Evaluation of the efficacy of an hyaluronic acid-based biogel on periodontal clinical parameters. A randomized-controlled clinical pilot study

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Summary

Hyaluronic acid (HA) is an ubiquitous form of non-sulphated glycosaminoglycan of the extracellular matrix of all mammalian connective tissues. It is mainly present during tissue's formation or during most of initial tissue's repair processess. Cell migration, adhesion and differentiation are only part of several unique biological characteristics of HA which have been under investigation in the past decades.
Aim of the study. **Evaluate the possible positive effect of an esterified form of HA on gingival tissues in mild chronic periodontitis patients, seeking for the reduction of all the periodontal disease clinical parameters PLI (Plaque Index), BOP (Bleeding on Probing), PPD (Probing Pocket Depth), GI (Gingival Index), PAL (Probing Attachment Level).**
Materials and methods. **The study is an open, intra-patient, controlled, single center pilot clinical trial including 19 adult patients with mild chronic periodontitis and shallow pockets (< 4 mm) in at least two different quadrants. One quadrant was treated with HA gel after regular toothbrushing (test), the other without (control).**
Results. **Although oral hygiene itself had a similar positive influence on the improvement of all the clinical indexes for test and control, the treatment with HA gel showed a greater effect almost always statistically significant. BOP in the HA gel treated areas had a decrease of 92.7% and GI of 96.5%, whereas controls 75.8% and 79.0% respectively. The difference of PPD in both areas was statistically significant ($p<0.01$) in favour of the HA gel treated zone. Also PAL and PI were reduced more with gel than with oral hygiene alone, although this did not reach a statistical significant difference.**
Conclusion. **It appears that an esterified gel form of HA has shown an effect in reducing the gingival inflammation when used as an adjunct to mechanical home plaque control and that it could be successfully used to improve the periodontal clinical indexes. This pilot study will gain substantial scientific significance when both a higher number of patients can be utilized and also by adding any possible further biological information, as with immunocytochemistry and histology.**

Key words: extracellular matrix, hyaluronan, periodontal disease.

Introduction

In normal conditions the gingival tissues carry out typical functions of fibrous tissues, although presenting features very similar to soft tissues.
The "ground substance", which is the supporting structure of the extracellular matrix and is formed by a highly structured net of proteoglycans in perfect equilibrium one another, gives the gingival tissues a typical firm consistency.
In this context, hyaluronic acid or *hyaluronan* (HA), an ubiquitous nonsulphated glycosaminoglycan, plays a fundamental role (2). In fact, it has been shown that among gingival diseases, periodontal disease is characterized by the loss of the normal gingival properties. Many studies show that the most important alterations are related to the reduction of the normal structural balance of the extracellular matrix (21,24).
In particular, the endogenous hyaluronan component results to be lacking within the epithelium and the gingival connective tissue with a consequent structural failure and loss of normal features of the gingiva (2,4,7). It was demonstrated that in patients with chronic periodontitis, there is a rapid loss of high molecular weight of hyaluronic acid due to enzymatic digestive processes (3). Hyaluronidase, an enzyme released by micro-organisms of bacterial plaque, plays an essential role in such mechanism (14). The supply of constituents that can be utilized by the regenerating tissues in order to re-establish their internal structure is, therefore, strictly necessary (10).
In the dental literature, HA has been shown to be bacteriostatic with respect to periodontal pathogens (18) and effective *in vitro* when both intramembranous and endochondral models of osteogenesis are utilized (12,17). More

recently HA appears to be effective for the treatments of gingivitis (19).
An HA derivative, in esterified form, by maintaining the characteristics of biocompatibility and biointeractivity of hyaluronic acid, (1,8), seems to be able to re-establish the ground substance's normal equilibrium. Furthermore, it possesses a very good bio-adhesiveness and fits tightly to the gingival mucosa, to be then rapidly incorporated into the epithelial layers (29). When compared to HA, the esterified form is more resistant to enzymatic biodegradation (20).
Based upon such premises, a benzylic ester of hyaluronic acid (HYAFF®, Anika Therapeutics Srl), for the treatment of gingival inflammation, has been developed in a gel form for clinical use. The aim of this clinical pilot study is to evaluate the efficacy of an esterified gel form of HA on the reduction of clinical indexes typically present in the initial stages of periodontal disease.

Materials and methods

The study is an open, intra-patient, controlled, single center pilot clinical trial including 19 adult patients with mild chronic periodontitis: 10 male (55,6%), 9 female (44,4%), with an age range of 20-75 (mean 41,9 ±15,1).
After ethical committee approval received, at initial screening visit, patients who fulfilled the selection criteria and with shallow pockets (< 4 mm) in at least two different quadrants, were included in the study. One quadrant was treated with HA gel (test), the other without (control).
Patients had to return for the control visits after 7, 14 and 21 days. The selection of study population has been based on the following criteria:

- patients affected by mild adult periodontitis;
- patients with shallow pockets (PPD <4mm), present in at least two different quadrants of the oral cavity (PSR 4);
- patients in good general health;
- no smokers;
- patients with given informed consent.

The exclusion criteria were:

- patients who did not collaborate and/or were not trustworthy;
- smokers;
- PPD >4mm.

Patients were withdrawn from the study for the following reasons: patient request, failure to return to two consecutive control visits, protocol violation, insufficient patient compliance, serious adverse event, other reasons which had to be justified.
Efficacy of the gel under investigation was assessed by measuring the following variables:

- Reduction of BOP (Bleeding on probing);
- Gain of PAL (Probing Attachment Level);
- Reduction of PPD (Probing Pocket Depth);
- Reduction of PLI (Plaque Index);
- Reduction of GI (Gingival Index);

Treatment Procedures
During the initial visit oral hygiene habits of the patients were assessed and an accurate exam of the oral cavity to detect any visible alteration of oral tissues was performed. Measurement of clinical indexes (BOP, PAL, PPD, PLI, GI) was also performed and patient was also questioned regarding root sensitivity and gingival pain. The two affected zones were randomly assigned by the investigator to be treated with HA gel or by normal oral hygiene procedures. Following initial assessment and after ensuring an adequate oral hygiene, HA gel was applied after scaling and root planning on the treated area (one quadrant) massaging the gingiva with a soft-bristles toothbrush for 2-3 minutes and then asking the patient to eliminate the excess by rinsing once with tap water. The other randomized selected quadrant (contralateral) was considered as control and treated by normal oral hygiene procedures. The treatment was repeated daily by the patient who has been adequately instructed by the investigator for a total period of three weeks. The patient was asked to return to the office after 7, 14 and 21 days to assess parameters for tolerability and efficacy.
Initial, control and final visits comprised: treatment compliance, assessment of concomitant medications, objective oral exam, gingival tenderness or pain, root sensitivity, evaluation of clinical indexes (BOP, PAL, PPD, PLI, GI). Control and final visits included respectively safety details as adverse events and overall judgement of the investigator about tolerability and efficacy of HA gel.

Statistics
No statistical considerations were made in order to define the sample size, considering a pilot study.
Baseline, demographic and anamnestic data and efficacy data were summarized by means of descriptive statistics such as mean, standard deviation, standard error, minimum and maximum value, frequency distributions.
Although it was not planned, statistical comparison between the two zones at baseline and at different visits after treatment, was performed by mean of Paired Wilcoxon test or Paired t-test. Before performing statistical analysis of data, the criteria for the identification of the population for the efficacy analysis were defined. The following three populations were identified:

1. Safety population: All patients who receive at least one application of HA gel;
2. Intention-to-Treat population (ITT): All patients of safety population who are assessed at least at one control visit;
3. Per-Protocol population (PP): All patients of Intention-to-treat population who have no major protocol violations.

Major protocol violations (that could affect efficacy) were classified following the definition here below:

1. Patients with poor compliance;
2. Patients with no (or not recorded) periodontal disease;
3. Patients without shallow pockets.

Data were transferred on a database by means of SAS/FSP software. Checks for inconsistencies and implausibility were made via computer.

Results

Study population
Of the 19 patients enrolled in the study eighteen were assessed at all visits, while one patient (5.3%) withdrew before completing the study because he did not return for the final visit. No patient was excluded from the study and no patient was excluded from the analysis of efficacy that was performed on all 19 individuals. (Safety, ITT and Per-Protocol population were the same).

Table 1 - Demography

	HA-gel
Number of patients	19
SEX	
Male	10 (55,6%)
Female	9 (44,4%)
AGE	
Mean	41,9
SD	15,1
ES	3,5
Range	20-75
N	19
WEIGTH (kg)	
Mean	68,2
SD	14,7
ES	3,4
Range	49,5-106,0
N	19
HEIGTH (cm)	
Mean	171,3
SD	7,0
ES	1,6
Range	150-180
N	19

Table 2 - Age distribution.

	HA-gel Number (%)
Number of patients	19
<18	0 (0,0)
18<30	5 (26,3)
30<40	3 (15,8)
40<50	5 (26,3)
50<60	4 (21,1)
60<65	1 (5,3)
>65	1 (5,3)

Protocol Deviations

Neither major nor minor protocol deviations were registered in the study. Consequently Safety, ITT and Per-Protocol population were identical.

Baseline Data

Demographic characteristic of the patients (mean, standard deviation, standard error, minimum and maximum value) are reported in Tables 1 and 2. Ten patients were males, 9 females with an average of 41.9±15.1 years. Three patients received some medications before entering the study, for a total of five drugs, while two patients received concomitant drugs also during the treatment period.

The two zones, test and control, appeared to be comparable for PAL, PPD, PI and GI. The same unbalance was present for BOP, with a greater average score in the test selected sites (39.6%), compared to the control sites (29.3%) (p=0.03). The two affected areas were randomly assigned by the investigator to be treated with HA gel or by normal oral hygiene procedures.

Although in favour of the control, this unbalance did not affect the comparability of the two groups.

Patient Compliance

All 19 patients (100%) applied the gel as prescribed. Oral hygiene compliance was very good in 94.7% of them.

Table 3 - Bop (bleeding on probing) during treatment.

BOP (Bleeding on Probing)	Baseline	T7 (after 7 days)	T14 (after 14 days)	T21 (after 21 days)
HA-gel				
Mean	39,6	20,8	5,2	2,9
SD	29,6	16,7	4,1	4,3
SE	6,8	3,8	1,0	1,0
Range	8,3-100	0-58,3	0-12,5	0-12,5
N	19	19	19	18
Control				
Mean	31,1	22,9	11,6	7,1
SD	21,2	16,3	10,5	6,8
SE	4,9	3,7	2,4	1,6
Range	8.3-83,3	4,1-54,1	0-33,3	0-29,1
N	19	19	19	18
Difference between treatments (*)	8,47	-2,09	-6,44	-4,18
Paired t-test	1,97	-0,50	-2,65	-2,84
p-value	0,06	0,62	0,02	0,01

(*) HA-gel/control

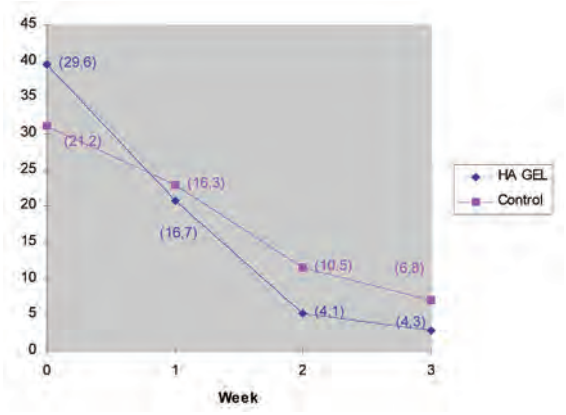

Figure 1 - Mean (SD) of BOP during the study.

to 22.9±16.3 % after one week, to reach the minimum value of 7.1±6.8 % at the end of the study (percent decrease = 75.8 %). Both at 14 and at 21 days, the difference between the two zones was statistically significant, with p=0.02 and p=0.01 respectively (Table 3, Figure 1).

PAL (Probing Attachment Level)

At baseline, the average of PAL was comparable in the two zones (2.2±0.7 and 2.0±0.5 respectively for test and control). This index decreased during treatment period in both areas, but even if the decrease was greater in the HA gel treated zones the difference between the two groups did not reach the statistical significance at any time. After 21 days the averages were respectively 1.9±0.8. and 2.0 ± 0.4 (Table 4, Figure 2).

Table 4 - PAL (Probing Attachment Level) during treatment.

PAL (Probing Attachment Level)	Baseline	T7	T14	T21
HA-gel				
Mean	2,2	2,1	2,0	1,9
SD	0,7	0,7	0,8	0,8
SE	0,2	0,2	0,2	0,2
Range	0,8-4,3	0,8-3,5	0,6-3,4	0-3,1
N	19	19	19	18
Control				
Mean	2,0	2,0	2,0	2,0
SD	0,5	0,5	0,5	0,4
SE	0,1	0,1	0,1	0,1
Range	1,1-2,7	1,2-2,6	1,2-2,6	1,3-2,6
N	19	19	19	18
Difference between treatments (*)	0,19	0,11	-0,01	-0,07
Paired t-test	1,44	1,04	-0,06	-0,55
p-value	0,17	0,31	0,95	0,59

(*) HA-gel/control

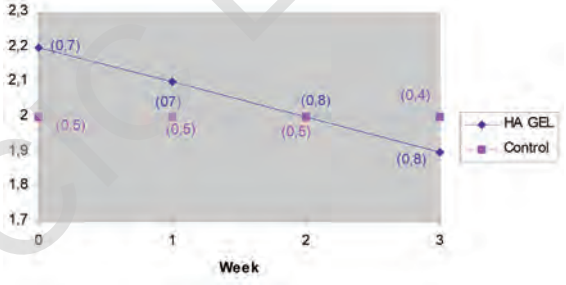

Figure 2 - Mean (SD) of PAL during the study.

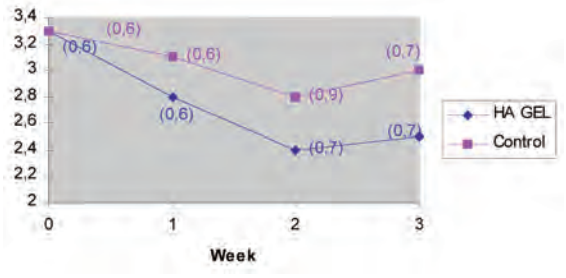

Figure 3 - Mean (SD) of PPD values during the study

BOP (Bleeding on Probing)

In the treated areas the average of BOP was 39.6±29.6 % at baseline, which decreased to 20.8±16.7 % after one week, then reaching the minimum value after 21 days: 2.9±4.3 % (percent decrease = 92.7 %). In the control areas the average was 31.1±21.2 % at baseline, decreased

PPD (Probing Pocket Depth)

At baseline the average of PPD was comparable in the two zones: 3.3±0.6 and 3.3±0.6 respectively for test and control. After seven days of treatment the index decreased with both treatments, but in the HA gel treated areas it reached a lower average than in the control ones: 2.8±0.6 vs.

Table 5 - PPD (Probing Pocket Depth) during treatment.

PPD (Probing Pocket Depth)	Baseline	T7 (after 7 days)	T14 (after 14 days)	T21 (after 21 days)
HA-gel				
Mean	3,3	2,8	2,4	2,5
SD	0,6	0,6	0,7	0,7
SE	0,1	0,1	0,2	0,2
Range	2,5-4,5	1,9-4,1	1,3-3,8	1,3-3,7
N	19	19	19	18
Control				
Mean	3,3	3,1	2,8	3,0
SD	0,6	0,6	0,9	0,7
SE	0,1	0,1	0,2	0,2
Range	2,35-4,3	2,3-4,4	0-4,2	2-4,4
N	19	19	19	18
Difference between treatments (*)	0,02	-0,29	-0,42	-0,47
Paired t-test	0,20	-3,29	-2,42	-3,36
p-value	0,85	<0,01	0,03	<0,01

(*) HA-gel/control

3.1±0.6 ($p < 0.01$). At the end of treatment the difference was still significantly in favour of the HA gel treated zone ($p<0.01$) (Table 5, Figure 3).

PLI (Plaque index)
At baseline the average of PLI was comparable in the two zones: 37.6±20.6 % and 37.0±22.6 % respectively for test and control. This index decreased during treatment in both areas, but even if the decrease was greater in the HA gel treated zone the difference between the two groups did not reach the statistical significance at any time. After 21 days the averages were respectively 4.6±4.3 % (percent decrease = 87.8 %) and 8.1±9.1 % (percent decrease = 78.1 %) (Table T6, Figure 4).

Table 6 - PLI (Plaque Index) during treatment

PLI (Plaque Index)	Baseline	T7 (after 7 days)	T14 (after 14 days)	T21 (after 21 days)
HA-gel				
Mean	37,6	12,7	4,3	4,6
SD	20,6	9,8	5,9	4,3
SE	4,7	2,2	1,4	1,0
Range	8,3-83,3	0-31	0-20,8	0-12,5
N	19	19	19	18
Control				
Mean	37,0	15,4	6,0	8,1
SD	22,6	16,4	10,7	9,1
SE	5,2	3,8	2,5	2,2
Range	12,5-83,3	0-70,8	0-45,8	0-37,5
N	19	19	19	18
Difference between treatments (*)	0,62	-2,72	-1,69	-3,46
Paired t-test	0,17	-1,11	-0,85	-1,66
p-value	n.s.	n.s.	n.s.	n.s.

(*) HA-gel/control

Table 7 - GI (Gingival Index) during treatment.

GI (Gingival Index)	Baseline	T7 (after 7 days)	T14 (after 14 days)	T21 (after 21 days)
HA-gel				
Mean	20,0	4,5	1,6	0,7
SD	18,8	6,6	3,0	2,1
SE	4,3	1,5	0,7	0,5
Range	0-75	0-25	0-8,3	0-8,3
N	19	19	19	18
Control				
Mean	21,0	10,2	3,6	4,4
SD	14,3	11,2	3,9	3,3
SE	3,3	2,6	0,9	0,8
Range	0-62,5	0-50	0-12,5	0-10
N	19	19	19	18
Difference between treatments (*)	-1,01	-5,68	-1,98	-3,68
Paired t-test	-0,20	-1,82	-2,92	-4,17
p-value	n.s.	0,09	0,01	<0,01

(*) HA-gel/control

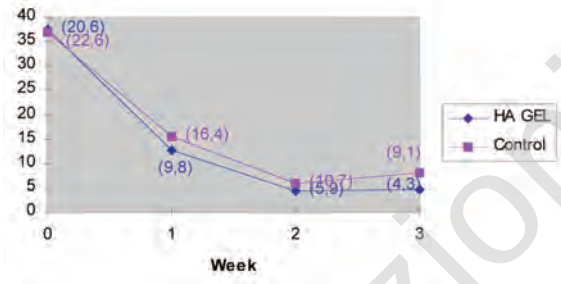

Figure 4 - Mean (SD) of Plaque Index during the study.

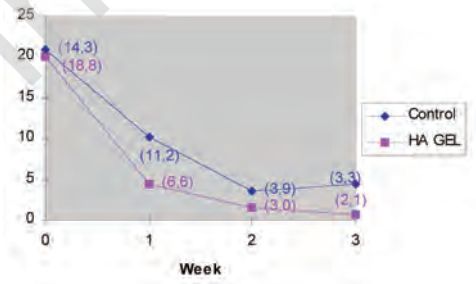

Figure 5 - Mean (SD) of Gingival Index during the study.

GI (Gingival index)
At baseline the Gingival Index was, on average, comparable in the two zones, with a mean value of 20.0±18.8 % in the one to be treated with HA gel and 21.0±14.3 % in the control. After one week the GI was reduced to 4.5±6.6 in the testy and to 10.2±11.2 % in the control. The very large variability in both groups did not allow this difference, strongly in favour of HA gel, to be significant. Statistical significance was reached at 14 and 21 days of treatment with p<0.01. At 21 days the percent reduction from basal average level in the HA gel areas was 96.5 % while in the control once it was 79.0 % (Table 7, Figure 5).

Discussion

In this study HA gel has been tested on 19 patients affected with mild chronic periodontal disease, with shallow pockets in at least two different quadrants , comparing the material with normal oral hygiene.
Based upon the obtained results it is important to notice that all the evaluated parameters for assessing the tolerability of HA gel, the principal aim of this study, are in favour of the biomaterial. No visible alterations of oral tissues were seen during the study in the treated areas with HA gel.
Both gingival reddening and pain were in favour of HA gel at all visits, although they do not reach statistical significance. Subjective judgement of patient on symptomatic evolution during the treatment period improved more in HA gel zones than in the control zones, reaching a positive result (100.0 %) at the end of treatment compared with the results (66.7 %) of the control zones. The difference between treated and control zone values are significant at all visits. (p<0.01).
Root sensitivity showed a higher decrease in HA gel treated zones and the taste of the product was positive at the end of the study for all patients.
Furthermore, investigator's overall judgement on tolerability of HA gel was completely positive and no adverse event occured, confirming the safety of the product.
All efficacy indexes had a decrease in the HA gel treated zone, a decrease which was significant in comparison with normal oral hygiene used in the control zones for BOP, PPD and GI.
In fact even if the oral hygiene had a positive influence on the outcome of all the clinical indexes measured in the study, the treatment with HA gel showed a greater effect

almost always statistically significant. BOP in the HA gel treated areas had a decrease of 92.7% and the correlated GI of 96.5%, respectively 75.8% and 79.0% in the control areas. The difference of PPD in both areas was statistically significant ($p<0.01$) in favour of the HA gel treated zone.
Also PAL and Pl diminished more with gel than with oral hygiene, although it does not reach a statistical significant difference. The investigator's overall judgement on efficacy of HA gel was positive in 84.3% of the treated patients.

Conclusion

In conclusion, it appears that the treatment with HA gel has shown an effect in reducing the gingiva inflammation and that it could be successfully used to improve the the periodontal clinical indexes.
Additionally, the effect of hyaluronan seems to be, from the patient's side, more beneficial by reducing, in the test sites, both staining and change of taste or calculus formation which occur with current traditional adjunctive therapy using products such as clorhexidine. This is a further help for the clinician for suggesting prolonged applications of the product without adverse reactions. Furthermore, the improvement of the clinical inflammatory signs on the test sites can be also explained from recent data (18) showing a bacteriostatic effect of hyaluronan on oral bacteria. Future research will be necessary in order to evaluate the histological changes where the new attachment is formed to understand its quality and characteristics.

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