Effect of an antibacterial dental varnish on the levels of prostanoids, leukotriene B_4, and interleukin-1β in gingival crevicular fluid

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The aim of this study was to investigate the effects of a chlorhexidine/thymol-containing dental varnish on the levels of prostaglandin E_2 (PGE_2), prostaglandin I_2 (PGI_2), leukotriene B_4 (LTB_4), and interleukin-1β (IL-1β) in gingival crevicular fluid (GCF). The material consisted of 15 adolescents undergoing treatment with fixed orthodontic appliances. Four buccal sites adjacent to bands or brackets and exhibiting a mild chronic gingival inflammation were selected in the upper quadrants of each patient. According to a split-mouth technique, the first and second quadrants were randomly treated with either a varnish (Cervitec®) containing 1% chlorhexidine diacetate and thymol (CHX/thymol) or a placebo varnish without active ingredients. The varnishes were applied immediately after the baseline registration, and follow-up examinations were carried out after 3, 8, and 30 days. GCF was sampled with the aid of a paper strip and the volume was determined using a Periotron® 8000. The concentrations of PGE_2, PGI_2, LTB_4, and IL-1β in GCF were assessed using radioimmunoassay and ELISA techniques. The results unveiled statistically significant reductions of PGE_2, PGI_2, and LTB_4 levels in GCF following the active varnish treatment when compared to baseline values. A slight drop in IL-1β levels was registered after both active and placebo varnish applications, but the differences were not significant. The results suggest that treatment with an antibacterial varnish decreases the levels of inflammatory mediators PGE_2, PGI_2, and LTB_4 in gingival crevicular fluid and further support the concept that topical application of a CHX/thymol-containing varnish is beneficial in patients with chronic gingival inflammation. □ *Chlorhexidine/thymol; gingival inflammation; inflammatory mediators; PGE_2; PGI_2*

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It is generally recognized that chlorhexidine (CHX), commonly administered through rinses or gels, can be used to control plaque development and thereby prevent gingivitis (1, 2). CHX-containing varnishes were introduced a decade ago and enhanced antibacterial effect has been suggested, probably due to a prolonged contact of the varnish on the teeth and the sustained release of chlorhexidine (3, 8). Thus, CHX-containing varnishes have been shown to suppress mutans streptococci colonization around bands and brackets in patients undergoing orthodontic treatment with fixed appliances (9–11). Chronic gingivitis is another frequent side effect of such appliances as a consequence of a local accumulation of dental plaque and it has been shown that topical CHX-varnish applications improve the gingival condition (12, 13). It is widely accepted that prostaglandins mediate the inflammatory response, resulting in an increased vascular permeability and dilatation (14, 15). Recently, we demonstrated that the levels of the inflammatory mediator prostaglandin E_2 (PGE_2) in gingival crevicular fluid (GCF) were reduced after antibacterial varnish applications, reflecting a diminished degree of gingival inflammation in subjects with fixed orthodontic appliances (13). The aim of the present investigation was to further explore the effect of chlorhexidine/thymol-containing varnish on the levels of the inflammatory mediators prostaglandin I_2 (PGI_2), leukotriene B_4 (LTB_4), and interleukin-1β (IL-1β) in GCF.

Materials and methods

Subjects

Healthy adolescents (11 male and 4 female, ranging from 14 to 20 years) undergoing active orthodontic treatment with fixed appliances were selected. Some of the participants were the same as those reported in an earlier study (13). The inclusion criteria were fixed appliances on at least 10 maxillary teeth and at least 4 buccal or interdental sites (two in each of the first and second quadrants, respectively) with a mild chronic gingival inflammation. Furthermore, the subjects had not taken any anti-inflammatory drugs during the last month before examination. Prior to the start of orthodontic treatment, 3–6 months before the study, the participants

were given instructions concerning oral hygiene and were asked to rinse daily with a 0.025% sodium fluoride solution. All patients consented to participate after verbal and written information.

Study design

A split-mouth study design was used and two mesiobuccal sites with clinically inflamed gingiva, from the first and second quadrants respectively, were selected. After baseline samplings, the sites were randomly treated with either an active antibacterial varnish or a placebo varnish. The subjects were then recalled 3, 8, and 30 days after baseline. The varnish contents were unknown for the participants. Approvals for the investigation were obtained from the Ethics Committee, University of Lund, Sweden and the Swedish Board of Welfare, Drug Division, Uppsala, Sweden.

Clinical procedures

Before the collection of GCF, supragingival plaque was carefully removed with an explorer and cotton pellets. The sites were then gently dried with air. A paper strip (Periopaper, ProFlow, Amityville, NY, USA) was inserted in the gingival sulcus for 15 s and the volume of the GCF sample was immediately determined using a Periotron® 8000 (ProFlow). Blood-contaminated strips were discarded. The strips were then transferred to small plastic tubes and eluted twice in 120 µl RIA buffer (0.9% NaCl, 0.01M EDTA, 0.3% bovine (γ-globulin 0.005% Triton X-100, 0.05% sodium azide; 0.0255M $NaH_2PO_4 \cdot H_2O$, 0.0245M $Na_2HPO_4 \cdot 7H_2O$; pH 6.8) containing 0.15 mM indomethacin (Sigma Chemical Co., St. Louis, MO, USA) by centrifugation (10,000 rpm for 5 min) and stored frozen at −70°C until analysis.

The test varnish (Cervitec®) contained 1% (w/w) chlorhexidine diacetate and 1% (w/w) thymol as active agents and, along with the placebo varnish (without chlorhexidine and thymol), was provided by the manufacturer (Vivadent/Vivacare, Schaan, Liechtenstein). Prior to application, the sites were dried using a gentle flow of air. The varnishes were applied with small brushes in a thin layer around each band or bracket and along the gingival margin. A standardized amount of each varnish (0.05 ml) was used and allowed to set for 20–30 s. The subjects were asked to avoid eating and drinking for 3 h after the treatments and to omit tooth-brushing the same day.

Laboratory procedures

The concentration of PGE_2, PGI_2 (6-keto-$PGF_{1\alpha}$) and LTB_4 was determined with the aid of commercial radioimmunoassay kits from DuPont (New England Nuclear Research Products, Boston, MA, USA) according to the manufacturer's protocol. The levels of PGE_2, PGI_2 and LTB_4 were expressed as pg/0.1 ml GCF. The PGE_2 antisera had 30% cross-reactivity with PGE_1. The level of IL-1β was determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits from Amersham Life Science Ltd (Amersham Place, Little Chalfont, Buckinghamshire, England). The level of IL-1β was expressed as pg/ml GCF.

Statistics

The data were subjected to Student's paired *t* test.

Results

The mean proportion of selected sites with bleeding on probing was 20% at baseline, thereafter decreasing to 10%, 5%, and 12% at the 3, 8, 30 day follow-ups at the CHX/thymol-treated quadrants. The volume of GCF at baseline and designated time intervals is presented in Table 1. A statistically significant reduction of the GCF volume was recorded after treatment with the CHX/thymol-containing varnish compared with baseline, while the placebo varnish did not significantly affect the GCF volume. The mean GCF volume of the active and placebo varnish treatments significantly differed at days 3, 8, and 30, whereas no significant difference was recorded at baseline. The concentrations of the selected inflammatory mediators PGE_2, PGI_2, LTB_4, and IL-1β in GCF, determined at baseline and follow-up examinations, are summarized in Table 2. The antibacterial varnish treatment significantly reduced the levels of arachidonic acid metabolites PGE_2, PGI_2, and LTB_4 at the 3 and 8 day follow-ups compared with baseline. After a period of 30 days, significant reductions of both PGE_2 and PGI_2 were still observed, while the LTB_4 level was back to baseline. The level of IL-1β dropped slightly after the applications of both active and placebo varnishes at the 3 and 8 day follow-ups, but the reduction was not significant. On comparing CHX/thymol and placebo varnish treatments, no significant differences in PGE_2, PGI_2, LTB_4, or IL-1β levels in GCF were observed on days 3, 8, and 30.

Discussion

In a previous study we reported that treatment with CHX/thymol-containing varnish diminished the level of the inflammatory mediator PGE_2 in GCF as well as reduced the number of sites with bleeding on probing and thereby improved gingival health (13). We therefore further investigated the effect of the antibacterial varnish on some other selected inflammatory mediators in GCF collected from partly the same participants. The patients exhibited a mild form of chronic gingival inflammation and although some bands cemented on molar teeth could exert a direct mechanical irritation of the gingiva, we considered plaque retention to be the main reason for the gingival condition. However, no efforts were made to

Table 1. Volume of gingival crevicular fluid (µl, mean ± SD) at baseline and after designated time intervals after treatments with a chlorhexidine/thymol-containing dental varnish (Cervitec®) and a placebo varnish in 15 adolescents with fixed orthodontic appliances

	CHX/thymol			Placebo			
Time	*n*	Mean	± SD	*n*	Mean	± SD	$P^{\S}$
Baseline	15	0.45	0.14	15	0.41	0.10	NS
3 days	15	0.25*	0.10	15	0.38	0.10	$P < 0.01$
8 days	14	0.19†	0.09	14	0.36	0.14	$P < 0.01$
30 days	15	0.25†	0.11	15	0.39	0.10	$P < 0.01$

The volume of GCF to each subject is based on two sites; CHX/thymol and placebo, respectively.
* † Significantly different from baseline, * $P < 0.01$; † $P < 0.001$; Student's paired *t* test.
$P^{\S}$ Differences between CHX/thymol and placebo at baseline and designated times; Student's paired *t* test. NS = not significant.

measure the degree of oral hygiene or to evaluate the degree of plaque accumulation during the experimental period. The novel finding of this study was that treatment with the CHX/thymol-containing varnish not only resulted in diminished levels of PGE_2 in GCF but also in a reduction of the inflammatory mediators LTB_4 and PGI_2 at the follow-ups, 3 and 8 days after baseline. In contrast, the level of the cytokine IL-1β was not significantly reduced by the antibacterial varnish treatment.

In agreement with previous findings (13) we demonstrate here that the CHX/thymol varnish treatment reduces the mean volume of GCF. This reduction was observed in relation to placebo treatments as well as within the CHX/thymol group as compared to baseline. In addition, the levels of PGE_2, PGI_2, and LTB_4 were significantly reduced after CHX/thymol varnish treatment compared to baseline. However, there was no significant difference between placebo and CHX/thymol varnish treatments on days 3, 8, and 30. Noticeably, in our previous study (13) we demonstrated a significant reduction of PGE_2 in the CHX/thymol-treated group compared to placebo exclusively day 8 at the follow-up. However, it has to be considered that the number of subjects in this study was reduced, which may be the reason why the difference between test and placebo was

Table 2. Concentration of selected inflammatory mediators (mean ± SD) in gingival crevicular fluid at baseline and designated time intervals after treatment with a chlorhexidine/thymol-containing varnish (Cervitec®) or a placebo varnish in 15 adolescents with fixed orthodontic appliances

	CHX/thymol			Placebo		
Variable/time	*n*	Mean	±SD	*n*	Mean	±SD
PGE_2, pg/0.1 ml						
Baseline	15	11.89	3.17	15	10.85	2.41
3 days	15	9.68‡	2.73	15	10.12	2.76
8 days	14	8.56*	2.22	14	9.27	2.13
30 days	15	8.51*	2.40	15	9.13	3.31
PGI_2, pg/0.1 ml						
Baseline	15	11.53	2.28	15	10.67	1.94
3 days	15	9.08*	2.39	15	9.18	1.73
8 days	14	9.23*	2.74	14	9.53	2.01
30 days	15	8.61‡	2.17	15	8.70	2.87
LTB_4, pg/0.1 ml						
Baseline	15	63.06	9.12	15	57.82	9.43
3 days	15	56.32‡	9.00	15	61.63	12.99
8 days	14	52.17*	9.95	14	56.92	9.56
30 days	15	60.02	16.25	15	55.80	7.51
IL-1β, pg/ml						
Baseline	15	176.4	116.3	15	151.7	131.5
3 days	15	134.4	144.1	15	112.7	114.6
8 days	14	135.6	159.5	14	127.1	132.5
30 days	15	151.9	145.0	15	166.2	132.2

‡ * † Significantly different from baseline, ‡ $P < 0.05$; * $P < 0.01$; † $P < 0.001$; Student's paired *t* test.
No significant difference between CHX/thymol and placebo at baseline and designated times.

not significant. In addition, the possibility of a certain cross-over effect cannot be neglected resulting in reduced level of inflammatory mediators after treatment with placebo.

In general, gingivitis in children, caused by Gram-positive species, is characterized by a redness and edema of a few interpapillary and marginal areas. Histologically, polymorphonuclear cells infiltrate and the neutrophil migration into the sulcus is marked by a rise in the level of LTB_4 in GCF (16). In our study, the level of LTB_4 was reduced only on days 3 and 8 after the active varnish treatment. The initial reduction of LTB_4 due to CHX/thymol treatment is compatible with the view that LTB_4 is involved in the earliest stage of gingivitis associated with enhanced vasodilation and permeability of the vessel. Thus, there is evidence that local application of CHX results in a diminished neutrophil recruitment, leading to improved gingival health. After 3–4 weeks without oral hygiene procedures, the microbial community shifts to a more Gram-negative flora and bleeding on probing begins to rapidly increase with concomitant enhancement of PGE_2 in GCF (16). This stage of inflammation is mainly characterized by monocyte activation due to an influence of lipopolysaccharides from the microbiotes in the plaque (16). In light of the antibacterial action of CHX it was logical to find that the levels of PGE_2 and PGI_2 were reduced following the antibacterial varnish treatments, which is also in agreement with our previous report (13).

The levels of PGE_2 and PGI_2 in GCF were still reduced 1 month after the treatment with the antibacterial varnish, which was not the case with LTB_4. This could be due to the fact that these inflammatory mediators are regulated by two different enzymes. The prostanoids, PGE_2 and PGI_2, are converted by the enzymes cyclooxygenase (COX-1 and COX-2), whereas LTB_4 is mediated by the enzyme lipooxygenase. The active varnish also contained thymol, which may further contribute to the decreased level of prostanoids. This assumption is based on the fact that thymol has a free phenolic hydroxyl group and that these compounds act in different degree as inhibitors of COX (17). However, it has been shown that another phenolic compound, triclosan, inhibits both cyclooxygenase and lipooxygenase and consequently decreases not only the formation of prostanoids but also LTB_4 (18). The question whether CHX and thymol have an additive or synergistic effect on arachidonic acid metabolites remains to be elucidated.

The cytokine IL-1β is mainly produced by monocytes, although various cells in the periodontal tissue, such as gingival fibroblasts, periodontal ligament cells, keratinocytes, also have the capacity to produce the proinflammatory cytokine (19–22). The level of IL-1β in GCF, compared to baseline, was not significantly affected by CHX/thymol treatment, although a decreasing tendency was noted. The possible alterations of IL-1β, however, have to be further investigated as well as the long-term effect of topical treatments with CHX/thymol regarding inflammatory mediators in GCF.

In conclusion, the present study further supports the concept that topical application of a CHX/thymol containing antibacterial varnish might be beneficial for patients with gingival inflammation.

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