Clin Orthop Relat Res (2015) 473:1913–1919
DOI 10.1007/s11999-014-3878-1

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

CrossMark

SYMPOSIUM: CURRENT APPROACHES TO THE MANAGEMENT OF LUMBAR DISC HERNIATION

Experimental Disc Herniation in the Rat Causes Downregulation of Serotonin Receptor 2c in a TNF-dependent Manner

Daniel Jonsson MS, Oscar Finskas MS, Yuki Fujioka MD, Anders Ståhlberg PhD, Kjell Olmarker MD, PhD

Published online: 21 August 2014
© The Association of Bone and Joint Surgeons® 2014

Abstract

Background During recent decades, the knowledge of the pathophysiology of disc herniation and sciatica has drastically improved. What previously was considered a strict biomechanical process is now considered a more complex interaction between leaked nucleus pulposus and the tissue in the spinal canal. An inflammatory reaction, with tumor necrosis factor (TNF) playing an essential role, has been demonstrated. However, the exact mechanisms of the pathophysiology of disc herniation remain unknown.

Questions/purposes In this study we use an animal model to investigate (1) if and/or how experimental disc herniation affects gene expression in the early phase (24 hours postsurgery) in the dorsal root ganglion; and (2) if TNF inhibition can reduce any observed changes.

Methods A rat model of disc herniation was used. Twenty rats were evenly divided into four groups: naïve, sham, disc herniation, and disc herniation with TNF inhibition. The dorsal root ganglion of the affected nerve root was harvested 24 hours after surgery and analyzed with a TaqMan Low Density Array® quantitative polymerase chain reaction assay. Gene expression levels in sham were compared with disc herniation to assess question 1 and disc herniation to disc herniation with TNF inhibition to assess question 2.

Results Experimental disc herniation caused a decrease in the expression of the serotonin receptor 2c gene ($p = 0.022$). TNF inhibition was found to reduce the observed decrease in expression of serotonin receptor 2c ($p = 0.037$).

Conclusions Our results suggest that a decrease in the expression of the serotonin receptor 2c gene may contribute to the pathophysiology of disc herniation. Further research on its involvement is warranted.

Clinical Relevance This pilot study gives a brief insight into cellular changes that may contribute to the pathophysiology of disc herniation. This knowledge may

One of the authors (KO) is founder and part owner of Orthopaedic Research & Development AB, Stayble Therapeutics and Sciaticon AB, Mölndal, Sweden. One of the authors (AS) is part owner of TATAA Biocenter, Gothenburg, Sweden. The institution of the corresponding author (KO) has received funding from AFA Insurance, Stiftelsen Olle Engkvist byggmästare, IngaBritt och Arne Lundbergs forskningsstiftelse, and the Spine Society of Europe Task Force on Research. One of the authors (AS) is supported by the Swedish Research Council and Swedish Society for Medical Research.
All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research*® editors and board members are on file with the publication and can be viewed on request.
Clinical Orthopaedics and Related Research® neither advocates nor endorses the use of any treatment, drug, or device. Readers are encouraged to always seek additional information, including FDA-approval status, of any drug or device prior to clinical use.
Each author certifies that his or her institution approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.
This work was performed at Musculoskeletal Research, Department of Medical Chemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

D. Jonsson, O. Finskas, Y. Fujioka, K. Olmarker (✉)
Musculoskeletal Research, Department of Medical Chemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 405 30 Gothenburg, Sweden
e-mail: kjell.olmarker@gu.se

Y. Fujioka
Department of Orthopaedic Surgery, Programs for Applied Biomedicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

contribute to the development of more and better treatment options for patients with disc herniation and sciatica.

Introduction

Low back pain (LBP), with or without sciatica, is extremely common, affecting as many as up to 80% of all people during their lifetime [13]. However well recognized the condition may be, the understanding of the exact pathophysiology remains unclear despite great progress over the last two decades. A review of LBP published in the *New England Journal of Medicine* [3] suggests a complex pathophysiology with many possible origins of pain, including ligaments, facet joints, paravertebral muscles, and intervertebral discs. The many possible causes of LBP are hard to distinguish clinically both by means of physical examination and radiological methods. Individualized treatment is therefore hard to achieve.

One specific etiology included in the LBP concept is disc herniation with sciatic pain. Experimental studies have shown that contrary to previous assumptions, disc herniation may produce symptoms not only by a mechanical deformation of the adjacent nerve root, but also by the mere presence of the nucleus pulposus [2, 17]. In this regard, it seems that the main role of the nucleus pulposus is to sensitize the nerve tissue to produce pain when mechanically deformed [2]. This effect seems to be related to disc-related proinflammatory cytokines, in particular tumor necrosis factor (TNF) [12, 14–16]. Although pharmaceutical inhibition seems to be efficient in reducing the nucleus pulposus-induced effects in experimental models [15], the clinical efficacy is still debated [5, 9].

To be able to gain an understanding of the molecular events after nucleus pulposus exposure, it is possible to assess changes in regulation of various molecules in the adjacent dorsal root ganglion. This previously has been performed in a similar model in the rat consisting of disc puncture alone without mechanical manipulation of the nerve root. This study focused on a number of inflammation-related genes after exposure to nucleus pulposus. On Day 1, Htr2a, Nos1, and Il2rg were upregulated and Plcb3 downregulated. On Day 3, Cysltr1 and Nos1 were upregulated. However, in the current study, we aimed to assess genes related to pain transmission with the addition of mechanical deformation of the nerve root, thus mimicking disc herniation with both a biochemical (nucleus pulposus) and a mechanical component.

The purposes of the current study were (1) to assess changes in the regulation of certain pain-related genes in the dorsal root ganglion in the initial phase (1 day post-surgery) of experimental disc herniation; and (2) to assess if any observed changes would be modulated by simultaneous pharmaceutical TNF inhibition.

A. Ståhlberg
Sahlgrenska Cancer Center, Department of Pathology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Materials and Methods

A newly developed rat model of disc herniation was used (Fig. 1) [4]. All animal procedures were carried out following the Principles of Laboratory Animal Care and were approved by the local animal research ethics committee. A total of 20 female Sprague-Dawley rats (body weight approximately 225 g [Charles River, Burlington, MA, USA]) were used and housed with free access to food and tap water. Room temperature was kept at 21° C. A light schedule with 12 hours of daylight starting at 6:00 AM and 12 hours of darkness starting at 6:00 PM was followed. The rats were anesthetized by inhalation of isoflurane. An intraperitoneal injection of 0.05 mg/kg Temgesic® (buprenorfin; RB Pharmaceuticals, Slough, UK) was administered preoperatively for postoperative analgesia.

Surgical Procedure

For the naïve group (n = 5), no surgery was performed

For the sham exposure group (n = 5), a skin incision of approximately 4 cm was made over the lumbar spinal processes. The thoracolumbar fascia was incised followed by dissection of the left spinal muscles at L3-L5, thus exposing the L3-L4 facet joint. The left facet joint of the L3-L4 vertebrae was removed, exposing the L4 nerve root, dorsal root ganglion of L3, and the intervertebral disc between L3 and L4. No further surgery was performed in this group. The spinal muscles were sutured and the skin closed with metal clips.

In the experimental disc herniation group (DH) (n = 5) [4], after exposure of the nerve root, dorsal root ganglion, and intervertebral disc as described in the sham group, the L3-L4 disc was punctured using a 0.4-mm injection needle. A small amount of air was injected into the disc to facilitate leakage of nucleus pulposus. Next, the needle was used to laterally diverge the L4 nerve root slightly, fixating the needle to the underlying vertebral body. The needle then was cut and left in this position, ensuring permanent displacement and thus mechanical deformation of the nerve root. The spinal muscles were sutured and the skin closed with metal clips.

In the experimental disc herniation and TNF inhibition group (DHI) (n = 5), the surgical procedure was the same as in the DH group, but with the addition of intraperitoneal administration of 4 mg/kg Remicade® (infliximab; Janssen

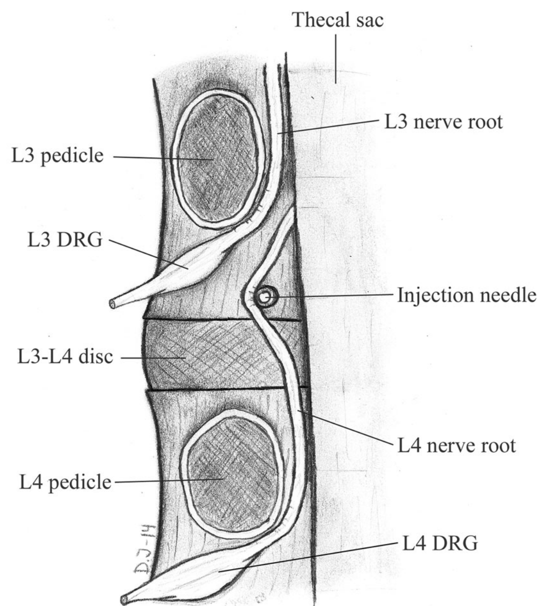

Fig. 1 Schematic drawing shows the disc herniation model. After puncturing the disc, the injection needle is used to carefully laterally displace the L4 nerve root from the thecal sac. The needle is secured in the underlying vertebral body and cut off, resulting in slight mechanical deformation of the nerve root. DRG = dorsal root ganglion.

Biologics BV, Leiden, The Netherlands) after disc puncture before diverging the nerve root.

Twenty-four hours after surgery the rats were reanesthetized and euthanized by cutting the heart, causing the rat to quickly bleed to death while still deeply sedated. The left L4 dorsal root ganglion was exposed, harvested, and placed in a solution with the RNAse inhibitor RNAlater (QIAGEN, Hilden, Germany). The dorsal root ganglions were then immediately frozen with liquid nitrogen and thereafter placed on dry ice and stored in a −80° C freezer.

Tissue Processing, RNA Purification, and Quantitative Polymerase Chain Reaction

The dorsal root ganglion was later homogenized using QIAzol® Lysis Reagent and TissueLyser® (QIAGEN). RNA was extracted using an RNeasy® Lipid Tissue Micro Kit (QIAGEN) following the standard protocol with addition of DNase treatment (as described in the RNeasy® Lipid Tissue Handbook). The purity and concentration of the RNA were confirmed using spectrophotometry (NanoDrop®; Thermo Scientific, Waltham, MA, USA). An absolute amount of 500 ng RNA from each sample was used to generate cDNA using a reverse transcriptase kit (iScript™ cDNA synthesis kit; Bio-Rad, Hercules, CA, USA) in a 20-μL reaction. Three control samples were also included in the reaction: (1) water only; (2) RNA-negative (all reaction components except sample RNA); and (3) RT-negative (all reaction components except reverse transcriptase). The temperature cycling conditions during the reaction were: 5 minutes at 25° C, 30 minutes at 42° C, 5 minutes at 85° C, then held at 4° C for a few minutes before once again being stored at −80° C. The cDNA was diluted with water to 100 μL. Quantitative real-time polymerase chain reaction (PCR) was then performed on the 20 samples and the control samples. The quantitative PCR was performed in three 384-well TaqMan Low Density Array custom plates (Applied Biosystems, Foster City, CA, USA), each plate analyzing all 24 of the genes (Table 1). All samples were run as quantitative PCR duplicates. cDNA corresponding to 30 ng total RNA was analyzed in each quantitative PCR. The plates were run in an Applied Biosystems 7900HT system (Applied Biosystems) at the Genomics Core Facility, University of Gothenburg. Of the 24 genes analyzed, 20 were detected in the samples. Control samples showed no signs of external interference or contamination of genomic DNA. The amplification curve of each gene was studied individually and a threshold level for detectible amplification was set manually in the exponential phase of the amplification curve. Reference genes were evaluated with NormFinder [1]. Actb, Gapdh, and Hprt1 were chosen as optimal reference genes. Cycle of quantification (Cq) for the genes analyzed was normalized, according to the MIQE guidelines, against the three chosen reference genes to compensate for any eventual difference in sample load [21]. Values were converted to log2-scale. Relative changes in expression between groups were calculated assuming 100% PCR efficiency [10, 11]. Statistical significance was tested with the Mann-Whitney U test. Statistical significance was defined as a p value of < 0.05.

Results

Experimental disc herniation induced a downregulation of the Htr2c gene (Table 2; Fig. 2). The expression of Htr2c was lesser in the DH group as compared with the sham group ($p = 0.022$). With the numbers available, no other regulation between sham and DH could be detected ($p > 0.05$).

The observed downregulation of the Htr2c gene in experimental disc herniation was reduced by pharmaceutical TNF inhibition (Table 2; Fig. 2). The expression of the Htr2c gene was greater in the DHI group as compared with the DH group ($p = 0.037$).

Discussion

Disc herniation and sciatica are common disorders. The pathophysiology of both the formation of the herniated disc

Table 1. Overview of genes analyzed with a focus on why they are relevant to this study*

Gene abbreviation	Full gene name	Properties/function
Grin2a	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A	Involved in, eg, long-term potentiation and activity-dependent increase in synaptic efficiency
Grin2b	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	Similar to that of Grin2a
Bdkrb1	Bradykinin receptor B1	Upregulated after tissue injury; mediates and regulates inflammatory response
Bdkrb2	Bradykinin receptor B2	Mediates chloride ion influx induced by bradykinin, involved in pain response
Grm1	Glutamate receptor, metabotropic 1	Involved in different psychiatric conditions and possibly also pain perception
Grm3	Glutamate receptor, metabotropic 3	G protein-coupled excitatory glutamate receptor
Grik1	Glutamate receptor, ionotropic, kainate 1	Excitatory glutamate receptor
Htr2a	5-hydroxytryptamine (serotonin) receptor 2A	Most known for its involvement in psychiatric conditions, but may also play a role in descending control of spinal nociceptive transmission.
Htr2c	5-hydroxytryptamine (serotonin) receptor 2C	Similar to that of Htr2a
Scn3a	Sodium channel, voltage-gated, Type III, alpha	Propagates action potentials in neurons and muscle; may play a role in hyperexcitability and neutropathic pain in spinal cord injury
Scn9a	Sodium channel, voltage-gated, Type IX, alpha	Important role in nociceptive signaling in primarily peripheral nerves
Il1a	Interleukin-1 alpha	Proinflammatory cytokine, may induce apoptosis
Il10	Interleukin-10	Cytokine produced primarily by monocytes. Modulates inflammation through effect on, eg, T- and B-cells
Il12b	Interleukin-12B	Cytokine involved in, eg, promoting T-cell development
Mapk1	Mitogen-activated protein kinase 1	Intracellular kinase, phosphorylates nuclear targets; involved in many intracellular pathways
Calca	Calcitonin-related polypeptide alpha	Small peptide hormone, potent vasodilator
Nos1	Nitric oxide synthase 1, neuronal	Catalyzes production of nitric oxide in neurons, which in turn can act as a neurotransmitter
P2rx3	Purinergic receptor P2X, ligand-gated ion channel, 3	ATP-gated cation channel; important for peripheral nociceptive response
Ngf	Nerve growth factor (beta polypeptide)	Promotes the growth and differentiation of, eg, sensory neurons
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	Reference gene, one of many enzymes involved in carbonhydrate metabolism
Hprt1	Hypoxanthine phosphoribosyltransferase 1	Reference gene, a transferase with a central role in the generation of purine nucleotides
18S	Ribosomal 18s and 28s RNA gene	Reference gene, ribosomal RNA
Rplp2	Ribosomal protein, large P2	Reference gene, subunit of the ribosome
Actb	Actin, beta	Reference gene, cytoskeletal component

* Information was collected from the NCBI Gene database. Properties/function were primarily collected from the gene description on Rattus norvegicus. On unsatisfysing and/or lacking information on the rat, human gene data were used.

as well as the subsequent induction of pain, both local and radiating, is, despite great progress during the last decades, still largely unknown. As a consequence, few efficient treatment options are today clinically available, although TNF inhibition is an option being suggested [5, 9]. In this pilot study, we found a downregulation of the Htr2c gene in experimental disc herniation in the rat that was reduced by TNF inhibition. This suggests that changes in serotonergic transmission may contribute to the changes in spontaneous pain behavior previously observed in this model [4], shedding further light on the complex pathophysiology of disc herniation and sciatica.

This study had several limitations. As a pilot study, the sample size was very small (five animals in each group). It is quite likely with such a small sample that we failed to detect differences in gene expression between groups. The genes presented in Fig. 2 represent examples of genes where this may be particularly relevant, because these genes showed trends when visually inspected in a chart but the statistical analysis failed to show any differences. We

Table 2. Changes in relative gene expression (all genes analyzed)

Gene	Mean relative expression ± 1 SD			
	Naïve	Sham	DH	DHI
Grin2a	0 ± 0.72	0.94 ± 1.39	1.29 ± 1.06	1.01 ± 1.17
Grin2b	0 ± 0.15	0.16 ± 0.73	0.68 ± 0.57	0.19 ± 0.35
Bdkrb1	Not expressed	-	-	-
Bdkrb2	0 ± 1.61	-0.28 ± 0.52	-0.61 ± 0.46	-0.16 ± 0.54
Grm1	Not expressed	-	-	-
Grm3	0 ± 0.74	0.12 ± 0.88	-0.86 ± 0.45	-0.50 ± 0.67
Grik1	0 ± 0.29	-0.10 ± 0.52	-0.48 ± 0.45	-0.45 ± 0.15
Htr2a	0 ± 0.59	-0.28 ± 0.34	-0.43 ± 0.62	0.07 ± 0.73
Htr2c	0 ± 0.52	-0.13 ± 0.84 ←*→	-1.20 ± 0.33 ←**→	-0.43 ± 0.46
Scn3a	0 ± 0.42	0.01 ± 0.24	0.15 ± 0.73	0.05 ± 0.35
Scn9a	0 ± 0.17	0.02 ± 0.08	-0.06 ± 0.25	-0.09 ± 0.06
Il1a	0 ± 1.78	1.78 ± 2.61	1.26 ± 2.25	2.63 ± 0.96
Il10	Not expressed	-	-	-
Il12b	Not expressed	-	-	-
Mapk1	0 ± 0.09	-0.06 ± 0.07	-0.17 ± 0.11	-0.29 ± 0.16
Calca	0 ± 0.13	-0.11 ± 0.17	-0.06 ± 0.26	0.03 ± 0.22
Nos1	0 ± 0.53	0.01 ± 0.46	0.01 ± 0.64	0.26 ± 0.24
P2rx3	0 ± 0.23	-0.18 ± 0.25	-0.13 ± 0.36	-0.16 ± 0.25
Ngf	0 ± 0.40	1.04 ± 1.42	1.92 ± 0.68	1.56 ± 0.43
Gapdh	Reference gene	-	-	-
Hprt1	Reference gene	-	-	-
18S	0 ± 0.30	0.21 ± 0.25	-0.13 ± 0.21	-0.04 ± 0.52
Rplp2	0 ± 0.20	0.11 ± 0.23	0.12 ± 0.18	0.18 ± 0.24
Actb	Reference gene	-	-	-

Expression level of naïve is used as baseline, 0, for each separate gene. Data were rounded to two decimals. Four genes, Bdkrb1, Grm1, Il-10, and Il12b, were not detectable. Three genes, Gapdh, Hprt1, and Actb, were used for normalization of the data. Asterisks mark changes of statistical significance (*p = 0.022, **p = 0.037). No other comparisons detected any changes (p > 0.05); DH = disc herniation group; DHI = experimental disc herniation and tumor necrosis factor inhibition group.

encourage future studies similar to ours to include these genes. Our data may then be used for making sample size calculations.

The newly developed rat model used in this study has been shown to increase spontaneous pain-related behavior [4], but studies specifically evaluating hyperalgesia have not yet been conducted, which is a limitation when comparing our results with others. Nevertheless, we believe this is the most clinically relevant model available, because the needle induces mechanical deformation of the clinically most commonly affected structure (the nerve root) [4]. Also, changes in gene expression, in this study primarily receptor genes, are believed to reflect a change in the number of receptors on the cell surface with downregulation causing lowered receptor-mediated signaling and vice versa. Although we do not know where, or even if, the changes observed are reflected on the neurons, this is something future studies should aim to assess. Two likely compartments would be the neural soma in the dorsal root ganglion itself and the axon terminal in the dorsal horn. It is also important to recognize that this study analyzed not only the neurons of the dorsal root ganglion, but the

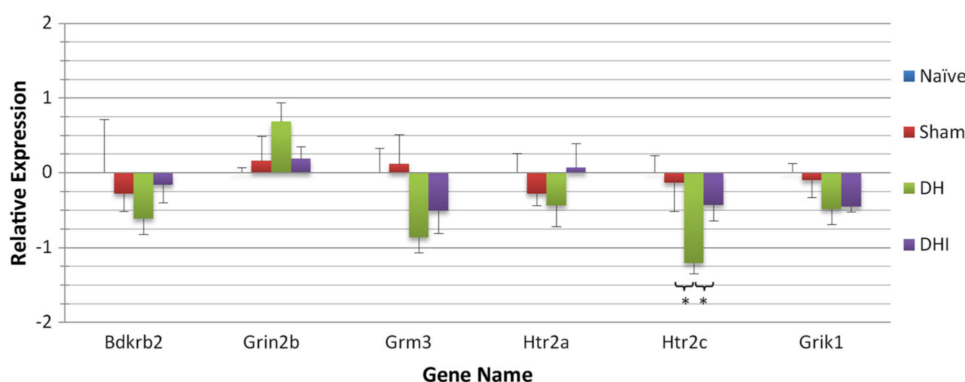

Fig. 2 Changes (mean ± SEM) in relative gene expression are expressed in log2-scale with the expression level of naïve being used as baseline, 0, for each separate gene. The asterisks mark the changes with statistical significance ($p < 0.05$); the changes in Htr2c expression between (1) sham and DH and (2) between DH and DHI.

supportive cells as well (including, eg, satellite cells). However, most genes analyzed code for receptors of different neurotransmitters that are likely expressed at much lower levels in supportive cells than in the neurons. As such, we believe this potential interference to be small and our results to mainly represent changes in the neurons of the dorsal root ganglion.

In this study we found that the Htr2c gene, coding for serotonin receptor 2c, was downregulated 24 hours after experimental disc herniation (Fig. 2; Table 2). There is some previous research on rat models regarding serotonin (5-HT) and its potential involvement in disc herniation/sciatica [7, 8, 18], but, to the authors' knowledge, this study is the first that has demonstrated its involvement in a rat model comprising both mechanical deformation of the nerve and the chemical component induced by disc puncture. Also worth mentioning here is that the dorsal root ganglion analyzed in our model has not been in direct contact with nucleus pulposus because the disc puncture in this model is performed on the spinal segment above (Fig. 1). It has previously been shown that exogenous application of 5-HT to the dorsal root ganglion in the rat causes increased pain transmission similar to application of nucleus pulposus [7, 8], thus suggesting a pronociceptive role for 5-HT. Although, interestingly, a recent study also shows that a selective serotonin reuptake inhibitor caused an increase in mechanical withdrawal thresholds in rats where nucleus pulposus had been applied to the dorsal root ganglion [18], thus suggesting that an increase in 5-HT leads to antinociception in disc hernia, which would better correlate with the downregulation we observed. Although, the improvements were likely caused by suppression of microglia, and not by a direct effect on the dorsal root ganglion. Of course, this discussion is very limited in that we only analyzed two serotonin receptors when there are several other subtypes of receptors potentially involved in its pain-modulating effects [6, 7, 19, 20, 22]. Our study strengthens the evidence for the involvement of 5-HT in the development of pain in disc herniation, but the exact mechanisms are in great need of further research.

The change in gene expression induced by experimental disc herniation observed in this study, the reduced Htr2c expression, was also found to be modulated by pharmaceutical TNF inhibition. The increased expression of Htr2c in DHI as compared with the DH group suggests that TNF inhibition prevents the downregulation of Htr2c. The interaction between TNF and serotonin in disc hernia has been previously demonstrated [8, 18]. Kobayashi et al. [8] described a synergistic effect between TNF and 5-HT in inducing pain-related behavior. Also, they observed a connection between the presence of nucleus pulposus, TNF, and serotonin with the expression of TNF and serotonin receptor 2a (5-HT2AR) in the dorsal root ganglion. Our study failed to detect any changes in the expression of Htr2a (coding for 5-HT2AR), suggesting that the changes might occur later than after 24 hours. Differences in animal models used or the low sample size in our study may also contribute to this discrepancy.

In conclusion, this study found a TNF-dependent downregulation of Htr2c in the dorsal root ganglion in a rat model of disc herniation. These results support previous research suggesting that changes in serotonergic transmission contribute to the pathophysiology of disc herniation/sciatica [7, 8, 18], but there are still many questions that need to be answered as to how it contributes. Future studies aiming to assess the effects of different serotonin receptors, for example by evaluating the effects of subtype-specific receptor agonists/antagonists in animal models of disc herniation, are warranted. The observed interaction with TNF also needs to be further investigated. Improved understanding of these events may contribute to the development of better and more effective treatment options for patients with disc herniation and sciatica.

References

1. Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004;64:5245–5250.
2. Byrod G, Rydevik B, Nordborg C, Olmarker K. Early effects of nucleus pulposus application on spinal nerve root morphology and function. Eur Spine J. 1998;7:445–449.
3. Deyo RA, Weinstein JN. Low back pain. N Engl J Med. 2001;344:363–370.
4. Finskas O, Blixt A, Fujioka Y, Olmarker K. New, clinically more relevant model for nerve root injury in the rat. Spine. 2013;38:1744–1748.
5. Freeman BJ, Ludbrook GL, Hall S, Cousins M, Mitchell B, Jaros M, Wyand M, Gorman JR. Randomized, double-blind, placebo-controlled, trial of transforaminal epidural etanercept for the treatment of symptomatic lumbar disc herniation. Spine. 2013;38:1986–1994.
6. Jeong CY, Choi JI, Yoon MH. Roles of serotonin receptor subtypes for the antinociception of 5-HT in the spinal cord of rats. Eur J Pharmacol. 2004;502:205–211.
7. Kato K, Kikuchi S, Konno S, Sekiguchi M. Participation of 5-hydroxytryptamine in pain-related behavior induced by nucleus pulposus applied on the nerve root in rats. Spine. 2008;33:1330–1336.
8. Kobayashi H, Kikuchi S, Konno S, Kato K, Sekiguchi M. Interaction of 5-hydroxytryptamine and tumor necrosis factor-alpha to pain-related behavior by nucleus pulposus applied on the nerve root in rats. Spine. 2011;36:210–218.
9. Korhonen T, Karppinen J, Paimela L, Malmivaara A, Lindgren KA, Bowman C, Hammond A, Kirkham B, Jarvinen S, Niinimaki J, Veeger N, Haapea M, Torkki M, Tervonen O, Seitsalo S, Hurri H. The treatment of disc-herniation-induced sciatica with infliximab: one-year follow-up results of FIRST II, a randomized controlled trial. Spine. 2006;31:2759–2766.
10. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonak J, Lind K, Sindelka R, Sjoback R, Sjogreen B, Strombom L, Stahlberg A, Zoric N. The real-time polymerase chain reaction. Mol Aspects Med. 2006;27:95–125.
11. 11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402–408.
12. Murata Y, Onda A, Rydevik B, Takahashi K, Olmarker K. Selective inhibition of tumor necrosis factor-alpha prevents nucleus pulposus-induced histologic changes in the dorsal root ganglion. Spine. 2004;29:2477–2484.
13. Nachemson A, Jonsson E. [Backache and Neck Pain—An Evidence-based Review of Causes, Diagnostics and Treatments] [in Swedish]. Stockholm, Sweden: SBU, Swedish Council on Health Technology Assessment; 2000.
14. Olmarker K, Larsson K. Tumor necrosis factor alpha and nucleus-pulposus-induced nerve root injury. Spine. 1998;23: 2538–2544.
15. Olmarker K, Nutu M, Storkson R. Changes in spontaneous behavior in rats exposed to experimental disc herniation are blocked by selective TNF-alpha inhibition. Spine. 2003;28:1635–1641; discussion 1642
16. 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;26:863–869.
17. Olmarker K, Rydevik B, Nordborg C. Autologous nucleus pulposus induces neurophysiologic and histologic changes in porcine cauda equina nerve roots. Spine. 1993;18:1425–1432.
18. Saito H, Wakai J, Sekiguchi M, Kikuchi S, Konno S. The effect of selective serotonin reuptake inhibitor (SSRI) on pain-related behavior in a rat model of neuropathic pain. *Eur Spine J.* 2014 Jun 5 [Epub ahead of print].
19. Sasaki M, Obata H, Saito S, Goto F. Antinociception with intrathecal alpha-methyl-5-hydroxytryptamine, a 5-hydroxytryptamine 2A/2C receptor agonist, in two rat models of sustained pain. *Anesth Analg.* 2003;96:1072–1078, table of contents.
20. Sommer C. Is serotonin hyperalgesic or analgesic? Curr Pain Headache Rep. 2006;10:101–106.
21. Stahlberg A, Elbing K, Andrade-Garda JM, Sjogreen B, Forootan A, Kubista M. Multiway real-time PCR gene expression profiling in yeast Saccharomyces cerevisiae reveals altered transcriptional response of ADH-genes to glucose stimuli. BMC Genomics. 2008;9:170.
22. Suzuki R, Rygh LJ, Dickenson AH. Bad news from the brain: descending 5-HT pathways that control spinal pain processing. Trends Pharmacol Sci. 2004;25:613–617.