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Laboratory Investigation

The Effect of GCSB-5 a New Herbal Medicine on Changes in Pain Behavior and Neuroglial Activation in a Rat Model of Lumbar Disc Herniation

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Objective : Lumbar disc herniation can induce sciatica by mechanical compression and/or chemical irritation. The aim of this study was to compare the effects of GCSB-5 (Shinbaro®) and NSAIDs on pain-related behavior and on the expressions of microglia, astrocytes, CGRP, TRPV1, IL-6, and CX3CL1 in a rat model of lumbar disc herniation.

Methods : 112 male Sprague-Dawley rats underwent implantation of nucleus pulposus to a dorsal root ganglion (DRG). Rats were divided into five groups as follows; a saline group (the vehicle control group) (n=27), a 10 mg/kg aceclofenac group (the aceclofenac group) (n=22), and 100, 300 or 600 mg/kg GCSB-5 groups (the GCSB-5 100, 300, or 600 groups) (n=21 for each group). Rats were tested for mechanical allodynia at 3 days after surgery and at 1 day, 3 days, 7 days, 14 days, 21 days, 28 days, 35 days, 42 days, 49 days, and 56 days after treatment commencement. Immunohistochemical staining of microglia (Iba1), astrocytes (GFAP), CGRP, and TRPV1, and PCR for IL-6 and CX3CL1 were performed on spinal dorsal horns and DRGs at 56 days after medication commencement.

Results : After 56 days of GCSB-5 300 administration, mechanical withdrawal thresholds were significantly increased (p<0.05), and immunohistochemical expressions of Iba1, GFAP, CGRP, and TRPV1 were reduced than other groups, but this difference was not statistically significant.

Conclusion : These results indicate GCSB-5 reduces mechanical allodynia and downregulates neuroglial activity and the expressions of CGRP and TRPV1 in the spinal segments of a rat model of lumbar disc herniation.

Key Words : GCSB-5 · Lumbar disc herniation · Neuropathic pain · Microglia · Astrocytes · Calcitonin gene-related peptide.

INTRODUCTION

Lumbar disc herniation can injure spinal nerve roots and cause severe radicular pain, characterized by hyperalgesia, allodynia, decreased conduction velocity, and histologic changes[34]. Previous studies have suggested that the clinical signs of lumbar disc herniation are caused by chemical factors released from the nucleus pulposus and by mechanical compression of lumbar nerve roots[2,21,32-34,48]. Proinflammatory cytokines, such as, tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), and interleukin-6 (IL-6), and nitric oxide and phospholipase A2 are known as chemical inducers of sciatica[3,4,15,20,33]. Nucleus pulposus contains TNF-α, and when nucleus pulposus is applied to a dorsal root ganglion (DRG) it induces pain-related behavior and causes morphological and functional changes in DRGs and spinal cords in rats[15,32-34,38]. Various medications are used to treat radicular pain. Non-steroidal anti-inflammatory drugs (NSAIDs) are generally used as analgesics, and most of the analgesic effects of anti-inflammatory drugs are due to blocking of the syntheses of inflammatory products from arachidonic acid. However, this interruption can generate undesirable adverse drug reactions, such as, gastrointestinal and cardiovascular problems[43].

GCSB-5 (Shinbaro®; Green Cross Corp., Yongin, Korea) is a medicine prepared from six herbs (Ledebouriellae Radix, Achyranthis Radix, Acanthopanacis Cortex, Cibotii Rhizoma, Glycine Semen, and Eucommiae Cortex), which are used in traditional East Asian medicine to treat osteoarthritis. A mixture containing fixed ratios of the six herbs was powdered and boiled

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in distilled water. The resulting extract was ultrafiltered to exclude the components with molecular weights exceeding 10000. The extract was then dried to a powder by freeze drying. A capsule of GCSB-5 contains 300 mg of the dried extract[25]. Although the active ingredients of GCSB-5 have not been completely identified, recent studies have described the analgesic and anti-inflammatory effects of Ledebouriellae Radix[24], the antioxidant and anti-inflammatory effects of Achyranthis Radix[14], and the antioxidant and analgesic effects of Eucommiae Cortex[12]. However, mechanistic evidence regarding molecular changes in spinal segments induced by GCSB-5 after lumbar disc herniation is lacking, and the comparative effects of GCSB-5 and NSAIDs on radicular pain have not been well studied.

This study was undertaken to compare the effects of aceclofenac (a NSAID) and GCSB-5 on pain-related behavior and on the expressions of Ionized calcium-binding adapter molecule 1 (Iba1), Glial fibrillary acidic protein (GFAP), calcitonin gene-related peptide (CGRP), transient receptor potential vanilloid 1 (TRPV1), IL-6, and CX3CL1 in a rat model of lumbar disc herniation.

MATERIALS AND METHODS

Animals

A total of 112 female Sprague-Dawley rats (200−220 g) were used. The animals were housed in plastic cages at room temperature under a 12-h light-dark cycle with free access to food and water. All experiments were conducted in a humane manner in accordance with the guidelines issued by the Institutional Animal Care and Use Committee of Yeungnam University, Korea.

Lumbar disc herniation procedures

Rats were anesthetized with an intraperitoneal injection of zoletil (Virbac; 50 mg/kg). With an animal placed prone, an incision of ~1 cm was made on the dorsal surface of the proximal tail for autologous nucleus pulposus harvesting. The disc between the second and third coccygeal vertebrae was incised, and nucleus pulposus was harvested by curette. A midline dorsal incision was then made over the lumbar spine, multifidus muscles were separated along the L4−S1 spinous processes, and left L5 nerve roots and DRGs were exposed by laminectomy. The harvested nucleus pulposus was then implanted next to the left L5 nerve root just proximal to DRG without applying mechanical compression. Similar amounts of nucleus pulposus were implanted in an identical manner in all animals.

Treatment after nucleus pulposus implantation

After nucleus pulposus implantation animals were divided into five treatment groups : a saline group (the vehicle control group) (n=27), a 10 mg/kg aceclofenac group (the aceclofenac group) (n=22), and 100, 300, and 600 mg/kg GCSB-5 groups (the GCSB-5 100, 300, and 600 groups) (n=21 for each group). Drugs were dissolved in 0.9% saline for administration. The drug concentrations used were based on clinical levels. Animals received 1.5 mL of the designated treatment solution orally once daily for 8 weeks beginning 3 days after surgery.

Pain behavior evaluation

Mechanical sensitivities of the plantar surfaces of ipsilateral hind paws were tested at 3 days after surgery and at 1 day, 3 days, 7 days, 14 days, 21 days, 28 days, 35 days, 42 days, 49 days, and 56 days after treatment commencement (Fig. 1). Mechanical allodynia was determined by measuring withdrawal response to mechanical stimulation of ipsilateral hind paws with von Frey filaments (North Coast Medical Inc., Gilroy, CA, USA), which had been calibrated in grams. Rats were placed in a clear plastic cage with a metal mesh floor, adapted to the testing environment for 30 minutes, and then the plantar surface of each hind paw was stimulated to cause slight filament bending for 5 seconds. Testing was started using a 1.4 g probe, and probe resistance was increased until a filament produced a consistent withdrawal response to more than 3 of 5 stimuli. Filaments were applied in increasing and decreasing thicknesses. 50% probability thresholds of mechanical paw withdrawal were then calculated. If no withdrawal response was elicited by the 26-g filament, a mechanical threshold of 26 g was assigned.

Immunohistochemical examination

To study microglial and astrocyte activations in dorsal horns and CGRP and TRPV1 expressions in DRGs, we euthanized 4−6

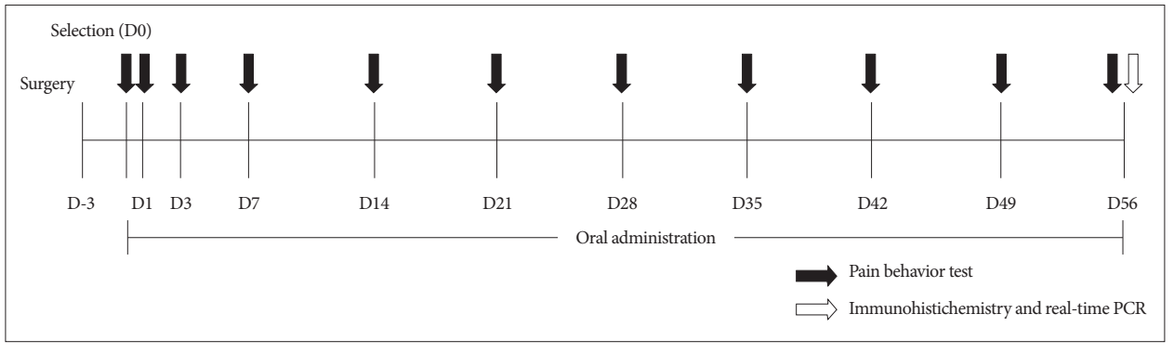

Fig. 1. Study flow schematic.

rats per group at 56 days after treatment commencement (Fig. 1). Under anesthesia, a catheter was inserted into the left ventricle, which was then rinsed with 500 mL of saline followed by 500 mL of 4% paraformaldehyde [in 0.1 N phosphate buffer (PB)]. The L5 spinal cord segment was removed, post-fixed for 2 days in the same fixative, and then were stored in 30% sucrose (in PB) for at least 24 hours. Transverse sections of spinal cord and of DRGs (30 and 20 μm thick, respectively) were prepared using a cryostat (Leica, Wetzlar, Germany) and stored in PB. All incubation and reaction procedures for multiple immunohistochemical staining were conducted at room temperature on a shaker. To enhance tissue penetration by antibodies, DRG sections were reacted with 50% ethanol for 30 minutes and rinsed with phosphate buffered saline (PBS) for 3×5 minutes and blocked to prevent nonspecific primary antibody reactions. Samples were then treated with 10% normal donkey serum (NDS; Jackson Immunoresearch, Westgrove, PA, USA). Tissue sections were incubated overnight in a mixture of primary antibodies, that is, mouse anti-ionized calcium-binding adapter molecule 1 (Iba1) (Wako, Osaka, Japan; 1 : 1000), mouse anti-glial fibrillary acidic protein (GFAP) (BD Pharmingen, San Jose, CA, USA; 1 : 100), anti-transient receptor potential vanilloid type 1 (TRPV1) (Neuromics, Edina, MN, USA; 1 : 5000), and anti-calcitonin gene related peptide (CGRP) (Enzo, Farmingdale, NY, USA; 1 : 200). After reaction completion, tissues were rinsed with PBS (3×5 minutes), treated with 2% NDS for 15 minutes, and incubated with cy3-conjugated donkey anti-mouse (Jackson Immunoresearch, PA, USA; 1 : 100), cy3-conjugated donkey anti-goat (Jackson Immunoresearch, PA, USA; 1 : 100), and Alexa 488-conjugated donkey anti-rabbit (Invitrogen, Eugene, OR, USA; 1 : 200) antibodies for 3 hours, rinsed with PBS, and mounted with Vectashield (Vector Lab, Burlingame, CA, USA). All antibodies were tested for sensitivity and specificity beforehand. The dilutions used were optimal, according to the manufacturers' recommendations. Immunofluorescent images were acquired using a cooled charge-coupled device (CCD) camera (Olympus DP71, Tokyo, Japan) attached to a light microscope (Olympus BX51, Tokyo, Japan).

Quantitative image analysis

To quantify immunostaining of Iba1 and GFAP in dorsal horns and CGRP and TRPV1 in DRGs, images were obtained of five spinal cord sections (for both Iba1 and GFAP) and of five DRG sections from L5 per rat. One image (898×660 mm) was taken with a CCD camera using the same shutter speed and digital gain of each spinal cord section. Images were encoded to blind the investigator before analysis. Pixels positive immunoreactive Iba1 or GFAP were identified by applying an appropriate threshold gray value, and area fractions (immunopositive area/total frame area) were calculated using image analysis software (Leica application suite V4.2, Leica Microsystems, Heerbrugg, Switzerland). Numbers of CGRP- and TRPV1-postive DRG cells were counted using the image analysis software at the same time.

Real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was performed on spinal cords and DRGs to assess the messenger RNA (mRNA) expressions of IL-6 and CX3CL1. 3−4 rats per group were euthanized at 56 days after treatment commencement and L5 spinal cord segments were divided into left (ipsilateral) and right (contralateral) halves, which included ventral and dorsal gray and white matter (Fig. 1). Total RNA was isolated from both halves of spinal cords and DRGs using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) and was conducted using 1 mg aliquots of total mRNA at 45°C using the High capacity cDNA reverse transcription kit (Applied Biosystems Inc., Foster City, CA, USA). Real-time PCR was performed using a Real Time ABI 7500 system (Applied Biosystems Inc., Foster City, CA, USA). Primers and a TaqMan probe were designed using ProbeFinder software (Universal Probe Library, UPL, Roche, Switzerland).

The following primers were used : 5'-TCCACTATCAACT GAACCAGGA-3' (sense) and 5'-TTGGGTCAGCACAGAAGT GT-3' (antisense) for CX3CL1 (GenBank accession number : NM134455), 5'-TATGAACAGCGATGACCACTG-3' (sense) and 5'-TTGCTCTGAATGACTCTGGCTT-3' (antisense) for IL-6, and 5'-ACCACCATGGAGAAGGCTGG-3' (sense) and 5'-CTCAGTGTAGCCCAGGATGC-3' (antisense) for hypoxanthine phosphoribosyl-transferase (HPRT, GenBank accession number : NM012583.2). HPRT gene was used as an internal reference control. The master mix used consisted of 10 μmol/L of UPL probe, 10 μmol/L of each primer (sense and antisense), 10 μL of 2× TaqMan master, and 2 μL of cDNA. All PCR reactions were run in duplicate. After preincubation at 95°C for 10 minutes, PCR was performed over 50 cycles of denaturation at 95°C for 10 s and annealing at 60°C for 30 s.

Statistical analysis

Outcomes were using descriptive analysis. Quantitative variables are presented as means (SDs). Paw withdrawal thresholds by time after treatment commencement, group, and interaction effects (time difference by group) were analyzed using repeated measures two factor analysis and multiple comparisons, and the results obtained were compared. One-way ANOVA was used to determine the significances of variable differences between D0 (the day of treatment commencement) and later times, and Scheffe test was used for multiple comparisons. *p*-values are presented for significant variables. All tests were 2-sided and *p*-values of <0.05 were considered significance. The analysis was conducted using IBM SPSS ver. 19.0.

RESULTS

Pain behavior

For rats in the GCSB-5 300 group, mechanical allodynia of ipsilateral hind paws was significantly attenuated at 56 days after treatment commencement (treatment day 56, TD56) than in vehicle control group ($p<0.05$). However, mechanical withdraw-

al thresholds were not significantly in the aceclofenac and vehicle control groups on TD56 (Fig. 2).

Microglia, Astrocytes, CGRP, and TRPV1

Immunohistochemical examination of in GCSB-5 groups for Iba1 and GFAP in dorsal horns, CGRP and TRPV1 in DRGs on TD56 revealed that numbers of microglia and astrocytes were attenuated in ipsilateral L5 dorsal horns and that CGRP and TRPV1 expressions were also attenuated in ipsilateral L5 DRGs versus vehicle controls. For all the proteins examined, the largest decreases were observed in the GCSB-5 300 group, but these were not statistically significant. In L5 dorsal horns, decreases in Iba1 immunoreactivity observed on TD56 were; 21% in the aceclofenac group and 43%, 73%, and 21% in the GCSB-5 100, 300, 600 groups versus the vehicle controls (Fig. 3). Corresponding decreases in GFAP immunoreactivity were 73%, 67%, 82%, and 63%, respectively (Fig. 4), decreases in CGRP were 21%, 54%, 77%, and 51% (Fig. 5), and decreases in TRPV1 were 7%, 38%, 55%, and 34%, respectively (Fig. 6).

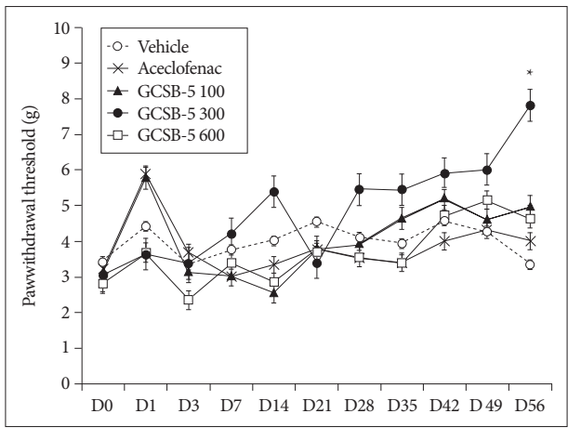

Fig. 2. Changes in mechanical withdrawal thresholds after drug or saline treatment in a rat model of lumbar disc herniation. GCSB-5 or aceclofenac was administered from 3 days after surgery. A significant increase in mean ipsilateral paw withdrawal threshold was found at 56 days after the commencement of GCSB-5 300 treatment versus vehicle controls. *Significantly different from vehicle group, $p<0.05$.

Expressions of IL-6 and CX3CL1 mRNA

The expressions of IL-6 mRNA in the aceclofenac and three GCSB-5 groups were not significantly different from those of vehicle controls on TD56, and at this time CX3CL1 immunoreactivity was also unaffected by aceclofenac or GCSB-5 (Fig. 7).

DISCUSSION

In the present study, we compared the effects of GCSB-5 and of aceclofenac (a NSAID) on pain-related behavior and neuroglial expression in a rat model of lumbar disc herniation. On TD56, mechanical withdraw thresholds in the GCSB-5 300 group were

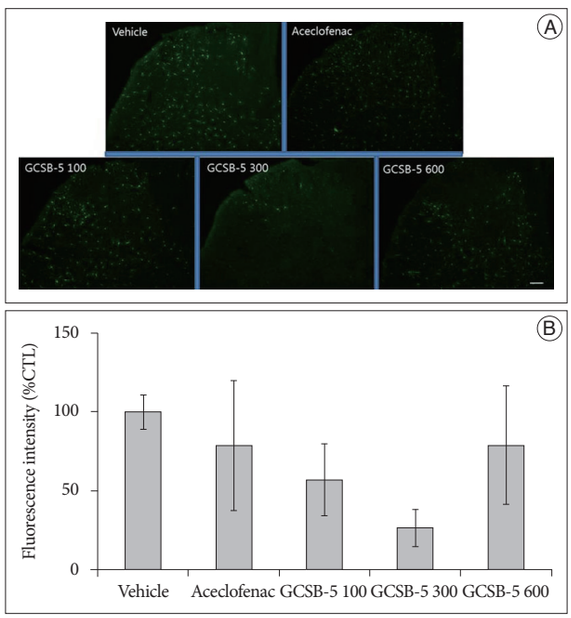

Fig. 3. Immunohistochemical expression of Iba1 in the ipsilateral L5 dorsal horn (A) and intensity of Iba1 immunoreaction versus vehicle controls (B) at 56 days after treatment commencement. A : Iba1-positive microglia numbers were lower in the ipsilateral dorsal horns of the GCSB-5 300 group than in the aceclofenac group or vehicle controls. B : the percentage decrease was greater in the GCSB-5 300 group than in the GCSB-5 100, GCSB-5 600, aceclofenac or vehicle control groups. Results are presented as mean±SEM. Bar=100 μm.

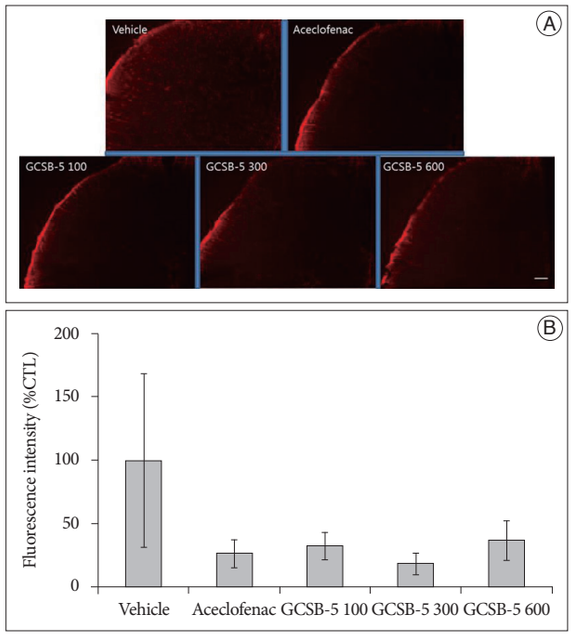

Fig. 4. Immunohistochemical expression of GFAP in ipsilateral L5 dorsal horns (A) and GFAP immunoreactivities versus vehicle controls (B) at 56 days after treatment commencement. A : GFAP-positive astrocyte numbers were lower in ipsilateral dorsal horns of the GCSB-5 300 group than in the aceclofenac or vehicle controls groups. B : Percentage decreases were greater in the GCSB-5 300 group than in the GCSB-5 100, GCSB-5 600, aceclofenac or vehicle control groups. Results are presented as mean±SEM. Bar=100 μm. GFAP : glial fibrillary acidic protein.

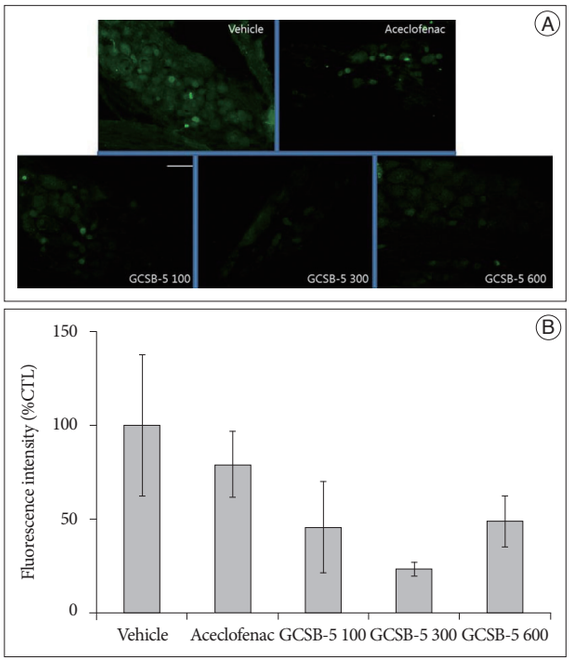

Fig. 5. Immunohistochemical expressions of CGRP in ipsilateral L5 dorsal root ganglions (DRGs) (A) and immunoreactivities of CGRP versus the vehicle controls (B) at 56 days after treatment commencement. A : CGRP expression was lower in ipsilateral DRGs in the GCSB-5 300 group than in the aceclofenac or vehicle control groups. B : Percentage decreases were greater in the GCSB-5 300 group than in the GCSB-5 100, GCSB-5 600, aceclofenac or vehicle control groups. Results are presented as mean±SEM. Bar=100 μm. CGRP : calcitonin gene-related peptide.

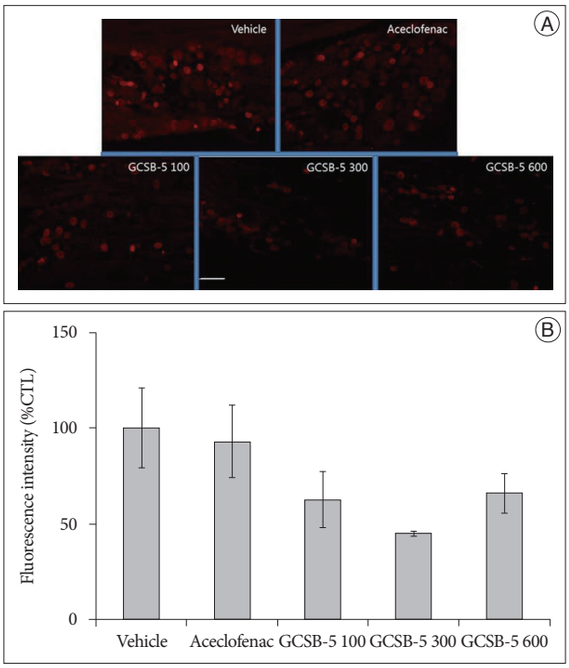

Fig. 6. Immunohistochemical expression of TRPV1 in ipsilateral L5 dorsal root ganglia (DRGs) (A) and TRPV1 immunoreactivities versus the vehicle controls (B) at 56 days after treatment commencement. A : TRPV1 expression was lower in the ipsilateral DRGs of the GCSB-5 300 group than in the aceclofenac or vehicle control groups. B : Percentage decreases in TRPV1 expression were greatest in the GCSB-5 300 group than in the GCSB-5 100, GCSB-5 600, aceclofenac or vehicle control groups. Results are presented as mean±SEM. Bar=100 μm. TRPV1 : transient receptor potential vanilloid 1.

significantly lower than in vehicle controls. Furthermore, the number of Iba1-positive microglia and GFAP-positive astrocytes in dorsal horns and CGRP and TRPV1 expressions in DRGs were lower in the three GCSB-5 groups than in the vehicle control group on TD56; however, these differences were not significant. IL-6 and CX3CL1 mRNA expressions were unchanged by aceclofenac or GCSB-5 versus controls.

Lumbar disc herniation is one of the most common causes of low back pain and sciatica, the latter of which is induced by mechanical and chemical factors[2,21,32-34,48]. It has been proposed that cytokines and chemokines play major roles in the chemical pathomechanisms of radicular pain[1,8,17], and it is known that spinal cord and DRG responses play important roles. Furthermore, proinflammatory cytokine and neuroglial cell expressions in the spinal cord and DRG have been implicated in the generation of radicular pain[31,41,45,47]. Radicular pain caused by lumbar disc herniation is characterized by hyperalgesia, spontaneous pain, and allodynia, and is normally treated using NSAIDs. However, the chronic nature of neuropathic pain requires the long-term use of NSAIDs, and this inseparable relationship unavoidably induces adverse drug reactions, such as, gastrointestinal and cardiovascular problems[43].

GCSB-5 is prepared from six herbs that are used to treat osteoarthritis in traditional East Asian medicine[26]. Thus, the safety of these herbs is supported by long histories of human use. In the present study, we found GCSB-5 300 administration ameliorated mechanical allodynia induced by autologous nucleus pulposus implantation to a nerve root on TD56. In addition, the expressions of Iba1, GFAP, CGRP, and TRPV1 on TD56 showed a tendency to be reduced in the three GCSB-5 groups. Microglia are the resident macrophages of the CNS and contribute to the development of chronic neuropathic pain by releasing mediators, such as, proinflammatory cytokines and chemokines, that influence pain signaling[28,42,46]. In previous studies, it has been suggested that nucleus pulposus application induces glial activity in spinal cords and that these activated glia play a crucial role in dorsal horn pain transmission[16,29,35]. In animal models of neuropathic pain, it has been reported that astrocytes are activated and express GFAP[11,23,31], and recently, it was suggested that injury-induced GFAP upregulation plays a role in the maintenance of neuropathic pain states[23]. In the present study, on TD56, numbers of Iba1-positive microglia and GFAP-positive astrocytes in dorsal horns showed a tendency to be reduced in the three GCSB-5 groups, and similar results were also obtained for the expressions of CGRP and TRPV1. Recently, it was reported that the effects of nucleus pulposus on nerve roots are closely associated with cytokines such as TNF-α, and cyclooxygenase-2

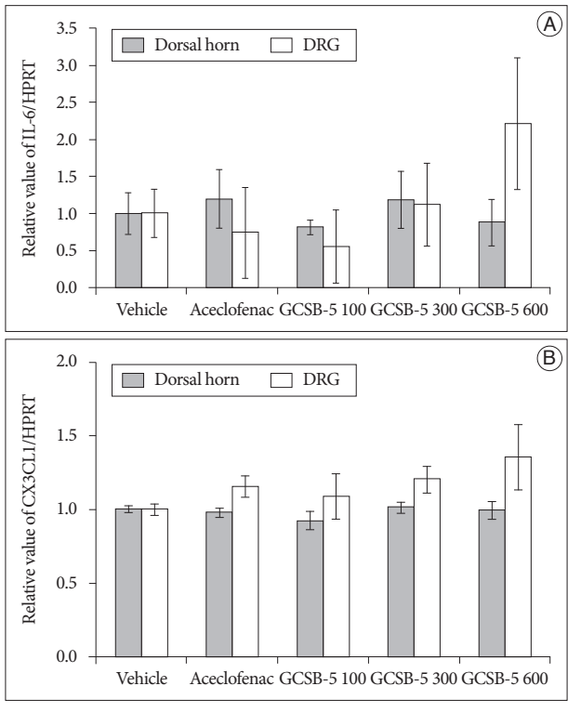

Fig. 7. Expressions of IL-6 (A) and CX3CL1 (B) mRNAs in ipsilateral L5 dorsal horns and dorsal root ganglia (DRGs). The mean expressions of the mRNAs of IL-6 and CX3CL1 in the aceclofenac and three GCSB-5 groups were not found to differ significantly from the vehicle control group at 56 days after treatment commencement. Results are presented as mean±SEM. IL-6 : interleukin-6, CX3CL1 : chemokine (C-X3-C motif) ligand 1/fractalkine, mRNA : messenger RNA.

(COX-2)[30,33]. TNF-α induces the productions of inflammatory neuropeptides such as substance P (SP) and CGRP, and induces the release of SP and CGRP in the spinal dorsal horn from peripheral terminals[9,13]. CGRP is a marker of sensory neurons involved mainly in pain perception. Moreover, TRPV1 receptors are molecular integrators of nociceptive stimuli at peripheral nerve endings, and spinal TRPV1 receptors have been shown to play important roles in nociceptive transmission modulation, especially under pathological conditions[6,27,36,39]. These observed downregulations of microglia, astrocytes, CGRP, and TRPV1 with time after GCSB-5 treatment commencement follow a course similar to that of pain behavior attenuation, and thus, may be responsible for the analgesic effect of GCSB-5, which suggests GCSB-5 could provide a treatment for radicular pain caused by lumbar disc herniation by reducing neuroglial expression in spinal segments.

It was previously reported that GCSB-5 exhibited anti-inflammatory potential in a rat model of osteoarthritis. At a molecular level, GCSB-5 have been reported to inhibit nitric oxide production[10,44], to reduce proinflammatory cytokine serum level[49], and to suppress COX-2 protein levels in macrophages[22], and in a recent animal study, GCSB-5 was found to have an anti-inflammatory effect on acute and chronic inflammation in arthritis[7]. In addition, in a study on its *in vivo* analgesic effects, it was concluded GCSB-5 ameliorated peripheral pain by increasing pain thresholds[24].

In the present study, we found no significant differences in the IL-6 mRNA expression in dorsal horns and DRGs between the vehicle control group and the three GCSB-5 groups. Furthermore, CX3CL1 mRNA expressions in dorsal horns and DRGs in the vehicle control group and the three GCSB-5 groups were not significantly different. Cells of the nucleus pulposus have been shown to be capable of producing several cytokines and chemokines, including IL-1α, IL-1β, TNF-α, IL-6, IL-8, and IL-10[1,18,19,37,40]. Chemokines constitute a family of proinflammatory cytokines with potent chemotactic activities for leukocytes *in vitro* and *in vivo*. They act at the initial stages of inflammatory response and draw inflammatory cells from blood into tissues. It has also been suggested chemokine production by herniated intervertebral disc tissue might explain the initiation of surrounding inflammation[5]. Park et al.[35], who used a nucleus pulposus implantation induced model of lumbar disc herniation, examined the longitudinal expression of CX3CL1 in spinal dorsal horns, and found CX3CL1 immunoreactivity gradually increased especially in laminae II and III of the ipsilateral dorsal horn. In addition, they observed strong CX3CL1 immunoreactivity in spinal neurons at 5 and 10 days after surgery but not observed in spinal cords at 10 and 30 days after surgery. In the present study, we examined IL-6 and CX3CL1 mRNA expressions only at 56 days after treatment commencement, and did not monitor these expressions with respect to time. We believe this is why we observed no differences in IL-6 and CX3CL1 mRNA expressions between the three GCSB-5 groups and the vehicle control group.

CONCLUSION

In conclusion, our results corroborate previous reports by showing that GCSB-5 administration ameliorates musculoskeletal pain-related behavior. To the best of our knowledge, this is the first report on neuroglial changes in lumbar spinal segments induced by oral GCSB-5 administration in a rat model of radicular pain. Our results indicate GCSB-5 downregulates neuroglial activity in the spinal dorsal horn and the DRG expressions of CGRP and TRPV1, and suggest these downregulations attenuate radicular pain. More detailed studies are required to elucidate the mechanisms responsible for the observed molecular changes and the attenuation of radicular pain elicited by GCSB-5. In addition, we suggest clinical trials be conducted to investigate the efficacy and safety of Shinbaro® in patients with low back pain and/or radicular pain.

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