INTRODUCTION: Recent progress in research on the effects of growth factors, such as insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGF-β) [1] and other members of the TGF-β superfamily [2], on intervertebral disc (IVD) cells introduced a new therapeutic approach to stimulate the anabolism of IVD cells to prevent disc degeneration or to restore a degenerated disc.

Growth and differentiation factor-5 (GDF-5), a member of the bone morphogenic protein (BMP) family, was originally found to be a factor responsible for skeletal alterations in brachypodism mice [3]. Recently, an analysis of GDF-5-deficient mice revealed the presence of disc degeneration as well as a loss of proteoglycan from the IVD. GDF-5 also stimulates PG and type II collagen expression in mouse IVD cells [4]. It is important to know whether GDF-5 stimulates extracellular matrix production and accumulation using cells that are phenotypically close to human IVD cells. Based on cell biology, isolating IVD cells from bovine tails and culturing those cells in alginate beads is considered to be a reasonable method to conduct such a study [5].

In order to explore the possibility for the in vivo application of recombinant human GDF-5 (rhGDF-5), this study was performed to determine if rhGDF-5 is effective in promoting the extracellular matrix metabolism of bovine nucleus pulposus (NP) and annulus fibrosus (AF) cells cultured in alginate beads.

METHODS: NP and AF cells from bovine tail IVDs (14- to 18-month-old) were enzymatically isolated and separately suspended in 1.2% low viscosity alginate beads. After one week of culture in complete medium (DMEM/F12 + 10% FBS), the cells were cultured for another 21 days in complete medium containing rhGDF-5 at 0 (Control), 100 or 200 ng/ml. On days 7, 14 and 21 after rhGDF-5 treatment, the contents of DNA and proteoglycan (PG), and the rate of PG (35S-sulfate uptake) and collagen synthesis (3H-proline uptake after peptic digestion) were assessed.

DNA Content: At various time points, the content of DNA in alginate beads was measured using the Hoechst 33258 dye method [6].

PG Synthesis: The rate of PG synthesis was assessed by adding 35S-sulfate (20 μCi/ml) to each medium during the last four hours of culture. After removing the medium in each case, the beads were dissolved and the two compartments (cell-associated matrix (CM) and further removed matrix (FRM)) were separated by mild centrifugation. Radiolabeled PGs were quantified by a rapid filtration assay [7].

Collagen Synthesis: The rate of collagen synthesis was assessed during the last 16 hours of culture by adding L-[2,3,4-3H]-proline (50 μCi/ml) to each medium. The beads were dissolved and the two compartments separated as described above. The collagen in the CM, FRM and medium were extracted with 0.5% acetic acid including peptic (100 μg/ml) digestion. The radiolabeled peptin-resistant protein, as a measure of collagen synthesis, was measured by rapid filtration assay after 25% trichloroacetic acid (TCA) precipitation.

PG Accumulation: The total PG content in each CM and FRM compartment was assessed using the DMBM method after papain digestion [6].

Statistical Analysis: The effects of culture period and treatment were analyzed by one-way ANOVA with Fisher LSD test as a post hoc test.

RESULTS:

DNA Content: The DNA content was significantly higher in both rhGDF-5-treated alginate beads than in the control beads at the 14- and 21-day time points in beads containing NP cells and only at the 21-day time points in beads containing AF cells. [NP (169% of control): p<0.01, AF (121%): p<0.05, 200 ng/ml, day 21].

PG Synthesis: rhGDF-5 at both concentrations significantly stimulated PG synthesis by NP cells at all time points but by AF cells at the 21-day time point [NP (238%), AF (124%), 200 ng/ml, day 21, p<0.01] (Fig. 1). NP cells with a long exposure to rhGDF-5 showed a greater response than AF cells (Fig. 1).

Collagen Synthesis: The rate of collagen synthesis was also significantly higher in both rhGDF-5-treated beads than in the control beads at all time points [NP (194%), AF (122%), 200 ng/ml, day 21, p<0.01] (Fig. 2). The effects of rhGDF-5 on collagen synthesis by NP and AF cells were generally similar to those on PG synthesis.

DISCUSSION: The results of this study showed that rhGDF-5 enhanced the cell proliferation and matrix synthesis and accumulation by both bovine NP and AF cells. The response to rhGDF-5 was greater by NP cells than by AF cells. While a longer exposure to rhGDF-5 induced a greater response in both cell types, a clear dose-dependency was not observed under the conditions tested. We have shown that another member of the TGF-β superfamily, osteogenic protein-1 (OP-1), stimulates matrix metabolism in a similar fashion using both rabbit and bovine IVD cells. OP-1 also induced a recovery of disc height after injection into the NP in a rabbit disc degeneration model [8]. Therefore, stimulation of the anabolic cascade by rhGDF-5 may prove useful as a therapeutic approach in delaying the progression of disc degeneration or in promoting repair of the degenerating human IVD. Further research on human IVD cells, including those cells from degenerated tissue, are critical before considering a human clinical study.


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