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Intrathecal Noggin administration in rats temporally ameliorates mechanical allodynia induced by a chronic constriction injury

Masayuki Hashimoto [a,*], Masao Koda [b], Takeo Furuya [b], Atsushi Murata [b], Masashi Yamazaki [c], Kazuhisa Takahashi [b]

[a] Department of Orthopaedic Surgery, Seikeikai Chiba Medical Center, 1-7-1, Minami-Cho, Chuo-Ku, Chiba 2600842, Japan
[b] Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, Japan
[c] Department of Orthopaedic Surgery, Tsukuba University Graduate School of Medicine, Japan

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ABSTRACT

Chronic intractable neuropathic pain after central or peripheral nervous system injury remains refractory to therapeutic intervention. Using microarray and RT-qPCR methods, we found that Noggin mRNA is downregulated in the lumbar enlargement 2 weeks after chronic constriction injury (CCI) in rats.

Eight-week-old female Sprague Dawley rats were used for the CCI model. Two weeks after CCI, rats underwent a laminectomy at L5 under halothane anesthesia, and a silicone tube connected to an osmotic minipump was inserted intrathecally for 14 days. Rats were administered Noggin ranging from 10 ng/ml to 10 μg/ml. Phosphate buffered saline (PBS) was used as a control. The time course of mechanical allodynia was assessed for 5 weeks using von Frey filaments. An ANOVA showed that rats administered Noggin at 2 μg/ml had significantly less mechanical allodynia compared with controls.

We next compared the effect of intrathecal administration (14 days) of Noggin (2 μg/ml), bone morphogenetic protein 4 (BMP4; 2 μg/ml), or BMP4 (μg/ml) + Noggin (μg/ml) with controls. Only Noggin administration significantly reduced mechanical allodynia in the CCI model.

Fluorescence immunohistochemistry indicated that Noggin administration decreased astrocyte accumulation in the dorsal horn compared with PBS after administration for one week. BMP4-driven conversion of oligodendrocyte progenitor cells (OPCs) to type 2 astrocytes is inhibited by Noggin Hampton et al. (2007) . We speculated that Noggin administration inhibits the conversion of OPCs to astrocytes, and decreases glial fibrillar acidic protein expression. This histological condition could decrease neuropathic pain.

1. Introduction

Neuropathic pain is one of the most refractory sequela of neurological injury. The search for novel therapeutic agents including drugs for the treatment of neuropathic pain is an area of intense laboratory and clinical research [1]. Several models of peripheral nerve injury have been developed in rodents to help study the mechanisms responsible for neuropathic pain. Among these are the spared nerve injury (SNI) model, in which 2 of the 3 peripheral branches of the sciatic nerve are transected, producing a distal partial nerve lesion [2]; the chronic constriction injury (CCI) model, in which loose ligatures cause compression and inflammation of the sciatic nerve, injuring mainly myelinated axons [3]; and the spinal nerve ligation (SNL) model of proximal axonal injury [4]. All of these models generate prolonged peripheral hypersensitivity to noxious and innocuous mechanical and cold stimuli. A cellular mechanism of the neuropathic pain state in the CCI model was reported more than a decade ago [5]. On microarray analysis, CCI has the smallest number of uniquely regulated genes compared with SNL or SNI [6]. CCI was chosen over other nerve injury models as the most generalizable, and because the deviation of gene expression in the CCI model is less than in the other 2 models.

Although changes in gene expression associated with chronic pain have been studied by microarray profiling [7–10,21], studies of CCI are scant [6,11]. In the present study, gene expression 14 days after CCI was compared with that in controls using an Agilent Rat Whole genome 4 × 44 K microarray, incorporated in the above study. On the microarray, 1136 genes were statistically significantly upregulated and 1709 genes were downregulated. After detailed consideration for gene ontology and gene function, we selected 6 upregulated genes and 2 downregulated genes for the following quantitative real-time RT-PCR experiments. IGF-1, tissue inhibitor of metalloproteinase-3 (TIMP-3), Pap, aquaporin-4 (Aqp4), CD38, and CD68 were significantly upregulated and Noggin and opioid receptor like-1 (Oprl1) were significantly downregulated. Generally, genes related with gliosis were upregulated, e.g., Timp-3 and Aqp4 are mainly upregulated on

* Corresponding author.
E-mail address: futre@tg7.so-net.ne.jp (M. Hashimoto).

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astrocytes [12,13]. CD38 is a leukocyte related antigen and CD68 is well-known as an ED1 antigen on microglia.

Noggin is a 26 kDa protein with a hydrophobic amino-terminal sequence and plays a role in normal dorsal development [14]. Mice lacking Noggin have defects in the projection of several groups of neurons, including initial ascending projections from the dorsal root ganglia (DRG) [15]. Overexpression of Noggin results in a significant increase in the number of neurons in the trigeminal and DRG [16]. Noggin plays a role in modulating sensory neuron number and axon guidance. These data suggest that Noggin administration modulates neuron number and axon outgrowth in the spinal cord, thereby reducing neuropathic pain. We also on focused Noggin antagonist, BMP4 from gliosis. BMP signaling suppresses oligodendrocyte development through a basic-helix–loop–helix transcription factor and promotes astrocytosis [20]. BMP promotes gliosis in demyelinating spinal cord lesions [25]. Inhibition of BMP4 by Noggin notably decreased the ratio of astrocytes to neuron numbers [24]. Therefore, we hypothesized that suppression of BMP signaling by Noggin suppresses astrocyte numbers.

In the present study, we focused our attention on Noggin, because Noggin is downregulated 14 days after CCI. Noggin is downregulated in the DRG after nerve injury [17]. These observations are consistent with the downregulation of Noggin after CCI. We hypothesized that administration of Noggin after CCI may ameliorate neuropathic pain. We administered Noggin intrathecally for 14 days after CCI using an osmotic minipump and evaluated mechanical allodynia using von Frey filaments weekly for 5 weeks. Our results showed that intrathecal administration of Noggin after CCI reduces mechanical allodynia, and thus may reduce neuropathic pain.

2. Materials and methods

2.1. Bennett chronic constriction injury (CCI) model

All rats were treated and cared for in accordance with the Chiba University School of Medicine guidelines pertaining to the treatment of experimental animals. The study was approved by the Animal Care and Use Committee of Chiba University Graduate School of Medicine (approval number 24–276). We used 18, 8-week-old adult female Sprague Dawley rats (Japan SLC, Hamamatsu, Japan), which were housed in individual cages and allowed food and water ad libitum. Rats were anesthetized with 1.5% of halothane in oxygen, delivered at 0.5 L/min. Sciatic nerve injury was induced using a previously reported CCI procedure with slight modifications. The right side biceps femoris and the gluteus muscles were divided to expose the sciatic nerve, around which 4 loose ligatures (6–0 nylon) were placed from a distal position on the femoral nerve to a proximal position. This model induces mechanical allodynia of the ipsilateral hind paw within the first week of the injury. Upon awakening, rats were housed in groups in our animal facility and maintained under conditions of constant temperature and humidity, and allowed food and water ad libitum.

2.2. Intrathecal Noggin injection

Fourteen days after CCI, rats were reanesthetized with 1.5% of halothane in oxygen, delivered at 0.5 L/min. After laminectomy at L5, a thin silicone tube was inserted into the subarachnoid space using a surgical microscope. The tube was connected to an osmotic minipump (model 2002; Alzet, Palo Alto, CA) containing 10 ng/mL, 2 μg/mL, or 10 μg/mL recombinant mouse Noggin (R & D Systems, Minneapolis, MN) based on previous reports [18] in phosphate-buffered saline (PBS), or PBS only. The infusion rate was 12 μL/day, resulting in delivery of 120 ng–120 μg Noggin/day. The infusion was continued for 14 days, and the total amount of Noggin administered was approximately 1.68 μg–1.68 mg/animal. Repeated-measures ANOVAs, and Bonferroni or Dunnett post hoc tests were applied to von Frey data and $p < 0.05$ was considered significant.

2.3. Intrathecal Noggin and bone morphogenetic protein 4 administration

Noggin is a potent inhibitor of bone morphogenetic protein (BMP) that exerts its function by binding to BMPs, preventing their interaction with their receptors. BMP4 production concomitantly decreases the BMP inhibitor Noggin, potentially resulting in a net increase in BMP signaling [19]. Conversely, recombinant human Noggin was used to suppress BMP action [20]. To block Noggin signaling in vivo, we selected BMP4 for the following experiments. Rats were divided into 3 groups, with intrathecal administration of either Noggin (2 μg/ml), BMP4 (2 μg/ml), or Noggin + BMP4 (2 μg/ml each) using an osmotic minipump for 14 days after CCI. Mechanical allodynia was measured for 42 days after implanting the minipump. The allodynic response was analyzed using repeated-measures ANOVAs with Bonferroni or Dunnett post hoc tests; $p < 0.05$ was considered significant.

2.4. Microarray experiments

Lacroix-Fralish et al. measured mechanical allodynia followed by analysis of global gene expression in the lumber spinal cord at two time points (7 days and 14 days) after surgery using the Affymetrix GeneChip. We decided to show microarray results at 14 days after surgery in the present study. The rats were deeply anesthetized with an intraperitoneal dose of pentobarbital (80 mg/kg; Abbott Laboratories, North Chicago, IL) and decapitated fourteen days after CCI. The lumbar enlargements of their spinal cords (1 cm) were rapidly excised and homogenized in TRizol (Invitrogen, Carlsbad, CA) to preserve RNA. The quality of the extracted RNA was checked using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The 260 nm/280 nm ratio was >2.00 in all 6 samples. Samples were electrophoresed using a 2100 Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). An Agilent Low RNA Input Linear Amplification PLUS kit was used for RNA amplification and labeling, and an Agilent Gene Expression Hybridization Kit and Wash Buffer Kit was used for hybridization. An Agilent DNA microarray scanner was used to scan chips. Feature Extraction Software (Agilent Technology) was used for quantification. Data was imported into Agilent GeneSpring software.

Intensity dependent (LOWESS) normalization was applied in the present study. This option is recommended for use in most 2-color experiments. Intensity dependent normalization is used to eliminate dye-related artifacts in 2-color experiments that cause the cy5/cy3 ratio to be affected by the total intensity of the spot. This normalization process attempts to correct for artifacts caused by nonlinear rates of dye incorporation and inconsistencies in the relative fluorescence intensity between some red and green dyes. Reliable genes from 3 array experiments were selected based on the data quality flags in the original data files. To identify the significantly up- and downregulated genes, we used a *t*-test with differences from controls of $p < 0.05$ considered to be significant.

2.5. Scatter plot

A scatter plot view is useful for examining the levels of expression of genes in 2 distinct conditions, samples, or normalization schemes. For instance, a scatter plot can be used to identify genes that are differentially expressed in one sample versus another. The vertical position of each gene represents its expression level in the current conditions, and the horizontal position represents its control strength. Genes that fall above the diagonal are over expressed and genes that fall below the diagonal are under expressed compared with their median expression level over the course of the experiments.