Animal Study

Assessment of a Discogenic Pain Animal Model Induced by Applying Continuous Shear Force to Intervertebral Discs

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Background: Chronic discogenic pain includes degeneration-driven changes under the mechanical macroenvironment of an internal disc, which leads to the progressive changes of biochemical microenvironment that induce abnormal ingrowth of the nociceptor. The propriety of the animal model reflecting the pathologic natural history has not been assessed.

Objectives: This study investigated the biochemical evidence of chronic discogenic pain by employing a discogenic pain animal model induced by shear force.

Study Design: Animal study utilizing rats in vivo model of a shear force device.

Methods: Fifteen rats were divided into 3 groups (n = 5/group) according to the period for which sustained dorsoventral shear force was applied (1 week or 2 weeks); the control group received the spinous attachment unit, without a spring. Pain data were collected using von Frey hairs on the hind paws. Growth factor and cytokine abundance was analyzed in the dorsal root ganglion (DRG) and plasma.

Results: After the shear force devices were installed, the significant variables were found to markedly increase in the DRG tissues of the 2-week group; however, they were not altered in the 1-week group. Specifically, interleukin (IL)-6, neurogrowth factor (NGF), transforming growth factor (TGF)-α, platelet-derived growth factor (PDGF)-β, and vascular endothelial growth factor (VEGF) were increased. Meanwhile, the plasma levels of tumor necrosis factor-α, IL-1β, IL-5, IL-6, IL-12, and NGF were increased in the 1-week group; whereas, TGF-α, PDGF-β, and VEGF were increased in the 2-week group.

Limitations: The limitations include the general limitations of quadrupedal animals, the poor precision and flexural deformation of shear force devices, inaccuracies regarding the evaluation of histological denaturation, and short intervention and observational periods.

Conclusions: This animal model effectively generated biochemical responses to shear loading with evidence of neurological changes induced without direct macrodamage to the outer annulus fibrosus. Chemical internals were induced by mechanical externals among the contributing factors of chronic discogenic pain.

Key words: Discogenic pain, dorsal root ganglion, intervertebral discs, low back pain, shear force, interleukin, growth factor, animal model

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Intervertebral discs (IVDs) are considered a source of chronic low back pain (LBP). A retrospective review of the clinical outcomes of spinal intervention in patients with chronic LBP revealed the prevalence of pain derived from IVDs, facet joints, and sacroiliac joints to be 42%, 31%, and 18%, respectively (1). Following the initial proposal of the concept of internal disc disruption (2), a consensus on chronic discogenic pain has been established over the past 35 years, and approximately 40% of patients with chronic axial LBP are now believed to be of discogenic origin (1).

To date, provocation discography has been identified as the most effective method for diagnosing chronic discogenic pain (3). However, apart from identifying the origin of the pain, there have been no characteristic findings regarding the pathologic status, prognosis, or underlying causes of IVDs. In addition, although various invasive procedures have been proposed for painful discs, there is a lack of clear evidence regarding their therapeutic efficacy or long-term benefits (4). Thus, basic research on animal models and human tissues are required to elucidate this poorly established pathophysiology.

Degeneration-driven changes in the mechanical macroenvironment of an internal disc can lead to the progressive changes of biochemical microenvironment that induce abnormal ingrowth of the nociceptor (5). Despite the time required for these environmental changes, spinal hypermobility contributes to the deterioration of disc degeneration as a single factor. Furthermore, mechanical changes caused by a single stab injury have been found to promote disc degeneration (6).

To emphasize that the human disc degeneration process proceeds over a long period of time, a model has been proposed for transmitting a continuous load force to the IVD (7). In terms of biomechanics, this model is more suitable than existing animal models, in which physical and chemical stimulation to IVD induces painful degeneration. However, although the study in which this model was proposed provided evidence through histological confirmation, it lacked biochemical evidence.

To this end, herein, it was hypothesized that the continuous application of static shear force would increase biochemical factors related to pain degeneration without direct macrodamage of outer annulus fibrosus in this discogenic pain animal model.

METHODS

Sprague–Dawley rats (n = 15, 15-17 weeks old, weighing 200-250 g; Orient Bio Inc, Seongnam, Republic of Korea) were separated into 3 groups according to the period for which sustained dorsoventral shear force was applied (Fig. 1). The simple randomization allocated animals based on random numbers. A custom-designed device (spinous attachment unit; SAU) without a loading spring was installed for each rat in the control group (n = 5). For experimental group 1 (n = 5), SAUs with a loading spring were installed for one week, while for experimental group 2 (n = 5), SAUs with a loading spring were installed for 2 weeks. The rats were housed in hanging wire cages in a room maintained on a 12-hour light-dark cycle at 22-25°C, with free access to food and water. The room temperature was kept at 23 ± 1°C with the humidity at 50 ± 5%. All animal experiments were approved by the Institutional Animal Care and Use Committee (approval #2014AN0163), and conducted in accordance with the relevant animal guidelines. The study was carried out in compliance with the Animals in Research: Reporting In Vivo Experiments guidelines.

The device was manufactured with the support of the inventor, based on a previously published article (7). The device was constructed using stainless steel and custom-designed to dorsoventrally apply a static shear load of up to 4 N. To this end, it used a coiled spring that was applied to the L5-L6 IV joints via a shear loading device attached to each rat's L5 and L6 vertebrae. The details of device and surgical protocol were presented in the supplementary data (Figs. S1-S3).

The rats were confined to a customized platform (30 × 30 × 30 cm) in a testing chamber made of transparent acrylic; their behavioral patterns were analyzed. Behavioral tests for mechanical allodynia in the 3 groups were performed one day before surgery, 7 days after surgery, and 8 (at which point the module was installed to generate shear load), 11, 14, 17, and 20 days after surgery. To examine the pain behavior in response to mechanical stimulation, the most sensitive part of the rat's hind paw was stimulated approximately 5-7 times using a series of calibrated von Frey filaments (3.92, 5.88, 9.80, 19.60, 39.20, 58.80, 78.40, and 147.00 mN, for 0.4 [equivalent to 0.00 from 1 g], 2.0, 4.0, 6.0, 8.0, and 15.0, respectively; Stoelting, Wood Dale, IL). This test normally does not cause pain, and a positive sign was marked on the filament that showed an avoidance response. The data were quantified, and in cases where the response score significantly increased compared to before surgery were taken to reflect mechanical allodynia.

The animals were sacrificed at one (n = 5) or 2 weeks (n = 5) in the 2 experimental groups, and at 2 weeks (n = 5) in the control group after the implantation of the SAU. Immediately after sacrifice, the lumbar segments (L3-S1) were removed and excess soft tissue was carefully washed to preserve IVD integrity; it was then placed in 10% neutral buffered formalin solution for 72 hours. Venous blood was collected for serological analysis and stored on ice for 45 minutes. After centrifugation at 2000 × g for 10 minutes at 4°C, serum was collected and stored in aliquots at -80 °C until further analysis. The following biochemical factors were analyzed: cytokines, such as interleukin (IL)-1β, IL-5, IL-6, IL-10, IL-12, tumor necrosis factor (TNF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF)-β. The bilateral sixth dorsal root ganglion (DRG) was removed. The protocol for Western blot analysis was applied in the method described in the previous study (8).

The Kruskal-Wallis test was used to assess the differences in cytokine and growth factor levels between the groups. The Mann-Whitney U test was used for intergroup comparisons of each factor. The surgeon, data collectors, and data analyzers were blinded to the experimental groups. For statistical analysis, IBM SPSS Statistics for Windows, Version 22.0 (IBM Corporation, Armonk, NY) was used, with $P < 0.05$ being taken to represent statistical significance.

RESULTS

Discogenic Animal Model

Fifteen rats were randomly assigned to 3 groups, and successfully underwent the surgical operations (Fig. 2). After the implantation of SAUs, all the rats survived without significant weight loss until they were sacrificed. During the immediate postoperative period (one week), as well as the planned follow-up period (additional one or 2 weeks), the rats showed no signs of noticeable discomfort or stress, such as loss of eating, drinking, or grooming. The experimental groups (n = 10) with shear load application continued to gain weight similar to the control group (n = 5).

Mechanical Allodynia

Before applying the shear force load (SFL), the hind paw withdrawal thresholds were nearly 13 N in both the control and experimental groups. The thresholds did not change significantly in the control group during the 2-week assessment (P = 0.21); however, a gradual decrease was observed in the experimental groups (P
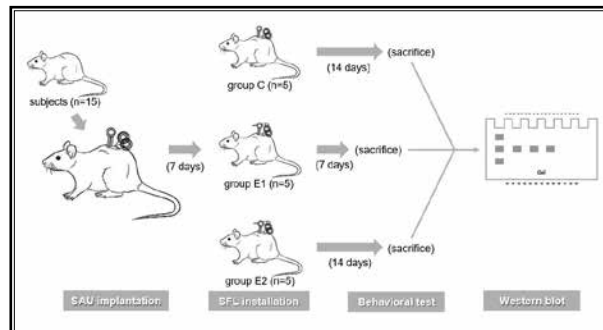

Fig. 1. *SAUs had a lower part of attachment saddle for vertebrae and an upper part for external connection. (a) The ESLDs were assembled in the SAUs as a test to check for defects in the instruments. (b) The SAUs were implanted into the L5 and L6 spinous processes in all 3 groups, including a control group. (c, d) After a 7-day recovery period of SAUs implantation, the external shear loading module was assembled. (e, f, g) The coil spring was designed to produce a shear force of 4 N. (h, i) The overall appearance of a rat in which the surgical wound was recovered and the implanted device was stable, and the external module was assembled and a shear force was applied. SAUs, spinous attachment units; ESLD, external shear loading device.*

Fig. 2. *Effect on SFL-induced mechanical allodynia in rats. Square: control (C); empty circle: experimental group with 1-week shear load (E1); closed circle: experimental group with 2-week shear load (E2). OP is the insertion of an SAU in L5 and L6 vertebral bodies. One week of healing at the surgical site, when the shear load module is coupled with the external connection part of the SAU, shear force begins to occur. Each value represents the mean with standard deviation (error bars) for the von Frey test. *P < 0.05. SFL, shear force load; OP, surgical operation; SAU, spinous attachment unit*

= 0.02). That is, a statistically significant difference appeared between the control and experimental groups from day 4 of SFL (Fig. 3). These results suggest that the SFL on the L5/L6 IVD resulted in mechanical allodynia.

Determination of Cytokines and Growth Factors

In the DRG tissues, significant differences were observed in the IL-6 ($P = 0.02$), neurogrowth factor (NGF; $P = 0.03$), TGF-α ($P = 0.03$), PDGF-β ($P = 0.01$), and vascular endothelial growth factor (VEGF; $P = 0.01$) levels between the 3 groups. In the intergroup comparisons for these factors, IL-6, NGF, PDGF-β, and VEGF showed significant increases after 2 weeks, without significant changes after one week; whereas, TGF-α was significantly increased after one week and continued to increase until the end of the study period (Fig. 4).

Within serum samples, significant differences were detected in TNF-α ($P = 0.04$), IL-1β ($P = 0.01$), IL-5 ($P = 0.01$), IL-6 ($P = 0.01$), IL-12 ($P = 0.04$), NGF ($P = 0.01$), TGF-α ($P = 0.04$), PDGF-β ($P = 0.02$), and VEGF ($P = 0.03$) between the 3 groups. In the intergroup comparisons for these factors, the levels of TNF-α, IL-1β, IL-5, IL-12, and NGF showed significant increases after one week and maintained these levels, or presented with

decreasing tendencies after 2 weeks. Meanwhile, the abundance of IL-6, TGF-α, PDGF-β, and VEGF were not significantly altered after one week, however, were increased in the following 2 weeks (Fig. 5).

Discussion

Biomechanical researches have suggested the presence of a significant relationship between the external force loaded on the spine and degeneration (9). Delivering mechanical stimulation to IVDs in animal models can cause cellular and structural changes. The axial compression load induces cell death in the IV limit and causes loss of tension in collagen fibers (10). In addition, a long-term continuous load can upregulate the digestion of IVDs and the expression of genes encoding anabolic molecules (11). These changes induce structural changes, such as the decomposition of annulus fibrosus. The animal model in this study differs from the structural damage model of IVDs in the induction of degeneration by the indirect intervention (i.e., mechanical environment) without direct injuries to IVDs. Models that cause chemical (i.e., chemopapain and chondroitinase ABC) and physical (i.e., drilling, resecting, stabbing, and incising) damage have been applied in attempts to prove that structural degenerative
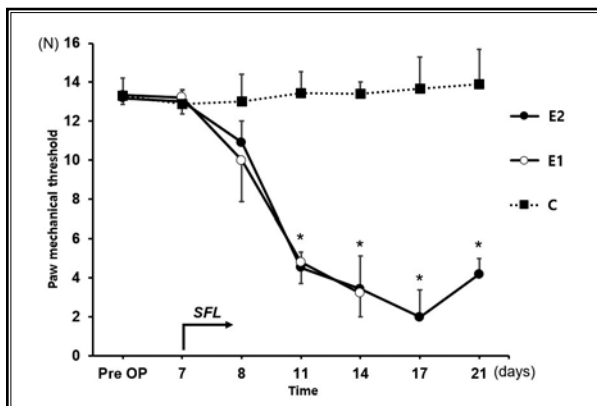

Fig. 3. *Western blot analysis and relative levels of cytokines and growth factors in the DRG tissues of 3 groups. C: control group; E1: experimental group with 1-week shear load; E2: experimental group with 2-week shear load. The quantities represented by gel bands are expressed as levels relative to α-tubulin. Western blots of TNF-α, IL-1β, IL-5, IL-6, IL-10, IL-12, NGF, TGF-α, PDGF-β, and VEGF. The data represent the box plots with outliers. *P < 0.05. DRG, dorsal root ganglion; TNF, tumor necrosis factor; IL, interleukin; NGF, neurogrowth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.*
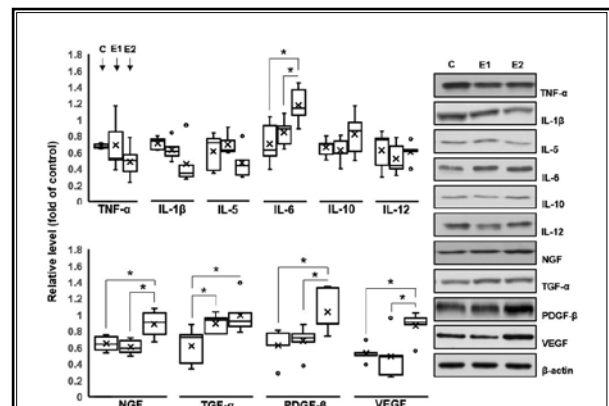
Fig. 4. *Western blot analysis and relative levels of cytokines and growth factors in the serum of 3 groups. C: control group; E1: experimental group with 1-week shear load; E2: experimental group with 2-week shear load. The quantities represented by gel bands are expressed as levels relative to α-tubulin. Western blots of TNF-α, IL-1β, IL-5, IL-6, IL-10, IL-12, NGF, TGF-α, PDGF-β, and VEGF. The data represent the box plots with outliers. *P < 0.05. TNF, tumor necrosis factor; IL, interleukin; NGF, neurogrowth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.*

changes occur through excessive inflammatory reactions (12). Histological features that accompany degenerative changes in the human body, such as a decrease in the height of the IVD due to a loss of proteoglycan, loss of cellularity, and the destruction of annulus, are insufficient to explain the pathophysiological mechanisms (13).

Most effects elicited by gradual accumulation of mechanical loads are imparted through pressure conditions, such as bending, postural change, and cyclical compression. For instance, studies using the bending of rat tails have shown that fibroblasts occurred on the bended side (i.e., where constructive pressure was transmitted); however, it has proved difficult to rule out direct disc damage (14). Furthermore, enforcing compulsory bipedal walking for quadrupedal animals was found not to guarantee a sufficient activity time (15). Moreover, applying cyclical pressure using an Ilizarov-type apparatus was found to result in an increase of proteoglycan content, contrary to expectation (16). Instability models that operate through surgical methods have shown more significant results. For mice, in which the segmental stability of the spine was gradually weakened by resecting its posterior structures (i.e., facet points, spinous processes, and interpersonal ligaments), evidence of degenerative changes was induced after 12 months (17).

The continuous shear force used in this animal model interfered with normal load delivery, based on the premise of the follower load concept for the stability of the lumbar spine. The effects of transmitting torsion to IVDs in animal experiments suggest that shear deformation contributes to microstructural damage (18). In addition, the transmittance of shear force to the lumbar spine may be a significant external force with respect to its anatomical structure and dynamic performance (19).

In a previous study (7), the histological features of animal applying this device have shown definitive signs of degeneration. The authors reported that with a continuous shear load of one week, disc degeneration occurred in 5 of 6 IVDs, which represents a relatively rapid change compared to other mechanical animal models. The difference in histological findings between weeks one and 2 was not significant; however, the degree of degeneration and the accumulation effect of shear force may have been different. Furthermore, it is possible that 2 weeks were insufficient to observe the accumulation effect (7). In addition, though the intervention was only targeted on a single segment, disc
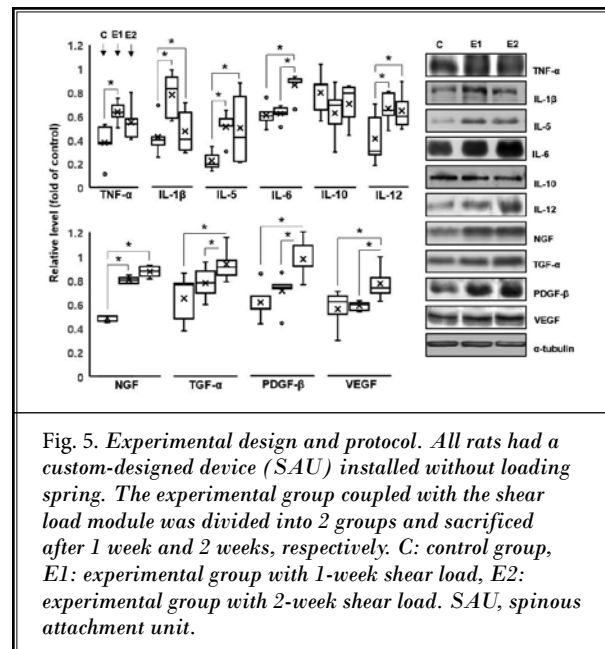

Fig. 5. *Experimental design and protocol. All rats had a custom-designed device (SAU) installed without loading spring. The experimental group coupled with the shear load module was divided into 2 groups and sacrificed after 1 week and 2 weeks, respectively. C: control group, E1: experimental group with 1-week shear load, E2: experimental group with 2-week shear load. SAU, spinous attachment unit.*

degeneration also appeared in adjacent segments; this was attributed to the complex function of the paravertebral muscles regarding the shear force applied to the local area (7).

The confirmation of the biochemical response in the present animal model supports the conventional consensus that the upregulation of proinflammatory mediators is closely related to the mechanism of discogenic pain. Many researchers have observed the upregulation of various proinflammatory molecules in animal models and human IVD tissues, including TNF-α, IL-1, IL-6, IL-8, nitric oxide-producing enzymes, prostaglandin E2, and NGF (20). Temporary inflammatory responses were observed in IVD-damaged animal models, including the production of TNF-a (20), IL-1β (21), IL-6 (20), monooxidase (22), and NGF (20). The continuous upregulation of proinflammatory mediators, including NGF, TNF-α, IL-1β, and IL-6, has been reported in models that deliver dynamic pressure loads in rat tails (23). Herein, levels of specific serum cytokines and growth factors, namely, TNF-α, IL-1β, IL-5, IL-12, and NGF, were increased at one week after the shear load, and maintained at 2 weeks. Meanwhile, IL-6, TGF-α, PDGF-β, and VEGF were only found to be increased at 2 weeks.

TNF-α is not involved in the initiation of IVD degeneration; however, it may be associated with the further progression of degeneration and clinical symptoms. TNF-α contributes to the upregulation of substrate matrix metalloproteinase (MMP) activity, stimulation

of cytokines, such as IL-1, IL-6, IL-8, and prostaglandin E2, reduction of proteoglycan synthesis, and the development of inflammatory overregulation (24). The expression of IL-1β has been found to increase in painful IVDs, and to mediate inflammatory response (25). Few studies have investigated IL-5 in relation to IVD disease. However, it is known to mediate eosinophil activation (26), and in this study, its preference was considered to be a reaction of cutaneous irritation by metal insertion in vivo. Insignificant changes in IL-10, known as an anti-inflammatory cytokine, are thought to be inhibited by unknown cascades. Increases in IL-10 have been reported in IVD herniation models and acute IVD injury (27). Unlike IVD herniation, which usually show a positive prognosis, the clinical chronicity of discogenic pain may be associated with failure to stimulate IL-10. Animal studies (12,28) have identified IL-10 as a possible treatment for delaying disc degeneration. A recent study (29) has shown that the mediating function of IL-10 in IVD disease is very complex. During disc degeneration, IL-10 decreases the number of cells in the nucleus pulposus (NP) and deteriorates the microenvironment. However, this is not specific and it has also been shown to be increased in disc herniation (30).

Elevated cyclic IL-6 levels in patients with degenerative IVDs is consistent with the observed differences in disc tissue cytokine levels, based on diagnosis (31). Cyclic IL-6 levels were found to be higher in the samples of patients whose discogenic pain was identified, compared to in those of patients with herniated IVDs (31). IL-6 can be spontaneously produced in vitro by human disc herniation and degenerative (i.e., non-ionic) discs, and genetic changes in IL-6 have been associated with increased risks of IVD disease with sciatica (32). Moreover, IL-6 stimulation of human NP cells downregulates extracellular matrix gene expression, and prostaglandin synthesis (33). In other animal models, IL-6 makes the DRG sensitive to painful stimuli (34). A clinical studies (35) has reported that serum IL-6 levels play a higher and broader role in chronic IVD disease (i.e., degenerative spondylosis and spinal stenosis) than in acute IVD disease (i.e., IVD herniation). Whether circulating IL-6 causes degenerative change and pain remains unknown, as does whether elevated cytokine levels result from deterioration and pain. Increased level of serum IL-6 have been associated with worse outcomes in the recovery from IVD herniation after one year (36). Although the effects of elevated serum cytokines in spinal stenosis or disc degeneration on clinical outcomes are unknown, elevated IL-6 levels may be associated with similar-to-worse outcomes. The results of this study also showed a delayed increase in serum IL-6, and a similar pattern was observed in the DRG tissue. These findings suggest that IL-6 played an exclusive role in neuropathological mechanisms.

NGF plays an important role in inflammatory pain (37). NGF levels increase dramatically in inflammatory tissues, and the enhanced delivery of NGF activates and sensitizes primary afferent nerves. In addition, painful discs have shown higher levels of NGF-positive nerve fibers than asymptomatic IVD (37). The direct application of anti-NGF antibodies to IVD have been shown to inhibit the neuropeptide markers of pain in mice (38). Moreover, painful discs have been shown to have nerve fibers that express NGF receptors and microvascular vessels that express NGF (37). Most DRG neurons inside the IVDs of mice are NGF-dependent (39). NGF-dependent calcitonin gene-related peptide-immunoreactive nerve fibers have been reported in humans (40). Here, NGF levels increased in serum from the first week, and in DRG tissue at the second week. As NGF can be expressed in disc nucleus cells, this could have contributed to the observed increase in NGF serum levels in the early phase. The growth factors clearly increased as the SFL accumulated. This may be the basis for the cellular adaptation to the environment (37). These results provide evidence that this animal model adequately reflects the pathological state of the spine that progresses throughout the whole lives of humans.

Increased serum growth factors are associated with recovery reactions. TGF-β is a powerful cell proliferation stimulator (41). In addition, it reduces the activity of nuclear MMP-2 and stimulates proteoglycan production (42). PDGF promotes the angiogenesis and growth of existing blood vessels. In the disc degeneration rabbit model, PDFG was found to significantly reduce disc degeneration, maintain disc structure, and promote biomechanical function by preventing apoptosis and increasing collagen-3 matrix production (43). Moreover, PDGF has been reported to inhibit IVD apoptosis and promote the expression of anabolic genes (44). The mediation of the neovascularization mechanism by VEGF and receptors has been closely related to inflammation, chronic back pain, and IVD degeneration (45). However, a recent study (46) has suggested that another pathological angiogenesis pathway other than VEGF exists during the degeneration of the IVD.

Here, in the DRG tissue of the experimental groups, IL-6, NGF, TGF-α, PDGF-β, and VEGF all showed statistically significant increases. TGF-α in-

creased at one week, which was maintained in the second week; meanwhile, IL-6, NGF, PDGF-β, and VEGF showed a statistically significant increase in the second week, however, did not show any significant change at one week. Previous studies (20,23) into discogenic pain have shown that among various biochemical factors, IL-6 and NGF are both significant factors that are inherently increased in tissues and serum, in relation to symptoms of IVD pain. However, unlike the results of previous studies, which were caused by direct IVD-damage, the effects observed in the current study were caused by continuous shear load (over 2 weeks). The observed 2-week increases in PDGF and VEGF levels also supported neuropathological changes. However, it is unclear why there were no significant changes of other cytokine levels at DRG tissues; TNF-α, IL-1β, IL-5, IL-10, and IL-12, which have appeared in acute tissue injury in other studies. The levels of TNF-α and IL-1β at DRG tissues, rather, exhibited a decreasing tendency in the second week. This pattern may have occurred due to a phenomenon caused by the transmission of pathochemical mediators due to the degeneration of IVD tissue, rather than due to damage to peripheral nerve endings; that is, unlike the biochemical changes of DRGs shown in the peripheral nerve injury model, here it seems that the signal transduction of nerve end terminals that sensed the degenerative changes of the extraneural tissues gradually induced an increase in growth factor expression. Unlike the induction of transient inflammatory injury in animal models by complete Freund's adjuvant injection, continuous shear force transmission may represent a different pathochemical mechanism for degeneration. In detail, it is caused by repeated injuries, within the limits of physical endurance, to IVD tissue. The delayed increase in the expression of growth factors at 2 weeks supports this phenomenon. Cytokines without a significant increase may be undetectable after exceeding a given injury threshold, due to an insufficient follow-up period, or due to unknown mediation procedures.

Limitations

The limitations of this study include the general limitations of quadrupedal animals, the poor precision and flexural deformation of shear force devices, and inaccuracies regarding the evaluation of histological denaturation, similarly to previous studies. Moreover, the intervention and observational periods were relatively short, and no consideration was given to post-biochemical reactions. Nevertheless, the present model achieved faster degenerative changes and related evidence than other models, and so may be advantageous. In addition, here the histological evaluation was not reconfirmed, which represents a further limitation. In previous studies, the degrees of degeneration in IVDs were confirmed to be mild and moderate, and one out of 6 IVDs did not show degenerative changes during the first week. However, this limitation could be minimized through a control setting, and it was judged that this analysis was not essential for evaluating the efficacy of animal models because the timing of histological and biochemical changes in IVD degeneration did not necessarily match.

CONCLUSIONS

The animal model employed here demonstrated biochemical reactions along with histological evidence for IVD pain. The model may be very similar to the theorical development of degenerative spinal disorders, and be appropriate for evaluating biochemical interventions. This model was able to induce denaturation relatively quickly, and it exhibited pain behavioral characteristics corresponding to clinical pain. The clinical perspectives to the results may be as follows: an experimental basis to refute the existing bias that tissue damage by vertical loads is preceded to degeneration, and a neurological basis for dermatomal symptoms due to an increase of inflammatory substances in DRG tissue accompanying disc degeneration without direct neural compression. Further research results from long-term observations are needed in the future, and various evaluations are required.

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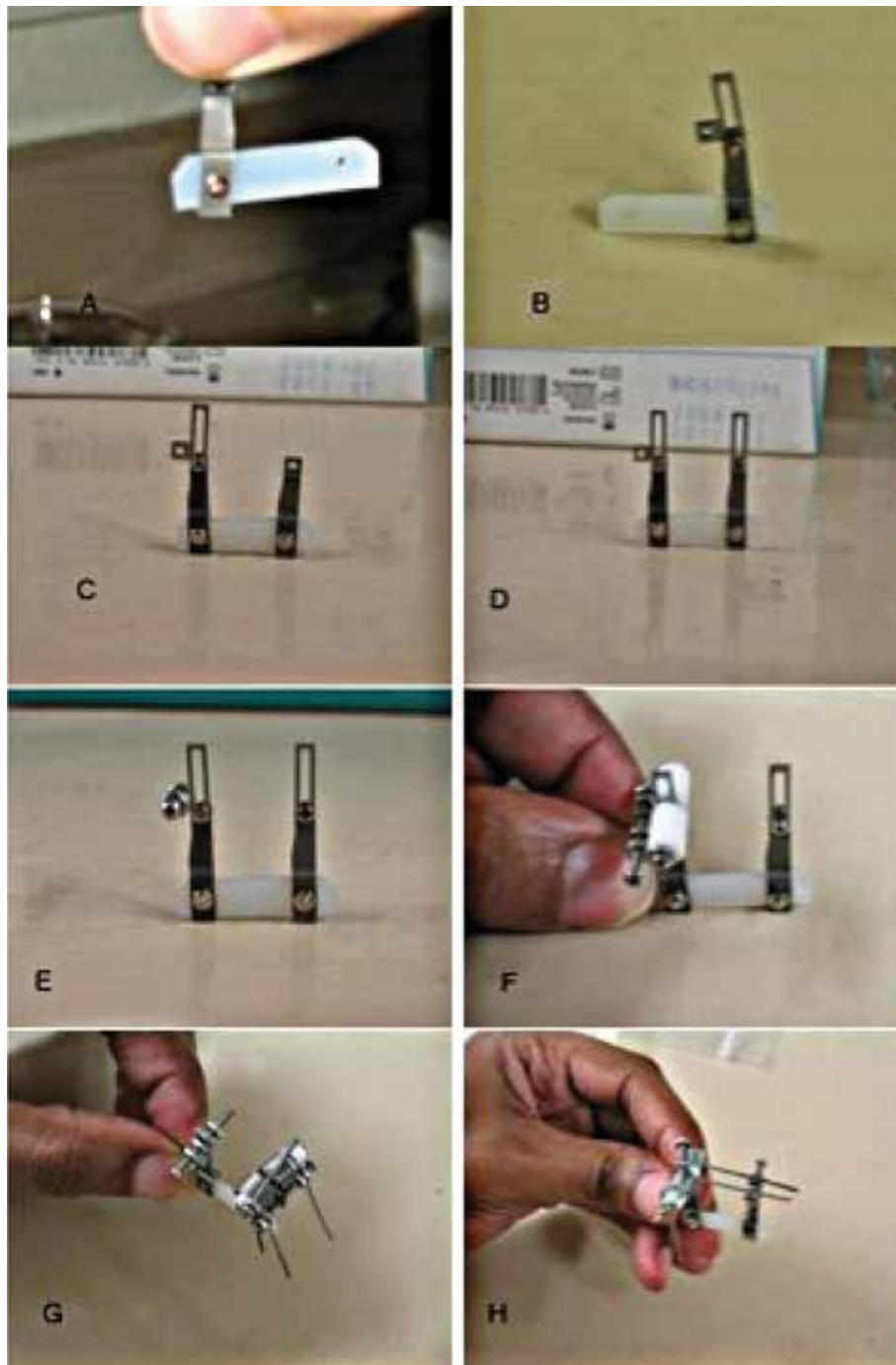
Fig. S1. *Assembly of the SAU and ESLD. (A) Attachment of the SAU. (B) Attachment of the L-shaped ESLD. (C) Attachment of the second SAU. (D) Attachment of the ESLD to the L-6 spinous process. (E) Attachment of the bolt to the extended portion of the L-shaped ESLD. (F) Attachment of the polymer-bolt piece to the L-5 ESLD. (G) Correct spring adherence to the extended portion of the L-shaped ESLD. (H) Spring loading on the entire apparatus. SAU, spinous attachment unit; ESLD, external shear loading device.*
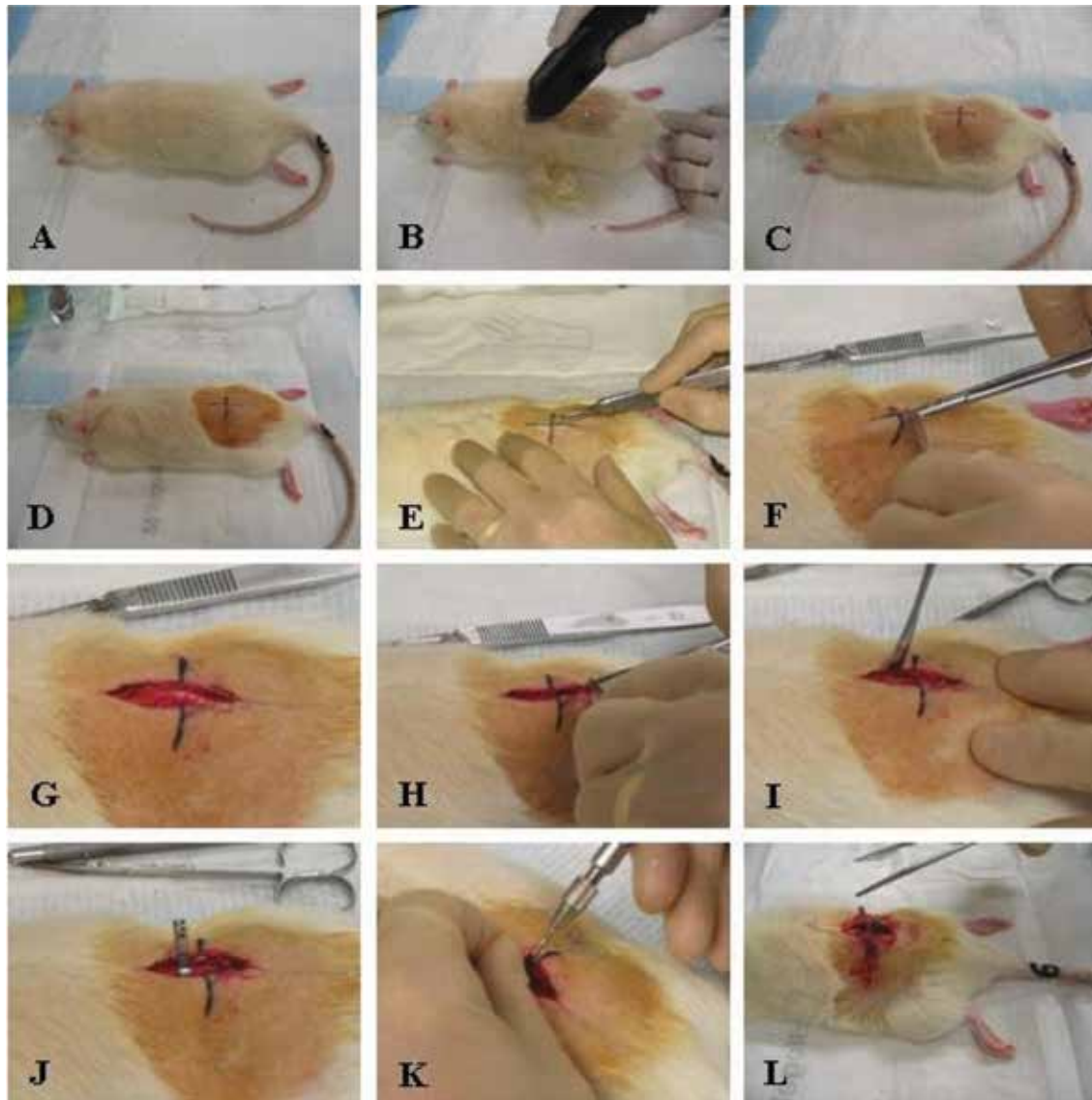
Fig. S2. *Surgical procedures for SAU implantation in rats. (A) a rat anesthetized with isoflurane; (B) hair removal from the surgical area; (C) identification of L6 spinous process by palpating the iliac crests and a guide line drawn over the spinous process of L6; (D) disinfection of the surgical area with iodine; (E, F) skin incision made over a guideline, approximately 2 cm; (G, H) incision of subcutaneous fascia; (I) incision of lumbo-dorsal fascia and separation of paraspinal muscle from both sides of the L5 and L6 spinous processes using Freer chisel; (J) placement of front SAU on L5 spinous process to be used as a drill guide; (K) drilling to secure the SAU to the spinous process; (L) completed implantation of the front SAU on L5 spinous process after the screw tightened. Procedure is identical for the rear SAU implantation on L6 spinous process. SAU, spinous attachment unit.*
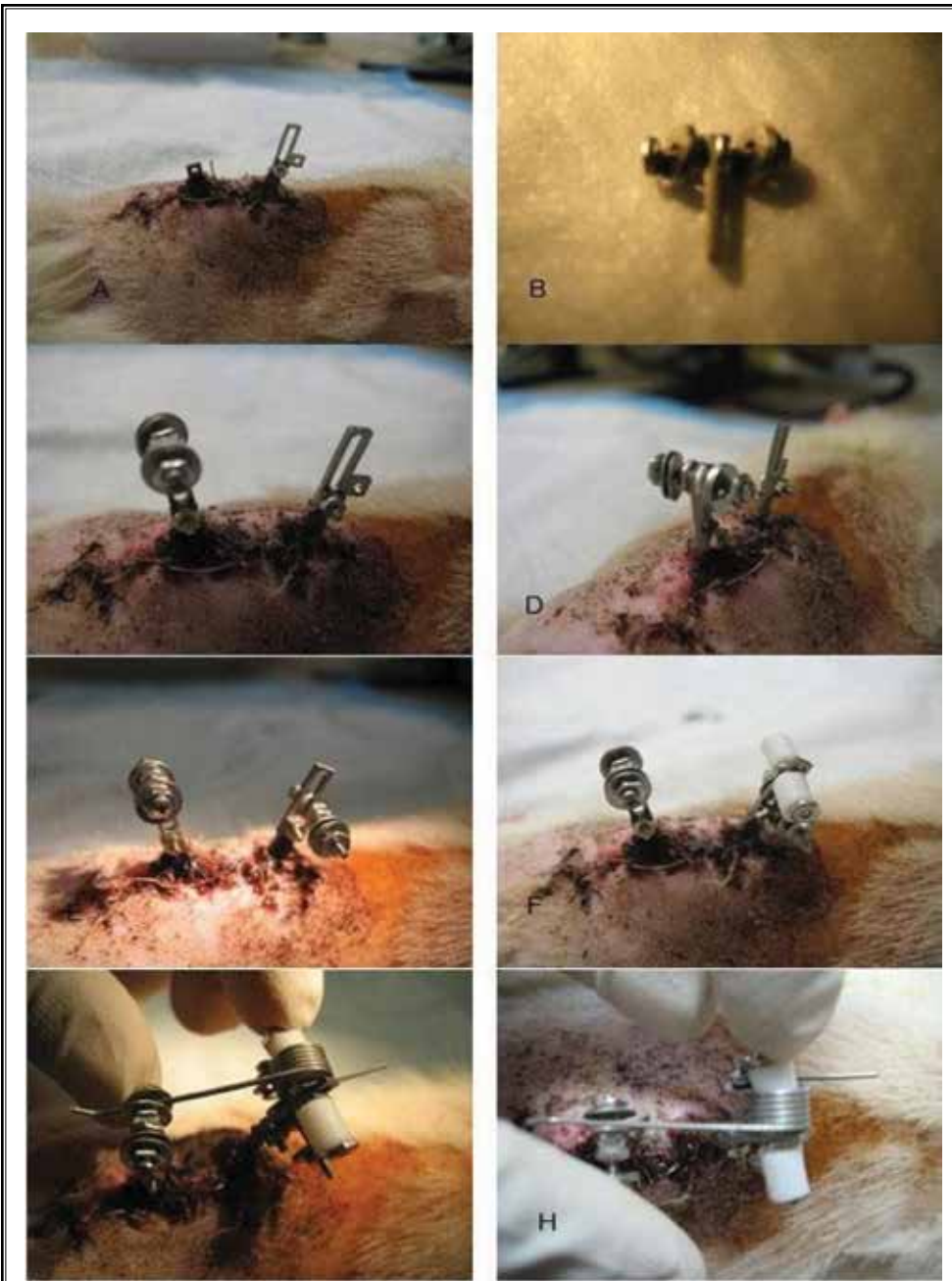
Fig. S3. *ESLD attachment to SAU. (A) Attachment of the L-shaped ESLD. (B) Assembly of the bolt. (C) Attachment of the bolt to the L6 spinous process. (D) Picture of the bolt attached to the L6 spinous process. (E) Attachment of the bolt to the extended portion of the L-shaped ESLD. (F) Attachment of the assembled polymer-bolt piece. (G, H) Loading of the spring onto the ESLD. ESLD, external shear loading device; SAU, spinous attachment unit.*