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Randomized controlled trial on the efficacy of new alcohol-free chlorhexidine mouthrinses after 8 weeks[1]

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Zimmer S, Korte P, Verde P, Ohmann C, Naumova E, Jordan RA. Randomized controlled trial on the efficacy of new alcohol-free chlorhexidine mouthrinses after 8 weeks.

Abstract: *Objectives:* To evaluate the efficacy of two alcohol-free antimicrobial mouthrinses in reducing plaque and gingivitis compared to an alcohol-containing rinse and toothbrushing alone. *Methods:* One hundred and sixty healthy volunteers were enrolled in the randomized controlled trial. Participants were randomly and equally assigned to four groups: (i) toothbrushing + rinsing (0.06% CHX + 0.025% NaF, alcohol-containing rinse, positive control); (ii) toothbrushing + rinsing (0.06% CHX + 0.025% NaF, alcohol-free experimental rinse); (iii) toothbrushing + rinsing (0.06% CHX + 0.03% CPC + 0.025% NaF, alcohol-free experimental rinse); (iv) toothbrushing alone (negative control). At baseline, Quigley-Hein plaque index (QHI), modified proximal plaque index (MPPI), and papillary bleeding index (PBI) were recorded. All subjects brushed their teeth as usual during the study. Additionally, groups 1–3 rinsed twice daily. Eight weeks after baseline, indices were recorded again. ANOVA with Bonferroni adjustment served for statistical analysis. *Results:* One hundred and fifty-five participants were included into final analysis (i: $n = 39$, 2: $n = 39$, 3: $n = 37$, 4: $n = 40$). Experimental rinses (ii, iii) reduced QHI and MPPI to a higher extent than the negative control (iv), whereas no significant difference to the positive control was found. QHI: (i) 36.6%, (ii) 32.3%, (iii) 36.8%, (iv) 21.6%; MPPI: (i) 11.9%, (ii) 12.2%, (iii) 13.6%, (iv) 3.5%. For PBI, no statistically significant difference was found between groups: (i) 80.2%, (ii) 77.8%, (iii) 76.5% and (iv) 78.8%. *Conclusions:* With respect to QHI and MPPI, toothbrushing in combination with any rinse was more effective than toothbrushing alone. No statistically significant differences were found between the alcohol-free and the alcohol-containing control rinses.

Key words: chlorhexidine; controlled clinical trial; mouthrinse; random allocation; single-blind-method

Introduction

Oral biofilm is an essential aetiological factor of caries and gingivitis (1, 2) and there is evidence that chronic gingivitis is an important risk factor for periodontitis and tooth loss (3, 4). Consequently, efficient plaque removal is an important measure to prevent these diseases. However, for Germany, epidemiological data show that on a public health scale, oral hygiene is still not satisfying (5). This might be due to the fact that mechanical oral hygiene is challenging and usually not resulting in plaque-free conditions (6). This in mind, chemical biofilm removal on a daily basis could be a useful adjunct to toothbrushing and interdental

hygiene measures. Daily use of an alcohol containing 0.06% chlorhexidine digluconate (CHX) mouthrinse has been shown to be an effective support of mechanical oral hygiene (7). Patients, however, are increasingly asking for alcohol-free rinses. Such products are especially required by consumers who want to abstain from alcohol, for example persons with previous alcohol addiction, pregnant women, children or members of some religious groups. To make sure that the lack of alcohol doesn't compromise the antiplaque activity of the alcohol-free mouthrinses, it was the aim of this study to evaluate the efficacy of two alcohol-free antimicrobial mouthrinses in reducing plaque and gingivitis compared with the alcohol-containing rinse as tested before (7) and toothbrushing alone. All tested mouth rinses were containing chlorhexidine digluconate 0.06% as antimicrobial agent. As it was speculated that the renunciation of alcohol could reduce the antimicrobial activity of the mouth rinse, cetylpyridinium chloride (CPC) 0.03% was added to one of the two newly developed alcohol-free rinses in order to compensate this possible disadvantage.

Study population and methodology

Trial design

This was a single centre, examiner-blind, randomized, controlled, four-arm, parallel group study design in 160 healthy subjects. The study was conducted according to ICH GCP regulatories (8). The trial was registered at the Clinical Trials Register of the National Institute of Health (NCT01811615, www.clinicaltrials.gov). Figure 1 shows the flow diagram for the subject enrolment.

Participants

In total, 160 healthy subjects aged 18–65 years and having a mean PBI per tooth ≥0.5 were enrolled in the study. Sample size was calculated on the basis of the following assumptions: power 0.8; alpha error: 0.05; delta-QHI between groups after 8 weeks: 0.3 (SD 0.5); one tailed analysis (Axum 7.0). Informed consent was obtained by each subject. Each subject had a minimum of 20 permanent natural teeth. Third molars, orthodontically banded, fully crowned or extensively restored or abutment teeth were not included in the tooth count. Subjects were excluded from the study if they were pregnant or breastfeeding, had diabetes type I or II, or severe periodontal disease, or wore removable dentures. Severe periodontal disease was defined as the presence of clinical attachment loss of more than 5 mm according to the periodontal disease classification of the American Academy of Periodontology (9). However, a subject was excluded only if this applied for a minimum of three teeth. Subjects were also excluded if any of the following conditions was present: use of antibiotics within 2 weeks prior the

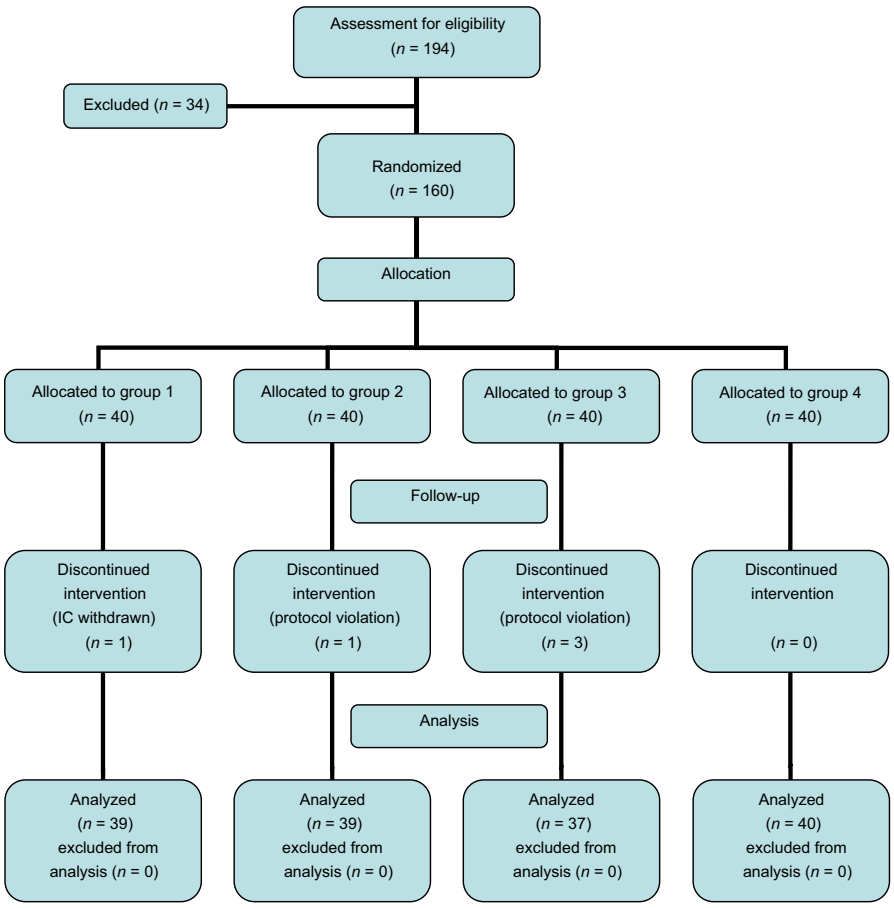

Fig. 1. Flow diagram.

first examination or use of any systemic medication which would have an effect on gingival conditions within 30 days prior to the screening visit, or recent history (within the last year) of alcohol or other substance abuse. Dental professionals, dental students and employees of the sponsor or members of their immediate families were also excluded from the study.

The study took place at the Dental School of the Witten/Herdecke University and was approved by the ethical review board of the Witten/Herdecke University (Approval# 22/2010).

Recruitment

Subjects were recruited between April 20th, 2010 and June 16th, 2010.

Screening and patient inclusion

In total, 194 were screened and 160 eligible participants were randomized.

Randomization

Using the stratification by sex and PBI (PBI/tooth ≥0.5 and <1.0 or PBI/tooth ≥1.0), the 160 eligible participants were allocated by block randomization to one of four groups with 40 subjects each. Block randomization was performed by a statistician not involved in the study (T.O.).

Interventions

Subjects within each group were randomly assigned to one of the following groups (n = 40 each): Group 1: twice daily tooth brushing and rinsing twice a day for 30 s with 10 ml of an alcohol-containing mouthrinse with 0.06% chlorhexidine digluconate (CHX) + 0.025% fluoride as sodium fluoride[2] , (positive control); Group 2: twice daily tooth brushing and rinsing twice a day for 30 s with 10 ml of an experimental alcohol-free mouthrinse with 0.06% chlorhexidine digluconate (CHX) + 0.025% fluoride as sodium fluoride; Group 3: twice daily tooth brushing and rinsing twice a day for 30 s with 10 ml an experimental alcohol-free mouthrinse with 0.06% chlorhexidine digluconate (CHX) + 0.03% cetylpyridinium chloride (CPC) + 0.025% fluoride as sodium fluoride; Group 4: twice daily tooth brushing alone.

To achieve standardized conditions, each subject used the same toothpaste without any antimicrobial ingredient[3] and a fresh toothbrush[4]. All participants received a short instruction on how to conduct the attributed oral hygiene measurements. The subjects were advised to brush their teeth in the morning and in the evening postprandial in the usual manner. No instructions concerning brushing technique and brushing duration were given. After using the brush, the subjects rinsed their mouth with tap water. The subjects of the rinsing groups waited for 30 min. Thereafter, they rinsed for 30 s with 10 ml of the assigned rinse. Afterwards, the subjects refrained from drinking, eating and rinsing for at least 30 min. To control the waiting and the rinsing time, each subject was provided with a digital stop watch.

During the study period, the use of other than the attributed oral hygiene tools strictly was prohibited (e.g. antibacterial mouthrinses, chewing gums, sweets with essential oils). Interproximal cleaning devices (floss, water pik, toothpicks) had been permitted if they were part of the usual oral hygiene routine of the included subjects. The subjects were requested not to brush their teeth 12 h (+5, −2 h) prior to the dental appointment. At the intermediate and final examination, the subjects were interviewed whether they had performed the oral hygiene measures as requested.

Smoking habits were recorded. At the screening visit, inclusion and exclusion criteria were checked and the following clinical assessments have been performed: Modified Proximal Plaque Index (MPPI) (10), Quigley-Hein Plaque Index (QHI) (11) modified by Turesky *et al.* (12) and Papillary Bleeding Index (PBI) (13). Data from the screening examination served as baseline. Four and 8 weeks after baseline, the indices were recorded again. Occurrence of discomfort in taste, discomfort in sensibility, gingival damage, gingival bleeding, staining of teeth and tongue, mouth burning and white plaque on tongue immediately after use was registered at baseline and after four and 8 weeks. Oral soft tissue examination was performed at each visit. All assessments of an individual subject in the course of the study have been conducted by the same investigator (P.K.) who had experience from previous clinical studies.

Blinding procedures

All personal instructions and delivering of the study products were performed by a study nurse not involved (A.P.) in the study examinations. The subjects were forbidden to tell the examiner their regimen during the study visits.

Outcomes

The primary endpoint was the comparison of the absolute values for MPPI, PBI and QHI after four and 8 weeks between groups. The secondary endpoint was the comparison of the improvements of MPPI, PBI and QHI after four and 8 weeks between groups. All adverse events occurring during the study period were recorded using a questionnaire and by clinical examination. The study was designed as an equivalence study. Following this, the working hypothesis was that plaque and gingivitis reduction by two non-alcoholic mouthrinse formulations is inferior to a chlorhexidine mouthrinse-containing alcohol.

Statistical methods

Statistical analysis was performed per protocol (n = 155). ANOVA with Bonferroni adjustment was used for statistical analysis of

[2]Corsodyl® Daily Defence Mouthwash/parodontax® Daily Mouthwash
[3]Dr. Best® Multi-Aktiv
[4]Dr. Best® plus medium

the indices. Logistic regression was performed to detect differences between groups with respect to smoking status. Chi square-test served to detect differences in side effects between groups.

Results

Baseline and follow-ups took place between 20th April 2010 and 27th August 2010.

In total, 155 participants were included into final analysis (1: $n = 39$, 2: $n = 39$, 3: $n = 37$, 4: $n = 40$). The mean age was 33.9 years (1: 33.8; 2: 34.0; 3: 33.8; 4: 33.9). After four and 8 weeks, the two experimental rinses (2, 3) showed no statistically significant difference to the positive control (4) with respect to QHI (Fig. 2, Table 1) and MPPI (Fig. 3, Table 1). For PBI, no statistically significant difference was found between groups (Fig. 4, Table 1). With respect to changes between baseline, intermediate and final examination, the positive control and the two experimental groups performed statistically significant better than the negative control (Table 2).

No statistical significant differences between groups were found with respect to smoking status (randomized subjects; smoker/non-smoker: 1. 10/30; 2. 9/31; 3. 10/30; 4. 10/30). Observed side effects were discoloration of teeth and tongue (Table 3). No statistically significant differences between groups were found for discoloration of the tongue. With respect to tooth staining, chi square-test revealed significant more occurrences in the three rinsing groups when compared to the negative control ($P < 0.001$). Gastrointestinal adverse events were found in all groups (group 1: 1 case, group 2: 2 cases, group 3: 7 cases, group 4: 1 case), in five cases (1 in group 2 and 4 in group 3), gastrointestinal infection was given as reason; in four cases, diarrhoea; and in one case, 'stomach burning' and meteorism, respectively. No serious adverse events were observed in the study.

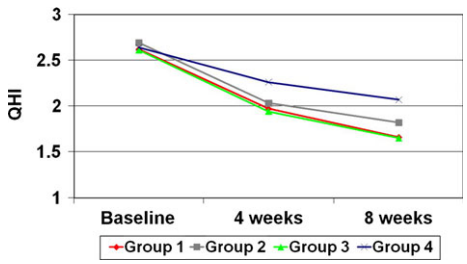

Fig. 2. Mean values of QHI at baseline, after four and 8 weeks.

Discussion

In the present study, the efficacy of two alcohol-free mouthrinses with antimicrobial activity in reducing interdental plaque and gingival bleeding was evaluated in comparison to an alcohol-containing product and a negative control. The products in group 1 and 2 contained 0.06% chlorhexidine digluconate (CHX) as antimicrobial agent. The alcohol-containing product in group 1 was found to be effective in an earlier study (7) and therefore served as positive control in the present trial. This product is commercially available. When compared to the alcohol-free product from group 2, no statistically significant difference was found with respect to any oral hygiene index (Tables 1 and 2). This was also true for group 3, where an experimental product with 0.06% chlorhexidine digluconate (CHX) + 0.03% cetylpyridinium chloride (CPC) was used. Discolorations of teeth and tongue were found in all three mouthrinse groups but without any significant difference between groups (Table 3). Over the study period of 8 weeks, some gastrointestinal adverse events were found in all groups. The reasons for some of these adverse events where attributed to gastrointestinal infection and the others were unclear. Bad taste as another common side effect of chlorhexidine digluconate was not found in this study.

The findings of the present study lead to two conclusions: Firstly, the removal of alcohol from the CHX-containing mouthrinse does not seem to have a negative effect on its

Table 1. **Mean values of QHI, MPPI and PBI at baseline, after four and 8 weeks**

	Group 1 (CHX/alc./pos. control) $n = 39$	Group 2 (CHX/no alc.) $n = 39$	Group 3 (CHX + CPC/no alc.) $n = 37$	Group 4 (neg. control) $n = 40$
QHI				
Baseline	2.62 (0.37)	2.69 (0.35)	2.61 (0.37)	2.64 (0.51)
4 weeks	1.97 (0.44) a ($P < 0.012$)	2.03 (0.44)	1.94 (0.35) b ($P < 0.006$)	2.26 (0.44) a,b
8 weeks	1.66 (0.48) a ($P < 0.0004$)	1.82 (0.46)	1.65 (0.35) b ($P < 0.0002$)	2.07 (0.45) a,b
MPPI				
Baseline	2.10 (0.19)	2.13 (0.18)	2.14 (0.18)	2.02 (0.29)
4 weeks	1.91 (0.22)	1.92 (0.17)	1.89 (0.18)	2.00 (0.23)
8 weeks	1.85 (0.22)	1.87 (0.22)	1.85 (0.24)	1.95 (0.25)
PBI				
Baseline	1.06 (0.40)	0.99 (0.37)	1.02 (0.39)	0.94 (0.31)
4 weeks	0.39 (0.26)	0.39 (0.27)	0.47 (0.35)	0.39 (0.30)
8 weeks	0.21 (0.13)	0.22 (0.22)	0.24 (0.16)	0.25 (0.20)

Means and standard deviations; in the same row of the table, groups with the same letters show significant differences at given *P*-values.

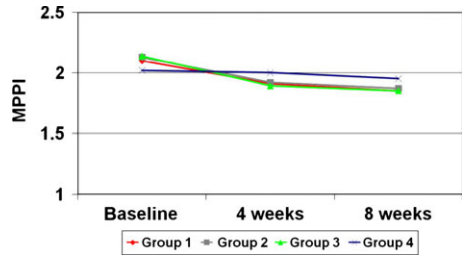

Fig. 3. Mean values of MPPI at baseline, after four and 8 weeks.

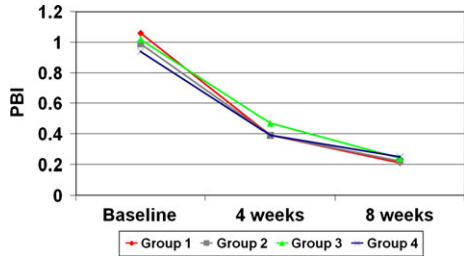

Fig. 4. Mean values of PBI at baseline, after four and 8 weeks.

efficacy and secondly, the addition of 0.03% CPC does not seem to result in an additional benefit. Therefore, the product containing 0.06% CHX alone without alcohol should be the preferred one from the three rinses tested in this study.

The proof of equivalence of CHX-containing alcohol-free mouthrinses for daily use in comparison to alcohol-containing ones has not yet been a subject of a clinical study. Insofar, the present study provides some new information. For CPC mouthrinses, this equivalence was shown recently (14).

The data of the present study also show that in addition to tooth brushing, rinsing with one of the tested solutions is more effective in reducing plaque after 8 weeks of use than tooth brushing alone. The results for interproximal bleeding (PBI) showed no statistically significant differences between the four groups. The reason might have been that the large reductions in PBI that were found after four and 8 weeks resulted in a nearly complete absence of bleeding in all groups. According to the so called Hawthorne effect (15), this might be attributed to the fact that all participants including those of the control group considerably improved their oral hygiene because of participating in this clinical study. The low PBI values at the end of the study gave no room for significant differences.

Alcohol in mouthrinses is used as a solvent for active and flavouring ingredients such as CHX, CPC and essential oils. Recently, risks arising from alcohol in mouthrinses have been discussed. It is obvious that alcohol-containing mouthrinses should not be used from patients suffering from alcoholic disease and because of the risk of swallowing from children under 12 years (16). In addition, the ingestion of mouthrinses has been observed in settings of restricted availability to alcoholic beverages such as hospitals, prisons or military establishments (17). This abuse should also be excluded. Beyond its ingestion, it is still under discussion whether alcohol, that is ethanol, may be harmful if used as a rinse. Swallowed alcohol

Table 2. **Mean improvements (in %) from baseline of QHI, MPPI and PBI after four and 8 weeks**

	Group 1 (CHX/alc./pos. control) $n = 39$	Group 2 (CHX/no alc.) $n = 39$	Group 3 (CHX + CPC/no alc.) $n = 37$	Group 4 (neg. control) $n = 40$
QHI				
Baseline-4 weeks	24.8% a ($P < 0.012$)	24.5% b ($P < 0.009$)	25.7% c ($P < 0.006$)	14.4% a,b,c
Baseline-8 weeks	36.6% a ($P < 0.0002$)	32.3% b ($P < 0.006$)	36.8% c ($P < 0.0002$)	21.6% a,b,c
MPPI				
Baseline-4 weeks	9.0% a ($P < 0.003$)	9.9% b ($P < 0.015$)	11.7% c ($P < 0.00007$)	1.0% a,b,c
Baseline-8 weeks	11.9% a ($P < 0.011$)	12.2% b ($P < 0.008$)	13.6% c ($P < 0.001$)	3.5% a,b,c
PBI				
Baseline-4 weeks	63.2% a	60.6%	53.9%	58.5% a
Baseline-8 weeks	80.2%	77.8%	76.5%	73.4%

In the same row of the table, groups with the same letters show significant differences at given *P*-values.

Table 3. **Occurrence of stain in teeth/tongue**

	Group 1 (CHX/alc./pos. control) $n = 39$		Group 2 (CHX/no alc.) $n = 39$		Group 3 (CHX + CPC/no alc.) $n = 37$		Group 4 (neg. control) $n = 40$	
	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks
Tooth staining	17	18	9	21	18	25	3	8
Tongue staining	12	18	11	22	16	23	8	14

is oxidized to acetaldehyde by the enzyme alcohol dehydrogenase. Acetaldehyde is known to be a carcinogen. In the oral cavity, it is assumed that the oral flora might produce acetaldehyde from alcohol in mouthrinses (18). According to Walsh, the production of acetaldehyde 'by normal commensal bacteria and fungi explains the well established link between oral hygiene and oral cancer' (18). However, there is a large body of evidence based on epidemiological data that alcohol-containing mouthrinses do not cause oral cancer (19, 20). The reason for this finding might be that the contact time of the mouthrinse with oral soft tissues is not long enough to induce acetaldehyde formation and that mouthrinses with antimicrobial activity are reducing oral microbials in number and metabolism and therefore reduce the acetaldehyde production. This view is supported by findings of Koschier *et al.* (21). Using an *in vitro* oral mucosa model, they simulated daily use of an alcohol-containing mouthrinse. The authors found no acetaldehyde or acetic acid production. In addition, the simulated daily use of the alcohol-containing mouthrinse had no apparent effect on the permeability of the mucosa model and no effect on the viability of the tissue construct or histopathology. Even if causality between alcohol in mouthrinses, oral cancer and possible other negative effects are not proven and probably not existing, there is rationale to abstain from alcohol in mouthrinses whenever possible. The present study has shown that this is possible at least for the tested product.

It can be concluded from the present study that the removal of alcohol from the CHX-containing mouthrinse does not seem to have a negative effect on its efficacy. Therefore, the product containing 0.06% CHX without alcohol should be recommended instead of the alcohol-containing one.

Clinical relevance

For certain groups of people, for example persons with previous alcohol addiction, pregnant women, children or members of some religious groups, alcohol-containing mouthrinses are not appropriate. Therefore, the effectiveness of alcohol-free mouthrinses should be investigated. With respect to plaque control, toothbrushing in combination with experimental alcohol-free and already marketed alcohol-containing antimicrobial mouthrinses was more effective than toothbrushing alone. No statistically significant differences were found between the alcohol-free and the alcohol-containing control rinses.

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Conflict of interest and sources of funding

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Contributions

SZ was responsible for the study design. SZ, EN and RAJ were responsible for writing the manuscript. PK carried out the experiments and the measurements, and PV and CO were responsible for data management and statistics.

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