Plaque inhibiting effect of combinations of chlorhexidine and the metal ions zinc and tin

A preliminary report

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Rinsing experiments with combinations of chlorhexidine, zinc- and stannous ions were performed in a group of students.

Chlorhexidine and zinc (0.2 % and 0.3 % respectively) gave a plaque inhibiting effect slightly better than chlorhexidine alone, whereas chlorhexidine and stannous ions in the same concentrations showed a reduced effect, presumably due to the low pH of the solution.

Pre-rinses with metal ions caused a marked reduction in the effect of chlorhexidine, whereas chlorhexidine first and metal ions afterwards gave approximately the same effect as chlorhexidine alone. The results indicate that the metal ions and chlorhexidine have the same receptor sites in the oral cavity, chlorhexidine exhibiting the stronger affinity when the ions were applied together.

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Chlorhexidine has a well established antiplaque effect, and the mechanisms involved are fairly well known (5). Chlorhexidine is retained on the oral mucosa for a prolonged period of time after a mouthrinse, and the slow release of the drug molecules is thought to adsorb to the oral microorganisms and reduce their metabolic activity and presumably interfere with their binding mechanisms to teeth. The retention of chlorhexidine in the mouth is electrostatic in nature, and it is assumed that carboxyl groups are essential (1, 3, 6).

More recently it has been shown that metal ions like the stannous and zinc ions exhibit plaque inhibiting activites (8, 11). At least the stannous ions are retained in the mouth in the same way as chlorhexidine, and it seems likely that this is also the case with zinc ions, even if this has not been directly demonstrated (2, 10).

The aim of the present study was to investigate whether any synergistic or antagonistic effects are observed when chlorhexidine and metal ions are applied together or in succession. Such data may be of practical interest, and can also give further information as to the retention mechanisms of metal ions and chlorhexidine.

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MATERIALS AND METHODS

Chlorhexidine-zinc combinations (Group 1)

Six dental students volunteered for the study. Prior to each experimental period, they all had their teeth thoroughly cleaned by the examiner (S.M.W.). During each test period (4 days), no mechanical oral hygiene procedures were allowed, and plaque formation was enhanced by sucrose rinses (1 minute rinse with 10 ml 15 % sucrose, 4 times daily) at least ½ hour prior to, or after, rinsing with the test solution(s). The test substanses were applied as 10 ml mouthrinses for 1 minute, twice daily – in a specific order, or in a combination (see Table 1). When two different test solutions were used, the second one followed *immediately* after the first one. At the end of each experimental period, the plaque score was evaluated by the examiner – according to the Plaque Index (Pl. I) (7). The study was carried out as a double blind test. The students were allowed a period of at least 3 days of habitual oral hygiene between each experimental period, to minimize possible carry-over effects.

The acetate salt of zinc was used because this salt is soluble in water, and the acetate ion has a low affinity for chlorhexidine and does not form any precipitates in the concentrations used.

Chlorhexidine-tin combinations (Group 2)

The experiment was carried out in the same way as the one described above, except that stannous ions were used instead of zinc. Because tin is known to have a higer effect than zinc, a fact based on data from relevant literature (8), the consentration of chlorhexidine for this study was 0.1 % to ensure a more sensitive system. Fluoride was used in combination with the stannous ion, because SnF_2 has a high solubility and fluoride does not react with chlorhexidine (4). The participants did not know which solutions were used for the different periods of the study.

Statistical analyses

In both groups statistical analyses of the results was carried out using the students' t-test for paired data (significance level: $p < 0.05$).

RESULTS

Chlorhexidine-zinc combination (Group 1)

The results of rinsing with different combinations of 0.2 % chlorhexidine and 0.3 % zinc acetate are given in Table 1.

Marked plaque inhibition was obtained when using chlorhexidine only, or when chlorhexidine was applied prior to zinc acetate. When the combination of chlorhexidine and zinc-acetate was used, an even stronger effect was observed. When used alone, zinck acetate, showed no statistical difference compared to the placebo solution.

Chlorhexidine-tin combinations (Group 2)

The results of rinsing with different combinations of 0.1 % chlorhexidine and 0.3 % stannous fluoride are given in Table 2.

Chlorhexidine and stannous fluoride both showed marked plaque inhibition when used separately, although stannous fluoride had less effect than chlorhexidine, as could be expected from previous studies (9). Similar results were found when chlorhexidine was used alone or prior to stannous fluoride. There was, however, a marked reduction in effect when stannous fluoride was used prior to chlorhexidine. When the combination of stannous fluoride and chlorhexidine was

Table 1. *Plaque Index values after rinsing with different test solutions*

Test persons	15 % sucrose 0.2 % CH	15 % sucrose 0.2 % CH 0.3 % Znac	15 % sucrose 0.3 % Znac 0.2 % CH	15 % sucrose 0.3 % Znac + 0.2 CH	15 % sucrose 0.3 % Znac	15 % sucrose 0.2 % NaCl
P.O.	0.23	0.10	0.73	0.07	0.63	1.64
K.A.S.	0.04	0.03	0.70	0.03	1.23	1.23
O.R.	0.20	0.25	0.74	0.16	0.94	1.63
K.E.O.	0.01	0.13	0.96	0.02	0.67	1.15
J.I.H.	0.12	0.25	1.09	0.05	– *	–
K.N.	0.12	0.06	1.44	0.07	– *	–
Mean	0.12	0.14	0.94	0.07	0.87	1.41
s.d.	0.09	0.09	0.29	0.05	0.27	0.26

* = Two students withdrew from the experiment.

Table 2.

Test persons	15 % sucrose 0.1 % CH	15 % sucrose 0.1 % CH 0.3 % SnF_2	15 % sucrose 0.3 % SnF 0.1 % CH	15 % sucrose 0.3 % SnF + 0.1 % CH	15 % sucrose 0.3 % SnF_2	15 % sucrose 0.2 % Nacl
B.C.	0.33	0.44	0.73	0.62	0.70	0.97
D.M.	0.17	0.16	0.29	0.44	0.63	1.27
M.C.	0.15	0.09	0.56	0.60	0.69	1.09
N.S.	0.24	0.13	0.56	0.48	1.08	1.19
S.R.	0.13	0.10	0.12	0.38	0.25	0.68
A.E.	0.28	0.22	0.71	0.82	0.81	1.08
K.R.	0.18	0.23	– *	–	–	–
Mean	0.21	0.20	0.50	0.56	0.69	1.05
s.d.	0.07	0.12	0.24	0.16	0.27	0.21

* = One participant withdrew from the experiment

used, the plaque inhibiting effect was reduced compared to using chlorhexidine only. Compared to the placebo rinse, all the different combinations had a statistically significant plaque inhibiting effect.

DISCUSSION

The present study showed that both chlorhexidine and stannous fluoride have a plaque inhibiting effect – and the results were similar to those previously reported (9). Zinc-acetate also exhibited some plaque inhibiting effect, although these values did not reach statistical significance at the 5% level. However, Skjørland et al. (1977) did show an effect when using zinc ions as a mouthrinse.

When using chlorhexidine only, or when using it prior to zinc-acetate, no statistical differences were observed. Similar results were shown in group 2, using stannous fluoride. However, when comparing chlorhexidine and a combination of chlorhexidine and the test substance, the results varied in the two groups. In group 1, the effect seemed to be enhanced with the combination compared with chlorhexidine by itself (although not statistically significant), while when using stannous fluoride, the effect was reduced. This may be explained by the fact that a 0.3% aqueous solution of stannous fluoride has a pH of 3.2. It has previously been reported that the clinical effect of chlorhexidine is markedly reduced at pH 3 (1). A competition between these two ions for the same receptor sites may also be a conceivable interpretation of our observations.

A 0.3% aqueous solution of zinc acetate has a pH of 6.3, which explains the difference between zinc and tin, as mentioned above. Combined with zinc the chlorhexidine exhibited its full effect, and in addition there was some effect which may depend on extra receptor sites for the zinc ions, not available to the chlorhexidine.

Another interesting finding was that zinc acetate or stannous fluoride used prior to chlorhexidine reduced the plaque inhibiting effect markedly compared to using the different solutions in the opposite order. The effect was close to that of metal-salts alone. These findings suggest that chlorhexidine and the divalent cations generally have the same receptor sites, and whichever substance used first is presumably blocking the receptors for the following substance.

Since the results were not identical when using a test substance by itself or as the first one in a sequence, it may be speculated that there are other specific receptor sites, not equally available to both substances. This specificity may be chemical or steric. The differences observed may be explained by postulating a model where all substances have affinity for one receptor site, but where this reseptor site is available to a higher degree for the small metal ions compared with the considerably larger chlorhexidine ions.

CONCLUSION

The present study showed that a synergistic effect was observed when using zinc ions and chlorhexidine together, while an antagonistic effect was observed when combining stannous ions and chlorhexidine. This latter effect was presumably caused by the low pH of SnF_2-solutions, reducing the retention of chlorhexidine in the mouth (1).

Rinsing with the chlorhexidine and the metal ions, in that sequence, no difference was observed from using chlorhexidine alone, while reversing the sequence lowered the effect markedly. This shows that the effect depends on which substance was used first, as they conceivably

competed for the same receptor sites. These findings indicate that chlorhexidine has the stronger affinity for these receptor sites, since when used in combination with zinc, the effect is on the same level as with chlorhexidine alone.

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