Introduction: Although pathophysiologic mechanisms of low back pain and/or leg pain are still controversial, intervertebral disc (IVD) degeneration or herniated IVD is thought to contribute to these pathologies. An ideal cure should be able to reduce pain and to repair or regenerate the affected IVDs. Recently, Osteogenic Protein-1 (OP-1) has been explored for treatment of disc diseases in animal models. We have developed a rat model, in which chronic mechanical compression to the tail induces IVD degeneration, and demonstrated that application of the degenerated nucleus pulposus (NP) tissues to the lumbar nerve roots enhanced hyperalgesia [1]. We showed that the application of the degenerated NP, into which OP-1 was injected, onto the nerve root did not induce mechanical hyperalgesia observed in the absence of OP-1 [2]. Further, OP-1 restored morphology of the disc and enhanced its extracellular matrix. These results suggest that intradiscal injection of OP-1 may have a potential as a therapy for the treatment of degenerative disc disease and discogenic pain. In addition, for safety reasons we evaluated if OP-1 leakage into the epidural space from the discs may induce ectopic bone formation or pain. We found that lumbar epidural application of OP-1 did not result in neural damage or ectopic bone formation, but rather attenuated mechanical hyperalgesia induced by the NP [3]. This indicated that epidural leakage of OP-1 during intradiscal injection to the patients might have no safety concerns and that epidural injection of OP-1 may be used as a treatment for lumbar disc herniation. However, the mechanisms of such OP-1 effects are still unknown. To further explore our findings the purpose of the present study was to evaluate the effect of epidural application of OP-1 on the expression and distribution of selected catabolic substances: interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α], substance P, substance P receptor NK-1, bradykinin, bradykinin receptors β1 [BDKRB1] and β2 [BDKRB2], bone formation and matrix synthesis induced in a herniated NP model.

Materials and Methods: The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee. Male Sprague-Dawley rats, 250 g each, were used. All surgical procedures were performed with the rats anaesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Rats were divided into six experimental groups. Sham group (n=4), amputation of the tail and partial laminectomy at L5 were performed to expose left L5 nerve root. NP group (n=4), the NP obtained from two IVDs of the amputated tail was relocated on the exposed L5 nerve root. NP+OP group (n=4), 0.2 μg of OP-1 was injected in 1 μl volume to the NP obtained from the amputated tail and this NP was placed on the nerve root. GS group (n=4), a gelatin sponge was applied on the exposed nerve roots. The weight of gel form was the same as that of the NP. GS+OP group (n=4), a gelatin sponge pre-soaked with 0.2 μg of OP-1 in 1 μl solution was placed on the nerve root. The operative fields were closed in layers with 4-0 nylon sutures. Naïve rats were used for the control (n=2). At one and six weeks postoperatively, the lumbar spine at L4 and 5 was harvested. The specimens were fixed, decalcified, embedded in paraffin, and sectioned at the L4-5 level in the axial plane at 6μm thickness. Alkaline phosphatase and safranin O/fast green staining were used to evaluate bone formation and matrix synthesis after epidural application of OP-1, respectively. For immunohistochemistry, the following antibodies were used: monoclonal rat anti-IL-1β, anti-TNF-α, and human/mouse/rat anti-alkaline phosphatase; polyclonal anti-bradykinin, anti-substance P, anti-BDKRB1, anti-BDKRB2, and anti-substance P receptor NK-1.

Results: In NP groups (no OP-1 treatment) a strong inflammatory reaction was observed at one and six weeks postoperatively which was characterized by an influx of leukocytes and elevation of tested pro-inflammatory substances, mainly neuromediators. Very high levels of substance P and its receptor NK-1 were detected in all areas of interest: spinal cord, nerve roots, dorsal root ganglia, as well as bone marrow, bone, and cartilage. Bradycin, constitutively expressed BDKRB2, IL-1β, and TNF-α were primarily detected in nerve tracts of the spinal cord (strongly positive axons and myelin sheath) and in some nerve fibers of the nerve roots. Inducible BDKRB1 appeared primarily in the nerve root. At six weeks, an overall intensity of all stains was reduced, although the size of inflamed area was similar to that identified at one week postoperatively. OP-1 injection into the NP led to a decrease in the level and size of inflammation and reduction in the intensity of immunostaining for all studied neuromediators as compared with NP only group. Thus, at one week of treatment substance P was decreased in spinal cord, nerve roots and ganglia, while in the vertebral body (bone marrow, bone and cartilage) it was negative or barely detectable. The most substantial changes in the appearance of the NK-1 receptor and bradykinin were detected in the OP-1 treated groups at six weeks postoperatively. The levels of bradykinin receptors were reduced in the OP-1 injected groups at both time points where BDKRB2 was not detectable in spinal cord and dura mater. Less apparent changes under the treatment with OP-1 (in comparison with NP group) were observed in the appearance and distribution of IL-1β and TNF-α. The pattern of staining of samples that received gelatin sponge without OP-1 was similar in the intensity and distribution of tested proteins to the sham operated lumbar spines. No signs of bone formation around dura mater and nerve roots were noticed when OP-1 was injected into the NP.

Discussion: This work is a continuation of the study on the mechanisms of the anabolic and anti-catabolic effects of OP-1 for cartilage and disc repair. The results obtained in this herniated NP model were similar to earlier studies on disc degeneration [2,4]; injection of OP-1 into the NP attenuated hyperalgesia already at one week postoperatively and this effect was primarily associated with significant inhibition of major neuromediators substance P and bradykinin and their receptors in tissues of their primary origin: spinal cord, nerve roots, and dorsal ganglia. Since some inflammatory reaction was still identified morphologically in the areas subjected to the NP injected with OP-1 at one and six weeks postoperatively we were not surprised to see lesser changes in the levels of pro-inflammatory cytokines IL-1β and TNF-α than in the levels of neuromediators. Importantly, at studied time points OP-1 injection did not induce noticeable ectopic bone formation at the subjected areas suggesting that the application of OP-1 for lumbar spine diseases might be a safe procedure to treat patients.