Biological repair of the degenerated intervertebral disc by the injection of growth factors

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Abstract The homeostasis of intervertebral disc (IVD) tissues is accomplished through a complex and precise coordination of a variety of substances, including cytokines, growth factors, enzymes and enzyme inhibitors. Recent biological therapeutic strategies for disc degeneration have included attempts to up-regulate the production of key matrix proteins or to down-regulate the catabolic events induced by pro-inflammatory cytokines. Several approaches to deliver these therapeutic biologic agents have been proposed and tested in a preclinical setting. One of the most advanced biological therapeutic approaches to regenerate or repair a degenerated disc is the injection of a recombinant growth factor. Abundant evidence for the efficacy of growth factor injection therapy for the treatment of IVD degeneration can be found in preclinical animal studies. Recent data obtained from animal studies on changes in cytokine expression following growth factor injection illustrate the great potential for patients with chronic discogenic low back pain. The first clinical trial for growth factor injection has been initiated and the results of that study may prove the usefulness of growth factor injection for treating the symptoms of patients with degenerative disc diseases. The focus of this review article is the effects of an in vivo injection of growth factors on the biological repair of the degenerated intervertebral disc in animal models. The effects of growth factor injection on the symptoms of patients with low back pain, the therapeutic target of growth factor injection and the limitations

of the efficacy of growth factor therapy are also reviewed. Further quantitative studies on the effect of growth factor injection on pain generation and the long term effects on the endplate and cell survival after an injection using large animals are needed. An international academic-industrial consortium addressing these aims, such as was achieved for osteoarthritis (The Osteoarthritis Initiative), may further the development of biological therapies for degenerative disc diseases.

Keywords Growth factor · Intervertebral disc · Disc degeneration · Biological repair

Introduction

The homeostasis of intervertebral disc (IVD) tissues is biologically regulated by the active maintenance of a balance between the anabolism and catabolism of disc cells. This is accomplished through a complex and precise coordination of a variety of substances, including cytokines, growth factors, enzymes and enzyme inhibitors, in a paracrine or/and autocrine fashion [1, 2]. Recent therapeutic strategies for disc degeneration have included attempts to up-regulate the production of key matrix proteins (e.g., aggrecan), or to down-regulate the catabolic events induced by the pro-inflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) [3–10].

Several approaches to deliver these therapeutic biologic agents, including protein injection and viral or non-viral gene transfer, have been proposed and tested in a preclinical setting [2, 11–13]. The most straightforward approach to regenerate or repair a degenerated IVD is the injection of anabolic factors; however, the half-life and solubility of the

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factors, the proper carrier, the presence of inhibitors, etc., are issues that need to be taken into consideration [13].

One of the most advanced biological therapeutic approaches to regenerate or repair a degenerated disc is the injection of a recombinant growth factor [14]. However, clinical studies need to confirm the efficacy and duration of action of these molecules and the possibility of adverse effects need to be addressed. The first investigational new drug study to test the safety and effect of osteogenic protein-1 [OP-1; otherwise known as bone morphogenetic protein-7 (BMP-7)] is underway.

Effects of growth factor on IVD cells

The stimulation of matrix synthesis by cytokines or growth factors alters IVD homeostasis by shifting cellular metabolism to the anabolic state [1]. This was shown for the first time by Thompson et al., who demonstrated that the rate of synthesis of proteoglycans (PGs) by IVD cells increases several-fold following the addition of transforming growth factor- β (TGF- β) and epidermal growth factor (EGF) [15, 16]. Insulin-like growth factor-1 (IGF-1) also stimulates IVD cell proliferation and matrix synthesis in vitro [17]. Using three-dimensional culture, Gruber et al. first showed that TGF- β stimulates the cell proliferation of human annulus fibrosus (AF) cells after 4 days exposure [16]. In another study, Gruber et al. reported that IGF-1 and platelet-derived growth factor (PDGF) were both able to significantly reduce the percentage of apoptotic AF cells induced by serum depletion in culture [18].

Members of the BMP family, OP-1 [19] and BMP-2 [20], have both been found to enhance the PG metabolism of IVD cells. OP-1 strongly stimulates the production and formation of the extracellular matrix by rabbit IVD cells [19], as well as by human IVD cells in vitro [21]. OP-1 was also found to be effective in the replenishment of a matrix rich in PGs and collagens after depletion of the extracellular matrix following exposure of IVD cells to IL-1 [22] or chondroitinase ABC [23]. Kim et al. reported that BMP-2 facilitates the expression of the chondrogenic phenotype by human IVD cells, increases PG synthesis and up-regulates the expression of aggrecan, collagen type I, and collagen type II mRNA, compared to untreated control levels [24]. Both rhBMP-2 and -12 increased human nucleus pulposus (NP) cell PG and collagen synthesis while having minimal effects on AF cells [25]. The authors suggested that BMP-12, which does not have the strong osteogenic potential of BMP-2, may be more suitable for use to induce disc repair.

Growth and differentiation factor-5 (GDF-5), another member of the BMP family, was also found to stimulate PG and type II collagen expression in mouse IVD cells [26]. Furthermore, recombinant human GDF-5 (rhGDF-5)

enhances cell proliferation and matrix synthesis and accumulation by both bovine NP and AF cells [27].

Clinically, the use of autologous growth factors to treat degenerative disc disease may be advantageous by avoiding regulatory complications. Wehling showed that the combination of autologous IL-1 receptor antagonist (IL-1ra), IGF-1 and PDGF proteins reduced the percentage of apoptosis and the production of IL-1 and IL-6 [28]. Platelet-rich plasma (PRP), which is a fraction of plasma that can be produced by centrifugal separation of whole blood in the operating room, contains multiple growth factors concentrated at high levels. PRP has been shown to be an effective stimulator of cell proliferation and PG and collagen synthesis, as well as PG accumulation, by porcine NP and AF cells cultured in alginate beads [29]. Chen et al. also reported that PRP induced human NP cell proliferation and differentiation, and also promoted tissue-engineered NP formation [30].

Growth factor injection: animal studies

Walsh et al. reported the in vivo effects of a single injection of several growth factors, including basic fibroblast growth factor (bFGF), GDF-5, IGF-1 or TGF- β , in the mouse caudal disc with degeneration induced by static compression [31]. These authors found that an injection of GDF-5 induced the expansion of the inner AF fibrochondrocytic population into the NP, while IGF-1 showed only a transient proliferative effect. They also reported that multiple injections of TGF- β induced fibrochondrocyte aggregation in the NP and that the disc height was increased in discs treated with GDF-5.

In the normal rabbit, a single intradiscal administration of OP-1 in vivo resulted in increased disc height and an increased PG content of the NP, neither of which was seen in the control saline injection group [32]. In an adolescent rabbit model of disc degeneration, an injection of recombinant human OP-1 (rhOP-1, 100 μ g/disc) restored the decrease in disc height and degenerative changes caused by an annular needle puncture [14]. As shown in Fig. 1a, the injection of OP-1, but not lactose, resulted in the restoration of disc height at 6 weeks; this restoration was sustained for the entire experimental period up to 24 weeks after the injection. The results of the MRI grading score showed significant differences between the OP-1- and the lactose-injected groups at the 8-, 12- and 24-week time points; this suggests an increased water content of the NP in the OP-1 group (Fig. 1b). The results clearly demonstrate the feasibility of restoring degenerative rabbit discs by a single injection of OP-1 into the NP. Using a newly developed dynamic viscoelastic property testing system, an intradiscal injection of OP-1 restored the biomechanical

Fig. 1 Changes in the intervertebral disc height index (DHI, **a**) and magnetic resonance imaging (MRI) grading (**b**) after anular puncture and osteogenic protein-1 (OP-1) injection (Modified from Masuda K et al. Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit anular puncture model. *Spine* 2006;31(7):742–754). Adolescent New Zealand White rabbits (3–3.5 kg) received an anular puncture with an 18G needle to induce disc degeneration. Four weeks later, either 5% lactose (vehicle control; 10 μ l) or osteogenic protein-1 (OP-1; 100 μ g in 10 μ l 5% lactose) was injected into the center of the nucleus pulposus (NP). By 4 weeks after the OP-1 injection, the mean %DHI of injected discs in the OP-1 group was significantly higher than that in the lactose control group ($P < 0.001$, repeated ANOVA). This significant difference in mean %DHI was maintained during the follow-up period ($P < 0.001$). MRI examinations were performed on all the spinal columns isolated from the rabbits ex vivo at the time of killing. When the degree of disc degeneration by MRI grading was compared between the lactose and OP-1 groups, a significant lower grading of MRI in the OP-1 group was observed ($P < 0.01$, Mann-Whitney U test)

properties of degenerated IVDs in the same animal model (Fig. 2) [33]. Four weeks after an injection, at all loading frequencies, the elastic modulus (E') in the OP-1 group was significantly higher than that in the lactose group and approached that of the non-punctured control disc. Similar trends were observed in the results for the viscous modulus (E''). The positive correlation between the PG content in the NP and the elastic modulus of the IVD suggests that

Fig. 2 Effects of treatment with osteogenic protein-1 (OP-1) or lactose on the dynamic viscoelastic properties and the biochemical properties of rabbit intervertebral discs (IVDs) (Miyamoto K et al. Intradiscal injections of osteogenic protein-1 restore the viscoelastic properties of degenerated intervertebral discs. *Spine* J 2006;6(6):692–703). After killing and removal of bone-disc-bone complexes 8 weeks post-injection, the dynamic viscoelastic properties of the IVDs were tested by applying a cycle of sinusoidal strain in uniaxial compression at six loading frequencies (0.05–2 Hz). In the OP-1-injected discs (closed circles), at all loading frequencies, the elastic modulus (E') was significantly higher than that in the lactose-injected discs and approached that of the non-punctured control discs (open circle) (OP-1 vs. non-punctured control, n.s., upper graph). The viscous modulus (E'') was significantly higher in the OP-1-injected discs (closed circle) than that in the lactose-injected discs (reverse triangle) (middle graph). No significant difference was observed in the loss tangent (E''/E') among the non-punctured control and the rhOP-1-injected and lactose-injected discs

biochemical changes induced by an injection of OP-1 may result in structural and mechanical restoration (Table 1).

The effect of OP-1 has also been validated in a chondroitinase-induced chemonucleolysis model in rabbits. Chondroitinase ABC (C-ABC), which has a narrow substrate spectrum and is without protease activity, has been suggested as an alternative to chymopapain for chemonucleolysis [34–41]. Although matrix regeneration in IVDs

Table 1 The in vivo effect of growth factors

Factor	Species	Model	Dose	Effect	Reference
IGF-1	Rat	Compression model	8 ng/8 μ l/disc	Clustering of inner anulus cells (single injection)	[69]
GDF-5	Rat	Compression model	8 ng/8 μ l disc	Clustering of cells, increase in disc height (single injection)	[69]
TGF- β	Rat	Compression model	1.6 ng/8 μ l disc	Proliferation of cells (multiple injections)	[69]
bFGF	Rat	Compression model	8 ng/8 μ l/disc	No response	[69]
OP-1	Rabbit	Normal	2 μ g/10 μ l/disc	Increase in disc height, initial PG content in the NP $\uparrow$	[32]
OP-1	Rabbit	C-ABC—Co-injection	100 μ g/10 μ l/disc	Increase in disc height, PG content in the NP	[70]
OP-1	Rabbit	Needle puncture	100 μ g/10 μ l/disc	Increase in disc height, improvement of MRI and histology grading scores, PG content in the NP and AF $\uparrow$ improvement of MRI and histology grading scores	[14]
GDF-5	Rabbit	Needle puncture	1 ng, 100 ng, 1 μ g, 100 μ g/10 μ l/disc	Increase in disc height, improvement of MRI and histology grading scores	[27]
OP-1	Rabbit	Needle puncture	100 μ g/10 μ l/disc	Increase in disc height, viscoelastic properties $\uparrow$	[33]
OP-1	Rabbit	After chemonucleolysis Degeneration by C-ABC—4 week after	100 μ g/10 μ l/disc	Increase in disc height, PG content in the NP and AF $\uparrow$	[47]
BMP-2	Rabbit	Anular tear (5 $\times$ 7 mm)	100 μ g/100 μ l/disc	More degeneration, vascularity and fibroblasts $\uparrow$	[54]
PRP	Rabbit	Nucleotomy	20 μ l PRP + Micro-sphere/disc or 5 μ l PRP + 15 μ l PBS	PRP + gelatin hydrogel microspheres Less degeneration, more PG PRP only, no effect	[55]

IGF-1 insulin-like growth factor; *GDF-5* growth differentiation factor 5; *TGF- β* transforming growth factor- β , *bFGF* basic fibroblast growth factor, *OP-1* osteogenic protein-1; *BMP-2* bone morphogenetic protein-2; *PRP* platelet-rich plasma; *C-ABC* chondroitinase-ABC

treated with C-ABC occurs earlier and to a greater extent than in those treated with chymopapain, disc height and PG content, as well as the biomechanical properties of the disc, do not fully return to normal [42]. The use of C-ABC has even been proposed as an animal model of disc degeneration in the rat tail [43, 44] and the goat disc [45, 46]. Considering the stimulatory effect on matrix production by OP-1 and the absence of protease activity in C-ABC to degrade the injected growth factor, an injection of rhOP-1 after chemonucleolysis with C-ABC may be useful to counteract the degradative effects of the enzyme and to induce the restoration of the IVD structure. In adolescent rabbits, C-ABC (10 mU) was first injected into IVDs to induce chemonucleolytic effects [47]. Four weeks following the injection of C-ABC, OP-1 (100 μ g/disc) or vehicle was injected and the disc height was measured for up to 12 weeks after the OP-1 injection. Although the disc height after the injection of C-ABC was significantly decreased (approximately 34%), the OP-1 injection induced a recovery of the disc height towards normal within 4 weeks after the rhOP-1 injection and gradually approached the

control level by 6 weeks (Fig. 3); this change was sustained for up to 16 weeks.

The effectiveness of this approach was confirmed in another study using rhGDF-5. The injection of rhGDF-5 (1 or 100 μ g per disc) into degenerated discs in the adolescent rabbit anular puncture model resulted in a restoration of disc height and improvements in MRI and histological grading scores (Fig. 4a) [27].

Normal discs from the adolescent rabbits (5–6 months) used in these studies still have a large number of notochordal cells [48, 49]. Although most notochordal cells in the NP were replaced with fibrochondrocytes after the needle puncture, the difference in cell populations needs to be taken into consideration when being used for preclinical information for human clinical trials. Yoon et al. have shown an age-related decrease in PG and water content and increased degenerative signs in sagittal MRI scans in their cross-sectional study using 1–4-year-old rabbits [50]. One can speculate that mature or older rabbits also have less diffusion through the endplate because of decreased vascularity, compared to adolescent animals. Therefore, the

Fig. 3 Effect of osteogenic protein-1 (OP-1) injection on the disc height after chemonucleolysis by chondroitinase-ABC (C-ABC) (With permission from Imai Y et al. Restoration of disc height loss by recombinant human osteogenic protein-1 injection into intervertebral discs undergoing degeneration induced by an intradiscal injection of chondroitinase ABC. Spine 2007;32:1197–205). Rabbit lumbar intervertebral discs (L2–5) were exposed through a posterolateral retroperitoneal approach and chondroitinase ABC (C-ABC) at 10 mU in 10 μ l of saline was injected into the center of the nucleus pulposus of three consecutive intervertebral discs (L2/3, 3/4, 4/5). A significant decrease of disc height in the lateral radiograph was observed at 4 weeks. Four weeks after the initial injection of C-ABC, the vehicle (lactose, 10 μ l) or rhOP-1 in lactose (100 μ g/10 μ l at each level) was injected. By 6 weeks after the rhOP-1 injection, the mean percent disc height index (%DHI) of the injected discs in the C-ABC/rhOP-1 group was significantly higher than in the C-ABC/lactose group ($P < 0.01$). This significant difference in mean %DHI was maintained during the follow-up period ($P < 0.01$)

possibility exists that the repair of the degenerated disc is age-dependent and that mature rabbits will not restore disc structure by growth factor injection as demonstrated in the young animals described above.

A study using 2-year-old rabbits showed that the intradiscal injection of rhGDF-5 induced a significant recovery of disc height during a 12-week observation period. The response to the GDF-5 injection was faster than that seen in the adolescent rabbits (Fig. 4b) [51]. Two weeks after the injection, the percent disc height index (%DHI) in the rhGDF-5-injected discs was significantly higher than that in the PBS-injected discs. By 8 weeks post-injection, the rhGDF-5-injected discs reached the DHI level of the non-punctured control discs. At 12 weeks after the injection, the MRI analysis revealed that the signal intensity in the NP of the rhGDF-5-injected discs was significantly higher than that of the PBS-injected discs. Biomechanical analyses indicated that the viscous and elastic moduli of the IVDs in the rhGDF-5-injected discs were significantly higher than those in the PBS-injected discs. These results indicated the effectiveness of an injection of rhGDF-5 in restoring the degenerative disc in the 2-year-old rabbit anular-puncture disc degeneration model, despite the

Fig. 4 Changes in the intervertebral disc (IVD) height index (DHI) after anular puncture and recombinant human growth and differentiation factor-5 (rhGDF-5) injection (Modified from Chujo T et al. Effects of growth differentiation factor-5 on the intervertebral disc- in vitro bovine study and in vivo rabbit disc degeneration model study. Spine 2006;31(25):2909–2917 and Chujo T et al. In vivo effects of recombinant human growth and differentiation factor-5 on the repair of the mature rabbit intervertebral disc. Spine J, 6(5):23S–24S, 2006 (North American Spine Society, 21st Annual Meeting Proceeding, abstract). **a** Four weeks after inducing disc degeneration by an anular needle puncture in New Zealand White rabbits (weighing 3.5–4 kg), the rabbits received an injection of phosphate buffered saline (PBS) (10 μ l) or rhGDF-5 (10 ng, 1 or 100 μ g, in 10 μ l PBS) and were monitored radiographically for up to 12 weeks after the injections. After killing, the specimens were assessed by magnetic resonance imaging (MRI) and histology. By 4 weeks after the rhGDF-5 injection, the mean %DHI of injected discs in the rhGDF-5 group (100 μ g) was significantly higher than in the PBS group ($P < 0.05$). After 12 weeks, the rhGDF-5 groups at two concentrations (1 and 100 μ g) demonstrated a significantly higher %DHI than the control group ($P < 0.01$). **b** In mature rabbits (2-year-old), 2 weeks after the injection, the %DHI for the rhGDF-5-injected discs was already significantly higher than for the PBS-injected discs ($P < 0.001$); this difference was sustained throughout the 12-week observation period. By 8 weeks post-injection, the rhGDF-5-injected discs achieved the DHI level of the non-punctured control discs

concern that age-related changes in cell activity might affect the efficacy of growth factor injection therapy (Fig. 4a, b).

Several possible mechanisms for the long term effects of growth factor injection warrant further investigation. First, the half-life of an injected protein in discs has been

considered to be short, in the order of minutes [52]. However, a recent collaboration with Stryker Biotech (Hopkinton, MA) revealed that the half-life of injected radiolabeled OP-1 was greater than the 1-month observation period (personal communication). A possible explanation for this includes the fact that the NP, as an injection site, is a confined space because of the surrounding AF. In those patients whose discograms do not reveal a clear leakage, the injected growth factor may be retained in the disc space after an injection. Second, rhOP-1 binds to collagen molecules in the NP and AF; this mechanism may be one explanation for its long acting effects [53]. Finally, the metabolic changes found in the cells following a single injection of a growth factor might be sustained and thus could induce long-term changes in disc structure.

However, the results from the use of other growth factors have shown some discrepancies with the above studies. Huang et al. injected saline (100 μ l) or BMP-2 alone or BMP-2 with coral grafts, immediately after a full-thickness annular tear (5 mm $\times$ 7 mm), in rabbit discs [54]. Interestingly, radiography revealed that degenerative changes were more frequent and severe in the animals treated with rhBMP-2 with or without the use of coral. Histological analyses also indicated that rhBMP-2 promoted hypervascularity and fibroblast proliferation of the intervertebral disc after an annular tear. These discrepancies may be due to a few factors. First, the annular tear model is acute and the application of BMP-2 was performed during an acute phase. Second, the injection volume was 100 μ l, while other groups used 10–20 μ l for injecting the rabbit disc; thus the injected material may have leaked out from the tear. It may be useful to compare the efficacy of BMP-2 under the same conditions used in the OP-1 and GDF-5 studies.

Nagae et al. also reported an interesting finding on the effects of PRP in the rabbit disc nucleotomy model [55]. Allograft PRP was injected into the NP of degenerated IVDs after impregnation into gelatin hydrogel microspheres to provide a slow release of the biological factors found in PRP, while other discs received PRP only or PBS. Gelatin hydrogel microspheres immobilize PRP growth factors physicochemically and release them in a sustained manner with the degradation of the microspheres. The progression of disc degeneration was remarkably suppressed in the group injected with PRP-microspheres, compared to the PBS and PRP only groups. The need for a sustained delivery system for growth factor injection may depend on the kind of growth factor injected, as Walsh also indicated [31]. In addition, when the defect in the NP is associated with its pathogenesis, such as post-discectomy, the use of such a scaffold or delivery system may provide a benefit to patients.

Does growth factor injection affect symptoms of pain in patients with low back pain?

Disc degeneration can be asymptomatic or symptomatic. The target of a biological therapy is a patient who has significant pain. Preclinical data from in vivo rat and rabbit experiments have provided evidence that an injection of growth factor can be effective in restoring structure, and can thus be used as a “structural modifying therapy.” However, more work needs to be done to prove that this approach is a “symptom-modifying therapy” capable of improving the pain symptoms that are associated with pathological changes. One hurdle to this attempt is the lack of an appropriate quantitative assessment of pain in rabbits or larger animals.

In rat models, some studies have indicated that the injection of a growth factor may reduce pain. Kawakami et al. applied intradiscal injections of OP-1 into biomechanically compressed discs and evaluated whether disc degeneration was reversed (Fig. 5) [56]. This study also evaluated hyperalgesia when the disc tissue was applied to lumbar nerve roots. Mechanical hyperalgesia was observed in the sham- and saline-injected groups, but not in the OP-1-treated group. Histologically, the content of the extracellular matrix was markedly increased. The results of the study indicated that an OP-1 injection into degenerative intervertebral discs resulted in an increased extracellular matrix and the inhibition of pain-related behavior. Although the rat NP compression model is not a discogenic pain model, but rather a radiculopathy model, the results suggested that changes induced by an injection of OP-1 may influence the biochemical properties of disc tissues and induce some pain relief. In a subsequent study, histological analyses of compressed and OP-1-injected discs revealed that there was reduced staining for aggrecanase, matrix metalloproteinase-13 (MMP-13), substance P, TNF- α and IL-1 β in the OP-1 treated discs [57]. In the rabbit annular puncture model, the injection of OP-1 also suppressed cytokine expression (IL-1 β , IL-6 and TNF- α) by NP and AF tissues (Fig. 6) [58]. Because these pro-inflammatory factors can induce a variety of pain markers, such as nerve growth factor, one can hypothesize that this therapeutic approach may have an effect on pain generation.

However, it remained to be determined if these changes are through the direct effect of OP-1 itself, or through a secondary effect from changes in biomechanical properties induced by OP-1. To investigate the direct effect of OP-1 on cytokine and matrix-degrading enzyme expression, the effects of OP-1 on the expression of IL-1 β , TNF- α , IL-6, MMP-3 and aggrecanase-1 by human NP and AF cells were investigated. The results of this study showed that OP-1 suppressed the IL-1-induced up-regulation of

Fig. 5 Effects of an intradiscal injection of OP-1 on the hyperalgesia induced by an application of the degenerative nucleus pulposus on the nerve root (With permission, Kawakami M et al. Osteogenic protein-1 (osteogenic protein-1/ bone morphogenetic protein-7) inhibits degeneration and pain-related behavior induced by chronically compressed nucleus pulposus in the rat. *Spine* 2005;30:1933–9). **a** To degenerate discs, the apparatus was used to chronically apply compression to the tail. Four weeks after surgery, some discs received OP-1 injection (0.2 $\mu\text{g}/1 \mu\text{l}$) and either continuous compression was applied (COP-1) or the compression was released (ROP-1). The sham group received the initial surgery and was kept without compression. The saline group received the compression treatment and at 4 weeks, the disc received a saline injection. **b** Rats in the saline group showed evidence of mechanical hyperalgesia in the ipsilateral hind paws from 2 days to 2 weeks after surgery. In rats in the COP-1 and ROP-1 groups, which were treated with intradiscal injection of OP-1, mechanical hyperalgesia was not observed

cytokine and catabolic enzyme mRNA expression by AF and NP cells [59]. These results suggest that OP-1 is not only an anabolic regulator, but also a catabolic regulator of the metabolism of IVD cells. In addition, the inhibition of aggrecanase may suggest that OP-1 may be able to delay the progression of disc degeneration.

Therapeutic target of growth factor injection

Although the clinical application of growth factor injection therapy to treat degenerative disc disease has been initiated, several important considerations need to be taken into account. First, the target population of this therapeutic approach is mainly an aged population, which has fewer cells in the IVD. It is also well known that the cell number is decreased in discs in an advanced stage of degeneration [60]. Without functional cells, an injection of growth factor

will not achieve a therapeutic effect. Therefore, for this type of biological therapy the appropriate stage of disc degeneration and age of the patients need to be defined. Alternatively, it may be possible that the transplantation of functional cells, such as autologous NP cells, which may be recovered from herniated tissues or mesenchymal stem cells, may be required [61–65].

Second, in an advanced stage of disc degeneration, the nutritional supply and removal of metabolic wastes are compromised by changes in the endplate, such as those caused by sclerosis. The importance of nutritional factors in the pathogenesis of disc disease is well recognized [66]. When growth factor injection therapy or cellular therapy is applied under such conditions, the increased demand for energy may negatively affect cell viability. To further the development of novel biologic therapies, an improvement in diagnostic methodologies to evaluate the environmental conditions in the IVD is essential. Rajasekaran et al. have

Fig. 6 Intradiscal injections of recombinant human bone morphogenetic protein-7 (BMP-7/OP-1) significantly suppressed the mRNA expression of cytokines and catabolic enzymes in the rabbit anular puncture model (With permission, Pichika R et al. Intradiscal injection of recombinant human bone morphogenetic protein-7 significantly suppressed the expression of cytokines and catabolic enzymes in the rabbit anular puncture model. Ortho Res Soc Trans 33:1389, 2008). Four weeks after the anular puncture, the rabbits received an injection of phosphate buffered saline (10 μ l, PBS group, $n = 12$ discs) or rhBMP-7 (100 μ g, in 10 μ l PBS, BMP-7 group) into the center of the NP. At the 12-week time point after the injection, AF and NP tissues were isolated and subjected to the quantitative PCR using the gene-specific primers for IL-1 β , TNF- α , IL-6, ADAMTS4 and ADAMTS5. A single injection of rhBMP-7 induced a significantly decreased expression of cytokines (IL-1 β , IL-6 and TNF- α , **a**) and ADAMTS4 mRNA (**b**)

reported a method to monitor the diffusion status of the endplate using contrast-enhanced MRI; this technique may help to define the indications for growth factor injection by revealing the in vivo conditions of the degenerated disc [67, 68].

Limitations of the current evidence on the effectiveness of growth factor therapy

Although the results described above showed some evidence of structural modifications in the rabbit disc

degeneration model, there are significant limitations to applying the results to the human situation. Studies using large animals, which have discs of a size similar to those of the human and do not contain notochordal cells, may be required to answer the remaining issues on nutritional supply. These efforts were hampered by a lack of appropriate animal models that give a quantitative measure of structural modification. The cost of studies using large animals and the ethical problems associated with these animals are other factors that delay this research. In addition, no clear data will be available for the effects of growth factor injection on pain relief using current techniques. Therefore, the development of quantitative large animal models and methodologies to assess pain in these animals are necessary to support further development in this area.

If, however, growth factor injection therapy is proven to be safe for other indications, (e.g., stimulation of bone formation), the judicious intradiscal application of growth factor therapy in a limited number of patients, who would otherwise undergo spinal fusion, may be one approach to obtain efficacy results in the human.

Conclusion

Abundant evidence for the efficacy of growth factor injection therapy for the treatment of IVD degeneration can be found in preclinical animal studies. This is especially true in the case of structural modification, however, the effects of growth factor injection on pain generation are not well known. Recent data obtained from animal studies on changes in cytokine expression following growth factor injection illustrate the great potential for patients with chronic discogenic low back pain. The first clinical trial for growth factor injection therapy has been initiated and the results of that study may prove its usefulness for treating the symptoms of patients with degenerative disc diseases. Considering the difficulty of repairing disc tissues in the advanced stages of disc degeneration, the prophylactic use of growth factor injection therapy, such as its application to discs adjacent to a fusion level, may be an alternative approach. Further quantitative studies on the effects of a growth factor injection on pain generation and the long-term effects on the endplate and cell survival using large animals are needed. An international academic-industrial consortium addressing these aims, such as was achieved for osteoarthritis (The Osteoarthritis Initiative, <http://www.oai.ucsf.edu/datarelease/>), may further the development of biological therapies for degenerative disc diseases.

Conflict of interest statement None of the authors has any potential conflict of interest.

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