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Comparative effects of different chlorhexidine mouth-rinse formulations on volatile sulphur compounds and salivary bacterial counts

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Abstract

Aim: To compare five different commercial mouth rinses with chlorhexidine (CHX) with respect to their anti-halitosis effect and anti-microbial activity on salivary bacterial counts, following a standardised research protocol. And secondly, to validate the study model proposed in the evaluation of patients suffering from halitosis.

Patients and Methods: Ten volunteers, with a healthy oral status, were enrolled in a double-blind, cross-over design, using sterile saline as negative control and five CHX-containing mouth rinses: 0.12% CHX alone (CHX+NO), plus alcohol (CHX+ALC), plus 0.05% cetylpiridinium chloride (CHX+CPC), plus sodium fluoride (CHX+NaF), and 0.05% CHX plus 0.05% CPC, plus 0.14% zinc lactate (CHX+Zn). The levels of whole-mouth volatile sulphur compounds (VSCs) were measured by means of a sulphide monitor at baseline, 1 and 5 h after rinsing with the assigned product. Baseline measurements also included an organoleptic assessment and the recording of the tongue-coating index. Aerobic and anaerobic salivary bacterial counts were also obtained by collecting unstimulated saliva samples at the same evaluation times, and processed by culturing techniques. Analysis of variance was used to evaluate whether significant differences existed among groups, at each evaluation point, or in changes between evaluations.

Results: No significant differences were detected at baseline, with VSC levels ranging between 190 and 227 parts per billion (p.p.b.) After rinsing, VSC levels were reduced with all products (except saline), after 1 h. Significant differences at 1 h were detected ($p = 0.04$), corresponding to a lower amount of p.p.b. (109) in (CHX+Zn) as compared with the other groups (except CHX+NO). At 5 h, VSC levels were lower for CHX+CPC and CHX+Zn (155 and 169, respectively), while the other groups showed levels higher than 220 p.p.b. With respect to aerobic salivary bacterial counts, CHX+CPC demonstrated the lowest percentage of survival (6% after 1 h and 18% after 5 h). For anaerobic bacterial counts, again CHX+CPC demonstrated the lowest percentage of survival (10% at 1 h and 23% at 5 h), together with CHX+ALC (18% of survival at 5 h). However, salivary counts and VSCs were only significantly correlated at baseline, but not after treatment.

Conclusion: Important differences can be expected from different CHX formulations, in relation to both their anti-halitosis effect and anti-microbial activity in saliva. Formulations that combine CHX and CPC achieved the best results, and a formulation combining CHX with NaF resulted in the poorest.

Key words: alcohol; cetylpyridinium chloride; chlorhexidine; halitosis; mouth rinses; salivary bacteria; volatile sulphur compounds

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Oral malodour, also called halitosis, is defined as a foul or offensive odour emanating from the oral cavity. Recent epidemiological studies have shown that the majority of halitosis cases have its origin in the oral cavity (Delanghe et al. 1999). The aetiology of oral halitosis has been mainly related to the putrefactive activities of Gram-negative anaerobic bacteria (Yaegaki & Sanada 1992, Scully et al. 1997, Loesche 1999, Ratcliff & Johnson 1999), in particular, the bacterial degradation of sulphur-containing amino acids such as methionine, cystine and cysteine, into volatile sulphur compounds (VSCs), mostly hydrogen sulphide, methyl mercaptan, and to a lower extent, dimethyl sulphide. This effect has been demonstrated by several authors by allowing the incubation of different microbial species in saliva and testing their capacity to produce VSC in vitro (saliva incubation test). Results from these studies indicated that whereas Gram-positive bacteria produce little or no malodour, most Gram-negative bacteria were potent producers of odoriferous compounds (Tonzetich & Carpenter 1971, Persson et al. 1990, Persson 1992, Kleinberg & Codipilly 1997, Niles & Gaffar 1997). Hence, oral micro-organisms, especially those derived from the Gram-negative anaerobic flora, are the main source of halitosis (McNamara et al. 1972).

To target these micro-organisms and thus to treat oral malodour, different topical anti-microbial agents have been used. Anti-bacterial compounds such as chlorhexidine (CHX), cetylpyridinium chloride (CPC), triclosan, essential oils, chlorine dioxide, zinc salts, hydrogen peroxide and sodium bicarbonate have been tested, either alone, in different combinations or together with the use of mechanical devices, for their efficacy to reduce oral malodour. These anti-microbial agents have been evaluated for their efficacy in the treatment of oral halitosis, either being vehicled in dentifrices (Brunette et al. 1998, Gerlach et al. 1998, Niles et al. 1999, Sharma et al. 1999, Olshan et al. 2000), or in mouth rinses (Pitts et al. 1981, 1983, Rosenberg et al. 1992, Bosy et al. 1994, De Boever & Loesche 1995, Giani et al. 1996, Kozlovsky et al. 1996, Frascella et al. 1998, Quirynen et al. 1998, Frascella et al. 2000, Suarez et al. 2000, van Steenberghe et al. 2001, Roldán et al. 2003, Winkel et al. 2003), using different study designs. However, most of this research assessed the short-term efficacy, because only one clinical study evaluated their long-term efficacy (more than 6 weeks) (Kozlovsky et al. 1996), and only a limited number of these studies have been designed as double-blind placebo-controlled randomised clinical trials (Roldán et al. 2003, Winkel et al. 2003).

CHX being the most studied anti-microbial agent in the treatment of gingivitis has also been tested for its efficacy in the treatment of oral halitosis. Results from a case series study in halitosis patients suggested a significant effect of CHX rinsing and tongue brushing after 1 week of treatment (Bosy et al. 1994, De Boever & Loesche 1995). More recently, a mouth-rinse formulation combining a low-dose CHX mouth rinse, with CPC and zinc lactate, was assessed as sole treatment, in a double-blind randomised placebo-controlled clinical trial with 40 halitosis patients. The results after 15 days demonstrated its efficacy by significantly reducing the halitosis outcome variables (Roldán et al. 2003, Winkel et al. 2003).

The lack of similar clinical trials studying the efficacy of the anti-halitosis effect of different anti-microbial compounds and the difficulties in gathering well-defined sample populations of pure halitosis patients advises the search of valid study models to assess the anti-halitosis effect of different products. In a manner similar to those models aimed at predicting plaque inhibitory and anti-plaque activities (Addy & Moran 1997). van Steenberghe et al. (2001) have developed the morning-breath model and have tested different CHX-based products in healthy volunteers after 12 days of plaque accumulation. However, it is not clear whether this ''morning-breath'' model is a good model to test oral halitosis.

We have proposed a study design based on the evaluation of salivary bacterial counts after a single use of the tested products, in a cross-over design (Elworthy et al. 1996, Addy et al. 1997). This method allows the evaluation of the anti-microbial activity in vivo (Addy & Moran 1997), the substantivity (Roberts & Addy 1981), the plaque inhibitory activity (Schiött et al. 1970) and, by adding the evaluation of VSC, it could be useful to predict the anti-halitosis capacity, in a morning-breath model.

The purpose of this investigation was to compare different commercial CHX-based products by testing their efficacy in reducing VSCs in mouth air, and aerobic and anaerobic salivary bacterial counts, by means of this proposed study model. As the second objective we aimed to assess the validity of this model.

Patients and Methods

Five commercial mouth rinses containing CHX were tested (Table 1). These products differed not only in the CHX concentration but also in the addition of other active ingredients, such as CPC, alcohol, sodium fluoride (NaF) or zinc lactate (Zn). Sterile saline was used as negative control.

Subjects

Ten students from the Faculty of Odontology in Madrid volunteered to participate in this study. Their age ranged between 25 and 40 years. All subjects underwent an oral examination before the study, including full-mouth periodontal probing and a caries evaluation. They all showed an overall healthy mouth status. They were selected to participate in the study after fulfilling two types of requirements.

Table 1. Commercial products tested in the study, with the main active ingredients and the acronyms used to identify them

Active agents	Commercial name	Acronym
0.12% CHX, 5% alcohol	PerioAid®*	CHX+ALC
0.12% CHX, 0.05% CPC	Perio-Aid tratamiento®*	CHX+CPC
0.12% CHX, NaF	Cariax Gingival®†	CHX+NaF
0.12% CHX	Clorhexidina Lacer®‡	CHX+NO
0.05% CHX, 0.05% CPC, 0.14% Zn	Halita®*	CHX+Zn

CHX, chlorhexidine; ALC, alcohol; CPC, cetylpiridinium chloride.
*Dentaid, Cerdanyola, Barcelona, Spain.
†Kin SA, Barcelona, Spain.
‡Lacer SA, Barcelona, Spain.

Pre-study criteria

- No antibiotic intake in the month prior to the beginning of the study.
- No antiseptic mouth rinse use in a week before the beginning of the study and during the study period.
- Subjects agreed to abstain from any oral hygiene measures during the morning throughout the investigation period.

All participating subjects agreed to comply with the following instructions during the assessment period:

- Avoid ingestion of food and liquids (except water). However, they were allowed to have breakfast in the morning immediately before the evaluation period.
- Refrain from any oral hygiene measures.
- Avoid the use of chewing gums or sweets.

Study design

The study was a randomised, double-blind (subjects, supervisor and laboratory staff), cross-over design. The washout period, between evaluations, was at least 1 week based on similar studies evaluating different CHX formulations (Addy et al. 1991, Harper et al. 1995). During the assessment periods, each subject used all test products and the negative control, in a randomised order, according to a computer-generated list. An external agent codified all the products in identical 15 ml bottles.

The assessment period started by obtaining saliva samples in the morning (at approximately 8.25 h). Subsequently, baseline halitosis measurements were registered, including organoleptic assessment of whole-mouth air, evaluation of the Winkel tongue-coating index (WTCI) and measurement of VSCs by means of Halimeter® (Interscan Corporation, Chatsworth, CA, USA). Subjects were then asked to rinse (not to gargle) with their assigned product under supervision for 1 min. Additional saliva samples were collected after 5 min and 1, 3 and 5 h, while new evaluations of VSC levels were rendered after 1, 3 and 5 h. The assessment period ended at 13:30 hours after the completion of the 5 h evaluation.

Halitosis outcome variables

Whole-mouth air was assessed organoleptically using a 0–5 scale (Rosenberg et al. 1991a, b), after 1 min of mouth closure, by two trained examiners located at a distance of 10 cm from the subject's mouth. Subsequently, the tongue coating was evaluated by means of the WTCI (Winkel et al. 2003) dividing the tongue in sextants, and every sextant was scored from 0 to 2. Finally, VSC levels in mouth air were assessed in parts per billion (p.p.b.), using a portable sulphide compound detector (Halimeter®, Interscan Corp., Chatsworth, CA) according to the following protocol. After an additional minute of mouth closure, 3 cm of a disposable straw was placed on the dorsal surface of the tongue and two consecutive measurements were obtained; the mean value was used for data analysis.

Saliva samples

Unstimulated saliva samples were obtained by requesting the volunteers to spit into a graded test tube (approximately 1 ml of saliva). Samples were processed in the laboratory within 30 min, following normal bacteriological procedures of dispersion (vortexing, 30 s), serial dilution in PBS and inoculation on two series of non-selective 5% horse blood agar plates (Oxoid no. 2, Oxoid Ltd., Basingstoke, UK) supplemented with haemin (5 mg/l) and menadione (1 mg/l). One series was incubated in air at 37°C for 24 h, and the other in an anaerobic atmosphere for 48 h. After the incubation period, counting was performed on the most suitable plates (those with 30–300 colonies).

Data analyses

VSC levels and total bacterial counts (in colony-forming units (CFU) per ml) for each sampling time and product were log transformed and averaged. Differences between two visits were calculated for VSC values, whereas for bacterial counts the percentage of survival was calculated by dividing CFU at 1 and 5 h, by CFU at baseline.

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed among groups with regard to: VSCs and log of bacterial counts for each evaluation time; changes in VSCs between evaluations; and percentages of survival for the different tested products. A multiple range test was used to identify the differences detected by the previous test. If skewness and kurtosis values were beyond normality, or significant differences in the variance were detected, a Kruskal–Wallis test was used instead. Previously, the components of the variance were analysed by the ANOVA test, and the possible influence of the baseline variables on the results was also evaluated. It was observed that baseline microbiological counts had an influence on microbiological results, but using the variable ''percentage of survival'' instead of bacterial counts to evaluate changes we controlled this.

Correlation coefficients were calculated by simple regression, in an attempt to assess the association between salivary bacterial counts and VSC levels, both at each evaluation time and between evaluations, as well as the association among VSC, organoleptic scores and WTCI at baseline.

The level of significance selected was $p < 0.05$.

In order to validate the use of a 7-day washout period for the assessment of halitosis parameters, ANOVA was used to compare the results among subjects with different order of product usage. The results showed no differences, which lead us to assume that the selected washout period was long enough.

Results

Baseline organoleptic scores were relatively low in all groups, with mean values ranging from 1.4 to 1.9 (standard deviation, SD = 0.5–0.8). No significant differences among the groups were detected.

Baseline VSC values ranged from 190.1 to 227 p.p.b. (Table 2). No significant differences were found among groups at baseline.

Baseline WTCI scores showed more heterogeneity, ranging from 2 to 10. The mean values significantly differed among the groups ($F = 2.54$, $p = 0.04$). The group CHX+CPC showed significantly lower WTCI scores at baseline (4.7, SD = 1.4), than the other groups, except CHX+NaF (5.2, SD = 1.4). This group also demonstrated a significantly lower WTCI score than CHX+NO. In the other groups, the mean WTCI score ranged from 6.3 to 6.9. However, the influence of the subject on the WTCI score was higher than the influence of the group, because highly significant differences were detected among subjects ($F = 3.25$, $p = 0.004$).

Table 2. VSC (mean and standard deviation (SD)) values per group and evaluation time

	VSC-baseline		VSC-1h		VSC-5h	
	mean	SD	mean	SD	mean	SD
CHX+ALC	227.0	71.3	196.1	43.8	221.9	50.4
CHX+NO	202.0	61.8	146.2	71.1	223.6	77.6
CHX+NaF	202.0	86.4	163.7	75.0	234.1	83.1
CHX+CPC	200.1	53.3	185.8	50.4	155.2	35.3
CHX+Zn	190.1	65.0	109.5	67.3	168.7	61.8
saline	217.6	146.2	230.0	155.1	236.2	159.2

VSC, volatile sulphur compounds; CHX, chlorhexidine; ALC, alcohol; CPC, cetylpiridinium chloride.

No correlation was observed between VSC and WTCI. By contrast, a statistically significant correlation was found between VSC and organoleptic scores ($r = 0.33$, $p = 0.01$).

Fig. 1 shows the variation in VSC levels throughout the assessment period. One hour after rinsing, significant differences were detected among groups ($F = 2.47$, $p = 0.04$), corresponding to a lower VSC value for the CHX+Zn group when compared with all the other groups, with the exception of the CHX+NO group. After 5 h, the scores for the CHX+Zn group were still significantly different (as detected by the multiple rank test (MRT)) than those of CHX+ALC, CHX+NO and CHX+NaF (Table 2).

When changes in VSC levels between baseline and 1 h were compared, the reduction of VSC in the CHX+Zn was significantly (MRT) higher than in the saline, CHX+NaF and CHX+ALC groups. From 1 to 5 h, all groups showed an increase in VSC values. In the overall change between baseline and 5 h, three groups showed a reduction (CHX+Zn, CHX+CPC and CHX+ALC), while the others showed an increase. The only significant (MRT) difference was observed between CHX+CPC and CHX+NaF.

Fig. 2 shows the evolution of the aerobic log-CFU bacterial counts in saliva.

At baseline, the log-CFU of saline and CHX+NaF groups were significantly (MRT) higher than those corresponding to CHX+ALC. At 1 h, reductions occurred in all groups. Differences among groups were significant ($F = 4.87$, $p = 0.001$), saline counts being significantly higher than the rest of the groups. Among the CHX groups, only CHX+NaF showed significantly higher counts than CHX+CPC.

At 5 h, the mean bacterial counts increased in all groups. However, significant differences were still detected between the saline and the rest of the groups ($F = 3.22$, $p = 0.01$). Among the CHX groups, the CHX+NaF showed significantly higher counts than CHX+CPC and CHX+Zn.

Changes between baseline and 1 h, calculated as percentage of survival at 1 h, were significantly different among groups (K–W = 13.29, $p = 0.02$). CHX+CPC demonstrated a percentage of survival (6.3%, SD = 9%) significantly lower than those of CHX+ALC (32.4%, SD = 37%) or saline (38.5%, SD = 16%). At 5 h, the percentage of survival ranged between 18.1% and 50.2%. Statistically significant (MRT) differences were detected between saline (50.2%, SD = 22%), and CHX+NO and CHX+CPC (18.1%, SD = 10%; and 18.4%, SD = 22%, respectively).

Fig. 3 shows the evolution of the anaerobic log-CFU bacterial counts in saliva.

At baseline, the log-CFU of saline was significantly (MRT) higher than those of CHX+ALC, and CHX+Zn groups. At 1 h, reductions occurred in all groups. Differences among groups were significant ($F = 8.37$, $p = 0.001$), saline counts being significantly higher than the rest of the groups. Among the CHX groups, CHX+NaF counts were higher than counts in CHX+CPC and CHX+ALC groups. CHX+NO showed significantly higher bacterial counts than CHX+CPC. At 5 h, the mean bacterial counts increased in all groups. The CHX+NaF showed significantly higher counts than the other CHX groups ($F = 5.65$, $p < 0.001$).

Changes between baseline and 1 h, calculated as percentage of survival at 1 h, ranged between 10.6% and 28.4% for all the CHX groups. They all showed significantly ($F = 5.44$, $p < 0.001$) lower values than the saline group (53.3%, SD = 28%). At 5 h, statistically significant differences ($F = 2.92$, $p = 0.02$) were shown between saline and CHX+NaF (49.3%, SD = 25% and 52.3%, SD = 25%, respectively), and CHX+ALC and CHX+CPC (18.8%, SD = 15% and 23.0%, SD = 22%, respectively).

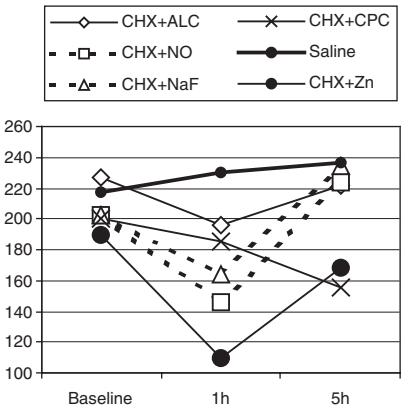

Fig. 1. Mean volatile sulphur compounds (in parts per billion) for each group and at every evaluation point.

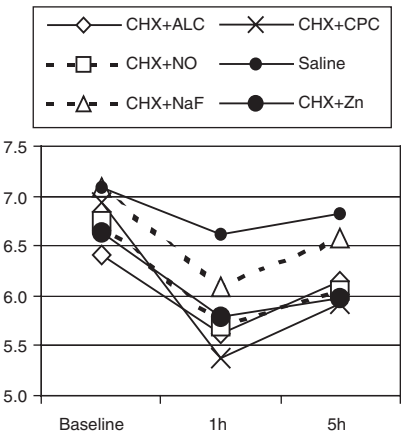

Fig. 2. Mean log of aerobic colony-forming unit for each group and at every evaluation point.

Fig. 4 shows the changes in the aerobic/anaerobic ratio in the different groups. This ratio was rather stable for saline and CHX–NaF; however, it showed a continuous reduction for CHX+NO and CHX+Zn groups, and an increase between baseline and 1 h, followed by a decrease reaching the baseline values at 5 h for CHX+ALC, and for the CHX+CPC groups no significant differences were detected among groups at any evaluation point.

VSC scores showed a tendency towards a positive correlation with both aerobic and anaerobic salivary counts at baseline ($r = 0.23$, $p = 0.08$ for aerobic, and $r = 0.21$, $p = 0.10$ for anaerobic).

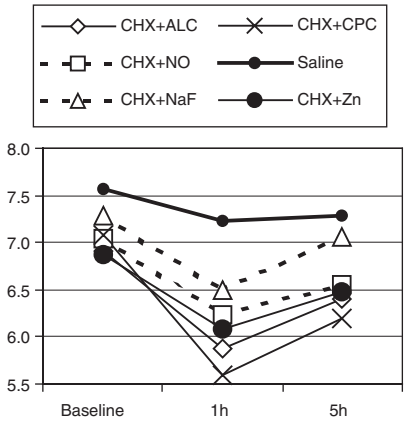

Fig. 3. Mean log of anaerobic colony-forming unit for each group and at every evaluation point.

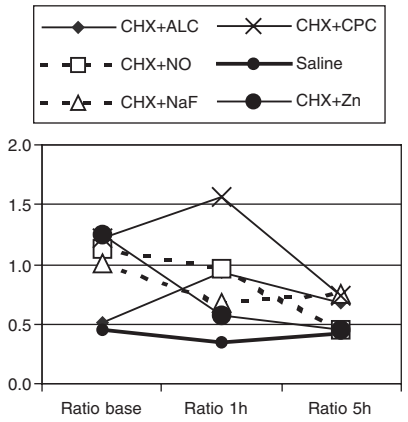

Fig. 4. Mean aerobic/anaerobic ratio for each group and at every evaluation point.

At 1 h, VSC and anaerobic CFU were significantly and positively correlated ($r = 0.32$, $p = 0.03$), while no relation was observed for aerobic counts. After 5 h, this significant correlation was lost.

Discussion

This investigation was aimed at testing a study model designed to compare different CHX-based mouth rinse formulations with regard to their capacity to reduce the number of bacteria in saliva and halitosis-related outcome variables, namely VSC, in expelled air. If proven valid, this study model could be used for testing both the anti-halitosis efficacy and the anti-microbial activity of any anti-microbial formulation for oral use. Although this model may have limitations as it uses healthy volunteers not suffering from oral halitosis, we have shown that the baseline VSC levels were high enough to adequately test the products and the instructions given to the subjects allowed us to reproduce a morning-breath model because they were not allowed to ingest liquids or food and did not brush or rinse their teeth.

This model, however, did not show any impact on the tongue-coating scores, because significant differences were found at baseline, reflecting a high individual variability. This observation is in agreement with a previous clinical study from our group, where VSC levels were significantly reduced by gargling with a mouth rinse; however, there was no effect on the tongue-coating scores (Winkel et al. 2003).

The evaluation of the different CHX-based mouth-rinse formulations demonstrated that the differences in formulation resulted in significant differences both in their anti-bacterial activity and their anti-halitosis efficacy. Although CHX+Zn was the most effective in reducing VSC levels after 1 h, this activity did not last up to the 5 h evaluation. At this evaluation time, CHX+CPC was the most effective in reducing VSC levels. This formulation (CHX+CPC) was also the most effective in reducing salivary bacterial counts, both after 1 and 5 h. Although both formulations contain CHX and CPC, the CHX+Zn formulation has a lower CHX concentration, which could account for lower anti-bacterial activity. However, the presence of zinc lactate could be responsible for the short-term higher anti-halitosis capacity. In contrast, CHX+NaF demonstrated a significantly lower activity than the rest of the CHX formulations, both with regard to its anti-bacterial activity (CFU changes) and its anti-halitosis efficacy (VSC reduction). This formulation has also demonstrated significantly lower anti-gingivitis efficacy in other studies, both in vitro and in vivo (van Steenberghe et al. 2001, Herrera et al. 2003). This lower activity might be because of the presence of NaF in the formulation, which might interfere with the activity of CHX.

In this study, we found a significant positive correlation between VSC levels and anaerobic CFU counts in saliva at 1 h. This finding is in agreement with previous reports, where odour scores were significantly correlated with total counts of bacteria (Pitts et al. 1981, De Boever & Loesche 1995, Hartley et al. 1995), lower aerobic/anaerobic ratio and lower percentage of anaerobic organisms (De Boever & Loesche 1995) in tongue samples. However, in spite of the significant efficacy of the CHX+Zn and CHX+CPC formulations in reducing the VSC levels, significant differences in the aerobic/anaerobic ratio among groups at the different evaluation points, could not be demonstrated. This was probably because of the short duration of the study (5 h). van Steenberghe et al. (2001) have also developed a study design to test the anti-halitosis activity of different anti-bacterial compounds. This model first assesses the halitosis-related outcome variables in the morning immediately after waking up (''morning breath''). Then it evaluates the inhibitory effect of different antiseptics in a cross-over design with periods of 12 days without mechanical plaque control. Using this model, these authors have compared different CHX-based mouth rinses (Corsodyl® (Glaxo SmithKline, Middlesex, UK), containing 0.2% CHX and alcohol; Cariax Gingival® (KIN, Barcelona, Spain) containing CHX 0.12% and sodium fluoride; and Halita® (Dentaid, Cerdanyola, Barcelona, Spain), containing 0.05% CHX, 0.05% CPC, 0.14% Zn). They reported that all tested products were able to improve the halitosis parameters (organoleptic ratings and VSC levels). However, only the 0.2% CHX formulation and the combination CHX–CPC–Zn were able to significantly reduce the oral microbial load, both in tongue and saliva samples. Therefore, the results obtained in the morning-breath study, and in the present study, are similar, demonstrating that formulations with a low concentration of CHX, CPC and zinc lactate, can be as effective as 0.12% or 0.2% CHX products, both in terms of their anti-microbial activity and their anti-halitosis efficacy.

CHX mouth rinses have also been evaluated for their anti-halitosis capacity in clinical studies. Two prospective studies (case series) have evaluated the efficacy of CHX at usual concentrations (0.12% and 0.2%) to control oral halitosis. The first study involved 16 subjects seeking treatment for oral malodour. The treatment regime included tongue brushing and rinsing with 0.12% CHX, twice a day, for 7 days. The results showed that the mean whole-mouth odour and VSC scores were reduced by 73.3% and 68.6%, respectively (De Boever & Loesche 1995). The second study involved 127

subjects mostly with complaints of oral halitosis. The treatment regime included rinsing with 0.2% CHX, twice a day, for 7 days. The results showed a significant reduction of VSC levels as well as reduction of anaerobic periodontal pathogens on the tongue (Benzoyl-DL-Arginine Naphthyl Amide (BANA) test) (Bosy et al. 1994).

Our research group has recently published the only clinical trial evaluating the efficacy of a CHX-based formulation in the treatment of halitosis. This study was designed as a dual-centre, double-blind, placebo-controlled, randomised clinical trial of 2-weeks duration (Roldán et al. 2003, Winkel et al. 2003). The results indicated that a specific formulation designed for the treatment of halitosis (Halita®) containing 0.05% CHX, 0.05% CPC and 0.14% Zn was efficient in reducing significantly both the organoleptic (53.6%) and VSC scores (58.6%). This formulation was also able to significantly reduce the total anaerobic counts, both in tongue and in subgingival samples.

These results agree with both the clinical and microbiological results obtained in the present investigation with the proposed study model. This model has been used to test the anti-bacterial activity of different mouth-rinse formulations in other studies (Roberts & Addy 1981, Elworthy et al. 1996, Addy et al. 1997). However, it is the first time that halitosis-related outcome variables were included in the model; thus, demonstrating its suitability for testing the possible anti-halitosis efficacy of different antiseptic formulations for oral use. The results from this investigation showed a weak correlation between the halitosis outcome variables and the salivary bacterial counts. This might indicate that the impact of a given mouth rinse in saliva does not reflect anti-halitosis activity. It is therefore suggested that other oral bacterial microflora, such as tongue bacterial counts, may be used in this proposed study model for testing anti-halitosis activity.

Conclusions

The results from this investigation show that in spite of using CHX anti-bacterial compounds, even at the same concentration, but with a different formulation, significant differences were demonstrated both in their anti-halitosis efficacy and in their anti-microbial activity in saliva. Formulations combining CHX and CPC achieved the best results, both for anti-microbial activity (CHX+CPC) and for anti-halitosis efficacy (CHX+Zn). Conversely, a formulation combining CHX with NaF showed significantly lower anti-halitosis and anti-microbial efficacy.

The proposed study design demonstrated its suitability to test the anti-halitosis activity of different anti-bacterial mouth-rinse formulations. However, the lack of correlation between this activity and the reductions in salivary bacteria advises the use of other microflora, such as the evaluation of the tongue-coating flora, in future studies.

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