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Antibacterial properties and side effects of chlorhexidine-based mouthwashes. A prospective, randomized clinical study

E. POLIZZI[1], G. TETÈ[2], F. BOVA[3], G. PANTALEO[4], G. GASTALDI[5], P. CAPPARÈ[6], E. GHERLONE[7]

[1]Head, Center for Dental Hygiene and Prevention, Department of Dentistry, IRCCS San Raffaele Hospital, Milan Italy and Dental School, Vita-Salute University, Milan, Italy
[2]DDS, Specialization School in Oral Surgery, Vita-Salute San Raffaele University, Milan, Italy
[3]Center for Dental Hygiene and Prevention, Department of Dentistry, IRCCS San Raffaele Hospital, Milan Italy and Dental School, Vita-Salute University, Milan, Italy
[4]PhD, Full Professor, UniSR-Social.Lab (Director), Faculty of Psychology, Vita-Salute San Raffaele University, Milan, Italy
[5]MD, DMD, Associate Professor, Dental School, Vita-Salute University and IRCCS San Rocco Hospital, Ome, Italy
[6]MD, MFS, Researcher, Dental School, Vita-Salute University and Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy
[7]MD, DMD, Full Professor and Chairman, Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy and Vita-Salute University, Milan, Italy

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ABSTRACT

Aim Chlorhexidine (CHX) is commonly used in clinical applications, including plaque control and gingivitis treatment. The im of this study was to evaluate the clinical properties, in terms of plaque control, bleeding and discoloration levels, of six CHX mouthwashes that differ in the following characteristics: CHX concentration, presence/absence of alcohol and presence/absence of an anti-discoloration system (ADS).

Materials and methods A single-center, prospective, double-blind randomized clinical trial was carried out on 78 consecutive patients. Six mouthwashes (CHX 0.12% alcohol free; CHX 0.20% alcohol free; CHX 0.12% alcohol free with ADS; CHX 0.20% alcohol free with ADS; CHX 0.12% with alcohol; CHX 0.20% with alcohol) were tested. Plaque Index (PI) and Bleedind Index (BI) were recorded, along with tooth staining (spectrophotometry) at T0 (beginning), at T1 (after 7 days), at T2 (after 14 days), and T3 (after 21 days). Data obtained were subjected to statistical analysis.

Results All CHX mouthwashes significantly reduced PI ($p<0.0001$), while only alcohol free CHX 0.20% significantly reduced BI ($p<0.0001$). Only the CHX 0.12% with alcohol and CHX 0.20% with alcohol mouthwashes showed a significant presence of extrinsic tooth staining ($p<0.05$).

Conclusions Within the limitations of the present study, the alcohol-free mouthwash CHX 0.20% allows a good control of the clinical indices, in particular the bleeding index.

INTRODUCTION

Chlorhexidine is widely used in daily clinical practice for its bactericidal and bacteriostatic properties are associated with high durability, it binds well to teeth and oral mucosa, and its progressive release can be maintained for up to 12 hours (1).

Its wide range of antimicrobial effects makes it the most effective, and hence the most extensively used, product available for the control of dental plaque, for the prevention of gingivitis and for the management of post-surgical infection (2). In particular, extensive use of Chlorexidine in the perioperative period is reported in many studies about oral implantology (3,4,5).

The collaboration between the hygienist and the dentist is very important to prevent dental infections, which have an impact not only in the oral cavity but also on systemic health (6).

Its antiseptic efficacy has been thoroughly investigated and is primarily attributed to its dicationic structure, which at the same time is responsible for some of its collateral effects, such as taste impairment and, above all, tooth and mucosal staining; this latter effect is the most commonly reported by long-term users of chlorhexidine-based products (7,8,9).

In recent attempts to minimize these unpleasant side effects, some formulations have added sodium metabisulfite and ascorbic acid to chlorhexidine (10). The resulting Anti-Discoloration System (ADS) substantially reduces negative side effects without diminishing the antiseptic efficacy of the mouthwash

(11). Another current problem is the presence of ethyl alcohol/ethanol in the chemical formulation of certain chlorhexidine-based mouthwashes. Ethanol is found in food and beverages, and is categorized as carcinogenic for humans by the International Agency for Research on Cancer (12). Concentrations of ethanol in mouthwashes vary from 3% to 26%; the use of ethanol derives from its ability to preserve products, its antiseptic properties and, consequently, its probable reinforcement of the antiseptic properties of ethanol-containing products (13).
The literature reports contrasting opinions on alcohol-containing mouthwashes. Recent studies have demonstrated that high alcohol content in prolonged-action mouthwashes can increase the risk of oral and oropharyngeal cancers (14,15). Further, ethanol has been related to other side effects, such as Burning Mouth Syndrome, dry mouth feeling and dysgeusia (16).
The aim of the present experimental study was to compare 6 mouthwashes in which 2 different chlorhexidine concentrations (0.12% and 0.20%) were tested in alcohol solution, without alcohol and with added ADS respectively. The reduction of bacterial plaque and gingival inflammation were the primary variables, discoloring was evaluated as secondary clinical variable.

MATERIALS AND METHODS

Study design

A single-center, prospective, double-blind randomized clinical trial was carried out on 78 consecutive patients. The trial was written following the CONSORT statement for improving the quality of RCT's.
The investigated treatments consisted of 6 mouthwashes (CHX 0.12% alcohol free; CHX 0.20% alcohol free; CHX 0.12% alcohol free with ADS; CHX 0.20% alcohol free with ADS; CHX 0.12% with alcohol; CHX 0.20% with alcohol). The experimental protocol was approved by the Local Ethical Committee. Each patient provided a written informed consent before participation.
Participants were selected among patients seeking care at the Center for Dental Hygiene and Prevention at the Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy. Patient eligibility for the study was determined on the basis of the inclusion/exclusion criteria.

Patient selection

Seventy-eight consecutive patients, 54 female and 24 male, aged between 20 and 50 years, were recruited. The inclusion criteria were:
- the absence of concomitant local or systemic pathologies;
- the absence of pregnancy or breast-feeding;
- no medical history of allergy;
- no intake of substances (including hallucinogenic drugs) characterized by a potential pharmacological interaction with the active ingredients to be tested;
- no intake of antibiotics and/or anti-inflammatory drugs in the 6 months prior to beginning of the study;
- no physical or mental disability such as might compromise normal domestic oral hygiene practice.

The exclusion criteria were:
- non-compliance with one or more of the inclusion criteria;
- smoking habit;
- the presence of fixed prostheses from upper right second premolar to upper left second premolar;
- the use of removable prosthesis;
- the presence of orthodontic brackets.

The patients were randomly assigned to 6 groups, each of which consisted of 13 subjects. Assignment of the mouthwashes to the groups was randomized by a computer-generated sequence and double-blind. The concealment of the allocation was preserved by sequentially numbered sealed envelopes.
The Groups were as follows.
- Group 1: Chlorexhidine Digluconate 0.12% without alcohol.
- Group 2: Chlorexhidine Digluconate 0.20% without alcohol.
- Group 3: Chlorexhidine Digluconate 0.12% without alcohol with ADS.
- Group 4: Chlorexhidine Digluconate 0.20% without alcohol with ADS.
- Group 5: Chlorexhidine Digluconate 0.12% in alcohol solution.
- Group 6: Chlorexhidine Digluconate 0.20% in alcohol solution.

To ensure standardization of dental hygiene procedures at home, each patient was provided with 2 x 250 ml bottles of mouthwash, a medium-bristle toothbrush and a toothpaste that did not contain chlorhexidine.
Patients were requested to rinse their mouths with the mouthwash for 1 minute twice daily half an hour after brushing their teeth. They were also asked not to consume discoloring drinks and food.
At the first check-up (T0), each patient completed a medical history questionnaire and underwent a session of professional oral hygiene, which identified plaque and bleeding scores (respectively, O'Leary Plaque Index (17) (PI), and Bleeding Index (BI) (18) by means of a periodontal probe (DP-10, Hu Friedy, USA).
To track modifications in dental surface color during the experimental period, we ascertained the pre- and post-treatment color of the maxillary right central incisor (the intrinsic technical characteristics of the spectrophotometer doe not allow, due to its size and the alignment required to perform the measurement, to operate on distal elements) by means of a spectrophotometer (SpectroShade™ MHT S.p.A. -

Medical High Technologies, Verona, Italy), which enables objective evaluations of chrome, color, value on the basis of the CIELAB system (Delta E) (19).
The subsequent check-ups at 7 (T1), 14 (T2) and 21 (T3) days monitored plaque index (PI) and bleeding index (BI) values, along with tooth color. Throughout the study, all patients were attended by a dental hygienist, who strongly encouraged correct home oral hygiene and who instructed all patients on the correct use of their mouthwash. Additionally, patients' compliance at home with the experimental protocol was evaluated by means of questionnaires that patients completed at each check-up.

Outcome measure

Primary outcomes were PI and BI; they were assessed according to Plaque Control Record and Gingival Bleeding Index respectively.

- Plaque Index (PI): it was assessed according to the Plaque Control Record (17). A dental hygienist, using a probe (PCP UNC 15, Hu Friedy, USA) sliding along the cervical surface of all teeth, detected the presence of plaque in six points of tooth surface (disto-buccal, mesio-buccal, buccal and lingual, mesio-lingual, disto-lingual). The number of surfaces with plaque divided by the number of available tooth surfaces and multiplied by 100 expresses the percentage of plaque presence.
- Bleeding Index (18). It detects the presence of gingival bleeding on gentle probing of six dental surfaces (disto buccal, mesio-buccal, buccal and lingual, mesiolingual, disto-lingual). A dental hygienist, using a probe (PCP UNC 15, Hu Friedy, USA) sliding along the cervical surface of all teeth, assigned a positive score when bleeding occurs within 10-15 seconds. The number of positive areas was divided by the number of those examined, and the result was multiplied by 100 to express the index as a percentage. The absence/reduction of Gingival Bleeding Index was interpreted as an improvement of the inflammatory condition.
- Delta E: the color difference pre- and post-treatment of the maxillary right central incisor, measured according to the CIELAB system (19).

Randomization

A computer generated list of random numbers was used to allocate the participants in the six groups. The randomization sequence was created using a specific statistical software (SPSS 17.0, SPSS Inc., Chicago, Illinois, Usa). A dental hygienist (blinded and calibrated at the baseline) performed patient enrolment, professional oral hygiene procedures and outcome assessment; another dental specialist performed assignment to each Group for mouthwash treatment; and each patient received the mouthwash in an anonymous bottle according to the randomization list.

Blinding

Treatments identity was blinded to the operator who performed patient enrolment and outcomes assessment, to the data analysts and to participants. Only the operator who performed group assignment was aware of the allocated group.

Statistical analysis

A dedicated software was used for statistical analysis (SPSS 17.0, SPSS Inc., Chicago, Illinois, Usa). Data were submitted to the Kolmogorov-Smirnov test for the assessment of normality of distribution.
Subsequently, each of the study's variables (PI, BI and Delta E) was submitted to multivariate analysis of variance and to Tukey post hoc test for multiple comparisons.
For all statistical analyses, statistical significance was fixed at α=0,05.
The initial null hypotheses were "no association exists between the different mouthwash formulations used and variation in PI and BI; no association exists between the different mouthwash formulations used and variation in dental tooth color".

RESULTS

Kolmogorov-Smirnov confirmed the normality of data distribution ($p>0.05$). The results of statistical analysis showed statistically significant differences in modifications both of plaque and bleeding indices ($p<0.05$) and of tooth color ($p<0.05$); accordingly, both the initial null hypotheses were rejected.
Specifically, multivariate analysis of variance between T0 and T3 showed significant differences in all 6 groups ($p<0.001$) with regard to PI, but only in group 2 ($p<0.001$) for BI.
With regard to PI, Tukey post hoc test for multiple comparisons with respect to T0 registered significant differences in all groups except for 1 and 3 ($p>0.05$), 1 and 5 ($p>0.05$) and 3 and 5 ($p>0.05$); whereas, at T3 the same test found statistically significant differences between 1 and 2 ($p<0.001$), 1 and 6 ($p<0.005$), 2 and 3 ($p<0.001$), 2 and 4 ($p<0.001$), 2 and 5 ($p<0.001$), 2 and 6 ($p<0.001$), 3 and 6 ($p<0.005$), 4 and 6 ($p<0.005$) and 5 and 6 ($p<0.001$).
With regard to BI, Tukey post hoc test for multiple comparisons with respect to T0 found statistically significant differences between 1 and 2 ($p<0.001$), 1 and 6 ($p<0.005$), 2 and 3 ($p<0.001$), 2 and 4 ($p<0.001$), 2 and 5 ($p<0.001$), 2 and 6 ($p<0.001$), 3 and 5 ($p<0.005$), 3 and 6 ($p<0.001$), 4 and 5 ($p<0.005$), and 4 and 6 ($p<0.001$). At T3, the test found statistically significant differences ($p<0.001$) exclusively between group 2 and the remaining 5 study groups.
For tooth color alterations, groups 5 and 6 differed, in a statistically significant manner ($p<0.05$), from the

GROUP	PI	BI
1	54%	9%
2	60%	38%
3	53%	14%
4	64%	100%
5	48%	15%
6	51%	17%
1 + 2 (CHX)	57%	23%
3 + 4 (CHX + ADS)	58%	57%
5 + 6 (CHX + ALCOOL)	49%	16%
1 + 3 + 5 (0.12%)	52%	13%
2 + 4 + 6 (0.20%)	58%	52%

TABLE 1 Percentage reductions in PI and BI.

other 4 groups, but revealed no statistically significant differences ($p<0.05$) between each other.
These results show that all the tested formulations were efficient in improving the periodontal indices here analyzed.
Mouthwashes containing 0.20% chlorhexidine reduced BI more significantly than did the 0.12% formulation, with an average reduction of 52% (Table 1); when combined with ADS, the 0.20% formulation achieved a mean reduction of 57% (Table 1).
Furthermore, all the tested formulations reduced PI effectively, although in this respect the 2 mouthwashes containing alcohol underperformed the remaining 4, with an average reduction of 49% (Table 1). The alcohol-containing mouthwashes worsen dental discoloring, and increased pigmentation irrespective of chlorhexidine concentration.
Comparison of T1 with T0 data indicates that all the tested mouthwashes demonstrated the efficacy of the chorhexidine molecule, in terms of the reduction both in PI and in BI.
The group 2 mouthwash was the most effective in reducing PI and it clearly demonstrated its ability to reduce BI.
As the data demonstrate, all 6 groups showed improvements in PI and BI, albeit with significant differences in and between the various phases of the treatment.
The pre- and post-treatment data for pigmentation, which we obtained with a spectrophotometer, showed changes in Delta E, and revealed that the patients of groups 5 and 6 were the most affected by such changes.
One patient in group 3 recorded slight pigmentation, as did 3 patients in group 4.
On the contrary, no patients in groups 1 and 2 were affected by color change.

DISCUSSION

Within the limits of the present study, comparative evaluation of all the mouthwashes here tested showed that they are all effective, but that their inhibition of plaque varies on the basis of the excipient and of chlorhexidine concentration.
The patient groups that received a chlorhexidine-based mouthwash with added ADS showed reduced efficacy in the reduction of both bacterial plaque and gingival inflammation.
Comparison of the 2 experimental concentrations (0.12% and 0.20%) tested shows that the 0.20% chlorhexidine concentration slightly outperforms its 0.12% equivalent as regards PI and BI parameters.
The same cannot be said for mouthwashes with added ADS.
The present study shows that the use of chlorhexidine-based mouthwashes without alcohol and without ADS produces a benefit almost equal to that of alcohol-boosted chlorhexidine in the lowering both of bacterial plaque and of gingival inflammation.
Patients who used the non-alcohol, non-ADS mouthwash did not record any color alterations.
Chohrexidine has for some time been the "gold standard" for the chemical control of bacterial plaque in fact, it is used in many areas of dentistry (20). In daily oral hygiene implant maintenance practices, the use of mouthwashes is a common practice; however, further studies are needed to determine whether alcohol has an effect on the stability of peri-implant tissue (21); Mokthar et al. point out that the material and then the type of abutment influences biofilm creation (22).
In a study by Polizzi et al. it is reported that the use of chlorhexidine in addition to SRP has led to clinical and microbiological benefits in the treatment of generalized chronic periodontitis (23).
Conditionally upon specific formulations, and above all in association with ethanol, the use of commercially available, chlorhexidine-based mouthwashes can induce side effects, such as dental pigmentation and taste impairment(24). Moreover, ethanol appears to increase the risk of neoplasia in the oral cavity; the current range of commercial mouthwashes includes chlorhexidine-based products without alcohol or with an added Anti-Discoloration System (ADS), the efficacy of which has been evaluated by various experimental studies (25).
A comparative study on patients treated with a chlorhexidine-based mouthwash with or without ADS found that the 2 formulations did not differ significantly in terms of plaque prevention, but with regard to dental discoloration; results for the ADS mouthwash showed distinctly lower levels of pigmentation (11).
A recent prospective clinical trial showed that compliance in post-periodontal surgery patients improved if the patients were treated with a chlorhexidine-based mouthwash with added ADS. Notably, however, this

study also found that the addition (or not) of ADS to the chlorhexidine-based mouthwash produced no differences in plaque control or in gingival inflammation in the post-surgical healing phase (10).
Contrasting results were found in another study, which compared 2 chlorhexidine-based moutwashes, one with ADS, the other with alcohol and without ADS. Data showed that the latter formulation inhibited plaque and reduced bacterial vitality better than did the former (26).
On the issue of alcohol-based mouthwashes, the literature expresses contrasting opinions; according to McCullough and Farah, mouthwash ethanol permeates oral lining mucosa and can easily cause damage by increasing the mucosa's permeability and thus rendering the mouth much more sensitive to other carcinogenic substances, such as nicotine (14). Alcohol concentrations in the various mouthwash formulations is highly variable, and can reach 26%.
When alcohol remains in the mouth for a long time, it is transformed into acetaldehyde, i.e. the alcohol undergoes degradation which leads to formation of acetaldehyde *in situ*, by means of enzymes in the oral cavity (27).
One study by Homann et al. demonstrated that cells exposed to acetaldehyde undergo notable functional alterations, even to the extent of cellular death (28). Numerous authors argue that alcohol-containing mouthwashes should be prescribed exclusively for short-term use, and that daily domestic use should include non-alcohol-containing products (29). It should be noted, however, that other studies have found no correlation between the use of alcohol-containing mouthwashes and the onset of cancers. To date, therefore, direct causality between the use of alcohol in mouthwashes and carcinogenesis in the oral cavity is not scientifically proven.

CONCLUSIONS

Within the limits of the present study, on the basis of these results, it can be hypothesized that chlorhexidine without alcohol and without ADS – at both the 0.12% and the 0.20% concentrations – unites mainstream antiseptic advantages with reduction of gingival inflammation; its use does not lead to unpleasant discoloring, nor to symptoms such as dry mucous lining or taste impairment.
The 0.20% concentration is advisable in cases of acute gingival inflammation, given that this formulation has superior control over bleeding.
Although pigmentation generally proves to be poorly tolerated by patients, it is normally reversible and in any case it shows after prolonged use (about 3 weeks), a period after which chlorhexidine is not normally prescribed.

Scientific rationale of the study:

This study aims to find out what would be the effect of six chlorhexidine-based mouthwashes with or without alcohol in the treatment of oral diseases, bleeding and gingivitis, paying particular attention to the discoloration caused by chlorhexidine.

Main findings

On the basis of these results, we can conjecture that chlorhexidine without alcohol and without ADS unites mainstream antiseptic advantages with reduction of gingival inflammation; its use does not lead to unpleasant discoloring, nor to symptoms such as dry mucous lining or taste impairment.

Practical implications

The concentration of 0.20% of mouthwashes is recommended in cases of acute gum inflammation, as this formulation has superior control over bleeding, however prolonged use causes discoloration.

Authors' contribution

EP conceived the idea; GT approved the study protocol
FB collected the data; GP, GP and EP perfomed statistical analysis and drafted the manuscript; GT and PC revised the manuscript; PC, FB and GT interpreted the data; EFG critical revisions carried out the final revision of the text and approved the idea.

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