RECOMBINANT HUMAN OSTEOGENIC PROTEIN-1 UPREGULATES EXTRACELLULAR MATRIX METABOLISM BY HUMAN ANNULUS FIBROSUS AND NUCLEUS PULPOSUS CELLS

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INTRODUCTION: Degeneration of intervertebral discs (IVDs) is a major cause of low back pain, a condition whose incidence increases with aging. The changes, which begin as early as the mid-teens [1], often involve a progressive decrease in proteoglycan (PG) content with subsequent dehydration of the nucleus pulposus (NP). These biochemical changes are thought to reflect an imbalance between the synthesis of PG and collagen molecules by NP and annulus fibrosus (AF) cells and their degradation in the extracellular matrix.

Osteogenic protein-1 (OP-1), also known as bone morphogenetic protein-7, is a member of the transforming growth factor-β superfamily capable of stimulating matrix synthesis by human chondrocytes [2]. Recombinant human OP-1 (rhOP-1) was recently shown to enhance repair of a chondral defect in sheep [3]. It was also found to stimulate PG and collagen synthesis by rabbit and bovine NP and AF cells [3, 4]. Furthermore, a recent report in rabbits suggested that it may be applied intradiscally for the treatment of disc degeneration [5]. Importantly, nothing is known about the effects of OP-1 on adult human IVD cells that are metabolically less active than those in the intervertebral disc.

The purpose of this study was to examine the effect of OP-1 on cell proliferation and PG synthesis by human NP and AF cells cultured in alginate gel.

MATERIALS AND METHODS:

Cell Preparation: Human lumbar IVDs were obtained from an adult patient under IRB approval who underwent anterior lumbar discectomy and fusion for scoliosis. The grade of degeneration was assessed as grade 1-2 by MRI based on the Thompson scale. The NP and AF were separated by blunt dissection and separately pooled. Cells were enzymatically released from each tissue by digestion with 0.4% pronase for 1 h and then with 0.025% collagenase P and 0.004% deoxyribonuclease II for 16 h. The isolated cells were encapsulated in 1.2% low-viscosity alginate at 2 million cells/ml as previously described [6] and cultured in complete media, DMEM/F12 supplemented with 10% FBS and 25 µg/ml ascorbic acid. The medium was changed daily.

Cell Culture: After 1 week of culture in complete medium, the cells were cultured for another 21 days in medium containing rhOP-1 (a gift from Stryker Biotech) at 0 (Control), 100 or 200 ng/ml. The medium was changed daily.

DNA Content: The content of DNA in alginate beads was measured using the Hoechst dye method and fluorometry [7].

PG Synthesis and Accumulation: The rate of PG synthesis in the presence and/or absence of OP-1 was assessed over the last 4 hours of culture on days 3, 7, 14 and 21 by adding 35S-sulfate (at a final concentration of 20 µCi/ml) to each medium. 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 [6]. The radiolabeled PGs were measured by a rapid filtration assay [8]. Total PG content in the beads was also assessed using the DMMB method after papain digestion [6].

RESULTS:

DNA Content: Initially, rhOP-1 did not have a significant effect on cell proliferation: in both cell type; the DNA content on day 14 was similar in beads cultured with or without rhOP-1. However, at later time points, i.e. on day 21, rhOP-1 caused a significant increase in the DNA content of the AF (100 ng: p < 0.01, 200 ng: p < 0.01 vs. Control).

PG Synthesis: In the control group, NP cells produced several times more PG at each time point than AF cells (3.3, 2.6, 1.7 and 4.3 fold on days 3, 7, 14 and 21 respectively). During the first week of treatment, rhOP-1 did not have a significant effect on PG synthesis. However, rhOP-1 significantly stimulated PG synthesis both on days 14 and 21 by both AF and NP cells (Fig. 1, day 14 [data now shown in Fig.]), NP: p < 0.05, AF: p < 0.05; day 21, NP: p < 0.01, AF: p < 0.01). The response to OP-1 at each time point was more pronounced in the AF than NP (Fig. 1, +rhOP-1 100 ng/ml; NP: 168.9%, AF: 264.1%; +rhOP-1 200 ng/ml; NP: 194.5%, AF: 252.4%. on day 21). The data expressed per µg DNA showed essentially the same results.

Matrix Accumulation: The addition of rhOP-1 at 200 ng/ml to the medium resulted in a marked increase in the content of PG in both the NP and AF beads (p < 0.01) (Fig. 2). A similar but less pronounced effect was observed at 100 ng/ml OP-1. This increase was minor during the first week, but dramatic after the second week of culture with rhOP-1.

DISCUSSION: In human IVDs the content of PG in the NP decreases with age and drops rapidly in degenerative disc disease [9]. Although cells in those individuals might be impaired, one cannot rule out the possibility that an intradiscal injection of growth factor, given early, would help induce regeneration of a degenerating IVD. The results of this study showed, for the first time, that rhOP-1 does enhance the production of PG by human NP and AF cells. Interestingly, AF cells that are more fibrochondrocytic, strongly responded to OP-1, suggesting that OP-1 might be beneficial not only for nucleus repair but for annulus repair as well. The intradiscal injection might promote biological repair or help prevent further degradation of not only NP, but also AF. The results presented here suggest that rhOP-1 could prove most useful as a therapeutic agent in promoting synthesis and repair of the matrix of both the AF and NP elements of degenerating human IVDs.


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