Antibacterial Effect of Two Toothpastes Following a Single Brushing

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Purpose: The aim of this double-blind, randomised cross-over study was to compare the antibacterial effect and the substantivity of two toothpaste formulations containing amine fluoride (AmF) or zinc chloride ($ZnCl_2$).

Materials and Methods: After a professional tooth cleaning, 20 volunteers refrained from all oral mechanical hygiene measures for the subsequent 24 hours (day 0). Subsequently, a plaque sample was taken from three teeth and analysed for vitality of the plaque bacteria by means of the vital fluorescence technique (VF_0; in %). After assessment of this baseline value the subjects had to brush their teeth for 2 minutes with 1.2 ml of the allocated toothpaste containing (a) 0.66% AmF or (b) 0.2% $ZnCl_2$. For the following 8 hours no oral hygiene measures were allowed. After 4 and after 8 hours further plaque samples were analysed for biofilm vitality (VF_4, VF_8). During the following 3 days the volunteers had to brush twice daily for 2 minutes with the allocated toothpaste. On day 4, plaque index was assessed using the criteria of Quigley and Hein (Turesky modification). After a washout time of 9 days the next test cycle with the other toothpaste was started.

Results: Both toothpastes reduced the biofilm vitality significantly at VF_4 and VF_8 compared with VF_0 ($p \leq 0.001$). While after 8 hours the vitality values for the $ZnCl_2$-toothpaste obtained significantly higher reductions (53%) than for the AmF-toothpaste (44%), results for plaque index were not significantly different (0.98 and 1.04 respectively).

Conclusions: Both toothpastes showed a significant and prolonged antibacterial effect up to 8 hours with a benefit in favour of the $ZnCl_2$ toothpaste.

Key words: amine fluoride, antibacterial agents, substantivity, toothpastes, zinc chloride

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Toothpastes are valuable in oral hygiene practices particularly in view of the additional benefits derived from constituents such as fluorides (Moran and Addy, 1984). In addition to the mechanical plaque removal capacity, toothpastes containing antibacterial ingredients should exert an inhibitory effect on remaining plaque and have substantivity to prevent plaque regrowth and metabolic activity respectively (Eley, 1999). Consequently, toothpastes can serve in the prevention of both caries and gingivitis. Metal ions, essential oils, amine fluoride (AmF)/stannous fluoride and triclosan, particularly in toothpastes, have already proven their antibacterial efficacy (Addy, 1986; Mandel, 1988). However, the complexity of toothpastes, with their different constituents (abrasives, detergents, flavourings, preservatives and humectants), may lead to mutual inactivation in clinical trials, although they have been proven to be antibacterial in simple in vitro systems (Addy et al, 1983). For example, the most efficient antibacterial agent, chlorhexidine, can be inactivated by the detergent sodium lauryl sulphate, which is an ingredient in many toothpastes (Barkvoll et al, 1989). However, an antibacterial effect can also be attributed to this detergent (Jenkins et al, 1991), so that

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Table 1 Study design

Visit	1	2	3	4	5	6	7
Day	0	7	8	11	21	22	25
Week	1	2	2	2	3	3	3
Working day	Mon	Mon	Tue	Fri	Mon	Tue	Fri
Medical history / randomising							
Supply with toothpaste and toothbrush							
Professional toothcleaning (PII=0)							
Brushing under supervision with allocated test paste							
Vitality (VF_0, VF_4, VF_8)							
Plaque index (PII)							
Questionnaire, safety							

the effectiveness of a specific agent in a complex toothpaste mixture is difficult to prove. Thus, new preparations should always be examined complete (Addy et al, 1990). For the agent zinc or zinc chloride ($ZnCl_2$), the antibacterial action has been proven both in vitro and in vivo (Afseth et al, 1983; Giertsen et al, 1989a; Giertsen et al, 1989b). Additionally, the efficiency in tartar control as well as an effect against halitosis were observed (van Steenberghe, 1997; Le Geros et al, 1999). The combination of zinc with other ingredients, e.g. sodium lauryl sulphate or fluorides, has been shown to intensify this effect (for review see Arweiler and Netuschil, 2000). AmFs have the advantage to combine the capability of remineralisation with an antibacterial effect on the mouth flora (Gülzow and Lang, 1967; Netuschil et al, 1995).

The aim of this study was to examine and to compare the antibacterial effect (over 8 hours) of two toothpastes containing either 0.66% AmF or 0.2% $ZnCl_2$.

MATERIALS AND METHODS

The study design consisted of a 4-week double-blind, cross-over, randomised, single-centre clinical trial according to the ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines for Good Clinical Practice as well as to the declaration of Helsinki. Ethical approval for the study was obtained from the ethical committee of the Albert-Ludwigs-University, Freiburg, Germany.

Volunteers

Twenty students from the dental clinic of the Albert-Ludwigs-University of Freiburg, Germany, gave signed and witnessed informed consent to participate. Written patient information was also provided. Exclusion criteria were the use of systemic antibiotics or other medicaments with ingredients exhibiting antibacterial action during the last 4 weeks before the study, less than 20 assessable teeth, crowns or restorations at sites of examination, prosthetic appliances or orthodontic treatment, known incompatibilities or allergy to toothpastes, age less than 18 years old and pregnancy.

Study design

Table 1 shows the design of the study. After taking a medical history, a professional tooth cleaning was performed at the beginning of a 1-week hygiene period in order to standardise oral conditions. A toothpaste containing sodium fluoride (Dr. Scheller Cosmetics AG, Eislingen, Germany) and one toothbrush (durodont dental® mittel, Dr. Scheller durodont GmbH, Eislingen, Germany), which the volunteers had to use during the hygiene phase and the wash-out period, were distributed.

At the beginning of each of the two test weeks (day 0), the teeth were stained with a disclosing solution (free of erythrosine; Mira-2-Ton, Hager & Werken, Duisburg, Germany) and a supragingival scaling and rubber cup polishing (PII=0) was performed. The volunteers were not allowed to practice any kind of oral hygiene for the next 24 hours. The next day (exactly after 24 hours) a plaque sample was taken from three teeth (16, 15, 36) with a sterile dental probe, smeared on a slide, stained with two fluorescent dyes and analysed by means of the vital fluorescence technique as described by Netuschil et al (1995). Vital bacterial cells were stained green by fluorescein diacetate (FDA) and dead cells received a red colouration from ethidium bromide (EB). Under the fluorescence microscope (Axioskop 2 plus, Zeiss, Göttingen, Germany) the percentage of the vital (green stained) bacteria compared with the total stained bacteria (red and green) was calculated (biofilm vitality; VF, %). This first sample represented the baseline value (VF_0). Subsequently, the volunteers brushed their teeth with the modified Bass technique for 2 minutes using the randomly allocated test toothpaste and a new toothbrush (durodont dental® mittel). After toothbrushing they expectorated the rest of the dentifrice and rinsed with water only once. To provide a standardised and exact dosage of the toothpaste, an amount of 1.2 ml was measured in a disposable syringe shortly before brushing. This procedure also had to be performed during home use. Further plaque samples were taken from other teeth (26, 25, 46 and 17, 14, 37) 4 (±1) hours and 8 (±1) hours after brushing to examine plaque vitality at these time points (VF_4, VF_8). During this time the volunteers were not allowed to practice any kind of oral hygiene.

In the evening of the same day, and in the morning and evening of the next 2 days they had to brush their teeth with the test paste, as described above. These tooth brushings were unsupervised, but the volunteers documented their brushing times in a diary. In the morning of day 4, the volunteers came without having brushed their teeth. After staining the teeth, the plaque index according to the Turesky et al (1970) modification of the Quigley and Hein plaque index was recorded for teeth 16, 36, 44, 11, 12, 21, 22, 31, 32, 41, 42 (Ramfjord teeth) and the mean values for each volunteer were calculated (PII).

For the following 9 days, the volunteers were allowed to practice their usual oral hygiene with the dentifrice and a standardised toothbrush for the wash-out period. After this wash-out period the next test cycle was started, in which each volunteer used the other test toothpaste.

Undesirable events and any side effects were recorded at every visit. The systemic use of any medicaments with antibacterial ingredients had to be notified and led to exclusion from the study.

Test products

One toothpaste ($ZnCl_2$) contained 0.2% $ZnCl_2$ and 0.316% sodium fluoride (1450 ppm F^-) and had an RDA value of 55. The other toothpaste (AmF) contained 0.66% AmF (500 ppm F^-) and 0.21% sodium fluoride (950 ppm F^-) as the main ingredients with an RDA value of 40. All test products as well as the toothbrushes were supplied by Dr. Scheller Cosmetics AG (Germany). All the pastes were filled in white, neutral tubes of 125 ml each. The coded tubes were distributed in a randomised order and provided with the number of the volunteer, so that after the return the pastes could be assigned to the respective volunteer.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package of Social Science/SPSS) 11.0. For each toothpaste the average vitality values of the supragingival plaque flora for the three time-periods (VF_0, VF_4, VF_8) and the clinical parameter (PII) were calculated. The data were checked for normal distribution by Kolmogorov-Smirnov test. Since the data records were not normally distributed, non-parametric tests were employed. Analysis of variance (ANOVA) showed significant differences between the groups and the time points. Wilcoxon-rank-sum test was performed to detect significant differences compared with baseline (VF_0) and between the two pastes.

RESULTS

All volunteers (10 female and 10 male), with an average age of 24.2 years (between 23 and 31 years), participated until the end of the study. Based on the given results (reductions of 42–53%; standard deviation between 3.5 and 5.5) and a level of significance of $\alpha = 0.05$, a power of 0.99 yielded for the study (http://www.stat.ucla.edu/calculators/powercalc/).

Fig 1 and Fig 2 present the mean results for the toothpastes. Both toothpastes achieved highly significant reductions ($p < 0.001$) in vitality of the plaque flora compared with their baseline values (VF_0). Paste $ZnCl_2$ showed a vitality reduction of 48% (VF_4) and

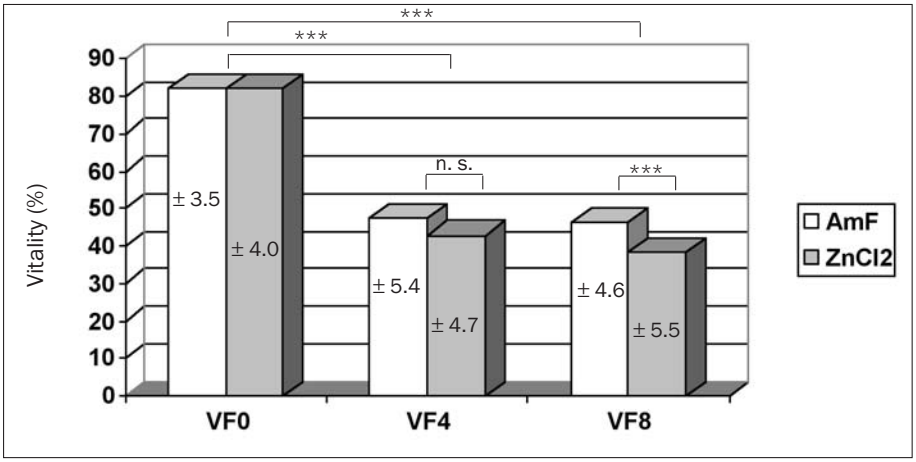

Fig 1 Mean vitality scores (VF; ± standard deviation) at the various time points, statistical comparison with VF_0 and between the toothpastes (by Wilcoxon test; ***: $p \leq 0.001$, n.s.: not significant).

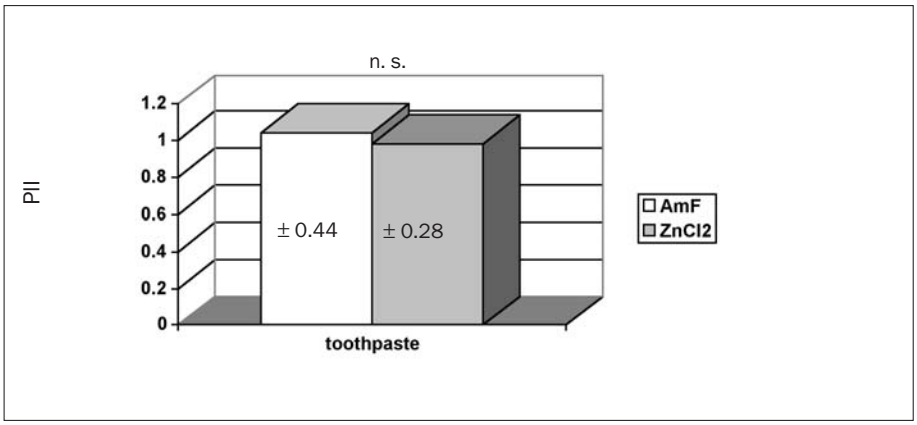

Fig 2 Mean values (± standard deviation) of the plaque index (PII), statistical comparison between the pastes (by Wilcoxon test; n.s.: not significant).

53% (VF_8), while paste AmF exhibited reductions of 42% and 44%. Comparing both toothpastes, the $ZnCl_2$ paste showed significantly higher reductions after 8 hours ($p < 0.001$). The PII for the $ZnCl_2$ paste was 0.98, and 1.04 for the AmF paste, which did not differ significantly.

At the end of each test cycle, the volunteers answered a questionnaire about flavour, taste disturbance, burning mouth, staining, and the condition of the gum during the application of the respective toothpaste (Table 2). Most of the 20 volunteers described the taste of both toothpastes as pleasant (AmF, n = 14; $ZnCl_2$, n = 15). Taste disturbance was noticed by five volunteers (25%) during the use of the AmF paste, and by seven volunteers (35%) using the $ZnCl_2$ paste. Irritation and burning was indicated once for the AmF paste, and twice for the $ZnCl_2$ paste. Fourteen volunteers (70%) would use the $ZnCl_2$ paste and 13 (65%) would use the AmF paste in the future.

DISCUSSION

A long-term antibacterial effect has been promoted as a positive property of toothpastes in general and, for example, has been claimed to last until the next toothbrushing for a triclosan/copolymer paste (Gaffar et al, 1994; Gaffar et al, 1997). In the clinical situation the question is if brushing twice daily is able to inhibit or reduce plaque regrowth.

In many long-term studies (3 to 6 months) the efficacy of antibacterial toothpastes was investigated using plaque indices as well as gingival indices (Shapira et al, 1999; Mankodi et al, 2002; Rosin et al, 2002; Adams et al, 2003). However, index data are superposed by mechanical brushing, which is necessary for ethical reasons. Because of this, most toothpaste studies have not proven superiority of any active toothpaste compared with corresponding placebo pastes or can only find them equivalent to other benchmark products (Shapira et al, 1999; Rosin et al, 2002). Therefore,

Table 2 Results of the questionnaire ('quality of life') (in total 20 volunteers, concerning flavour multiple choices)

	AmF n (%)	$ZnCl_2$ n (%)
Flavour		
Pleasant	14 (70)	15 (75)
Unpleasant	3 (15)	4 (20)
Biting	2 (10)	2 (10)
Mild	3 (15)	2 (10)
Bitter	/	/
Freshness		
Yes	16 (80)	18 (90)
No	4 (20)	2 (10)
Change of taste		
Yes	5 (25)	7 (35)
No	15 (75)	13 (65)
Unpleasant feeling, e.g. burning		
Yes	1 (5)	2 (10)
No	19 (95)	18 (90)
Condition of the gingiva after use		
Well kept	3 (15)	3 (15)
Tightened	3 (15)	3 (15)
Unaltered	12 (60)	12 (60)
Irritated	0 (0)	1 (5)
Other	2 (10)	1 (5)
Discolouration of the teeth		
Yes	0 (0)	0 (0)
No	20 (100)	20 (100
Would you use the toothpaste regularly?		
Yes	13 (65)	14 (70)
No	7 (35)	6 (30)
Remarks	• very smooth teeth, like a thin layer on the teeth • unpleasant/bitter aftertaste (n = 2)	• very dry mouth • astringent (n = 2)

long-term brushing studies are not well-suited to investigating the efficacy of antibacterial ingredients.

The choice of an appropriate study design for the evaluation of antibacterial properties of toothpastes is difficult because toothbrushing with toothpastes has both a mechanical and an antibacterial effect. Studies that use toothpastes in the form of 'slurries' (a mixture of the toothpaste with water) are suitable for examining the efficacy of active ingredients, because the influence of mechanical plaque removal is eliminated. Although this mixture should imitate the dilution of the paste in the mouth through saliva, it cannot represent the real conditions of brushing. In the present study, the design was chosen to evaluate the toothpastes under almost normal conditions.

The present study recorded bacterial vitality before and during two time periods following a single brushing with a toothpaste formulation. This method followed, with slight modifications, the pattern of other studies examining toothpastes and their ingredients by recording the magnitude and duration of salivary bacterial counts (Moran et al, 1988; Jenkins et al, 1990a) or by examining bacterial vitality after a single brushing (Arweiler et al, 2002a; Gaffar et al, 1994). For antimicrobials these methods are accepted as measurements of their substantivity (Roberts and Addy, 1981). Moreover, a good correlation between plaque inhibition and the effect on salivary bacterial counts (Addy et al, 1989, 1990; Jenkins et al, 1990b) as well as between plaque inhibition and the effect on bacterial vi-

tality (Arweiler at al, 2002a) has already been reported.

A 'not affected' plaque sample taken before brushing served as a control. Thus, comparisons could be made between baseline and both toothpastes, and no placebo paste was needed similar to other studies (Binney et al, 1996; Moran et al, 2001).

For two further reasons the study was performed without a placebo paste. Firstly, the choice of a control for toothpaste studies is still a subject of debate. Commonly, the following controls had been proposed: true placebos, e.g. water, which are, however, only of limited value; a formulation minus the active ingredient $ZnCl_2$; a formulation minus the active ingredient AmF; or a benchmark commercial product (Addy et al, 1992). However, the use of every control would have advantages and disadvantages.

Secondly, it was important to position the two newly formulated (AmF) toothpastes relative to an established one ($ZnCl_2$) (Arweiler et al, 2002b). Moreover, an antibacterial effect compared with a placebo could already be assumed for both toothpastes.

Both toothpastes were able to reduce the number of vital bacterial cells in the plaque biofilm that remained after toothbrushing, and they also exhibited an antibacterial effect on the attached plaque for the next 8 hours (substantivity). Possibly, not only the main active ingredients of the toothpastes, $ZnCl_2$ and AmF, are responsible for this action, but also a synergism with other ingredients that are included in the pastes is possible (Jenkins et al, 1991). Further ingredients with an antibacterial potential were propylene glycol in the AmF toothpaste and sodium lauryl sulphate and divers parabens in the $ZnCl_2$ toothpaste.

An antibacterial action and a good retention for zinc and zinc salts have been proved previously (Saxton, 1986; Moran et al, 1988; Günbay et al, 1992).

Moreover, a good correlation between vitality values from the present study and the results of a previous study with the same design was found, in which a 0.2% $ZnCl_2$ paste, with only a few modifications of the ingredients, was used (Arweiler et al, 2002a).

Generally, since toothbrushing removed most of the plaque mechanically, the assessment of plaque index should not be overestimated in this kind of study. Four days of brushing seems to be too short to determine an effect on plaque formation, which, however, inevitably results from an antibacterial action against the dental biofilm. In the present study, the assessment of the plaque index 12 hours after the last brushing served as a secondary evaluation parameter. However, the inhibition of plaque formation by zinc has been shown in other clinical studies (Saxton, 1986; Günbay et al, 1992). In spite of these positive results, the use of zinc in clinically active amounts is limited for many manufacturers due to the known astringent effect of the zinc ion. This correlated with the subjective data of the volunteers according to the flavour and the condition of the gum after the use of the zinc paste.

AmFs are incorporated in toothpastes more often than zinc because they exert an excellent caries preventive efficacy. In several investigations the antibacterial action of AmFs as well as the superiority of AmFs over inorganic sodium fluoride in their caries preventive action (fluoride uptake by enamel, resistance of enamel to decalcification by acid) could be confirmed (Gülzow, 1983; Dolan et al, 1974; Schneider and Mühlemann, 1974). Other reports have shown that AmFs lead to an inhibition of anaerobic bacterial glycolysis and of acid production in vitro (Hermann et al, 1958; Shern et al, 1970; Gehring, 1983). Due to their surface activity, the AmFs are rapidly dispersed in the oral cavity and wet all surface areas. Particularly, tooth surfaces are covered with a homogenous molecular layer (Hassel et al, 1971). It is assumed that this continuous film prevents washing off by saliva. The AmFs are thus available as an active agent for a prolonged period of time.

Similar to the $ZnCl_2$ toothpaste, not all volunteers liked the taste of the AmF paste. Some volunteers complained about a bitter aftertaste, which was covered with a very strong anise taste only for a short time. It was interesting that some volunteers noticed a thin layer/film on their teeth, which points to the aforementioned surface-active properties of the AmFs.

Comparing both toothpastes, it was shown that the $ZnCl_2$ paste had a stronger effect on the vital bacterial cells than the AmF dentifrice. However, it should be mentioned that the AmF concentration in the present study (0.66%) was lower than in the studies of Gülzow (1983) (1%) or Mühlemann (1967) (1.15%), and in the commercially available Elmex® toothpaste containing about 1.65% pure AmF.

In the literature, there are no data with which to compare the vitality values. A similar study examined the change in bacterial vitality of toothpastes used as slurries without any mechanical effect (Arweiler et al, 2002a). Besides a chlorhexidine solution (0.1%; Chlorhexamed® fluid), the toothpastes Colgate Total® and Parodontax® yielded an antibacterial effect up to 12 hours (Arweiler et al, 2002a). After 8 hours Chlorhexamed® reduced the vitality to 47% according to the baseline values, the toothpaste Colgate Total® and Parodontax® showed a reduction of 38% and 32% respectively. However, the results are only partly comparable since one study evaluated slurries that react-

ed only chemically with the dental biofilm, while in the other study the biofilm was removed to a certain extent due to toothbrushing.

CONCLUSIONS

The results of this study demonstrate the marked and prolonged reduction in bacterial vitality by both toothpastes, which is remarkable even 8 hours after a single toothbrushing. While both toothpastes showed similar results in plaque index and at VF_4, the $ZnCl_2$ paste seemed to be superior to the AmF paste only at VF_8. Both dentifrices are safe and acceptable, and two-thirds of the volunteers said they would use the toothpastes regularly in the future.

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