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Effect of ozone on non-cavitated fissure carious lesions in permanent molars. A controlled prospective clinical study

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ABSTRACT: ***Purpose***: To investigate, with a randomized controlled clinical study, the effect of ozone on non-cavitated initial occlusal fissure caries compared with untreated contra-lateral control lesions (split mouth) considering the patient's current caries risk. ***Methods***: Forty-one patients with 57 pairs of lesions were enrolled in the study (mean age 7.7 ± 2.2 years; upper jaw n=29, lower jaw n=28). Gaseous ozone (HealOzone) was applied once for 40 seconds to the randomly assigned test molar of each pair without the use of remineralizing solutions. Lesion progression or reversal was monitored by the laser fluorescence system DIAGNOdent for up to 3 months and the deterioration or improvement compared between the ozone-treated lesions and the untreated control lesions (in pairs). This was done for the whole study population and a subgroup of patients with high current caries risk (lesion pairs n=26). ***Results***: After 3 months, explorative data analysis revealed that the ozone-treated lesions showed significantly more caries reversal or reduced caries progression than the untreated control lesions within the group of patients at high current caries risk (Wilcoxon-Test, P= 0.035). There was no statistical significance examining the whole study population. From the data it can be concluded that ozone application significantly improved non-cavitated initial fissure caries in patients at high caries risk over a 3-month period. (*Am J Dent* 2005;18:223-228).

CLINICAL SIGNIFICANCE: Application of ozone gas to non-cavitated initial fissure caries reverses caries or reduces caries progression in patients with high caries risk for up to 3 months.

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Introduction

Caries is known to be a multifactorial infectious disease caused by acidogenic and aciduric microorganisms.[1] The process of de- and remineralization is dynamic and it is possible to shift the equilibrium towards remineralization by elimination of the caries specific ecological niche flora comprising primarily of acidogenic and aciduric microorganisms and their by-products. A reversal of demineralized but non-cavitated enamel and dentin has been reported.[2-4] These findings are especially important as recent epidemiological oral health data has highlighted the importance of initial carious lesions in the prevention of future cavitated lesions.[5]

Ozone is a powerful oxidant and highly potent antimicrobial agent that has been used for over a century for treatment of sewage and disinfection of drinking-water, food preservation and equipment sterilization.[6-9] A new ozone delivery system (HealOzone[a]) allows the application of high concentrations of gaseous ozone (2100 ± 200 ppm at a flow rate of 615 ccs per minute) to a precise area on the tooth surface under controlled conditions. It has been suggested that this process be used as a promising non-invasive approach in the management of dental caries.[10-15] Ozone has been shown to significantly reduce the total microbial load within a carious lesion on tooth roots as well as isolated strains of *Streptococcus mutans* and *Streptococcus sobrinus in vitro* and *in vivo*.[16,17] The elimination of these particular microorganisms is expected to cause a shift in the microbial flora towards less acid-tolerant and less acidogenic microorganisms, which inhibit the recolonization of the cariogenic microorganisms over a certain time period.[18,19] It was proposed that tooth remineralization might be promoted with the assistance of salivary minerals and fluorides resulting in a tooth surface that was more resistant against future acid attacks.[20] Ozone-induced oxidation may also result in reduction of carbohydrates and acids within the carious lesion and might be another cariostatic effect.[21-24]

To determine the clinical effect of ozone on carious lesions over time, their arrest or progression must be monitored indirectly. The laser-based DIAGNOdent[a] as a tool with good to excellent sensitivity and reproducibility (*in vitro* and *in vivo*) has been suggested for the monitoring of occlusal carious lesions.[25-29] Based on clinical validation, the borderline reading for operative intervention was recommended at a peak value of 30.[26]

To assess the efficacy of any clinically applied preventive strategy, the currently existing caries risk of the individual patients must be assessed as it might influence its efficacy. Conventionally-used caries risk predictors are based on the caries experience of the primary and permanent teeth in the past (dmfs-/DMFS-index)[30-33] or quantify discolored fissures and white spot lesions on permanent molars (Dentoprog-value).[33-36] However, the changes in DIAGNOdent values measured at a reproducible spot of an untreated lesion over a certain time period might be especially useful for determining the current existing caries risk of individuals within this study. Therefore, this clinical study aimed to explore the effect of ozone application on non-cavitated occlusal fissure caries lesions in permanent molars without the aid of remineralizing solutions, in comparison to untreated control lesions within a split mouth design and take into consideration the patient's existing caries risk. The development of the lesions was indirectly monitored using the laser-based DIAGNOdent.

Materials and Methods

Study design and sample selection - This randomized controlled prospective clinical trial (RCT) used a split mouth single blind design. Ethical approval and informed consent were obtained. Healthy patients with no evidence of developmental dental hy-

Table 1. Patients profile regarding number, gender, age, number of lesion pairs, caries experience in the past (dmfs-/DMFS-index) and caries prediction value (Dentoprog-value in percent, DPW%).

Number of patients	n=41
Males	n=20
Females	n=21
Age	Mean 7.7 ± 2.2 years (min. 5 years; max. 13 years)
Number of pairs	n=57
	Upper jaw n=29; Lower jaw n=28
	First permanent molars n=51
	Second permanent molars n=6
dmfs	Mean 22.8 ± 16.1 (min. 0; max. 57) Median 24
DMFS	Mean 0.6 ± 1.0 (min. 0; max. 4) Median 0
DPW (%)	Mean 56.1 ± 24.5 (min. 4.3; max. 91.3) Median 58.2

pomineralization were admitted to the study when two contra-lateral permanent molars showed macroscopically non-cavitated occlusal fissure caries lesions and measurements with the laser fluorescence system DIAGNOdent showing peak values of between 10 and 30 after professional cleaning with an airflow device. This range of readings excluded those lesions, which required operative care according to recently published guidelines for the clinical use of the laser-based device.[26] Teeth with any form of restoration, fissure sealants or orthodontic bands were also excluded from the study. At random, one of the two contra-lateral molars was assigned to receive ozone treatment whereas the other served as an untreated control tooth. As the control tooth is located contra-lateral to the test tooth within the same mouth (*i.e.* exposed to the same microflora), it can be assumed that its initial carious lesion develops comparably to the test lesion. Forty-one patients (males n=20, females n=21) aged between 5 and 13 years (mean age 7.7 ± 2.2 years) with 57 pairs of molars (upper jaw n=29, lower jaw n=28; pairs of first permanent molars n=51, pairs of second permanent molars n=6) were enrolled in the study. There were no patient drop-outs within the study period of 3 months. The patients' profiles are shown in Table 1. To define a subgroup with high caries risk, only patients with the following criterion were included: stable or deteriorating DIAGNOdent values of the control lesions between the baseline evaluation and the 3-month evaluation, which is considered to represent a steady state or deterioration of caries.

Baseline clinical examination - During the baseline examination, the past caries experience of each patient was assessed by the dmfs- and DMFS-Index and a caries prediction value (Dentoprog-value expressed in percent) was calculated for each patient, which is based on the number of sound primary teeth and initial caries in first molars[35] (Table 1). The patients' oral hygiene was assessed by recording the plaque and gingival status present at the distal surfaces and the plaque accumulation at the occlusal surfaces of each molar under examination using a periodontal probe. This evaluation was performed at baseline and after 3 months and graded after Ekstrand *et al*[37] (Table 2). All the study participants live in a nonfluoridated area and used fluoridated toothpaste (for children, age< 6 years, F^- 500 ppm; age ≥ 6 years, F^- 1000 ppm). During the study, no additional fluoridated or antimicrobial mouthrinses or medications were used nor were special oral hygiene instructions given to the patients or their parents.

Study procedure - The lesion site within the occlusal fissure system of each tooth was recorded in a drawing so that it could be located again during further re-examinations. One molar was

Table 2. Assessment of the patient oral hygiene according to Ekstrand *et al*[37] by recording the plaque and gingival status present at the distal surfaces and the plaque accumulation at the occlusal surfaces of each molar under examination using a periodontal probe.

Plaque status (distal surface of examined molars)	
0	No plaque on probe
1	Thin plaque on probe
2	Thick plaque on probe
Gingival status (distal of the molars under examination)	
0	No gingival examination
1	Gingival examination but no bleeding on probing
2	Gingival inflammation with bleeding on probing
Visible plaque recording (occlusal surface of examined molars)	
0	No visible plaque
1	Visible plaque but difficult to recognize
2	Visible plaque but easy to recognize

Fig. 1. Clinical application of ozone to a first permanent molar using a silicone cup *via* a handpiece that is connected to the ozone delivery system HealOzone.

randomly assigned to receive ozone therapy while the other molar of the pair served as the corresponding untreated control.

DIAGNOdent values were assessed after cleaning the occlusal surfaces with airflow (Prophyflex[a]) and subsequent rinsing with copious amounts of water.[26-28] Two separate measurements were taken using probe tip A at the preselected site and the average recorded. These DIAGNOdent values were assigned as baseline reference values.

Gaseous ozone was then applied to the occlusal surface of the randomly assigned test tooth once for 40 seconds using a silicone cup *via* a handpiece connected to the ozone delivery system (HealOzone[a]) according to the manufacturer's instructions (Fig. 1). In this study the use of additional remineralizing solutions, as suggested by the manufacturer, was omitted to ensure that only the effect of ozone was determined. The contra-lateral control tooth was left totally untreated.

After 1, 2 and 3 months, DIAGNOdent values were re-evaluated for the ozone-treated and control lesions by a calibrated blinded examiner. Again, measurements were taken in duplicate and the average recorded. At baseline and after 1, 2 and 3 months, digital photographs of the occlusal surfaces of test and control teeth were taken for documentation (magnification x2).

Assessment of reproducibility, repeatability and statistical analysis - Statistical analysis was performed using SPSS soft-

Table 3. Profile of the whole study population in comparison to the subgroup of patients with high current caries risk regarding the selection parameter, the number of lesion pairs, the dmfs-/DMFS-index and the Dentoprog-value (DPW%) (mean ± standard deviation, SD) as well as the Wilcoxon-test for dependent samples concerning the deterioration or improvement of DIAGNOdent values of the ozone-treated lesions in comparison to the control lesions over the 3-month period.

Study group	Selection parameter	Lesion pairs (n)	dmfs (mean±SD)	DMFS (mean±SD)	DPW (%)	Ozone *vs* control (3 mo.) Wilcoxon
All	No selection	57	22.8 ± 16.1	0.6 ± 1.0	56.1 ± 4.5	P> 0.05
High caries risk	DDV control (3mo) < +3	26	28.3 ± 13.9	0.6 ± 1.1	63.9 ± 9.1	P= 0.035

ware[c] (Version 11.5). The examiners had been extensively calibrated prior to the study through discussions about the working mechanism and the handling of the DIAGNOdent device including practical exercises for a period of 1 month. The intra- and inter-examiner reproducibility was assessed at the end of the 1-month exercises and calculated using Cohen's unweighted kappa statistic and Spearman's correlation coefficient.[38] Kappa values were calculated using the *in vivo* determined cut-off levels for different caries states.[26] For analysis of the repeatability of the DIAGNOdent, the similarity of the duplicate measurements was assessed by calculating the standard deviations of the differences between the pairs of measurements.[38]

To determine the deterioration or improvement of each lesion, differences were calculated (DIAGNOdent difference values, DDV) between the baseline reference DIAGNOdent value and the respective re-evaluation value. Positive changes represent an improvement of lesions while negative changes represent a deterioration of lesions.

According to the split mouth design, each test tooth and its corresponding control tooth were handled as dependent samples. Therefore, the DDV were compared between the ozone-treated teeth and their dependent control teeth using the Wilcoxon-Test for dependent samples. The level of significance was set at $\alpha = 0.05$. When comparing different groups of patients (*e.g.* regarding their past caries experience), the Mann-Whitney U-Test for independent samples was used.

Results

Repeatability and reproducibility - In regard to the repeatability of measurements from the laser fluorescence system DIAGNOdent, an average standard deviation of 2.4 between the pairs of measurements was calculated. The DIAGNOdent method revealed good intra-examiner reproducibility (Cohen's κ= 0.76 and 0.72; Spearman's rho= 0.94 and 0.96) as well as good inter-examiner reproducibility (κ= 0.61; rho= 0.75).

Oral hygiene in the ozone-treated teeth and the contra-lateral control teeth - At the beginning of the study and after 3 months the status of oral hygiene in the ozone-treated teeth and their contra-lateral controls was evaluated. These examinations revealed no significant difference in any of the stated categories between the ozone-treated teeth and their controls at baseline or after the 3-month period, as calculated by the Wilcoxon-test for dependent samples (P> 0.05). Therefore, each pair of lesions (test lesion and respective control lesion) met essentially equal oral hygiene conditions during the study period, which underlines the suitability of the untreated contra-lateral tooth as the control.

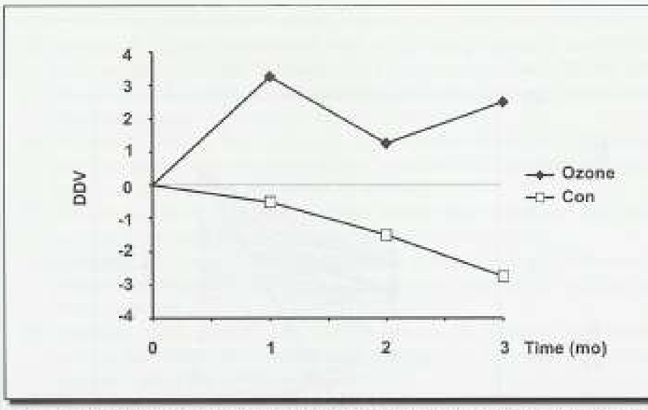

Fig. 2. Evaluation of the ozone effect on non-cavitated initial fissure carious lesions in a subgroup of patients with high current caries risk. The DIAGNOdent difference values (DDV) are the differences between the DIAGNOdent values obtained 1, 2 and 3 months after the treatment and the respective baseline values. Positive DDV represent an improvement of lesions and negative ones a deterioration of lesions. These DDV (medians) are separately depicted for the ozone-treated teeth and the untreated control teeth in the graphic. It represents the development of the lesions over the 3-month period for the subgroup of patients with high current caries risk (n=26 pairs of lesions).

Monitoring the effect of ozone treatment using DIAGNOdent - The effect of the ozone treatment on initial caries lesions was evaluated using the DIAGNOdent-system. Initially, the differences between the values obtained at 1, 2 and 3 months after the treatment and the respective baseline values both for the ozone-treated teeth and the untreated control teeth were calculated. These differences were named DIAGNOdent difference values (DDV). As a next step, the DDV calculated for the ozone-treated teeth were compared to the DDV determined for the control teeth. It should be stated that in this procedure each single ozone-treated tooth was compared to its respective dependent contra-lateral control tooth and that after 3 months the Wilcoxon-test for dependent samples was calculated to evaluate statistical significance.

Effect of ozone on the whole study population and a subgroup with high caries risk - No significant difference between the test and the control teeth was found examining the whole study population (Wilcoxon-test, P> 0.05) (Table 3). No adverse effects were observed during the study. As the individual caries risk is known to influence lesion arrest or progression, the effect of ozone in a subgroup of patients at high current caries risk was also tested. This subgroup was defined by identifying only those patients out of the whole study population who showed steady or deteriorating DIAGNOdent values (DDV < 3) in their control lesions over 3 months (which is considered to represent a steady state or deterioration of caries). The patients of this subgroup also showed significantly higher previous caries experience assessed by the dmfs-index (Mann-Whitney U-test, P= 0.049) and a significantly higher future caries prediction-value assessed by the Dentoprog-value (P= 0.031) than the rest of the study population. Further, they also showed a significantly poorer oral hygiene as expressed by the plaque status at the distal surfaces and the visible plaque recording at the occlusal surfaces[37] after 3 months (Mann-Whitney U-test, P< 0.01). No significant difference in the general gingival status was detected (Mann-Whitney U-test, P> 0.05).

As a next step, we evaluated if ozone exerted an effect in this subgroup. The median DDV of the ozone-treated lesions and the control lesions are depicted graphically in Fig. 2 demon-

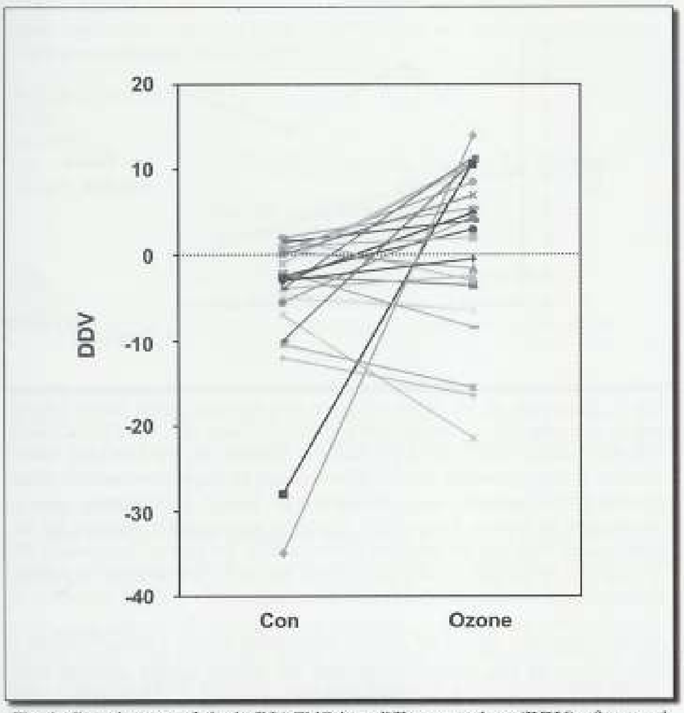

Fig. 3. Complete set of single DIAGNOdent difference values (DDV) of test and control lesions of the subgroup of patients after 3 months (n=26 pairs of lesions). Each single lesion pair is connected by a different shaded line. The statistical significance of the data according to the Wilcoxon-Test is symbolized by an asterisk (P= 0.035).

strating higher DDV medians (*i.e.* improvement of DIAGNOdent values) for the ozone-treated lesions compared to their controls. The complete set of the DDV of the subgroup after 3 months is summarized in Fig. 3. In this figure, each single lesion pair is connected by drawing a line between the ozone-treated lesion and the respective control. In most of the cases, ascending lines from the control lesion on the left to the ozone-treated lesions on the right side are shown indicating a lesion-improving effect of ozone. Investigating this subgroup statistically, the Wilcoxon-test for dependent samples revealed a significant difference between the test and control lesions after 3 months in favor of the ozone-treated lesions (Wilcoxon-test, P= 0.035) (Table 3). These data suggest that the application of ozone gas on non-cavitated fissure caries lesions has an improving effect in patients at high caries risk as monitored by the laser-based DIAGNOdent.

Discussion

While the rate of dental caries has shown a remarkable decline in children and adolescents during the last two decades, the proportion of fissure carious lesions in permanent molars still accounts for over 80% of their total caries experience.[39] Due to the potential reversibility of carious lesions in their non-cavitated status, the development of treatment modalities aiming at total reversal has a significant priority in clinical dentistry. Based on the understanding that caries is an infectious disease, recently, ozone has been proposed as a pharmacological approach to treat caries mainly by elimination of caries-associated microflora but also by oxidation and therefore decreasing carbohydrates and acids within the caries lesion.[16,17,21,22] Subsequently, ozone might be considered as a method of promoting the remineralization process, enabling a *restitutio ad integrum* in cases of initial non-cavitated lesions. Most dentists currently use ozone, treating lesions during the first visit, and then 4 weeks later, they re-ozone and seal these pits and fissures. Clearly, remineralization of non-cavitated lesions, followed by further disinfection before sealing, should be preferable to the conventional drilling and filling approach. This paper provides further evidence for the remineralization of these carious lesions in patients at high caries risk.

The use of additional remineralizing solutions after the ozone application, as suggested by the manufacturer of the ozone delivery system, was omitted in order to ensure that only the effect of ozone was determined. In this study the only remineralizing solution was the patient's saliva. A split mouth design was chosen with test and corresponding contra-lateral control tooth within the same patient to eliminate bias deriving from patients with quantitatively and qualitatively different bacterial loads[40,41] or differences in the mineralization state after eruption of test and control teeth.[42] Further on, the choice of control should be valuable as the included ozone-treated and corresponding control lesions show comparable lesion severity and could be proven to meet essentially the same oral hygiene conditions. The monitoring of the lesions over a period of 3 months was performed indirectly using the laser based DIAGNOdent-system that has been suggested for occlusal caries detection and monitoring purposes after intensive efforts for its clinical and histological validation.[25-27,29,43,44] In order to minimize the reported specific source of measurement deviation due to fissure staining,[25,26] the cleaning of the fissure systems by airflow was done as standardized as possible. In the beginning of the study the technique could be shown to perform well regarding the reproducibility and repeatability.

When considering the whole study population, no significant difference between test and control lesions could be found over the 3-month period. It is possible that, although no additional oral hygiene instructions were given, the study procedure itself could have resulted in an increase in the patient's oral hygiene motivation (*i.e.* more intensive use of fluoridated toothpaste). This may be of relevance since the subsequent lesion improving effect[45,46] (both test and control) would make it difficult to detect a further impact of ozone treatment within this study design. For the whole study population, comprising also low risk patients, one single ozone application without additional use of remineralizing solution might not be sufficient to result in a significant effect when compared with a control which profits from good oral hygiene.

Bearing in mind that oral hygiene with fluoride as well as the individual caries risk is known to influence lesion arrest or progression,[32,33,47,48] this study assessed also the effect of ozone considering the current caries risk of each patient. Presuming that lesions of high risk individuals performed worse than lesions of patients with minor caries risk, we determined the current caries risk by the deterioration or improvement of the DIAGNOdent values of the untreated lesions over the study period. Thus, we defined a subgroup of patients comprising the individuals out of the whole study population who showed steady or deteriorating DIAGNOdent values in their control lesions over 3 months (which is considered to represent a steady state or deterioration of caries). This procedure was proved to be correct, as the group of patients with high current caries risk was shown to have significantly higher dmfs- and

Dentoprog values as well as worse oral hygiene (general and occlusal plaque index) than the rest of the study population. Therefore, a covering effect due to increased oral hygiene, as suggested for the whole study population, would be eliminated.

Within this subgroup the explorative data analysis revealed that the ozone-treated lesions showed statistically more caries reversal or reduced caries progression than the untreated control lesions after 3 months (Wilcoxon-test). Several studies reported at international meetings differed from the one presented here in methods of assessment of lesion behavior, in the degree of severity of the lesions included and in differing time of ozone applications. But all studies give promising results regarding the remineralization enhancing effect of ozone application and are in agreement that no adverse effects associated with the ozone treatment were observed.[11,15] It could be hypothesized that repeated ozone applications in combination with additional remineralizing solutions as reported at the above-mentioned meetings, might serve to further improve the outcome of the treatment described in the present study. Although confirmatory studies further investigating these findings are required, the data in this explorative study suggest ozone as a novel therapeutic approach to the management of non-cavitated initial fissure carious lesions in patients at high caries risk.

a. KaVo, Biberach, Germany.
b. Nikon, Tokyo, Japan.
c. SPSS Inc., Chicago, IL, USA.

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- Effect of air-particle abrasion on the retention and texture of the maxillary complete denture.
  A.M. Husham, M.H.K. Al-Bazirgan
- Clinical performance of posterior compomer restorations over four years.
  N. Krämer, F. García-Godoy, C. Reinelt & R. Frankenberger
- Removal of oral biofilm by sonic phenomena.
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- Evaluation of a self-etching adhesive in deciduous and permanent dentition.
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- The impact of ozone treatment on enamel physical properties.
  P. Celiberti, P. Pazera & A Lussi
- Capacity of coronal dentin to increase fiberglass post retention: A pull-out test.
  C. D'Arcangelo, G. Domenico Prosperi, P. Passariello, S. Caputi & V.A. Malagnino
- Effect of cavity form variety on adhesion to cavity floor.
  R. Kishikawa, A. Koiwa, H. Chikawa, E. Cho, N. Inai & J. Tagami