The effect on early plaque formation, gingivitis and salivary bacterial counts of mouthwashes containing hexetidine/zinc, aminefluoride/tin or chlorhexidine

Arthur F. Hefti and Brigitte Huber
Department of Preventive Dentistry, Dental Institute University of Basel, Switzerland

Hefti AF and Huber B: The effect on early plaque formation, gingivitis and salivary bacterial counts of mouthwashes containing hexetidine/zinc, aminefluoride/tin or chlorhexidine. J Clin Periodontol 1987; 14: 515–518.

Abstract. This study was designed to investigate the effectiveness of mouthwashes containing hexetidine/zinc (HZA) or tin (ASF) in inhibiting plaque formation and gingivitis in humans. 24 dental students and assistants participated in the study (latin square design) with 7-day test periods. They rinsed twice daily for 1 min with one of the following formulations: HZA = 750 ppm hexetidine/750 ppm zinc acetate, ASF = 100 ppm aminefluorid/310 ppm stannous fluoride, CHX = 0.1% chlorhexidine and M = negative control. Plaque accumulation was determined planimetrically and gravimetrically. Gingivitis was evaluated with the papillary bleeding index. Total colony forming units and *S. mutans* counts were estimated from saliva samples.
The results showed that HZA and CHX almost completely inhibited plaque accumulation and gingivitis. ASF was less effective than HZA and CHX but still reduced plaque significantly compared to the negative control. Furthermore, CHX reduced salivary *S. mutans* counts.

Key words: Clinical study – cationic agents plaque reduction – S. mutans.

Accepted for publication 2 October 1986

The primary rôle of dental plaque in the development of caries, gingivitis and periodontitis has been recognized for almost 80 years. Axelsson & Lindhe (1981) showed that over a 6-year period these diseases are prevented by the adoption of adequate oral hygiene procedures. Complete plaque removal is a prerequisite to oral health, but at the same time it involves difficult and time-consuming skills. Therefore, one seeks chemical alternatives. The effectiveness of chlorhexidine (CHX) in inhibiting plaque accumulation has been extensively tested (for review see: Hull (1980), Loesche (1976), Tonelli et al. (1983)). However, untoward side-effects, e.g., staining of teeth (Eriksen et al. 1985), as well as transient modifications of the gustatory sensation (Schaupp & Wohnaut 1978), impeded a widespread application of the cationic antiseptic.

Substitutes with similar effectiveness and fewer side-effects have been proposed (Saxer & Mühlemann 1983, Southard et al. 1984). Recently, Mühlemann (1984) published observations on mouthwashes which combine metal ions with bis-pyrimidinamine (hexetidine) or diamine-HF-olaflur (aminefluoride). These solutions inhibited plaque growth as effectively as CHX and much better than hexetidine alone, aminefluoride alone and metal ions alone.

The aim of the present study was to investigate the effect on plaque formation, gingivitis development and salivary bacterial counts of mouthwashes containing hexetidine/zinc or aminefluoride/tin. This was compared with chlorhexidine and a negative control preparation.

Material and Methods

Test panel

24 dental students and assistants (9 female, 15 male) participated in the clinical study. They had full dentitions without clinically and radiographically detectable caries lesions and were without medical history. Their age ranged from 19 to 40 years. 12 subjects from the same sample volunteered for the measurement of salivary bacterial counts. All participants were informed on the purpose of the study and on the possibility of tooth staining.

Experimental design and test solutions

Prior to each test period, the participants received a thorough scaling and professional tooth cleaning in order to remove calculus, plaque and extrinsic stain. The study was designed as a double-blind latin square trial (Chilton 1955) with 7-day test periods. Thus, each mouthwash was used by each subject during 1 test period. Intervals of 3 weeks ensured that the effects from the previous treatment did not carry over

into the period of the next treatment.

The participants refrained from all mechanical oral hygiene measures during the experimental periods. Instead, they rinsed twice daily, before breakfast and after dinner. 1-min rinsings with 10 ml of the following solutions were prescribed:

- 0.1% chlorhexidine (CHX);
- 750 ppm hexetidine/750 ppm zinc acetate (HZA);
- 100 ppm aminefluoride/310 ppm stannous fluoride (ASF);
- negative control (M).

The negative control was flavoured with quinine to mimic a bitter taste and furthermore contained 0.01% saccharine and 3% alcohol in distilled water.

Papillary-Bleeding-Index (PBI)

At the beginning and the end of each test period, gingival inflammation was measured with the PBI (Saxer & Mühlemann 1975). All assessments were performed by the same examiner throughout the study.

Planimetric and gravimetric plaque determination

At the end of each test phase, plaque on the labial surfaces of 13 to 23 and 33 to 43 was disclosed with a 1% malachite green solution and photographed. The stained area was evaluated on projected slides as discribed by Plüss et al. (1975). Furthermore, plaque was carefully scrubbed from the buccal surfaces of selected teeth (16, 21, 24, 36, 41, 44). The collected material was placed onto preweighed papers, freeze-dried and then the dry-weight was determined.

Salivary bacterial counts

Immediately before the clinical examinations at the end of a test period, two 5 ml portions of paraffin-stimulated saliva were collected. The first 5 ml were discarded. Processing of the second 5 ml was performed within 3 h. The samples were dispersed for 10 s by sonication (Branson S-2 with microtip) in an ice-bath and serially diluted in 10-fold steps in phosphate buffered saline. 1 ml of selected dilutions were then mixed with 9 ml of trypticase soy agar (TSA) and plated in petri dishes. The medium was allowed to solidify before a further portion of 10 ml of agar was poured over the surface. The plates were incubated at 37°C for 72 h. For *Streptococcus mutans* counts, 0.1 ml of the dilutions were plated on Gold agar (GA, Gold et al. 1973). The plates were anaerobically incubated for 72 h at 37°C. Colonies were counted with a darkfield colony counter.

Statistics

Plaque weights, plaque areas, PBI-sums and bacterial counts were analyzed by two-way analysis of variance (ANOVA). The standard error of the mean (s_x) was calculated from the residual variation, and the Newman-Keuls sequential method was used to assign levels of significance to the differences between treatments. Bacterial counts were log-transformed because of the large variability of individual results.

Results

Effect of treatments on gingivitis

Mouthwashes CHX and HZA decreased the mean PBI-sum after 1 week of use by 16.0 and 16.1, respectively, ASF by 13.2 (Table 1). In contrast, probands on M showed a pronounced increase of 27.9 units. The effect of ASF, CHX and HZA was significantly different from M ($p<0.001$), but differences between active solutions were not significant.

Plaque formation

Planimetric evaluation (Table 2a)

The mean tooth surface covered by plaque was 26.1 mm² after 1 week of rinsing with M. HZA and CHX were equally effective (4.4 and 4.2 mm²). Both solutions inhibited plaque formation better than ASF or M ($p<0.001$).

Gravimetric evaluation (Table 2b)

The smallest mean plaque weight was measured after use of HZA or CHX. A large difference was observed between HZA, CHX on the one hand and ASF or M on the other ($p<0.001$). ASF inhibited plaque growth significantly better than M ($p<0.05$).

Table 1. Treatment effects on gingivitis. Average values from 24 PBI-sum determinations with a pooled standard error s_x

	Treatment			
	CHX	HZA	ASF	M
PBI-sum: s_x: 1.25	-16.0^a	-16.1^a	-13.2^a	27.9^b

Values with different indices are significantly different from each other ($p<0.001$)
ASF: aminefluoride/stannous fluoride.
CHX: chlorhexidine.
HZA: hexetidine/zinc acetate.
M: negative control.

Table 2. Treatment effects on (a) average plaque extent measured planimetrically on front teeth and (b) average plaque dry weights

	Treatment			
	CHX	HZA	ASF	M
(a) surface (mm²): s_x: 1.1	4.4^c	4.2^c	13.5^b	26.1^a
(b) weight (mg): s_x: 0.072	0.595^c	0.259^d	2.027^b	2.788^a

Plaque was collected on buccal surfaces of 16, 21, 24, 36, 41, 44. Average values from 24 subjects with a pooled standard error s_x. *Values with different indices are significantly different from each other ($p<0.05$) (for abbreviations see Table 1).*

Table 3. Treatment effects on salivary bacterial counts (log-transformed) after a 7-day rinsing period

	Treatment			
	CHX	HZA	ASF	M
Total colony forming units (CFU)				
log CFU: s_x: 0.187	6.90^b	6.32^a	7.13^b	7.27^b
S. mutans				
log CFU: S_x: 0.278	4.11^a	4.97^b	5.07^b	5.15^b

Average values of 12 determinations with a pooled standard error s_x. *Values with different indices are significantly different from each other ($p<0.05$) (for abbreviations see Table 1).*

S. ivary bacterial counts (Table 3)

Rinses with HZA were associated with a lower CFU number than with the negative control M. CHX and ASF had a weak effect on total CFU, but the difference was statistically not significant.

A significant difference in *S. mutans* levels was observed between subjects rinsing with CHX and other mouthwashes. In contrast, *S. mutans* counts were not affected by HZA or ASF.

Discussion

The results of this clinical study indicate that mouthwashes containing hexetidine and zinc acetate (HZA) or amine-fluoride and stannous fluoride (ASF) were effective in inhibiting the accumulation of plaque and the development of gingivitis over a 7-day experimental period. At a frequency of 2 rinses per day, the effect of HZA was comparable with that of a 0.1% chlorhexidine solution. This finding is in agreement with an observation of Saxer & Mühlemann (1981), who compared the antiplaque effect of stannous fluoride, hexetidine and a combination of stannous fluoride and hexetidine with a fluoride control mouthwash. They found a 90% plaque area reduction in volunteers refraining from toothbrushing, but rising 3 times per day for 1 week. This value is in the same order of magnitude as that we calculated from Table 2.

The effect on plaque formation of ASF was significantly smaller as compared to CHX and HZA, which was particularly evident from the determination of plaque dry-weights. The finding contrasts with a report of Saxer & Mühlemann (1983) who found a better plaque inhibition with ASF than with 0.1% CHX. The discrepancy may be attributed to the mouthwashes, which differed slightly in their compositions.

A pronounced gingivitis reduction was observed in the ASF-group, although the antiplaque effect of the mouthwash was rather weak. The anti-inflammation activity may be explained by the adstringent effect on mucosal tissues of metal salts (Laghi 1951).

The efficacy of chlorhexidine mouthwashes is dependent on the concentration, volume, duration of exposure and frequency of rinses per day. A 0.2% solution used twice daily for 30 s is accepted in order to inhibit plaque growth almost completely, but a 0.1% solution was shown to be almost equally effective when the rinsing time and volume were increased (Cumming & Löe 1973). In the present study, an almost complete elimination of plaque was achieved with the lower CHX concentration.

Longitudinal studies which assessed plaque bacteria from tooth surfaces (Hardie et al. 1977) or from saliva (Klock & Krasse 1979), associated *S. mutans* with coronal caries development. With respect to the specific plaque bacteria hypothesis as formulated by Loesche (1979), a mouthwash should reduce the number of *S. mutans* below a critical threshold level. Results from an in vitro study (Mörmann & Mühlemann 1983) suggested a specific effect of HZA on *S. mutans*. This, however, was not confirmed by the present in vivo study. Only CHX significantly diminished the absolute as well as the relative (data not shown) number of *S. mutans* bacteria in saliva. This observation adds to earlier studies which successfully investigated the use of CHX in reducing *S. mutans* levels in plaque or saliva (Zickert et al. 1982, Köhler et al. 1983).

Yellow-brown tooth staining was common with all active rinsing solutions in this study. This side-effect is well-known and may be attributed to the cationic nature of the antiseptics (Eriksen et al. 1985).

Acknowledgment

This work was supported in part by GABA AG/Ltd., Therwil (Switzerland).

Zusammenfassung

Der Effekt von Mundspülungen mit Hexetidin/Zink, Aminfluorid/Zinn oder Chlorhexidin auf die frühe Plaquebildung, die Gingivitis und die Gesamtkeimzahl

Ziel dieser Studie war, den Einfluss von Mundspülungen mit Hexetidin/Zink und Aminfluorid/Zinn auf Plaquebildung, Gingivitis und Keimzahl im Speichel zu untersuchen. 24 Probanden nahmen am Versuch teil. Während der 7-tägigen Testperioden spülten sie zweimal täglich während 1 Minute mit folgenden Lösungen: HZA = 750 ppm Hexetidin/750 ppm Zinkacetat, ASF = 100 ppm Aminfluorid/310 ppm Zinnfluorid, CHX = 0.1% Chlorhexidin und M = Negativkontrolle. Die Plaquebildung wurde planimetrisch und gravimetrisch, Gingivitis mit dem Papillen-Blutungs-Index untersucht. Aus Speichelproben wurde die Gesamtkeimzahl sowie *S. mutans* bestimmt.

Die Resultate ergaben eine starke Hemmung der Plaquebildung und Gingivitis durch HZA und CHX. ASF wirkte weniger ausgeprägt doch signifikant besser als die Negativkontrolle. CHX reduzierte die *S. mutans* Kolonienzahl.

Résumé

Effets de bains-de-bouche contenant de l'hexetidine/zinc, du fluorure d'amines/étain

References

Axelsson, P. & Lindhe, J. (1981) Effect of controlled oral hygiene procedures on caries and periodontal disease in adults – results after 6 years. *Journal of Clinical Periodontology* **8**, 239–248.

Chilton, N. W. (1955) The latin square design in clinical experimentation. *Journal of Dental Research* **34**, 421–428.

Cumming, B. R. & Löe, H. (1973) Optimal dosage and method of delivering chlorhexidine solutions for the inhibition of dental plaque. *Journal of Periodontal Research* **8**, 57–62.

Eriksen, H. M., Nordbø, H., Kantanen, H. & Ellingsen, J. E. (1985) Chemical plaque control and extrinsic tooth discoloration. A review of possible mechanisms. *Journal of Clinical Periodontology* **12**, 345–350.

Gold, O. G., Jordan, H. V. & Houte, J. van (1973) A selective medium for *Streptococcus mutans*. *Archs oral Biol.* **18**, 1357–1364.

Hardie, J. M., Thomson, P. L., South, R. J., Marsh, P. D., Bowden, G. H., McKee, A. S., Fillery, D. & Slack, G. L. (1977) A longitudinal epidemiological study on dental plaque and the development of caries – interim results after two years. *Journal of Dental Research* **56**, 90–98.

Hull, P. S. (1980) Chemical inhibition of plaque. *Journal of Clinical Periodontology* **7**, 431–442.

Klock, B. & Krasse, B. (1979) A comparison between different methods for prediction of caries activity. *Scandinavian Journal of Dental Research* **87**, 129–139.

Köhler, B., Bratthall, D. & Krasse, B. (1983) Preventive measures in mothers influence the establishment of the bacterium *Streptococcus mutans* in their infants. *Archives of Oral Biology* **28**, 225–231.

Laghi, C. (1951) Die Wirkung einiger Ätzmittel auf die Gingiva. *Med. Diss.* Zürich.

Loesche, W. J. (1979) Clinical and microbiological aspects of chemotherapeutic agents used according to the specific plaque hypothesis. *Journal of Dental Research* **58**, 2404–2412.

ou de la chlorhexidine sur la formation de la plaque dentaire, la gingivite et le nombre de bactéries salivaires

La présente étude a été entreprise pour mesurer l'efficacité de bains-de-bouche contenant de l'hexetidine/zinc (HZA) ou du fluorure d'amines/étain (ASF) à inhiber la formation de la plaque et la gingivite chez l'humain. Vingt-quatre étudiants et assistants en médecine dentaire ont participé à cette étude comprenant des périodes tests de sept jours. Ils se rinçèrent la bouche deux fois par jour pendant une minute avec une des solutions suivantes: HZA: 750 ppm hexetidine/750 ppm acétate de zinc, ASF: 100 ppm fluorure d'amines/310 ppm fluorure d'étain, CHX: 0.1% chlorhexidine (contrôle positif) et M: contrôle négatif. L'accumulation de plaque a été déterminée planimétriquement et gravimétriquement, et la gingivite par l'indice de saignement papillaire. Le nombre total d'unités formant une colonie ainsi que le nombre de *S. mutans* one été calculés dans les échantillons salivaires.

Les résultats ont montré que l'HZA et la CHX inhibaient presque complétement l'accumulation de plaque et la gingivite. L'ASF était moins efficace que l'HZA et la CHX mais réduisait néanmoins significativement la plaque par rapport à la solution contrôle négatif. La CHX avait également réduit le nombre de *S. mutans* salivaires.

Mörmann, J. E. & Mühlemann, H. R. (1983) Synergistic inhibitory effect of zinc and hexetidine on in vitro growth and acid production of *Streptococcus mutans*. *Journal of Dental Research* **62**, 135–137.

Mühlemann, H. R. (1984) 25 Jahre Schweizer Plaque-Pharmakologie. *Acta Parodontologica* **13**, 21–31.

Plüss, E. M., Engelberger, T. R. & Rateitschak, K. H. (1975) Effect of chlorhexidine on dental plaque formation under periodontal pack. *Journal of Clinical Periodontology* 2, 136–142.

Saxer, U. P. & Mühlemann, H. R. (1975) Motivation und Aufklärung. *Schweizerische Monatsschrift für Zahnheilkunde* **85**, 905–919.

Saxer, U. P. & Mühlemann, H. R. (1981) Plaque-inhibiting effect of zinc ions and fluoride, hexetidine and their combinations. *Zahn-, Mund- und Kieferheilkunde* **69**, 730, abstract 28th ORCA-Congress.

Saxer, U. P. & Mühlemann, H. R. (1983) Synergistic antiplaque effects of a zinc fluoride/hexetidine containing mouthwash. A review. *Helvetica odontologica acta* **27**, 1–16.

Schaupp, H., Wohnaut, H. (1978) Geschmacksstörungen durch Mund-desinfizientien. *HNO* **26**, 335–341.

Southard, G. L., Boulware, R. T. B., Walborn, D. R., Groznik, W. J., Senior, S. L. & Yankell, S. L. (1984) Sanguinarine – a new antiplaque agent. In: Cohen, Spec. Suppl. no 5, *Continuing Education*, pp. 72–75.

Tonelli, P. M., Hume, W. R. & Kenney, E. B. (1983) Chlorhexidine: A review of the literature. *The Journal of the Western Society of Periodontology* **31**, 5–10.

Zickert, I., Emilson, C. G. & Krasse, B. (1982) Effect of caries preventive methods in children highly infected with *S. mutans*. *Archives of Oral Biology* **27**, 861–868.

Address:

Arthur Hefti
Zahnärztliches Institut
Abteilung für Präventivzahnmedizin
CH-4051 Basel
Switzerland