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The effect of systemic administration of ibuprofen in the experimental gingivitis model

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Abstract

Background: Studies in humans have indicated that systemically administered flurbiprofen and ibuprofen may reduce gingivitis. De novo plaque formation is enhanced at tooth surfaces adjacent to inflamed gingivae.

Objective: The aim of the present clinical trial was to evaluate the effect of systemic administration of ibuprofen on gingivitis and plaque build-up.

Material and Methods: Eleven subjects were recruited for the study and were given oral hygiene instruction, scaling and professional mechanical tooth cleaning (PTC). At the end of a preparatory period (Day 0), the participants were told to abstain from all mechanical plaque control measures during a 2-week experimental period but to rinse with an assigned mouth rinse (positive control: 0.1% chlorhexidine digluconate; negative control: saline) or administer ibuprofen (tablets of 200 mg twice daily). Mouth rinsing was performed twice a day (after breakfast and in the evening), for 60 s with 10 ml. Re-examination was performed after 14 days of experiment. After a 2-week ''wash-out'' period, the participants received a new PTC and a second 14-day experimental period was initiated. The experimental and ''wash-out'' periods were repeated until all volunteers had been involved in all three regimens. *Dental plaque* was scored using the Quigley & Hein Plaque Index system and *gingivitis* according to the Gingival Index (GI) system. *Supragingival plaque* was collected and prepared for dark-field microscopy. One hundred bacterial cells were counted and classified into six different groups: coccoid cells, straight rods, filaments, fusiforms, spirochetes and motile rods. *Gingival crevicular fluid* (GCF) was collected from the same sites that were sampled for plaque. The volume of GCF collected in each strip was measured and analysed regarding content of lactoferrin and albumin.

Results: During the period when the panelists rinsed with saline they accumulated large amounts of plaque and developed marked signs of gingivitis. When they rinsed with chlorhexidine digluconate, small amounts of plaque formed and few sites received GI score $\geqslant 2$. After the 2 weeks of ibuprofen administration, the panelists presented with significantly fewer sites that scored GI $\geqslant 2$ but had formed similar amounts of plaque as during the negative control period.

Conclusion: It is suggested that ibuprofen administered via the systemic route has an effect on gingivitis but not on de novo plaque formation.

Key words: experimental gingivitis; ibuprofen; non-steroid anti-inflammatory drug

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The inflammatory lesion in gingivitis represents a host response to biofilms present in the dento-gingival region. Thus, findings from studies in humans and experimental animals demonstrated that plaque build-up resulted in gingivitis – determined by clinical as well as histological means – while mechanical removal of plaque enabled the establishment of healthy gingivae (e.g. Egelberg 1965, Löe et al. 1965, Attström & Egelberg 1971, Lindhe & Rylander 1975, Payne et al. 1975, Lindhe et al. 1978, 1989, Sato et al. 1993). It was further documented that the regular use of mouth rinses containing chlorhexidine not only retarded plaque formation and prevented gingivitis but had, in addition, the potential to resolve an established plaque and the associated soft-tissue lesion (Löe & Schiött

1970a, b). Findings from extensive oral health programmes that included the use of chlorhexidine in mouthwash and dentifrice formulations documented marked reductions in plaque and gingivitis (e.g. Flötra et al. 1972, Lang et al. 1982, Briner et al. 1989, Gjermo 1989).

In studies by e.g. Saxton (1973), Hillam & Hull (1977); Quirynen et al. (1991) and Ramberg et al. (1994), it was observed that the condition of the gingiva played an important role in de novo plaque formation. Thus, Ramberg et al. (1994) reported that on tooth surfaces adjacent to healthy gingival units substantially less amounts of plaque formed during a 4-day period of no mechanical tooth cleaning than on tooth surfaces facing inflamed gingiva. It was suggested (Saxton 1973, Hillam & Hull 1977, Ramberg et al. 1994) that the exudates present in the dento-gingival region of inflamed gingiva contained substances that favoured the initial adherence of bacteria on the tooth surface and promoted bacterial growth.

Non-steroid anti-inflammatory drugs (NSAIDs) are known to interfere with the cyclooxygenase pathway of arachidonic acid metabolism and inhibit the formation of important metabolites such as prostaglandins and leukotrienes. Studies in humans demonstrated that some systemically administered NSAIDs, such as flurbiprofen and ibuprofen, reduced gingivitis in various models (Heasman & Seymour 1989, Heasman et al. 1993, 1994, Taiyeb & Waite 1993), while other NSAIDs (e.g. naproxen, suldinac and acetylsalicylic acid) had no apparent effect (Vogel et al. 1984, Johnson et al. 1990).

Based on the above findings, it is suggested that the reduction of gingivitis that is achieved through the use of systemic flurbiprofen or ibuprofen may reduce gingival fluid flow and hence may also influence the build-up of plaque.

The aim of the present clinical trial was to evaluate the effect of systemic administration of ibuprofen on gingivitis and de novo plaque formation.

Material and Methods

Study design

The study was planned as a controlled, single-blind clinical trial with a cross-over design. Eleven subjects, aged 20–41 (mean 25.6 years), were recruited for the study. Each volunteer had to fulfil the following criteria: (i) be in good general health, (ii) exhibit no sign of destructive periodontal disease, (iii) have a minimum of 24 teeth and $\geqslant 6$ teeth in each jaw quadrant, (iv) not being engaged in ongoing restorative dental treatment, (v) not been exposed to antibiotic treatment in the previous 3 months, (vi) not use anti-inflammatory drugs, (vi) not use tobacco products and (vii) not use oral antiseptic products. Exclusion criteria included e.g. pregnancy or breast-feeding, involvement in other clinical studies and extensive fixed partial dentures. All subjects received verbal and written presentation of the study and signed written informed consent forms. The trial was approved by the local investigational review board at the Göteborg University.

The volunteers were subjected to a screening examination to assess the status of their dentition, were given oral hygiene instruction, supragingival scaling and professional mechanical tooth cleaning (PTC; Axelsson et al. 1976). PTC was performed with the use of an abrasive applied on rubber cups and brushes and was repeated twice a week during a 2-week preparatory period.

At the end of the preparatory period (Day 0), all subjects were given a final PTC and asked to abstain from all mechanical plaque control measures during the course of a 2-week experimental period but to rinse with an assigned mouth rinse or administer ibuprofen according to protocol.

The following products were tested:

ibuprofen (IBUP) (Ibumentin®; tablets 200 mg, Nycomed AB, Lidingö, Sweden);
chlorhexidine; (CHX) (0.1% chlorhexidine digluconate; Hexident, Ipex AB, Solna, Sweden); *positive control*; *saline* (CTRL) (Fresenius Kabi Norge AS, Halden, Norway); *negative control*.

Mouth rinsing was performed twice a day during the positive and negative control periods (after breakfast and in the evening), for 60 s with 10 ml of the assigned preparation. During the test period, the volunteers were administered 1 tablet of ibuprofen (200 mg) twice a day (after breakfast and in the evening). Immediately following the Day 14 examination, the participants received a new PTC and were instructed to perform proper mechanical plaque control measures. After a 2-week ''wash-out'' period, the participants received a final PTC and a second 14-day experimental period was initiated. The experimental and ''wash-out'' periods were repeated until all volunteers had been involved in all three regimens.

Clinical examinations

Dental plaque

Dental plaque was disclosed with erythrosin (Rondell Red®, Nordenta, Enköping, Sweden) and scored at six surfaces (disto-, mid-, mesio-buccal and disto-, mid-, mesio-lingual) of each tooth according to the Turesky modification of the 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 each tooth according to the Gingival Index (GI) system (Löe 1967). The examinations were performed on Days 0 and 14.

Microbiological examination

Plaque samples

Each site selected for plaque sampling was isolated with cotton rolls and gently dried with air. The supragingival plaque was collected using sterile Gracey curettes (LM Dental®, Turku, Finland).

Samples were obtained from the following tooth surfaces:

Day 0: mesio-buccal surfaces of 16, 24, 33 and 41;
Day 14: mesio-buccal surfaces of 14, 23, 31 and 46.

Dark field microscopy

The plaque samples were pooled and transferred to a glass vial containing saline and 0.5% gelatin and analysed within 30 min. from sampling in a dark field microscope. One hundred bacterial cells were counted and classified according to six different morphological groups: coccoid cells, straight rods, filaments, fusiforms, spirochetes and motile rods (Listgarten & Hellden 1978).

Gingival crevicular fluid (GCF) examination

GCF samples were obtained immediately after plaque sampling on Days 0 and 14. GCF was collected from the same sites that were sampled for plaque. The sites were dried and isolated with cotton rolls; a Periopaper® (ProFlow Inc., Amityville, NY, USA) was gently inserted into the gingival crevice until mild resistance was felt 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 pooled, stored in a sterile 1 ml Eppendorf tube (Sarstedt, Nümbrecht, Germany) 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* (Alb and Lf) (in such eluates of GCF was assessed using a sandwich ELISA technique (Adonogianaki et al. 1994): one plate for the analysis of Alb, and a second plate for the analysis of Lf. 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 (OD) value for each sample.

The amount of Lf (× 1000) per unit Alb was calculated.

Statistical analysis

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

Results

Plaque scores

The mean individual QHI score obtained at the end of the experimental periods (Day 14) were 1.2 (SD 0.5) (CHX), 3.1 (0.5) (IBUP) and 2.9 (0.4) (CTRL). The amount of plaque formed in the CHX was significantly smaller than that formed in IBUP and CTRL ($p < 0.05$).

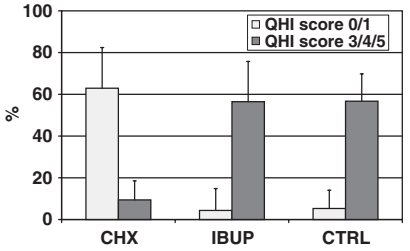

Fig. 1. Frequency distribution (mean and SD) of various plaque scores (QHI) on Day 14. QHI, Quigley & Hein Index; CHX, chlorhexidine; IBUP, ibuprofen; CTRL, control.

The proportion of surfaces with small (QHI scores 0/1) and large (QHI scores 3/4/5) amounts of plaque is presented in Fig. 1. The percentage of OHI scores 0/1 was significantly higher ($p < 0.05$) in CHX (63%) than in the remaining two study groups (IBUP: 4% and CTRL: 6%). The proportion of surfaces that harboured large amounts of plaque was significantly higher in the IBUP (59%) and CTRL (58%) groups than in the CHX (9%) group.

Bacterial morphotypes

The examination of plaque samples obtained on Day 0 disclosed that there were no significant differences between the three groups regarding the distribution of various bacterial morphotypes (Fig. 2). In samples obtained on Day 14, however, the proportions of filaments and fusiforms were significantly smaller in CHX (20%) than in IBUP (29%) and CTRL (30%) ($p < 0.05$). In addition, a significantly smaller proportion of spirochetes and motile rods occurred in CHX (9%) than in IBUP (14%; $p < 0.05$) and CTRL (16%; $p < 0.01$). No significant differences were observed between IBUP and CTRL in terms of proportions of various bacterial morphotypes on Day 14.

Gingivitis

The mean individual GI scores calculated from measurements made on Day 0 varied between 0.52 and 0.54 in the three treatment groups. In other words, at the start of the experimental periods all three groups had close to identical and comparatively low levels of gingivitis. This is further illustrated by the fact that the proportion of sites with overt signs of gingivitis (GI score $\geqslant 2$) was small and similar in the three groups; i.e. 4% (CHX), 3% (IBUP) and 2% (CTRL) (Fig. 3).

After 2 weeks of no mechanical tooth cleaning (Day 14), the proportions of sites with GI score $\geqslant 2$ were in all groups significantly higher than on Day 0. In the CHX group, the proportion of sites with GI score $\geqslant 2$ had increased

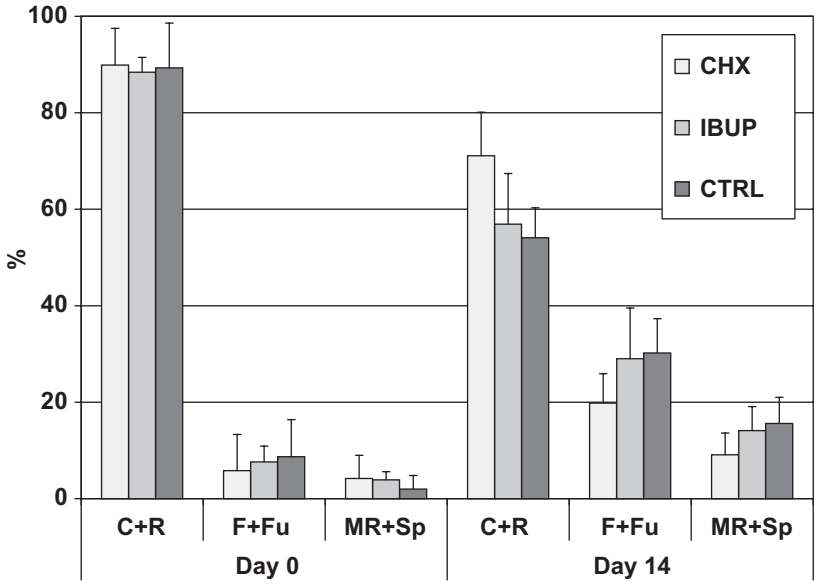

Fig. 2. Percentage distribution (mean and SD) of different groups of bacteria found in plaque samples harvested on Days 0 and 14. C+R, cocci and rods; F+Fu, filaments and fusiforms; MR+Sp, motile rods and spirochetes. CHX, chlorhexidine; IBUP, ibuprofen; CTRL, control.

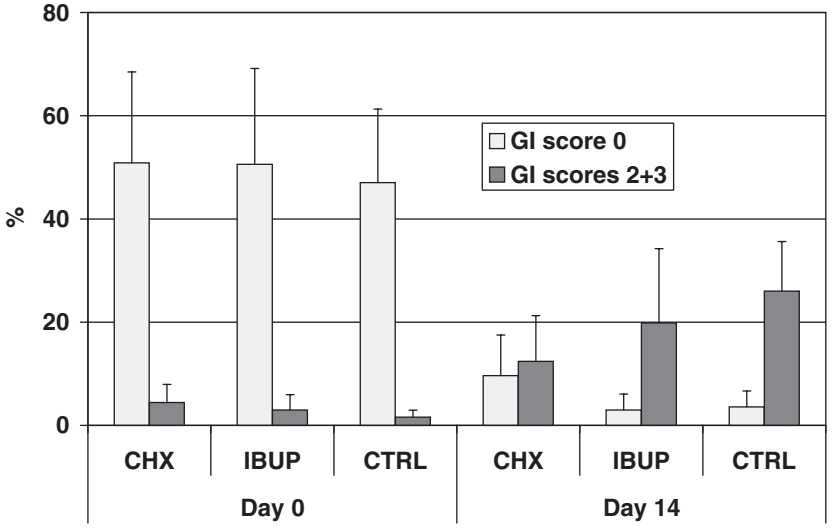

Fig. 3. Frequency distribution (mean and SD) of various GI scores on Days 0 and 14. GI, Gingival Index; CHX, chlorhexidine; IBUP, ibuprofen; CTRL, control.

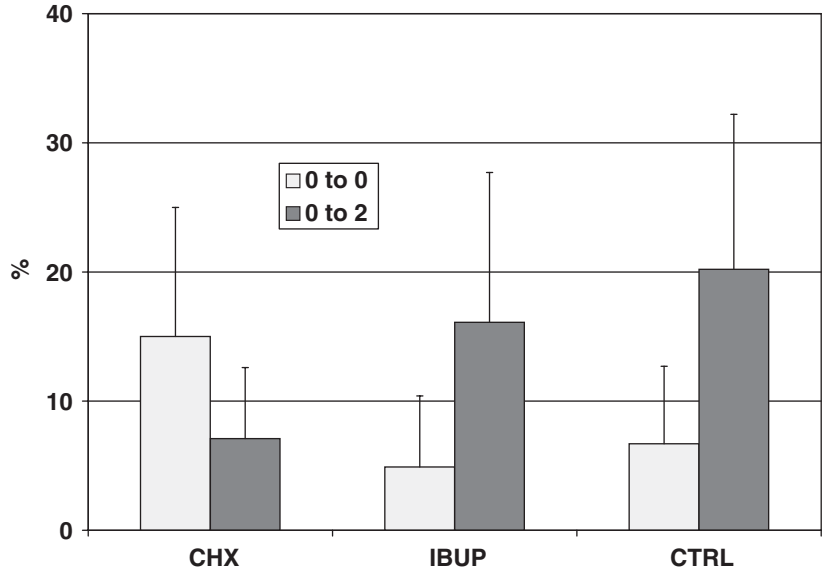

Fig. 4. Gingival sites that on Day 0 received a GI score 0, and on Day 14 either remained unchanged or had become inflamed (GI score $\geqslant 2$). GI, Gingival Index; CHX, chlorhexidine; IBUP, ibuprofen; CTRL, control.

from 4% to 12% (+8%), while the corresponding increase in the remaining two groups was 17% (IBUP) and 25% (CTRL). The increase in the proportion of inflamed gingival units was significantly smaller in CHX than in IBUP and CTRL ($p<0.01$) and significantly smaller in IBUP than in CTRL ($p<0.05$).

A further analysis of the GI score categories revealed that the percentage of sites that on Day 0 were identified as being healthy (GI score 0) but on Day 14 had become inflamed (GI score $\geqslant 2$) varied between treatments. Thus, in CHX the proportion of such changing sites was 7% while in IBUP it was 16% and 20% in CTRL (Fig. 4). Also, in this comparison, the increase was less pronounced in CHX than in IBUP and CTRL ($p<0.01$).

GCF

Volume: The flow of gingival fluid increased in all three groups between Days 0 and 14 (Fig. 5). Thus, the mean increase in the GCF volume was 0.07 µl in CHX and IBUP while the corresponding change in CTRL was 0.11 µl.

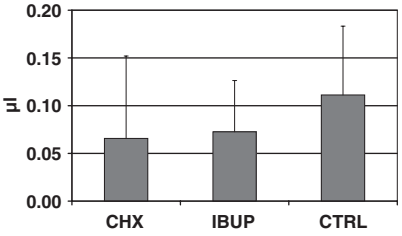

Fig 5. Increase of gingival crevicular fluid (GCF; volume; µl) (mean & SD) between Days 0 and 14. CHX, chlorhexidine; IBUP, ibuprofen; CTRL, control.

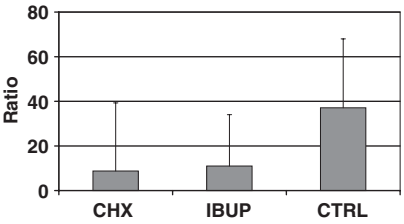

Fig. 6. Increase in the lactoferrin/albumin ratio (mean and SD) in the GCF sampled on Days 0 and 14. GCF, gingival crevicular fluid; CHX, chlorhexidine; IBUP, ibuprofen; CTRL, control.

Lf: Between Days 0 and 14 the amount of Lf in the crevicular fluid samples increased in all three treatment groups (Fig. 6) This is illustrated by the enhanced Lf/Alb ratio. The increase in this ratio amounted in IBUP to 13.4 compared with 38.1 in the negative control group (CTRL) and 9.5 in the positive control group (CHX).

Discussion

The present clinical trial examined whether gingivitis and de novo plaque formation were influenced by systemically delivered ibuprofen. The findings from examinations performed prior to and after 2 weeks of no mechanical tooth cleaning demonstrated that the administration of the anti-inflammatory drug (i) reduced gingivitis (GI scores, gingival fluid volume and composition) while (ii) neither the amount of plaque formed (QHI scores) nor the distribution of various bacterial morphotypes in the biofilm appeared to be influenced by the daily use of ibuprofen.

De novo plaque formation

The observation that suppression of gingivitis had no apparent effect on de novo plaque formation may not be

consistent with data reported previously. Thus, in clinical studies by e.g. Saxton (1973), Hillam & Hull (1977), Goh et al. (1986), Quirynen et al. (1991), Ramberg et al. (1994, 1995) and Daly & Highfield (1996) it was noted that the initial phases of the build-up of a dental plaque were influenced by the condition of the adjacent gingival tissue. The apparent discrepancy between the current findings and the results of the earlier studies is most likely explained by differences in the duration of the plaque formation intervals. Hence, in the studies referred to above, plaque was sampled and/or scored after a few hours (Saxton 1973), 1 day (Goh et al. 1986), 3 days (Daly & Highfield 1996), 4 days (Quirynen et al. 1991, Ramberg et al. 1994), 7 days (Hillam & Hull 1977) of no mechanical tooth cleaning, while in the present trial the biofilm was examined first after 2 weeks of ''experimental gingivitis''. There are reasons to suggest that the effect of gingival inflammation on plaque formation may be discernible only during an early phase of plaque build-up and that once biofilm formation has begun; its further growth seems to be independent of the amount of crevicular fluid that is available in the adjacent gingival sulcus.

Suppression of gingivitis

In the present clinical trial, the ''experimental gingivitis in man'' model as described by Löe et al. (1965) was used and two different control periods – one negative and one positive – were included. During the negative control period, the panelists rinsed with saline and accumulated large amounts of plaque and developed marked signs of gingivitis. Thus, at the end of this period 58% of all tooth surfaces received a QHI score of 3, 4 or 5 and $>25\%$ of gingival sites exhibited a GI score of $\geqslant 2$. During the positive control period, the panelists rinsed with chlorhexidine digluconate, formed small amounts of plaque and exhibited few sites ($<10\%$) with a GI score $\geqslant 2$. Throughout the main test period, the participants administered a low dose of ibuprofen (200 mg twice daily) but did not use a mouth rinse. During this 2-week regimen, they formed similar amounts of plaque (59% of surfaces received a QHI score 3/4/5) as during the negative control period but presented on Day 14 with significantly fewer sites that scored GI $\geqslant 2$.

Based on the above findings, it is suggested that ibuprofen administered via the systemic route may have a small but clinically discernible effect on gingivitis. The validity of this proposal is supported by data describing gingival fluid flow. Thus, while during the negative control regimen the GCF value increased from 0.11 to 0.22 µl, in positive control as well as in the test periods, the corresponding increase was only 0.07 µl. Further, the increase in the amount of Lf – indicative of the presence and amounts of neutrophilic leucocytes (Adonogianaki et al. 1994) – in GCF was three times greater in the CTRL group than in the IBUP group.

The finding that systemic administration of an NSAID may control gingivitis is in agreement with data presented previously. Heasman et al. (1993) examined the effects of systemically administered flurbiprofen (50 mg twice daily) in human volunteers who abstained from mechanical tooth cleaning for a period of 3 weeks. They reported that the NSAID significantly ($p<0.001$) inhibited the proportion of sites that developed gingival redness (from 42% new units in a placebo group to 21% new units in the flurbiprofen group). Further, the mean percentage of sites that bled on probing increased in the control group from zero at baseline to 0.7 at 2 weeks and 3.4 at 3 weeks, while there ''was a total absence of gingival bleeding throughout the trial in the flurbiprofen group''.

In the current study, the number of sites that at baseline were clinically healthy (GI score 0) but at 2 weeks exhibited signs of marked inflammation (GI score $\geqslant 2$) amounted to 25% in the negative control group and 17% in the IBUP group. Thus, the effect on gingivitis by ibuprofen in the present sample appears more modest than that accomplished under similar conditions by the systemic administration of flurbiprofen. The difference in treatment outcome may in part also be explained by the amount of NSAIDs used in the two trials. Thus, in the study by Heasman et al. (1993) 50 mg × 2 of flurbiprofen was given to the panelists. This amount is within the recommended dose range to provide an optimal antiphlogistic effect (Insel 1996). In the current trial, the daily dose of ibuprofen was limited to 400 mg as this amount is regarded as sufficient to secure an anti-inflammatory outcome (Giannessi et al. 1993).

Non-steroidal anti-inflammatory compounds have also been used as adjuncts in the treatment of gingivitis/periodontitis. Johnson et al. (1990) examined the effect of Naprosyn® (Roche Pharmaceuticals, Nutley, NJ, USA) on gingivitis and its resolution following treatment. One hundred and twenty patients with generalized gingivitis (mean GI score $\geqslant 1.5$) were recruited and divided into two groups: one received Naprosyn® (250 mg × 2/day) and the second group a placebo drug. Examinations regarding plaque, gingivitis and sulcular bleeding were performed at baseline and after 28 days. A full-mouth prophylaxis was subsequently provided and the patients were re-examined on day 30. Naprosyn® had no effect on parameters examined on day 28 but ''enhanced the resolution of gingival inflammation following removal of microbial plaque''. Similar findings were presented by Heasman et al. (1994), who studied the effect of systemically administered flurbiprofen used as an adjunct to toothbrushing in the resolution of gingivitis. Forty-seven volunteers abstained from mechanical plaque control measures for a period of 21 days and developed generalized gingivitis. At this interval, 23 subjects were prescribed flurbiprofen (100 mg daily) while 24 subjects received a placebo. In both groups, tooth-cleaning measures were resumed and subjects were re-examined after 1 week. GI values in both groups were reduced between Days 21 and 27 but ''the flurbiprofen group showed a small, yet statistically significant greater improvement than the placebo subjects''.

Similar findings were presented by Taiyeb & Waite (1993), who studied the effect of systemic ibuprofen used as an adjunct to basic mechanical therapy in patients with chronic periodontitis. Seventeen subjects were divided into two groups: one test and one control group. The subjects in the test group received a 2-week treatment with ibuprofen (200 mg × 4/day). All 17 patients were given oral hygiene instruction and two quadrants were in addition exposed to scaling and root planing. Examinations were performed at baseline and subsequently every 2 weeks during a 2-month interval. It was observed that the ibuprofen administration had an effect on clinical parameters describing periodontitis. Thus, at the 2-week examination there was ''significantly greater reduction in gingival

bleeding, colour and pocketing''...''in the test compared with the control group. ''The beneficial effects were less evident thereafter''.

Conclusion

Findings from the present clinical trial as well as data reported from studies on the prevention and treatment of gingivitis/periodontitis in humans suggest that NSAIDs administered via the systemic route may have an effect on the inflammatory lesions in the gingiva. In this context, however, it must be realized that (i) there seems to be no long-term effect on plaque by the suppressed gingival inflammation, (ii) the outcome of antimicrobial measures, mechanical as well as chemical, in the prevention and treatment of gingivitis is more predictable than that obtained by the systemic administration of anti-inflammatory compounds.

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