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Optimal rinsing time for intra-oral distribution (spread) of mouthwashes

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Abstract

Aim: To investigate the intra-oral spread of an erythrosine mouthwash in relation to the rinsing period.

Material and Methods: Thirty subjects were randomly divided into two equal groups and asked to rinse with 10 ml erythrosine mouthwash for cumulative periods of 15, 30 and 60 s (Group I) and 30, 60 and 90 s (Group II). Each rinsing session was followed by new plaque measurements. After rinsing plaque was finally assessed using the erythrosine mouthwash applied by means of a cotton swab.

Results: In Group I there was a difference observed between the 15 s rinsing period and those of 30 and 60 s. Explorative analysis for Group I suggested that differences in both jaws and approximal sites on both vestibular and lingual surfaces appeared to have contributed to the overall difference seen between 15 and 30 s rinsing periods. Also, pre-molars and front teeth seem to have contributed to this observed difference. No differences were noted between rinsing sessions and cotton swab application for Group II or Groups I+II combined.

Conclusions: Rinsing for 30 s appeared to be sufficient for all plaque-covered surfaces of the dentition to come into contact with the mouthwash.

Key words: disclosing agents; erythrosine; mouthwash; plaque

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Stability of the periodontal condition is associated with effective plaque control (Axelsson et al. 1991). This, in turn, is best accomplished through meticulous mechanical plaque removal. Despite the availability of various oral hygiene devices, even the most careful patient will not always completely remove all plaque. Evidence indicates that the degree of motivation and skill required for the effective use of various oral hygiene products may be beyond the ability of the majority of patients (Johansson et al. 1984, Wilson 1987). Therefore, for decades it was the goal of periodontal research to seek for chemical agents that would inhibit plaque formation. The use of mouthwashes was introduced for chemical plaque control (Mandel 1988) and it aims at improving the efficacy of self-performed mechanical plaque removal.

Rinsing with chlorhexidine (CHX) is one of the most undisputed ways to restrain plaque growth. Although numerous studies have been performed in this field, there is not much information available about the optimal rinsing time with this agent. Traditionally, rinsing for 60 s with 10 ml 0.2% CHX twice daily was considered to be sufficient for plaque inhibition (Löe & Schiøtt 1970). Segreto et al. (1986) showed comparative plaque inhibition after rinsing for 60 s with 15 ml 0.12% or 0.2% CHX. Keijser et al. (2003) reported that rinsing for 30 s with 15 ml 0.12% CHXwas equally effective in terms of plaque inhibition to rinsing for 60 s with 10 ml 0.2% CHX. A recent study (van der Weijden et al. 2005) assessed the plaque inhibiting effect of a 0.2% CHX solution with three different rinsing times (15, 30 and 60 s) during 72 h non-brushing period. No significant differences in plaque scores were observed between the three rinsing periods. The authors emphasized the need for additional research towards shorter rinsing times suggesting that 60 s rinsing time might not be necessary in order to achieve satisfactory plaque inhibition.

Clinical experience has shown that not all individuals are rinsing in the same manner. Uninstructed patients may hold fluid in their mouth and bow the head from side to side or perform another action that cannot force the fluid between the teeth (Wilkins 1999). This could affect the intra-oral distribution and the efficiency of the mouthwash. In order to achieve the most favourable action of a mouthwash, steps for teaching the patients have been developed (Wilkins 1999).

By incorporating a disclosing agent in a mouthwash solution, all places in the mouth, where plaque is present, are theoretically exposed for the staining agent. This, in turn, gives an indication of the intra-oral distribution of the mouthwash. Several disclosing agents have been developed in order to make plaque visible to the naked eye (Tan 1981, Kipioti et al. 1984). Erythrosine is one of the most widely known disclosing agents today. It was the purpose of this study, to investigate the intra-oral distribution of an erythrosine containing mouthwash in relation to the rinsing time.

Material and Methods

Thirty healthy individuals were recruited for the purpose of the study. The subjects were required to be periodontally healthy, with no history of allergic reaction to erythrosine and at least 28 evaluable teeth. Orthodontic appliances were not allowed. The subjects were informed about the purpose of the study and were requested to sign an informed consent prior to entering the trial.

The subjects were instructed to refrain from any means of oral hygiene for a period of 48 h prior to their appointment so that plaque could freely accumulate on all tooth surfaces. They were randomly allocated into two groups (15 subjects each) and were subjected to a series of subsequent rinsing episodes with 10 ml of a 0.9% erythrosine mouthwash as follows: the subjects of the first group (Group I) were instructed to rinse initially for 15 s after which the plaque was scored (Quigley & Hein 1962 index modified by Turesky 1970 and further modified by Lobene 1982). Next, they rinsed for another 15 s (to reach a total rinsing time of 30 s) again followed by plaque scoring. Then they rinsed for another 30 s (total rinsing time of 60 s) and new plaque scores were obtained. The remaining 15 subjects of Group II were requested to rinse for three consecutive periods of 30 s (cumulative rinsing time of 30, 60 and 90 s) each of which was succeeded by assessment of the amount of disclosed plaque. Finally, in both groups, subsequent to the last rinsing session, all available plaque was disclosed by applying the erythrosine by means of a cotton swab whereafter plaque scores were again obtained.

Immediately after each rinsing session with erythrosine the individuals rinsed with a single sip of water for approximately 5 s to remove excess erythrosine. Each subsequent full-mouth plaque assessment lasted approximately 10 min. Plaque measurements were performed by one and the same examiner (M. M. D.).

At the end of the experiment subjects received a thorough professional prophylaxis in order to remove all plaque and the disclosing agent from the tooth surfaces.

Statistical analysis

The study was designed in such a manner that with a sample size of 15 participants a true difference of 0.06 with a standard deviation of 0.07 in the plaque index scale (Turesky modification of the Quigley–Hein index) with $>80\%$ power and with an α error of 0.05 could be observed.

Mean plaque scores were calculated. Initial analysis of the primary outcome variable (full-mouth scores) included comparisons of all rinsing sessions within each group to one another. In addition, each rinsing session was compared with the results obtained with the cotton swab. Wilcoxon's non-parametric tests were used to compare differences between different rinsing times. Bonferonni's correction was applied for multiple testing. In total, 15 tests were performed for this main outcome variable and a factor of 15 was used (Table 1). *P*-values <0.05 were considered statistically significant.

For explorative analysis, plaque scores were calculated for the different jaws, tooth types (front, pre-molars and molars) and different tooth surfaces (buccal, lingual and approximal; Tables 2a and c). For this analysis, however, no inferences on statistical significance were made.

Results

Table 1 shows the results of the plaque assessments for the two groups, both separately and combined, after the rinsing with erythrosine and application of erythrosine by means of a cotton swab. For Group I the 15 s rinsing session differed significantly from the 30 and 60 s rinsing sessions ($p = 0.03$ and 0.045, respectively). In search of the origin of the difference between 15 and 30 s explorative analyses were performed. Tables 2a gives additional information on the mean plaque scores for the different jaws and tooth surfaces of Group I. The *p*-values of the explorative analysis between different rinsing sessions and the cotton swab application and between different areas or surfaces per session are shown in Tables 2b. Within each rinsing session the amount of plaque stained on the vestibular surfaces was less as compared with that of the palatal/lingual surfaces ($p<0.01$). Also, the amount of plaque stained on the mid-vestibular/lingual surfaces was less than that on the approximal surfaces ($p<0.01$). The *p*-values suggest that, with the exception of the mid-lingual surfaces, all other surfaces in both jaws appeared to have contributed to the overall difference seen between 15 and 30 s rinsing periods. The mean plaque scores for the different tooth categories are shown in Tables 2c. The *p*-values of the comparisons between the different rinsing sessions and cotton swab and between different tooth categories per rinsing session or after the application of the cotton swab are described in Tables 2d. Pre-molars and front teeth seem to have contributed to the observed difference between the 15 and 30 s rinsing session.

Table 1. Total plaque scores and statistical analysis for both groups and different rinsing sessions

	Group I ($N = 15$)	Group II ($N = 15$)	Group I+II ($N = 30$)
Rinsing time (s)			
15	2.63 (0.38)	–	–
30	2.70 (0.37)*	2.49 (0.46)	2.60 (0.47)
60	2.72 (0.39)*	2.52 (0.48)	2.62 (0.44)
90	–	2.55 (0.46)	–
Cotton swab	2.69 (0.40)	2.53 (0.46)	2.61 (0.54)

Standard deviations are in parentheses.

The *p*-values of the performed tests have been adjusted by using Bonferonni's correction for multiple tests (15 comparisons).

*Significantly different in comparison with 15 s rinsing session (Wilcoxon's signed-rank test, $p<0.05$).

Table 2a. Mean plaque scores of the upper and lower jaw and different tooth surfaces of Group I ($N = 15$)

	Upper jaw	Lower jaw	Vestibular surfaces		Lingual surfaces	
			mid	approximal	mid	approximal
Rinsing time (s)						
15	2.60 (0.45)	2.67 (0.38)	2.85 (0.47)	3.24 (0.49)	1.75 (0.53)	2.35 (0.42)
30	2.70 (0.41)	2.70 (0.40)	2.93 (0.52)	3.31 (0.51)	1.78 (0.57)	2.41 (0.42)
Cotton swab	2.71 (0.45)	2.66 (0.41)	2.89 (0.45)	3.31 (0.50)	1.76 (0.61)	2.42 (0.44)

Standard deviations in parentheses.

Table 2b. *p*-values of the explorative analysis (Wilcoxon's signed-rank tests) between the different rinsing sessions and the cotton swab for upper and lower jaw and different tooth surfaces (Group I, $N = 15$)

	Upper jaw	Lower jaw	Vestibular surfaces		Lingual surfaces	
			mid	approximal	mid	approximal
Test between rinsing times						
15–30 s	0.006	0.025	0.030	0.005	0.245	0.011
15 s–cotton swab	0.004	0.638	0.310	0.006	0.363	0.055
30 s–cotton swab	0.249	0.029	0.489	0.875	0.299	0.834

Test between areas or tooth surfaces	Upper jaw *versus* lower jaw	Vestibular *versus* lingual	Mid (vestibular+lingual) *versus* approximal (vestibular+lingual)
15 s	0.378	0.001	0.001
30 s	0.713	0.001	0.001
Cotton swab	0.513	0.001	0.001

Table 2c. Mean plaque scores for different tooth categories of Group I ($N = 15$)

	Molars	Pre-molars	Front
Rinsing time			
15 s	2.75 (0.49)	2.67 (0.46)	2.53 (0.39)
30 s	2.78 (0.48)	2.73 (0.45)	2.63 (0.39)
Cotton swab	2.76 (0.51)	2.63 (0.42)	2.63 (0.43)

Standard deviations in parentheses.

Table 2d. *p*-values of the explorative analysis (Wilcoxon's signed-rank tests) between the different rinsing sessions and the cotton swab for different tooth categories (Group I, $N = 15$)

	Molars	Pre-molars	Front
Test between rinsing times			
15–30 s	0.166	0.011	0.005
15 s–cotton swab	0.727	0.147	0.012
30 s–cotton swab	0.421	0.656	0.629

Test between tooth categories	Molars *versus* pre-molars	Molars *versus* front	Pre-molars *versus* front
15 s	0.112	0.069	0.156
30 s	0.477	0.177	0.280
Cotton swab	0.310	0.307	0.433

In Group II plaque scores obtained after rinsing for 30 s with erythrosine did not differ significantly from those after rinsing for 60 or 90 s or after the application of the cotton swab (Table 1).

When Groups I and II were combined (Table 1), rinsing with erythrosine for 30 s resulted in a mean plaque index of 2.60. After having rinsed for another 30 s (in total 60 s) the plaque index was found to be 2.62. After the application of the cotton swab the mean plaque score was 2.61. No significant differences were observed when the different rinsing sessions were compared with the cotton swab.

Discussion

Erythrosine is a disclosing agent that received the FDA approval (Arnim 1963) and has been used for many years as means of motivation and evaluation of the effectiveness of the oral hygiene (Tan 1981).

Most of the knowledge on the use of mouthwashes and the possible factors that could determine their effectiveness originates from the field of CHX. Retention studies have demonstrated that higher retention of CHX is associated with higher initial concentrations (Bonesvoll 1977). By increasing the concentration of the active agent it is possible to reduce the time of application (Bonesvoll et al. 1974, Bonesvoll 1977, 1978). Little is known, however, about the optimal rinsing time with CHX. In a recent study, Keijser et al. (2003) found no significant differences in efficacy when a product was used for 30 s as compared with the 60 s.

The choice for erythrosine in the present study was made because it readily stains the plaque. Therefore, its incorporation in the mouthwash can be used in order to get a visual assessment of the oral spread of the mouthwash. The concept of the use of a disclosing agent as means of determining the effectiveness of a treatment approach or delivery system is not new. Pitcher et al. (1980) used a disclosing agent in order to examine the effectiveness of the penetration of a mouthwash or local irrigation into the periodontal pocket. Similarly, Eakle et al. (1986) used erythrosine in order to evaluate the effectiveness of the penetration of a water irrigator in pockets of patients suffering from moderate and advanced periodontitis.

One of the findings of the present investigation was that the amount of stained plaque on the lingual surfaces was consistently lower than on the buccal. This is in agreement with previous observations (Lang et al. 1973, Ramberg et al. 1992, van der Weijden et al. 1998, Claydon et al. 2002, Danser et al. 2003). One possible explanation for this finding could be the inability of the mouthwash to reach the lingual surfaces. If this

would have been the case one should expect that the plaque disclosure by means of a cotton swab would have revealed this difference. However, no differences between the plaque scores achieved by rinsing or by application with a cotton swab were found. This suggests that presumably other factors contribute to lower plaque scores lingually such as the natural cleansing mechanisms (movements of the tongue or salivary flow) that could prevent somehow the plaque growth (Claydon et al. 2002).

The patients in the present study were subjected to rinsing regimens that differed with respect to time. In order to explore the effects of the rinsing time on the intra-oral spread of the mouthwash two groups of patients provided data on rinsing for 15, 30, 60 and 90 s. There was a statistically significant difference when the 15 s rinsing session was compared with the rinsing for 30 s and more. This suggests that 15 s of rinsing is not adequate to reach all surfaces covered with plaque. Although statistically significant, one can question the clinical relevance of this difference since its magnitude (0.07–0.09) was small if one considers the 0–5 scale of the modified Quigley & Hein index used in this study. This was supported by van der Weijden et al. (2005) who found no differences in terms of plaque inhibition after 3 days, when subjects were requested to rinse for 15, 30 or 60 s with a 0.2% CHX mouthwash.

It is recognized that subsequent plaque scores obtained after each rinsing period represent a cumulative effect of the mouthwash in the mouth. It is not known, however, whether this effect is equal to that observed after letting the subjects rinse once for a total period of time. Considering the fact that subjects had to rinse with water after each rinsing period one may assume that some amount of erythrosine has been lost. Although data on the substantivity of this agent are lacking one cannot exclude the possibility of loosing some amounts of erythrosine in the period between two subsequent rinsings. Furthermore, it is not known whether the results obtained with this agent are applicable to all agents with antibacterial properties. CHX is one of the most extensively tested agents. In a recent study by van der Weijden et al. (2005) did, however, observe that 15 s would be enough with CHX when studying this effect on 3-day plaque accumulation. This underlines the results of the present study.

Erythrosine is usually applied by means of a cotton swab in order to avoid interference with parts of the mouth other that the regions of interest and decrease the non-esthetic coloration. Care was taken in the present study to ensure that all areas of the dentition had been reached by the cotton swab to justify its use as positive control. After the application of cotton swab, rather interestingly, a small non-significant numerical drop of the amount of disclosed plaque was observed when compared with the results after the last rinsing in both groups. It seems reasonable to assume that the cotton swab application possibly results in some (clinically not relevant) plaque removal when applied onto the tooth surface.

In conclusion, the present study showed that rinsing for 30 s is sufficient in order for plaque-covered surfaces of the dentition to come into contact with the mouthwash.

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