The clinical application of hyaluronic acid in gingivitis therapy

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Objective: The efficacy of a topical application of hyaluronic acid (HA) was tested for treating gingivitis. **Method and materials:** Sixty nonsmoking outpatients in good general condition, with clinical signs of gingivitis, were included in the study. Forty patients (HA group, 20 men, 20 women; age: 32.8 ± 11.3 years) used a spray containing HA 5 times daily over a period of 1 week. The control group consisted of 20 patients (10 men, 10 women; age: 31.3 ± 9.3 years). The clinical parameters DMF-T (decayed, missed, filled teeth) index, approximal plaque index, sulcus bleeding index, papilla bleeding index, and gingival crevicular fluid were measured at baseline (T1), after 3 days (T2), and after 7 days (T3). **Results:** A reduction in the sulcus bleeding index of the HA group (T1: 72.9 ± 19.5%) to 50.3 ± 21.1% was noted at T2, and at T3 the sulcus bleeding index was 40.7 ± 23.0%. The papilla bleeding index values of the HA group were 1.6 at T1, 1.0 at T2, and 0.7 at T3. The gingival crevicular fluid showed significant reductions in the HA group. At T1 the recorded mean value was 16.3, at T2 it was 11.8, and at T3 it was 7.9. Only insignificant changes were observed in the respective indices of the control group. There were no significant alterations in the plaque values of either group throughout the study period. **Conclusion:** The results obtained by this study demonstrate that the topical application of an HA-containing preparation represents a potentially useful adjunct in the therapy of gingivitis, although its use does not diminish the need for plaque reduction as a primary therapeutic measure. *(Quintessence Int 2005;36:531-538)*

Key words: gingivitis, gingivitis therapy, hyaluronic acid

Inflammatory diseases of the gingiva and the periodontium represent the most frequent pathologic changes of the oral cavity. The high prevalence of gingivitis in Western industrialized countries underlines the urgent need for preventive action.[1] Although gingivitis does not always lead to periodontitis, it is well known that periodontitis is always preceded by gingivitis[2]; therefore, maximum attention has to be given to gingivitis therapy as a strategy for preventing periodontitis.

Clinically healthy gingiva is characterized by a dynamic equilibrium[3] of dental plaque bacteria and immunologic tissue responses. Various enzymes of oral microorganisms, such as hyaluronidases and chondroitinases, are capable of making the gingival epithelial barrier more permeable by changing the structure of the extracellular matrix, thereby facilitating the subsequent passage of bacteria into the gingival tissue. Furthermore, various enzymes, such as collagenases or chon-

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droitinases, are able to break up connective tissue structures. Bacterial metabolites like exo- and endotoxins (lipopolysaccharides), as well as ammonia, hydrogen sulfide, indole, or fatty acids, are frequent, active participants in the destructive process. As a result of a simultaneous increase in plaque formation and a change in the bacteria population in favor of anaerobic bacteria, the adjacent gingival connective tissue is characterized by vasodilatation of the gingival vessels and subsequent infiltration of neutrophil granulocytes. The most important immunologic reaction mechanisms include the release of interleukin-1, tumor necrosis factor x, and prostaglandin release, in particular prostaglandin E_2 (PGE_2).[4] These alterations are generally clinically detectable by redness, or by the presence of the edematous swelling associated with pseudopockets.[3] Various systemic or metabolic disorders, bacterial invasion, heavy smoking, or specific long-term medications are either confirmed or potential risk factors, both for the development and the severity of gingivitis.[5–10]

Although hyaluronic acid (HA) is known to play a decisive role in the course of forced repair processes, and has been found in the epithelium of the oral mucosa,[11] it has rarely been used as a therapeutic measure in periodontics.[12,13] Various medical specialties have, however, reported the successful application of HA in treating temporomandibular joint disorders.[14,15]

One of the most important functions of HA is the regulation of water homeostasis in tissues. This serves as a barrier to the diffusion of macromolecules and modulates cellular functions, including cell aggregation, cell division, and cell proliferation.[16–18] Additionally, high molecular weight HA is capable of inhibiting the phagocytic activity of macrophages, whereas low molecular weight HA is known to stimulate this activity.[19,20] Polypeptide growth factors have further been shown to influence HA production.[21,22] Reports by various authors support the assumption that HA is critically important in the localization of blood vessels, wound healing, and tissue regeneration.[12,16,23]

In addition to the receptor for hyaluronan-mediated motility (RHAMM), CD44 is the most important known cell surface receptor for HA, and is actively involved in cell-cell interaction.[24–30] Free extracellular HA may be degraded by macrophages, cells of the lymphatic system, or by enzyme activity.[31–34]

Of special interest in the field of pharmacotherapy are HA's anti-inflammatory and tissue regenerative properties, as well as its anti-edematous effects.[12,16,35,36] The aim of this study was to determine the effect of an HA-containing solution in the treatment of gingivitis. The effect of its topical application was tested in patients with clinical signs of acute gingivitis. Particular emphasis was placed on the investigation of the effect of the solution on possible alterations of various clinically relevant periodontal parameters.

METHOD AND MATERIALS

A total number of 60 outpatients (30 men, 30 women; age: 32.3 ± 10.6 years) were included in the study. All test subjects volunteered to participate in the study and were randomly allocated to a test group (HA group, 20 men, 20 women; age: 32.8 ± 11.3 years) and a control group (10 men, 10 women; age: 31.3 ± 9.3 years). Test subjects who smoked, had systemic diseases, such as diabetes mellitus and cardiovascular diseases, were pregnant, took ongoing medications, or had taken antibiotics within the past 6 weeks were excluded from the study. All patients showed clinical signs of gingivitis (reddening, loss of stippling, bleeding on probing, elevated flowrates of gingival crevicular fluid [GFC]), but none of the patients had signs of periodontitis. To exclude the possibility of an underlying periodontal disease, each patient underwent periodontal pocket probing at 6 sites of all teeth, and radiographs were obtained at the time of each examination.

The HA-containing substance (Gengigel, Merz Dental) used in this study was applied as a spray (50 mL). The active ingredient of the biotechnologically produced solution is sodium hyaluronate-based and does not contain a propellant. A high mean molecular weight HA preparation (approximately 8 × 10^6 Da) was selected for the exogenous

application of the dental medication to ensure effective binding to the mucous membrane.[18] Further ingredients of the spray include aqua, xylitol, alcohol, polyethyleneglycol-40 hydrogenated castor oil, polyvinyl alcohol, aromatics, and dichlorobenzyl alcohol.

A detailed medical history was obtained for all test subjects following allocation to a group. The patients of both groups were instructed not to change their oral hygiene habits during the study period. Patients of the test group (HA group) were instructed in the proper handling of the spray. Each time the spray was used, the patient was to apply each drizzle from the buccal right (first and fourth quadrant) and from the buccal left (second and third quadrant), respectively. Another drizzle was applied from labial to the dental front and then from oral to all four quadrants. The patient was to hold his or her breath while applying the spray. The spray was to be used 5 times a day. They were further instructed to then rinse the entire oral cavity gently, and to spit the remaining solution out after an exposure time of approximately 5 to 10 seconds. The HA group used the test spray throughout the entire 7-day study period. The control group did not use a placebo solution, because technical difficulties encountered by the manufacturer prevented the production of this solution. The members of the control group therefore did not receive any detailed information on the aim of the investigation. In the course of the study each patient was examined at 3 different times: the time from the initial (T1) to the second examination (T2) was 3 to 4 days, the interval from T1 to the third and final appointment (T3) was 7 days.

At baseline, the patients received detailed information and underwent a comprehensive dental examination, including x-ray documentation. The DMF-T (decayed, missed, filled teeth) index was evaluated in all patients to guarantee an equal distribution of oral health situations in both groups. The modified approximal plaque index (API; Lange), the sulcus bleeding index (SBI; Mühlemann/Son), the papilla bleeding index (PBI; Saxer/Mühlemann), and the GCF flowrates were assessed at baseline, as well as at each follow-up examination.

The API was evaluated based on a yes/no decision following plaque staining (Oral-B). Assessment with the SBI was performed up to approximately 30 seconds after gentle probing of the entrance of the gingival sulcus with a periodontal probe (PCPUNC 15, HuFriedy) with a yes/no decision. The values obtained with the API and the SBI were indicated in percentages.

To enable a differentiated assessment of the severity of the gingival inflammation, the PBI was determined at the Ramfjord teeth using a periodontal probe. The occurrence of bleeding observed after careful probing in the region of the papillae mesial or distal of a Ramfjord tooth was recorded. Probing was done in the first and third quadrant (teeth 16 and 36) from oral, and in the second and fourth quadrant (teeth 21, 24, 41, and 44) from vestibular. The findings were recorded after a waiting period of approximately 30 seconds.

The GCF flowrates were also determined at the Ramfjord teeth. The sulcus fluid sample was obtained using a standard sterile strip of filter paper (Periopaper, Harco). After calibration of the Periotron device (Harco) the end of the strip was held with forceps and inserted into the gingival sulcus for a period of 5 seconds. Insertion of the strip was achieved from buccal/mesial for teeth 16 and 36, and from oral/distal for teeth 21, 24, 41, and 44. After removal from the sulcus, the filter paper strips were placed between the sensors of the Periotron device for assessment.

All assessments were made by the same dental professional, who was unaware which group the individual patient was part of.

The Wilcoxon test for paired samples was used to compare the periodontal parameters recorded in each group at the different time points; the Wilcoxon test for unpaired samples was used for group comparisons. The probability level was taken as $\leq 5\%$.

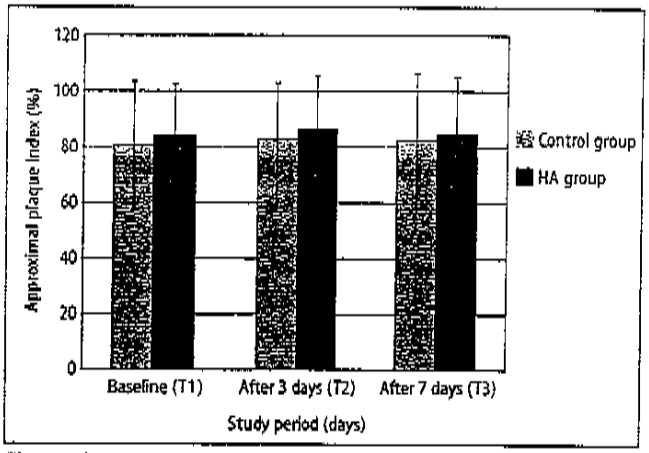

Fig 1 The approximal plaque index (API) of the hyaluronic acid and the control group during the study (baseline, T1; after 3 days, T2; after 7 days, T3). No significant differences were observed between the groups, or occurred in the course of the study period (mean ± SD).

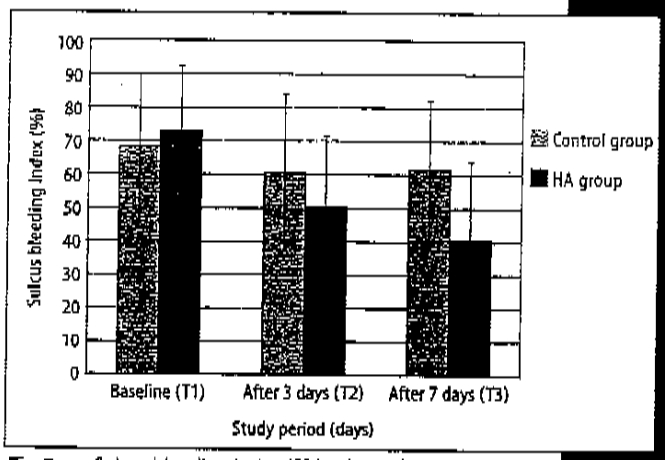

Fig 2 Sulcus bleeding index (SBI) values of the hyaluronic acid (HA) and the control group during the study (baseline, T1; after 3 days, T2; after 7 days, T3). The SBI of the HA group was reduced significantly by approximately 30% in the course of the study, but remained nearly stable in the control group (mean ± SD).

RESULTS

The dental examination at the onset of the study showed comparable findings on the oral status in the patients of both groups. The DMF-T index value recorded at baseline (T1) was 12.5 ± 6.7 in patients in the HA group, and 12.1 ± 7.3 for those in the control group. The values noted for API were 83.8 ± 18.6% in the HA group, and 80.4 ± 23.1% in the control group; and the SBI values were 72.9 ± 20.0%, and 68.0 ± 21.9%, for patients in the HA group and the control group, respectively. There were no significant differences between the groups.

The API values recorded for both groups remained nearly unchanged during the 1-week study period (Fig 1). However, the SBI value of the HA group (T1: 72.9 ± 19.5%) was reduced to 50.3 ± 21.1% at T2, and to 40.7 ± 23.0% by the end of the study (T3). Thus, a reduction of 22.5% occurred during the interval from T1 to T2, at a reduction of 32.2% from T1 to T3. The alterations in the SBI of the HA group at the different examination times, or compared to those of the control group, were statistically highly significant at $P < .0001$. Although the SBI of the control group shows minor fluctuations in the course of the investigation period, it remained essentially stable (T1: 68.0 ± 21.9%; T2: 60.4 ± 23.5%; T3: 61.4 ± 20.6%; Fig 2).

The PBI of the test group decreased significantly. The following values were obtained: at T1 median 1.6 (25th quartile: 1.3; 75th quartile: 2.1); at T2 median 1.0 (25th quartile: 0.7; 75th quartile: 1.4); and at T3 median 0.7 (25th quartile: 0.3; 75th quartile: 1.3). The difference from T1 to T2 was 0.5 degrees, and from T1 to T3 the difference was 0.8 degrees (each has a significance of $P < .0001$). Conversely, the measurement of PBI values in the control group showed only statistically insignificant changes (median T1: 1.3; T2: 1.2; T3: 1.3; Fig 3).

The values for the GCF flowrates showed significant alterations in the HA group. During the interval from T1 to T2 the values (median) decreased from 16.3 (25th quartile: 4.1; 75th quartile: 25.4) to 11.8 (25th quartile: 2.9; 75th quartile: 15.3), and to 7.9 at T3 (25th quartile: 1.6; 75th quartile: 14.0 ($P < .0001$; Fig 4). The following values were obtained for the

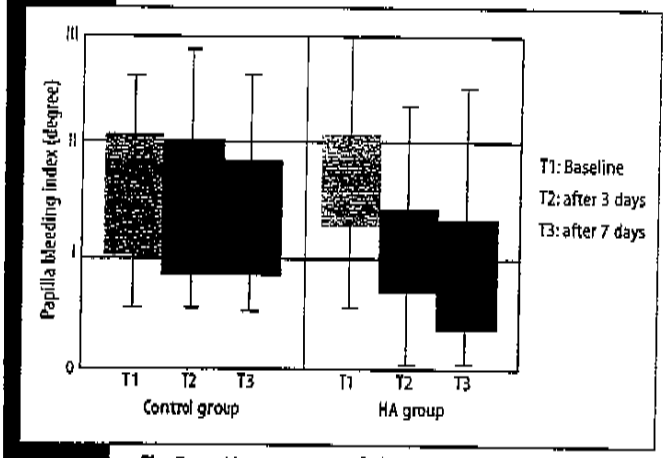

Fig 3 Measurement of the papilla bleeding index (PBI) values within the study period (baseline, T1; after 3 days, T2; after 7 days, T3) in the hyaluronic acid (HA) and the control group (median, 25th, and 75th quartile). There was a significant reduction in the PBI of the HA group.

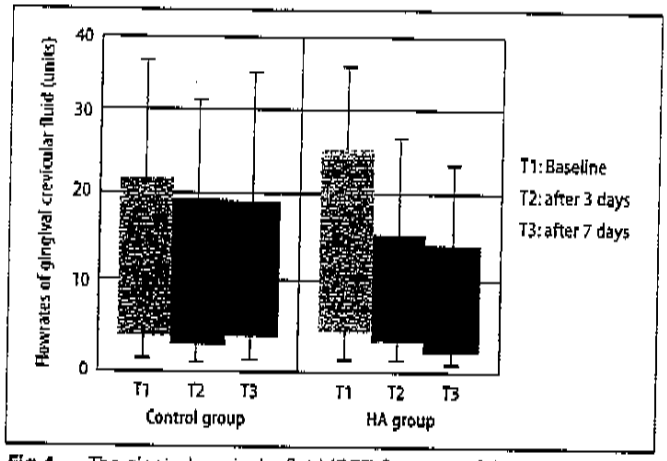

Fig 4 The gingival crevicular fluid (GCF) flowrates of the hyaluronic acid (HA) and the control group during the study (baseline, T1; after 3 days, T2; after 7 days, T3). The GCF of the control group remained constant, and a significant reduction was observed in the GCF of the HA group (median, 25th, and 75th quartile).

Ramfjord teeth: tooth 16: T1 = 10.5, T3 = 5.5; tooth 21: T1 = 10.0, T3 = 4.5; tooth 24: T1 = 13.0, T3 = 8.0; tooth 36: T1 = 15.5, T3 = 7.0; tooth 41: T1 = 12.5, T3 = 6.5; tooth 44: T1 = 16.5, T3 = 8.0. The values recorded for the control group remained largely unchanged (Fig 4).

At the end of the study, all patients were asked for their subjective assessment and for their experiences with the HA-containing spray. All but one (39 out of 40) patient described the taste of the spray as pleasant, the handling to be simple, and rated their oral health status as positive. One patient evaluated the taste of spray as being too sweet. No adverse side effects were observed.

DISCUSSION

In the present study, an HA preparation (Gengigel) was tested over a period of 7 days in patients with mild inflammatory forms of a periodontal disease (gingivitis). At the time of the baseline examination, no significant differences were observed in sex distribution, age, DMF-T index, API, or SBI in the test subjects of both study groups, allowing the assumption of homogeneity of the patient population. To avoid distortions of the results due to the Hawthorne effect, at the onset of the study the patients were asked to maintain their oral hygiene habits. Because the API remained nearly stable in both groups throughout the study period, a relevant distortion of the results by the Hawthorne effect could be excluded.

The HA group showed a significant, successive improvement in the SBI over the study period, characterized by an improvement of 20% recorded from the first to the second examination, then by the further improvement of only 10% observed at the time of the third examination. The effect of the spray therefore appears to manifest itself more markedly within the first 3 to 4 days after onset of the treatment. The slight fluctuations noted in the SBI values of the control group do not represent a clinically significant improvement, because in addition to being too small, they remained essentially stable from the time of the second to the third examination. Possible reasons for this development may be both minor measurement inaccuracies, and reductions in the amount of plaque

accumulation in the periodontal regions as the result of a mild Hawthorne effect.

Distinct and continuous improvements were recorded for the PBI and the GCF of the HA group. Positive changes in both the PBI and the GCF were again observed to be most pronounced during the time from the first to the second examination. Comparable to the SBI, the changes noted in the control group for both parameters were of no clinical relevance.

After 1 week of HA application, the gingiva of the HA group was characterized by an improvement of 30% for the SBI, of nearly 1 degree for the PBI, and of up to 5 units for the GCF.

Patient acceptance of the spray was very high. Undesirable side effects associated with the medication have not been reported or detected clinically to date.[15,37]

Rabasseda[12] also reported a rapid, beneficial effect of an HA-containing preparation in gel form on gingivitis—in 9 out of 10 patients, the clinical signs of gingivitis decreased within 2 to 10 days. Furthermore, a significant therapeutic effect was observed in a second clinical double-blind study in patients with marginal periodontitis. Pomowski et al[13] reported significant improvements not only in the SBI, but also in the API. In contrast, no significant difference in plaque accumulation was found between the test group and the control group in the present study.

Hyaluronic acid has been described as a possible ligand-mediating binding of the periodontitis-associated germ *Treponema denticola*. A reduction in its binding capacity to gingival tissue would therefore lead to a decrease in the risk of infection.[38] The exogenous application of HA in an animal experiment showed the medication to be suitable for both direct pulp cappings and vital amputations[39]; the authors observed the differentiation of fibroblasts and odontoblasts after 1 week, and the formation of a new layer of reparative dentin after 2 weeks.

The amount of HA in inflamed tissue is invariably increased.[40] Local HA synthesis is further known to be elevated in inflammatory reactions, eg, in rheumatoid arthritis.[37,41] The production of HA by gingival fibroblasts is markedly increased in the presence of elevated bacterial lipopolysaccharide concentrations.[21,42–45] This process is induced by increased PGE_2 release. However, primarily low molecular weight HA molecules have been implicated in the process. It has been speculated that an increase in hyaluronidase activity of the micro-organisms may be the cause of the diminished molecular weight of HA. The enzymatic degradation leads to weakening of the tissue structures, which facilitates the passage of other metabolites and toxins into the injured gingiva. Our study does not answer the question of whether the extrinsic HA undergoes the same degradation. It is equally uncertain if the long-term medication has possible side effects on the oral mucosa. It is not possible with the current data to clarify these questions; further studies are required.

CONCLUSIONS

1. The results of the present study demonstrate that the anti-inflammatory and reparative potentials of exogenously applied HA lead to a significant improvement of the clinical inflammatory parameters in the treatment of gingivitis.
2. The plaque values of the probands did not change throughout the measurement period. Therefore, the increase of inflammation parameters was not based on a reduction of plaque.
3. The HA in the spray form tested in this study proved to be a potentially useful adjuvant in the treatment of gingivitis. However, it will take further clinical investigation to determine the long-term success of this method.

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