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Decoy strategy targeting the brain-derived neurotrophic factor exon I to attenuate tactile allodynia in the neuropathic pain model of rats

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ABSTRACT

The mechanism underlying neuropathic pain is still largely unclear. Recently, much attention has been focused on the role of brain-derived neurotrophic factor (BDNF) as a neuromodulator in the spinal cord. We previously reported that the expression of *Bdnf* exon I mRNA was remarkably up-regulated in the dorsal root ganglion (DRG) neurons with the rat L5 spinal nerve ligation (SNL) model. In the present study, we investigated whether neuropathic pain response would be reduced by the inhibition of the *Bdnf* exon I in the rat SNL model. We identified the promoter region of exon I and synthesized the decoy ODNs targeting the region. Reverse transcription-polymerase chain reaction analysis confirmed that the decoy ODN treatment reduced SNL-induced *Bdnf* exon I mRNA up-regulation in ipsilateral L4 and L5 DRGs. Furthermore, post-treatment with the decoy ODNs significantly attenuated SNL-induced tactile allodynia. This study suggested that decoy ODNs targeting the *Bdnf* exon I might provide a novel analgesic strategy for the treatment of neuropathic pain.

1. Introduction

Neuropathic pain is a debilitating pain that is characterized by spontaneous pain, allodynia, and hyperalgesia. Despite considerable research during the past decades, the mechanism of neuropathic pain is still largely unknown. Furthermore, neuropathic pain responds poorly to most of the commonly used analgesic drugs.

The brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, may act as a survival factor for the dorsal root ganglion (DRG) neurons [1] and may also serve as a neurotransmitter and/or neuromodulator within the dorsal horn of the spinal cord [2]. Some evidence has revealed that the *Bdnf* mRNA expression in DRG neurons was up-regulated in animal pain models, in conditions such as sciatic axotomy [3], inflammation [4] and spinal nerve ligation (SNL) [5]. In addition, increased BDNF levels are transported anterogradely to the central terminals in the spinal dorsal horn [6] and involved in spinal plasticity and central sensitization [7].

The rat *Bdnf* gene consists of nine exons (exons I through IX), which are transcribed into splice-variant mRNAs separately, and all exons are regulated by distinct promoters [8]. In our previous study, among all variants, the *Bdnf* exon I variant showed the greatest response to peripheral inflammation in rats and nerve growth factor (NGF) stimulation in cultured rat DRG neurons [9]. Subsequently, we examined the expression profiles of the *Bdnf* transcripts in bilateral L4 and L5 DRGs two weeks after an L5 SNL with rats [10]. The L5 SNL induced increased expression of the *Bdnf* exon I variant in the ipsilateral DRG, indicating that the *Bdnf* exon I variant is likely to play an important role in processing SNL-induced pain information. Therefore, we hypothesized that inhibition of the *Bdnf* exon I variant would block the neuropathic pain response to peripheral nerve injury.

For the specific knockdown of the *Bdnf* exon I variant, we used the decoy oligodeoxynucleotide (ODN) strategy. This strategy involves the intracellular delivery of double-stranded ODNs corresponding to a specific promoter region, resulting in the inhibition of sequence-specific transcription factors and the transactivation of the target gene [11]. In this study, we aimed to detect the promoter region of exon I, to synthesize decoy ODNs and to investigate the effect of the decoy ODNs on tactile allodynia in a rat neuropathic pain model.

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2. Materials and methods

2.1. Animals

Male Wistar rats (CLEA Japan Inc., Tokyo, Japan) weighing 190–220 g at the time of surgery were used. Animals were housed individually in cages under a 12–12 h light–dark cycle with free access to food and water. The experimental rats were allowed to acclimatize to the facility for 5–7 days prior to surgery. All animals were treated in accordance with the Ethical Guidelines for Investigation of Experimental Pain in Conscious Animals issued by the International Association for the Study of Pain [12] and all procedures were approved by the Animal Care and Use Committee of Okayama University Medical School.

2.2. Surgical procedures

All the rats were placed under anesthesia with pentobarbital sodium (40 mg/kg intraperitoneally). Additional inhalation anesthesia with isoflurane was given as needed. L5 SNL was performed using a modification of the procedure described previously [13]. Briefly, the left transverse process of the L6 vertebra was gently removed, and the left L5 spinal nerve was exposed and tightly ligated with 6-0 silk thread. Subsequently, a polyethylene intrathecal catheter was inserted from the interspace between L6 and S1 and passed inward cranially to have the tip of the catheter locating near L4 and L5 DRGs. After appropriate hemostasis was confirmed, the wound was closed.

2.3. Identification of the promoter region of the Bdnf exon I and synthesis of decoy ODNs targeting the Bdnf exon I

Promoter sequences for the exon I of human, rat and mouse genomic gene were aligned, and polymerase chain reaction (PCR) primers were designed on the conserved sequences as forward primers S1, S2, S3 and reverse primer AS1 (Table 1). The promoter regions with different length (S1–AS1, S2–AS1 and S3–AS1) were amplified with each primer pair using human or rat genomic DNA, and inserted into pGL4.14 firefly luciferase reporter plasmid (Promega, Madison, WI, USA) after confirmation by DNA sequencing. C6 rat glioma cells were grown to 70% confluent in 48-well plates. The pGL4.14 plasmid with each promoter region (0.18 μg) was cotransfected with 0.02 μg of *Renilla* luciferase plasmid pTK-hRG (Promega), as an internal control, into the C6 cells using Effectene (Qiagen, Cologne, Germany). Seventy-two hours after transfection, the activity of firefly luciferase and *Renilla* luciferase were measured using the Dual-Luciferase Reporter Assay kit (Promega) on Labosystems Luminoskan RT (Thermo Scientific, Waltham, MA, USA), and normalized for the variation in transfection efficiency.

Target sequence between primer S1 and S2 was divided into seven fragments with overlapping. The fragments were synthesized as sense and antisense strand DNAs, and double-stranded DNAs were constructed by annealing of each pair (Table 2). Luciferase assay was performed under the same condition of pGL4.14 plasmid with the promoter region S1–AS1, in presence of each DNA fragment (50 nM). Twenty-four hours after transfection, the luciferase activity was measured using the Dual-Luciferase Reporter Assay kit, and normalized for the variation in transfection efficiency.

Table 1
Primer pairs used for detecting *Bdnf* exon I promoter region.

Promoter region	Primer name	Sequence (5′–3′)
S1–AS1	S1	Forward: CCATTAGAGCAAACGCAGTCATA
	AS1	Reverse: CCACCACTTGGTGTGACTTATG
S2–AS1	S2	Forward: CATTTGATCATCACTCACGACC
	AS1	Reverse: CCACCACTTGGTGTGACTTATG
S3–AS1	S3	Forward: TTACCTGGCGACAGGGAAATCT
	AS1	Reverse: CCACCACTTGGTGTGACTTATG

Table 2
Sequence of decoy ODNs.

Decoy ODN	Sequence of the sense strand (5′–3′)
A	CCATTAGAGCAAACGCAGTCATAACTTCATTCAAC
B	TCAACTCAAGCCGCTTGAGAGCTTGGCIIACACCG
C	ACCGGTTCCTGTGGGCAACTAGTGGCTCGCCCGGG
D	CGGGTGCCTCTCGCCTAGTCATCAGTCCCTAAGAG
E	AGAGGAAAAGGGAAAGTTGTGGGGCTGATGCGCTC
F	GCTCTTCGATTCACGCAGTTGTTCCCTAGAACAAG
G	ACAAGTCACTCCTGCTCCATCACCCCTCCCCCCCC
S	TAGTATTTAGTGAGTTAGATGCAGGATATTAGTCG

S; control ODN with a scrambled sequence.

2.4. Intrathecal administration of decoy ODN

For preparation of decoy ODNs, single-stranded sense and antisense ODNs were purchased from Tsukuba Oligo Service (Ibaraki, Japan). Single-stranded ODNs were then annealed for 6 h, during which time the temperature was reduced from 94 to 25 °C.

The rats were randomly divided into four groups before administration of the decoy ODNs which were selected according to the result of the luciferase assay. The experimental groups (decoy C, E and F group) received 42 μg (14 μl volume) of decoy ODNs once a day for three consecutive days, at 5, 6 and 7 days after the L5 SNL. The same dose of double-stranded scrambled ODN was used as a negative control (decoy S group) (Table 2). The double-stranded ODNs were dissolved in phosphate-buffered saline and injected without the use of viral vectors or lipid formulations. The drugs were flushed with 12 μl of saline (dead volume of the catheter).

2.5. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR)

Rats were sacrificed by decapitation under deep anesthesia with a pentobarbital overdose at 7 days after the L5 SNL. Ipsilateral L4 and L5 DRGs were dissected rapidly and dipped immediately in RNAlater (Qiagen GmbH, Hilden, Germany). RNA was isolated and purified from individual DRGs with an RNeasy Lipid Tissue Mini Kit (Qiagen Inc., Valencia, CA). cDNA was synthesized by reverse transcription of each sample with a Ready-To-Go T-primed First-Strand Kit (Amersham Biosciences, Piscataway, NJ).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed as previously described [10]. The sequences of forward and reverse primers for *Bdnf* exon I transcript were developed from sequences available through the National Center for Biotechnology Information (NCBI) (GenBank ID: EF125675). Beta-actin was used as the reference gene. Primer sequences for beta-actin were developed from sequences available at NCBI (GenBank ID: NM_031144). Sequences of primers for *Bdnf* exon I transcript and beta-actin are shown in Table 3.

Real-time RT-PCR was performed with a LightCycler (Roche Diagnostics, Mannheim, Germany) using SYBR Green detection (Takara Bio Inc., Shiga, Japan). Amplification conditions consisted of an initial denaturation of 10 s at 95 °C followed by 45 cycles of 5 s at 95 °C and 20 s at 60 °C. Total rat RNA was amplified with each primer pair, and serial dilutions of each PCR product were used as templates to generate a standard curve.

Table 3
Oligonucleotide sequences of primers for real-time RT-PCR.

Target cDNA	Sequence (5′–3′)	Amplicon size (bp)
Bdnf exon I	Forward: AGTCTCCAGGACAGCAAAGC Reverse: CGTGGACGI II GCTTCTTTC	130
Beta-actin	Forward: CTAAGGCCAACCGTGAAAAG Reverse: ACCCTCATAGATGGGCACAG	170

2.6. Behavioral assessment

Behavioral tests were performed before surgery and at 1, 3, 5–10, 12, and 14 days after the L5 SNL. For assessment of tactile allodynia, the sensitivity to mechanical stimulus was tested by determining hind paw withdrawal response to von Frey filaments (North Coast Medical, Morgan Hill, CA, USA). The rats were placed individually in a plastic cage ($13 \times 10 \times 15$ cm^3) with an elevated wire mesh bottom, allowing full access to the plantar surface of both hindpaws. The mechanical stimuli were applied to the medial plantar aspect of each hindpaw with one of a series of nine von Frey filaments (0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0 and 15.0 g). Each trial was started with a von Frey force of 2 g for 1–2 s. Stimuli were presented at intervals of at least several seconds, allowing for apparent resolution of any behavioral responses to previous stimuli. On the basis of the response pattern and the force of the final filament, the 50% paw withdrawal threshold (PWT) was determined using the up-down method of Dixon [14] and calculated using the formula described by Chaplan et al. [15]. If the strongest filament did not elicit a response, the threshold was recorded as 15.0 g. The behavioral assessment was performed by an investigator who was blind to the treatment type of ODNs.

2.7. Statistical analysis

For the data of luciferase assays and RT-PCR analysis, results are presented as mean ± SD. Statistical significance was determined using the Student's *t*-test. A *P*-value <0.05 was considered significant.

For the data of behavioral tests, tactile withdrawal thresholds with von Frey filaments are non-parametric variables. Between testing days, the data were analyzed by Friedman analysis of variance (ANOVA) for repeated measurements, followed by Wilcoxon matched pairs signed rank test when appropriate. The data between groups on a given testing day were analyzed by Kruskal–Wallis ANOVA, followed by Mann–Whitney *U* test when appropriate. Differences were considered statistically significant at $P < 0.05$. In order to facilitate comparison with other papers in the field, data are presented as mean ± SEM.

3. Results

3.1. Identification of the upstream promoter region of the Bdnf exon I

To identify the proximal promoter region of the *Bdnf* exon I, we generated three promoter deletion constructs: S1–AS1, S2–AS1 and S3–AS1 (Table 1). The highest transcriptional activity was observed with the S1–AS1 promoter region, 7.7-fold higher than the promoterless pGL4.14 basic vector. In contrast, deletion of the S1–S2 region dramatically decreased transcriptional activity, indicating that this region probably contained positive regulatory elements essential for the activation of the *Bdnf* exon I variant (Fig. 1A).

3.2. Synthesis of decoy ODNs

The promoter region between primer S1 and S2 was divided into seven fragments (A–G) with overlapping (Table 2). Luciferase activity of the reporter plasmid with the promoter region S1–AS1 was examined in presence of a double-stranded decoy ODN. Compared to the basal luciferase activity of pGL4.14 basic vector, the rat S1–AS1 promoter region with the scrambled decoy ODN (decoy S) showed 10.0-fold higher activity. However, the rat S1–AS1 promoter activity was repressed by the other seven decoy ODNs (A–G) compared to that with decoy S (Fig. 2). Among all decoy fragments, three kinds of fragments (C, E, and F) strongly repressed the promoter activity (3.7-, 3.9-, and 3.7-fold, respectively). The effective suppression was also observed in the human S1–AS1 promoter activity with these three fragments (data not shown). We adopted the three fragments (C, E, and F) as decoy ODNs in *in vivo* study.

3.3. Expression of the Bdnf exon I transcript in the DRGs after decoy ODN treatment

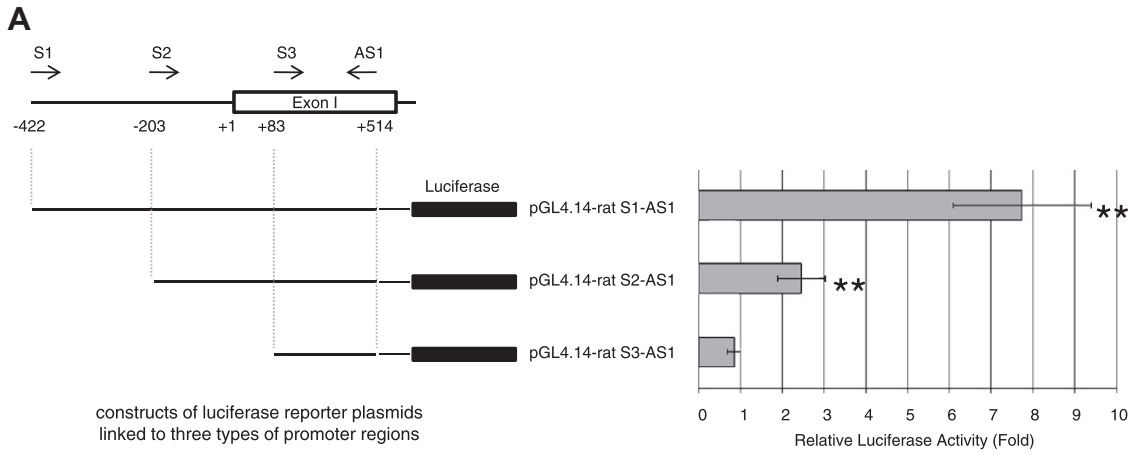
Using RT-PCR analysis, we measured the *Bdnf* exon I mRNA transcript in DRGs after either decoy S or decoy C administration with the L5 SNL model. In both groups, the expression of exon I mRNA significantly increased in the ipsilateral L4 and L5 DRGs compared to the contralateral side. In the decoy C group, however, the up-regulation of exon I was obviously suppressed compared to the value of the decoy S group (22.6 and 17.1% reduction of the expression ratio in the ipsilateral L4 and L5 DRG, respectively) (Fig. 3). In the ipsilateral L4 DRG especially, the decoy C treatment significantly reduced the up-regulated exon I mRNA level compared to the decoy S, while only a slight inhibition was observed in the contralateral side.

3.4. Behavioral assessments

At baseline, there was no significant difference in mechanical withdrawal thresholds across groups. After the L5 SNL, all rats developed tactile allodynia with ipsilateral PWT <4.0 g before the decoy ODNs injection, while contralateral PWT did not vary significantly from baseline values (Fig. 4). The response was maintained at least for 14 days in control group. The 50% PWTs in control group were 12.3 ± 1.1 g at baseline, and 3.3 ± 0.4, 2.9 ± 0.4, 3.2 ± 0.4, 1.7 ± 0.6, 2.1 ± 0.3, 2.7 ± 0.1, 3.1 ± 0.4, 2.8 ± 0.3, 3.2 ± 0.3, and 2.7 ± 0.4 g at 1, 3, 5, 6, 7, 8, 9, 10, 12, and 14 days after surgery, respectively. In experimental groups, mechanical allodynia was significantly attenuated compared to control group after the decoy ODNs injection. In decoy C group, the decoy ODN administration strikingly increased ipsilateral PWTs at 6, 7, 8, 9, and 10 days (4.5 ± 0.3, 7.2 ± 1.6, 8.2 ± 1.1, 7.8 ± 1.2, and 4.7 ± 0.6 g, respectively) after the L5 SNL. In decoy E group at 7 days (3.1 ± 0.2 g) and in decoy F group at 7 and 8 days (4.4 ± 0.7 and 5.0 ± 0.8 g, respectively) after the SNL, ipsilateral PWTs were higher than those in control group (Fig. 4A). There were no significant changes in PWT in the contralateral hindpaw after decoy ODNs injection (Fig. 4B). No motor deficiency was observed during the whole experimental period.

4. Discussion

In the present study, we found significant promoter activity in a 219-bp fragment including the 5′ flanking region of the *Bdnf* exon I. Dividing the region into seven fragments, we synthesized decoy ODNs targeting the *Bdnf* exon I variant. RT-PCR analysis showed that the up-regulated exon I mRNA level in the ipsilateral DRGs after the SNL was reduced by intrathecal administration of the decoy ODN. We also investigated the effects of the decoy ODN treatments on mechanical allodynia in the rat L5 SNL model. Results presented the first evidence that intrathecal post-treatment with decoy ODNs to the *Bdnf* exon I promoter attenuated mechanical allodynia after nerve injury.



B

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-527  gatcctcccc tcctagccta tacctcttcg ctcagagatc tagggctcgc agggcggacc tagctgcaca cagaggctgt
                                  Decoy A                                 Decoy B
-447  taactctcat ttgagaagcc ataagccatt agagcaaacg cagtcataac ttcattcaac tcaagccgct tgagagcttg
                Decoy C                          Decoy D                                Decoy E
-367  gcttacaccg gttcctgtgg gcaactagtg gctcgcccgg gtgcctctcg cctagtcatc agtccctaag aggaaaaggg
                                Decoy F                          Decoy G
-287  aaagttgtgg ggctgatgcg ctcttcgatt cacgcagttg ttccctagaa caagtcactc ctgctccatc acccctcccc

-207  ccccatttga tcatcactca cgaccacgtc cgctggagac ccttagtcat ggtgggggag gggcacgaac ttttctaaga

-127  agtttccttt ttaccaaggg agtcacagtg agttggtcac gtaactggct cagagaggct gccctggccc cctcccctcg

                                                          +1
 -47  ccccctcccc tcgccccctc cccgctgcgc ttttctggta ttcttatTAA AGCGGTAGCC GGCTGGTGCA GGAAAGCAAC

  34  AAGTTCCCCA GCGGTCTTCC CGCCCTAGCC TGACAAGGCG AAGGTTTTCT TACCTGGCGA CAGGGAAATC TCCTGAGCCG

 114  AGCTCATCTT TGCCACAGCC CCAGGTGTGA CCTGAGCAGT GGGCAAAGGA GCGGCGTGCA AATTGGATTA TTTGTATGGG

 194  GGTACTCTGA AACTCCCTCA CTTTTTCTGG GAACTTTTTG TGCTAGGGCG CAGTGACAGG CGTTGAGAAA GCTGCTTCAG

 274  GAAACGCCCG CTATATAGCA GGGCAGTTGG ACAGTCATTG GTAACCTCGC TCATTCATTA GAATCACGTA AGAACTCAAA

 354  GGGAAACGTG TCTCTCAGAA TGAGGGCGTT TGCGTAAATC TATAGGTTTT TCAACATCGA TGCCAGTTGC TTTGTCTTCT

 434  GTAATCGCCA AGGTGGATGA GAGTTGAAGC TTGCGGATAT TGCAAAGGGT TATTAGATTC ATAAGTCACA CCAAGTGGTG

 514  GGCGATCCAC TGAGCAAAGC CGAACTTCTC ACATGATGAC TTCAAACAAG ACACATTACC TTCCAGCATC TGTTGGGGAG

 594  ACGAGATTTT AAGACACTGA GTCTCCAGGA CAGCAAAGCC ACAATGTTCC ACCAGGTGAG AAGAGTGATG ACCATCCTTT
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Fig. 1. Analysis of the proximal promoter region of the rat *Bdnf* exon I. (A) Genomic structure of the rat *Bdnf* exon I and its 5′ flanking region are shown in top. Exon is represented as a box and introns are shown as lines. Nucleotide numbering starts from the transcriptional initiation site (GenBank ID: EF125675). Arrows indicate primers (S1, S2, S3 and AS1) listed in Table 1. Schematic illustrations below indicate rat *Bdnf* exon I promoter-luciferase reporter constructs. The promoter activities of various constructs were determined by dual luciferase assay. Relative activity to empty vector (pGL4.14) is shown on the right side. The data were represented as the mean ± SD ($n = 8$). $^{**}P < 0.01$ vs. empty vector. (B) Sequence alignment of the rat *Bdnf* exon I and its 5′ flanking region are shown. The 5′ flanking region is in lower case letters; exon I is in upper case letters. The underlined parts represent the sequence of decoy oligodeoxynucleotides (Decoy A–G) listed in Table 2. Nucleotide numbers relative to the transcription start site (+1) are indicated on the left side of the sequence. The ATG translational start site is boxed.

It has been suggested that the promoter linked to exon I contributes to regulation of the *Bdnf* mRNA expression by means of sciatic axotomy, dorsal rhizotomy and peripheral inflammation [16], which is consistent with our previous studies exhibiting up-regulation of the *Bdnf* exon I variant in SNL and inflammatory pain models [9,10]. According to our previous data, the expression of the *Bdnf* exon I mRNA remarkably increased in the ipsilateral L4 and L5 DRGs after the L5 SNL. On the other hand, BDNF has emerged as an important regulator concerned with the synaptic plasticity mechanism underlying learning and memory in the adult central nervous system (CNS) [17], indicating that inhibition of BDNF protein or all of the variant mRNAs might cause a serious CNS side effect. We think that the *Bdnf* exon I plays a pivotal role in the development of neuropathic pain and the specific knock-down of the *Bdnf* exon I might contribute to a selective therapeutic effect on pain response without CNS side-effect.

The rat *Bdnf* gene contains eight noncoding exons (exon I–VIII) and one coding exon (exon IV). Each of the nine exons links to separate promoters [18] and several transcription factors regulating BDNF have been characterized [8], while the specific *Bdnf* exon I transcription factor is still unknown. Our result showed that a 219-bp fragment, located between 422 and 204 bp upstream of rat exon I mRNA start site, was sufficient for promoter activity. This region is well conserved between rat and human (86.3%). We think this site is essential for basal promoter activity. The region was divided into seven overlapping fragments, and then seven kinds of 35-bp decoy ODNs were synthesized as listed in Table 2. Among them, decoy C, E, and F (located at −361 to −327, −299 to −265,

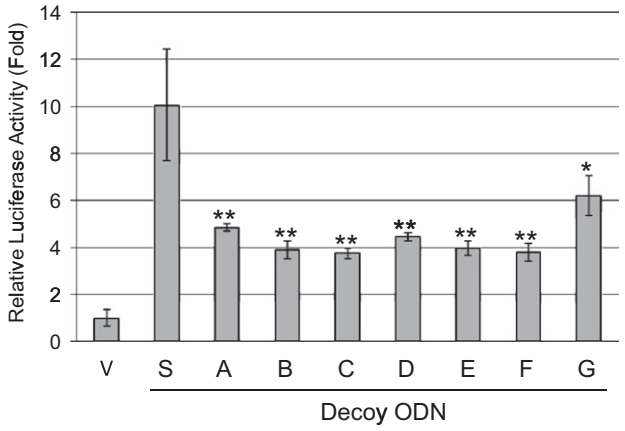

Fig. 2. Repressive effect of decoy ODNs on promoter region of the rat *Bdnf* exon I. In presence of each double-stranded decoy ODN (S, A–G), the promoter activities of pGL4.14 plasmid with the promoter region S1–AS1 were determined by dual luciferase assay. V: empty vector. Relative activity to empty vector is shown. The data were represented as the mean ± SD ($n = 4$ per group). $^{*}P < 0.05$ vs. decoy S and $^{**}P < 0.01$ vs. decoy S.

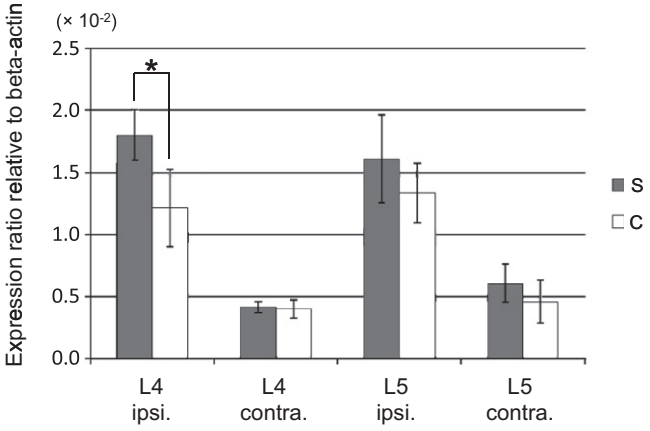

Fig. 3. Expression of the *Bdnf* exon I mRNA in the DRGs after decoy ODN treatment. The *Bdnf* exon I mRNA transcript in bilateral L4 and L5 DRGs was measured separately using RT-PCR analysis. Beta-actin was used as a reference gene. The data were represented as the mean ± SD ($n = 4$ per group). $^{*}P < 0.05$ vs. decoy S.

and −268 to −234 upstream of rat *Bdnf* exon I start site, respectively) demonstrated the most efficient repression of the transcriptional activity of the *Bdnf* exon I promoter region. The sequence of decoy C is highly conserved between rat and human (91.4%).

As shown in Fig. 3, RT-PCR analysis confirmed that intrathecal injection of the decoy ODN reduced the *Bdnf* exon I mRNA up-regulation in ipsilateral DRGs on day 7 after the SNL. Furthermore, the decoy ODN treatments significantly attenuated SNL-induced mechanical allodynia compared with the scrambled control ODN (Fig. 4). These results support our hypothesis that the specific knockdown of the *Bdnf* exon I contributes to attenuation of the neuropathic pain response.

It is reported that the *Bdnf* mRNA expression rapidly increases in DRG neurons at two days after axotomy, and BDNF immunoreactivity also increases in DRGs and their central terminals in the dorsal horn at four days after nerve injury [19]. The *Bdnf* mRNA up-regulation seems to be maintained at least for 14 days after axotomy. Consistent with the findings, we previously observed increased *Bdnf* exon I mRNA level at 14 days after the L5 SNL [10]. In this study, ipsilateral PWTs increased after injection of decoy ODNs as shown in Fig. 4A, indicating that the onset of these decoy ODNs is within 24–48 h and the anti-allodynic effect maintains for a maximum of five days. Lee et al. showed that intrathecal treatment of NF-κB decoy attenuated pain behaviors induced by chronic constriction injury of the sciatic nerve, though it had to be injected 24 h before the operation [20]. In our experiment, decoy ODNs were administered after the development of neuropathic pain response. Post-treatment has an important implication because pre-treatment of analgesic agents would be impossible in most clinical settings. To our knowledge, there is no study that has demonstrated the anti-allodynic effect of post-treatment with decoy ODNs as a tool for neuropathic pain management.

Previous authors concluded that the production and maintenance of mechanical hyperalgesia depended on impulses from injured afferents [21]. In contrast, recent investigators have suggested that input from the uninjured nerve fibers plays a critical role in the production and maintenance of mechanical hyperalgesia in neuropathic pain [22,23]. Furthermore, Coull et al. have reported that microglia-derived BDNF triggers allodynia in the rat peripheral nerve injury model [24]. In the present study, the physiological mechanism of the decoy ODN's effect was not investigated in detail. Therefore, additional studies are required to determine which site of nociceptive pathways the decoy ODN is acting on.

In summary, we synthesized decoy ODNs targeting the *Bdnf* exon I and confirmed the transcriptional suppression of the *Bdnf* promoter using luciferase assay *in vitro* and RT-PCR analysis *in vivo*. Intrathecal administration of the decoy ODN attenuated

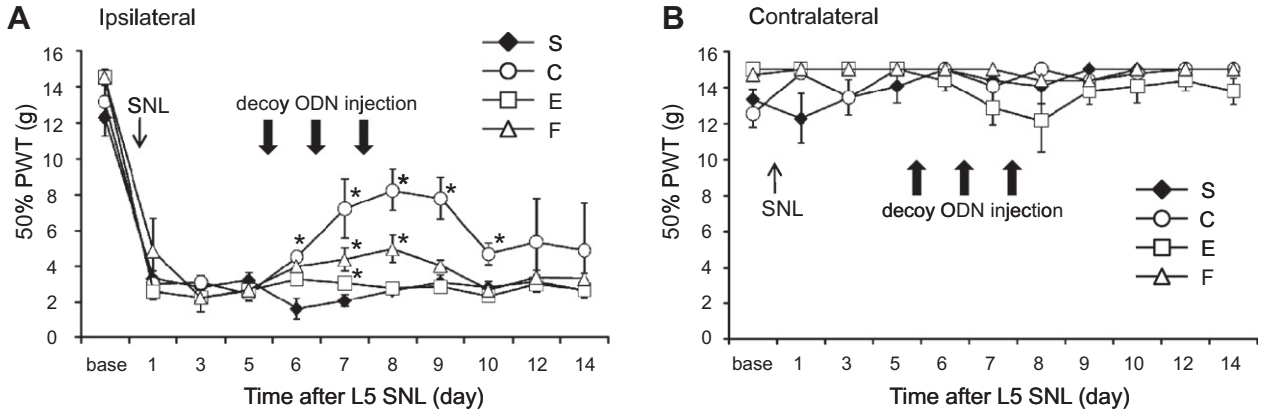

Fig. 4. Time course of (A) ipsilateral and (B) contralateral 50% paw withdrawal threshold (PWT) to mechanical stimuli. Data are expressed as mean ± SEM. ($n = 5$ per group). $^{*}P < 0.05$ vs. decoy S group.

neuropathic pain response in the rat L5 SNL model. This study indicates that a decoy ODN to the *Bdnf* exon I might provide a novel analgesic strategy for the treatment of neuropathic pain.

Conflict of interest

None declared.

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