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Basic Science

Administration of ONO-2506 suppresses neuropathic pain after spinal cord injury by inhibition of astrocytic activation

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Abstract

BACKGROUND CONTEXT: Spinal cord injury (SCI) results in not only motor dysfunction but also chronic neuropathic pain. Allodynia, an abnormal sensation that evokes pain against non-noxious stimuli, is a major symptom of post-SCI neuropathic pain. Astrocytic activation is a cause of post-SCI neuropathic pain and is considered a key treatment target. However, no effective treatment for these problems is available to date. ONO-2506 is a novel agent that suppresses astrocytic activation by inhibition of S100B production from astrocytes. Recently, it has been demonstrated that ONO-2506 inhibits secondary injury and improves motor function after SCI.

PURPOSE: This study aimed to investigate the effect of ONO-2506 on post-SCI neuropathic pain.

STUDY DESIGN: Animal study of a rat model of spinal cord contusion.

METHODS: A total of 22 male Sprague-Dawley rats aged 6 weeks were used. Incomplete SCI was created at T10 level. Animals were divided into two groups: Saline group and ONO-2506 group. Nine animals in each group were finally included for this study. Intraperitoneal administration of ONO-2506 (20 mg/kg) or saline was continued daily for 1 week following SCI. Recovery of hind limb motor function was assessed using the Basso, Beattie, and Bresnahan (BBB) score. Mechanical and thermal allodynia of hind paws were evaluated by the withdrawal threshold using a von Frey filament and the withdrawal latency using the plantar test device. At 6 weeks after SCI, sagittal sections at the injured site and axial sections at L 4/5 were evaluated by fluorescent immunohistochemistry staining using S100B and glial fibrillary acidic protein (GFAP) antibodies.

RESULTS: The improvement course of BBB scores was similar between the two groups. However, the withdrawal thresholds for mechanical stimuli and the withdrawal latency for thermal stimuli were significantly higher in the ONO-2506 group than in the Saline group over 6 weeks after SCI. The histologic assessments at the injured site demonstrated a significant reduction in the cross-sectional area of the cysts and a high fluorescence intensity area of S100B and GFAP in the ONO-2506 group. By correlation analysis, a high absolute value of the correlation coefficient was confirmed between the intensity of S100B expression at the injured site and the allodynia severity.

FDA device/drug status: Not applicable.

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CONCLUSION: Administration of ONO-2506 attenuated post-SCI neuropathic pain in a rat model of incomplete SCI. Histologic results support that the inhibition of S100B production and subsequent suppression of astrocytic activation contributed to the reduction in neuropathic pain.

Keywords: Spinal cord injury; Neuropathic pain; Astrocyte; S100B; Allodynia; ONO-2506; Arundic acid

Introduction

Neuropathic pain occurs in 53% of patients who experienced spinal cord injury (SCI) [1,2]. Severe, chronic pain represents a physical and psychological burden to patients even with incomplete injury, resulting in long-term deterioration of activities of daily living and quality of life [3]. However, no effective treatment for these problems is available to date [4].

At the injured site of the spinal cord, a secondary injury due to immune reaction occurs within a week after the primary mechanical damage, which exacerbates neurologic disorders. In this immune reaction, astrocytes play an important role through aberrant proliferation and activation, called reactive astrogliosis. Reactive astrocytes display pro-inflammatory and neurodegenerative phenotypes with morphologic characteristics such as expanded cytoplasm and extended processes. They produce various pro-inflammatory cytokines and neurotoxic agents, which induce neuronal hyperexcitability, neuronal cell death, and formation of a glial scar, which obstructs regeneration of neuronal axons [5,6]. Recently, reactive astrogliosis is considered to be one of the major causes of post-SCI neuropathic pain including allodynia, which is an abnormal sensation that evokes pain against non-noxious stimuli [7]. Reactive astrogliosis has recently attracted attention as a new therapeutic target [8,9].

S100B, a member of a family of EF-hand Ca^{2+} binding proteins, is expressed primarily by astrocytes in the central nerve system. Its production and secretion are enhanced in reactive astrocytes. This protein shows neurotoxic effects at high extracellular concentrations [10]. In addition, S100B secreted into the extracellular space promotes further astrocytic activation, causing a S100B autocrine loop [11]. Therefore, inhibiting S100B production by astrocytes can be an effective approach for modulation of inflammation due to astrocytic activation.

ONO-2506 is an agent that suppresses astrocytic activation by inhibiting S100B production from astrocytes. In brain trauma and ischemic brain injury, ONO-2506 has been demonstrated to reduce lesion expansion and improve neurologic function, and the effects on recovery of motor function after SCI have already been reported [12−14]. Based on these findings, we hypothesized that ONO-2506 is also effective to attenuate neuropathic pain after SCI. The purpose of this study was to investigate the effect of ONO-2506 on post-SCI neuropathic pain.

Materials and methods

Experimental animals

All animal experiments in this study were approved by the committee on the Ethics of Animal Experiments in our university. A total of 22 SD rats (male, 6-week-old) were used (weight range; 180−240 g). They were divided into two groups: Saline group (control group; n=12) and ONO-2506 group (n=10).

Spinal cord injury

The animals were anesthetized by subcutaneous injection of 2 mg/kg midazolam (Astellas Pharma, Tokyo, Japan), 0.15 mg/kg medetomidine (Nippon Zenyaku Kogyo, Fukushima, Japan), and butorphanol tartrate (Meiji Seika, Tokyo, Japan). In addition, preoperative antibiotics (50,000 U/kg penicillin G, Meiji Seika, Ltd., Tokyo, Japan) were administered subcutaneously. A posterior midline incision was made and the laminae of the T9−11 vertebrae were exposed, followed by laminectomy at T10 vertebra without disruption of the dura mater. Incomplete SCI was created with a contusion by dropping a 10 g weight from 1.2 cm height onto the exposed spinal cord at T10 level [15,16]. After SCI, the muscles and skin were sutured using nylon sutures. The rats were allowed to recover with free access to water and food after surgery. Postoperative antibiotics were administered for 3 days.

Drug administration

ONO-2506 [(R)-(-)-2-propyloctanicacid (arundic acid)] was kindly provided by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). In the ONO-2506 group, the animals received daily intraperitoneal injection of ONO-2506 (20 mg/kg) for 1 week following SCI, whereas saline was injected in the control group. The dose and the route of drug administration were decided based on previous studies [13,17].

Locomotor function

Recovery of hind limb motor function was evaluated using the Basso, Beattie, and Bresnahan (BBB) score [18]. The BBB scores were measured before and 1, 3, 7, 14, 21, 28, 35, and 42 days after SCI. An average score of both limbs was recorded. Animals were excluded from the analysis when the score was higher than 1.5 or urinary retention

was observed on the next day of SCI. Finally, nine animals in each group were included.

Mechanical allodynia

To assess mechanical allodynia, the withdrawal threshold was measured using von Frey filaments (Muromachi Kikai Co., Ltd, Tokyo, Japan: bending forces 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0 g). After 30 minutes of acclimation in plastic cages on a metal mesh floor, the filaments were applied to the center of plantar surface of each hind paw using a modification of the up-down method [19]. The trials were started with the 2.0 g filament. A positive response was recorded when an animal quickly withdrew its paw. Each filament was applied 5 times at 10-second intervals. The withdrawal threshold was defined as the lowest bending force at which positive responses were observed at least three times. When an animal showed less than 3 responses at the force of 15 g, the threshold was considered as 15 g. The threshold was recorded as an average of both hind paws. This testing was performed before SCI and weekly until 6 weeks after SCI.

Thermal allodynia

Thermal allodynia after SCI was assessed using the plantar test instrument (Ugo Basile, Varese, Italy) based on Hargreaves' method [20]. After 30 minutes of acclimation in plastic cages on the acrylic glass floor, infrared radiation (intensity: 50) was applied to the center of plantar surface of each hind paw. Withdrawal latency was recorded automatically when the irradiated hind paw was moved. An automatic cutoff time of 15 seconds was used to prevent possible tissue damage. The mean latency of three trials was recorded and an average of two hind paws was considered as the withdrawal latency of each animal. This testing was performed before SCI and weekly until 6 weeks after SCI.

Tissue preparation

At 6 weeks after SCI, the animals were deeply anesthetized with a combination of the three agents described above and transcardially perfused with 4% paraformaldehyde. Subsequently, the injured sites of the thoracic spinal cord and the lumber spinal cord (L4/5) were collected, post-fixed in 4% paraformaldehyde for 1 week, stored in 30% sucrose for 3 days, embedded in frozen section compound (FSC) 22 (Leica), and stored at −80°C. The thoracic cord samples were sectioned into serial 10 μm sagittal sections and the lumber cord samples were sectioned into 20 μm transverse sections with a cryostat (Leica).

Immunohistochemistry

After blocking with 10% goat serum for 30 minutes, the sections were incubated with mouse anti-S100B (1:1,000, S2532, Sigma) and rabbit anti-GFAP (1:200, G9269, Sigma) primary antibodies for 2 hours at room temperature. Subsequently, the sections were incubated with goat anti-mouse IgG Alexa Fluor Plus 555 antibodies (1:1,000; Thermo Fisher Scientific) and goat anti-rabbit IgG Alexa Fluor Plus 488 antibody (1:1,000, Thermo Fisher Scientific) for 1 hour at room temperature. Finally, the sections were mounted with ProLong Diamond mountant with DAPI (Thermo Fisher Scientific).

Histologic analysis

The stained sections were observed under a BZ-X700 fluorescence microscope (Keyence, Osaka, Japan) with the same settings. Histologic analysis was performed with BZ-X Analyzer software (Keyence). In sagittal 7,000 μm sections of the injured site, the area of high fluorescence intensity region of S100B and GFAP high fluorescence intensity (threshold; S100B: >20, GFAP: >25) and cross-section area of the cyst were measured. The average value of three sections was recorded (nine samples per group). In axial sections of L4/5, the mean intensity of S100B and GFAP at the dorsal horn was calculated. The average value of both sides with three sections was recorded (three samples per group).

Statistical analysis

JMP pro 13 software (SAS Institute, Cary, NC) was used for statistical analysis. All results are presented as mean± standard deviation (SD). Wilcoxon rank sum test was performed to compare values of behavioral and histologic results between the two groups. The correlation between the behavioral and histologic data of individual animals was analyzed using Spearman's rank correlation analysis (n=18).

Results

Locomotor function

Evaluation of BBB scores demonstrated a similar improvement in the course of locomotor function between the ONO-2506 and the Saline groups (Fig. 1A). The day after SCI, animals in the both groups showed paraplegia (BBB scores-Saline group: 0.33±0.43, ONO-2506 group: 0.44±0.53). Paralysis recovery continued consistently to forelimb-hindlimb coordinated stepping without dragging at week 6, on average (BBB scores-Saline group: 18.7± 2.2, ONO-2506 group: 19.2±2.3). Although there were no significant differences between the two groups through the 6 weeks after SCI, the recovery was earlier in the ONO-2506 group than the Saline group during weeks 1 to 3 after SCI.

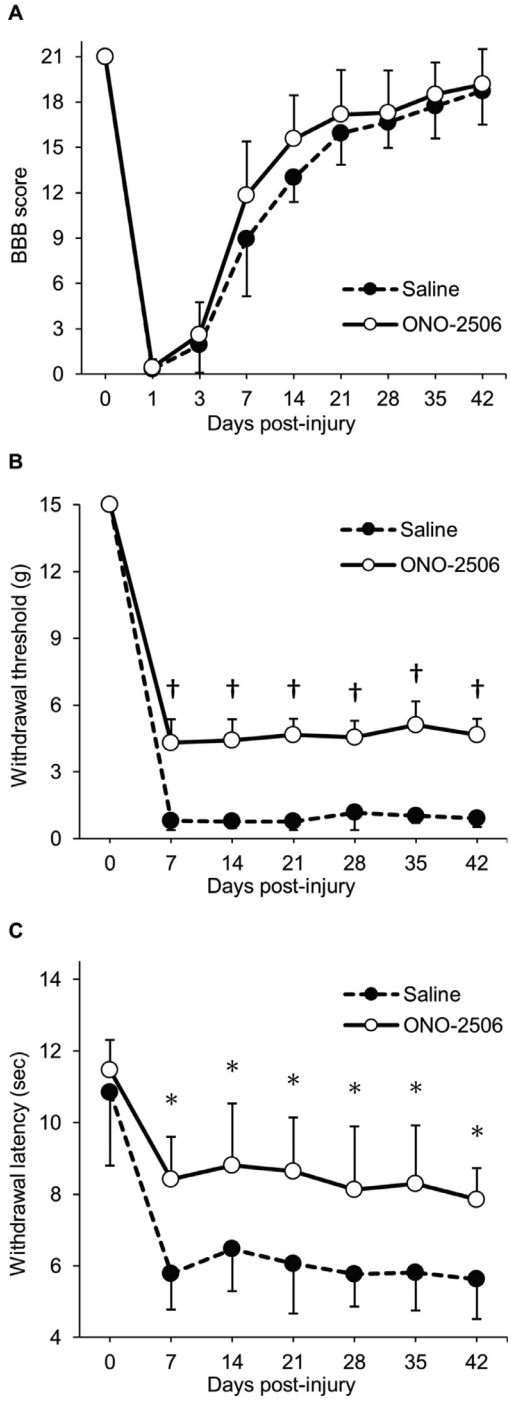

Fig. 1. Graphs of locomotor function and sensory function after spinal cord injury (SCI).

(A) BBB scores. The animals in both groups showed paraplegia at day 1, followed by progressive recovery to about 19 on average at week 6. There was no significant difference between the scores of the two groups through 6 weeks after SCI. (B) Withdrawal thresholds to mechanical stimuli by von Frey filaments. The thresholds in both groups showed a remarkable decrease at week 1 and persisted for 6 weeks. The ONO-2506 group demonstrated significantly higher thresholds than the Saline group over the entire 6 weeks after SCI. (C) Withdrawal latencies to thermal stimuli by the plantar test. The latencies in both groups decreased at week 1 and were maintained for 6 weeks. The ONO-2506 group showed significantly higher latencies than the Saline group over the entire 6 weeks after SCI. Mean± standard deviation, †p<.001, *p<.05.

Mechanical allodynia

The withdrawal threshold to mechanical stimuli by von Frey filaments decreased after SCI in both groups, indicating the appearance of mechanical allodynia (Fig. 1B). Withdrawal thresholds in the Saline and ONO-2506 group 1 week after SCI were 0.80±0.41 and 4.3±1.1, respectively. Mechanical allodynia persisted at almost the same degree for 6 weeks in both groups. Withdrawal thresholds of the Saline and ONO-2506 group at week 6 were 0.91± 0.39 and 4.7±0.7, respectively. Withdrawal thresholds in the ONO-2506 group was significantly higher than in the Saline group over the entire 6 weeks after SCI (p<.001).

Thermal allodynia

Withdrawal latencies to heat stimuli by plantar test decreased at 1 week after SCI in both groups (Fig. 1C). Withdrawal latencies in the Saline group and ONO-2506 group at 1 week after SCI were 5.8±1.0 and 8.4±1.2, respectively. Thermal allodynia also persisted for 6 weeks and the withdrawal latencies in the Saline group and ONO-2506 group at week 6 were 5.6±1.1 and 7.8±0.9, respectively. Withdrawal latencies in the ONO-2506 group was significantly longer than in the Saline group through the 6 weeks after SCI (p<.05).

Immunohistochemical assessment

Astrocytes at the insured site

At 6 weeks after SCI, the formation of cysts was observed at the injured sites in both groups (Fig. 2A). The cross-sectional area of the cysts in the Saline group and the ONO-2506 was 2.4±1.1 mm^2 and 1.4±0.6 mm^2, respectively. The values in the ONO-2506 group were significantly lower than those in the Saline group (p<.05; Fig. 3A). In the Saline group, widespread increase of S100B fluorescence intensity was observed around the cysts. In contrast, in the ONO-2506 group, the high-intensity region was confined to the outer edge of the cysts (Fig. 2A). The area of S100B high-intensity region in the Saline and the ONO-2506 group was 0.49±0.20 mm^2 and 0.16±0.10 mm^2, respectively. The value in the ONO-2506 group was significantly lower than that in the Saline group (p<.005; Fig. 3B). The GFAP fluorescence intensity was also assessed to evaluate astrocytic activation. The localization of GFAP positive cells was similar to that of S100B in both groups (Fig. 2A). The area of GFAP high-intensity region in the Saline and ONO-2506 groups was 0.90±0.37 mm^2 and 0.60±0.44 mm^2, respectively. The value in the ONO-2506 group was significantly lower than that in the Saline group (p<.005; Fig. 3C). At high-magnification images, GFAP positive astrocytes in the Saline group showed expanded cytoplasm and extended cell processes, matching with localization of S100B positive cells. In contrast, most of the astrocytes in the ONO-2506 group showed normal morphology with low S100B intensity (Fig. 2B).

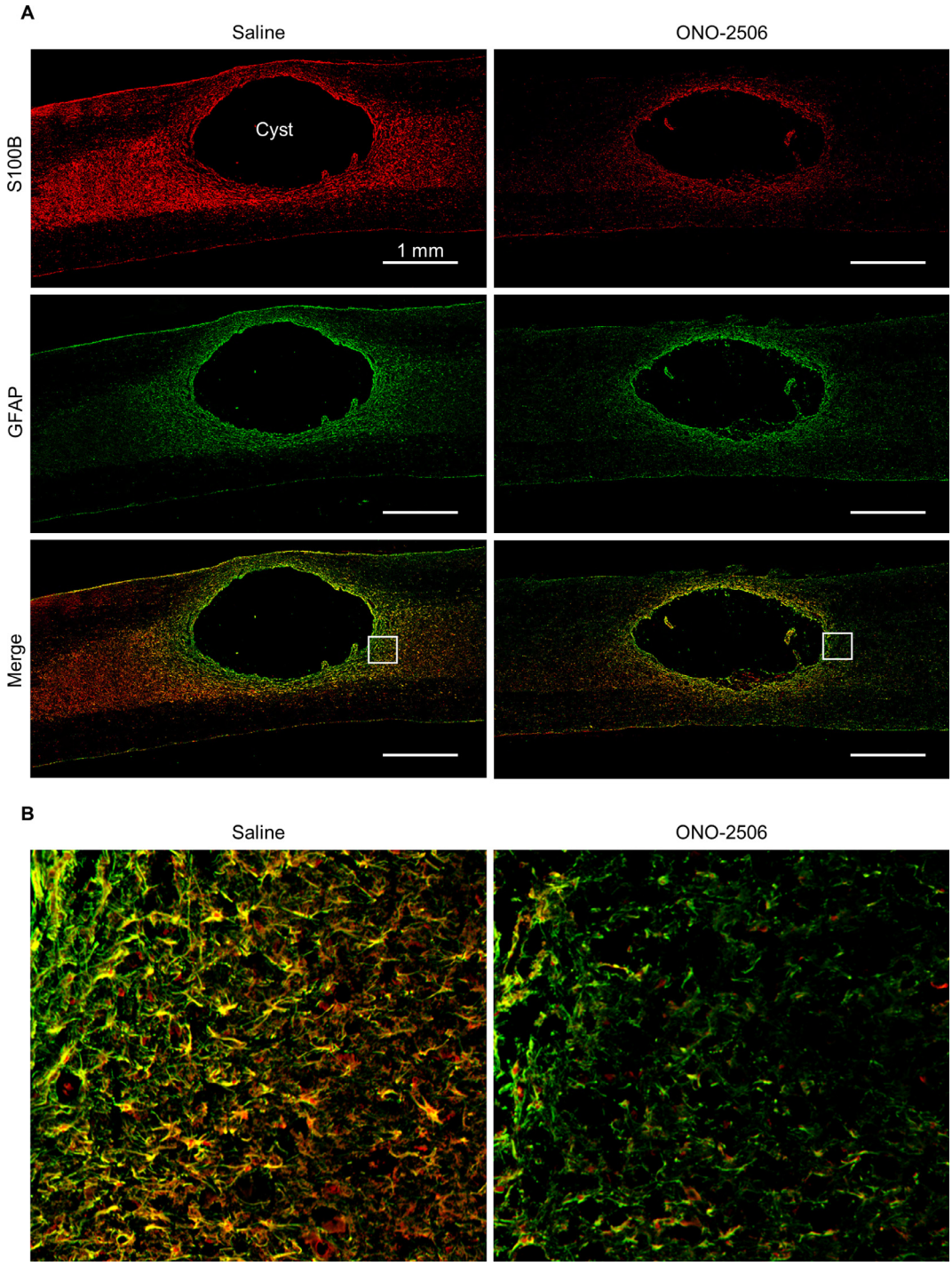

Fig. 2. Immunohistochemical evaluation of the injured site. (A) Fluorescent immunostaining for S100B (red) and GFAP (green) in the sagittal section of the spinal cord at the T10 level. Cyst formation was observed in both groups. The high-intensity region of S100B and GFAP was distributed extensively around the cyst in the Saline group. In contrast, the high-intensity region was confined to the outer edge of the cyst in the ONO-2506 group. (B) High magnification of merged images of S100B and GFAP at the part indicated by the square in the figure above. In the Saline group, astrocytes positive for both S100B and GFAP with expanded cytoplasm and extended cell processes were observed extensively. By contrast, in the ONO-2506 group, astrocytes showed normal morphology with low S100B intensity.

Collectively, these results indicated that ONO-2506 inhibited the expression of S100B in astrocytes and reduced the resultant reactive astrogliosis and cystic formation at the injured site.

Astrocytes at the lumber dorsal horn

Next, astrocytes at the lumbar dorsal horn in both groups were evaluated. The astrocytes at the lumbar dorsal horn did not show a reactive phenotype such as cytoskeletal

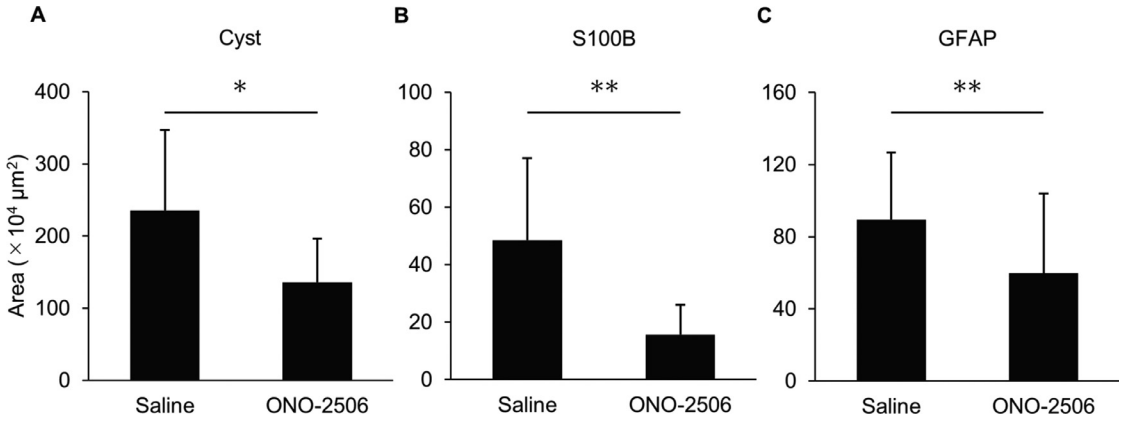

Fig. 3. Quantitative analysis of immunohistochemistry at the injured site. (A) The cross-sectional area of the cyst. (B) The area of the S100B high-intensity region. (C) The area of the GFAP high-intensity region. All values are significantly lower in the ONO-2506 than in the Saline group. Mean±standard deviation, *p<.05, **p<.005.

changes and increased GFAP expression in both groups (Fig. 4A). The mean intensity of S100B at the dorsal horn in the Saline and ONO-2506 groups was 64.8±36.0 and 77.7±52.4, respectively. There was no significant difference in S100B intensity between the two groups (Fig. 4B). The mean intensity of GFAP in the Saline and ONO-2506 groups was 31.5±7.4 and 27.0±4.3, respectively. There was no significant difference in GFAP intensity between both groups (Fig. 4C). These results suggested that SCI at T10 level did not cause astrocytic activation at the lumber dorsal horn and the effect of ONO-2506 was mainly exerted on the astrocytes at the SCI level.

Correlation analysis between behavioral and histologic results

The results of the correlation analysis between the behavioral and the histologic values are shown in Tables 1 and 2. BBB scores at days 3, 7, and 14 after SCI correlated only with the area of GFAP high-intensity lesion, whereas BBB scores at days 21, 28, 35, and 42 did not show a correlation with any histologic results. In contrast, the withdrawal threshold in the von Frey test and withdrawal latency in the Plantar test were negatively correlated mainly with the area of the S100B high-intensity lesion. Notably, the Spearman's rank correlation coefficient between S100B area and withdrawal latency in Plantar test was −0.73. These results indicate that increased S100B expression of astrocytes at the injured site is critically involved in the deterioration of allodynia.

Discussion

Our study revealed that ONO-2506 administration had significant attenuating effects on both mechanical and thermal allodynia. At the injured site of the spinal cord, S100B production and the resultant activation of astrocytes decreased due to ONO-2506 treatment. On the other hand, at the dorsal horn of L4/5, no effects related to astrocyte activity were observed. These results demonstrated that ONO-2506 suppresses post-SCI neuropathic pain by suppression of secondary injury associated with astrocytic activation at the injured site. Additionally, a strong correlation was confirmed between the intensity of S100B expression at the injured site and the allodynia severity. Collectively, ONO-2506 can be a rational therapeutic agent for post-SCI neuropathic pain.

S100B plays a pivotal role in secondary injury attributed to astrocytic activation in the central nervous system. Reactive astrocytes at the injured site play a role in the increase in S100B production. This causes an elevation of the extracellular concentration of S100B, which induces nitric oxide (NO)-dependent neuronal death [21]. In addition, S100B promotes astrocytic activation by an autocrine loop dependent on the receptor for advanced glycation endproducts in astrocytes, enhancing the expression of pro-inflammatory factors such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin 1-beta, interleukin 6, and tumor necrosis factor-α [22]. In transgenic mice overexpressed S100B, the infarct region and neuronal deficit have been reported to deteriorate after cerebral ischemia [23]. In S100B knockout mice, it has been reported that neuronal dysfunction due to cerebral infarction was suppressed [24]. Based on the relationship between cerebral infarction volume and S100B concentration in blood, it is widely recognized as a useful clinical biomarker [25,26]. Moreover, S100B levels in serum and cerebrospinal fluid have been reported to closely related to the severity of SCI [27].

ONO-2506 acts on astrocytes selectively and suppresses astrocytic activation by inhibiting S100B production. In this respect, ONO-2506 is different from other antiastrocytic agents such as propentofylline, which inhibits adenosine reuptake [28], and pentamidine, which blocks the interaction between S100B and p53 [29]. In vitro, ONO-2506 has been shown to suppress the expression of inflammatory cytokines such as COX-2 in astrocytes by inhibiting excess production

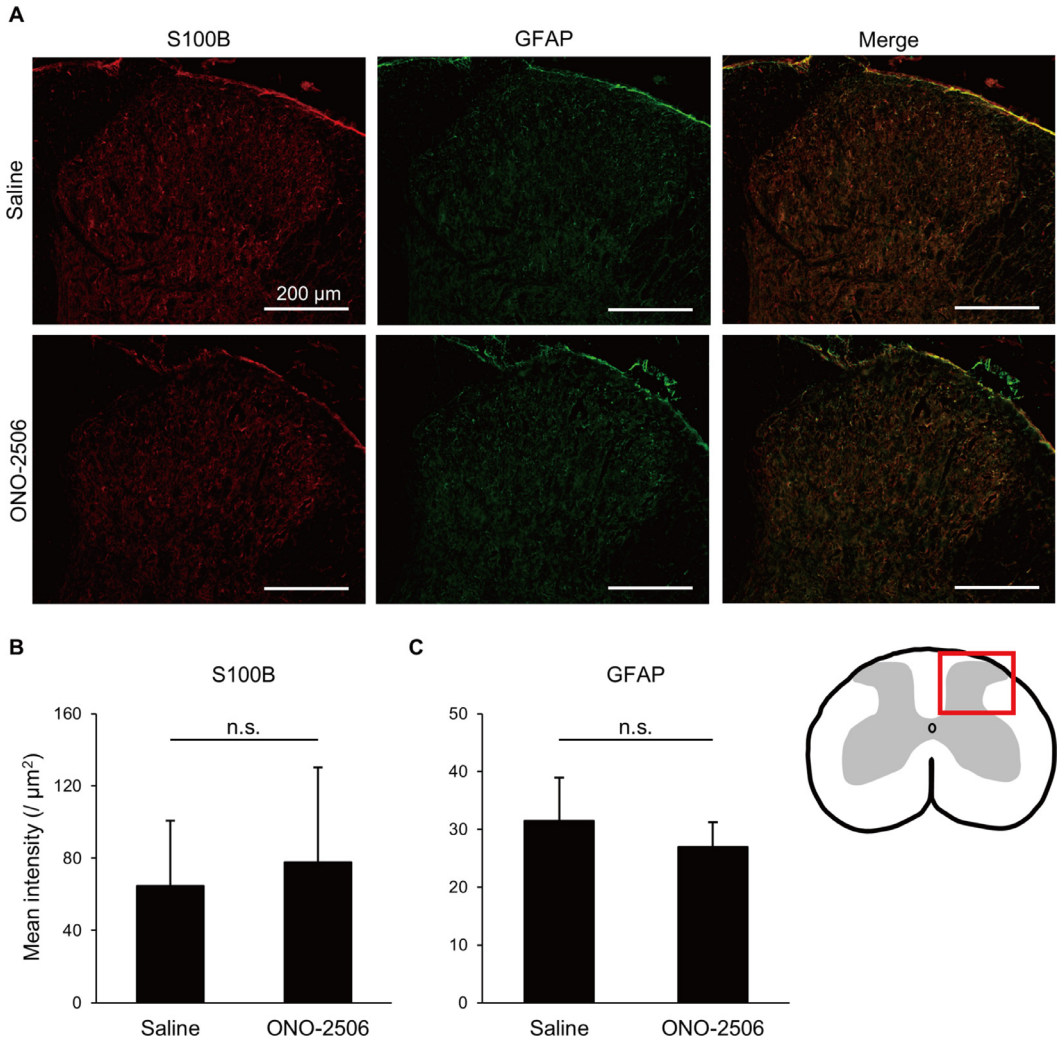

Fig. 4. Immunohistochemical evaluation of the lumber dorsal horn. (A) Fluorescent immunostaining for S100B (red) and GFAP (green) at the lumber dorsal horn. The astrocytes did not show a reactive phenotype such as cytoskeletal changes and increased GFAP expression in both groups. (B) The mean intensity of S100B and GFAP at the dorsal horn. There was no significant difference in S100B and GFAP intensity between the two groups. Mean±standard deviation.

Table 1
Correlation between histologic results and BBB score at day 3, 7 and 14

	Days investigated BBB score	Spearman's rank correlation coefficient (ρ)
Cyst area	Day 3	0.34
	Day 7	−0.07
	Day 14	−0.36
S100B area	Day 3	−0.41
	Day 7	−0.37
	Day 14	−0.41
GFAP area	Day 3	−0.63*
	Day 7	−0.68*
	Day 14	−0.58*

BBB score, Basso, Beattie and Bresnahan score; GFAP, glial fibrillary acidic protein.
* $|\rho|>0.5$.

Table 2
Correlation between results of histology and allodynia tests (withdrawal thresholds by von Frey test and withdrawal latency by plantar test) at day 42

	Allodynia tests	Spearman's rank correlation coefficient (ρ)
Cyst area	von Frey test	−0.36
	Plantar test	−0.36
S100B area	von Frey test	−0.54*
	Plantar test	−0.73*
GFAP area	von Frey test	−0.30
	Plantar test	−0.55*

GFAP, glial fibrillary acidic protein.
* $|\rho|>0.5$.

of S100B [10]. In a co-culture system of astrocytes and neurons, ONO-2506 also attenuates NO-induced neuronal death by suppressing S100B production and iNOS expression of astrocytes [10,30]. Through these effects, ONO-2506 not only reduces lesion expansion and improves neurologic function after traumatic and ischemic brain injury, but also ameliorates motor function after SCI [12−14,31]. The safety and tolerability of ONO-2506 has been confirmed in a clinical trial on acute ischemic stroke [32].

We evaluated allodynia using a model of incomplete SCI by relatively weak contusion energy. With this model, even in the Saline group, the BBB score recovered to about 19 on average after 6 weeks of injury. A 10 g weight dropped from a height of 1.2 cm was considered to provide lower damage to the spinal cord compared to 200 kdyn force by commercially available SCI devices, affecting the BBB scores that was higher than other publications [33]. Probably for this reason, the effect of ONO-2506 on motor functional recovery, which has been reported in the previous study [13], was not observed in this study. However, allodynia was observed in both groups from the week following SCI. After ONO-2506 administration for 1 week after injury, mechanical and thermal allodynia was significantly suppressed compared to the Saline group from the following week of injury, and the effect sustained over the entire 6-week period after SCI. These facts mean that ONO-2506 suppresses neuropathic pain by acting within 1 week after SCI and the effect lasts during the following period.

Histologic evaluation demonstrated that ONO-2506 inhibited S100B production and subsequent activation of astrocytes at the injured site. An extensive expression of S100B and GFAP around the cysts was significantly decreased in the ONO-2506 group. On the other hand, a strong correlation between S100B expression at the injured site and allodynia severity was confirmed, whereas GFAP expression highly correlated with BBB scores at days 3, 7, and 14. These results suggest that the increase of S100B at the injured site is critical for the development of a disorder in the sensory nervous system after SCI. Taken together, ONO-2506 attenuated pain behaviors by reducing sensory neuronal damage around the injured site, which is achieved by the inhibition of S100B production. In addition, the decreased production of other neurotoxic substances by the widespread distribution of reactive astrocytes may also contribute to the pain relief.

ONO-2506 administration also reduced the formation of the cysts and the glial scar around the injured site. Since the glial scar prevents regeneration of neuronal axons [34], these effects may have promoted regeneration of secondary afferent nociceptive neurons or descending serotonergic neurons, which can lead to alleviate neuropathic pain. Besides, the cyst is formed through phagocytosis of dead cells by macrophages whose activity is influenced by astrocytic activation [35,36]. Suppression of astrocytic activation by ONO-2506 may have been involved in modulating the action of macrophages and following cyst formation. Unlike with the S100B and GFAP positive area, the cross-sectional area of the cyst was not correlated with pain behavior. This suggests that inflammatory changes due to astrocytic activation at the injured site are more strongly implicated in sensory function than the physical damage of axons and disturbance of axonal regeneration by the cysts and glial scar.

By contrast, reactive astrogliosis was not observed at the L4/5 dorsal horn in both groups. This result is different from previous reports [5,37,38], but it may be attributable to the fact that relatively low contusion energy was applied to the spinal cord in this study compared to previous reports. Our results suggest that the change caused at the injured site is a key component in neuropathic pain after SCI rather than the changes caused at the lumber dorsal horn.

A phase Ⅲ clinical study for acute ischemic stroke was terminated because of lack of efficacy. As to the potential side effects represented by depression [39], the definite relationship between the appearance or aggravation of depression and ONO-2506 administration is not evidenced. However, screening and careful observation of patients at risk for depression are recommended. As to the lack of efficacy in the phase Ⅲ clinical study, the low-dose administration (2−12 mg/kg) might have affected the results. Therefore, in this study, we selected the dose of 20 mg/kg which has been reported to be more effective than 10 mg/kg for secondary injury in SCI [13]. The findings of this study are expected to be reproduced in clinical trials at higher dose.

There are several limitations in this research. First, we did not examine axonal regrowth or sprouting at the injured site histologically. Second, the detailed mechanisms of the inhibitory effect by ONO-2506 on astrocytic S100B production have not been clearly elucidated. Finally, we did not assess the changes at a higher level including the thalamus, which can also be related to pain after SCI. Future studies are required to address these limitations.

Conclusions

Administration of ONO-2506 attenuated neuropathic pain after SCI in a rat model of incomplete SCI. The decrease in fluorescence intensity of S100B and GFAP at the injured site suggests that the inhibition of S100B production and subsequent suppression of astrocytic activation contributed to the reduction of neuropathic pain. As S100B expression at the injured site strongly correlates with allodynia, ONO-2506 could be a rational therapeutic agent against post-SCI neuropathic pain.

Acknowledgments

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