs of original articles: 10.1016/j.jdent.2007.11.008, 10.1016/j.jdent.2008.11.003

Muller et al.[5] found 5% NaOCl superior to gaseous Ozone in eliminating microorganisms organised in a cariogenic biofilm. As stated above, this study[5] reported less than one log reduction of bacteria after using Ozone gas above biofilms in culture media, which was only a similar reduction to that achieved by using 0.2% chlorhexidine or photoactivated disinfection. However, it should have been noted that Ozone is a potent oxidant and will form a redox reaction with reductants in a culture media. In addition, the authors did not bubble the Ozone into the biofilm. Ozone should be delivered under pressure into a lesion by pressing the delivery tube onto the surface so that Ozone can penetrate the lesion and/or biofilm.

Nagayoshi et al.[13] used a much higher dose of Ozone than Hems et al.[14] and found nearly the same antimicrobial activity (against *Enterococcus faecalis* and Streptococcus mutans) and a lower level of cytotoxicity of Ozonated water as compared to 2.5% NaOCl. They stated ''Ozone is known to act as a strong antimicrobial agent against bacteria, fungi, and viruses. In the present study, we examined the effect of Ozonated water against *Enterococcus faecalis* and Streptococcus mutans infections *in vitro* in bovine dentin. After irrigation with Ozonated water, the viability of *E. faecalis* and *S. mutans* invading dentinal tubules significantly decreased. Notably, when the specimen was irrigated with sonication, Ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite (NaOCl). We also compared the cytotoxicity against L-929 mouse fibroblasts between Ozonated water and NaOCl. The metabolic activity of fibroblasts was high when the cells were treated with Ozonated water, whereas that of fibroblasts significantly decreased when the cells were treated with 2.5% NaOCl. These results suggest that Ozonated water application may be useful for endodontic therapy''.[13]

Hems et al.[14] concluded that ''Ozone had an antibacterial effect on planktonic *E. faecalis* cells and those suspended in fluid, but little effect when embedded in biofilms. Its antibacterial efficacy was not comparable with that of NaOCl under the test conditions used.'' Unfortunately these authors used an extremely low dose of Ozone in their experiments. The concentration of Ozone in water mentioned in the paper was only 0.68 ppm. This concentration was immediately after production, which will have reduced further by the time it was used. The dose of NaOCl used was enormous in comparison to the Ozone dose. Surprisingly, immediately following Ozone sparging 1 mL of this broth had ozone inactivation by a transfer into 9 mL of neutralizing broth. This neutralization does not appear to have been similarly used with the NaOCl. Given the above methodology in this paper, and the low dose and time of application of Ozone used, it is surprising that Ozone was as effective as was reported.

Moreover, another study[15] has found that the irrigation of infected human root canals with Ozonated water, 2.5% NaOCl, 2% chlorhexidine or the application of gaseous Ozone was not sufficient to inactivate *E. faecalis*. The methodology used was obviously draconian as no tested agent had any antimicrobial effect. It is highly probable that the Ozone (oxidant) reacted preferentially with reductants in the Brain Heart Infusion used for the inoculation in a simple redox reaction rather than with the bacterial strain. Unfortunately, this paper did not report the concentration of ozone in the water and did not state if any special method was used to increase the solubility of ozone in water.

More recent papers have also supported the antimicrobial efficacy of ozone when used in sufficient dose.[16] Virtej et al.[17] compared the antimicrobial performance of 4 systems used as root canal irrigants. Seventy instrumented and initially sterile roots with open access cavities and containing a paper point were carried by one volunteer in the oral cavity for 1 week. After removal, samples were taken for microbiological analysis. The root canals were then disinfected with the Endox Endodontic System, MTAD, 3% sodium hypochlorite (NaOCl), or HealOzone, and, thereafter, samples were repeated for microbiological analysis. The roots were then sealed and incubated for a further week, after which bacterial growth was again determined. After disinfection, there was a significant decrease in the absolute bacterial count between each disinfection method and the positive control group. There was no statistically significant difference between the 3% NaOCl, MTAD, and HealOzone groups. The Endox device showed the least antibacterial effect with significant differences to MTAD and HealOzone. Bacterial regrowth after 1 week of incubation was detected in all samples of the control group, whereas test groups showed several bacteria-free samples. They[17] concluded that Ozone has great potential in endodontic antimicrobial use and that MTAD and HealOzone seem to be as effective as 3% NaOCl in reducing mixed bacterial infection in the root canal system. I would speculate that the antimicrobial effect of the Ozone would have been even greater if it had been used as I recommend.[16] I personally feel that conventional irrigation (including NaOCl) should be used during cleaning and shaping and Ozonated water (preferably also with Ozone gas) should be used as the final irrigant with ultrasonication.[16]

Cardoso et al.[18] concluded that the Ozonated water, used as an irrigant agent, immediately significantly reduced the number of *Candida albicans* and *Enterococcus faecalis* in root canals in human teeth.

Ozone is effective when it has been prescribed in sufficient concentration, used for an adequate time and delivered correctly into root canals after the traditional cleaning, shaping and irrigation has been completed.[16] Clearly, Ozone will not be successful if too little dose of Ozone is delivered or the Ozone is not delivered appropriately.

Ozone is one of the most powerful antimicrobial agents we could use in medicine or dentistry[2] and as failure of root canal therapy is mainly caused by microorganisms, it is not surprising that there are enormous advantages to kill these pathogens. Hundreds of peer reviewed published research papers have proven the antimicrobial effectiveness of Ozone as a gas and/or as ozonated water.

3. Use of ozonated oils as medicaments

Ozonated oils were also not mentioned as possible medicaments in endodontics.

An investigation[19] evaluated histologically and histobacteriologically the response of periradicular tissues to the endodontic treatment of infected root canals performed in a

single visit or in two visits using either Ozonated oil or calcium hydroxide in camphorated paramonochlorophenol (CMCP) as an intracanal medication. After 6 months, the animals were sacrificed and the specimens were processed for histological and histobacteriological analyses. The root canals treated in a single visit showed a success rate of 46%. When a calcium hydroxide/CMCP-based interappointment intracanal medication was used, 74% of the cases were categorized as successful. In cases where Ozonated oil was used as the intracanal medication, a success rate of 77% was observed.

Siqueira et al.[20] evaluated the antibacterial activity of the Ozonated oil and calcium hydroxide pastes against bacterial species commonly associated with the aetiology of periradicular diseases. Of the tested medicaments, Ozonated oil was the most effective against the evaluated bacterial species.

4. Biocompatibility of Ozone in root canal therapy

It should be noted that sodium hypochlorite, especially in the high concentrations used (up to 5%), does not have the high level of biocompatibility of aqueous Ozone on human oral epithelial cells, gingival fibroblast cells and periodontal cells.[3,7,13,21,22]

The effect of aqueous Ozone on the NF-κB system was not mentioned. The transcription factor NF-κB plays a crucial role in inflammatory/immune processes and apoptosis. NF-κB is also thought to be of primary importance in the regulation of periodontal/periapical inflammatory reactions and the pathogenesis of periodontal diseases and apical periodontitis. A recent paper[22] reported that aqueous Ozone exerts inhibitory effects on the NF-κB system, suggesting that it also has anti-inflammatory and immune-modulatory capacities.

5. Oxidant ability of Ozone

The oxidant ability of Ozone[23] deserved more discussion. Pyruvic acid (Ka = 3.20 mmol) contributes substantially to the decreased pH values associated with active carious lesions.[24] Pyruvic acid is oxidatively decarboxylated to acetate and carbon dioxide on reaction with ozone[23] as in the following equation:

$$CH_3COCO_2^- + O_3 \rightarrow CH_3CO_2^- + CO_2 + O_2$$

Remineralization of incipient carious lesions can be encouraged by buffering plaque fluid by the production of acetate, or other high pK_a acids found in resting plaque.[25]

6. Management of root caries

The paper described three studies[26–28] that successfully treated root caries with the HealOzone.

The paper incorrectly criticizes the outcome measurement of these studies for having used subjective tactile hardness for the clinical severity score as being a methodological concern. However hardness is the most reliable clinical measure of lesion severity. The authors in the above studies also clearly reported the reproducibility of their methods.

In his well designed, randomised, double blinded, controlled clinical trial, Holmes[27] published ''The reproducibility of the data was tested at the 12-month recall. One week after assessment by the first dentist, 15 subjects (30 PRCL's) were recalled and examined by a third dentist. There was a good agreement in the classifications of hardness and severity of PRCL's (kappa = 0.80). Twenty subjects with 40 PRCL's were also re-assessed 24 h later, at the final 18-month recall visit, by the usual dentist who had used the same criteria throughout the study period. Two lesions that were marked as soft were re-assigned to leathery on the second visit (kappa = 0.95)''.

Baysan[28] published ''The intra-examiner reproducibility of the clinical examinations, the ECM and DIAGNOdent readings were assessed by repeating the examinations on 22 teeth in 10 subjects. Repeat examinations were performed on separate days. The intra-examiner reproducibility of the ECM and DIAGNOdent readings and the clinical examinations, were assessed by repeating examinations on 22 teeth in 10 subjects at 1 and 3 months. There was perfect agreement in the classifications of hardness, texture, colour, cavitation, size, severity of lesions and distance from the gingival margin. The ECM readings had excellent agreement (kappa = 0.86, $P < 0.0001$). There was a fair agreement between first and second measurements of the DIAGNOdent readings (kappa = 0.50). The intraclass correlation coefficient[41] between the first and second measurements of size was 0.99 (95% CI 0.96–1.00). For the distance to the gingival margin, this parameter was 0.99 (95% CI 0.99–1.00).'' Some of this data was subsequently published[29] but it might have been useful to also quote another paper[30] in the review[1] which was published in 2004 before the termination date of the review. This clinical severity score used in the above studies[27–30] has been validated with respect to the microflora present with these root carious lesions and I feel that hardness is the best indicator we can use to reflect root caries lesion activity.[31–37]

7. Electrical Caries Monitor (ECM)

The ECM can be a useful special test to objectively quantify the severity of the root caries index[31] used in these studies[28,29] and was implied to be a methodological concern.

A study[38] related the Electrical Caries Monitor (ECM) readings with the same clinical criteria used to define primary root carious lesions in the above studies[27,28]. Primary root carious lesions were classified according to colour, texture, hardness, cavitation, size and severity before ECM readings were recorded. ECM readings for all five classes of severity and all three classes of hardness of lesions were significantly different from each other and from sound root tissue ($P < 0.05$). There was a significant correlation for ECM readings and cavitation ($P < 0.05$). There was a clear logarithmic relationship between ECM end values and size ($P < 0.05$). The ECM is capable of distinguishing the severity of primary root carious lesions since it is a less invasive but accurate method of detecting carious lesions when compared to tactile methods.[38]

8. Data analyses

The paper[1] stated ''Data analysis: In all these studies, the data analyses were conducted at the level of the lesion. The researchers assumed that there was no variability between subjects, i.e., all these teeth were analysed independently as if they were from different subjects. This approach may produce a significant result; however, the literature demonstrates its lack of validity.''

It is very clear in the study by Holmes that each subject had one test and control lesion. Holmes states ''A total of 89 subjects (age range 60–82, mean + S.D., 70.8 + 6 years), each with two leathery primary root carious lesions were recruited. The two lesions in each subject were randomly assigned for treatment with ozone or air, in a double-blind design, in a general dental practice. Clearly analysis of the lesion is the same as analysis of the patient as each patient only had one test lesion. Baysan[29] similarly had one test and control lesion in each group. In other words, these studies used the unit of analysis as the lesion which was the same as per patient as each subject had only one test lesion.

9. Blinding and placebo ozone treatment

The paper states ''Blinding: All these studies lack an appropriate method of blinding as no placebo ozone treatment was given and thus subjects and/or their parents were not blinded to the study. The exception is Holmes'' Clearly Holmes[27] did complete a fully double blind study and used a placebo ozone treatment. Holmes[27] states ''The lesions were assigned into two groups by a dentist, using a computer generated random table; Group 1 lesions were treated with 40 s of ozone, and Group 2 lesions were left as controls. Following initial oral hygiene instruction, subjects were given ozone or air treatment. The treatment method was explained and demonstrated. Two dentists were involved in this study; the first assessed the primary root carious lesions and the second dentist assigned them to Group 1 or 2 with a computer generated random table. The first dentist then carried out the treatment for 40 s, applied the mineral wash, dispensed the remineralising products, and instructed the subjects. A double blind system was employed, and the ozone treatment was applied by a different operator than the one recording the clinical criteria used to define the severity of the lesions''.

10. Ozone reversal of open caries

Again this study[39] was incorrectly criticized for using hardness but hardness of caries is our best clinical tool to reflect the activity of dentine caries as discussed above.[31–37].

This study[39] reported the treatment of open carious lesions with ozone in anxious children. Ninety four percent of the children were treatable and 93% lost their dental anxiety. The hardness values improved significantly in the ozone-treated test lesions after 4, 6, and 8 months compared with baseline while the control lesions had no significant change in hardness at any recall interval.[38]

11. ''Caries Balance'' concept

The ''Caries Balance'' concept from John Featherstone[40] is excellent. I believe that the balance between pathological and preventive factors can be swung in the direction of caries intervention and prevention by the active role of the dentist and his/her auxiliary staff and that Ozone has a key part to play in this process.

Ozone's place is for us to use its proven powerful antimicrobial efficacy and undoubted potent oxidant ability, to reduce cariogenic microorganisms and provide beneficial effects against organic acids in lesions, in conjunction with our existing management strategies for dental caries to tip the ''caries balance''. Double blind randomized controlled clinical trial data has proven the significant reversal of shallow primary root carious lesions and we all want more trials to evaluate even more possible uses of ozone in dentistry, but I believe ozone's prime place is as an adjunct rather than an alternative to conventional treatments.

Disclosure

Professor Edward Lynch is a consultant and principal investigator for research grants from CurOzone USA (Aurora, Ontario, Canada) administered by Queens University Belfast, Northern Ireland.

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0300-5712/$ – see front matter

doi:10.1016/j.jdent.2008.11.002