Intra-articular Injection of HB-IGF-1 Sustains Delivery of IGF-1 to Cartilage through Binding to Chondroitin Sulfate

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Introduction: Insulin-like growth factor-I (IGF-1) stimulates cartilage growth and repair but is not a practical therapy due to its short half-life in vivo and potential side effects when delivered systemically. We previously generated a new heparin-binding IGF-1 fusion protein (HB-IGF-1) by adding the heparin-binding domain of heparin-binding epidermal growth factor-like growth factor (HB-EGF) to the amino-terminus of IGF-1. In vitro experiments have shown that HB-IGF-1 produces long-term delivery of bioavailable IGF-1 to bovine cartilage explants. We hypothesized that HB-IGF-1 was retained in cartilage through binding heparan sulfate proteoglycans in the matrix and at the cell surface. In the present study, we tested this hypothesis by measuring the binding affinities of isolated HB-IGF-1, binding of HB-IGF-1 to cells lacking heparan sulfate, and release of bound HB-IGF-1 following enzymatic treatment of cartilage explants. We then tested whether HB-IGF-1 binds human cartilage and whether intraarticular injection of HB-IGF-1 allows sustained in vivo delivery to rat knee cartilage.

Materials and Methods: Protein Production: Both HB-IGF-1 and control IGF-1 were expressed with Xpress and 6x-His tags in E. coli and purified by Ni-NTA affinity and reverse-phase chromatography. Bovine Cartilage with Enzyme Treatment: Cartilage disks (3 mm diam, 0.5 mm thick) from calf femoropatellar grooves were cultured in serum-free DMEM with 500 nM HB-IGF-1 or IGF-1 for 2 days. At Day 2, disks were washed and treated with either medium alone, 100 U/M BC (0.4 U/mL), or heparitinase (0.036 U/mL). At Day 4, IGF released to the medium was detected by ELISA, and IGF remaining in the tissue was detected by Western analysis. Heparitinase treatment following chondroitinase treatment did not release any additional IGF, indicating no steric interference. CHO cell binding: Mutant CHO cells lacking heparan sulfate (strain pgsD-677) and wildtype CHO (K1) cells were incubated in serum-free F12 medium with either 10 µg HB-IGF-1 or IGF-1 for 3 h, washed, and analyzed by Western. Surface Plasmon Resonance (SPR): Chondroitin sulfate (CS) (CS-C, shark cartilage), heparan sulfate (HS) (bovine kidney), and heparin (porcine intestinal mucosa) were biotinylated and bound to a streptavidin-coated Biacore chip. The interaction of HB-IGF-1 with CS, HS, and heparin was measured using BIAevaluation software v4.1 and floating Rmax as a local parameter. Human Cartilage Binding Assay: Cartilage disks (3 mm diam, 0.7 mm thick) were harvested from femoropatellar grooves of post-mortem 26-year old (Collins Grade 0) and 42-year old females (Grade 2) and cultured in 1% ITS serum-free HGDME supplemented with 500 nM HB-IGF-1 or X-IGF-1. After 48 h (Day 0), disks were washed with PBS and incubated in IGF-1 free medium. Disks were collected on Days 0, 1, 2, and 4 and analyzed for IGF-1 bound by Western blot. Procedures were approved by the Office of Research Affairs, Rush Univ. and the Committee on Use of Humans as Experimental Subjects at MIT. Intra-articular Injection in Rat: 10 µg HB-IGF-1, 10 µg IGF-1, or saline alone was injected into the knee joints of 2-month-old male Sprague-Dawley rats. After one day, joint tissues were harvested and extracted. Portions of extracts with equal total protein were analyzed by Western. All animal procedures were approved by the Harvard Medical Area Standing Committee on Animals.

Statistical Analysis: Data are presented as mean±SEM. Data were log-transformed when necessary and a general linear model was used with p<0.05 considered significant. Pairwise comparisons were made by Tukey post-hoc tests.

Results: SPR analysis showed that while IGF-1 does not bind to GAGs (response units <10), HB-IGF-1 binds to heparin, HS, and CS. As expected, heparin and HS binding was strongest (Kd = 46.8 and 20.8 nM respectively), but HB-IGF-1 also bound to HS (Kd = 196 nM) (Table 1). To identify whether HB-IGF-1 is retained in cell monolayers by HS, we tested retention to mutant CHO cells that lack HS. Surprisingly, HB-IGF-1 bound the mutant cells as well as wild-type cells, indicating that HB-IGF-1 is not required for cell-surface binding (Fig 1A). We then tested whether HS or CS is required for HB-IGF retention in cartilage explants and found that treatment with chondroitinase ABC released significantly more HB-IGF-1 than treatment with heparitinase (Fig 1B). Human knee cartilage obtained from a Grade 0 joint was shown to bind HB-IGF-1 up to four days after incubation; binding of IGF-1 was not detectable (Fig 2). Similar results were seen with cartilage from a Grade 2 joint. One day after intra-articular injection in a rat knee, HB-IGF-1 was retained in articular cartilage, whereas IGF-1 was undetectable (Fig 3, Articular Cartilage). HB-IGF-1 was detectable despite stronger immunoreactivity of IGF-1 (Fig 3, Protein Standards). Neither type of IGF-1 was detected in patellar tendon extracts (Fig 3, Tendon), consistent with better delivery to the CS-rich cartilage.

Discussion: HB-IGF-1 is retained in rat knee cartilage in vivo longer than IGF-1 after intra-articular injection and is shown to bind human cartilage ex vivo. Although HB-IGF-1 does indeed bind most strongly to HS, the enhanced retention of HB-IGF-1 by cells and in cartilage appears primarily due to binding of CS, which is ~500-1000 times more abundant in cartilage than HS. Control IGF-1 is not able to bind CS or HS. Binding may be related to the amount of sulfation, with heparin > HS > CS, although specific interactions due to structural domains are likely to be involved as well based on the strong binding to HS. Taken together, this suggests that CS may act as a reservoir for HB-IGF-1 in cartilage, possibly acting as a source for later delivery to cell surface IGF-1 receptors and/or to higher affinity binding on heparan sulfate chains closer to the cell surface. Ongoing studies in vivo are focused on biosynthesis markers following intra-articular delivery. In conclusion, HB-IGF-1 may be a new therapeutic for sustained and relatively specific local delivery of IGF-1 to cartilage, precluding side effects seen with other forms of IGF-1 delivery.

Table 1. Kinetic binding constants for HB-IGF-1. * vs. CS, p<0.02. n=3.

<table>
<thead>
<tr>
<th>Protein</th>
<th>kD (1/Ms)</th>
<th>koff (s-1)</th>
<th>KD (nM)</th>
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<tr>
<td>CS</td>
<td>4.25x10^-4±1.1x10^-4</td>
<td>2.4x10^-3±2.7x10^-4</td>
<td>139±13.4</td>
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<td>HS</td>
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<td>2.75x10^-3±3.05x10^-4</td>
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<td>heparin</td>
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<td>1.41x10^-3±2.7x10^-4</td>
<td>46.8±11.2</td>
</tr>
</tbody>
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Figure 1. (A) HB-IGF-1 binding to wildtype (WT) and mutant CHO cells lacking heparan sulfate (-HS). (B) HB-IGF-1 released following enzyme treatment of cartilage explants. * vs. No enzyme, p<0.01, n=4.

Figure 2. Grade 0 human cartilage incubated with HB-IGF-1 or IGF-1.

Figure 3. Rat cartilage extracts one day post intra-articular injection.

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