Pseudopolysaccharides for the treatment of Osteoarthritis: *in-vitro* degradation and *in-vivo* residence time

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Introduction: There is significant controversy surrounding the use of hyaluronic acid (HA) viscosupplementation for the treatment of knee OA. The problem with current formulations is that they have low residence time and on average remain within the joint space for less than 24 h as a consequence of the high turnover rate of HA by the lymph system and degradation by hyaluronidase.[1, 2]

HA is susceptible to degradation which occurs in response to mechanical, ultrasonic, pH, thermal, free radical, and enzymatic stress[3]. The most prevalent means of HA degradation is digestion by the hyaluronidase enzymes. There are three different known types of hyaluronidases, which act by dissimilar mechanisms. Mammalian hyaluronidase (EC 3.2.1.35), also known as endo-β-*N*-acetyl-*D*-hexosaminidases, degrades HA to even-numbered oligosaccharides (4 or 6) with glucuronic acid residues as the non-reducing ends. The second type which consists of endo-β-glucuronidase (EC 3.2.1.36) from leeches and other parasites, acts by a mechanism that is currently unknown. Bacterial hyaluronidase (EC 4.2.2.1) act on HA via a β-elimination reaction to yield tetrasaccharides and hexasaccharides, or disaccharides as the final products.

Herein, we report the use of a novel pseudopolysaccharide designed to overcome this enzymatic degradation since the backbone does not have the cleavable functions that HA contains.

Experimental:

In-vitro resistance to hyaluronidases of synthetic polymers.

To test this enzymatic resistance, we incubated polymeric solution of our polymer (Mw of 3MDa) at 0.5 %wt/wt with bovine hyaluronidase at 300 µg/mL. To confirm the activity of the enzyme, the same experiment was repeated with a 0.5%wt/wt HA solution (from Acros Chemical 5MDa Mw). The molecular weights were measured after 24h using SEC to detect any degradation.

In-vivo Intra articular injection into the elbow joints of rabbits.

To test in an *in-vivo* setting, four 24-week-old male New Zealand white rabbits received an injection of pseudopolysaccharides in the left elbow joint by a 20 gauge needle under fluoroscopic guidance. Once injected with the pseudopolysaccharide, the rabbits were allowed free cage activity for 9 days. For the control group data all right rabbit elbows (n =4) were used. After 9 days all the rabbits were sacrificed and the synovial fluid of both right and left elbows collected. A gel electrophoreses using the following conditions: 0.5% agarose, V = 50V, t = 8h, stained for 2 days with cationic dye "Stains All", and destained for 1.5 days was done on these samples to determine the presence of the pseudopolysaccharides in the elbows after 9 days.

Results and Discussion:

As shown in Figure 1, the pseudopolysaccharide did not undergo any hyaluronidase degradation *in-vitro* since the molecular weight remained the same after 24h. On the other hand, the molecular weight of HA was drastically decreased from 5 M to less than a million. These results show that the synthetic polymers are not degraded by hyaluronidase even at high concentrations. Furthermore, the *in-vivo* experiments confirm the presence of the pseudopolysaccharide after nine days (Figure 2).

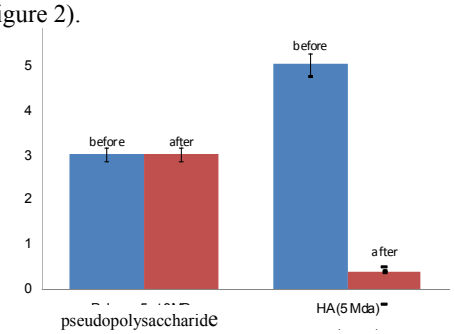

Figure 1. SEC results on pseudopolysaccharide and HA. (n=3).

Figure 2. gel electrophoreses results: a and d) untreated rabbit elbow (right elbow), b and c) treated rabbit elbow (left elbow) with pseudopolysaccharides (purple dot).

Conclusion:

These results show that the synthetic pseudopolysaccharides are not degraded by hyaluronidase even at high concentrations. Furthermore, the in-vivo experiments confirm that the pseudopolysaccharide remains in the joint at least for 9 days compare to HA.

These formulations were also found to be non-cytotoxic up to 2% wt/wt (data not shown). These results support the further evaluation of these pseudopolysaccharides as new viscosupplements for the treatment of OA. Additional characterization studies are underway.

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