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The effect of a mouth rinse containing phenolic compounds on plaque formation and developing gingivitis

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Abstract

Background: The clinical effect of Listerine®, a mouth rinse containing a mixture of phenolic compounds, is ascribed to its bactericidal properties. However, phenolic compounds are also known to interfere with the inflammatory process.

Objective: The purpose of this clinical trial was to evaluate the effect of regular mouth rinsing with Listerine® on plaque and gingivitis during a 2-week period of no mechanical oral hygiene.

Material and Methods: Twenty-one subjects were recruited for the study. On Day 0 of each 2-week experimental period, the participants were told to abstain from all mechanical plaque-control measures but to rinse twice a day with 10 ml of the assigned solution (test: Listerine®, positive control:0.1% chlorhexidine (CHX), negative control: saline) for 60 s. Each experimental period was preceded by a 2-week period including oral hygiene instruction, scaling and professional mechanical tooth cleaning. Examinations included assessments of plaque and gingivitis (Days 0 and 14), sampling of plaque and collection of gingival crevicular fluid (GCF) (Days 0, 7 and 14). From the supragingival plaque samples, six different morphotypes of bacteria were counted using dark-field microscopy. The GCF collected was analysed with respect to the content of lactoferrin and albumin.

Results: During the experimental periods, it was observed that significantly less plaque formed and less gingivitis developed when the participants rinsed with the Listerine® mouthwash than with saline solution. However, significantly more plaque formed during the Listerine® than during the CHX rinse period, while there was no significant difference in the development of gingival bleeding between the Listerine® and the CHX rinse regimens. Significantly smaller proportions of motile rods and fusiforms were found in the List and CHX groups than in the control (Ctrl) group. The increase of the lactoferrin/albumin ratio in the List group was significantly smaller than that in the Ctrl group but significantly larger than in the CHX group.

Conclusion: It was suggested that the effect of Listerine® on gingivitis is more pronounced than on plaque formation. This indicates that the phenolic compound may have anti-inflammatory effects.

Key words: chlorhexidine; experimental gingivitis; lactoferrin; Listerine®

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Because of their low toxicity and antibacterial properties, phenolic compounds are used in antiseptics and disinfectants, but also in mouth rinse preparations. Short- and long-term clinical studies have indicated that the daily use of Listerine® (Pfizer AB, Täby, Sweden), a mouth rinse that contains different phenolics such as thymol, eucalyptol, menthol, and methyl salicylate, may retard plaque buildup and reduce gingivitis (Fornell et al. 1975, Gordon et al. 1985, Axelsson & Lindhe 1987, Mankodi et al. 1987, Depaola et al. 1989, Overholser et al. 1990, Brecx et al. 1992, Ross et al. 1993). The effect of Listerine® on plaque was ascribed to its bactericidal properties that were

documented in vitro as well as in vivo (Ross et al. 1989, Jenkins et al. 1994, DePaola et al. 1996, Pan et al. 2000). Phenolic compounds, however, are also known to interfere with the inflammatory process (Dewhirst 1980, Azuma et al. 1986). Thus, Axelsson & Lindhe (1987) observed that Listerine® used as a mouth rinse twice daily for 6 weeks had a limited influence on plaque but was as effective in reducing gingival inflammation as a rinse that contained chlorhexidine digluconate. In other studies with a similar design (Siegrist et al. 1986, Brecx et al. 1990), however, the regular utilization of Listerine® failed to retard plaque-associated gingivitis.

The purpose of the present clinical trial was to further evaluate the effect of regular mouth rinsings with a preparation containing phenolic compounds on plaque and gingivitis during a 2-week period of no mechanical oral hygiene.

Material and Methods

Study design

This trial was designed as a cross-over, randomized, single-blind study. Twenty-one healthy subjects aged 20–42 (mean 27 years) were recruited. Each volunteer had to fulfil the following criteria: (i) good general health, (ii) no sign of destructive periodontal disease, (iii) a minimum of 24 teeth; six teeth in each quadrant, (iv) no antibiotic treatment during a 3-month period prior to the start of the trial, (v) no regular medication with anti-inflammatory compounds, (vi) no use of tobacco products and (vii) no regular use of oral antiseptics. All subjects received verbal and written description of the study design, and signed informed consent forms. The study was approved by the regional ethical review board at Göteborg University.

Each volunteer was subjected to a screening examination to assess the overall status of his/her dentition. The panellists were given careful case presentation, oral hygiene instruction, and received meticulous supragingival scaling and professional mechanical tooth cleaning (PTC; Axelsson & Lindhe 1974). The PTC was performed with the use of an abrasive paste, rubber cups and brushes, and was repeated twice a week during a 2-week preparatory period.

On a given day (Day 0), all subjects were given PTC and were asked to abstain from all mechanical plaque control measures during the course of the experimental period but to rinse with one out of three mouth rinses. Mouth rinsing was performed twice a day (after breakfast and in the evening), for 60 s with 10 ml of the assigned product. On Days 0, 7 and 14, all participants were subjected to examinations which included assessments of plaque and gingivitis, as well as sampling of plaque and collection of gingival crevicular fluid (GCF).

Immediately after the Day 14 examination, all participants received PTC and were instructed to perform proper plaque control measures during the subsequent 2 weeks. After this ''wash-out'' period, the subjects received a new PTC, after which a second 14-day rinsing period was initiated. The third rinsing period was accordingly initiated after a 2-week ''wash-out'' period. Clinical re-examinations were repeated as described above.

The following mouth rinse preparations were tested:

(1) Listerine® (0.06% thymol, 0.04% menthol, 0.09% eucalyptol, 0.05% methyl salicylate, 27% ethanol) (Pfizer AB, Täby, Sweden);
(2) Hexident® (chlorhexidine digluconate, 1 mg/ml (Ipex AB, Solna, Sweden);
(3) Saline (0.7% sodium chloride) (Fresenius Kabi Norge AS, Halden, Norway).

Examination variables

Dental plaque

Dental plaque was disclosed with erythrosin (Diaplac®, Nordenta AB, Enköping, Sweden) and scored at the disto-, mid-, mesio-buccal and disto-, mid-, mesio-lingual surfaces of all teeth except third molars, according to the criteria of the modified Quigley & Hein Plaque Index System (QHI; Quigley & Hein 1962, Turesky et al. 1970). The examination was performed on Day 14.

Gingival inflammation

The degree of gingival inflammation was scored at six sites (disto-, mid-, mesio-buccal and disto-, mid-, mesio-lingual) of all teeth except third molars according to the criteria of the Gingival Index System (GI; Löe 1967). The examinations were performed on Days 0 and 14.

Plaque composition

After the sampling sites had been dried and isolated with cotton rolls, supragingival plaque was sampled with a sterile curette (Gracey®, LM Dental, Turku, Finland).

Samples were obtained from the following tooth surfaces:

Day 0: mesio-buccal surfaces of 16, 24, 33 and 41;

Day 7: mesio-buccal surfaces of 11, 26, 34 and 43;

Day 14: mesio-buccal surfaces of 14, 23, 31 and 46.

The plaque samples were pooled and transferred into a glass vial containing saline and 0.5% gelatin, and analysed within 30 min. in a *dark-field microscope* as described by Listgarten & Hellden (1978). One hundred bacterial cells were counted and classified into six different morphological types, namely coccoid cells, straight rods, motile rods, filaments, fusiforms and spirochetes.

GCF

GCF samples were obtained on Days 0, 7 and 14 immediately after plaque sampling and from the same sites that were exposed to plaque sampling. The sampling sites were dried and isolated with cotton rolls. A paper strip (Periopaper®; ProFlow Inc., Amityville, NJ, USA) was gently inserted into the orifice of the gingival crevice and kept in place for 30 s.

The volume of GCF collected in each strip was determined in a chairside Periotron 8000® (ProFlow Inc.) and expressed in µl/sample. The four strips from each patient and examination interval, respectively, were then pooled, stored in a sterile 1 ml Eppendorph tube and frozen at -70°C until further processing.

The pooled GCF samples were eluted into 1 ml of phosphate-buffered saline with 0.1% bovine serum albumin (BSA) for 60 min. at room temperature and further diluted in a phosphate-buffered saline with 0.1% BSA and 0.05% Tween 20.

The amount of *albumin and lactoferrin* in such eluates of GCF was assessed using a sandwich ELISA technique (Adonogianaki et al. 1994): one plate for the analysis of albumin, and a second plate for the analysis of lactoferrin. The

amount of the two different proteins was determined and expressed in ng/ml buffer. Standard curves for each assay were computed and sample concentrations were calculated using an optical density value for each sample. The amount of lactoferrin ($\times 1000$) per unit albumin was calculated.

Statistical analysis

Subject mean values were calculated for all clinical and microbiological parameters and GCF. Analysis of variance and the Student–Newman–Keuls (SNK) test were applied to evaluate whether there were significant differences between the treatment groups ($p < 0.05$).

Results

Dental plaque

The mean individual QHI scores representing Day 14 of each experimental period of no mechanical tooth cleaning are presented in Fig. 1. The mean QHI score for the Listerine® (List) period was 2.08 compared with 2.87 for the control (Ctrl) ($p < 0.05$) and 1.36 for the chlorhexidine (CHX) period ($p < 0.05$). The difference in plaque accumulation following the use of List was statistically significantly less than Ctrl ($p < 0.05$). In addition, significantly more plaque had formed in the List than in the CHX group ($p < 0.05$).

The percentage frequency distributions of QHI plaque scores 2–5 for the three study groups on Day 14 are presented in Fig. 2. In the List group, the percentage of surfaces with that magnitude of plaque was 79.4%, while in Ctrl and CHX the corresponding proportions were 90.0% and 47.5% (CHX < List < Ctrl; $p < 0.05$).

Figure 3 reports the mean percentage of different bacterial morphotypes present in plaque sampled at the various examination intervals. On Day 0, there were no significant differences in the mean percentage of the various morphotypes between the three groups. During the course of the experiment, the percentage of coccoid cells and straight rods decreased in all groups, while the proportions of filaments, fusiforms and motile rods increased. The percentage of spirochetes remained low throughout the 2 weeks of study. On Day 7, the proportion of coccoid cells was significantly larger ($p < 0.05$) in the List (46.6%) than in the Ctrl group (34.6%) but not significantly different from the CHX group (49.2%). Between Days 7 and 14, the reduction of the percentage of coccoids was significantly lower in the List and CHX groups (7.1% and 1.4%, respectively) than in the Ctrl group (11.7%; $p < 0.05$). Further, in samples from Day 14, the percentage of motile rods was significantly lower in List (8.8%) and in CHX (6.1%), than in Ctrl (14.0%) ($p < 0.05$). The percentage of fusiforms was low in all groups on Day 0 (0.3–1.1%). On Day 14, significantly smaller proportions of fusiforms were found in the List and CHX groups (5.4% and 5.1%, respectively) than in the Ctrl group (9.7%) ($p < 0.05$).

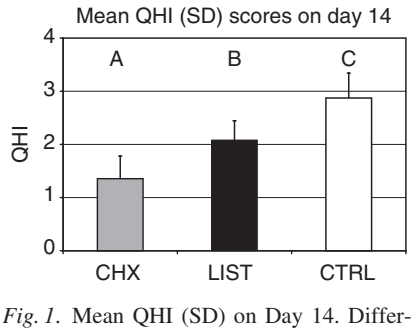

Fig. 1. Mean QHI (SD) on Day 14. Different letters indicate a statistically significant difference (SNK test). SD, standard deviation; QHI, Quigley & Hein Index; CHX, chlorhexidine; LIST, Listerine®; CTRL, control; SNK, Student–Neumann–Keuls.

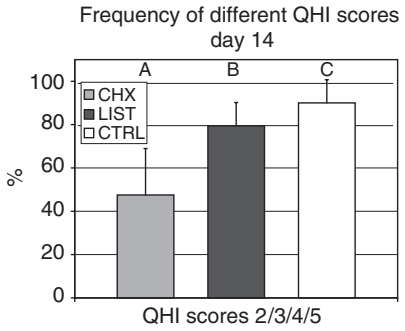

Fig. 2. Frequency distribution (SD) of various plaque scores (QHI) on Day 14. Different letters indicate a statistically significant difference (SNK test). SD, standard deviation; QHI, Quigley & Hein Index; CHX, chlorhexidine; LIST, Listerine®; CTRL, control; SNK, Student–Neumann–Keuls.

Gingivitis

The mean GI scores calculated from the measurements made on Day 0 varied between 0.43 (List) and 0.47 (CHX). There were no significant differences regarding gingival inflammation between the three groups at the start of the experimental periods. On Day 0, there were no significant differences between the three study groups either regarding the proportions of gingival units scored

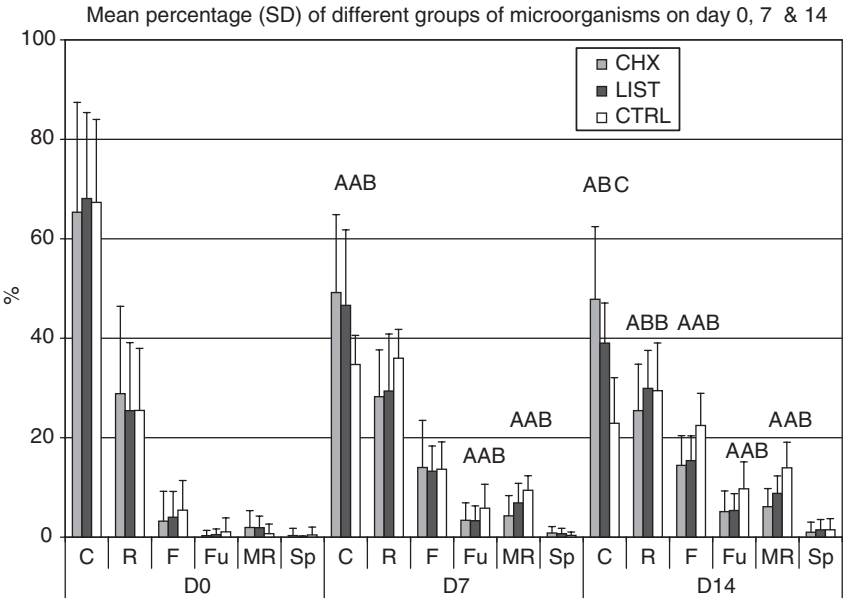

Fig. 3. Percentage distribution (SD) of different groups of bacteria in plaque samples on Days 0 and 14. Different letters indicate a statistically significant difference (SNK test). SD, standard deviation; C, Cocci; R, rods; F, filaments; Fu, fusiforms; MR, motile rods; Sp, spirochetes; CHX, chlorhexidine; LIST, Listerine®; CTRL, control; SNK, Student–Neumann–Keuls.

GI = 0 or regarding the percentage of units scored GI = 2 (Fig. 4).

On Day 14, the mean percentage of healthy gingival units (GI = 0) was 12.3% in the List group, compared with 3.5% in Ctrl ($p < 0.05$) and 18.7% in the CHX group ($p < 0.05$). The proportions of gingival units that bled on probing (GI = 2) were 10.7% (List), 23.2% (Ctrl) and (CHX) 13.5%, respectively. The difference in inflamed units between List and Ctrl was statistically significant ($p < 0.01$) while no significant difference was observed between List and CHX.

Figure 5 presents the mean percentages of sites that were considered healthy (GI = 0) at Baseline and on Day 14 had remained healthy or become inflamed (GI = 2). In the List group, 17.0% of the sites remained healthy after 2 weeks of no mechanical tooth cleaning, while in the Ctrl group the corresponding percentage was 5.8% ($p < 0.01$). The mean percentage of sites that remained healthy in the CHX group (25.3%) was significantly ($p < 0.05$) higher than in the List group.

The mean proportion of sites that underwent a shift from GI = 0 to GI = 2 between Baseline and 2 weeks was on the average 10.0% (List), 18.6 (Ctrl) and 7.6% (CHX). In this respect, there was a significant difference between List and Ctrl ($p < 0.05$) but not between List and CHX.

The mean GI scores at sites with different QHI scores on Day 14 are presented in Fig. 6. At surfaces scored QHI = 0, the mean GI scores were 0.85 (List), 0.90 (Ctrl) and 0.82 (CHX). The amount of gingivitis present adjacent to surfaces with small (QHI = 1+2) and large amounts of plaque (QHI = 3+4+5) was similar in the List group (1.00 and 1.08, respectively) and the CHX group (0.93 and 1.04) but significantly lower than in the Ctrl (1.17 and 1.24) ($p < 0.05$).

Table 1 shows the mean lactoferrin/albumin ratio (Lf/Ab) ($\times 10^3$) on Days 0, 7 and 14. There was no difference in the mean Lf/Ab at Day 0 of the three experimental periods. The increase of the Lf/Ab ratio between Days 0 and 14 was statistically significant for the List (14.8) and the Ctrl groups (28.1) but not

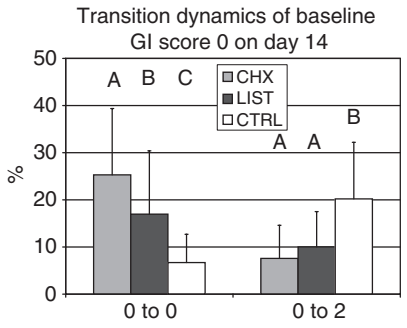

Fig. 5. Frequency distribution (SD) of sites that on Day 0 received GI score 0 and Day 14 either remained unchanged or had become inflamed (GI score ⩾2). Different letters indicate a statistically significant difference (SNK test). SD, standard deviation; GI, Gingival Index; CHX, chlorhexidine; LIST, Listerine®; CTRL, control; SNK, Student–Neumann–Keuls.

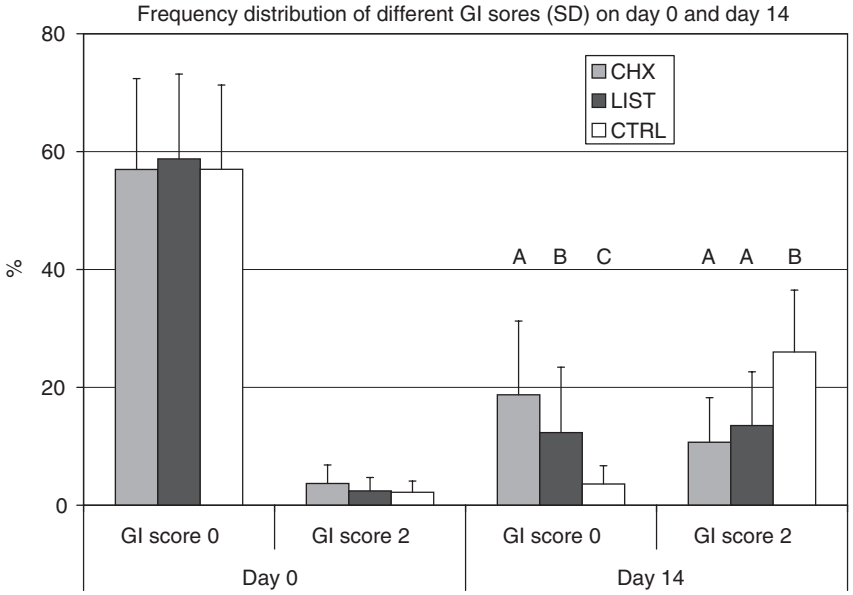

Fig. 4. Frequency distribution (SD) of various GI scores on Days 0 and 14. Different letters indicate a statistically significant difference (SNK test). SD, standard deviation; GI, Gingival Index; CHX, chlorhexidine; LIST, Listerine®; CTRL, control; SNK, Student–Neumann–Keuls.

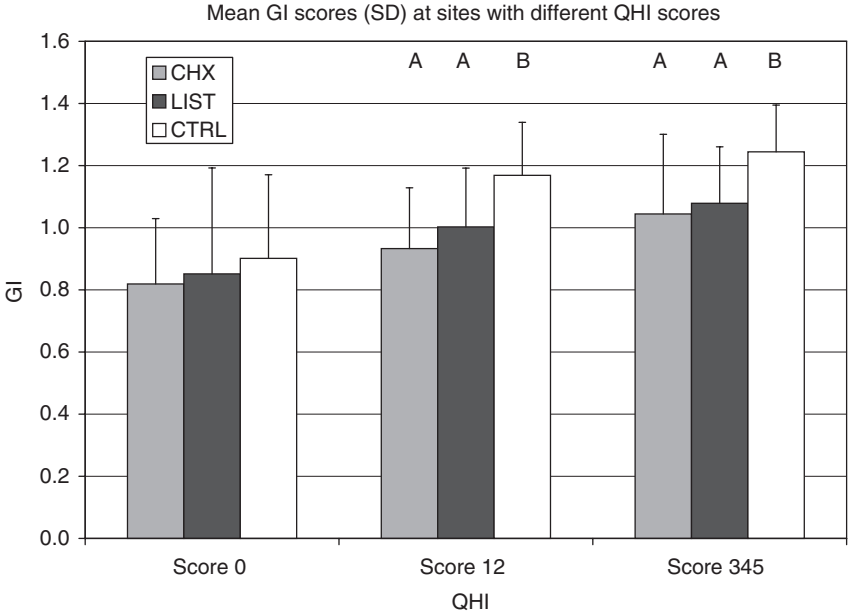

Fig. 6. Mean GI (SD) at sites with different QHI scores on Day 14. Different letters indicate a statistically significant difference (SNK test). SD, standard deviation; QHI, Quigley & Hein Index; GI, Gingival Index; CHX, chlorhexidine; LIST, Listerine®; CTRL, control; SNK, Student–Neumann–Keuls.

Table 1. Lactoferrin/albumin (SD) ratio

	Day 0	Day 7	Day 14	Days 14–0
List	18.7 (12.1)	32.5 (13.4)	33.5 (16.7)	14.8 (21.6)
Ctrl	19.9 (12.2)	31.5 (18.6)	48.0 (27.3)	28.1 (15.2)
CHX	19.9 (12.9)	24.8 (15.9)	25.8 (23.8)	5.8 (26.3)

SD, standard deviation; CHX, chlorhexidine; Ctrl, control.

for the CHX group (5.8). In addition, the mean increase in the List group was significantly smaller than that in the Ctrl group but significantly larger than in the CHX group ($p < 0.05$).

Discussion

In the present study, it was observed that during a 2-week period of no mechanical tooth cleaning, significantly less plaque formed and less gingivitis developed when the participants rinsed with the Listerine® mouthwash than with the saline solution (negative control). However, significantly more plaque formed during the Listerine® than during the CHX period (positive control), while the change in the condition of the gingiva in the two periods was less conspicuous. Thus, the percentage of sites in the List group that shifted from GI score 0 to 2 (Baseline–Day 14) was 10% while the corresponding shifts in Ctrl was 19% and in 7% CHX. Further, the total percentage of sites with GI score 2 on Day 14 was 14% in the Listerine® group, 23% in the Ctrl group, and 11% in the CHX group. These findings indicate that Listerine® may interfere with the development of plaque-associated gingival inflammation.

In some respects, the current findings are in agreement with Moran et al. (1991), who reported that the amount of plaque formed during a 19-day period of no tooth cleaning was significantly larger when the subjects rinsed with Listerine® than during a corresponding regimen when a CHX mouth rinse was used. Furthermore, Moran et al. (1991) reported that the increments of gingivitis were somewhat lower during the CHX than during the Listerine® regimen. Axelsson & Lindhe (1987) studied the effect of CHX and Listerine® mouth rinses used as adjuncts to regular oral hygiene measures and stated that CHX-containing mouth rinses were more effective than Listerine® in reducing plaque but not in resolving gingivitis. Similar findings were described by Gordon et al. (1985) and Overholser et al. (1990). Further, Charles et al. (2004) reported that Listerine® mouth rinse and a CHX mouth rinse had similar antiplaque and antigingivitis activity. Siegrist et al. (1986) and Brecx et al. (1990, 1992), on the other hand, reported that a 0.12% CHX mouth rinse was superior to Listerine® in maintaining low plaque and gingivitis scores in the absence of mechanical plaque control.

The reason for the conflicting results obtained in the studies referred to may be because of different study design. Thus, studies that showed a superior effect of CHX in retarding plaque formation and gingivitis development (Siegrist et al. 1986, Brecx et al. 1990, Moran et al. 1991) were of short duration, about 3 weeks. The studies by Axelsson & Lindhe (1987), Overholser et al. (1990) and Charles et al. (2004), on the other hand, were mainly designed to evaluate the effect of mouth rinse solutions used as adjuncts to regular oral hygiene measures during periods ranging between 6 weeks and 6 months. The findings from these studies indicate that the effect of Listerine® on the gingival condition may be more pronounced when the patients rinse after the plaque deposits have been mechanically disrupted. Thus, the limited ability of Listerine® to penetrate the plaque biofilm (Netuschil et al. 1995) may in part explain its comparatively small effect on plaque formation in studies utilizing the experimental gingivitis model. The effect of Listerine® on gingivitis may partly be because of the anti-inflammatory properties of the phenolic compounds incorporated in the rinse. In vitro studies by Dewhirst (1980) indicated that phenolic compounds have anti-inflammatory and prostaglandin synthetase inhibitor activity. Azuma et al. (1986) demonstrated in a neutrophil chemotaxis assay that phenolic compounds act as scavengers of free oxygen radicals and hence have an effect on leucocyte activity. Further, Firatli et al. (1994) showed in an in vitro study that the antioxidative effect of Listerine®, expressed as percentage inhibition of spontaneous oxidation, was greater than that of CHX and cetylpyridinium chloride. Hence, the authors suggested that the anti-inflammatory potential of Listerine® is related to an antioxidative mechanism and is partly because of the inhibition of free oxygen radical activity and the interaction with the biological membranes.

In the present study, the lactoferrin content in GCF was evaluated as it may serve as an indicator of the number of neutrophilic granulocytes present in the gingival exudate. Lactoferrin is an iron-binding protein mainly derived from neutrophilic granulocytes, and has antibacterial potential (Spitznagel et al. 1974, Ellison & Giehl 1991). In the studies referred to, it was observed that during the course of experimental gingivitis the lactoferrin levels, contrary to the serum-derived acute-phase protein levels, significantly increased. Therefore, in the present study lactoferrin was considered to be a suitable marker to characterize the initial phase of developing gingival inflammation. Albumin, which was used as a standard, is produced in the liver, circulates in the serum, and is minimally modulated or absorbed in the gingiva (Wilson et al. 2003). Thus, the amount of albumin present in the gingival exudate may act as an indicator of extravasation (Adonogianaki et al. 1995, 1996). In the present study, the Lf/Ab ratio at GCF samples collected on Day 14 was lower in the Listerine® than in the Ctrl group, albeit somewhat higher than in the CHX group. This finding corroborates the clinical findings regarding the proportion of sites in the three groups that scored GI = 0 and GI = 2 on Day 14.

Results from the dark-field examinations revealed that concomitant with the buildup of plaque, important shifts occurred regarding the proportion of different bacterial morphotypes. Thus, the percentage of coccoid cells was reduced while motile rods and fusobacteria increased. This is in agreement with findings from previous studies (Sekino et al. 2005). Some important differences between the three study periods were observed. Thus, in the CHX and List groups, the reduction in coccoid cells and the increase in motile rods on days 7 and 14 were less pronounced than in the Ctrl group. This may indicate that the difference with respect to gingivitis development in the three groups was associated not only with the amount but also with the quality of the dental biofilms formed.

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