

Fibrin Glue Reduces the Dissolution Rate of Sodium Hyaluronate

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ABSTRACT

Sodium hyaluronate (HA) is known to modulate wound healing and interact with inflammatory reactions. High concentrations of extracellular HA are for example correlated to scarless wound healing. Topical treatment with HA has, however, limited effect due to the rapid clearance of HA in the tissue. In an effort to prolong the dissolution rate and enhance the effect of topically administered HA, HA was incorporated in a cross linked fibrin clot and placed in NaCl. The concentration of HA in the NaCl solution was analysed after 30', 60', 4h, 8h, and 24h. It was found that the dissolution rate of HA incorporated in cross linked fibrin was dramatically decreased in vitro, especially when the HA-fibrin mixture was put at rest and not exposed to a mechanical stress. The findings indicate a new possibility for slow release of HA after topical administration.

INTRODUCTION

Sodium hyaluronate (Hyaluronic acid, HA) is a large water soluble polymer polysaccharide which is found in the extracellular space of all tissues. It has the same simple chemical structure in all species and is an important component of the extracellular matrix. The degree of polymerization of native HA is in the order of 10^4 and the average molecular weight ranges from 100,000 to 5 million. In physiological salt solutions the molecule forms a flexible random coil. The sponge-

like HA molecule binds large amounts of solvent volume. It can be calculated that in a solution containing 0.16-0.5 mg HA per 1 ml solvent, all the solvent space is occupied (3). At higher concentrations the individual coils start to overlap and are thus compressed. This leads to a rapid increase in viscosity of the solution, and with increasing concentrations the elastic properties also increase.

The visco-elastic properties of HA have led to its clinical use as a spacer and to facilitate operative procedures in the field of eye surgery (15). HA has, however, also been demonstrated to be biologically active. It is known that tissues with high concentrations of HA are able to heal wounds through regeneration without formation of scar tissue and shrinkage (1,10,17,18).

It has further been shown that topical application of HA qualitatively improves wound healing by reducing or preventing the occurrence of post operative adhesions after tendon surgery (2,21,24) and spinal surgery (20).

Therapeutical effects of HA are, however, limited due to its rapid turnover and short half-life. The molecule is very soluble in balanced physiological saline solutions (3) and is rapidly cleared from most tissues through the lymphatic system (5,13,23). HA that enters the blood is cleared by receptor-mediated uptake in the liver (4,11,12) and has a half life of only 2-6 minutes (6,7). The rapid clearance after topical administration is believed to be one of the reasons why sodium hyaluronate has not been as effective as postulated in influencing wound healing and reducing postoperative scar formation (9,14,19,24).

The wound healing process begins with the formation of a haemostatic clot containing platelets, red blood cells and fibrinogen. Clotting starts with conversion of fibrinogen into fibrin, which is polymerised into a fibrin monomer network. A cross linking reaction is then initiated by factor XIII to form a fast γ -cross linkage followed by a slower α -cross linkage (16). Fibrin clots form a three-dimensional network which theoretically could trap the HA molecules, and thereby decreasing the dissolution rate which would in turn extend the possible time for providing a biological effect of locally administered HA. Fibrin glue has previously been used as a

carrier of cancellous bone (22) and for administration of antibiotics (8).

The concept of a slow delivery of HA by incorporating it in a fibrin clot is especially interesting as it opens up new possibilities for wound treatment by combining the two natural wound healing components HA and cross linked fibrin. The possibility of reducing the dissolution rate of HA by incorporating it in a cross linked fibrin clot was tested in the following experiment.

MATERIALS AND METHODS

Tisseel^R (Immuno AG, Vienna, Austria) is a two-component fibrin glue delivered in two deep-frozen syringes. One syringe contains 75-115 mg/ml fibrinogen, 2-9 mg/ml plasma fibronectin, 10-50 U factor XIII, 40-120 µg/ml plasminogen and 3000 KIU/ml aprotinin. The other syringe contains 500 U/ml thrombin and 40 mM CaCl₂. The contents of the two syringes are mixed during the application, using a Duploject^R system.

The deep-frozen syringes were thawed in a water bath which was kept at 37°C. The fibrinogen contained in one syringe of the fibrin glue was mixed 1:1 with HA, 10 mg/ml (Healon^R, Kabi Pharmacia, Uppsala, Sweden) by passing it back and forth one hundred times between two syringes. The thrombin in the other syringe was left unmixed, resulting in a final concentration of 0.25% HA. One hundred mg of the final composition was placed at the bottom of a petri dish. One minute (groups A and C) and 10 minutes (groups B and D) later 10 ml of 0.145 M NaCl was added. The petri dishes were either put in a shaking bath with a frequency of 1 Hz and set at 37°C (groups A and B), or at rest at 37°C (groups C and D).

The corresponding controls were HA (Healon^R) diluted 1:4 with 0.145 M NaCl, leading to the same final concentrations of HA as in the experimental groups (0.25%). The controls were treated in the same manner as the experimental groups with and without shaking, groups AC, BC, CC and DC, respectively.

Samples from the NaCl solution in the petri dishes were taken after 30', 60', 4h, 8h and 24 h for analysis of HA. The HA concentrations were determined with the HA Test 50 (Kabi

Pharmacia AB, Uppsala, Sweden).

RESULTS

The NaCl solutions were almost immediately saturated in the control groups and there was no further increase of HA up to 24 hours. There were no differences between the groups that were put at rest or shaken in the control groups, Fig. 1 and 2.

In the experimental groups the dissolution rate depended on whether the fibrin clots were allowed to set for 10 minutes before adding the NaCl solution, and whether they were put at rest or shaken, Fig. 1 and 2. Group D, in which the fibrin glue/HA composition was allowed to set for 10 minutes before adding the NaCl solution and which was not shaken, was the most effective in decreasing the dissolution rate. To express how fast HA was dissolved was the time calculated until 30% of the HA was found in the NaCl solution. The dissolution rate was dramatically decreased in all the experimental groups compared to the controls. Ten minutes of setting time decreased the dissolution rate compared to one minute setting time, but putting the mixture to rest had a greater impact on the dissolution rate than the setting time. The results are summarized in table 1.

Table 1.

Time calculated in minutes until 30 % of the final amount of HA was dissolved for each of the groups.

Group	Experimental				Control			
	A	B	C	D	AC	BC	CC	DC
Minutes	15	36	336	462	3	3	3	3

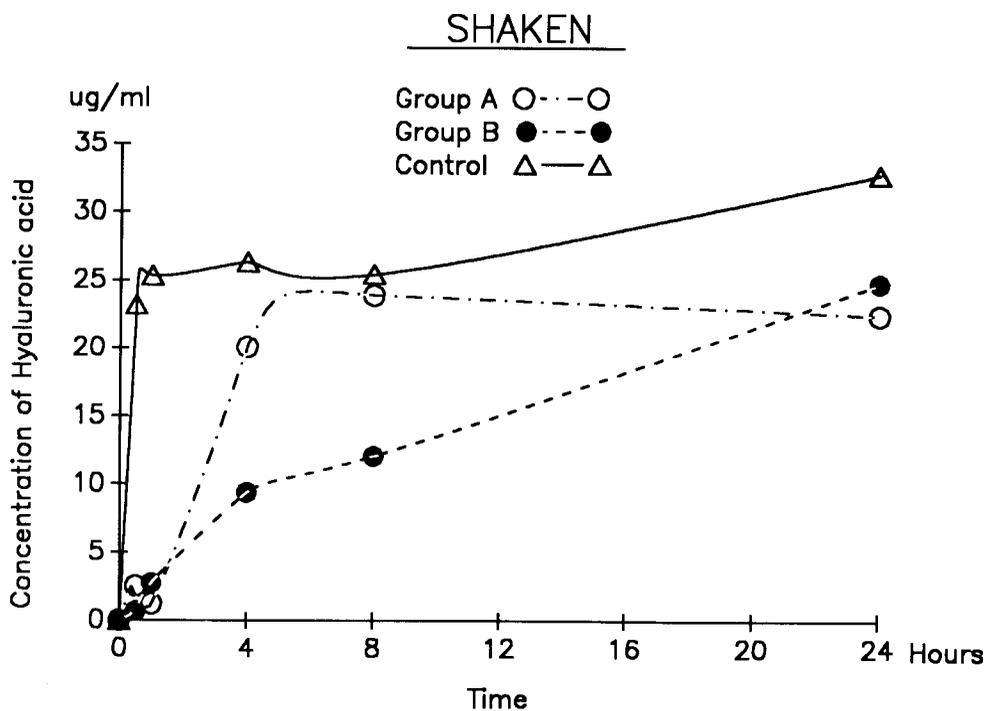


Figure 1.

The concentration of HA at different times after addition of NaCl to the mixture of HA and fibrin. The mixture was allowed to set for one minute before addition of saline in group A and during 10 minutes in group B. The control was a mixture of HA and NaCl resulting in the same final concentration of HA in the mixture as in the experimental groups. The mixtures were put in a shaking bath at a frequency of 1 Hz.

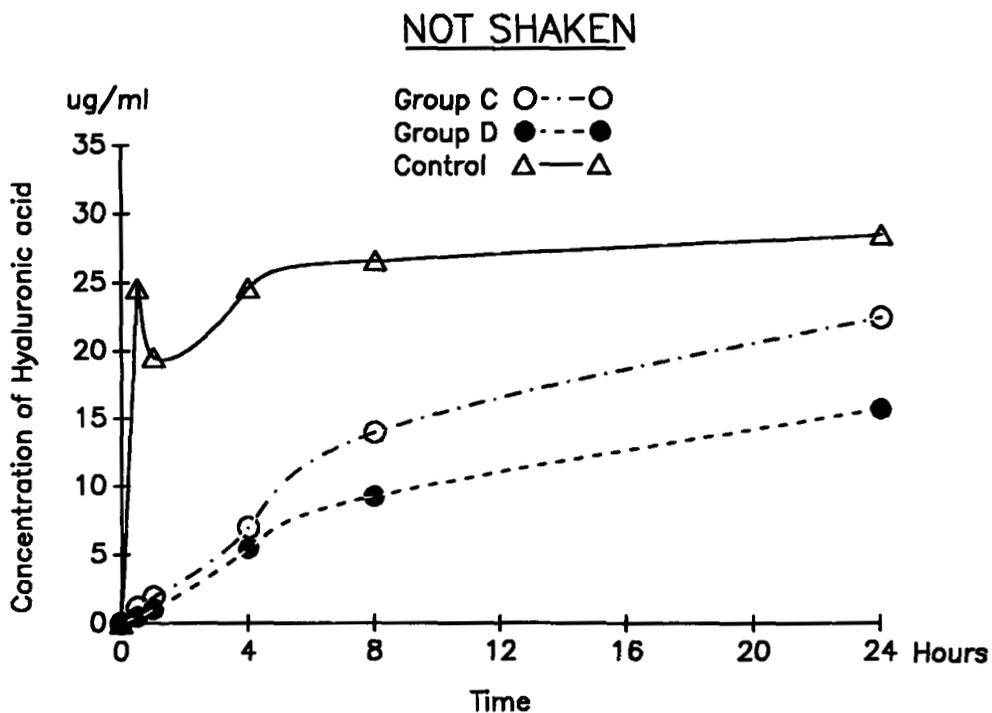


Figure 2.

The concentration of HA at different times after addition of NaCl to the mixture of HA and fibrin. The mixture was allowed to set for one minute before addition of saline in group C and for 10 minutes in group D. The control was a mixture of HA and NaCl resulting in the same final concentration of HA in the mixture as in the experimental groups. The mixtures were kept at rest during the experiment.

DISCUSSION

HA dissolved up to 150 times slower in a saline solution when it is incorporated in a fibrin clot. The results indicate that fibrin glue can prolong the possible time for providing a biological effect of topically administered HA by decreasing the dissolution rate. This opens up a new approach for local therapeutic treatment with HA to promote wound healing and for anti adhesion purposes. The dissolving rate was dependent upon how long the fibrin glue was allowed to set before the solvent was added, which indicates that cross linking of fibrin is important in keeping HA within the clot. It is possible that further prolongation of the setting time would lead to a further decrease in dissolution rate. It is additionally demonstrated that the mixture of HA and fibrin should be at rest if a prolongation of the dissolution rate is wanted. It is therefore possible that it will be necessary to immobilize a wound when it is treated with the combination of HA and cross linked fibrin.

In vivo studies have confirmed these in vitro findings and demonstrated that scarless wound healing can be induced by a combination of HA and fibrin glue (25). Further studies are needed to evaluate whether the dissolution rate is dependent on the molecular weight and mixing ratios of HA and fibrin glue in order to have an even better effect on the induction of scarless wound healing.

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