

# Three different rinsing times and inhibition of plaque accumulation with chlorhexidine

G. A. Van der Weijden,  
M. F. Timmerman, A. G. A. Novotny,  
N. A. M. Rosema and A. A. J. Verkerk

Department of Periodontology, Academic  
Center for Dentistry Amsterdam, ACTA,  
Amsterdam, The Netherlands

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## Abstract

**Aim:** This study assessed the plaque inhibiting effect of a 0.2% chlorhexidine (CHX) solution (Corsodyl®) with three different rinsing times following a 72 h non-brushing period.

**Material and Methods:** The clinical investigation was a single-blind, randomised study involving 90 volunteer students (40 male and 50 female, mean age 23.2 years). Subjects were randomly allocated to one of three groups for which the protocol only differed with respect to the duration of rinsing. At the start of the trial, all participants received a dental prophylaxis to remove all plaque deposits. Subjects refrained from all mechanical oral hygiene procedures, but rinsed two times per day for the allocated duration with CHX mouth rinse over a period of 72 h. The chlorhexidine preparation was of 0.2% concentration used at a dose of 10 ml for either 15, 30 or 60 s. After 72 h, the Quigley & Hein plaque index (PI) from all volunteers was recorded at six sites per tooth. All participants received a questionnaire to evaluate their perception of rinsing duration.

**Results:** After 72 h, the mean whole-mouth PI was 1.33, 1.18 and 1.24, respectively, for the 15, 30 and 60 s rinsing group. The difference in plaque scores between the three groups was not statistically significant. Results from the questionnaire showed a significant difference between the groups for their perception of rinsing duration.

**Conclusions:** No significant difference was observed in the level of plaque after 72 h of non-brushing whether the subjects rinsed for 15, 30 or 60 s with 0.2% chlorhexidine.

Key words: chlorhexidine; clinical trial; mouth rinse; plaque

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The role of dental plaque in the aetiology of dental diseases is well recognised with many excellent reviews (Bowden & Edwardsson 1994, Shibly et al. 1995). While there are other factors to consider, thorough and regular plaque removal will result in profound reductions of these diseases. The concept of effective mechanical plaque control is intellectually simple, but there are many barriers to its successful implementation (Needleman 1998).

For many years, researchers have been searching for an effective chemical mouth rinse, that prevents oral plaque growth, to replace mechanical plaque

control. Until now chlorhexidine (CHX) seems to be the most effective chemical agent. The toxicity of CHX is low and therefore used for many medical and dental applications (Addy 2003). Several studies have shown that rinsing twice per day with a CHX solution inhibits plaque formation and helps to prevent inflammation of the gingiva and dental caries (Löe & Schiøtt 1970, Gründemann et al. 2000). CHX has found many short- to medium-term uses in the control of the oral flora and plaque accumulation, particularly when mechanical cleaning is suspended, difficult or inadequate (Jones 1997, Addy 2003).

It is generally accepted that the mode of action of chlorhexidine is dependent on initial adsorption to surfaces (Addy & Renton-Harper 1997). CHX epitomises the term substantivity, showing antimicrobial activity in the mouth for at least 7 h (Addy & Wright 1978) and probably more than 12 h (Schiøtt et al. 1970, Gjermo 1974). Bonesvoll et al. (1974a) have shown that the retention of CHX is affected by concentration, pH and volume of the mouth rinse. They also tested three different rinsing times: 15, 30 and 60 s using CHX solutions at room temperature and having two different concentrations (0.1% and 0.2%).

All subjects ( $n = 5$ ) rinsed with 10 ml of the test solution for the requisite time. Approximately half of the quantity retained during a 60 s rinse will be bonded to receptor molecules in the first 15 s. The inter-individual variation was rather large. For example, one person retained the amount after 15 s as did another after 60 s.

A recent study (Keijser et al. 2003) compared two mouth rinses with different concentrations and rinsing times of CHX (Oral-B® (Oral-B Laboratories, Rijswijk, The Netherlands), 0.12% CHX and Corsodyl®, 0.2% CHX). During 72 h, subjects rinsed with CHX twice a day and refrained from any other form of oral hygiene. One group rinsed with 15 ml of 0.12% CHX for 30 s, and the other group rinsed with 10 ml of 0.2% CHX for 60 s. Their study showed that the plaque inhibition in both groups was comparable. Results of the questionnaire indicated that the subjects preferred the shorter rinsing time. The latter result emphasises the need for investigations into whether shorter rinsing times can be sufficient for effective plaque control.

The purpose of the present study, therefore, was to assess the plaque inhibiting effect of a 0.2% CHX solution (Corsodyl®) with three different rinsing times following a 72 h non-brushing period, this being 60 s as proposed by the manufacturer and two shorter rinsing times of 30 s and 15 s.

## Material and Methods

### Subjects

A group of 90 healthy dental students (40 male and 50 female; age range 18–41 years, mean age 23.2 years) participated in this study. The selection criteria were: no removable or fixed dental prosthetics, no extensive cervical restorations and a minimum of five evaluable teeth per quadrant. At the start of the study, all subjects were given oral and written instructions and information about the product and purpose of the study.

### Design

The study was designed as a single-blind, three-group parallel experiment. During the 72 h period any form of oral hygiene other than the rinsing regimen was prohibited as mentioned in the instructions. Each group rinsed twice a

day (once in the morning and once in the evening before sleeping) with a 0.2% CHX mouth rinse (Corsodyl®, GlaxoSmithKline, Zeist, The Netherlands). The first group rinsed for 15 s, the second group rinsed for 30 s and the third group rinsed for 60 s. All subjects were randomly assigned into one of the three different groups.

All test subjects received a professional prophylaxis at the start of the study, with the purpose of making the dentition 100% free of plaque and calculus. This was realised by using hand instruments, an air-scaler (Sonicflex, KaVo Dental GmbH & Co. KG, Biberach, Germany) and rotating cups and brushes (Hawe-Proply #0220, Hawe-Neos Dental Dr. H.V. Weissenfluh AG, Bioggio, Switzerland) with polishing paste (Superpolish #361, Hawe-Neos Dental). After the calculus was removed, the plaque was stained with an erythrosine disclosing solution applied with cotton buds. All visible plaque was removed. Next the erythrosine disclosing solution was used again to make sure all the plaque was removed. Finally, unwaxed floss (Johnson and Johnson, GABA B.V., Almere, The Netherlands, distributor) was used for a professional interdental cleaning.

At this moment, all subjects received one bottle of 0.2% CHX and were instructed to rinse twice a day with 10 ml per rinsing time. After reaching the exact rinsing time the subjects had to expectorate the mouth rinse. Rinsing with water or any other fluid after this procedure was not allowed as was any form of mechanical oral hygiene during the experimental period. A written instruction on how to use the mouth-rinse was included. The subjects were also given a stop-watch to keep track of the precise assigned rinsing time. To check for compliance, the subjects were asked to register the time at which they rinsed every day.

At the second visit (72 h later), all teeth were disclosed and plaque was recorded by one examiner (N.A.M.R.) at six sites per tooth using the Quigley & Hein (1962) plaque index (PI) as modified by Turesky et al. (1970) and further modified by Lobene et al. (1982). All measurements were carried out under the same circumstances using the same batch of disclosing solution. Finally, the participants were requested to give an opinion about their perceived temporal experience – in terms of short and long – rinsing times. Therefore, a

visual analogue scale (VAS) was used, where the subjects had to mark a point on a 10 cm long uncalibrated line. On the left (0 cm), the value was “very short” and on the right (10 cm), it was “very long”.

### Statistical analyses

The plaque scores were used as the main response variable. All analyses comparing differences (PI, VAS-scores) between the three groups were performed using the Kruskal–Wallis test. Explorative analysis were performed using the Mann–Whitney test comparing data between groups. 95% confidence intervals were calculated for the difference in plaque scores between groups.  $p$ -values  $\leq 0.05$  were considered as statistically significant.

## Results

All subjects completed the study. Table 1 presents the mean results for the PI. The 15 s CHX group showed an average plaque score of 1.33, the 30 s CHX group an average score of 1.18 and the 60 s CHX group 1.24. The statistical analysis showed no significant difference in plaque scores between the three groups. Further analysis by separating the data by tooth surface, tooth type and upper and lower jaw showed no significant differences between groups. Table 2 provides a summary of the explorative analysis of differences of the overall plaque index between groups and 95% confidence intervals of the differences. Comparing the groups in pairs also revealed no statistically significant differences.

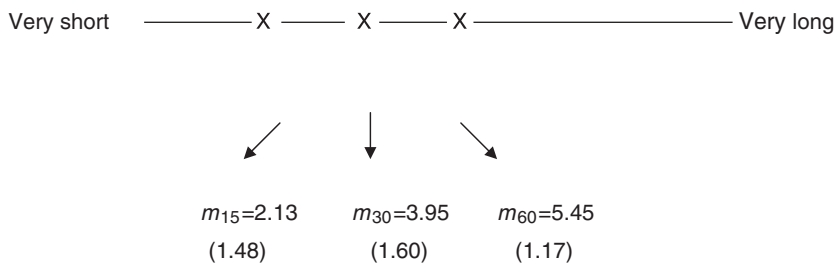
Figure 1 illustrates the results of the questionnaire. The average scores of the subjective opinions about the length of the rinsing time indicated that the 60 s CHX rinsing was considered acceptable, it being neither very short nor long (5.4). The two shorter rinsing times were perceived as such with 15 s receiving a score of 2.2 and 30 s a 3.9.

Table 1. Results of the Quigley & Hein (1962) plaque index (PI) (scale 0–5)

Rinsing time (s)	PI*
15 ( $n = 30$ )	1.33 (0.48)
30 ( $n = 30$ )	1.18 (0.41)
60 ( $n = 30$ )	1.24 (0.36)

Standard deviations in parentheses.

\*Kruskal–Wallis test,  $p = 0.5660$ .



\*Kruskal-Wallis test,  $p < 0.0005$

Fig. 1. Average results of the questionnaire present of the visual analogue scale score line with the two extremes being very short on the left and very long on the right. Standard deviations in parentheses.

Table 2. Summary of the explorative analysis of differences between the groups and the 95% confidence interval (CI) of the difference

Groups	Mean difference	SE difference	$p$ -value*	95% CI
15–30 s CHX	0.15	0.12	0.377	– 0.08–0.39
15–60 s CHX	0.09	0.11	0.102	– 0.13–0.31
30–60 s CHX	0.06	0.10	0.434	– 0.35–0.47

CHX, chlorhexidine; SE, standard error.

\*Mann–Whitney test.

Table 3. Explorative analysis of differences concerning the outcome of the questionnaire between the groups

Groups	Mean difference	SE difference	$p$ -value*
15–30 s CHX	1.82	0.40	$< 0.0005$
15–60 s CHX	3.32	0.35	$< 0.0005$
30–60 s CHX	1.50	0.36	$< 0.0005$

CHX, chlorhexidine; SE, standard error.

\*Mann–Whitney test.

Statistical analysis indicated that these scores did differ significantly (Table 3).

## Discussion

The present study was designed to determine the plaque inhibiting effect of three different rinsing times with a 0.2% CHX solution. The results showed that, starting from a plaque-free dentition, there was no significant difference in 72 h plaque development whether the subjects rinsed twice daily for 60 s, 30 s or 15 s.

Bonesvoll et al. (1974b) showed that a CHX depot is formed in the mouth during CHX mouth rinsing. This property of keeping an adequate concentration in the mouth for a prolonged period of time seems to be a factor of

importance in plaque inhibition (Gjerme et al. 1974, Bonesvoll & Gjerme 1978). Jenkins et al. (1988) suggested that the plaque inhibitory action is derived from the CHX absorbed to the tooth surface rather than its oral retention or initial bactericidal effect. It is possible that the CHX molecule attaches to pellicle by one cation, leaving the other free to interact with bacteria attempting to colonise the tooth surface. The process of plaque prevention would therefore occur at the tooth surface itself by tooth-bound CHX (Jones 1997, Addy 2003).

Bonesvoll et al. (1974a) showed that there is a rapid binding of CHX in the mouth during the first 15 s of rinsing. They observed that, compared with a 60 s rinse, approximately half of the CHX was already retained after a 15 s rinse and approximately 75% within

30 s. Although rinsing for a longer time period than 15 s does increase CHX retention, the present study has shown that this may not be necessary for the plaque inhibiting effect. Following the line of reason, as suggested by Jenkins et al. (1988) that the tooth-bound CHX is responsible for plaque growth inhibition, 15 s is enough to achieve an adequate retention of CHX onto the tooth surface.

The present results are in agreement with those of Keijser et al. (2003). In their two-group parallel design, they compared the plaque inhibiting effect of a 60 s rinse of 0.2% CHX with a 30 s rinse of 0.12% CHX during 72 h without any other form of oral hygiene. No statistically significant difference could be found between the two groups with regard to the slowing down of plaque formation on the teeth. It was concluded that, to be effective, a 30 s rinsing time is sufficient for an 18 mg dose of CHX in a 0.12% solution. The panellists appreciated the shorter rinsing time as did the panellists in the present study. The latter results emphasise the need for investigations into shorter rinsing times for effective plaque control, because a shorter rinsing time could have a positive effect on compliance.

In summary, the present study did not reveal a significant difference in plaque development whether the subjects rinsed for 60, 30 or 15 s. Further studies are needed to establish whether shorter rinsing times will be sufficient for effective plaque and gingivitis control with CHX over a longer period of use. A consideration is that a shorter rinsing time could have a positive effect on compliance.

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Address:  
 Fridus Van der Weijden  
 Department of Periodontology  
 Academic Center for Dentistry Amsterdam,  
 ACTA  
 Louwesweg 1  
 1066 EA Amsterdam  
 The Netherlands  
 Fax: +31 20 51 88 512  
 E-mail: ga.vd.weijden@acta.nl

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