Effect of Dentifrices with Antimicrobial Agents on Mutans Streptococci in Saliva and Approximal Dental Plaque in Orthodontic Patients

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Purpose: The aim of the present investigation was to study the effect of daily use of fluoride dentifrices containing various antimicrobial agents on mutans streptococci (MS) in saliva and approximal dental plaque.

Materials and Methods: Fifty-nine healthy adolescents, 12–14 years old, undergoing orthodontic treatment with fixed appliances and harbouring high levels of MS in saliva and preferably also in interdental plaque, were randomly distributed into four groups, using dentifrices with: 1) zinc lactate (n = 16), 2) amine fluoride-stannous fluoride (n = 13), 3) triclosan (n = 15), and 4) no antimicrobial agent (control; n = 15). Changes of MS scores versus baseline were determined after 1, 3 and 6 months, using the Dentocult® SM Strip mutans test.

Results: At the 6-month sampling occasion, the subjects using dentifrice with either amine fluoride-stannous fluoride or triclosan showed a tendency to lower MS scores in interdental plaque ($p < 0.05$). In saliva and in the 1- and 3-month plaque samples, no changes of MS were detected in any of the four groups.

Conclusion: This 6-month clinical study showed that dentifrices with various antimicrobial agents only result in small or no changes of the MS scores in saliva and approximal dental plaque in orthodontic patients.

Key words: amine fluoride, caries prevention, dentifrice, mutans streptococci, stannous fluoride, triclosan

Oral Health Prev Dent 2007; 5: 223-227. *Submitted for publication: 24.04.06; accepted for publication: 10.10.06.*

Treatment with fixed orthodontic appliances with bands, brackets and wires is considered to negatively interfere with oral hygiene requirement and favour dental plaque accumulation, especially at interdental sites (Øgaard et al, 1988; Travess et al, 2004). These conditions may jeopardise the ecology of different microorganisms in the oral environment. An accumulation of cariogenic microorganisms, such as mutans streptococci (MS), in the plaque may contribute to a potential risk of developing early enamel caries lesions during the orthodontic treatment (Eriksen and Dimitrov, 2003). After bonding, the MS level has been found to increase up to 2–3 times within a few months (Jordan and LeBlanc, 2002).

Today's lifestyle with frequent intake of sugar among adolescents makes it important to suppress the cariogenic microflora (Macpherson et al, 1990; Zero, 2004). A number of antimicrobial agents, e.g. chlorhexidine, amine fluoride, stannous fluoride and triclosan, have been shown to inhibit plaque formation, bacterial metabolism and numbers of MS (Baehni and Takeuchi, 2003; Marsh, 2004). Such agents applied either professionally or in dentifrice and mouthwash solutions, may be useful in order to minimise the caries risk. The aim of the present investigation was therefore to study if daily use of dentifrice containing

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Table 1 Antimicrobial agents and main ingredients in the four dentifrices studied

Dentifrice (number of subjects)	Antimicrobial agents, concentration	Other main ingredients*	Brand name Manufacturer
Zinc lactate (n=16)	Zinc lactate, 0.73%	Glycerin, silica, sodium lauryl sulphate	Blend-a-med Classic Procter & Gamble, Austria
AmF-SnF_2 (n=13)	Amine fluoride and stannous fluoride, 0.14% F	Sorbitol, silica, silica hydroxyethylcellulose	Meridol® Gaba International, Switzerland
Triclosan (n=15)	Triclosan, 0.3%	Sorbitol, silica, glycerin, tetrapotassium	Blend-a-med Zeit Active Procter & Gamble, Austria
Control (n=15)		Sorbitol, silica, trisodium phosphate, sodium lauryl sulphate	Blend-a-med Milde Frisch Procter & Gamble, Austria

*All dentifrices contained 1450 ppm F as NaF or as AmF-SnF_2 dentifrice.

various antimicrobial agents reduces the number of MS in saliva and approximal plaque in adolescents with fixed orthodontic appliances.

MATERIALS AND METHODS

Subjects

Fifty-nine healthy 12–14-year olds, with high salivary counts of MS, according to the method described by Jensen and Bratthall (1989), were selected from a group of children referred to Department of Orthodontics, Halmstad, Sweden. The orthodontic treatment was carried out in both arches with fixed appliances. Brackets were bonded with a light-cured composite material (Transbond™, 3M, Monrovia, California, USA), and molar bands with a glass ionomer cement (AquaCem®, Dentsply De Trey, Weybridge, UK). The study was approved by the Regional Ethics Committee, Lund University, Sweden. Informed consent to participate was given by the parents of the children.

Dentifrice groups

The selected children were randomly distributed into four groups: 1) zinc lactate group, 2) amine fluoride-stannous fluoride group (AmF-SnF_2), 3) triclosan group, and 4) control group. The main ingredients of the dentifrices are shown in Table 1. The fluoride content was similar in the four products (approximately 1450 ppm F). All children were instructed to brush their teeth twice a day for 2 minutes with approximately 1.5 cm (approximately 1 g) of dentifrice each time. Dentifrice and new brushes were delivered throughout the study. The compliance was carefully checked by one author (KM).

During the entire test period, all children had regular check-ups at the Public Dental Health Clinic and received regular instructions in oral hygiene. All orthodontic patients in the County of Halmstad are recommended to rinse daily with a 0.05% NaF solution.

Sampling method

Sampling was carried out at baseline (BL) and after 1, 3 and 6 months. MS in saliva and approximal plaque was determined using a chair-side method (Dentocult® SM Strip mutans, Orion Diagnostica, Helsinki, Finland). After isolation and gentle air-blow of the approximal area, a small Paint On brush (Vivadent, Schaan, Liechtenstein) was slightly wetted in sterile saline and then inserted between the first permanent molar and the second premolar in each quadrant. Thus, four samples were collected from each subject. The brush was thereafter immediately stroked twice (back and forward) across the plastic strip. The saliva samples were collected during one minute of paraffin chewing by the subjects, and then inoculated on a Dentocult® SM Strip mutans. All plaque and saliva strips were incubated at 37°C for 2 days, according to the manufacturer's instructions.

Table 2 Changes of the MS level compared with baseline in saliva recorded as a decrease (-), an increase (+) or as no change (0) at 1, 3 and 6 months

	Zinc lactate (n = 16)			AmF-SnF_2 (n = 13)			Triclosan (n = 15)			Control (n = 15)		
Changes	-	0	+	-	0	+	-	0	+	-	0	+
1 month	5	8	3	5	6	2	7	5	3	4	8	3
3 months	3	10	3	5	7	1	7	5	3	3	10	2
6 months	7	7	2	4	6	3	5	7	3	4	7	4
1–6 months	15	25	8	14	19	6	19	17	9	11	25	9

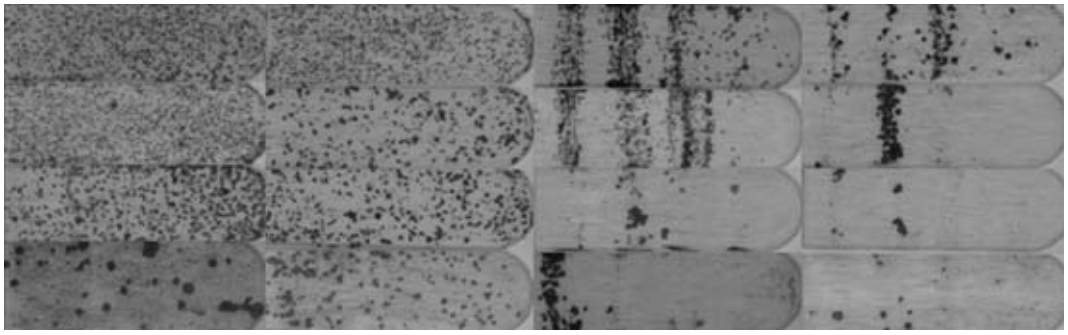

Fig 1 MS in saliva and in approximal dental plaque at baseline and after 1, 3 and 6 months in a subject brushing with a dentifrice containing either triclosan or a mixture of amine fluoride and stannous fluoride (AmF-SnF_2).

Scoring method

The cultivated strips were stored at room temperature (22°C) and kept on sheets for later scoring. When all saliva and plaque samples had been collected from a patient, the strips at BL, 1, 3 and 6 months were compared using a stereomicroscope. Thus, all four samples were evaluated at the same occasion. The level of MS in saliva and in the interdental plaque was recorded as the number of 'decreased', 'unchanged' or 'increased' CFU comparing BL with 1, 3 and 6 months (Fig 1). If the number of CFU on the strips differed ± 20%, a decrement (score -) or an increment (score +) was recorded, or an unchanged (score 0) score was determined, and calculated statistically with chi-square test. This scoring system resulted in a count of +, -, or 0 scores for the saliva samples (n = 59) and for the approximal sites (n = 59 x 4 = 236).

Statistics

When analysing the MS scores, the patient was used as a unit for the saliva samples and each site for the plaque samples. All data (i.e. BL vs. 1 month, BL vs. 3 months and BL vs. 6 months), within each dentifrice group, were compared using a chi-square test. For the reliability of the readings, 30% of the strips were analysed twice; the Pearson correlation coefficient was 0.94.

RESULTS

The results of the MS scores of the saliva samples are presented in Table 2. There was no statistically significant difference in any of the four groups after 1, 3 or 6 months. If all sampling occasions are combined (1 + 3 + 6 months), there were more samples showing a decreased (score -) than an increased (score +) score. However, most of the saliva samples were unchanged (score 0).

The results of MS scores of the approximal plaque samples are presented in Table 3. There were more samples showing a decrease (score -) for AmF-SnF_2 and triclosan than an increase (score +). An opposite trend was found for the zinc lactate and control groups. When analysing the data with the chi-square test, only

Table 3 Changes of the MS level compared with baseline in approximal plaque recorded as a decrease (-), an increase (+) or as no change (0) at 1, 3 and 6 months

	Zinc-lactate (n = 64)			AmF-SnF_2 (n = 52)			Triclosan (n = 60)			Control(n = 60)		
Changes	-	0	+	-	0	+	-	0	+	-	0	+
1 month	17	27	20	17	16	19	20	24	16	15	22	23
3 months	13	27	24	14	16	22	19	24	17	13	28	20
6 months	12	18	34	20*	12	20	24*	24	12	6	24	30
1–6 months	42	72	78	63	44	61	63	72	45	34	74	73

* $p < 0.05$

the 6-month samples for the AmF-SnF_2 group and the triclosan group showed small, but statistically significant decreases ($p < 0.05$). However, at 1 and 3 months, no significant changes compared with baseline were found. Representative strips from the AmF-SnF_2 group and the triclosan group are shown in Fig 1.

No patient complained about the taste of any of the four dentifrices and no adverse effect was found. When calculating of the amount of dentifrice used during the 6 months, it could be concluded that all subjects had followed the instructions given, i.e. brushing twice a day using approximately 1 g of paste each time.

DISCUSSION

The present study was designed to evaluate the effect on MS in saliva and approximal plaque in adolescents with fixed orthodontic appliances after brushing with dentifrice containing different antibacterial agents over 6 months. Orthodontic patients were chosen since it has been shown that treatment with fixed appliances increase the number of MS in the oral cavity (Jordan and LeBlanc, 2002). There is a general consensus that regular use of fluoride dentifrice is a safe and efficient preventive regimen in young individuals (Twetman et al, 2003). However, in orthodontic patients there may be a need for dentifrice that contains an antimicrobial agent to prevent tooth demineralisation and gingivitis (Øgaard et al, 2005)

The method of scoring MS in saliva and at interdental plaque using Dentocult® SM Strip mutans has been used in previous studies (Jensen and Bratthall, 1989). However, the scoring method was modified in the present study. According to Twetman and Petersson (1998, 1999), the evaluation of colonisation of MS was done in two categories, those lower than 20 CFU and those higher than 20 CFU. However in the present study, we found most chair-side strips were colonised with >20 CFU. Therefore we decided to evaluate the result after a modified method based on changes of MS colonization up to 6 months, counting >20% of CFU as an increase or <20% of CFU as a decrease compared with the CFU counts at baseline. Those strips showing ± 20% of CFU thus were evaluated as unchanged. By using this modified technique, the results were independent of the uncertainty in calculating exactly the CFU, but we were still able to determine if the strips harboured great variations of CFU even with high MS colonisation.

In the AmF-SnF_2 group, there was a slight decrease of MS in approximal plaque after 6 months ($p < 0.05$; Table 3). Both SnF_2 and AmF have been used as anticaries agents since the 1950s and the antibacterial effect of SnF_2 is well documented (Boyd and Chun, 1994). Long-term evaluation of SnF_2 gel has also been shown to reduce gingivitis and decalcifications of white spot lesions in adolescents undergoing orthodontic treatment (Boyd, 1994). It has been demonstrated that a combination of AmF and SnF_2 reduce the viability of oral bacteria and inhibit acid production and bacterial accumulation on tooth surfaces (Axelsson, 1993; Embleton et al, 2001).

In the triclosan group, there was a slight decrease of approximal MS after 6 months' use as for the AmF-SnF_2 group ($p < 0.05$; Table 3). Triclosan has a broad antimicrobial spectrum and affects both Gram-positive and Gram-negative bacteria by damaging the cell-wall membrane (Baehni and Takeuchi, 2003). Triclosan is active against MS in combination with xylitol and shows anti-inflammatory properties (Bradshaw et al, 1993; Renton-Harper et al, 1996; Jannesson et al, 2002; Brading et al, 2004). However, dentifrice that contained triclosan showed only a slight reduction of MS in saliva after 6 months compared with baseline

(Renvert and Birkhed, 1995), in agreement with the present results.

To conclude, the results of the present 6-month study indicate that daily use of fluoride dentifrices containing either AmF-SnF_2 or triclosan might have a slight effect of number of MS at approximal sites in patients undergoing fixed orthodontic treatment. No effect was found after 1 and 3 months. Further studies should therefore be conducted before dentifrices that contain antimicrobial agents can be recommended to this group of caries-risk patients.

ACKNOWLEDGEMENTS

We are grateful to the staff of the Orthodontic Specialist Clinic, Halmstad, and the Public Dental Service, Laholm, County of Halland. We also thank Dr. Amir Baigi, Research and Development, Central Hospital, Halmstad, for statistical advice.

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