rhGDF-5 INJECTIONS CREATE REGENERATIVE EFFECTS IN RABBIT INTERVERTEBRAL DISCS EXPERIMENTALLY DEGENERATED USING CHONDROITINASE-ABC

INTRODUCTION:
Back pain is one of the most prevalent causes of disability in the world, with 85% of the U.S. population experiencing some form of back pain in their lifetime. Intervertebral disc (IVD) injuries and disorders, mainly herniated discs and degenerative discs disease, are the major contributors to back pain among the entire population. In recent years, the pathology of disc herniation and degenerative disc disease have become somewhat understood, and researchers are attempting to reverse and cure changes in the disc.

In the early 1990’s, spine researchers first began to test the effect of growth factors on intervertebral disc cells, concluding that the confined space of the intervertebral disc was ideal for such growth factor injection therapies. Since then, several studies have been published pertaining to the use of growth factor injections into both normal and degenerated intervertebral discs.

The current study uses a rabbit model to prove the efficacy of rhGDF-5 (recombinant human Growth and Differentiating Factor-5) in repairing intervertebral discs degenerated by chondroitinase-ABC (C-ABC) chemonucleolysis. C-ABC is currently used as a chemonucleolytic agent for inducing intervertebral disc degeneration by specifically degrading extracellular matrix (ECM) components such as chondroitin sulfate, dermatan sulfate, chondroitin, and hyaluronic acid. Degradation of these components leads to a decrease in the glycosaminoglycan molecules’ ability to hold water. rhGDF-5 has been shown to produce an increase in cell proliferation as well as increases in aggrecan and type II collagen gene expression specifically degrading ECM components such as aggrecan and type II collagen in vivo. We hypothesize that rhGDF-5 will impede the degeneration process caused by C-ABC, exhibited by disc height increases, MR images, and histology.

METHODS:
Thirty discs in 10 New Zealand White (NZW) rabbits were used. The experimental group of 8 rabbits received C-ABC (1.5 U/30 ml/disc) into 3 disc levels in each animal (L2/3, 3/4 and 4/5). The other 2 rabbits (6 discs) received phosphate buffered saline (PBS) solution as a control group (PBS only).

Three weeks after the C-ABC injection, 18 discs from 6 rabbits of the experimental group were given 10ml injections of rhGDF-5 (rhGDF-5 group) supplied by DePuy Biologics. The dose was randomly chosen as 100mg, 1mg, or 10ng. The remaining 2 rabbits (6 discs) in the experimental group were given 10mg injections of rhGDF-5. All animals were terminated 12 weeks after the rhGDF-5 injection date (15 weeks after the initial PBS or C-ABC injections). Weekly radiographs were taken, and the disc heights were measured starting the day of surgery. To assess changes in disc height, the Disc Height Index (DHI) system was used per the literature. DHI measurements were recorded from digital radiographs using commercially available software.

The DHI measurements from all discs taken before any injection(s) were averaged and used as the initial disc height. In subsequent weeks after injection(s), the average of the DHI’s for a given treatment group were divided by the initial average to give a %DHI of initial value. The averages of the different treatment groups were then compared to determine the effect of the different treatments. The entire measurement process was repeated one month after completing the first set of measurements to provide more accurate measurements and calculation of intraobserver error. A one-way ANOVA and Tukey’s post-hoc analysis were used for statistical significance (p<0.05 taken as significant).

Prior to sacrifice, MRI was performed using T2-weighted scans. Immediately following sacrifice, the discs were harvested and H&E histological staining was performed.

RESULTS:
The data indicates that intervertebral discs treated with rhGDF-5 demonstrate disc height recovery. The %DHI of initial for discs treated with rhGDF-5 is significantly (p<0.05) greater than %DHI of initial for discs receiving only chondroitinase-ABC. However, the rhGDF-5 treated discs were statistically (p<0.05) smaller than the PBS only discs. Figure 1 shows the %DHI of initial data for all treatment groups, including ±1 SD. No significant difference was seen between discs treated with varying dosages of rhGDF-5. After 15 weeks, the PBS injected discs remained around their initial height (104.5%). The C-ABC only discs had disc heights 54.5% of initial, while discs treated with rhGDF-5 recovered to 77.4% of initial.

DISCUSSION:
The results of our study show that rhGDF-5 injections have a regenerative effect on chemonucleolytically degenerated intervertebral discs. Increases in disc height exhibited here are comparable to other studies using growth factor injections to repair experimentally degenerated discs. MRI and some histological slides suggest that various cell types may have been signaled in the disc space. Lack of MRI signal intensity suggests that a large proliferation of annulus cells may have caused disc height increases, but poor hydration. A potential followup study would involve improved methods to better analyze the cellular material contained in the disc spaces at sacrifice.

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