Intradiscal Injection Of Simvastatin Results In Radiologic, Histologic, And Genetic Evidence Of Disc Regeneration In A Rat Model Of Degenerative Disc Disease

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Disclosures:

Introduction: Bone morphogenetic proteins (BMPs) are a group of multipotent growth factors that have been shown to stimulate chondrogenesis and production of the extracellular matrix in intervertebral discs (IVDs) [1]. We previously demonstrated that simvastatin (SIM), a commonly prescribed cholesterol lowering drug, up-regulates BMP-2 expression in mammalian cells and reverses degenerative characteristics of IVDs in a rat model of degenerative disc disease [2,3]. In this study, we expanded our previous work and aimed to further explore the potential of SIM as a disc regeneration therapy using various concentrations of the drug in two different drug delivery vehicles.

Methods: Animals. 272 Sprague-Dawley rats (3 months old) were used in this study.

Surgical procedure. Palpation was used to determine the correct Co5/Co6 disc level. A 21-gauge needle was used to induce injury at both the Co5/Co6 and Co7/Co8 levels. Fluoroscopy was used to visualize needle penetration. The needle was rotated 180 degrees and held in place for 5 seconds.

Simvastatin treatment. Six weeks after the stab injury, 2 μL simvastatin at three different doses (5, 10, 15 mg/mL) in either saline or a hydrogel carrier was injected into the injured discs using a 31-gauge needle. Rats were then assessed 2, 4, 8, 12 and 24 weeks after drug delivery with imaging, histological and molecular biological analyses.

Statistical analysis. All data are summarized as mean ± standard deviation by treatment group and follow-up time. A p-value of less than 0.05 was considered statistically significant. Statistically significant differences were determined using SPSS software.

Results: MRI. 126 rats underwent MRI (6/treatment group). Based on weeks 2 and 4 data, the time-averaged mean MRI index was significantly lower (i.e. increased degeneration) for saline alone, for 15 mg/mL SIM in saline, and hydrogel alone than that of stab controls. On the other hand, 5 mg/mL SIM in hydrogel and in saline groups showed higher time-averaged MRI indices. Long-term MRI indices were lower for saline only and for 15 mg/mL SIM (saline and hydrogel vehicle). Conversely, these values were higher for treatment with 5 mg/mL SIM in hydrogel. A representative example is shown in Figure 1.

Histology. 144 disc spaces were prepared for histological analysis. Histological grades averaged over time showed significantly higher grades (worse outcomes) than those for normal controls in the following groups: hydrogel only, 10 mg/mL and 15 mg/mL SIM in saline, and 15 mg/mL SIM in hydrogel. Of the treatment groups that were followed to week 24 (saline only, 15 mg/mL SIM in saline, 5 mg/mL SIM in hydrogel, and 15 mg/mL SIM in hydrogel), treatment with 5 mg/mL SIM in hydrogel was the only treatment that resulted in histological grades lower (better) than normal controls. Representative examples are displayed in Figure 2.

Quantitative rtPCR. Genetic expression of BMP-2 as a ratio to control is displayed in Figure 3. As expected and shown in our previous work, it was up-regulated at all doses in both saline and hydrogel carriers. Importantly, BMP-2 expression decreased with time in the saline carrier groups and increased with time in the hydrogel + 5 mg/mL SIM group. Similarly, mRNA expression of aggrecan was consistently elevated in the hydrogel + 5 mg/mL SIM group. The differentiation index (DI = collagen type II/collagen type I), which is considered to be a measure of active chondrogenesis, was calculated based on their mRNA levels. The highest DI occurred in the hydrogel + 5 mg/mL SIM group at 24 weeks. Although elevated DI was observed in the saline + SIM groups, hydrogel + SIM groups had elevated DI more consistently.

Discussion: The results support our previous finding that SIM can regenerate IVD matrix via up-regulation of BMP-2. The escalating dosage regimen revealed that high doses (10 and 15 mg/mL) of SIM may be toxic to disc cells. Importantly, the rat discs treated with 5 mg/mL SIM in the hydrogel carrier were radiographically normal through the 24-week study period and had average histologic grades better than normal controls. Quantitative rtPCR results indicate that, in this treatment group, BMP-2 and aggrecan expression gradually increased over time and the DI reached highest among other treatment groups at 24-week time point. These data suggest that the use of a hydrogel as a drug delivery carrier allows the drug to be slowly released and maybe clinically beneficial. Together, the present study demonstrated the promise of our approach to treating degenerative disc disease and warrants further investigation using large animals.
Significance:

Acknowledgments: The authors gratefully acknowledge funding support provided by the National Institutes of Health. The project described was supported by Grant Number R01 AR056649 from NIAMS/NIH.

Figure 1. Hydrogel + 5 mg/mL SIM increases MRI index at 8 weeks after treatment.

Figure 2. Simvastatin improves histological grades at 24 weeks. (A) Normal disc (B) Gel + 5 mg/mL SIM (C) Gel + 15 mg/mL SIM.