Effect of a triclosan-containing dental gel on the levels of prostaglandin I_2 and interleukin-1β in gingival crevicular fluid from adolescents with fixed orthodontic appliances

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Sköld-Larsson K, Yucel-Lindberg T, Twetman S, Modéer T. Effect of a triclosan-containing dental gel on the levels of prostaglandin I_2 and interleukin-1β in gingival crevicular fluid from adolescents with fixed orthodontic appliances. Acta Odontol Scand 2003;61:193–196. Oslo. ISSN 0001-6357.

The effect of a triclosan-containing (0.3%) dental gel on inflammatory mediators in gingival crevicular fluid (GCF) was evaluated in 14 healthy adolescents undergoing orthodontic treatment with fixed appliances. A double-blind randomized split-mouth study design was used with color-coded experimental and placebo gels. The gel was self-applied for 5 min twice daily for 14 days in custom-made soft plastic trays. Clinical data (visible plaque index (VPI) and gingival bleeding index (GBI) and samples of GCF were collected at baseline and after 1, 2, 4, and 6 weeks. The concentrations of prostaglandin I_2 (PGI_2) and interleukin-1β (IL-1β) were determined by radioimmuno- and enzyme-linked immunosorbent assays, respectively. No clinical effects of the gel applications regarding amount of plaque or gingival bleeding were unveiled. Neither the experimental nor the placebo gel applications caused any statistically significant alterations in the inflammatory mediators, PGI_2 and IL-1β, compared to baseline. In conclusion, the present study did not reveal any beneficial effects of the triclosan-containing gel regimen on mild gingivitis in adolescents with fixed orthodontic appliances. □ *Interleukin-1β; orthodontics; prostaglandin; triclosan*

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Orthodontic treatment with fixed appliances is associated with extensive plaque accumulation that might jeopardize oral health, enamel demineralization and gingivitis, which are frequently reported side effects (1, 2). In prevention, local applications of antibacterial agents and fluoride have been suggested in addition to thorough oral hygiene instruction. The most frequently used and evaluated antibacterial drug in this respect is without doubt chlorhexidine (3, 4). In recent years, however, the broad spectrum antimicrobial agent triclosan has been introduced in dentistry and a reduction in supragingival plaque, gingivitis, hypersensitivity and caries has been documented in various clinical trials (5). Triclosan has been added to toothpaste and is also available as a mouth rinse. The mechanisms of action are not fully clear, but it has been suggested that the diphenyleter derivate interferes with metabolic pathways over the bacterial cell membrane, and compromises the functional integrity without inducing cell lysis (6, 7). Furthermore, it is anticipated that the agent may block fatty acid production through enzyme affection (8). Previously, it was generally assumed that triclosan's effect on gingival inflammation was due to its antimicrobial and anti-plaque effect, but in vitro studies have now suggested that triclosan may have a direct anti-inflammatory effect on the gingival tissues (9–11). A novel preparation, namely a 0.3% triclosan gel, has recently been developed for self-administration. Such a regimen might be especially beneficial as targeted action for subjects at temporary high risk of developing caries and gingivitis, such as orthodontic patients. The aim of the present study was therefore to evaluate the effect of a triclosan-containing dental gel on the gingival conditions of patients undergoing treatment with fixed orthodontic appliances, and assessed by the levels of selected prostaglandins and interleukins, in gingival crevicular fluid. The hypothesis was that the active treatment would decrease the levels of inflammatory mediators compared to the corresponding placebo gel treatment.

Material and methods

Subjects

The study group comprised 14 healthy, non-medicating and non-smoking adolescents (6 boys and 8 girls) with a mean age of 16.5 years (14–19 years) invited from the patient stock at the Department of Orthodontics, Medical and Dental Center, Halmstad, Sweden. Consent was obtained from the participants and from their parents after verbal and written information had been given. The inclusion criteria were ongoing orthodontic treatment with fixed maxillary appliances on at least 10 teeth and clinical signs of gingivitis in at least two buccal sites in each quadrant adjacent to their bands and brackets. The total expected orthodontic treatment time was 12–15 months

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DOI 10.1080/00016350310003242

Table 1. Composition of the experimental and placebo gels

	Experimental gel (%)	Placebo gel (%)
Crystal mint	0.30	0.30
Glycerine	20	20
Sodium CMC	2	2
Sodium fluoride	0.243	0.243
Sodium hydroxide (pH adjuster 6.8)	15.2	15.2
Sodium lauryl sulfate	0.60	0.60
Sorbitol	15	15
Triclosan (Irgacare MP)	0.30	–
Water and colour dispersion	44.7	45
Total	100	100

and the selected subjects had had their appliances for at least 4 months. The participants were inhabitants in a community with low fluoride levels in the drinking (tap) water (<0.1 ppm) and they brushed their teeth twice daily with fluoridated toothpaste. The local ethics committee at Lund University approved the study design.

Study design

The study had a randomized double-blind split-mouth design. After inclusion and baseline registration, the subjects were instructed to apply a standard amount of a color-coded experimental or placebo gel in a custom-made tray for a period of 14 days. The experimental gel was randomly assigned to the 1st or 2nd quadrant with the placebo gel on the opposite side. The gels were self-applied twice a day for 5 min each in custom-made soft plastic trays covering the posterior parts (canine to second molar) of the left or right upper quadrant, leaving the incisors untreated. The left and right sides of the trays were marked with the same color as the gel that should be applied therein. Immediately after each gel application, the participants were asked to rinse the oral cavity thoroughly in tap water. Both the test persons and the investigators were blinded to the active and placebo gel. Follow-up clinical registrations were carried out after 7 and 14 days (end of gel treatment) and then 2 and 4 weeks after termination of the treatment. The compliance was checked regularly during the 2-week treatment period. The experimental gel was colorless and contained 0.3% triclosan as the active ingredient and was provided along with the placebo gel from the Colgate Palmolive Technology Center, Piscataway, NJ, USA. The composition of the gels is given in Table 1.

Clinical registrations

The amount of dental plaque and degree of gingival inflammation adjacent to the brackets were scored on all maxillary teeth with the visible plaque index (VPI) and gingival bleeding index (GBI), respectively (12). A sample of gingival crevicular fluid (GCF) from two selected sites in each quadrant was collected using paper strips (Perio-paper, ProFlow, Amenityville, NY, USA) inserted in the gingival sulcus at the mesiobuccal corner for 15 s after gentle drying with compressed air. The volume of GCF (μl) was immediately determined with a Periotrone® 8000 (ProFlow). The strips were then transferred into small eppendorf tubes containing 120 μL assay buffer (0.9% NaCl, 0.01M EDTA, 0.3% bovine γ-globulin, 0.005% Triton-X-100, 0.05% sodium azide, 0.0255M NaH_2PO_4, 0.0245M Na_2HPO_4, pH 6.8) and 0.15 mM indomethacin (Sigma Chemical Co., St Louis, Mo., USA) and kept frozen at −70°C until further processing.

Laboratory procedures

The concentration of PGI_2, measured as 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), was determined with commercial radioimmunoassay kits from PerkinElmer Life Sciences (New England Nuclear Research Products Boston, Mass., USA). The level of IL-1β was analysed with an enzyme-linked immunosorbent assay (Amersham Life Science Ltd, Amersham Place, Little Chalfont, Buckinghamshire, England) as previously described (13, 14). The protein levels in GCF samples were determined using Bio-Rad DC Protein assay (Bio-Rad Laboratories).

Statistical method

The data obtained during treatment as well as the post-treatment values were compared with baseline with ANOVA for repeated samples.

Results

Compliance with triclosan-containing gel treatment was excellent and no subjective or objective adverse effects were noted. The mean value of VPI at baseline was 31%, and 10% of the participants had a GBI of >20%. Neither the experimental nor the placebo gel affected the clinical GBI and VPI indices at any of the follow-ups (Table 2). The mean volume of GCF was 0.40 μL ± 0.10 (*s*) in the experimental and placebo quadrants at baseline (range 0.3–0.8 μL) and the mean values remained unchanged during the follow-ups. The concentrations of PGI_2 and IL-1β in the GCF samples at baseline and at the designated follow-ups are presented in Table 3. No significant differences were found between the two separate sites in the experimental or placebo quadrants and mean values of each quadrant were calculated. Treatment with the triclosan-containing gel did not result in any statistically

Table 2. Clinical variables VPI (%), GBI (%) and volume of GCF (μl) expressed as mean ± *s* at baseline and after the 2-week treatment period with a triclosan-containing dental gel or a placebo gel in adolescents with fixed orthodontic appliances (*n* = 14)

	Experimental gel			Placebo gel		
Time	VPI	GBI	GCF	VPI	GBI	GCF
Baseline	30	36	0.40 ± 0.10	32	40	0.40 ± 0.10
2 weeks	28	30	0.36 ± 0.14	30	34	0.39 ± 0.10

s = standard deviation.

significant alterations of the inflammatory mediators compared to baseline or in comparison with the placebo gel treatments at any follow-up. A tendency towards decreased PGI_2 values after 1 week of treatment of the experimental gel with a *P* value of 0.08 was noticed, however. The data were also re-calculated in relation to the volume of GCF (output) and content of protein, but with the same insignificant results (data not shown).

Discussion

The present study was undertaken to examine the effect of a triclosan gel on the gingival health of adolescents with slightly affected periodontal status due to treatment with fixed orthodontic appliances. The double-blind split-mouth design is widely used in clinical dentistry since it allows the patient to be his/her own control. The inflammatory mediators, PGI_2 and IL-1β, were chosen as the major outcome measure in this study because these are thought to be sensitive and appropriate markers of the degree of gingival inflammation (10, 15, 16). With the exception of mild gingivitis adjacent to the brackets, the patients exhibited non-compromised dental health. Although the level of oral hygiene was far from optimal in some subjects during the study period, it must still be emphasized that the present findings might not be representative of a population with more frequent or severe forms of gingivitis.

The main finding was that neither the experimental nor the placebo gel altered the concentration of inflammatory mediators in GCF significantly compared with baseline. The values were in general slightly lower in the experimental quadrants, but we were unable to clinically verify that triclosan could inhibit the formation of PGI_2 and IL-1β, mediators of gingival inflammation. A recent short-term study with the present gel has suggested a significant clinical effect on moderate gingivitis cases (17), while others have found that triclosan possesses limited activity as an antigingivitis ingredient in dentifrices and mouth rinses (18, 19). According to Bruhn et al. (20), fluoride toothpaste containing 0.3% triclosan and essential oil reduced gingival inflammation in patients with periodontitis during a 28-week period, but no effect was found after 18 and 8 weeks. Apart from patient selection, there are several possible explanations why triclosan did not reduce the production of inflammatory mediators: (i) the triclosan concentration in the gel was to low, (ii) the agent was not sufficiently released from the gel (biologically inactive), or (iii) the treatment regimen with 2 applications a day for 14 days was inadequate. At present, we can only speculate, but it could be that a combination of all the explanations mentioned above contributed to the lack of effect of triclosan. A carry-over effect of the active gel into the placebo-treated quadrant was unlikely. Had this been the case, a reduction of the levels of mediators in GCF would have been obvious on both sides. It was interesting to compare the present findings with our previous results with chlorhexidine-containing varnish, obtained in a similar experimental set-up (14), since chlorhexidine remains the gold standard with which other antiplaque and gingivitis agents are compared. In that study, both gingival bleeding and PGI_2 and IL-1β values were significantly decreased by the varnish treatments as a clear indication of its clinical efficacy.

Table 3. Concentrations of PGI_2 (pg/ml) and IL-1β (pg/ml) in GCF at baseline and designated intervals during and after treatment with a triclosan-containing dental gel or a placebo gel in adolescents with fixed orthodontic appliances (*n* = 14). The dotted line indicates the end of the 2-week treatment period

	Experimental gel		Placebo gel	
Time	PGI_2 Mean ± *s*	IL-1β Mean ± *s*	PGI_2 Mean ± *s*	IL-1β Mean ± *s*
Baseline	81.5 ± 48.3	204.3 ± 171.9	83.3 ± 30.7	261.8 ± 113.5
1 week	67.3 ± 25.9	201.4 ± 140.0	72.7 ± 22.3	248.1 ± 130.9
2 weeks	79.1 ± 24.9	125.9 ± 112.7	82.2 ± 27.7	185.9 ± 114.4
4 weeks	79.6 ± 39.1	145.6 ± 117.8	84.4 ± 43.6	137.1 ± 103.6
6 weeks	72.6 ± 22.2	168.7 ± 115.1	86.9 ± 33.3	136.9 ± 99.5

s = standard deviation.

In conclusion, the hypothesis could not be verified. Treatment with a triclosan-containing dental gel for 2 weeks did not affect mediators of gingival inflammation or gingival health more than a placebo preparation in adolescents with mild gingivitis adjacent to fixed orthodontic appliances. However, further studies in extended study groups and possibly with more severe forms of gingivitis are needed before any firm conclusions can be drawn. At present, however, there is limited evidence for its clinical use.

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Received for publication 19 December 2002
Accepted 28 March 2003