

Treatment of gingivitis with hyaluronan

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Abstract

Objectives: Hyaluronic acid (hyaluronan) is a glycosaminoglycan with anti-inflammatory and antiedematous properties. It was evaluated in a gel formulation for its effect in the treatment of plaque-induced gingivitis.

Method: In a randomised double-blind study, 50 male subjects with plaque-induced gingivitis were divided into two groups and used a verum or placebo gel twice daily additionally to oral hygiene for a 3-week treatment period. Clinical indices (API, Turesky index, PBI) and crevicular fluid variables (peroxidase, lysozyme) were determined at baseline and after 4, 7, 14 and 21 days, respectively.

Results: Significant improvements could be found for all clinical variables in both groups. The verum group showed significant improvement in the study area for the plaque indices beginning with day 4 ($P = 0.011$) and the PBI beginning with day 7 ($P = 0.001$) in comparison with the placebo group. The crevicular fluid variables were significantly improved in the centre of the studied inflammation area in the verum group. Here all studied sites had significant decreases in peroxidase (176.72–128.75 and 188.74–128.75 U/L) and lysozyme (1.27–0.27 and 1.30–0.33 mg/L) activities after 7, 14 and 21 days (P between 0.034 and <0.001), whereas in the placebo group only one site showed a significant decrease for lysozyme (1.74–0.75 mg/L) after 7 and 21 days ($P = 0.048$ and 0.025).

Conclusions: These data suggest that a hyaluronan containing gel has a beneficial effect in the treatment of plaque-induced gingivitis.

Key words: clinical trial; crevicular fluid; gingivitis; hyaluronan

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Hyaluronic acid (hyaluronan) is a glycosaminoglycan with 200–10 000 disaccharides and a molecular weight exceeding 10^6 Da (Toole 1990). Hyaluronic acid has been studied as a metabolite or marker of inflammation in the gingival crevicular fluid (Embery et al. 1982, Last et al. 1985, Smith et al. 1996) as well as a significant factor in growth, development and repair of tissues (Pogrel et al. 1996). Hyaluronic acid is a component of the connective tissue and was detected in the gingiva (Bartold et al. 1981, Giannobile et al. 1993). Glycosaminoglycans are able to interact with tropocollagen to form the interactive region between ground substance and fibrous protein (Comper & Laurent 1978). The concentration of hyaluronic acid is increased during wound healing

(Fraser & Laurent 1989, Longaker et al. 1991), embryonic development (Kujawa et al. 1986, Toole & Gross 1971) and tumour development (Knudson et al. 1989). High molecular weight hyaluronic acid stimulates early healing of rat long bones (Sasaki & Watanabe 1995) and reparative dentine formation (Sasaki & Kawamata-Kido 1995). Hyaluronic acid elevates *in vitro* the mesenchymal cell migration and differentiation (Pilloni & Bernard 1992) as well as many functions of blood cells with special interest for the inflammatory response, e.g. phagocytosis and chemotaxis (Weigel et al. 1986). Hyaluronic acid has been used in radio-epithelitis (Liguori et al. 1997), osteoarthritis of the knee (Adams et al. 1995, Huskisson & Donnelly 1999, Wu et al. 1997)

and rheumatoid arthritis (Matsuno et al. 1999). The anti-inflammatory effect may be due to the action of exogenous hyaluronan as a scavenger by draining prostaglandins, metalloproteinases and other bio-active molecules (Laurent et al. 1995). The anti-edematous effect may also be related to the osmotic activity. Only a few studies could be found focussing the interest on results after the treatment with hyaluronan in dentistry. Pagnacco et al. (1997) executed a clinical trial treating 60 men and women for unspecific gingivitis. They found significant improvements for clinical data in the verum and placebo group. Significant differences between the groups with better results in the verum group were found for gingival bleeding after four weeks. Vangeli-

sti et al. (1997) documented the differences between verum and placebo rating the inflammation with a self-created scale (good, doubtful, poor). They found better results in the verum group in this preliminary trial.

The inflammatory process is linked with an increase in activity of tissue destructing enzymes like proteases (Sorsa et al. 1994) and mostly leucocyte-derived lysosomal enzymes (Fine & Mandel 1986). The lysozyme activity of the crevicular fluid is increased in gingivitis and periodontitis (Brandtzaeg & Mann 1964). The peroxidase activity is increased in gingivitis (Smith et al. 1984).

The aim of this study was to verify the anti-inflammatory effect of a hyaluronic acid gel in the therapy of gingivitis by a randomised double-blind study.

Material and methods

Fifty unselected male volunteers with plaque-induced gingivitis (mean age 29.9 ± 10.5 years) gave written informed consent and participated in the randomised double-blind study after approval of the ethic commission of the Medical Faculty of the University of Rostock. The size of the sample was fixed after model calculations for API, peroxidase and lysozyme activities using $\alpha = 5\%$ for the level of significance and a test power of 80% (Jones & Payne 1997), where an improvement of API in

peroxidase and lysozyme activities had been specified. Finally, using estimates of standard deviations for the variables the sample size was calculated with the computer program 'inquiry advisor 4.0'. The criteria for inclusion were: healthy, no antibiotic therapy in the last 6 months, no prophylaxis, 28 teeth. The study period was 3 weeks. The volunteers used a verum (with 0.2% hyaluronan, Merz Dental, Lütjenburg, Germany, group 1) or a placebo gel (without hyaluronan, group 2) twice a day. Both gels contained water, xylitol, cellulose gum, denatured alcohol, PEG-40 hydrogenated castor oil, polyvinyl alcohol, polycarbophil, dichlorobenzyl alcohol, aroma, CI 42090. Both gels had the same physical consistency, equal colours and taste. The participants placed 1 mL of the gel in the inflamed area (study area) of the buccal gingiva of four frontal or premolar teeth in the maxilla as indicated by the examiner. The volunteers were advised to take a constant length of 1.5 cm of the gel which was found to correspond to 1.0 mL gel. Tooth brushing or eating was avoided for the next 3 h. No restriction of the individual oral hygiene procedures was given.

The clinical examination was done by only one examiner (R.P.) without knowledge of the group membership. It consisted of determination of the Turesky plaque index (Turesky et al. 1970), approximal-plaque-index (API, Lange 1986) and the papilla-bleeding-index

(PBI, Saxer & Mühlemann 1975) at baseline, after 4, 7, 14 and 21 days. The plaque indices were modified and exclusively determined at the buccal tooth areas. These indices were calculated for the whole mouth as well as for the four teeth of the study area to be treated with the gel.

At every appointment, crevicular fluid samples were taken. The crevicular fluid was collected at the buccal surfaces from the two central teeth of the study area. Periopaper (Pro Flow™, Amityville, NY, USA) was introduced into the sulcus for 2 min under relative dryness. The stripes were eluted in 120 μ L of 0.5 M NaCl and stored at -18°C . Separate eluates were used for biochemical analysis.

The biochemical analysis of the activities of lysozyme and total peroxidase was done after 6–10 weeks of sample storing. Lysozyme was analyzed using the lysoplate method (Osserman & Lawlor 1966). Plates with agarose gel supplemented with *Micrococcus luteus* (Boehringer-Mannheim, Mannheim, Germany) were inoculated with the samples and incubated. After 24 h the diameter of the lysed areas was measured. Hen egg white lysozyme (Boehringer-Mannheim) was used as a standard. The determination of the peroxidase activity was performed according to Mansson-Rahemtulla et al. (1988). 5,5'-Dithiobis-2-nitrobenzoic acid in phosphate buffer pH 5.6, reduced to nitrobenzoic acid by mercaptoethanol, was used as substrate, potassium thiocyanate served as cosubstrate. The spectrophotometric analysis was performed at a wavelength of 412 nm.

The statistical analysis was performed after exclusion of extreme values. A variable was considered as an extreme value in the following case: $v < x - 3s$ or $v > x + 3s$. The Student *t*-test or the Mann-Whitney *U*-test, respectively, was used for testing differences between the verum and placebo groups. The *t*-test or *U*-test, respectively, was selected after checking the variables for normal distribution by the Kolmogorov-Smirnov test. Within-group changes were checked statistically using the Friedman-Wilcoxon test. A level of $\alpha \leq 0.05$ was considered to be significant.

Results

Clinical variables of the whole mouth

The API, the Turesky index and the PBI for the whole mouth did not differ sig-

Table 1. Clinical variables of the whole mouth examination

Index	Appointment Day	Group 1			Group 2			<i>t</i> -test <i>P</i>
		<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	
API %	baseline	25	43	34	25	34	43	n.s.
	4	25	36	28	25	32	40	n.s.
	7	25	27	25	25	20	27	n.s.
	14	25	23	22	25	15	19	n.s.
	21	22	15	19	24	13	20	n.s.
Friedman test <i>P</i>		<0.001			<0.001			
Turesky	baseline	25	1.08	0.55	25	1.17	0.81	n.s.
	4	25	0.95	0.55	25	0.94	0.78	n.s.
	7	24	0.56	0.41	25	0.62	0.62	n.s.
	14	25	0.53	0.52	25	0.48	0.55	n.s.
	21	21	0.31	0.36	24	0.41	0.56	n.s.
Friedman test <i>P</i>		<0.001			<0.001			
PBI	baseline	25	1.12	0.56	25	1.33	0.74	n.s.
	4	25	1.02	0.55	25	0.86	0.78	n.s.
	7	25	0.70	0.53	25	0.55	0.54	n.s.
	14	25	0.55	0.49	25	0.40	0.37	n.s.
	21	21	0.39	0.36	24	0.36	0.42	n.s.
Friedman test <i>P</i>		<0.001			<0.001			

nificantly between the groups at any examination. The mean values and the standard deviations as well as the test results are given in Table 1. A significant improvement could be documented for all three clinical variables (API, Turesky index and PBI) in the verum and placebo groups. The level of significance was always reached. Comparing the PBI between the appointments there were always significant differences in the verum group, while in the placebo group no significant result could be found between the 14th and 21st day.

Clinical variables in the study area

During the study, significant improvements could be found for all clinical variables in both groups. These changes were without any exception in the verum group. In the placebo group the first significant differences occurred beginning with the 7th day.

In the study area the clinical variables were without statistically significant difference at baseline. Beginning at day 4, both plaque indices were significantly different between the two groups with better results in the verum group. At the PBI, significantly better results were recorded in the verum group beginning with the 7th day. The clinical results of the study area are given in Table 2.

Results for the crevicular fluid in the study area

The lysozyme and peroxidase activities of the two central collection sites of the crevicular fluid in the study area were analysed separately. The two sites in the centre of the study area were coded A and B, and the results are presented in Table 3. Comparing the verum and placebo groups, no significant difference could be found for peroxidase activity. Lysozyme activity was significantly different between both groups at the 7th ($P=0.015$) and 21st day ($P=0.039$). Applying the Wilcoxon test, significant reductions could be found for activities of peroxidase ($P=0.010$ and <0.001) and lysozyme ($P<0.001$ and <0.001) in the verum group at both study sites in the centre of inflammation. The decreases in activities could be found mostly starting with the 7th day compared to the baseline results ($P=0.001$ and >0.001 for peroxidase, $P=0.001$ and $P=0.002$ for lysozyme, applying the Wilcoxon test). Also the decreases between the 4th and 7th day were highly

Table 2. Clinical variables of the study area

Index	Appointment Day	Group 1			Group 2			t-test P
		n	\bar{x}	s	n	\bar{x}	s	
API %	baseline	25	99	5	25	100	0	n.s.
	4	25	92	12	25	99	5	
	7	25	59	12	25	87	13	
	14	25	31	11	25	77	10	
	21	22	3.4	9	24	75	15	
Friedman test P		<0.001			<0.001			
Turesky	baseline	25	1.28	0.54	25	1.33	0.55	n.s.
	4	25	1.05	0.33	25	1.30	0.48	
	7	25	0.66	0.29	25	1.17	0.44	
	14	25	0.31	0.17	25	1.01	0.48	
	2	22	0.068	0.11	24	0.96	0.50	
Friedman test P		<0.001			<0.001			
PBI	baseline	25	1.52	0.51	25	1.30	0.54	n.s.
	4	25	1.20	0.33	25	1.30	0.54	
	7	25	0.74	0.28	25	1.08	0.37	
	14	25	0.38	0.16	25	0.95	0.40	
	21	22	0.068	0.11	24	0.94	0.40	
Friedman test P		<0.001			<0.001			

significant for all four variables in the verum group ($P=0.001$ and $P<0.001$ for peroxidase, $P<0.001$ and $P=0.001$ for lysozyme, applying the Wilcoxon test). The peroxidase and lysozyme activities decreased significantly and continuously until the 21st day. The significance levels are given in Table 3. In the placebo group there was a significant decrease only for lysozyme activity at site B comparing the results at baseline with the 7th ($P=0.048$) and 21st day ($P=0.025$), respectively.

Discussion

The use of hyaluronan in the treatment of inflammatory processes is established in orthopaedics, dermatology and ophthalmology. Significant improvements of clinical parameters could be found in the treatment of osteoarthritis of the knee (Adams et al. 1995, Huskisson & Donnelly 1999), in rheumatoid arthritis (Matsuno et al. 1999), radio-epithelitis (Liguori et al. 1997) and cataract surgery (Caporossi et al. 1995). In the field of dentistry, preliminary clinical trials have been done by Vangelisti et al. (1997) and Pagnacco et al. (1997).

This trial was done to study the effect of the topical application of a hyaluronan-containing gel in the treatment of plaque-induced gingivitis. The idea was to follow the health improvements of the patients with clinical indices as well as with paraclinical variables in the crevicular fluid. The study was undertaken as

a double-blind trial. The variables in the crevicular fluid have the advantage that they are less influenced by subjective impressions of the examiner. Peroxidase and lysozyme activities are related to inflammatory processes (Klebanoff 1991, Miyasaki 1991). The study period of 3 weeks follows the recommendations of Chilton & Fleiss (1986) to undertake trials regarding gingival inflammation with a study period longer than 2 weeks. In this study, two plaque indices and one inflammation index were used. The approximal-plaque-index gives information about the approximal region, the Turesky plaque index considers the plaque quantity on the buccal or labial tooth surfaces. The results of these two indices are influenced not only by oral hygiene, but also by the degree of inflammation (Quirynen et al. 1991, Ramberg et al. 1994). The enhanced stream of crevicular fluid functions as a nutritional reservoir for the plaque bacteria to further plaque growth (Daly & Highfield 1996). Thus, the highly significant improvements of the two plaque indices for the whole mouth during the study can be attributed to the training effect due to the inclusion into the study as well as to the reduction of the inflammation. The plaque indices are significantly improved in both groups; the final results as calculated for the whole mouth are not different for verum and placebo. The effect on clinical indices was equal in both groups, as indicated in Table 1.

Regarding the study area, the differ-

Table 3. Variables of the crevicular fluid in the centre of inflammation

Index	Appointment Day	Group 1			Group 2			t-test P
		n	\bar{x}	s	n	\bar{x}	s	
Peroxidase A U/L	baseline	25	176.72	68.72	25	170.84	186.73	n.s.
	4	25	179.02	120.80	25	157.59	84.57	n.s.
	7	25	124.26	30.41	25	140.33	61.55	n.s.
	14	25	146.43	51.61	25	154.06	85.61	n.s.
	21	22	128.75	61.65	25	168.71	98.18	n.s.
Friedman test P								0.010
Wilcoxon test P								n.s.
baseline - 7			0.001					-
baseline - 21			0.020					-
7-14			0.034					-
Peroxidase B U/L	baseline	25	188.74	97.13	25	158.91	92.56	n.s.
	4	25	165.81	56.40	25	164.29	102.41	n.s.
	7	25	125.68	30.32	25	134.95	59.57	n.s.
	14	25	138.85	36.82	25	160.26	76.95	n.s.
	21	22	128.75	61.65	25	138.90	58.00	n.s.
Friedman test P								0.001
Wilcoxon test P								n.s.
baseline - 7			<0.001					-
baseline - 14			0.001					-
baseline - 21			0.001					-
Lysozyme A mg/L	baseline	25	1.27	0.89	25	1.66	2.11	n.s.
	4	25	1.38	1.13	25	1.89	3.65	n.s.
	7	25	0.56	0.47	25	1.15	1.07	0.015
	14	25	0.67	0.63	25	1.01	2.00	n.s.
	21	22	0.27	0.43	25	0.69	0.88	0.039
Friedman test P								0.001
Wilcoxon test P								n.s.
baseline - 7			0.001					-
baseline - 14			<0.001					-
baseline - 21			<0.001					-
Lysozyme B mg/L	baseline	25	1.30	0.98	25	1.74	2.20	n.s.
	4	25	1.21	0.92	25	1.29	1.53	n.s.
	7	25	0.64	0.89	25	0.93	1.20	n.s.
	14	25	0.48	0.52	25	1.08	1.58	n.s.
	21	22	0.33	0.50	25	0.75	1.05	n.s.
Friedman test P								0.001
Wilcoxon test P								0.046
baseline - 7			0.002					0.048
baseline - 14			0.001					-
baseline - 21			<0.001					0.025

ence in total improvement minus improvement due to oral hygiene is equal to the benefit by the verum improvement when the result occurs exclusively in the verum group. At baseline no significant differences existed in the degree of inflammation between both groups. Regarding the buccal gingiva at four anterior teeth, the API and the Turesky index are already significantly better in the verum group in very early phases of the study. The very low API at the end of the study results from the fact that 19 out of the remaining 22 volunteers were absolutely free of plaque in the study area. These results are confirmed by the Turesky index. Because the equal influence of the training effect in the verum and placebo group was demonstrated analysing

the indices of the whole mouth, these better results in the verum group can be attributed to the hyaluronan-containing gel. Studies on the influence of extrinsic hyaluronan on dental plaque metabolism could not be found. No significant difference could be found between the groups comparing the results for the papilla-bleeding-index (PBI) for the whole mouth. There was a continuous significant decrease of PBI in the verum and placebo groups starting very early at the 4th day of the study. We addressed these changes to the training effect during the study. In the study area significant differences between verum and placebo regarding the PBI could be seen beginning with the 7th day of the study. Here, also, better results were obtained

in the verum group. The findings could be attributed to the anti-edematous and scavenger effect of prostaglandins and metalloproteinases (Laurent et al. 1995). Studies focussing on the absorption of hyaluronan through the gingival epithelium or skin could not be found.

The peroxidase and lysozyme activities in the crevicular fluid were significantly reduced only in the verum group during the study period. Significant differences between the verum and placebo group could be seen for the lysozyme activity at the 7th and 21st day of the study.

In the present study the verum group showed significant improvements in clinical and para-clinical data during the 21 days. It could be demonstrated that these changes could be related to the anti-inflammatory and anti-edematous effect of hyaluronan. The influence of hyaluronan in a gel formulation at the gingival inflammation is slightly stronger than a placebo.

Conclusion

The above study suggests a beneficial effect of a hyaluronan-containing gel during the therapy of plaque-induced gingivitis proven by clinical and para-clinical variables.

Zusammenfassung

Behandlung der Gingivitis mit Hyaluronan

Ziele: Hyaluronsäure (Hyaluronan) ist ein Glycosaminoglycan mit entzündungshemmenden und ödemhemmenden Eigenschaften. Sein Effekt in der Behandlung der plaque-induzierten Gingivitis wurde in einer Gelapplikation überprüft.

Methoden: In einer randomisierten doppelten Blindstudie wurden 50 Männer mit einer plaque-induzierten Gingivitis in zwei Gruppen aufgeteilt. Sie nutzten zusätzlich zur oralen Hygiene während einer dreiwöchigen Behandlungsperiode entweder das Wirkstoffgel oder ein Placebogel zweimal täglich. Die klinischen Indices (API, Turesky Index, PBI) und Variable der crevikulären Flüssigkeit (Peroxidase, Lysozym) wurden zur Basis und nach 5, 7, 14 und 21 Tagen bestimmt.

Ergebnisse: Signifikante Verbesserungen für alle klinischen Variablen in beiden Gruppen konnten gefunden werden. Die Wirkstoffgruppe zeigte eine signifikante Verbesserung im Studiengbiet für die Plaqueindices beginnend am Tag 4 ($p=0,011$) und den PBI beginnend mit Tag 7 ($p=0,001$) verglichen mit der Placebogruppe. Die Variablen der crevikulären Flüssigkeit wurden in der Wirkstoffgruppe im Zentrum des untersuchten Entzündungsgebietes signifikant verbessert. Alle untersuchten Flächen hatten einen signifi-

kanten Abfall in der Peroxidase- (176,72 zu 128,75 und 188,74 zu 128,75 U/l) und Lysozymaktivität (1,27 zu 0,27 und 1,30 zu 0,33 mg/l) nach 7, 14 und 21 Tagen (p zwischen 0,034 und <0,001), während in der Placebo-gruppe nur eine Fläche einen signifikanten Abfall für Lysozym (1,74 zu 0,75 mg/l) nach 7 und 21 Tagen zeigte ($p=0,048$ und 0,025). Schlussfolgerungen: Die Daten deuten darauf hin, dass ein Hyaluronan enthaltendes Gel einen verbessernden Effekt in der Behandlung der durch Plaque induzierten Gingivitis hat.

Résumé

Traitement de la gingivite par l'acide hyaluronique

Objectifs: L'acide hyaluronique (hyaluronan) est un glycosaminoglycane possédant des propriétés anti-inflammatoires et anti-oedémateuses. Il a été évalué dans une formule en gel pour ses effets dans le traitement de la gingivite due à la plaque.

Méthode: Lors d'une étude randomisée en double aveugle, 50 sujets masculins présentant une gingivite due à la plaque furent divisés en 2 groupes et utilisèrent un gel verum ou placebo deux fois par jour en complément de l'hygiène buccale pendant 3 semaines. Les indices cliniques (API, indice de Turesky, PBI) et des variables du fluide gingival (peroxydase, lysozyme) furent déterminés au départ et après 4, 7, 14, et 21 jours respectivement.

Résultats: Des améliorations significatives purent être montrées pour toutes les variables cliniques dans les deux groupes. Le groupe verum présentait une amélioration significative dans le cadre de l'étude pour les indices de plaque dès le quatrième jour ($p=0,011$) et pour le PBI dès le septième jour ($p=0,001$) par rapport au groupe placebo.

Les variables du fluide gingival étaient significativement améliorées au centre de la surface enflammée étudiée dans le groupe verum. Ici, sur tous les sites étudiés, il y avait une diminution de l'activité de la peroxydase (176,72 à 128,75 et 188,74 à 128,75 U/l) et du lysozyme (1,27 à 0,27 et 1,30 à 0,33 mg/l) après 7, 14, et 21 jours (p entre 0,034 et <0,001), alors que dans le groupe placebo, seul un site présentait une diminution significative du lysozyme (1,74 à 0,75 mg/l) après 7 et 21 jours ($p=0,48$ et 0,025).

Conclusions: Ces données suggèrent qu'un gel contenant de l'acide hyaluronique a un effet bénéfique dans le traitement de la gingivite due à la plaque.

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