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# 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

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**Study Design.** To study the therapeutic efficacy of intradiscal injection of osteogenic protein-1 (OP-1) to reduce degeneration and associated discogenic pain.

**Objective.** To evaluate if intradiscal injection of OP-1 can reverse disc degeneration and reduce hyperalgesia, a pain-related behavior.

**Summary of Background Data.** We showed that induction of hyperalgesia was higher in rats exposed to compressed nucleus pulposus (NP). It has been reported that intradiscal injection of OP-1 stimulates synthesis of proteoglycans and collagen in normal intervertebral discs.

**Methods.** Rats were divided into several groups. In the sham group, the rings of an Ilizarov-type apparatus were only applied to the tail without compression. In the compressed NP group, the apparatus was used to apply chronically compression to the tail. Four weeks after surgery, the NP group was subdivided into 3 groups: saline-treated and OP-1-treated, which was divided into 2 groups (*i.e.,* the continuous compression OP-1 [COP-1] group, in which compression was continuously applied to the tail for 4 weeks after OP-1 treatment and the release compression OP-1 [ROP-1] group, in which compression was released at treatment. Either physiologic saline or OP-1 was injected into the instrumented NP. The treated NP was harvested and applied to the left lumbar nerve roots 4 weeks after injection. Hyperalgesia was measured up to 3 weeks after surgery. The degree of disc degeneration and the appearance of the extracellular matrix in the intervertebral discs were evaluated by histology.

**Results.** Mechanical hyperalgesia was observed in the sham and saline groups, but not in the OP-1 treated group. In the saline group, NP cells became spindle-shaped. In the OP-1 group, the NP cells became swollen with vacuolated cytoplasm, and the content of the extracellular matrix was markedly increased.

**Conclusion.** OP-1 injection into degenerative intervertebral disc resulted in the enhancement of the extracellular matrix and the inhibition of pain-related behavior.

**Key words:** disc degeneration, regeneration, hyperalgesia, osteogenic protein-1, histology. **Spine 2005;30: 1933–1939**

Back pain is a major cause of disability and has a socioeconomic impact because of a direct cost of the treatments and indirect costs such as loss of productivity. There are 2 mechanisms of intervertebral disc degeneration that may contribute to back pain: loss of disc structure and mechanical properties, and a release of mediators that may sensitize nerve endings.[1] Conventional and current treatments for pain caused by disc degeneration include medication, physical therapy, intradiscal electrothermal therapy, and surgeries such as artificial nucleus pulposus (NP) replacement, intervertebral disc prostheses, and spinal fusion.

The biochemistry of the intervertebral disc plays an important role in its mechanical properties.[2] Imbalance in organ homeostasis leads to intervertebral disc degeneration. Although the direct relationship between disc degeneration and back pain needs to be studied, it is thought that repair or regeneration of the degenerated intervertebral disc with the suppression of pain is a key for biologic manipulation in a future treatment option.

To intervene biologically in disc degeneration and pain, it is necessary to develop an animal model of disc degeneration that would allow the measuring of pain and would make possible the application of the anabolic factors able to overcome degenerative processes induced in the intervertebral disc. Recently, it has been reported that pain-related behaviors could be measured in the experimental rat models of lumbar nerve root irritation.[3–8] In addition, models of disc degeneration have been developed in small animals such as rats and mice. In these models, the tail is instrumented with an Ilizarov-type apparatus[9] or an external compression device.[10] Based on the method of Iatridis[9] and Mente[11] *et al*, we developed an animal model of disc degeneration, in which 2 tail intervertebral discs were immobilized and chronically compressed with an Ilizarov-type apparatus.

For the assessment of pain-related behaviors,[12] hyperalgesia and the hypersensitivity to noxious stimuli were measured. Thus, we have evaluated radicular pain of the

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hind paw induced by the application of NP on the lumbar nerve roots. NP tissues are harvested from the chronically compressed discs and applied to lumbar nerve roots. We showed that the induction of hyperalgesia was higher and of a longer duration in animals exposed to the compressed NP tissue compared to the control animals (normal NP tissue).[13]

Growth factors, such as fibroblast growth factor and transforming growth factor-$\beta$, have stimulated cell proliferation and matrix synthesis of intervertebral discs *in vitro*.[14] A member of the transforming growth factor-$\beta$ superfamily, osteogenic protein-1 (OP-1) or bone morphogenetic protein-7, stimulates proteoglycan and collagen synthesis in rabbit intervertebral disc cells cultured in alginate beads.[15] It has also been reported that intradiscal injection of OP-1 stimulates proteoglycan and collagen synthesis in normal intervertebral discs,[16,17] and, furthermore, the injection of OP-1 following chondroitin ABC-induced chemonucleolysis results in the recovery of disc height in the rabbit.[17,18] The therapeutic efficacy of intradiscal injection of OP-1 in the reduction of degeneration and associated discogenic pain remains to be shown. The hypothesis of the present study is that intradiscal injection of a growth factor, such as OP-1, into degenerated disc results in repair or regeneration of the affected disc and abolishment of discogenic pain. The purpose of the present study was to evaluate if intradiscal injection of OP-1 can reverse compressed NP degeneration and reduce pain-related behavior induced by the application of the degenerative NP on the nerve root.

## ■ Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee. The study is based on 34 male Sprague-Dawley rats, each weighing about 250 g. The rats were housed in an animal room with a 12-hour light/12-hour dark cycle, and had free access to food and water.

**Surgical Protocol.** All surgical procedures were performed with the rats anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Animals were divided into 4 experimental groups. In the sham group (n = 8), 2, 0.8-mm diameter Kirschner wires were inserted percutaneously through the third and fifth coccygeal vertebrae. Each wire was fixed separately to a specially designed aluminum ring, consisting of 2, 30-mm diameter external rings. In the compressed NP group (n = 24), the 2 rings were linked with 4 rods to immobilize and chronically apply compression on the Kirschner wires until the tail showed maximum angular deformity. Four weeks after surgery, those animals were divided into 3 subgroups. In the saline and OP-1 groups (n = 8 and 16, respectively), 1 $\mu$L physiologic saline or 0.2 $\mu$g/1 $\mu$L OP-1 was injected into the NP of the instrumented vertebrae, respectively. The injection of saline or OP-1 was performed with a Hamilton syringe (Hamilton Co., Reno, NV). OP-1 treated animals were divided into 2 subgroups. In the COP-1 group (n = 8), compression was continuously applied to the tail after OP-1 treatment. In the ROP-1 group (n = 8), compression was released at treatment. All tails in all 4 groups were amputated 8 weeks after primary treatment. The experimental design used in the present study is summarized in Figure 1. For histologic examination, which is described later, 2 rats were used as naïve controls.

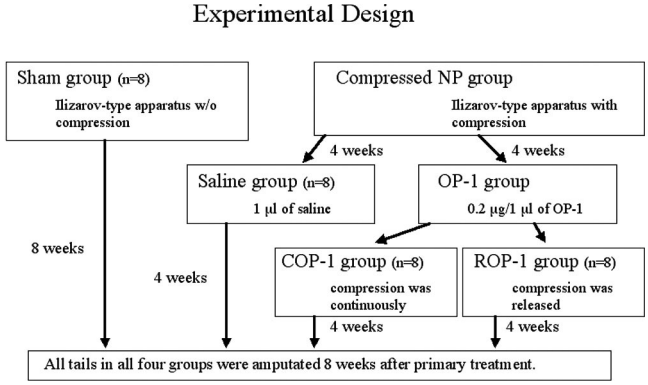

Figure 1. Summary of the study design of experimental groups.

**Evaluation of Mobility in the Fixed Vertebrae.** Eight weeks after fixation of the tail with an Ilizarov-type apparatus, the mobility of the fixed vertebrae was manually evaluated in the saline, COP-1, and ROP-1 groups.

**Pain Evaluation.** To evaluate pain, tissue was retrieved from treated NP after amputation of the tail using 5 rats in each group. The NP tissue was applied to the left L4 and L5 nerve roots after partial laminectomy. After surgery, all wounds were irrigated and washed with preservative-free sterile saline. The operative fields were closed in layers with 4-0 nylon sutures. Radicular pain was assessed by behavioral measurements described later.

**Behavioral Observations.** Motor function and reflex responses to both mechanical and thermal noxious stimuli to both hind paws were measured in all rats before surgery, and up to 3 weeks after surgery. Behavioral observations were made at the same time during the day. The same examiner performed all observations and was blinded to the treatment group being observed.

**Motor Function.** Rats were placed on the floor in an open field, and their gait patterns were observed. Motor function was assessed using a previously described scale, based on gait pattern and hind paw deformities.[19]

**Mechanical Withdrawal Threshold and Thermal Withdrawal Latency.** Using methods reported previously,[4,5] reflex responses to noxious mechanical and thermal stimuli to both hind paws were assessed quantitatively for all rats. Briefly, for the measurement of mechanical withdrawal threshold, rats were allowed to crawl freely under a piece of cloth. Once the rat had settled, the mechanical withdrawal threshold was measured on the dorsal surface of the hind paw, between the fourth and the fifth metatarsal bones, using a specially designed apparatus made from a 50-mL syringe, a needle with a 2-mm dull tip, and plumbs. The threshold at which the hind paw was withdrawn was expressed in grams. The placement of the stimuli was varied slightly from one trial to the next to avoid sensitizing the skin. Each trial was repeated 3 times at least 5 minutes apart, and the average of the results was determined. Noxious thermal stimulation was applied to the paw using a radiant heat

source (Heat Stimulator 535; Department of Bioengineering, University of Iowa, Iowa City, IA).

Each rat was placed unrestrained under a clear glass floor and allowed to habituate before testing. Radiant heat, which was allowed to increase steadily from 40°C, was focused on the planter surface of a hind paw when it was in contact with the glass. The latency at which the hind paw was withdrawn was recorded. Three trials were conducted on each hind paw at least 5 minutes apart, alternating between the left and right, and the average of the results was determined. The percentage difference in withdrawal threshold or withdrawal latency from noxious stimuli between the ipsilateral and contralateral hind paws was calculated using the appropriate formula:

$$[(\text{ipsilateral threshold or latency} - \text{contralateral threshold or latency})/\text{contralateral threshold or latency}] \times 100$$

All values are presented as percentage differences. Negative percentages reflect hyperalgesia, whereas positive percentages reflect hypoalgesia.

**Histologic Examination.** Eight weeks after insertion of the Kirschner wires, the tail was amputated, and the instrumented vertebrae were resected, including 2 in the naïve control rats, 3 in the sham group, 3 in the saline group, 3 in the ROP-1 group, and 3 in the COP-1 group. After fixation in 10% neutral buffered formalin, the specimens were decalcified in 10% ethylenediamino-tetraacetic acid solution and then embedded in paraffin wax. The specimens were sectioned longitudinally in the sagittal plane at 5 $\mu$m and processed for histology. Sections were stained with hematoxylin and eosin to evaluate degeneration of the intervertebral disc. Toluidine blue and safranin O fast-green[20] staining were used to evaluate changes in the extracellular matrix of the intervertebral discs. Two independent examiners assessed histologic findings obtained from these specimens in a blind manner. Evaluation of the results was performed with the Nikon Eclipse 600 microscope (Nikon, Tokyo, Japan) with a Spot 2 camera and Metamorph software (Universal Imaging Corporation 228 , Downingtown, PA).

**Statistical Analysis.** Values are presented as means ± standard deviation. Data obtained from behavioral measurements were analyzed by analysis of variance and the Student *t* test. Probability values $<0.05$ were considered statistically significant.

## ■ Results

Rats from every experimental group showed no stress reaction, such as vocalization or struggling. There was no infection at the insertion site of the Kirschner wires in rats in the sham and compressed NP groups.

### *Mobility in the Fixed Vertebrae*

Manual examination of the fixed tail after removal of the Ilizarov-type apparatus revealed that the instrumented vertebrae in the saline group were fixed and did not show any mobility of the vertebrae. However, the instrumented vertebrae in the COP-1 and ROP-1 groups had mobility after removal of the apparatus (Table 1).

**Table 1. Mobility of the Instrumented Vertebrae After Removal of the Apparatus**

| Group | Mobility 8 Wks After Fixation |
|---|---|
| Saline (n = 8) | Fixed |
| COP-1 (n = 8) | Mobile |
| ROP (n = 8) | Mobile |

### *Motor Function*

All rats in the sham, saline, COP-1, and ROP-1 groups showed normal gait (score 0) during the experimental period.

### *Sensitivities to Mechanical Noxious Stimuli*

Rats in the sham group showed evidence of mechanical hyperalgesia in the ipsilateral hind paws for 4 days after surgery. Rats in the saline group showed evidence of mechanical hyperalgesia from 2 days to 2 weeks after surgery ($P < 0.05$). However, the mechanical hyperalgesia observed in the saline group was higher and of longer duration than that in the sham group ($P < 0.05$). On the other hand, in the COP-1 and ROP-1 groups, there were no significant differences in responses to noxious mechanical stimuli between right and left hind paws. Mechanical hyperalgesia was not observed in rats treated with intradiscal injection of OP-1 (Figure 2).

### *Sensitivities to Thermal Noxious Stimuli*

All rats in these groups showed evidence of normal response to thermal noxious stimuli (Figure 3).

### *Histologic Examination*

Examination of hematoxylin and eosin-stained sections revealed the existence of diffuse NP cells in the matrix in both naïve and sham animals (Figure 4). NP cells were arranged in concentric arcs, with the concave side as the center in animals in the saline group. There was a tendency for these cells to become spindle-shaped at the

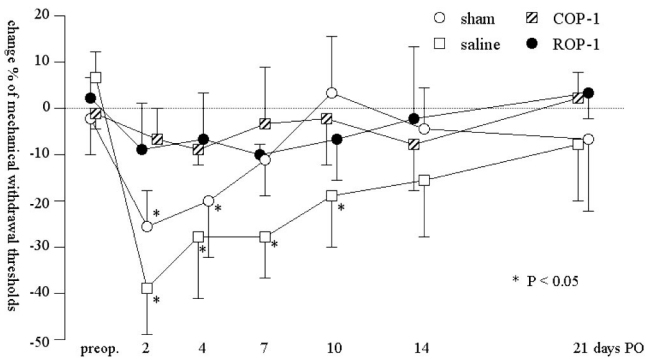


Figure 2. Rats in the sham group showed evidence of mechanical hyperalgesia in the ipsilateral hind paws at 4 days after surgery. However, rats in the saline group showed evidence of mechanical hyperalgesia from 2 days to 2 weeks after surgery. The mechanical hyperalgesia observed in the saline group was higher and of longer duration than that in the sham group. On the other hand, in the COP-1 and ROP-1 groups, which were treated with intradiscal injection of OP-1, mechanical hyperalgesia was not observed.

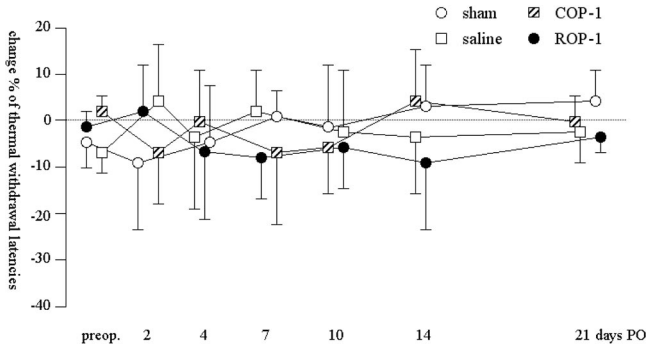


Figure 3. All rats in these groups showed evidence of normal response to thermal noxious stimuli.

convex side of the disc. The original large cluster of cells was disassociated into islands (Figure 4).

In COP-1 and ROP-1 animals, NP cells were swollen with vacuolated cytoplasm. The cellularity of NP was decreased in the COP-1 and ROP-1 groups (Figure 4). Figures 5, 6 represent the histologic appearance of the rat discs stained with either toluidine blue or safranin O, respectively. With both stains, control discs revealed normal histology. Untreated control discs (Figure 6-1) showed normal morphology, elongated NP, and light safranin O stain within the NP. A few cells within anulus fibrosis were also stained with safranin O. Because discs were obtained from skeletally immature rats, endplate cartilage was also evident in tissue sections. This endplate cartilage displayed normal safranin O staining. Sham operated discs (Figure 6-2) morphologically looked similar to the control discs, but safranin O stain was more intense in the endplate cartilage. The compressed discs (Figure 6-3) displayed morphologic and histologic changes, including a loss of elongated shape and a decrease in size of NP, a displacement with regard to the anulus fibrosis, and a substantial loss of proteoglycans in NP and endplate cartilage, as detected by reduced safranin O staining.

Treatment with OP-1 (COP-1 and ROP-1) restored the morphology of the discs; the size of NP was significantly enlarged in comparison to control and compressed discs, and represented normal oval shape. Vacuolated cytoplasm of the NP incorporated both stains (*i.e.*, safranin O and toluidine blue). Safranin O staining appeared very intense in the NP, endplate cartilage, and anulus fibrosis. In the anulus fibrosis of the OP-1-treated discs, safranin O stain was increased, indicating the accumulation of sulfated proteoglycans in this compartment. The intensity of staining in all these areas was much stronger than in control discs. No significant differences were found between the 2 types of OP-1 treatment. Staining with toluidine blue (Figure 5) confirmed histologic results described for safranin O.

## ■ Discussion

We have reported that chronic compression of tail intervertebral discs resulted in disc degeneration and that the NP obtained from the intervertebral discs led to the induction of severe mechanical hyperalgesia.[13] In the present study, we found by histologic assessment that OP-1 injection counteracted continuous compression load and induced the synthesis of the extracellular matrix in these intervertebral discs. Mechanical hyperalgesia, which is thought to be a pain-related behavior, was not observed in animals whose lumbar nerve roots were exposed to the compressed NP treated with OP-1. Me-

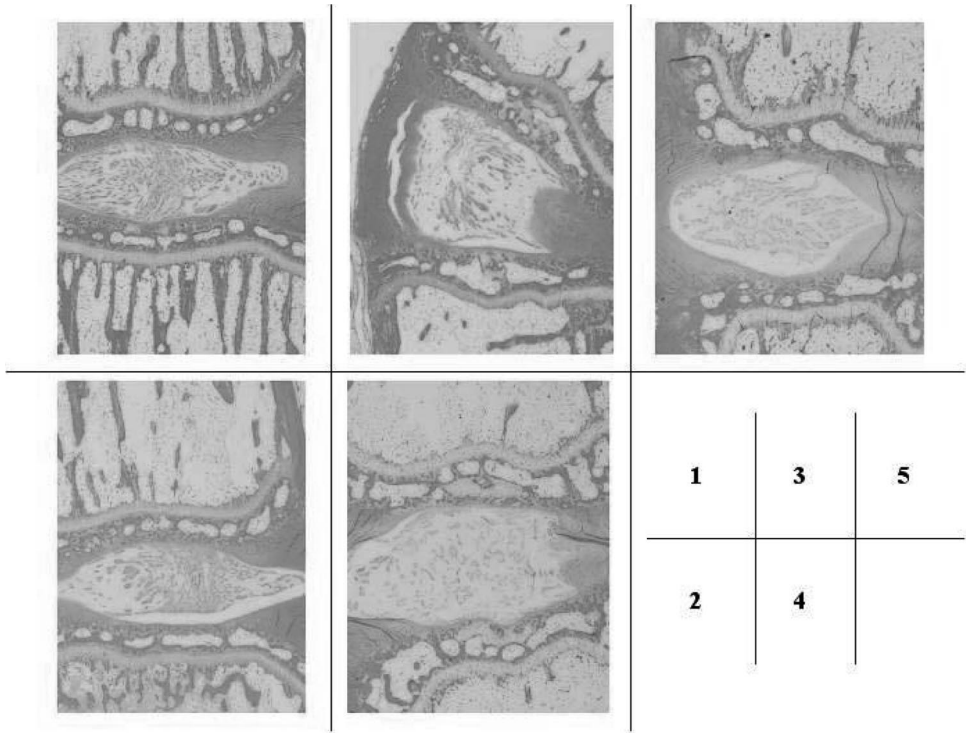


Figure 4. Examination of hematoxylin and eosin-stained sections revealed the existence of diffuse NP cells in the matrix in both control and sham animals. NP cells were arranged in concentric arcs, with the concave side as the center in animals in the saline group. There was a tendency for these cells to become spindle-shaped at the convex side of the disc. The original large cluster of cells was disassociated into islands. In COP-1 and ROP-1 animals, NP cells were swollen with vacuolated cytoplasm. The cellularity of NP was decreased in the COP-1 and ROP-1 groups. **1**, Naive control animal. **2**, Sham group. **3**, Saline group. **4**, COP-1 group. **5**, ROP-1 group.

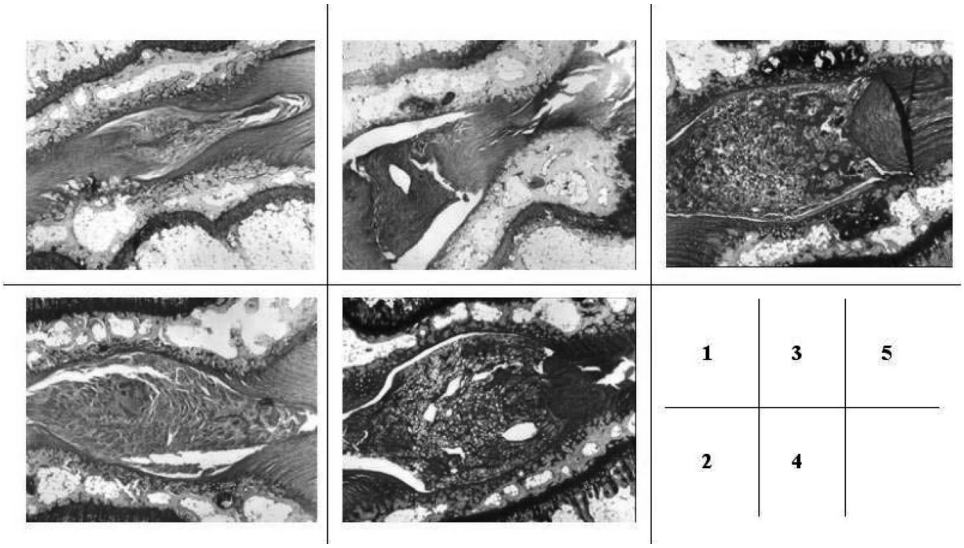


Figure 5. Toluidine blue staining of the paraffin embedded disc sections. **1**, Naïve control group. **2**, Sham operated disc. **3**, Saline group. **4**, COP-1 treated disc. **5**, ROP-1 treated disc.

chanical hyperalgesia was induced by compressed NP treated with saline, but not by those treated with OP-1.

OP-1 is the only bone morphogenetic protein that does not induce catabolic events in adult articular cartilage. Its anabolic activity in adult articular cartilage has been reported. OP-1 stimulates the synthesis of the majority of the extracellular matrices, including proteoglycan and collagen type II, with no induction of type I collagen and cell proliferation.[21] In the present study, the enlargement of the extracellular matrix in the vertebral discs treated with OP-1 was observed by histology, and may be caused by an increase in proteoglycan and collagen synthesis.

Several studies have identified inflammatory mediators and autoimmune reactions in lumbar disc herniation. As inflammatory mediators, biologically active substances in the arachidonic acid cascade, such as phospholipase $A_2$ ($PLA_2$),[5,22–24] and inflammatory cytokines, such as interleukin-1$\beta$ (IL-1$\beta$)[5,23,25,26] and tumor necrosis factor-$\alpha$ (TNF-$\alpha$),[27–29] are related to pathophysiologic mechanisms of painful radiculopathy in lumbar disc herniation. One appealing hypothesis is that leakage of these agents may produce excitation of the nociceptors, direct neural injury, nerve inflammation, or enhancement of sensitization to other pain-producing substances, leading to nerve root pain.[30] Inflammation is a key pathophysiologic mechanism of pain induced by herniated disc.

In our recent study, we have found that the application of degenerative NP to the nerve roots enhances mechanical hyperalgesia. We showed no significant differences in $PLA_2$, IL-1$\beta$, and TNF-$\alpha$ among the naïve control, sham, and compressed NP groups using immunohistochemistry,[31] and a lower pH in the treated NP

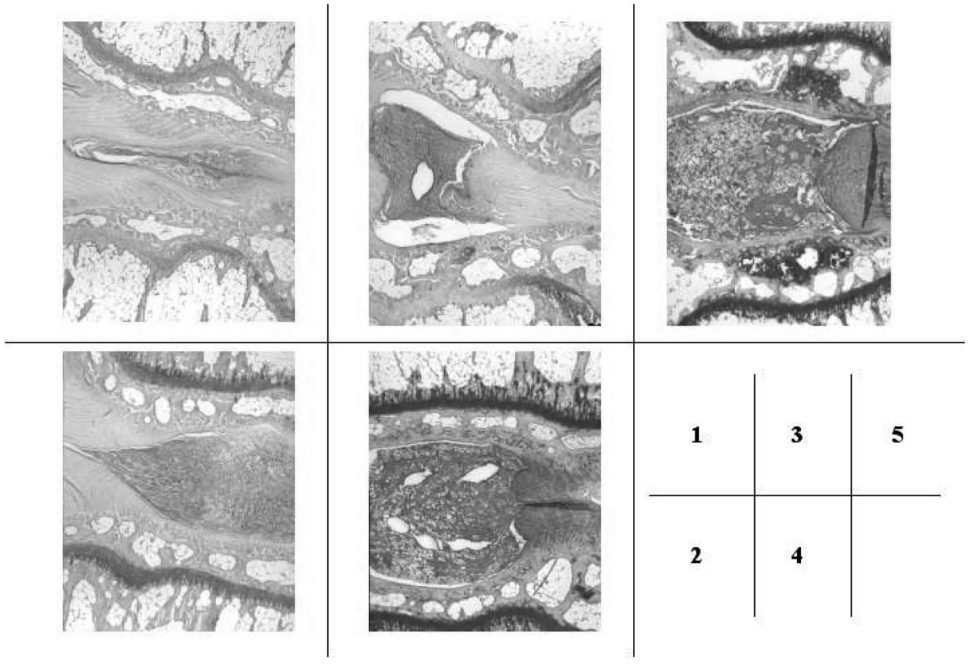


Figure 6. Safranin O fast-green staining of the tissue sections of the rat tail discs. **1**, Naïve control. **2**, Sham operated discs. **3**, Saline group. **4**, COP-1 treated disc. **5**, ROP-1 treated disc.

tissue in the compressed NP group.[32] In this experimental model, in which mechanical compression was chronically applied to the tail intervertebral discs, production of inflammatory substances such as $PLA_2$, IL-1$\beta$, and TNF-$\alpha$ did not increase in the NP cells, but a lower pH in the intervertebral disc was observed. A lower pH is thought to be related to pain.[33,34] A lower pH of the NP tissue around the nerve root may enhance the hyperalgesia. Intradiscal injection of OP-1 to the instrumented vertebrae resulted in an increase in the extracellular matrix. This effect may improve nutrition of the disc and may prevent a decrease in pH levels. On the other hand, OP-1 is thought to suppress the IL-1 effect and reduce inflammation.[21] Regarding intervertebral disc cells, Takegami *et al* [35] have reported that OP-1 enhances matrix replenishment by intervertebral disc cells previously exposed to IL-1. Therefore, it is possible that OP-1 acts as an anti-catabolic factor by inhibiting biologic activities or production of proinflammatory mediators, such as interleukins. These effects may contribute to the abolishment of the hyperalgesia.

Low back pain is important in patients with lumbar disc degeneration. The animal model cannot evaluate axial pain such as low back pain. The mechanism of discogenic pain is thought to be sensitization of nerve endings in the anulus fibrosus by the release of mediators.[1] However, it is very difficult to evaluate the mechanisms of axial pain or discogenic pain secondary to disc degeneration in the rat applying behavioral measurements. Therefore, in the present study, we used a model in which nerve roots were irritated by the NP and evaluated pain induced by the disc material.

The results of the current study indicate a role for OP-1 in pain reduction. Although it has been suggested that NP itself induces nerve injury, Lidslot *et al* [36] clearly showed that NP had a toxic effect on the axons by blocking axonal outgrowth *in vitro*. There are also some reports that OP-1 induces or enhances dendritic formation or neuron growth.[37,38] Collectively, these findings suggest that OP-1 may stimulate neural protection and regeneration after nerve injury. Based on our studies, OP-1 may also have an antitoxic effect on the nerve root, which injury was induced by degenerative discs.

However, there is a number of limitations that have to be addressed in future studies: (1) biologic assessment of disc degeneration and regeneration has to be considered; (2) OP-1 concentration in the injected disc should be clarified; (3) the interaction between OP-1, arachidonic acid cascade, and cytokines in tissues obtained from discs and around the nerve root after the application of NP has to be examined; and (4) biomechanical examination of the discs is also critical, as well as neurochemical and morphologic assessments of the nerve root. We began to investigate biologic, morphologic, and biomechanical mechanisms of the effects induced by recombinant OP-1 on the compressed intervertebral discs of rat tail.

## ■ Conclusions

OP-1 injection into the intervertebral disc, in which mechanical compression was applied in the tail, resulted in the inhibition of the mechanical hyperalgesia, induced by the NP. OP-1 enhanced the replenishment of the extracellular matrix by the NP cells. Not only the activation of the NP cells after intradiscal injection of OP-1, but also the effect of OP-1 itself may be associated with the inhibition of pain-related behavior. From a clinical perspective, it is important to activate or regenerate intervertebral disc cells with no pain induction to treat disc degeneration. Our current results suggest that intradiscal injection of OP-1 has the potential to be a therapy for the treatment of discogenic pain.

### ■ Key Points

- Chronically applied compression of tail intervertebral discs resulted in disc degeneration with the induction of severe hyperalgesia (indicative of a pain-related behavior) in the NP.
- An increase of the extracellular matrix in these vertebral discs was observed by histology after injection of OP-1, despite continuous compression load.
- OP-1 injection into the intervertebral disc caused inhibition of mechanical hyperalgesia induced in the NP by mechanical compression applied to the tail.
- Not only the activation of the NP cells after intradiscal injection of OP-1, but also OP-1 itself may be related to the inhibition of pain-related behavior.
- Our current study suggests that intradiscal injection of OP-1 has the potential to be a therapy for the treatment of pain induced by degenerative NP.

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## References

1. Buckwalter JA, Martin J. Intervertebral disk degeneration and back pain. In: Weinstein JN, Gordon SL, eds. *Low Back Pain: A Scientific and Clinical Overview*. Rosemont, IL: American Academy of Orthopaedic Surgeons; 1996:607–23.
2. Urban JP, McMullin JF. Swelling pressure of the lumbar intervertebral discs: Influence of age, spinal level, composition, and degeneration. *Spine* 1988;13: 179–87.
3. Kawakami M, Weinstein JN, Spratt KF, et al. Experimental lumbar radiculopathy. Immunohistochemical and quantitative demonstrations of pain induced by lumbar nerve root irritation of the rat. *Spine* 1994;19:1780–94.
4. Kawakami M, Tamaki T, Hayashi N, et al. Possible mechanism of painful radiculopathy in lumbar disc herniation. *Clin Orthop* 1998;351:241–51.
5. Kawakami M, Matsumoto T, Kuribayashi K, et al. mRNA expression of interleukins, phospholipase A2, and nitric oxide synthase in the nerve root and dorsal root ganglion induced by autologous nucleus pulposus in the rat. *J Orthop Res* 1999;17:941–6.

6. Hashizume H, DeLeo JA, Colburn RW, et al. Spinal glial activation and cytokine expression after lumbar root injury in the rat. *Spine* 2000;25: 1206–17.
7. Deleo TA, Hashizume H, Rutkowski MD, et al. Cyclooxygenase-2 inhibitor SC-236 attenuates mechanical allodynia following nerve root injury in rats. *J Orthop Res* 2000;18:977–82.
8. Olmarker K, Storkson R, Berge OG. Pathogenesis of sciatic pain: A study of spontaneous behavior in rats exposed to experimental disc herniation. *Spine* 2002;27:1312–7.
9. Iatridis JC, Mente PL, Stokes IA, et al. Compression-induced changes in intervertebral disc properties in a rat tail model. *Spine* 1999;24:996–1002.
10. Lotz JC, Chin JR. Intervertebral disc cell death is dependent on the magnitude and duration of spinal loading. *Spine* 2000;25:1477–83.
11. Mente PL, Stokes IA, Spence H, et al. Progression of vertebral wedging in an asymmetrically loaded rat tail model. *Spine* 1997;22:1292–6.
12. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 1988;33:87–107.
13. Kawakami M, Hashizume H, Nishi H, et al. Comparison of neuropathic pain induced by the application of normal and mechanically compressed nucleus pulposus to lumbar nerve roots in the rat. *J Orthop Res* 2003;21: 535–9.
14. Gruber HE, Fisher EC Jr, Desai B, et al. Human intervertebral disc cells from the annulus: Three-dimensional culture in agarose or alginate and responsiveness to TGF-beta1. *Exp Cell Res* 1997;235:13–21.
15. Masuda K, Takegami K, An H, et al. Recombinant osteogenic protein-1 upregulates extracellular matrix metabolism by rabbit annulus fibrosus and nucleus pulposus cells cultured in alginate beads. *J Orthop Res* 2003;21: 922–30.
16. Takegami K, Masuda K, An H, et al. In vivo administration of Osteogenic protein-1 increases proteoglycan content and disc height in rabbit intervertebral disc. *Ortho Res Soc Trans* 2000;25:338.
17. An H, Eugene J-MAT, Masuda K. Biological repair of intervertebral disc. *Spine* 2003;28:S86–92.
18. Takegami K, Masuda K, Kumano F, et al. Osteogenic potein-1 is most effective in stimulating nucleus pulposus and annulus fibrosus cells to repair their matrix after chondroitinase ABC-induced chemonucleolysis. Ortho Res Soc Trans 1999;24:201.
19. Chatani K, Kawakami M, Weinstein JN, et al. Characterization of thermal hyperalgesia, c-fos expression, and alterations in neuropeptides after mechanical irritation of the dorsal root ganglion. *Spine* 1995;20:277–90.
20. Rosenberg L. Chemical basis for the histological use of Safranin-O in the study of articular cartilage. *J Bone Joint Surg* 1971;53A:69–82.
21. Chubinskaya S, Kuettner KE. Regulation of osteogenic proteins by chondrocytes. *Int J Biochem Cell Biol* 2003;35:1323–40.
22. Franson RC, Saal JS, Saal JF. Human phospholipase A2 is inflammatory. *Spine* 1992;17:S129–32.
23. Kang JD, Stefanovic-Racic M, McIntyre LA, et al. Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. *Spine* 1997;22:1065–73.
24. Saal JS, Franson RC, Dobrow R, et al. High levels of inflammatory phospholipase A2 activity in lumbar disc herniations. *Spine* 1990;15:674–8.
25. Miyamoto H, Saura R, Harada T, et al. The role of cyclooxygenase-2 and inflammatory cytokines in pain induction of herniated lumbar intervertebral disc. *Kobe J Med Sci* 2000;46:13–28.
26. Takahashi H, Suguro T, Okajima Y, et al. Inflammatory cytokines in the herniated disc of the lumbar spine. *Spine* 1996;21:218–24.
27. Igarashi T, Kikuchi S, Shubayev V, et al. Exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Molecular, histologic, and behavioral comparisons in rats. *Spine* 2000;25:2975–80.
28. Olmarker K, Rydevik B. Selective inhibition of tumor necrosis factor-alpha prevents nucleus pulposus-induced thrombus formation, intraneural edema, and reduction of nerve conduction velocity: possible implications for future pharmacologic treatment strategies of sciatica. *Spine* 2001;26:863–9.
29. Olmarker K, Larsson K. Tumor necrosis factor alpha and nucleus-pulposus-induced nerve root injury. *Spine* 1998;23:2538–44.
30. Goupille P, Jayson MI, Valat JP, et al. The role of inflammation in disk herniation-associated radiculopathy. *Semin Arthritis Rheum* 1998;28: 60–71.
31. Kawakami M, Hashizume H, Matsumoto T, et al. Experimental lumbar radiculopathy induced by the intervertebral disc and clinical relevancy [in Japanese]. *Rinshoseikeigeka* 2004;39:431–8.
32. Hashizume H, Kawakami M, Matsumoto T, et al. A mechanism of the neurogenic pain induced by a degenerative nucleus pulposus associated with a pH change [in Japanese]. *J Jpn Orthop Assoc* 2003;77:S1070.
33. Maves TJ, Gebhart GF, Meller ST. Continuous infusion of acidified saline around the rat sciatic nerve produces thermal hyperalgesia. *Neurosci Lett* 1995;194:45–8.
34. Nachemson A. Intradiscal measurements of pH in patients with lumbar rhizopathies. *Acta Orthop Scand* 1969;40:23–42.
35. Takegami K, Thonar EJ, An HS, et al. Osteogenic protein-1 enhances matrix replenishment by intervertebral disc cells previously exposed to interleukin-1. *Spine* 2002;15:27:1318–25.
36. Lidslot L, Olmarker K, Kayama S, et al. Nucleus pulposus inhibits the axonal outgrowth of cultured dorsal root ganglion cells. *Eur Spine J* 2000;9:8–13.
37. Lein P, Johnson M, Guo X, et al. Osteogenic protein-1 induces dendritic growth in rat sympathetic neurons. *Neuron* 1995;15:597–605.
38. Le Roux P, Behar S, Higgins D, et al. OP-1 enhances dendritic growth from cerebral cortical neurons in vitro. *Exp Neurol* 1999;160:151–63.