Biotechnological value of the hyaluronic acid in periodontal treatment

GIURGIU MARINA CRISTINA1, PAUNICA STANA1, GEORGETA MANIU1, DUMITRIU HORIA TRAIAN1, DUMITRIU ANCA SILVIA1
1 Department of Periodontology, Faculty of Dental Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
2 Department of Information Technology Mathematics and Physics, Petroleum-Gas University of Ploiesti, Romania
Corresponding author: Marina Cristina Giurgiu giurgiu.marina@yahoo.com

Abstract

The hyaluronic acid (HA) is a basic component in the structure of the gingiva which maintains tissue integrity and also has anti-inflammatory properties. It is among the non-collagenous components of the matrix which is degraded during periodontal diseases. The study showed the benefits of association of hyaluronic acid gel in surgical treatment of periodontal disease by evaluating the two common periodontal indices directly related by gingival status and salivary pH. After treatment with HA gel, decrease in mean for plaque index was 73%, for bleeding index 72.55% (p<0.0001) and for salivary pH 1.15%. Moreover, by monitoring the value of the salivary pH, the results showed that after treatment the difference is statistically non-significant in the case of pH. Only the value of PI has a significant influence on the value of the pH (p<0.01) only before the treatment.

Keywords: hyaluronic acid gel, salivary pH, periodontal disease.

Introduction

Hyaluronic acid (HA) is a polysaccharide, a non-sulphated glycosaminoglycan which is found in connective tissues, in synovial fluid and vitreous humor serum (C. CERMELLI & al. [1]). It has distinct physicochemical properties which underlie its application in ophthalmology (kerato-conjunctivitis, ophthalmological surgery) (C. CERMELLI & al. [1], K. L. GOA & al. [2]), in treatment of osteoarthritis (H. MATSUNO & al. [3]), in rheumatoid arthritis (H. MATSUNO & al [3]), in dermatology (R. MOSELEY & al. [4]) and in cosmetic intervention, in gynecology, in treatment of asthma (C. CERMELLI & al. [1]).

HA is a basic compound of the extracellular matrix and plays a major role in maintaining the integrity of gingival chorion (H.T. DUMITRIU [5]). It contributes significantly to tissue hydrodynamics, cell migration and proliferation (J. BANSAL & al. [6]). Due to its viscosity and elasticity HA acts as a lubricating and shock absorbing (K. L. GOA & al. [2], H.T. DUMITRIU [5]). It has anti-inflammatory and antiedematous properties (H. JENTSCH & al. [7], N. ROSOIU & al. [8]). HA has a bacteriostatic effect on bacterial strains, including Aggregatibacter actinomycetemcomitans, Prevotella spp. depending on its molecular weight (P. PIRNAZAR & al. [9]) and shows the ability to interfere with viral replication, such as herpes simplex virus, enteroviruses, Coxsackievirus (C. CERMELLI & al. [1]). In addition, HA is effective in promoting osteoblast-like cell differentiation and bone formation during the repair of bone defects, indicating that it may be use in the treatment of various bone defects (K. TANAKA & al. [10]).

Periodontal disease is an inflammatory destructive disease which, if untreated may lead to destruction of the alveolar bone support, loss of tooth implantation and, finally tooth avulsion.
The crucial role in determining the onset of the disease is held by the bacteria in the bacterial plaque, especially the mature subgingival plaque, together with local and systemic factors. Due to bacterial plaque deposit on the dental surface immediately after brushing, the gingiva is practically speaking in a permanent state of chronic inflammation (H.T. DUMITRIU [5]). The first clinical sign of chronic inflammation is gingival bleeding, which is initially provoked and spontaneous in more advanced stages of the disease. This is why the plaque and the bleeding indices are important in determining the gingival status.

The subgingival plaque becomes more complex when periodontal pockets exist (A. S. DUMITRIU & al. [11]). The bacteria found in dental plaque produce a variety of enzymes including hyaluronidase that contribute to the disease process (H.T. DUMITRIU [5], F.A. CARRANZA & al. [12]). In periodontal pocket there is a high level of hyaluronidase and this changes the permeability of junctional epithelium attached to radicular cementum (H.T. DUMITRIU [5]).

It has been recognized that saliva is a mirror of the body’s health as it contains proteins, hormones, antibodies and other molecules (B.P. PREETHI & al. [13]). Saliva plays an important antibacterial role both by its flow and by its composition. Changes in salivary composition and flow rates may compromise the integrity of the soft and hard tissues in the oral cavity (M.I. ROCKENBACH & al. [14]). The imbalance in levels of free radicals, reactive oxygen species and antioxidants in saliva play an important role in the onset and development of dental caries (M. BATTINO & al. [15]). If the bicarbonate level in the saliva increases, this will not only increase salivary pH and buffer capacity, and facilitate remineralization, but will also exert ecological effects on the oral flora. Higher salivary pH will suppress the tendency for acid tolerating microorganism to grow, particularly streptococci and Candida albicans (L.J. WALSH [16]).

HA being a natural constituent present in human conjunctive tissues, the use of it has less adverse effects, it is nonimmunogenic.

Material and Method

40 patients were selected, aged 35–59, non-smokers and without general conditions. All subjects suffered from periodontal disease with at least four periodontal pockets ≥ 6 mm, with alveolar bone vertical resorption on radiographic exam. Three parameters were monitored before and four weeks after periodontal treatment with hyaluronic acid gel: pH, plaque index (PI) and bleeding index (BI). Treatment consisted of subgingival curettage of periodontal pockets followed by HA topical gel. Revaluation of indices was done 4 weeks after the treatment. PH was determined on unstimulated saliva with GC Saliva-Check Buffer (the pH test strip was placed into the sample of resting saliva for 10 sec., and then the colour of the strip was compared with the testing chart available in the package; the interpretation: highly acidic pH[5-5.9], moderately acidic pH[6.0-6.6], healthy saliva pH[6.7-7.8]. Interpretation of values for plaque and bleeding indices were very good for PI<15%, BI<10%, good for PI 10-40%, BI 15-40% and bad for value after 40% in both cases.

Results

After periodontal treatment associated with HA a favorable post surgical evolution was noticed, with no signs of gingival inflammation and with improvement of the indices under study.
The paired T-test is used to determine the significance of the difference in mean for each of the observed characteristics (pH, PI and BI) before and after the medical treatment with HA. The study shows that the difference is statistically non significant in the case of pH, but significantly lower (p<0.0001) for both PI and BI (figure 1).

**Figure 1.** Mean values of pH, PI and BI before and after treatment

The mean value of pH decreases only with 1.15%, from 6.94 - the observed mean value before the treatment, to 6.86 - the observed mean value after treatment (figure 2).

**Figure 2.** Patient distribution according pH values before and after treatment

The mean value of PI decreases with 73%, from 57.69% - the observed mean value before the treatment, to 15% - the observed mean value after treatment (figure 3).
Figure 3. Patient distribution according to PI value before and after treatment

The mean value of BI decreases with 72.55%, from 57.51% - the observed mean value before the treatment, to 15.78% - the observed mean value after treatment (figure 4).

Figure 4. Patient distribution according to BI value, before and after treatment

To study the influence of the PI and BI over the pH before and after the treatment with HA, the Chi-square test for marginal probabilities in the contingency tables was used. The study shows that the value of PI has a significant influence on the value of the pH (p<0.01) only before the treatment. Before treatment pH was normal [6.7-7.8] 64.7% and moderately acid [6-6.6] 35.3%.

After the treatment: in 83% of the cases of patients with moderately acid pH, the level of the pH became normal; for the others, the level of pH increased towards the lower value of the interval of the pH normal range. More than 80% of the subjects with PI less than 50% and also, more than 75% of the subjects with extreme values of the PI (60-80%), register a normal value for the pH (in the interval [6.8-7.8]). More than 90% of the patients with PI in the interval [50-60%] register a value of the pH in the interval [6-6.6], hence moderately acid.
Before the treatment there is no significant influence of BI over pH, and there is no significant influence of PI or BI over pH after the treatment with HA (figure 5, figure 6).

**Figure 5.** Plaque index (PI) distribution according pH values, before treatment.

**Figure 6.** Plaque index (PI) distribution according pH values after treatment.

**Discussion**

Test T was used to determine if the difference between mean statistic values obtained for the sample before and after treatment is significant from a statistic point of view for each of two characteristics under study: PI and BI. The study showed that the observed difference is significant from a statistic point of view (p<0.0001) both for PI and BI, which means that the treatment influences these values in a significant way. There is a 73% decrease in the mean value of the PI and 72.55% for the mean value of the BI.

The Chi-square test for marginal probabilities in the contingency tables was used to determine if PI influences the significance of BI.

The analysis was done a) before treatment and b) after treatment.

**Case a)**

At the first stage (Case 1) the subjects were grouped in 4 groups corresponding to PI values - [20% - 40%), [40% - 60%), [60% - 80%), [80% - 100%]. Further on the patients in each
group were divided into sub-classes corresponding to the observed value of BI (5 sub-groups: [0%-20%), [20% - 40%), [40% - 60%), [60% - 80%), [80% - 100%]). The study aimed to verify whether individuals belonging to one of the classes of values for BI are influenced by the observed PI. 

According to the test used and based on the observed data (the statistically calculated value is 31.72, which is higher than the table index value $\chi^2_{12,0.01} = 24.72$), it is observed that PI value significantly influences BI value. The distribution of BI values before treatment is as follows:

- 40% of the patients with PI values in the interval [20%-40%) have BI values in the interval [0%-20%), 40% have BI values in the interval [20%-40%) and 20% have a high BI value in the interval [80% - 100%];
- 25% of the patients with PI values in the interval [40% - 60%) have BI values in the interval [40% - 60%), 25% in the interval [60%-80%) and 50% have BI values in the interval [80%-100%];
- 14.28% of the patients with PI values in the interval [60%-80%) have BI values in the interval [20% - 40%), 28.56% in the interval [40%-60%), 14.28% in the interval [60%-80%) and 42.85% have BI values in the interval [80%-100%];
- 75% of the patients with a high PI value in the interval [80%-100%] have a BI value in the interval [20%-40%); 25% have BI values in the interval [80%-100%].

A similar analysis (Case 2) was carried out reducing the number of classes for the observed BI values to two: [0%-50%, [50%-100%] According to the test used, based on the observed data, the statistically calculated value was 12.10, which is higher than the table index value. Therefore, the conclusion is the same: PI values significantly influence BI values. The distribution of BI values according to PI values before treatment is the following:

- 100% of the patients with PI value in the interval [20%-40%) have the BI value in the interval [0%-50%);
- 25% of the patients whose PI value is in the interval [40%-60%) have the BI value in the interval [0%-50%), 75% of them have BI values in the interval [50%-100%];
- 71% of the patients whose PI value is in the interval [60%-80%) have the BI value in the interval [0%-50%), 28 % of them have BI values in the interval [50%-100%];
- 75% of the patients whose PI value is in the interval [80%-100%] have the BI value in the interval [0%-50%), 25 % of them have BI values in the interval [50%-100%].

Case b)
At the first stage (Case a) the subjects were grouped in 4 groups/classes corresponding to PI values - [0% – 20%), [20% - 40%), [40% - 60%), [60% - 80%). Further on the patients in each group were divided into sub-classes corresponding to the observed values of BI (3 sub-groups: [(0%-20%), [20% - 40%), [40% - 60%]). The statistically calculated value was 31.45 which is obviously higher than the table index value $\chi^2_{5,0.01} = 22.46$. Therefore, the value of PI significantly influences the value of BI ($p<0.001$).

After treatment the distribution of BI values depending on PI is the following:

- 76.92% of the patients with low PI value (in the interval [0%-20%)) have a low BI value (in the interval [0%-20%)) and 23.07% have a BI value in the interval [20%-40%);
- 100% of the patients with PI values in the interval [20%-40%) have a BI value in the interval [20%-40%);
50% of the patients with PI values in the interval [40%-60%) have BI values in the interval [0%-20%), the other 50% have BI values in the interval [40%-60%);

100% of the patients with PI values in the interval [60%-80%) have a BI value in the interval [0%-20%).

If observed BI values before treatment were in the interval [0% -100%), after treatment observed values vary in the interval [0%-50%). Thus, in case 2 the number of classes for BI observed values has been reduced to two: [0%-25%), [25% - 50%). According to the test used the statistically calculated value was 11.97 which is obviously higher than the table index value \( \chi^2_{0.01} = 11.34 \), therefore the conclusion is the same: PI values significantly influence BI values.

The distribution of BI values according to IP after treatment is the following:

- 84.61% of the patients with PI values in the interval [0%-20%) have BI values in the interval [0%-25%) and 15.38% have BI values in the interval [25%-50%);
- 25% of the patients with PI values in the interval [20%-40%) have BI values in the interval [0%-25%) and 75% have BI values in the interval [25%-50%);
- 50% of the patients with PI values in the interval [40%-60%) have BI values in the interval [0%-25%) and 50% have BI values in the interval [25%-50%];
- 100% of the patients with PI values higher than 60% have BI values in the interval [0%-25%).

In the last part of the study test T was used to determine if the difference between the statistically obtained mean values at the sample level before and after treatment is significant from a statistic point of view for the pH. The study has demonstrated that the observed difference is insignificant from a statistic point of view as far as pH is concerned, which means that treatment with HA does not significantly influences the observed values of the pH. There was a decrease of 1.15% in the mean value of the pH, from 6.94- mean value before treatment- to 6.86 – mean value after treatment, both values being in the inferior range of normal values.

The Chi-square test for marginal probabilities in the contingency tables was used to study if PI, BI respectively, significantly influences the observed values of the pH. The subjects were grouped in two groups according to the observed values of the pH: normal values [6.7- 7.8] and moderately acid values [6-6.6]. Each group was divided into sub-groups corresponding to 7 intervals of observed values of the PI. It was the aim of the study to determine if the observed value of a subject’s PI determines that this subject belongs to one of the two classes of pH values.

It was observed that the PI value has a significant influence on the observed value of the pH (p<0.01) only before treatment with HA.

Over 80% of the subjects with observed PI value lower than 50%, and also over 75% of the patients with PI values in the interval [60%-80%] have normal pH values (in the interval [6.8-7.8]).

Over 90% of the patients with PI values in the interval [50%-60%] have a moderately acid value of the pH (in the interval [6-6.6]).

It was observed that in the case of 83% of the patients with moderately acid pH before treatment the pH level has become normal, in the other 17% of cases the pH level has slightly increased towards the inferior limit of normal pH values.

The study has demonstrated that there is no statistically significant influence of the BI on the pH before treatment with HA, and also that there is no significant influence of the BI on the pH or of the PI on the pH after the treatment with HA.
Conclusion

The surgical periodontal treatment associated with HA resulted in quicker healing of the wound of all subjects. Moreover, after treatment the values of PI and BI got better and they were between normal limits.

The study shows that after periodontal treatment with hyaluronic acid gel the value of pH tends to neutral and also supports the efficacy of hyaluronic acid in periodontal regeneration.

References


