The Effect of Parodontax® on the MMP-8 Concentration in Gingivitis Patients

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Summary The aim of the study was to evaluate the efficacy of Parodontax® (GlaxoSmith-Kline, Bühl, Germany) on the signs gingival inflammation and the enzyme activity of matrix metalloproteinase-8 (aMMP-8) in the gingival crevicular fluid.

After approval by the ethics commission, a total of 50 volunteers participated in the study; group 1 (n = 25, age: 43 ± 12 years) with moderate gingivitis (BOP +) and group 2 (n = 25, age: 29 ± 11 years) with clinically healthy gingival conditions (BOP –). After obtaining anamnestic data, the dental examination included assessment of oral hygiene (Quigley & Hein 1962), gingival inflammation (Saxer & Mühlemann 1975), probing pocket depth and clinical attachment level. Gingival crevicular fluid was collected from both groups. A quantitative assessment of aMMP-8 in the gingival crevicular fluid samples was performed (DentoAnalyzer, Dentognostics GmbH, Jena, Germany). Study participants were instructed to use only Parodontax®. After three weeks, all parameters were measured again.

The aMMP-8 values of group 1 were significantly reduced after the use of Parodontax® toothpaste and mouthwash ($p < 0.001$; baseline median 41.25 ± 38.16 ng/ml, final post-treatment median 7.73 ± 7.58 ng/ml aMMP-8 eluate; group 2: baseline median 3.75 ± 3.16 ng/ml, final post-treatment median 3.73 ± 1.54 ng/ml aMMP-8 eluate). Gingival inflammation and plaque accumulation were reduced.

It was shown that Parodontax® was effective in reducing the enzymatic activity of inflammation.

Introduction

Inflammatory periodontal diseases are among the most common diseases in the industrialized nations. For instance, the Fourth German Oral Health Study (DMS IV) from 2006 showed a dramatic increase in periodontal lesions. According to this source, 52.7% of adults suffer from moderate forms of periodontitis and 20.5% from severe forms. Among the elderly, however, 48.0% exhibited moderate and 39.8% severe forms of periodontitis (Micheelis & Schiffner 2006).

Gingivitis is one of the most frequent periodontal diseases, and is closely associated with dental plaque bacteria and their metabolites, as shown in numerous experimental and clinical studies (Mühlemann & Son 1971, Page 1986, Lauterbach 1998). According to data from the German Periodontology Society (DGP), 80% of all Germans suffer from some degree of gingivitis (Pecanov-Schröder et al. 2004).

Because this disease is so common, the industry has constantly marketed new, improved oral hygiene products, the efficacy of which has been tested both clinically and in vitro

(Renggli 1990, Axelsson 1993, Bernimoulin & Deschner 1995, Arweiler et al. 2002).

During the pathogensis of periodontitis, the inflammatory response of the host tissue to the extant bacterial biofilm leads to the release of matrix metalloproteinase-8 (MMP-8), a tissue-destroying enzyme with an average zymogenic mass of 75 kilodalton (Kinane 2000, Hellwege 2003, Sorsa et al. 2006).

The MMPs are a family of endopeptidases with over 20 zinc-dependent proteolytic enzymes, which are classified into the following subgroups based on their different characteristics: basal membrane collagenases, interstitial collagenases, stromelysins, matrix lysines, and membrane-bound MMPs (Deutzmann et al. 2007). These enzymes possess the ability to restructure tissue by catabolizing the extracellular matrix. However, even under physiological conditions, the extracellular matrix can undergo catabolism, e. g., during organogenesis, or during other pathological conditions in inflammatory processes (Galis & Khatri 2002).

MMP activity is regulated by cytokines, growth factors, and eicosanoids, for instance, interleukin-1 (IL-1), tumor necrosis factor (TNF)-α, transforming growth factor (TGF-α), epidermal growth factor (EGF), and prostaglandin E2 (PGE2) (Müller-Ladner & Gay 2006).

Further, tissue-resident TIMPs (tissue inhibitors of matrix metalloproteinases) can effect an inhibitory potential on MMP activity through a non-covalent bond to the MMP. Under physiological conditions, TIMPs and MMPs are in equilibrium. As periodontitis develops, this equilibrium is lost and shifts in favor of MMP-8. This results in an increased level of aMMP-8 and hence enhanced collagen breakdown in the tissue affected (Overall 1994, Brew et al. 2000, Ehlers et al. 2008). Non-steroid, synthetic MMP inhibitors, such as chemically modified tetracycline, are highly important for periodontal treatment (Ryan et al. 1996).

Depending on specificity, aMMP can break down certain components of the extracellular matrix, e. g., collagen, fibronectin, laminin, or elastin (Chandler et al. 1995); aMMP contains a group of various MMPs. Due to their high structural instability, the fragments produced by proteolytic collagen breakdown degrade further into gelatin components.

Besides type I to III fibrillar collagen, MMP-8 has the aggrecan protein as a substrate, and is the most important detectable MMP in the crevicular fluid during periodontal degradation processes (Birkedal-Hansen 1993, McCulloch 1994).

The activity of human MMP-8 is decisively influenced by the individual's immunological status. Moreover, MMP-8 has become established both clinically and scientifically as a highly specific biomarker for the degree of inflammation of the periodontium (Sorsa et al. 2004, Kinney et al. 2007, Prescher et al. 2007). In addition to damaging periodontal tissue, it is also associated with bone degeneration (Sorsa et al. 1999, Ma et al. 2000, Netuschil 2009).

The aim of the present clinical study was to determine the inhibitory effect of Parodontax® on the degree of gingival inflammation, plaque accumulation, and aMMP-8 concentration, in order to optimize the therapy of patients suffering from gingivitis.

Material and methods

This study included a total of 50 adult volunteers of both sexes (36 women, 14 men, age: 36.14 ± 13.51 years) with no general or systemic diseases. The participants were divided into 2 groups: group 1 included participants (n = 25, age: 43.08 ± 12.01 years) with slight to moderate gingivitis (presence of bleeding on probing), and group 2 comprised only gingivally healthy participants (n = 25, age: 29.20 ± 11.31 years) without bleeding on probing (BOP 0%). Because group 2 contained periodontally healthy volunteers, the average age was lower. In selecting the participants, a medical history was first taken to check the inclusion/exclusion criteria. All study participants had to be of age, and the exclusion criteria were as follows: antibiotic therapy within three months prior to treatment, diabetes mellitus types I and II, smoking, systemic diseases, allergy to antibiotics, and pregnancy.

Only the plaque-induced form of gingivits was examined; that is, hormonally caused (puberty or pregnancy gingivitis) or medication-caused gingivitis, and gingivitis associated with systemic disease and diabetes were not considered.

The examination was conducted after explaining the study to participants and obtaining their signed informed consent. The ethics commission approved the study (No. 837.198.08 [6195]), and the evaluation was performed anonymously. The guidelines of the Helsinki Declaration were observed.

Following explanation and informed consent, the clinical examination was conducted, including documentation of the status of the dentition and orthopantographs (indication was met). For the aMMP-8 measurement, one periodontium per quadrant was chosen. At each of the four periodontia, a GCF sampling strip (GCF = gingival crevicular fluid) was mesially or distally inserted into the gingival sulcus for 30 s (Fig. 1).

After collecting the crevicular fluid, the samples were processed as follows: the GCF sampling strips were placed in a reaction flask (elution flask) containing 800 µl of elution solution and eluted. The elution solution consisted of HEPES buffer (2-[4-[[2-hydroxyethyl]]-1-piperazinyl]-ethane sulfonic acid) with nonionic detergent and protein. 0.1% bromonitrodioxane (BND) was added as a preservative.

Elution was performed to extract the crevicular fluid from the GCF sampling strip. In the present study, the GCF strip was placed in the elution solution for exactly 30 seconds and steadily swirled. The resultant mixture of dissolved substances and elution agent were pipetted out of the reaction flask and placed in the test set-up.

Subsequently, quantitative analysis was performed in the DentoAnalyzer (Dentognostics; Jena, Germany), as described previously elsewhere (Prescher et al. 2007, Sorsa et al. 2009).

Further examinations were conducted to determine clinical periodontal parameters: probing depth, recession, attachment

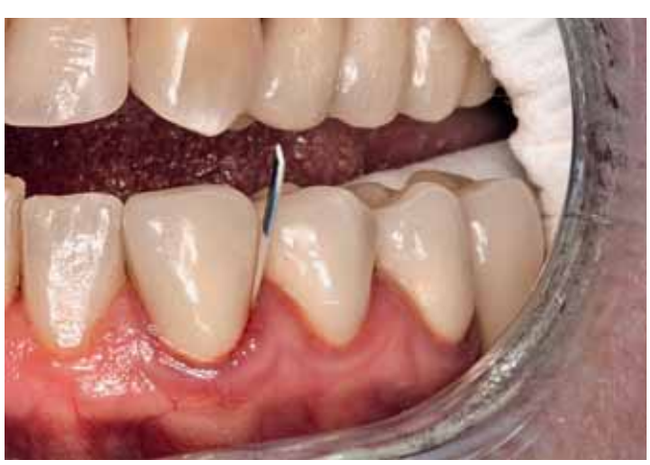

Fig. 1 GCF sampling strip in situ for evaluating the aMMP-8 concentration in crevicular fluid.

level, degree of mobility, percussion, senstitivity test, papillary bleeding index (PBI) according to Saxer & Mühlemann (1975), and the Quigley-Hein plaque index (PI) (Quigley & Hein, 1962). Measurements were done with a periodontal probe (Hu-Friedy; Chicago, IL, USA), that is, a color-coded periodontometer of the Qulix series (PCPUNC156). To record the plaque index, study participants were asked to chew a plaque disclosing tablet (Produits Dentaires S. A., Vevey, Switzerland), distribute it throughout the oral cavity, and finally rinse out twice. After the plaque had been stained, its amount was evaluated.

Using the previously recorded tooth status and OPGs, the DMFT index was calculated, and the number of missing, endodontically treated, and crowned teeth, as well as fillings and implants were counted and documented. The OPG was also used to evaluate the dental findings. In addition, a distinction was made between gingivitis and chronic periodontitis. Participants were then asked about which oral hygiene aids they used to clean their teeth: conventional or electric toothbrush, dental floss, interdental brush.

Once the baseline examination was complete, each participant was given Parodontax® fluoridated toothpaste and Parodontax® mouthwash concentrate (GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, Bühl, Germany) with instructions to use each three times a day (morning, midday, and evening).

Three weeks after the baseline examination, a control examination was performed in which the aMMP-8 concentrations and other clinical parameters were again recorded.

The statistical evaluation was carried out by the Institute for Medical Biometry, Epidemiology and Informatics (IMBEI) at the University Medicine Department of Johannes Gutenberg University, Mainz, Germany. A numerical key was used to enter the data in the statistics program SPSS 15.0 for Windows and the statistical analysis was performed.

The main question as to the anti-inflammatory effect of Parodontax® on the concentration of aMMP-8 was tested group-specifically using the Wilcoxon signed-rank test for related samples. Due to multiple tests, a Bonferroni correction was performed and each group was analyzed to a level of 2.5%.

For each of the periodontal parameters – probing depth, recession, attachment level, papillary bleeding index, plaque index, and aMMP-8 concentration – the arithmetic mean was taken.

For parametric data, which are normally distributed, means and standard deviations were calculated; for the non-normally distributed (non-parametric) data, medians and quartiles were presented. In addition, for discrete parameters, absolute and relative frequencies were given.

Results

The aMMP-8 concentration in ng/ml eluate was determined in all participants at baseline and after three weeks of oral hygiene care using the Parodontax® products. The median baseline aMMP-8 concentration in group 2 was 3.75 ng/ml aMMP-8 eluate (first quartile = 3.00 ng/ml, third quartile = 5.36 ng/ml), and the final median was comparable (3.73 ng/ml aMMP-8 eluate; first quartile = 2.99 ng/ml, third quartile = 4.25 ng/ml) (Fig. 2). In contrast, the baseline median in group 1 was 41.25 ng/ml aMMP-8 eluate (first quartile = 23.13 ng/ml, third quartile = 97.64 ng/ml), and after three weeks using the Parodontax® products, it was 7.73 ng/ml aMMP-8 eluate (first quartile = 3.08 ng/ml, third quartile = 13.00 ng/ml) (Fig. 3). Because there were multiple tests, a Bonferroni correction was performed, and each group was analyzed to a level of 2.5%. The group 2 p-value was 0.161, and the group 1 p-value was < 0.001. Thus, the reduction of aMMP-8 concentration in group 1 was significant. In group 2, the average papillary bleeding index (PBI; Saxer & Mühlemann, 1975) at baseline was 0.85 ± 0.71, and at the follow-up it was 0.80 ± 0.12.

The average PBI in group 1 at baseline was 3.34 ± 0.30, but it had dropped to 1.14 ± 0.53 at the end of the study, which was statistically significant ($p < 0.001$) (Fig. 4). In group 2, the average value of the Quigley-Hein plaque index (PI) was 0.90

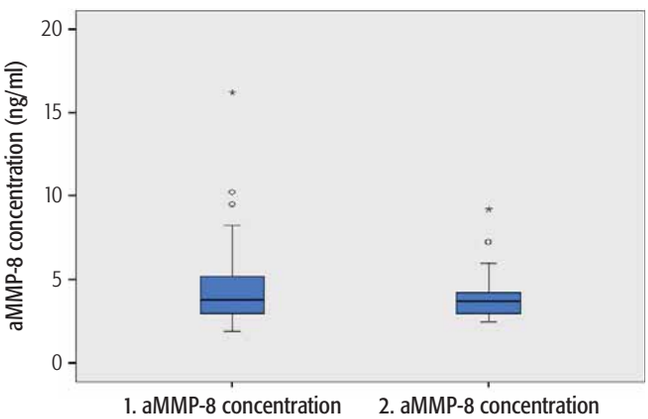

Fig. 2 Baseline and final aMMP-8 concentrations (ng/ml) in group 2 (healthy subjects). The graph shows medians ($p = 0.161$).

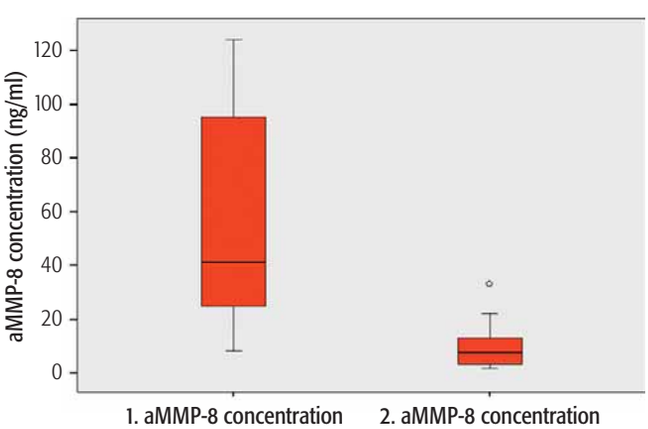

Fig. 3 Baseline and final aMMP-8 concentrations (ng/ml) in group 1. The graph shows medians ($p < 0.001$).

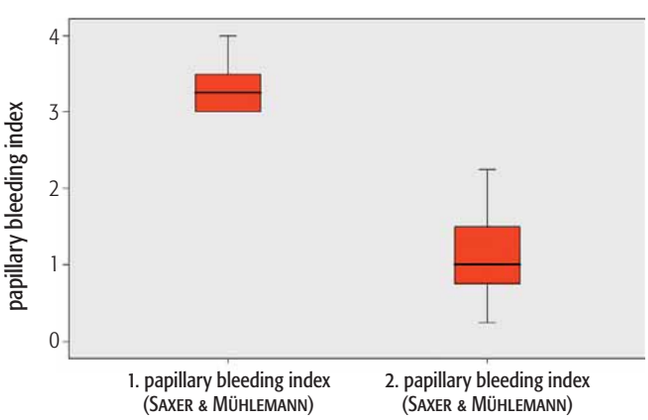

Fig. 4 Baseline and final papillary bleeding indices in group 1. The graph shows medians ($p < 0.001$).

± 0.59 at baseline. Three weeks later, the average PI was 0.33 ± 0.40. The average baseline PI in group 1 was 1.37 ± 0.86, which decreased to 0.54 ± 0.56 by the final examination.

Discussion

The present clinical study was conducted to examine the effect of Parodontax® fluoridated toothpaste combined with Parodontax® mouthwash concentrate on the degree of gingival inflammation, amount of plaque, and concentration of aMMP-8.

Group 2 showed good baseline values in terms of the Quigley-Hein plaque index (1962) and the papillary bleeding index (PBI; Saxer & Mühlemann 1975), which were attributable to the good to excellent oral hygiene. Under these conditions, it was already clear at baseline that improvement in this group would only be slight.

Although numerous studies have examined the efficacy of Parodontax® on plaque, degree of gingival inflammation, and pH of saliva, they only considered clinical parameters and did not use enzyme diagnostics. In a single-blind, placebo-controlled 4-week study, 50 participants were instructed to use only the Parodontax® products or the placebo twice a day. The experimental group exhibited a marked reduction of the sulcus bleeding index (SBI) and the approximal plaque index (API): the SBI dropped from 33.4% to 18.6%, and the API from 40.8% to 23.9%. In the control group, both the SBI and the API increased: from 20.4% to 29.1% and from 35.8% to 37.2%, respectively (Willershausen et al. 1991). A subsequent study confirmed these findings (Willershausen et al. 1993).

These clinical studies substantiate the positive influence of Parodontax® on the plaque and bleeding indices. Many other clinical studies also support the efficacy of Parodontax® in patients with gingivitis. For instance, in a study by Berghoff (1969) on gingivitis patients who used Parodontax®, the patients (n = 207) were instructed to brush their teeth with Parodontax® toothpaste after every meal and then rub the toothpaste into their gums. Despite the short observation period, gingival bleeding disappeared almost entirely in 25–30% of all patients.

In a 20-day examination, de Rysky (1988) proved the anti-inflammatory effect of Parodontax® toothpaste in a group of 40 patients who were suffering from gingivitis. The patients were instructed to brush their teeth twice a day with Parodontax® toothpaste and leave the toothpaste in the mouth for a few minutes.

In a 4-week double-blind study with 168 gingivitis or periodontitis patients, Parodontax® toothpaste yielded much better results in terms of bleeding, swelling, redness, and taste, than did a placebo toothpaste without herbal active ingredients (Murai & Emling 1988).

These studies demonstrate that the use of Parodontax® by gingivitis patients can lead to definite improvement. The current clinical study – in which gingival inflammation was clearly reduced – confirms these earlier results. In terms of the unusual taste, attributable to the particular ingredients and special combination of mineral salts and herbal extracts, participants in the present study also mentioned the salty flavor to require some accustomization, but after a certain period of using this toothpaste, the participants described it as pleasantly refreshing.

In another double-blind study on 1,255 patients (Jaschouz, 1991), which took place in cooperation with dental practices in Switzerland, the use of Parodontax® showed pronounced improvement in the BOP value, which decreased in the test group from 27.7% to 18.3%.

In 22 gingivitis patients in a placebo-controlled, double-blind, randomized study, Saxer et al. (1994) compared the effects of Parodontax® and a fluoridated toothpaste. During the 4-week observation period, the patients were to brush their teeth twice a day with the respective toothpaste and rub it into the free gingiva in the evening. Compared to the fluoridated toothpaste, Parodontax® effected a significant reduction in bleeding on probing.

In a 6-month double-blind study on 128 patients, Yankell et al. (1993) examined the influence of Parodontax® vs a placebo toothpaste on the clinical parameters plaque index, gingival index, and bleeding on probing at baseline and then at 3-month intervals. Compared to the placebo, Parodontax® toothpaste significantly reduced the plaque and bleeding indices.

Panutti et al. (2003), in a further double-blind trial, studied the possible influence of Parodontax® toothpaste on the gingiva. After three weeks, a significant reduction of the gingival index was documented for the test group.

In a 4-day crossover study on eight participants, Arweiler et al. (2002) observed the antimicrobial effects of various oral hygiene products, including that of Parodontax® toothpaste. At baseline, all participants received professional tooth cleaning and instructions to rinse twice a day exclusively with Parodontax® toothpaste slurry or a chlorhexidine (Chlorhexamed-Fluid) 0.1% mouthwash. In the Parodontax® group, a plaque reduction of 12–30% was found, and the chlorhexidine group showed 40–64% fewer plaque accumulation.

However, long-term use of chlorhexidine products is associated with some undesirable side-effects, such as tooth and mucous-membrane discoloration, desquamation and ulceration of the gingiva, and alterations in the sense of taste (Pucher & Daniel 1992, Yates et al. 1993). Thus, Parodontax® mouthwash provides certain advantages compared to chlorhexidine rinses.

In a placebo-controlled, double-blind clinical study with 60 subjects in which the effect of Parodontax® toothpaste was compared to that of Crest® toothpaste, the Parodontax group exhibited a significant improvement in bleeding on probing after two months vs the Crest and placebo groups. The Löe gingivitis index (Löe, 1967) also substantiated a clear superiority of Parodontax® vs Crest® and the placebo toothpaste (Yankell & Emling 1988).

Similarly, Saxer et al. (1995) compared the effect of Parodontax® with a new, not yet commercially available toothpaste containing herbal extracts. In the first four weeks of the 12-week study, all 60 participants used the new toothpaste. After this 4-week period, two groups were formed, where one group continued using the new toothpaste and the other used Parodontax® toothpaste. Compared to baseline findings, both groups exhibited a 40% reduction in the bleeding and gingivitis indices at the end of 12 weeks.

aMMP-8, which can be considered a highly significant biomarker both clinically and scientifically for the degree of periodontal inflammation (Sorsa et al. 2004, Kinney et al. 2007), was quantitatively evaluated in the present study using Dento-Test aMMP-8. After using Parodontax®, patients in the test group exhibited a significant reduction in aMMP-8 concentration, while in the control patients the aMMP-8 concentration did not change between baseline and the second, final examination. Moreover, both the papillary bleeding index and the plaque index decreased markedly in the test group.

In summary, it was shown that the use of Parodontax® fluoridated toothpaste combined with Parodontax® mouthwash concentrate significantly reduced the aMMP-8 concentration in the crevicular fluid of patients with slight to moderate gin-

givitis, and also decreased the bleeding and plaque indices. Therefore, Parodontax® products can be seen as adjuvant oral hygiene measures in the treatment of gingivitis patients, and can also be used successfully for daily prophylaxis. Nevertheless, in all clinical studies on the optimization of oral hygiene measures and improvement of inflammation-related periodontal diseases, patients' cooperation and implementation of optimal oral hygiene techniques must always be taken into consideration.

Résumé

La présente étude clinique se proposait de déterminer l'efficacité du Parodontax® (GlaxoSmithKline, Bühl, Allemagne) sur la réduction de l'inflammation et de l'activité enzymatique (aMMP-8) dans le liquide gingival. Après une expertise concluante et un vote positif de la commission d'éthique, 50 adultes au total ont pris part à l'étude: groupe 1 (n = 25, âge: 43 ± 12 ans), avec une inflammation gingivale faible à modérée (BOP +) et groupe 2 (n = 25, âge: 29 ± 11 ans), avec un tissu gingival sain (BOP –). Un bilan dentaire a été effectué pour chacun des participants, incluant état dentaire, indice de saignement des papilles (Saxer & Mühlemann 1975), indice de plaque (Quigley & Hein 1962), profondeur des poches, récession et niveau d'attache clinique. Au début de l'étude, on a mesuré l'activité des enzymes aMMP-8 (DentoAnalyzer, Dentognostics GmbH, Allemagne) dans le tissu gingival des individus des groupes 1 et 2. Par la suite, durant trois semaines, les participants ont utilisé exclusivement un dentifrice et une solution buccale avec adjonction d'herbes (Parodontax®). A la fin de l'utilisation des produits Parodontax®, l'activité des enzymes aMMP-8, ainsi que les autres paramètres dentaires ont été de nouveau relevés. Les résultats du groupe 1 ont montré une réduction significative de la concentration en aMMP-8 ($p < 0{,}001$; valeur initiale moyenne 41,25 ± 38,16 ng/ml, valeur finale moyenne 7,73 ± 7,58 ng/ml aMMP-8-Eluat; groupe 2: valeur initiale moyenne 3,75 ± 3,16 ng/ml, valeur finale moyenne 3,73 ± 1,54 ng/ml aMMP-8-Eluat). On a également observé une amélioration de l'indice de saignement et de l'indice de plaque.

Par conséquent, les produits Parodontax® peuvent réduire l'activité enzymatique des collagénases en cas de gingivite.

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