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Influence of alcohol in mouthwashes containing triclosan and zinc: an experimental gingivitis study

J. M. Almerich[1], B. Cabedo[1], J. C. Ortolá[1] and J. Poblet[2]

[1]Department of Stomatology, Faculty of Medicine and Dentistry, University of Valencia, Valencia; [2]Lácer Personal Health, Lácer, S.A., Barcelona, Spain

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Abstract

Objectives: An experimental gingivitis model was used to analyse the influence of alcohol in mouthwashes containing 0.15% triclosan and zinc chloride on the formation of supragingival plaque, the development of gingivitis and the appearance of adverse events.

Material and Methods: Using a double-blind crossover design, 30 subjects underwent two consecutive experimental phases with two 0.15% triclosan and zinc chloride mouthwashes, differentiated mainly by their excipient (hydroalcoholic or aqueous). In each phase, the subjects discontinued all oral hygiene measures and were treated solely with the randomly assigned experimental mouthwash for 21 days. Each experimental phase was preceded by a 14-day washout period in which, after receiving a complete oral prophylaxis, the subjects were instructed to perform thorough oral hygiene procedures. Gingivitis and plaque levels were assessed at the start and end of both the experimental phases.

Results: The evolution of the gingivitis and plaque indices showed no statistically significant differences between both treatments. The mean gingival index increased from 0.30 to 0.38 with the non-alcoholic mouthwash and from 0.32 to 0.42 with the hydroalcoholic mouthwash. The respective changes in plaque index were from 0.88 to 1.93 and from 0.94 to 1.92. The hydroalcoholic mouthwash produced a larger number of fresh symptoms ($p = 0.033$), oral itching being the most reported.

Conclusions: Alcohol does not influence the effectiveness of a 0.15% triclosan and zinc chloride mouthwash against gingivitis development and supragingival plaque formation. The absence of alcohol significantly decreases the incidence of adverse events produced by the mouthwash.

Key words: alcohol; gingivitis; plaque; triclosan; zinc

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Dental plaque is a key factor in the aetiology of gingival inflammation. It is therefore extremely important to encourage patients to perform accurate oral hygiene procedures aimed at removing dental plaque and consequently, preventing gingival inflammation. The use of antiseptic chemical agents may supplement oral hygiene programmes and compensate for motivation deficits in patients.

The antiplaque potential of multiple antimicrobial agents has been assessed. Among these agents, chlorhexidine has shown the highest inhibitory effect on plaque formation and gingivitis development (Gjermo 1989), but its adverse effects, particularly tooth staining, restrict its long-term application.

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is an antimicrobial agent, which lacks the local effects of cationic agents that occur with chlorhexidine. Clinical studies have shown that the use of triclosan in toothpastes inhibits plaque accumulation moderately (Saxton 1986, Saxton et al. 1988, Jenkins et al. 1989, Palomo et al. 1989, Deasy et al. 1991, Moran et al. 1992, Lindhe et al. 1993, Svatun et al. 1993, Binney et al. 1997, Owens et al. 1997) and that its daily use may improve healing following non-surgical treatment for advanced periodontal disease (Furuichi et al. 1999).

Zinc salts have also shown to have a moderate inhibitory effect against pla-

que and gingivitis (Harrap et al. 1983, Saxton 1986, Saxton et al. 1986, 1988, Saxton & van der Ouderaa 1989).

Many studies have shown that long-term use of toothpastes containing triclosan, combined with a zinc salt, inhibits plaque and supragingival calculus formation, controlling gingivitis effectively (Saxton 1986, Saxton et al. 1987, Svatun et al. 1987, 1989a, b, 1990, Saxton & van der Ouderaa 1989, Stephen et al. 1990, Fairbrother et al. 1997) and with no significant side effects (DeSalva et al. 1989, Zuckerbraun et al. 1998).

The combination of triclosan with a zinc salt is also used in mouthwashes, showing reductions in plaque formation and gingivitis development (Abello et al. 1990, Rustogi et al. 1990, Cummins 1991, Deasy et al. 1992, Jenkins et al. 1993, Schaeken et al. 1996, Moran et al. 1997).

The formulation of mouthwashes containing triclosan and zinc salts has traditionally included alcohol (Schaeken et al. 1994) as a solvent for other substances. Alcohol has a weak antibacterial effect at the concentrations used, which range from 10% to 30% by volume. However, it cannot be regarded as a neutral substance insofar as it entails possible side effects. The publication of studies relating the presence of alcohol in mouthwashes with their possible carcinogenic effects (Weaver et al. 1979, Wynder et al. 1983, Mashberg et al. 1985), together with the alterations detected in aesthetic restorations (Weiner et al. 1997, Settembrini et al. 1995), make it necessary to carry out clinical trials to demonstrate the therapeutic effectiveness of the substances considered to be active when not associated with alcohol.

Löe et al. (1965) performed the first clinical study on experimental gingivitis. This study showed that withdrawal of all oral hygiene measures resulted in gross plaque accumulation and the development of clinically observable gingivitis in all subjects in a period of time ranging from 10 to 21 days. The situation returned to the initial levels when brushing was resumed.

Schaeken et al. (1994) analysed the effect of mouthwashes containing zinc and triclosan by developing experimental gingivitis in patients over 21 days using a partial mouth design.

Based on the development of experimental gingivitis during 21 days, this study compares the preventive effect on gingivitis and supragingival plaque of two mouthwashes with a formulation based on 0.15% triclosan and zinc chloride, which differed mainly in their excipient (hydroalcoholic or aqueous). The aim was to assess whether a 0.15% triclosan and zinc chloride mouthwash is equally effective in an aqueous or hydroalcoholic solution, and to compare the frequency of adverse events with the two treatments.

Material and Methods

Subjects

Thirty subjects over 18 years old, who gave their written informed consent before the start, were included in the study. The following exclusion criteria were applied:

- Periodontal pockets deeper than 4 mm.
- Fewer than 20 natural teeth at baseline.
- Periodontal surgery during the previous 3 months in the area of the teeth to be studied.
- Treatment with antibiotics or any other medication, which might affect the periodontal condition (e.g. phenytoin, NSAIDs, calcium antagonists, etc.), within the 30 days prior to baseline.
- History of hypersensitivity or specific oral allergy to any of the ingredients of the products to be assessed.
- Use of antiseptics for chemical control of dental plaque within the 30 days prior to baseline.
- Wearers of orthodontic appliances.
- Pregnancy or breast-feeding.
- Systemic diseases, especially chronic ones, which might interfere with the collection of representative data on the pathology under study (gingivitis).

The study was authorized by the Clinical Research Ethics Committee of *Hospital General Universitario de Valencia* (Spain).

Design

This was a double-blind crossover study with random distribution of both experimental mouthwashes.

After a baseline clinical evaluation, the subjects who fulfilled the selection criteria received a thorough oral prophylaxis (tartar removal and polishing with a rubber cup and prophylactic paste). They were also instructed to maintain excellent oral hygiene for 14 days (pre-experimental phase) by brushing their teeth for a minimum of 2 min. three times a day. The aim of this phase was to assure the least possible presence of plaque, as well as the practical non-existence of gingivitis, before the start of the experimental phase.

After these 14 days, the subjects discontinued all oral hygiene procedures and for a period of 21 days (first experimental phase) were treated solely with one of the mouthwashes under study, which had previously been randomly assigned. The subjects rinsed their mouths with 10 ml of the assigned mouthwash twice a day for about 1 min. in the morning (after breakfast) and in the evening (after dinner). They also had to avoid rinsing afterwards with water, as well as eating or drinking within 30 min. after using the mouthwash.

Upon completing the first experimental phase, the subjects again received a complete oral prophylaxis and maintained excellent oral hygiene for another 14-day pre-experimental (washout) period. The second experimental phase was then carried out for a further 21 days, during which the subjects again discontinued all oral hygiene procedures and were treated with the mouthwash not used in the first experimental phase.

Experimental products

All the subjects that fulfilled the selection criteria were randomized to follow double-blind crossover treatment with both experimental mouthwashes: a *non-alcoholic mouthwash* (Gingilácer® Mouthwash; Lácer, S.A., Barcelona, Spain) (0.15% triclosan, 0.1% zinc chloride, 0.04% vitamin E and 1% xylitol) and a *hydroalcoholic mouthwash* (Gingilácer® Hydroalcoholic Mouthwash; Lácer, S.A.) (0.15% triclosan, 0.2% zinc chloride, 0.2% allantoin and 15.7% ethanol). The concentrations of both experimental mouthwashes are expressed as weight/volume. At the outset of each experimental phase, the subjects were supplied with a bottle containing 500 ml of the assigned mouthwash.

To verify treatment compliance, the subjects returned the unused medication to the researchers at the end of each experimental phase. Use of less than 60% of the scheduled amount was rated as an unsatisfactory compliance.

For homogeneity, and to prevent any interference with subsequent assessment of the response to the experimental products, all the subjects received a toothbrush (Lácer® Medium Toothbrush; Lácer, S.A.) and a tube of toothpaste (Lácer® Toothpaste; Lácer, S.A.) (1.89% Sodium monofluorophosphate and 0.2% Aldioxa) for each washout period.

The subjects were carefully instructed that it was forbidden to use any product (toothpaste, mouthwash, etc.) other than those supplied during the study.

Clinical evaluation

Gingivitis and supragingival plaque levels were assessed at days 0 and 21 of each experimental phase by means of the Löe–Silness (Löe & Silness 1963) and Turesky-modified Quigley–Hein (Turesky et al. 1970) indices, respectively. The same researcher recorded all the measurements.

The assessments were carried out on the mesial, distal, vestibular and lingual faces of the six teeth suggested by Ramfjord (1959).

At the end of each experimental phase, all the subjects were systematically asked whether they had noticed any discomfort or new symptom.

All feasible measures were taken to ensure blinding of the experimental mouthwashes, but these had a different taste, depending on the presence or absence of alcohol. To avoid any comment by the subjects concerning the possible inclusion of alcohol in the mouthwash used, one researcher recorded the gingival and plaque indices and another person questioned the subjects about any discomfort or new symptom.

Statistical analysis

Non-parametric tests were used to compare gingival and plaque indices: Mann–Whitney's test for baseline homogeneity and Friedman's repeated measures test to compare the evolution of the treatments. The chi-squared test was used to compare the frequency of adverse events. The significance level of the statistical tests was set at $\alpha = 0.05$. For descriptive purposes, the results of the gingivitis and supragingival plaque indices are presented as the mean and standard deviation and the median and inter-quartile range. Statistical analysis was carried out by the Biostatistical Department of Bioclever (Biomedical Systems Group, S.A., Barcelona, Spain) using the SAS® 6.12 software for Windows.

Table 1. Descriptive statistics for gingivitis and supragingival plaque indices at baseline and after a 21-day period of experimental treatment with each mouthwash

Index	Baseline		After treatment		*p*-value*
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Gingivitis					0.386
Non-alcoholic mouthwash†	0.30 (0.13)	0.33 (0.21–0.39)	0.38 (0.21)	0.37 (0.21–0.50)	
Hydroalcoholic mouthwash‡	0.32 (0.14)	0.33 (0.20–0.41)	0.42 (0.18)	0.41 (0.29–0.54)	
Supragingival plaque					0.804
Non-alcoholic mouthwash†	0.88 (0.24)	0.89 (0.71–1.08)	1.93 (0.13)	1.91 (1.83–2.00)	
Hydroalcoholic mouthwash‡	0.94 (0.21)	0.96 (0.83–1.12)	1.92 (0.16)	1.87 (1.79–2.08)	

*Significance of differences (compared with baseline) between groups was tested using Friedman's repeated measures test. †$n = 28$. ‡$n = 29$. SD, standard deviation; IQR, inter-quartile range.

Table 2. Distribution of adverse events reported during the experimental phases

Adverse events	Non-alcoholic mouthwash ($n = 29$)	Hydroalcoholic mouthwash ($n = 29$)
No	**21**	**13**
Yes*	**8**†	**16**‡
Oral itching	7	13
Oral soreness	—	2
Aphthous ulcer	1	—
Dry mouth	—	1

Significant differences in the appearance of adverse events (No/Yes) between treatments: chi-squared = 4.549; $p = 0.033$.
*No subject reported more than one adverse event during the use of each mouthwash.
†27.6% (95% CI: 12.7–47.2).
‡55.2% (95% CI: 35.7–73.5).

Results

Thirty subjects ranging in age from 18 to 32 years (mean age: 21.3 ± 4.0 years) and with a female predominance (22 women and eight men) were included in the study.

One subject did not complete either of the two experimental phases because of a change of address and the compliance of another subject was below the minimum requirement (it was 49% of scheduled consumption) in one of the two experimental phases (that of the non-alcoholic mouthwash). The effectiveness analysis was carried out on the subjects who correctly completed the experimental phases: 28 with the non-alcoholic mouthwash and 29 with the hydroalcoholic mouthwash. The 29 subjects who received a treatment dose were included in the safety analysis.

Both treatment groups were basally homogeneous with regard to gingival and plaque indices.

Descriptive statistics for gingivitis and supragingival plaque indices are summarized in Table 1, together with the results of the statistical analysis. The trend for both indices showed no statistically significant difference between the two treatments.

On being asked systematically, 16 subjects (55.2%) reported some adverse event during treatment with the hydroalcoholic mouthwash, a figure that was reduced to half with the non-alcoholic mouthwash (eight subjects, 27.6%) (Table 2). These differences were statistically significant (*p*-value of the chi-squared test = 0.033). Attributability of the adverse events to the non-alcoholic mouthwash was considered probable or possible in seven cases (seven reports of oral itching) and to the hydroalcoholic

mouthwash in all cases. Treatment discontinuation was not required in any case.

Discussion

After 21 days of use as the sole oral hygiene procedure, the supragingival plaque accumulation and the development of gingivitis did not differ significantly between two mouthwashes, which both contained 0.15% triclosan and zinc chloride and differed mainly in their excipient (hydroalcoholic or aqueous).

Unlike those observed in the experimental gingivitis model with a 3 weeks evolution (Löe et al. 1965, Theilade et al. 1966), on completing both experimental phases practically all our subjects had a gingival index lower than 1. Furthermore, the mean gingival indices obtained at the end of the experimental phases (0.38 and 0.42) were below 0.5, which according to the clinical experience of some authors (Bosman & Powell 1977), has been shown to be the likely score of patients who maintain good oral hygiene through their own efforts.

The experimental gingivitis model is usually implemented for a period of 14–21 days with no application of mechanical oral hygiene measures (Wennström 1988). In the present study, we opted for 21 days, as a period in which all subjects develop clinically detectable gingivitis (Löe et al. 1965, Theilade et al. 1966). This was intended to eliminate the risk that a shorter duration of the experimental phase could mask the real effects of the mouthwashes analysed.

The aim of this study was to evaluate the influence of alcohol by comparing two triclosan mouthwashes rather than to show the effectiveness of mouthwashes containing this antiseptic. Other authors have already shown the effectiveness of triclosan, whether formulated with a zinc salt (Cummins 1991, Schaeken et al. 1994, 1996, Kjærheim et al. 1996) or with a polyvinylmethyl ether maleic acid (PVM/MA) copolymer (Abello et al. 1990, Rustogi et al. 1990, Deasy et al. 1992, Kjærheim et al. 1996, Moran et al. 1997). For this reason, a placebo group was not included.

After a 21-day single treatment period, our study recorded increases of 26.7% and 31.3% in the gingival index, and 119% and 104% in the plaque index. Especially for gingivitis, these increases are better than those observed by Schaeken et al. (1994) with two triclosan/zinc mouthwashes (differing mainly in their ethanol and humectants content) using the partial mouth experimental gingivitis model. The use of different types of indices does not allow a direct comparison of our results with those obtained by other authors. Nevertheless, the aforementioned percentage increases are clearly lower than those recorded in various 21-day experimental gingivitis studies, both for placebo mouthwashes (Hugoson et al. 1974, Reddy & Salkin 1976, Siegrist et al. 1986, Gusberti et al. 1988, Brecx et al. 1990, Schaeken et al. 1994) and for mouthwashes containing such antiseptic substances as carbamide peroxide (Reddy & Salkin 1976), hydrogen peroxide (Gusberti et al. 1988), amyloglucosidase and glucoseoxidase (Hugoson et al. 1974), a phenolic compound (Siegrist et al. 1986, Brecx et al. 1990), bloodroot extract (Siegrist et al. 1986) or a solution of amine fluoride and stannous fluoride (Brecx et al. 1990). In relation to chlorhexidine, a more effective antimicrobial agent, our results differ in that, on the one hand, they are better (Brecx et al. 1990, Schaeken et al. 1994) or similar (Siegrist et al. 1986, Gusberti et al. 1988) for gingivitis development, while on the other hand they are worse (Brecx et al. 1990, Schaeken et al. 1994) or better (Siegrist et al. 1986, Gusberti et al. 1988) for supragingival plaque accumulation. Since there are evident differences in plaque accumulation and gingivitis development between young and old individuals (Holm-Pedersen et al. 1975), the foregoing comparisons are confined to studies using populations of a similar age to that of ours (18–32 years).

Although the efficacy of non-alcoholic triclosan mouthwashes has been shown previously (Kjærheim et al. 1996, Arweiler et al. 2001), we are not aware of any prior comparative evaluation of triclosan formulations differing mainly in the fact of their excipient being alcoholic or non-alcoholic.

With regard to the adverse events recorded in our study, these highlight the improvement associated with the use of the non-alcoholic mouthwash (27.6% adverse events, compared with 55.2% for the hydroalcoholic mouthwash). These adverse events, which in no case led to treatment discontinuation, may be attributed essentially to the presence of alcohol, as they coincided almost exactly with the 21-day period of use of the hydroalcoholic mouthwash.

The presence of alcohol in mouthwashes is a controversial subject in the literature. Cases of acute ethanol intoxication in children resulting from the accidental ingestion of mouthwashes have been reported (Goepferd 1983, Tipton & Scottino 1985). Even though very rare, these cases make it advisable to limit the use of alcohol in mouthwashes.

The long-term use of mouthwashes containing alcohol has adverse effects on the longevity of composites. The degree of deterioration of composites seems to bear a certain relationship to the concentration of alcohol in the mouthwash, as alcohol causes a chronic dissolution of the resin matrix which leads to a softening of the composite surface and lowers its resistance to wear (McKinney & Wu 1982, 1985, Penugonda et al. 1994, Weiner et al. 1997). Composite colour variations have also been reported (Settembrini et al. 1995).

Different studies have been conducted for many years in order to clarify a matter of controversy: the possible relationship between the chronic use of alcoholic mouthwashes and oral cancer. In 1979, a study of 200 patients with squamous cell cancer already showed a clear relationship with the consumption of tobacco and alcoholic beverages, but 11 cases had no such history. Of these, 10 had used mouthwashes many times daily for over 20 years, raising doubts as to the possible carcinogenic effect of alcohol in mouthwashes, although the authors emphasized the fact that mouthwashes contained other possible irritants, such as thymol, phenol, eucalyptol, methyl-salicylates and boric acid (Weaver et al. 1979). Later studies did not reach any clear conclusion as any connection that might exist between mouthwashes and the development of oral cancer is masked by the use of cigarettes and alcoholic beverages (Blot et al. 1983).

Newcombe & Arendorf (1983) gave an alternative explanation of such a possible association: mouthwash use could be a response to halitosis caused by an early cancer or precancerous lesions, or associated with several factors implicated in the aetiology of oral cancer, other than smoking and alcohol ingestion, namely, carious teeth, frac-

tured restorations, ill-fitting dentures and poor oral hygiene.

Many authors have concerned themselves with the subject without being able to establish a clear relationship (Wynder et al. 1983, Mashberg et al. 1985, Young et al. 1986, Kabat et al. 1989). However, Winn et al. (1991) identified a small but significant relationship between the regular use of mouthwashes with a high alcohol content (25% or more) and the development of oral cancer. Although this does not provide sufficient evidence to consider mouthwashes with an alcoholic excipient as carcinogenic, it does support the recommendation that individuals who use mouthwashes regularly should select one with an alcohol content of less than 25% (Zunt et al. 1991).

Lastly, we should mention that a more recent rigorous methodological review and analysis of the available evidence (1976–1994) does not support a causal association between mouthwash use and oral cancer (Elmore & Horwitz 1995).

If we heed the comments of the National Cancer Institute researchers (Blot 1992), it is too early to draw conclusions on the possible carcinogenic effect of the alcoholic excipient contained in mouthwashes.

Nonetheless, the minimal risk of side effects stemming from the alcohol content of mouthwashes ought to be avoided, especially when available non-alcoholic formulations show a favourable response in therapeutic equivalence studies such as the one reported here. With possible reservations because of other minor differences between the two experimental mouthwashes, the results of this study suggest that alcohol does not influence the effectiveness of a 0.15% triclosan and zinc chloride mouthwash in the development of gingivitis and the formation of supragingival plaque. Moreover, the absence of alcohol significantly decreases the incidence of adverse events produced by the mouthwash.

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Address:
José M. Almerich
University of Valencia
Faculty of Medicine and Dentistry
Gascó Oliag, 1
46010 València
Spain
E-mail: jose.m.almerich@uv.es