Potential Biologic Therapies for the Intervertebral Disc

Christopher Evans
J Bone Joint Surg Am. 88:95-98, 2006. doi:10.2106/JBJS.E.01328

Potential Biologic Therapies for the Intervertebral Disc

By Christopher Evans, PhD, DSc

Biology offers several strategies for restoring the degenerating disc, including the use of recombinant or natural proteins that increase matrix accumulation and assembly, enhance the number of disc cells, or in other ways lead to restoration of the native healthy disc. Recombinant bone morphogenetic protein-7 (osteogenic protein-1) shows promise in this regard. Other growth factors, as well as cytokine antagonists such as the interleukin-1 receptor antagonist, are also good candidates. Because disc degeneration is a chronic, progressive disorder occurring over many years, it is likely that growth factors and other therapeutic proteins will need to be present in the disc for extended periods of time. The intradiscal injection of recombinant or natural proteins is unlikely to fulfill this requirement. In this scenario, the delivery of genes that encode the protein in question may provide a better delivery system. Kang and associates have pioneered this strategy, demonstrating the responsiveness of disc cells to in situ genetic modification.

The success of protein and gene therapy requires the presence of an adequate number of responding cells. Disc degeneration is accompanied by a decline in cellularity. Restoring cell numbers could be achieved by either stimulating the division and inhibiting the death of endogenous cells or by introducing new cells into the disc. The latter strategy may be more successful, especially if the endogenous cells of a degenerating disc are unresponsive or otherwise abnormal. When pursuing this strategy, there are several important reasons why it is better to introduce progenitor cells than to attempt to harvest and reintroduce mature disc cells. Progenitor cells of the mesenchymal lineage, available from bone marrow, fat, and other convenient sources, could be useful. However, although the presumption exists that these types of cells can differentiate into disc cells, this has never been demonstrated. One impediment to confirming differentiation into a disc cell is our inability to identify these cells; there are no robust molecular, biochemical, or biologic markers. The serious study of disc-cell biology at this level would be most rewarding.

Advances in molecular and cellular biology are beginning to influence the clinical practice of orthopaedics[1]. Novel, biologically based therapeutic agents—also called biologics—promise to improve the management of a variety of orthopaedic conditions[2], including intervertebral disc degeneration[3]. Broadly speaking, biologics include any molecules appropriated from the body and used therapeutically in a native or modified form. According to this definition, prostaglandins, steroids, and their analogs should be included; however, these molecules are more commonly viewed as pharmacologic agents. Likewise, chondroitin and glucosamine sulfates are categorized as neutraceuticals, and even hyaluronic acid, widely used in the treatment of osteoarthritis, is rarely considered a biologic. This review focuses on the three most commonly discussed types of biologic therapy—protein therapy, gene therapy, and cell therapy.

Therapeutic Targets and Strategies

Biologic therapies are intended to regenerate the disc and eliminate pain. Intervertebral disc degeneration is quite complex, involving altered nutrition, disturbances in the biophysical environment, quantitative and qualitative changes in matrix turnover, loss of cells, neurophysiologic abnormalities, and altered biomechanics. Which of these processes are primary and which are contingent is not clear; moreover, there appears to be a strong genetic contribution. Such complexities confuse the search for reasonable therapeutic targets that can be usefully modulated by biologic means. Nevertheless, despite our imperfect knowledge, several approaches can be suggested (Table I).

One strategy for preventing, arresting, or reversing intervertebral disc degeneration aims to increase the accumulation of extracellular matrix by enhancing its synthesis and/or inhibiting its degradation. A variety of growth factors promote the accumulation of matrix, and certain cytokines have the opposite effect—they inhibit matrix synthesis as well as accelerate its catabolism. Despite considerable research, identification of the important mediators of degradation triggered by catabolic cytokines is incomplete. Candidates include various types of proteinases, such as matrix metalloproteinases, members of the ADAM (a disintegrin and metalloprotease) family, and cathepsins. The highly acidotic conditions existing within the nucleus pulposus could strongly favor catheptic action. Nonenzymic mechanisms involving molecules such as nitric oxide, peroxynitrite, and superoxide are also candidates, but the extent to which they are restrained by the anoxic environment of the disc is unknown. In addition to the ability to manipulate the concentrations and activities of cytokines, growth factors, proteinases, and radicals, opportunities exist

TABLE I Issues for Biologic Treatment of Intervertebral Disc Degeneration*

Pathologic target?
Matrix turnover?
Cell death?
Pain?
Other
Type of therapeutic?
Anabolic (e.g., BMP, FGF, IGF, PDGF)
Cytokine antagonist (e.g., IL-1Ra, TNFsR)
Proteinase inhibitor (e.g., TIMPs, Serpins, PAIs)
Transcription factor (e.g., NF-κB, AP-1, Sox)
Antioxidant
Analgesic
Other
Mode of delivery?

*BMP = bone morphogenetic protein, FGF = fibroblast growth factor, IGF = insulin-like growth factor, PDGF = platelet-derived growth factor, IL-1Ra = interleukin-1 receptor antagonist, TNFsR = tumor necrosis factor soluble receptor, TIMPs = tissue inhibitors of metalloproteinases, Serpins = inhibitors of serine endopeptidase (serine protease), and PAIs = plasminogen activator inhibitors.

to regulate matrix turnover at the level of gene expression, particularly transcription, given that a number of key transcription factors (such as Sox-4, 5, and 9; NF-κB; and AP-1) have been identified.

Because intervertebral disc degeneration is associated with reduced cellularity, restoration may be aided by treatments that protect against cell death or promote mitosis. Several of the growth factors that promote matrix accumulation also serve as survival factors, and many are mitogenic. Whether cell death primarily reflects environmental stresses, responses to radicals, or other stimuli is not known.

Also unknown is whether restoration of the disc would also eliminate the pain that is associated with intervertebral disc degeneration. Pain might have to be considered a separate, albeit related, therapeutic target (Table I).

Protein Therapy

Cells of the anulus fibrosus and nucleus pulposus respond to a number of different cytokines[3]. Several growth factors, including bone morphogenetic protein-2 (BMP-2), BMP-7 (also known as osteogenic protein-1 [OP-1; Stryker, Kalamazoo, Michigan]), growth and differentiation factor-5, transforming growth factor-β (TGF-β), and insulin-like growth factor-1 (IGF-1) stimulate matrix production, while interleukin-1 (IL-1) and tumor necrosis factor (TNF) inhibit the synthesis of matrix and enhance its catabolism. Thus, a rationale exists for administering growth factors or cytokine antagonists to the degenerating disc.

Several recombinant growth factors and cytokine antagonists are already in clinical use for the treatment of musculoskeletal conditions (Table II); a few of them, such as BMP-2, BMP-7, and parathyroid hormone, have a direct application in the orthopaedic arena. Although none are yet approved for the treatment of intervertebral disc degeneration, an application has been filed with the United States Food and Drug Administration for the intradiscal injection of recombinant human BMP-7. This filing is based on preclinical data demonstrating that nuclear and anular cells respond to BMP-7 by dividing and also by increasing their synthesis of aggrecan and collagen. Moreover, intradiscal injection of BMP-7 increases disc height in normal rabbits[4] and slows loss of disc height in a lapine model of intervertebral disc degeneration[5].

The effectiveness of growth factors such as BMP-7 might be enhanced by the coadministration of additional proteins with synergistic actions. For example, as discussed by Sobajima et al.[6], combinations of TGF-β, BMP-2, and IGF-1 act collaboratively to stimulate proteoglycan synthesis in the disc. The addition of molecules with antioxidant properties or the ability to inhibit matrix degradation might provide further benefit. In the latter context, an obvious candidate is the interleukin-1 receptor antagonist (IL-1Ra), the recombinant form of which is already in clinical use as the drug Kineret (anakinra) for the treatment of rheumatoid arthritis.

Although recombinant proteins are often effective clinically, they are very expensive. The TNF antagonists used to treat rheumatoid arthritis, for instance, cost well over $10,000 per year; BMPs used to promote bone growth cost approximately $5,000 per application. If the effective treatment of intervertebral disc degeneration were to require the administration of several different recombinant growth factors, antioxidants, and cytokine antagonists, the cost and complexity would become prohibitive. One solution is to inject cocktails of native, rather than recombinant, proteins obtained from a convenient, autologous source such as blood. This is the basis for administering autologous conditioned serum.

When peripheral blood is withdrawn and incubated with etched glass beads, leukocytes within the aspirate enrich the plasma with anti-inflammatory cytokines, such as IL-1Ra, IL-4, and IL-10, as well as growth factors, including fibroblast growth factor-2, TGF-β, and hepatocyte growth factor. After clotting, centrifuging, and filtering, the autologous conditioned serum, which is marketed in Europe as Orthokine, is returned to the body. It has been used successfully, by way of local injection, for the treatment of muscle injuries, human and equine osteoarthritis, and radiculopathy[2]. The use of Orthokine in the intervertebral disc has not been reported, but is worthy of consideration given its impressive safety record and rich mixture of growth factors, cytokine antagonists, and, possibly, additional helpful agents.

Gene Therapy

Rapid biologic clearance is one limitation of protein therapy, whether administered by injection of purified, recombinant proteins or autologous conditioned serum. This is an important consideration for a chronic condition such as intervertebral disc degeneration. Gene transfer has the ability to pro-

TABLE II Recombinant Proteins in Clinical Use for the Treatment of Musculoskeletal Conditions*

Medical Condition	Protein Used	Mode of Administration
Rheumatoid arthritis		
	Enbrel (etanercept) (bivalent TNFsR:IgG Fc)	Subcutaneous
	Remicade (infliximab) (partially humanized anti-TNF antibody)	Intravenous
	Humira (adalimumab) (fully humanized anti-TNF antibody)	Subcutaneous
	Kineret (anakinra) (interleukin-1 receptor antagonist)	Subcutaneous
Delayed bone-healing		
	Infuse bone graft (BMP-2) (Medtronic Sofamor Danek, Memphis, Tennessee)	Surgically implanted
	OP-1 (BMP-7) (Stryker, Kalamazoo, Michigan)	Surgically implanted
Osteoporosis		
	Forteo (teriparatide) (PTH 1-34)	Intramuscular

*TNFsR = tumor necrosis factor soluble receptor, IgG Fc = the constant fragment of immunoglobulin G, BMP = bone morphogenetic protein, OP-1 = osteogenic protein-1, and PTH = parathyroid hormone.

vide the sustained, local endogenous synthesis of therapeutic proteins that have undergone authentic posttranslational processing. It is particularly useful for the delivery of intracellular proteins such as transcription factors, although the identification of protein transduction domains that facilitate the uptake of extraneous proteins provides additional opportunities for the acute therapeutic use of intracellular proteins.

As well as being a useful delivery system, gene transfer provides the potential for the regulated synthesis of gene products. The inclusion of inducible promoters whose activities are sensitive to oxygen tension, pH, or mechanical stimuli might be particularly useful when treating intervertebral disc degeneration. Gene therapy has wide potential applications in orthopaedics[7]; data from the first clinical trial of gene therapy for arthritis have just been published[8].

Intradiscal gene therapy holds considerable promise for the treatment of intervertebral disc degeneration[6,9], both in terms of regenerating damaged tissue and in treating pain. Genes may be transferred to the disc by in vivo or ex vivo strategies by way of viral or nonviral vectors[7]. Most of the progress has been made in rabbit models, in which a recombinant adenovirus or adeno-associated virus has been used to transfer complementary deoxyribonucleic acid (cDNA) by direct injection. Remarkably long periods (one year or greater) of transgene expression have been achieved, even when using highly antigenic vectors and transgene products. The immunologic isolation of the disc and the low rate of cell division within it presumably account for these findings. Whether the degenerating disc offers similar protection is unknown.

Transgenes shown to increase the accumulation of matrix by disc cells include those that encode the tissue inhibitor of metalloproteinase-1, TGF-β, BMP-2, and the transcription factor Sox-9. Data from the first evaluation of gene therapy in an animal model of intervertebral disc degeneration are just beginning to emerge, and those data suggest that the transfer of a BMP-2 cDNA to the disc by the direct injection of a recombinant adenovirus vector reverses the early loss of disc height[10].

Noncoding sequences of nucleic acids also hold therapeutic potential[11]. For instance, it is possible to administer oligonucleotides that contain the response elements within the promoter regions of genes. These decoys bind their cognate transcription factors, thus making them unavailable to drive expression of the genes in question. They are widely and successfully used experimentally both in vitro and in vivo, and a phase-I clinical trial is under way in which oligonucleotide decoys for the transcription factor NF-κB are injected into the joints of subjects with rheumatoid arthritis.

Several types of ribonucleic acid (RNA) molecules may also be used to regulate gene expression for therapeutic purposes. Antisense RNA, for example, contains sequences that are complementary to the target messenger RNA molecules to which they bind and thereby inactivate. One antisense RNA drug, Vitravene (fomivirsen), is on the market for the treatment of cytomegalovirus infections of the eye.

Lack of specificity has restricted the development of other promising antisense strategies. An alternative RNA-based approach makes use of ribozymes. These are RNA molecules with the ability to catalyze the degradation of target RNA molecules in a sequence-specific manner; a number of them are in preclinical development for various indications. The recent discovery of RNA interference[12], however, has provided a powerful new avenue for drug development that has displaced much of the enthusiasm for antisense molecules and ribozymes. Small, interfering RNA (siRNA) molecules are produced from larger double-stranded precursors through the action of an enzyme known as Dicer. In a precise, sequence-specific, and highly efficient manner, the siRNA molecule binds to and cleaves its target mRNA molecules.

The relatively short biologic lifetimes of RNA molecules limit their use in the treatment of chronic conditions such as intervertebral disc degeneration. However, sequences that encode these molecules may be incorporated into viral vectors and expressed for extended periods of time by the gene-transfer methods indicated above.

Cell Therapy

Given that intervertebral disc degeneration is associated with loss of cells, there is the potential to reverse degenerative processes by the introduction of cells with the ability to regenerate disc tissue. In one embodiment of this approach, the cells would be genetically modified to improve their regenerative capabilities, thereby implementing a form of ex vivo gene therapy. The development of cell therapies is impeded by poor knowledge of disc-cell biology.

Of the potential sources of cells for cell-based therapies, autologous disc cells seem the least promising. These cells would presumably be harvested from the patient's own degenerating disc and, in addition to the need for an intrusive recovery procedure, the cells may be abnormal and poorly suited for repair. Mesenchymal stem cells, which can be readily obtained from autologous sources such as bone marrow, are better candidates. Although the presumption exists that these cells can differentiate into disc cells, this has not been demonstrated. Because both the various cell types found within the human intervertebral disc and mesenchymal stem cells lack robust phenotypic markers, it is difficult to study the differentiation of disc cells from their presumptive progenitors. Serious study of disc-cell biology at this level would be most rewarding.

The clinical use of either adult disc cells or progenitor cells will require the ex vivo expansion of the cells. Although this is feasible, it is expensive—a matter of considerable importance in the present health-care environment. The use of allogeneic progenitor cells would offer a more cost-effective approach. This possibility arises because of claims that mesenchymal stem cells can be successfully allografted. If so, a universal donor line of these cells, genetically modified or not, could be established and used directly in all suitable patients. The first human clinical trial involving allografted mesenchymal stem cells is about to begin, so preliminary data on the success of allografting should become available soon.

Regardless of their origin, the survival of the transplanted cells could be a limiting factor. The interior of the degenerating disc provides a harsh environment that is acidic, hypoxic, and poor in nutrients. The transplants may have to be preconditioned, possibly by genetic manipulation, if they are to survive and restore matrix under these unfavorable conditions.

Conclusions

Biology has much to offer orthopaedics in general and the treatment of intervertebral disc degeneration in particular. Protein, gene, and cell therapies for a variety of indications are being developed and might be used singly or in combination for the treatment of intervertebral disc degeneration. To be clinically useful, such modalities need to be not only effective, but also safe and affordable. Within these constraints, there is room for considerable optimism about their eventual clinical use.

Corresponding author:
Christopher Evans, PhD, DSc
Center for Molecular Orthopaedics, Harvard Medical School, 221 Longwood Avenue, BLI-152, Boston, MA 02115. E-mail address: cevans@rics.bwh.harvard.edu

The author did not receive grants or outside funding in support of his research for or preparation of this manuscript. The author received payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity (Dr. Evans is on the scientific advisory board of TissueGene Inc. and Orthogen AG). No commercial entity paid or directed, or agreed to pay or direct, any benefits to any research fund, foundation, educational institution, or other charitable or nonprofit organization with which the author is affiliated or associated.

doi:10.2106/JBJS.E.01328

References

1. Evans CH, Rosier RN. Molecular biology in orthopaedics: the advent of molecular orthopaedics. J Bone Joint Surg Am. 2005;87:2550-64.

2. Evans CH. Novel biological approaches to the intra-articular treatment of osteoarthritis. BioDrugs. 2005;19:355-62.

3. Phillips FM, An H, Kang JD, Boden SD, Weinstein J. Biologic treatment for intervertebral disc degeneration: summary statement. Spine. 2003;28(Suppl 15):S99.

4. An HS, Takegami K, Kamada H, Nguyen CM, Thonar EJ, Singh K, Andersson GB, Masuda K. Intradiscal administration of osteogenic protein-1 increases intervertebral disc height and proteoglycan content in the nucleus pulposus in normal adolescent rabbits. Spine. 2005;30:25-31.

5. An HS, Masuda K. Relevance of in vitro and in vivo models for intervertebral disc degeneration. J Bone Joint Surg Am. 2006;88(Suppl 2):88-94.

6. Sobajima S, Kim JS, Gilbertson LG, Kang JD. Gene therapy for degenerative disc disease. Gene Ther. 2004;11:390-401.

7. Evans CH, Ghivizzani SC, Herndon JH, Robbins PD. Gene therapy for the treatment of musculoskeletal diseases. J Am Acad Orthop Surg. 2005;13:230-42.

8. Evans CH, Robbins PD, Ghivizzani SC, Wasko MC, Tomaino MM, Kang R, Muzzonigro TA, Vogt M, Elder EM, Whiteside TL, Watkins SC, Herndon JH. Gene transfer to human joints: progress toward a gene therapy of arthritis. Proc Natl Acad Sci USA. 2005;102:8698-703.

9. Wehling P. Transfer of genes to intervertebral disc cells: proposal for a treatment strategy of spinal disorders by local gene therapy. Joint Bone Spine. 2001; 68:554-6.

10. Larson JW 3rd, Levicoff EA, Gilbertson LG, Kang JD. Biologic modification of animal models of intervertebral disc degeneration. J Bone Joint Surg Am. 2006;88(Suppl 2):83-7.

11. Kalota A, Shetzline SE, Gewirtz AM. Progress in the development of nucleic acid therapeutics for cancer. Cancer Biol Ther. 2004;3:4-12.

12. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. Nat Rev Genet. 2002;3:737-47.