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SHORT COMMUNICATION

NL Hoenderdos
NAM Rosema
DE Slot
MF Timmerman
U van der Velden
GA van der Weijden

The influence of a hydrogen peroxide and glycerol containing mouthrinse on plaque accumulation: a 3-day non-brushing model

Authors' affiliations:
N. L. Hoenderdos, N. A. M. Rosema, D. E. Slot, M. F. Timmerman, U. van der Velden, G. A. van der Weijden, Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Amsterdam, The Netherlands

Correspondence to:
N. L. Hoenderdos
Department of Periodontology ACTA
Louwesweg 1
1066 EA Amsterdam
The Netherlands
Tel.: +31 (0) 20 5188 548
Fax: +31 (0) 20 5188 512
E-mail: N.Hoenderdos@acta.nl

Abstract: *Aim:* To evaluate the inhibition of plaque growth by an experimental mouthrinse (BioXyl®) based on hydrogen peroxide/glycerol. *Design:* It was a double-blind, randomized study involving 40 volunteers in good general health. At the start of the trial, all participants received a dental prophylaxis to remove all plaque deposits. During the next 3 days subjects refrained from any mechanical oral hygiene procedure, except for the allocated mouthrinse being either the hydrogen peroxide (H_2O_2; 0.013% H_2O_2/0.004% glycerol) or the placebo without H_2O_2. At the third day of appointment, plaque levels were assessed at six sites per tooth. *Results:* The test group had a mean overall plaque score of 2.66 and the placebo group of 2.70. The difference in plaque scores between the two groups was not statistically significant. *Conclusions:* The results of this pilot study showed that there was no statistically significant difference between the H_2O_2/glycerol group and the placebo group with respect to plaque inhibition within this study design.

Key words: clinical trial; glycerol; hydrogen peroxide; mouthrinse; plaque

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Introduction

Plaque is known to be the initiating factor in the development of gingivitis when in contact with the gingival tissues. The biofilm character of dental plaque is the key for survival of the microflora and also to their role in oral health and disease. The

biofilm serves to moderate metabolic activities and to protect the flora against the harsh environment of the mouth (1).

Tooth-brushing is the most widespread mechanical means of personal plaque control in the world and represents the cornerstone of good oral hygiene practice (2). Enthusiastic use of the toothbrush is not, however, synonymous with a high standard of oral hygiene (3). Despite the availability of various oral hygiene devices, even the most meticulous patient will not always completely remove all plaque. An alternative or adjunctive method of plaque control would be desirable by the use of chemotherapeutic agents (4).

Hydrogen peroxide (H_2O_2) was first isolated in 1818 by reacting barium peroxide with nitric acid (5). Hydrogen peroxide, alone or in combination with salts, has been used in dentistry for over 70 years for instance in the treatment of peri-coronitis caused by anaerobic microorganisms. Peroxide that creates an aerobic atmosphere is destructive to these microorganisms. No hazard is likely to be associated with the long-term use of H_2O_2 at concentrations found in oral care products (5).

The contact time of H_2O_2 in the oral cavity is short (6). Hydrogen peroxide is relatively unstable and in the oral cavity rapidly decomposed into water and oxygen as a result of the activity of salivary catalase (7). This results in a rapid recovery of the bacterial population to normal levels consequently plaque growth is unaffected (8). Degradation of glycerol can act as a stabilizing agent thus slowing down the oxidation process caused by H_2O_2 (9).

The following hypothesis was formulated for this study: an experimental mouthrinse with 0.013% H_2O_2 in combination with a placebo mouthrinse with 0.004% glycerol dissolved in demineralized water has a statistically significant inhibitory effect on *de novo* plaque formation in healthy patients.

Material and Methods

Subjects

Forty (non-dental students) subjects were recruited to participate voluntarily in the study. Subjects were recruited at universities and colleges in and around Amsterdam and were in good general health without a medical history or medication that might interfere with the study outcome. All subjects were dentate with at least five scorable teeth per quadrant. Subjects were excluded if they had fixed or removable orthodontic appliances, removable prosthesis or pockets >5 mm. After thorough explication of the procedures informed consent was obtained.

Procedure

This double-blind randomized study was based on a 3-day non-brushing plaque accumulation model that assessed the effect of the test product on *de novo* plaque formation. The test mouthrinse contained 0.013% H_2O_2 and 0.004% glycerol dissolved in demineralized water (BioXyl®, ClearWater Revival B.V., Amsterdam, Holland). The placebo mouthrinse did not contain H_2O_2 but only contained 0.004% glycerol dissolved in demineralized water.

At baseline (day 0) all subjects received a thorough dental prophylaxis to remove all stain, calculus and plaque. Subjects were randomly assigned to the test and control groups. Written instructions as well as verbal instructions by a dental hygienist were given on the use of the mouthrinse. The subjects rinsed at home unsupervised. The rinsing instructions were as follows: rinse with approximately 15 to 20 ml of fluid for 20 s. Afterwards expectorate the fluid and refrain from any eating or drinking for the next 30 min.

All bottles with mouthrinse were preweighed. Subsequently, all subjects rinsed for the first time in presence of a dental hygienist. Subjects refrained from brushing for 3 days. During these days the subjects rinsed two times per day with the allocated mouthrinse. During the experiment the subjects had to refrain from any other form of oral hygiene.

After 3 days subjects returned to the clinic and plaque was recorded. All measurements were performed by one and the same experienced blinded examiner (NAMR) under the same conditions. The examiner has been trained and calibrated in the plaque scoring system and has applied this in other studies (10–13). All returned mouthrinse bottles were weighed to calculate the amount of mouthrinse used and to check compliance.

Clinical parameter

The Plaque Index used in this study was developed by Quigley and Hein (14) and modified by Turesky *et al.* (15) and Lobene *et al.* (16). This index is recognized as a reliable index for measuring plaque using an estimate of the area of the tooth covered by plaque. The technique of scoring plaque on the labial, buccal and lingual surfaces provided a comprehensive method for evaluating anti-plaque procedures, such as toothbrushing and flossing as well as chemical anti-plaque agents (17). This index emphasizes the difference in plaque accumulation in the gingival third of the tooth. Furthermore, the modification of the original index by using six surfaces of each tooth (distal–buccal, buccal, mesial–buccal, distal–lingual,

lingual and mesial–lingual) implicated that more weight was placed on the interproximal surfaces. Each of the six tooth surfaces was given a score of 0, 1, 2, 3, 4, or 5 according to criteria [0 = no plaque, 1 = separate flecks of plaque at the cervical margin of the tooth, 2 = a thin continuous band of plaque (≤1 mm) at the cervical margin of the tooth, 3 = a band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth, 4 = plaque covering at least one-third but less than two-thirds of the crown of the tooth, 5 = plaque covering two-thirds or more of the crown of the tooth].

Data analyses

The sample size of 40 was calculated *a priori* in such a way that with an alpha of 0.05, a difference of 0.27 (between groups) of the plaque index (PI) could be identified with 80% power, based on a pooled SD of 0.3 derived from previous studies.

Individual mean plaque scores were calculated and an overall mean was calculated by group. The Mann–Whitney *U*-test was used to test for differences between the two groups. *P*-values <0.05 were considered as statistically significant.

Results

Thirty nine of 40 subjects, aged between 19 and 42 years (mean age: 24.5 years), completed the study. One subject failed to meet her second appointment due to illness unrelated with the study products. Table 1 shows the mean plaque scores for rinsing with the H_2O_2 containing mouthrinse as well as the placebo mouthrinse.

Statistical analysis showed no significant differences in overall plaque scores between the two mouthrinses. For clear interpretation of the data, Table 1 shows 95% CI. This provided insight in the magnitude of the differences between both groups. With respect to the amount of mouthrinse that was used during the study protocol no statistically significant difference between groups could be observed (Table 2).

Table 1. **Mean (SD) plaque scores of the two mouthrinse groups, the mean difference and the 95% confidence interval of the difference**

Overall	*n* = 39	Mean	Mean difference	95% CI of the difference
H_2O_2	19	2.66 (0.29)	0.036	[−0.16; 0.23]
Placebo	20	2.70 (0.32)		
P *		0.440		

*Mann–Whitney *U*-test.

Table 2. **Mean (SD) weight of mouthrinse bottles (in grams)**

Overall	*n* = 39	Base	End	Difference
H_2O_2	19	578.37 (9.15)	432.84 (24.54)	145.53 (21.13)
Placebo	20	587.15 (5.48)	441.05 (21.59)	146.10 (1.34)
P *		0.978		

Mann-Whitney U-test.

No adverse events were reported by the subjects and the examiners.

Discussion

Hydrogen peroxide and other oxygenating agents such as perborates are disinfectants which, when acted on tissue- and bacterial-derived enzymes, release oxygen with an associated effervescence. Hydrogen peroxide is recognized as a germicidal agent with a relative weak effect against gram-positive and gram-negative organisms. It is particularly useful against anaerobes (18). Oxygenating agents such as H_2O_2 have anti-inflammatory properties (19). Hydrogen peroxide is used widely in professional and self-administered oral health care products such as dentifrices and mouthrinses. Low concentrations neither damaged hard or soft oral tissues nor do they pose a significant risk of adverse long-term effects (20).

The present study was designed to determine the plaque-inhibiting effect of 0.013% H_2O_2 combination with 0.004% glycerol compared with a placebo. It used a 3-day non-brushing model that allowed for plaque accumulation. This design was previously used to assess the effect of various mouth-washes (11, 21–25).

This 3-day model has also proven to be useful to discern between 'rapid' and 'slow' plaque formers (10, 26, 27). The results of the present study showed that after a non-brushing period of 3 days, an experimental mouthrinse containing H_2O_2/glycerol used twice daily for 20 s, had no significant effect on the inhibition of plaque growth. The present study did not provide an explanation for outcome; however, one can hypothesize as to what could be the cause of this lack of efficacy.

After 3 days of plaque accumulation with no oral hygiene measures, Simonsson *et al.* (28) demonstrated considerable variation in the rate of plaque formation. This confirmed earlier studies on experimental gingivitis in man. Löe *et al.* (29) and Theilade *et al.* (30) showed that plaque formation varied to a great extent between different individuals. When Quirynen & van Steenberghe (31) studied the early plaque growth in 15 young adults going through a 4-day period of no oral hygiene, they reported that the growth rate was rather slow during the

first 24 h with an exponential increase during the following 3 days. The rate of supragingival plaque growth appeared not to be constant with time. During the 'nights rest' the plaque growth rate decreased up to 50%. Clear differences between 'slow' and 'rapid' plaque formers seemed to be closely correlated to irregularities of the tooth surfaces (32). Also the inflammatory status of the marginal gingiva had an important effect on early, supragingival plaque accumulation (33). Other factors such as gingival fluid, salivary components and diet may also contribute to the observed differences. However, as in the present study the allocation to the test and control rinse was randomized, 'slow' and 'rapid' plaque formers 'by chance' should be equally distributed over the two groups.

Previous studies have shown that mouthrinses containing 1.5% H_2O_2, used once or twice daily, could be a beneficial adjunct for the prevention and control of gingival inflammation (34–36). However, they did not indicate conclusively that this concentration was the optimal therapeutic level of H_2O_2. Gusberti *et al.* (37) concluded that mouthrinses containing 1% H_2O_2 did not provide meaningful anti-plaque or anti-gingivitis benefits. The review of Marshall *et al.* (38) also noted that efficacy of H_2O_2 was not associated with use of H_2O_2 at <1%.

The concentration H_2O_2 that was used for the present experimental mouthrinse was much lower as the above-mentioned concentrations. It remains the question whether this low concentration was the cause of the absence of a significant effect on the inhibition of plaque growth. The manufacturer considered the addition of glycerol sufficient to improve stability and ensured an anti-plaque effect. However, the plaque growth inhibiting effect of 0.013% H_2O_2 combined with 0.004% glycerol was found to be insignificant within the present study design.

The anti-plaque efficacy of a chemotherapeutic agent is also related to the retention time in the oral cavity. Such 'substantivity' involves the ability of the agent to be retained in the oral cavity for protracted periods, with subsequent sustained release of the active molecules at effective levels (39). The amount of time that H_2O_2 was present in the oral cavity following its introduction from dentifrices was generally <1 min (6). Consequently, little if any H_2O_2 would be available from twice daily use that would be effective enough to inhibit plaque growth (6).

To control the implementation of instructions all subjects rinsed for the first time in presence of a dental hygienist. Subjects subsequently rinsed six times unsupervised at home in the course of the next 72 h. An attempt was made to standardize the usage of rinses by time and amount of fluid. All returned mouthrinse bottles were weighed to calculate the amount of mouthrinse used and to check compliance. The average used mouthrinse per subject was 146 ml (Table 2) which corresponded to the instructions that were given. In conclusion, the hypothesis that an experimental mouthrinse with 0.013% H_2O_2 in combination with 0.004% glycerol has a statistically significant inhibitory effect on *de novo* plaque formation is rejected.

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