Role of satellite cell-derived L-serine in the dorsal root ganglion in paclitaxel-induced painful peripheral neuropathy

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Abstract

Paclitaxel is one of the most commonly used anti-neoplastic drugs for the treatment of solid tumors. Unfortunately, its use is often associated with dose-limiting painful peripheral neuropathy and subsequent neuropathic pain that is resistant to standard analgesics. However, there are few clinically available drugs or drug classes for the treatment of paclitaxel-induced neuropathy due to a lack of information regarding the mechanisms responsible for it. In this study, we examined the involvement of L-serine in paclitaxel-induced hyperalgesia/allodynia and decrease in sensory nerve conduction velocity (SNCV). We used a preclinical rat model of paclitaxel-induced painful peripheral neuropathy. Response to von Frey filaments, SNCV, 3-phosphoglycerate dehydrogenase (3PGDH) expression, and L-serine concentration were examined. Effects of L-serine administration were also investigated. Paclitaxel treatment induced mechanical allodynia/hyperalgesia and reduction of SNCV. Paclitaxel also decreased the L-serine concentration in the dorsal root ganglion (DRG) but not in the sciatic nerve or spinal cord. In addition, paclitaxel decreased expression of 3PGDH, a biosynthetic enzyme of L-serine, in the DRG. Immunohistochemistry showed that 3PGDH was localized in satellite cells but not in neurons in the DRG. Intraperitoneal administration of L-serine improved both paclitaxel-induced mechanical

allodynia/hyperalgesia and the reduction of SNCV. These results suggest that satellite cell-derived L-serine in the DRG plays an important role in paclitaxel-induced painful peripheral neuropathy. These findings may lead to novel strategies for the treatment of paclitaxel-induced painful peripheral neuropathy.

Key words: L-serine, dorsal root ganglion, paclitaxel, peripheral neuropathy, 3-phosphoglycerate dehydrogenase

Abbreviations: SNCV, sensory nerve conduction velocity; 3PGDH, 3-phosphoglycerate dehydrogenase; DRG, dorsal root ganglion; PGP9.5, protein gene product 9.5; PFA, paraformaldehyde; PBS, phosphate buffered saline; OPA, *Ortho*-phthalaldehyde; NAC, *N*-acetylcysteine; HPLC, high-performance liquid chromatography; EDTA, ethylenediamine tetraacetic acid; BBB, blood-brain barrier; BNB, blood-nerve barrier

Introduction

Because advances in cancer therapies have increased the life expectancy of cancer patients, more work is needed to improve patients' quality of life (QOL). Paclitaxel is one of the most effective and commonly used anti-neoplastic drugs, but its use often causes peripheral neuropathy and subsequent neuropathic pain that is resistant to standard analgesics (Quasthoff and Hartung, 2002). The symptoms of paclitaxel-induced neuropathy are mostly sensory and peripheral in nature, consisting of mechanical allodynia, cold allodynia, ongoing burning pain, tingling, and numbness (Holmes et al., 1991; Rowinsky et al., 1993; Forsyth et al., 1997). Unfortunately, paclitaxel-induced pain and sensory abnormalities can become chronic, persisting for months or years following the termination of paclitaxel therapy (van den Bent et al., 1997; Dougherty et al., 2004). Thus, paclitaxel-induced peripheral neuropathy is a dose-limiting side effect that diminishes QOL. However, there are few clinically available drugs for the treatment of chemotherapy-induced neuropathy due to a lack of information regarding the mechanisms responsible for it.

Emerging evidence indicates that the nonessential amino acid L-serine plays a critical role in neuronal development and function. L-serine is a building block of proteins and a precursor for synthesis of L-cysteine, phosphatidyl-L-serine,

sphingolipids, nucleotides, and the neuromodulators D-serine and glycine (Snell, 1984; Snyder and Kim, 2000; Furuya and Watanabe, 2003). L-serine biosynthesis from the glycolytic intermediate 3-phosphoglycerate (i.e., the phosphorylated pathway) involves three sequential reactions initiated by 3-phosphoglycerate dehydrogenase (3PGDH) (Ichihara and Greenberg, 1957; Snell, 1984). *In vitro* studies have shown that exogenously added L-serine promotes neuronal survival and differentiation of sensory ganglia, hippocampal neurons, and cerebellar Purkinje cells (Savoca et al., 1995; Mitoma et al., 1998; Furuya et al., 2000). When neurons are cultured in the absence of L-serine, levels of sphingolipids and phosphatidylserine are dramatically decreased (Mitoma et al., 1998). These data indicate that L-serine has crucial neurotrophic effects. Interestingly, neurons do not produce L-serine themselves, and instead receive it following anabolic metabolism in nearby glial cells (Furuya et al., 2000; Yamasaki et al., 2001; Yamashita et al., 2003). However, the role of L-serine in paclitaxel-induced peripheral neuropathy has not been examined.

Here, we focused on the L-serine biosynthesis system in the dorsal root ganglion to clarify the mechanisms underlying paclitaxel-induced painful peripheral neuropathy. We used a preclinical rat model of paclitaxel-induced painful peripheral neuropathy developed by Polomano et al. (Polomano et al., 2001). This model produces

mechanical allodynia/hyperalgesia and cold allodynia in rats similar to that observed in human patients. We examined changes in the L-serine biosynthesis system following paclitaxel administration and whether L-serine treatment improved painful peripheral neuropathy.

Experimental Procedures

Animals

The experiments were approved by the Sapporo Medical University Animal Care Committee (No.08-028) and were in performed accordance with the ethical guidelines of the National Institutes of Health. Experiments were conducted in adult male Sprague–Dawley rats (160-200 g at the start of the experiments, Japan SLC, Hamamatsu, Japan). The rats were housed in a temperature-controlled (21 ± 1ºC) room with a 12-h light/dark cycle and were given free access to food and water.

Paclitaxel-induced painful peripheral neuropathy model

Paclitaxel (Sigma, St. Louis, MO) was dissolved in Cremophor EL/dehydrated ethanol at 6 mg/mL, and was prepared by diluting with 0.9% NaCl to a final concentration of 2 mg/kg in a volume of 1.0 mL. Rats were given intraperitoneal (ip) injections of paclitaxel (2 mg/kg) or vehicle (1.0 mL of Cremophor EL/dehydrated ethanol diluted in 0.9% NaCl) on 4 alternate days (days 1 [D1], 3 [D3], 5 [D5], and 7 [D7]), according to the method described by Polomano et al. (Polomano et al., 2001). Body weight was recorded before and after the initiation of paclitaxel or vehicle treatment. To examine the effects of L-serine on paclitaxel-induced peripheral neuropathy, some paclitaxel-treated rats received ip injection of either L-serine (0.01, 0.03, or 0.1 mmol/kg in 0.9%

NaCl) or vehicle (0.9% NaCl) daily for 28 consecutive days, starting on the day of the first injection of paclitaxel. L-serine and vehicle were administered in a volume of 1.0 mL. Behavioral testing, neurochemical analysis, and electrophysiological analysis were performed before (D0) and on days 8 (D8), 15 (D15), 22 (D22), 29 (D29), 36 (D36), and 43 (D43) following the initiation of paclitaxel or vehicle treatment.

Behavioral testing

Mechanical sensitivity was assessed as previously described (Flatters and Bennett, 2006). The animals were placed on an elevated wire mesh floor, confined beneath overturned cages made of clear plastic, and allowed to acclimatize for 20 min. Three von Frey filaments with bending forces of 4 g, 8 g, and 15 g were applied 10 times to the mid-plantar skin of the left hindpaw for 5 s per application. Withdrawal responses to the von Frey filaments of the hindpaw were counted and expressed as an overall percentage response. Paclitaxel-induced responses to 4 g are commonly described as allodynia. Responses to 15 g are commonly described as hyperalgesia, and responses to 8 g are intermediate (Flatters and Bennett, 2004).

Measurement of sensory nerve conduction velocity

After each behavioral assessment, sensory nerve conduction velocity (SNCV) in the tail was determined using digital equipment for stimulation and recording (Neuropak 2; Nihon-Kohden, Kyoto, Japan) as described previously (Pisano et al., 2003). Animals were lightly anesthetized with an intraperitoneal pentobarbital injection. The recording needle electrodes were inserted into the tail 2 cm and 8 cm distally from the anus, whereas the stimulating electrodes were placed 12 cm distally from the anus. To determine the latency, electrical stimulation was repeated 50 times at a frequency of 1 Hz. The latency of the averaged summation of potentials recorded at the two sites was determined (peak-to-peak), and the nerve conduction velocity was calculated by dividing by the distance (6 cm). The assessments were performed under strictly standardized conditions with the rats warmed with heat pads for at least 10 min before the nerve conduction measurements in order to obtain skin temperatures of 36-37°C. Conduction velocity measured in these methods is considered to originate from large myelinated A-fibers.

Immunohistochemistry

We used the following polyclonal antibodies: guinea pig anti-3PGDH (1.0 μg/mL; gift from Dr. Masahiko Watanabe, Hokkaido University, Sapporo, Japan), rabbit anti-protein gene product 9.5 (PGP9.5) (1:4000, RA95101, UltraClone, Yarmouth, UK),

mouse anti-CD31 (1:100, 10R-CD31gRT, Fitzgerald, North Acton, MA), and rabbit anti-S-100ß (1:100, RY330, Yanahara Institute, Fujinomiya, Japan). Previous studies have confirmed the specificity of the anti-3GPDH antibody (Yamasaki et al., 2001; Yamashita et al., 2003). Rats were deeply anesthetized with 50 mg/kg ketamine ip and perfused transcardially with 4% paraformaldehyde (PFA). The left L4 DRG was removed and immersed in 4% PFA for 2 h for postfixation. The DRGs were then cryoprotected in 25% sucrose overnight at 4ºC. DRGs were placed in Tissue-Tek embedding medium (Sakura, Tokyo, Japan) and rapidly frozen. Frozen sections were cut at 20 μm using a sliding cryostat (Sakura) and thaw-mounted onto gelatin-coated slides for processing. The tissue sections were washed in phosphate buffered saline (PBS) and incubated for 1 h at room temperature in a blocking solution (10% normal donkey serum in PBS). Sections were then incubated in a mixture of primary antibodies overnight. After rinsing, the sections were incubated in Alexa Fluor 488-, Alexa 597-, and Alexa 647-labeled species-specific secondary antibodies at a dilution of 1:500 (Invitrogen, Carlsbad, CA) in PBS-T for 2 h at room temperature. Images were taken with a confocal laser scanning microscope (Digital Eclipse C1; Nikkon, Tokyo, Japan). For each image, a single stack was obtained, which was acquired with line-by-line sequential scanning to prevent bleed-through and cross-excitation of fluorophores.

Western blot analysis

Rats were deeply anesthetized with urethane, and the bilateral L3-5 DRGs were rapidly removed and homogenized in PBS and a protease inhibitor cocktail (Sigma) on ice. The crude homogenates were centrifuged at 700 × *g* for 10 min at 4ºC. Supernatants were collected, and protein concentrations were determined using the DC protein assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4%) and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA). Membranes were then incubated with guinea pig anti-3PGDH antibody (1 μg/mL) or rabbit anti-actin antibody (1:5,000; A2066, Sigma) in PBS containing 10% skim milk overnight at 4ºC. Immunoreaction was visualized with an enhanced chemiluminescence plus chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ).

Measurement of D- and L-serine

Rats were deeply anesthetized with urethane. The spinal cords, sciatic nerves and L3/4/5 DRGs were rapidly removed and frozen at –80ºC. Samples were homogenized in methanol on ice. The amount of methanol added was equivalent to 10 times the weight of the samples. The homogenates were centrifuged at 15,000 × *g* for 10 min at 4ºC. The

supernatants were diluted in a 10-fold volume of distilled water, and 1/4 volume of 5 mM *Ortho*-phthalaldehyde (OPA)/*N*-acetylcysteine (NAC) solution was added. After standing at room temperature for 2 min, 20 μL of the reaction mixture was injected onto a high-performance liquid chromatography (HPLC) system. The HPLC system consisted of two EP-300 pumps (Eicom Corporation, Kyoto, Japan), a DG-300 mobile phase degassing unit (Eicom), an ATC-300 column oven (Eicom), and an FLD-370 fluorescence detector (Eicom). Samples and standard solutions were automatically derivatized and injected onto the HPLC system. Diastereomeric derivatives of D- and L-serine in OPA/NAC were separated on an SC-5ODS reversed phase column (Eicom), and the fluorescence was monitored with an excitation wavelength of 340 nm and an emission wavelength of 445 nm. A mixture of 14% (v/v) methanol and 86% (v/v) 0.1 M phosphate buffer (pH 6.0) containing 5 mg/L EDTA-2Na was used as the mobile phase. After the elution of D- and L-serine derivatives, late eluting peaks were flushed out with a mixture of 80% (v/v) methanol and 20% (v/v) 0.05 M phosphate buffer (pH 6.0) containing 5 mg/L EDTA-2Na. The mobile phase flow rate was set at 0.5 mL/min, and the column temperature was set at 30°C.

Statistical Analysis

Data are expressed as the means ± S.E.M. For western blot analysis, the intensity of

bands was determined using an image densitometer (NIH Image 1.63; National Institutes of Health, Bethesda, MD) and was normalized to the intensity of ß-actin. Data were analyzed using the Mann-Whitney U test or Kruskal-Wallis test followed by Bonferroni's test. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using Statview 5.0 software (Abacus Concepts, Berkeley, CA) and NP Multi (Nagata T, Tokyo, Japan).

Results

General health of the animals

The average body weights in the vehicle- and paclitaxel-treated groups at the start of the experiment were 167 ± 4 g and 175 ± 2 g, respectively. Animals in both groups continued to gain weight normally, and average body weights in the vehicle- and paclitaxel-treated groups at the end of the study period (D43) were 403 ± 6 g and 407 ± 13 g, respectively. There was no significant difference in average weight gain between the two groups during 43-day study period. All rats survived to the end of the experiment without showing ill health including alopecia or diarrhea.

Effects of paclitaxel treatment on mechanical sensitivity and SNCV

At D0, before the first paclitaxel or vehicle injection, there was no significant difference between the two groups in responses to 4 g, 8 g or 15 g von Frey stimulation. The overall percentages of withdrawal responses to 4 g and 15 g von Frey stimulation in the paclitaxel-treated group were significantly higher than those in the vehicle-treated group at D22, D29 and D36. The response to 8 g stimulation in the paclitaxel-treated group was also significantly higher than that in the vehicle-treated group at D22 and D29 (Fig. 1A). These results indicated the development of paclitaxel-induced

mechanical allodynia/hyperalgesia.

Consistent with previous reports (Pisano et al., 2003; Tredici et al., 1998; Ono et al., 1979), SNCV gradually increased with body weight gain throughout the observation period. SNCV was comparable in the paclitaxel- and vehicle-treated groups at D0. Following treatment, a significant reduction of SNCV in the paclitaxel-treated group was observed compared to that in the vehicle-treated group at D15 (34.6 ± 0.7 vs 38.4 ± 0.9 m/s, $P < 0.01$), D29 (39.5 ± 0.9 vs 42.8 ± 1.1 m/s, $P < 0.05$), and D36 (41.6 ± 0.5 vs 43.0 ± 0.6 m/s, $P < 0.05$) (Fig. 1B).

Effects of paclitaxel treatment on amount of L-serine

The following measurements of serine and 3PGDH were done with a separate series of rats. Before obtaining a tissue sample at each time point, mechanical sensitivity and SNCV were measured. Paclitaxel- or vehicle-treated rats used for serine and 3PGDH analysis showed mechanical sensitivity and SNCV comparable to those in rats used for behavioral and SNCV analysis (data not shown).

As shown in Fig. 2A, we were able to simultaneously measure L-serine and D-serine, separately. Retention times of D-serine and L-serine were 11.45 min and 12.5 min, respectively. Paclitaxel treatment did not affect L-serine concentration in the

sciatic nerve or spinal cord (Fig. 2B, C). On the other hand, paclitaxel treatment significantly decreased L-serine concentration in the DRG compared to vehicle treatment at D15 (5.9 ± 0.42 vs 10.0 ± 0.99 ng/mg, P<0.01) and D22 (7.7 ± 0.5 vs 9.5 ± 0.5 ng/mg, P < 0.01) (Fig. 2D). D-serine concentrations were very low level. D-serine concentrations in the spinal cord, sciatic nerve and DRG at D0 were 45.8 ± 4.3, 23.1 ± 2.1 and 56.6 ± 3.5 pg/mg, respectively, in the vehicle-treated rats and 43.6 ± 2.1, 25.3 ± 5.1 and 51.3 ± 4.1 pg/mg, respectively, in the paclitaxel-treated rats. In both groups, D-serine concentrations in the spinal cord, sciatic nerve and DRG were not significantly changed throughout the observation periods (data not shown).

Effect of paclitaxel treatment on 3PGDH expression

Next, we examined DRGs to determine whether paclitaxel altered the expression of 3PGDH, an initial enzyme in L-serine biosynthesis from 3-phosphoglycerate. Western blot analysis showed that 3PGDH protein levels in DRGs from paclitaxel-treated rats were significantly decreased compared to those in the vehicle-treated group at D15 (3PGDH/ß-actin, 2.5 ± 0.2 vs 3.9 ± 0.3, P < 0.01) and D22 (3.0 ± 0.3 vs 4.6 ± 0.2, P < 0.01) (Fig. 3). The time course of changes in 3PGDH expression was consistent with that of changes in L-serine in DRGs.

Localization of 3PGDH in DRGs

Immunohistochemistry was performed to examine the localization of 3PGDH in DRGs. Intense immunostaining for 3PGDH was observed as a large ring-like structure surrounding most of the somata of DRG neurons, as indicated by immunostaining for PGP9.5, a marker of neurons (Fig. 4A). Using double immunostaining, we observed that immunostaining for 3PGDH overlapped with immunostaining for S100ß, a marker of satellite cells, indicating that 3PGDH was localized in satellite cells (Fig. 4B) as described previously (Yamashita et al., 2003). In addition, immunostaining for 3PGDH was adjacent to immunostaining for CD31, a marker of blood vessels (Fig. 4A). These results indicated that 3PGDH was localized in satellite cells, which are located between blood vessels and somata of DRG neurons.

Effects of L-serine on paclitaxel-induced mechanical allodynia/hyperalgesia and reduction in SNCV

Next, we examined whether L-serine administration improved the paclitaxel-induced mechanical allodynia/hyperalgesia and the reduction in SNCV. Figure 5A shows the effects of L-serine on paclitaxel-induced mechanical allodynia/hyperalgesia. All three

doses of L-serine used in this study significantly improved paclitaxel-induced allodynia. Administration of 0.1 mmol/kg L-serine significantly decreased the response to 4 g von Frey stimulation at D22, D29 and D36 compared to that in rats treated with vehicle (0.9% NaCl). Administration of 0.01 and 0.03 mmol/kg L-serine also significantly decreased the response to 4 g von Frey stimulation at D22/D36 and D29/D36, respectively. In addition, 0.1 mmol/kg L-serine significantly decreased the response to 8 g von Frey stimulation at D29 and the response to 15 g at D29 and D36. On the other hand, neither 0.03 mmol/kg nor 0.01 mmol/kg L-serine decreased the responses to 8 g and 15 g von Frey stimulation. Figure 5B shows effects of L-serine on paclitaxel-induced reduction of SNCV. Administration of 0.1 mmol/kg L-serine significantly increased SNCV compared to that in vehicle-treated rats at D15, D22, D29 and D36. Administration of 0.03 mmol/kg L-serine also significantly increased SNCV at D22, D29 and D36, and 0.01 mmol/kg L-serine increased SNCV only at D22.

In vehicle-treated rats, but not paclitaxel-treated rats, administration of 0.1 mmol/kg L-serine, which was the highest dose used in this study, did not have any significant effects on responses to von Frey filaments and SNCV compared to the effects of saline (Fig. 6).

At the doses of L-serine used in this study, rats continued to gain weight

normally and did not show any undesirable behavioral effects.

Discussion

In this study, paclitaxel treatment caused mechanical allodynia/hyperalgesia and a reduction of SNCV, as previously described (Polomano et al., 2001; Persohn et al., 2005). Our new findings were: (i) that paclitaxel decreased the L-serine concentration in the DRG but not in the sciatic nerve or spinal cord; (ii) that paclitaxel decreased the expression of the biosynthetic enzyme for L-serine, 3PGDH, in the DRG; (iii) that administration of L-serine was followed by recovery from mechanical allodynia/hyperalgesia and led to an increase in SNCV. In addition, immunohistochemistry showed that 3PGDH was localized in satellite cells but not neurons in the DRG, as previously described (Yamashita et al., 2003). These results suggest that satellite cell-derived L-serine in the DRG likely plays an important role in paclitaxel-induced painful peripheral neuropathy.

We found that the L-serine concentration was decreased in the DRG but not in the sciatic nerve or spinal cord of paclitaxel-treated rats. The central nervous system and the peripheral nervous system are segregated from systemic circulation by special barrier systems, namely, blood-brain barrier (BBB) and blood-nerve barrier (BNB), respectively (Abbott et al., 2006; Bernacki et al., 2008). On the other hand, DRGs have different permeability characteristics compared to most other neural structures.

Accordingly, substances that are excluded by the BBB or BNB readily penetrate the DRG when injected intravenously (Olson, 1968; Abram et al., 2006). A previous study showed that the tissue concentration of paclitaxel was higher in DRGs than in the spinal cord and peripheral nerve axons following repeated administration of paclitaxel (Cavalletti et al., 2000). In addition, we found that paclitaxel decreased the expression of 3PGDH in DRGs, with a similar time course as changes in the L-serine concentration (Fig. 2D and 3). Our immunohistochemical study showed that 3PGDH was expressed in satellite cells but not in neuronal cell bodies and was located between blood vessels and somata of DRG neurons (Fig. 4A). Therefore, it appears that satellite cells are more susceptible to systemically administered paclitaxel than the somata of DRG neurons.

Since paclitaxel-induced mechanical allodynia/hyperalgesia and a reduction of SNCV developed following a decrease in L-serine and since treatment with L-serine improved changes in behavior and SNCV, our results indicate that satellite cell-derived L-serine likely plays an important role in paclitaxel-induced painful peripheral neuropathy. The detailed mechanisms by which decreased L-serine levels evoke abnormal sensation and reduction of SNCV remain to be elucidated. We observed that hyperalgesia/allodynia appeared after decrease in L-serine level/3PGDH expression

and that abnormal sensation improved after restoration of decreased L-serine level/3PGDH expression. Previous studies have shown paclitaxel-induced functional and morphological/strucural alterations in peripheral sensory neurons. Paclitaxel treatment increases abnormal spontaneous discharges in the A- and C-fibers of primary afferent neurons (Xiao and Bennett, 2008). *In vitro* studies have shown that paclitaxel alters intracellular signal transduction mechanisms (Boehmerle et al., 2006). Morphological studies have shown that paclitaxel increases the prevalence of atypical (swollen and vacuolated) mitochondria in peripheral sensory axons (Flatters and Bennett, 2006) and decreases epidermal innervation of peripheral neurons (Siau et al., 2006). Because glia-derived L-serine is a building block of proteins that maintain neuronal function and development (Snell, 1984; Snyder and Kim, 2000; Furuya and Watanabe, 2003), a decrease in the supply of L-serine from satellite cells may evoke abnormal sensation secondary to structural and/or morphological changes in peripheral sensory neurons rather than directly changing neuronal signaling. In addition, administration of L-serine may restore structural and/or morphological changing in peripheral sensory neurons, normalizing mechanical sensitivity and SNCV. These putative neuronal effects of L-serine may be involved in the inconsistency in the time courses between behaviors and L-serine level/3PGDH expression after paclitaxel

treatment.

In addition, recent studies have reported that intravenously administered paclitaxel induced changes including Activating Transcription Factor 3 expression and enhanced Glial Fibrillary Acidic Protein expression in satellite cells with a higher dose of paclitaxel (36 mg/kg cumulative dose) (Jimenez-Andrade et al., 2006; Peters et al., 2007) than the dose we used in this study (8 mg/kg cumulative dose). Therefore, our results and those of previous studies suggest that satellite cells in the DRG may play a role in the development of paclitaxel-induced painful peripheral neuropathy.

In conclusion, our study suggests that a decrease in satellite cell-derived L-serine contributed to paclitaxel-induced painful peripheral neuropathy. The findings of this study may lead to novel strategies for the treatment of paclitaxel-induced painful peripheral neuropathy in human patients with cancer.

Conflict of interest statement

Tomohiro Kiya and Tomoyuki Kawamata have applied for a patent, L-serine as a treatment drug for peripheral neuropathy, from this result. Other two authors report no conflicts of interest.

Acknowledgment

This work was supported by Grants-in-Aid for Scientific Research (19390407 to T Kawamata, 19791075 to T Kiya, and 20390418 to A Namiki) from the Japan Society for the Promotion of Science, Tokyo, Japan and by the Program for Developing the Supporting System for Upgrading Education and Research from Sapporo Medical University School of Medicine, Sapporo, Japan. The funding sources had no involvement in the study design; in the collection, analysis or interpretation of the data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

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Figure legends

Figure 1.

Effects of paclitaxel treatment on mechanical sensitivity of the hindpaw (A) and sensory nerve conduction velocity (SNCV) of the tail nerve (B). (A1), (A2), and (A3) show the response frequency to 4 g, 8 g and 15 g von Frey filaments, respectively. The percentage responses to 4g von Frey filament were 0 at D22 and D36. Data are expressed as the mean ± S.E.M of 6 animals ** $P < 0.01$, * $P < 0.05$, compared to vehicle at each time point.

Figure 2.

L-serine measurement using high-performance liquid chromatography. *A ,* left and right panels shows a chromatogram of 30 pmol of amino acids (standard solution).and a representative chromatogram of a DRG tissue sample, respectively. *B-D*, time course of L-serine concentration in the spinal cord (*B*), sciatic nerve (*C*), and dorsal root ganglion (DRG) (*D*). Data are expressed as the mean ± S.E.M of 6 animals. ** $P < 0.01$, compared to vehicle at each time point.

Figure 3.

Time course of 3-phosphoglycerate dehydrogenase (3PGDH) expression in the dorsal root ganglion (DRG). The upper and lower panels are representative bands of 3PGDH in vehicle-treated rats and paclitaxel-treated rats, respectively. The bar graph shows the statistical summary of the densitometric data, which are expressed relative to ß-actin. Data are expressed as the mean ± S.E.M of 6 animals. ** $P < 0.01$, compared to vehicle at each time point.

Figure 4.

Immunohistochemical analysis of 3-phosphoglycerate dehydrogenase (3PGDH) localization in the dorsal root ganglion (DRG). *A*, a confocal representative image of triple immunostaining for 3PGDH, PGP9.5, and CD31 in the DRG at low magnification. *Red*, 3PGDH; *green*, PGP9.5; *blue*, CD31. *B*, a confocal representative image of double immunostaining for 3PGDH and S100ß in the DRG at high magnification. Bars indicate 20 µm.

Figure 5.

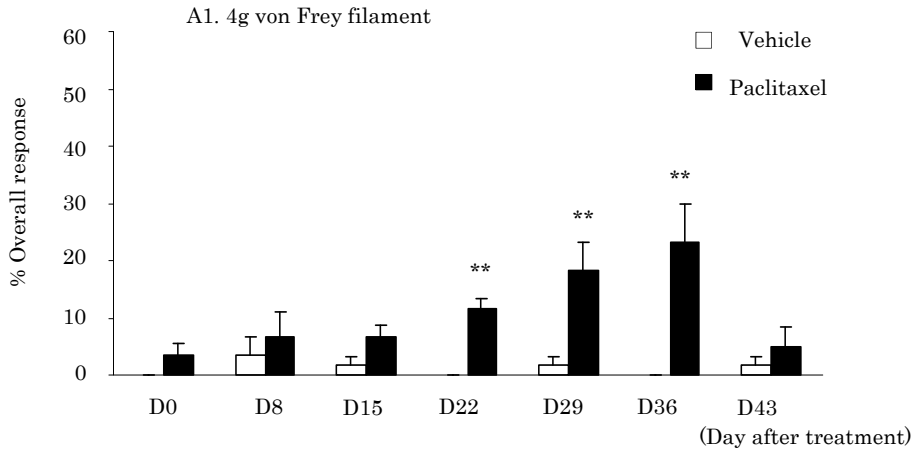
Effects of L-serine treatment on paclitaxel-induced mechanical allodynia/hyperalgesia (A) and the reduction in sensory nerve conduction velocity (SNCV) (B). (A1), (A2), and

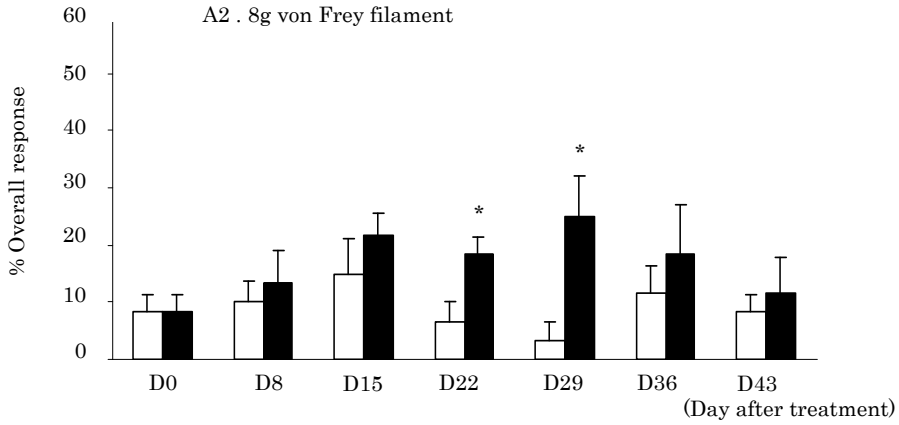
(A3) show the response frequency to 4 g, 8 g and 15 g von Frey filaments, respectively. All animals received paclitaxel. The percentage responses to 4g von Frey filament were 0 at D0 except for L-serine 0.1 mmol/kg treatment. In addition, The percentage responses to 4g von Frey filament were 0 at D15 and D29 in L-serine 0.1 mmol/kg treatment. Data are expressed as the mean ± S.E.M of 6 animals. ** $P < 0.01$, * $P < 0.05$, ompared to vehicle at each time point.

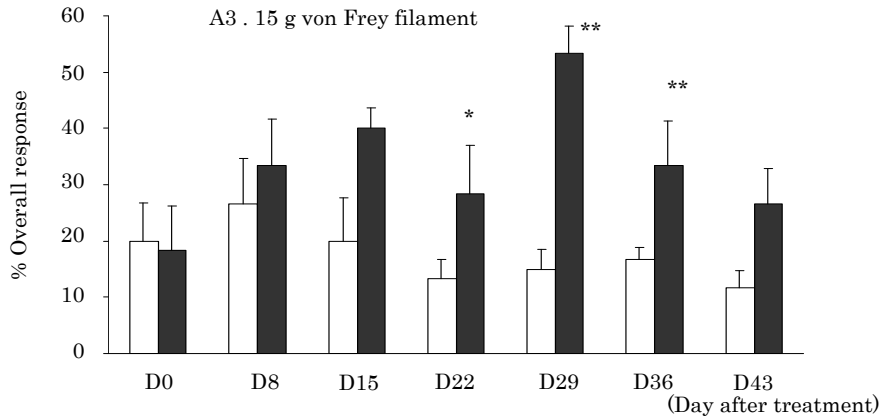
Figure 6.

Effects of 0.1 mmol/kg L-serine treatment on mechanical sensitivity of the hindpaw (A) and sensory nerve conduction velocity (SNCV) of the tail nerve (B) in the vehicle-treated rats. (A1), (A2), and (A3) show the response frequency to 4 g, 8 g and 15 g von Frey filaments, respectively. All animals received vehicle (Cremophor EL/dehydrated ethanol). The percentage responses to 4g von Frey filament were 0 at D0 and D22 in L-serine treatment and at D0 in 0.9% NaCl treatment. Data are expressed as the mean ± S.E.M of 6 animals ** $P < 0.01$, * $P < 0.05$, compared to vehicle at each time point.

A. Mechanical sensitivity







B.SNCV

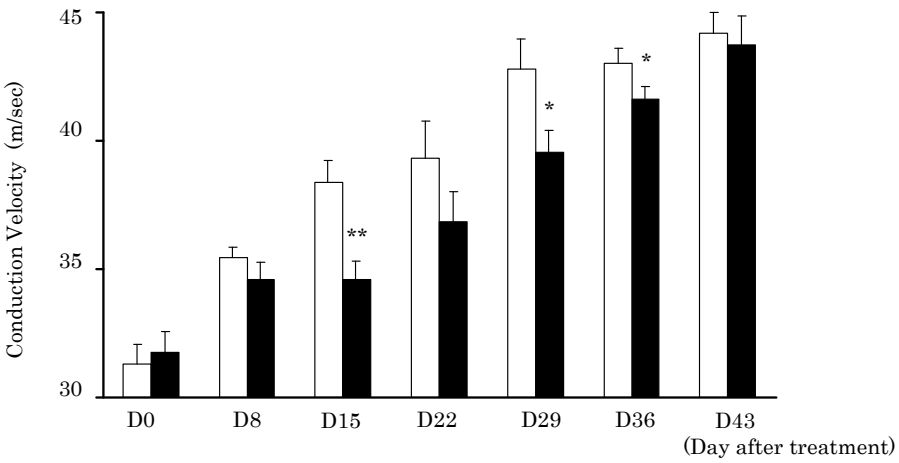
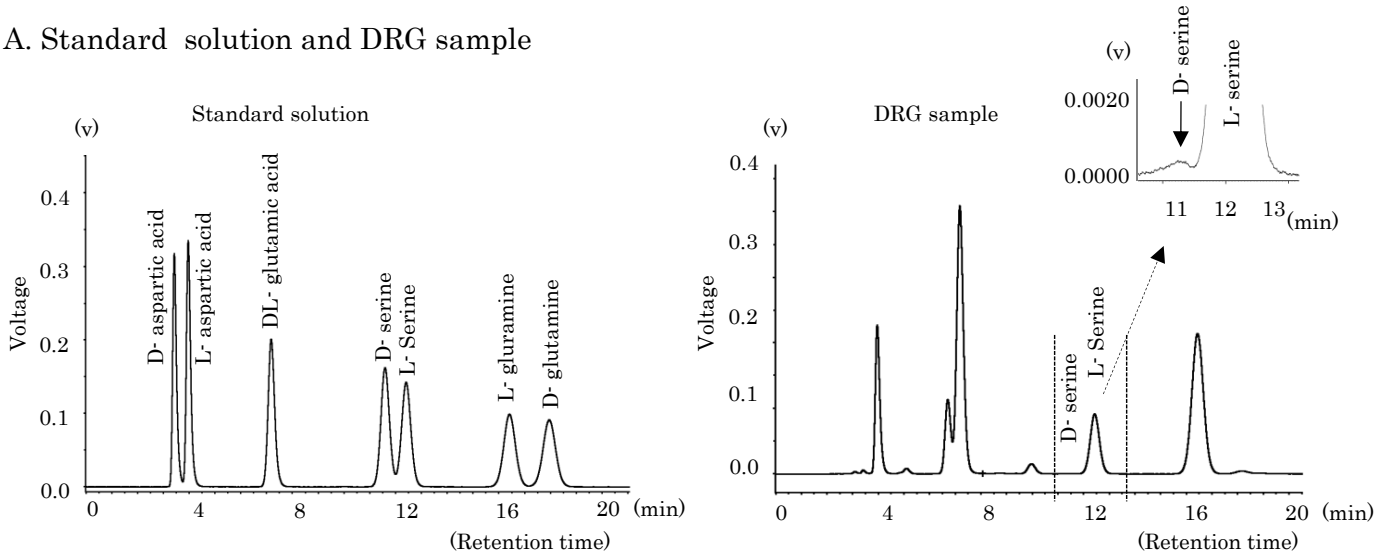
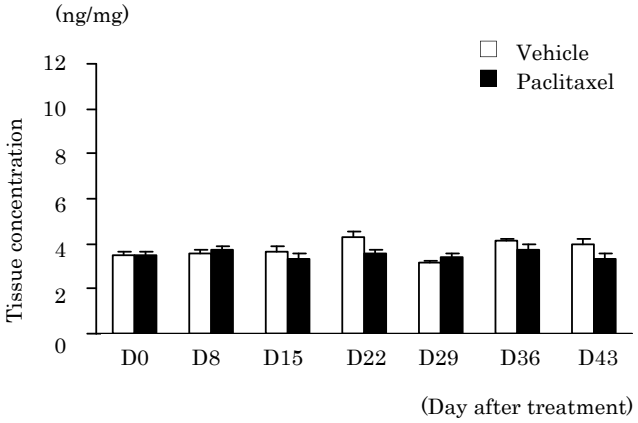


Figure 1

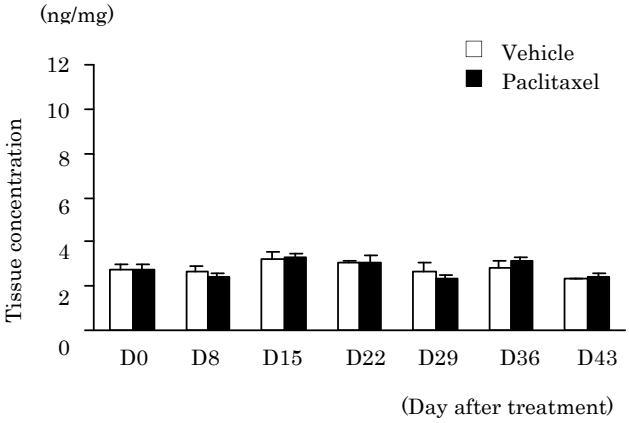
A. Standard solution and DRG sample



B. Spinal cord



C. Sciatic nerve



D. DRG

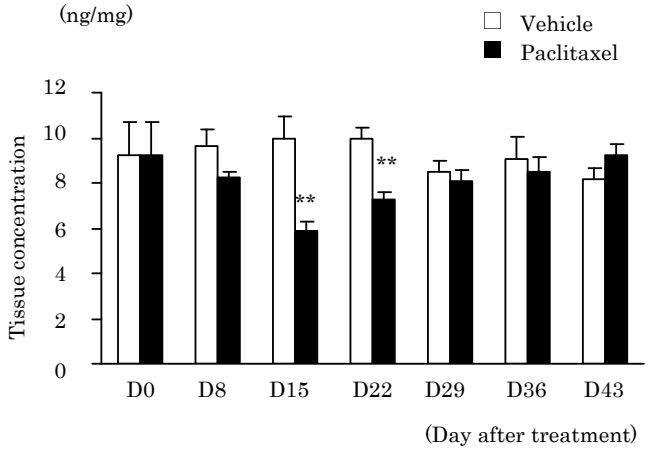


Figure 2

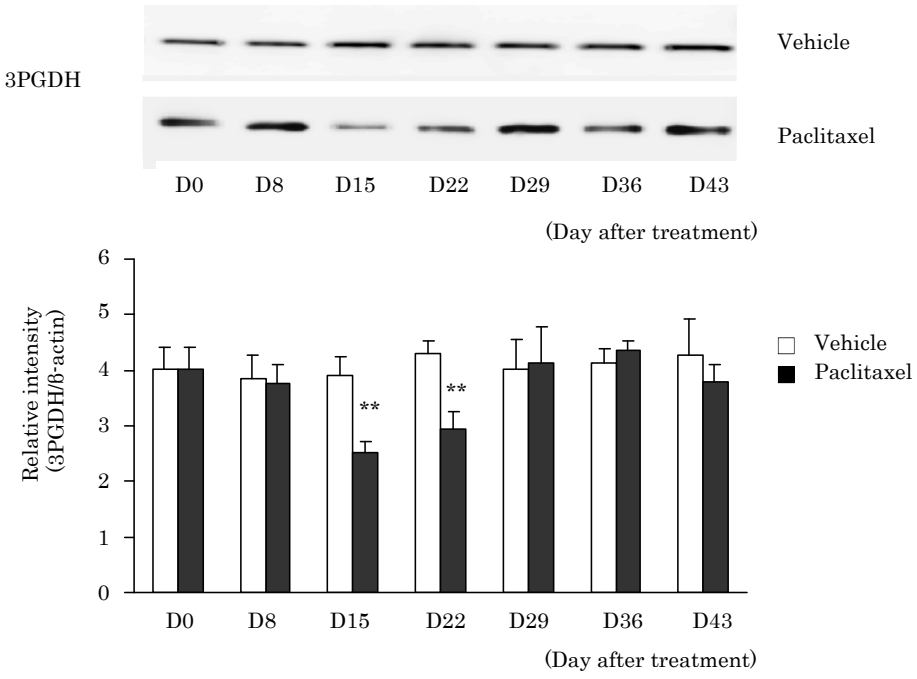

3PGDH
Vehicle
Paclitaxel
D0
D8
D15
D22
D29
D36
D43
(Day after treatment)
Relative intensity
(3PGDH/β-actin)
6
5
4
3
2
1
0
**
**
Vehicle
Paclitaxel
D0
D8
D15
D22
D29
D36
D43
(Day after treatment)

Figure 3

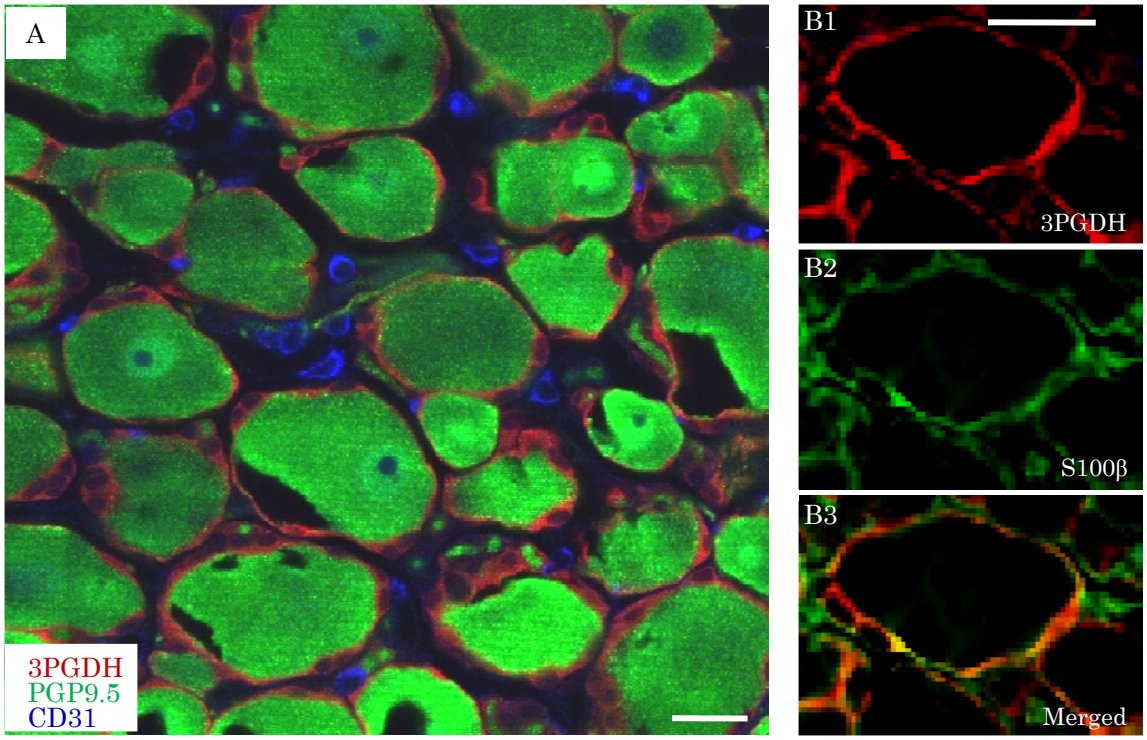
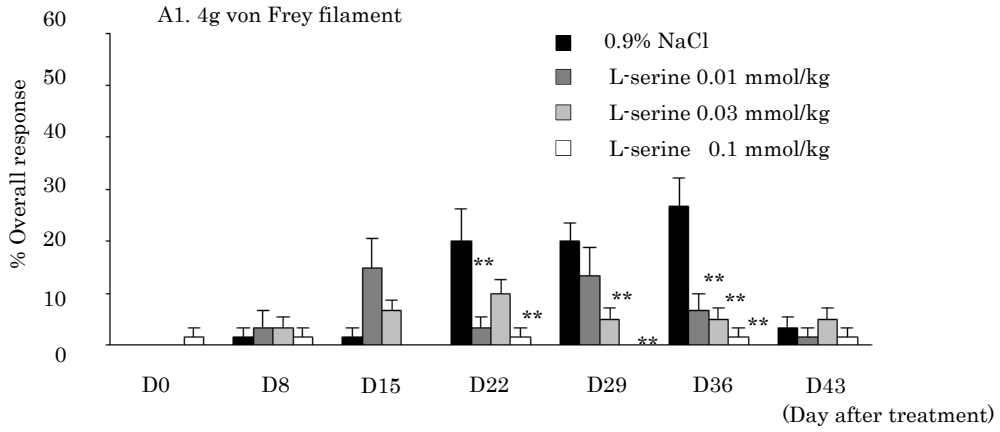
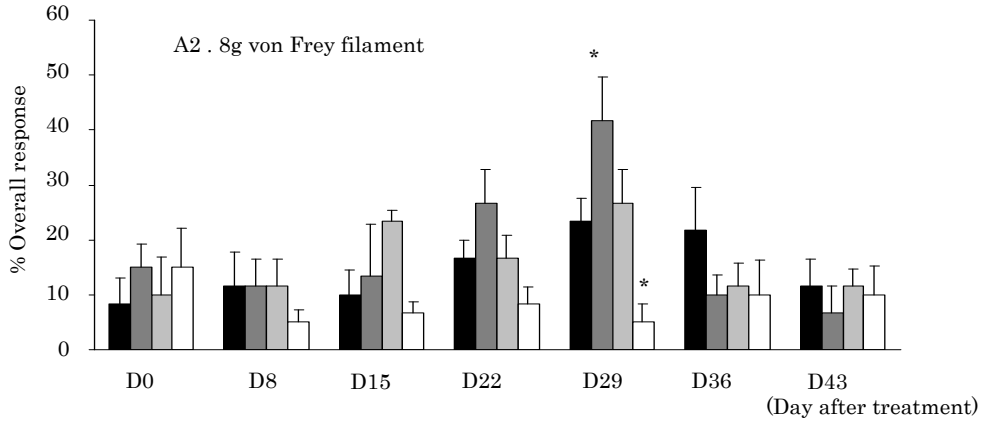
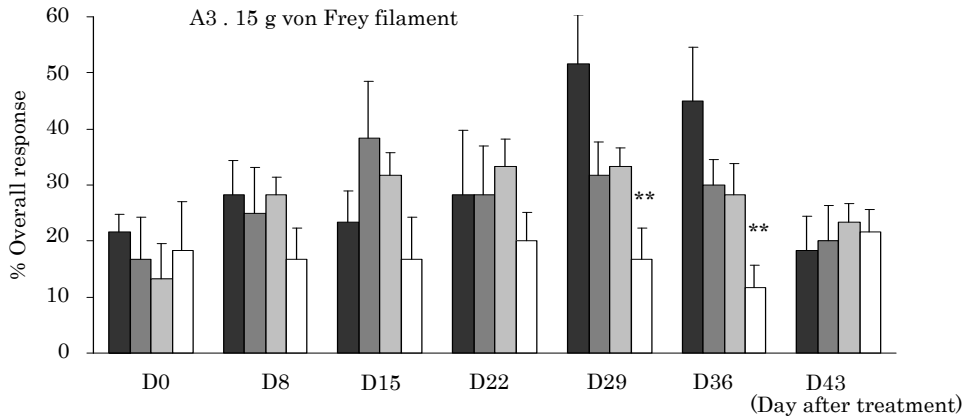


Figure 4

A. Mechanical sensitivity







B.SNCV

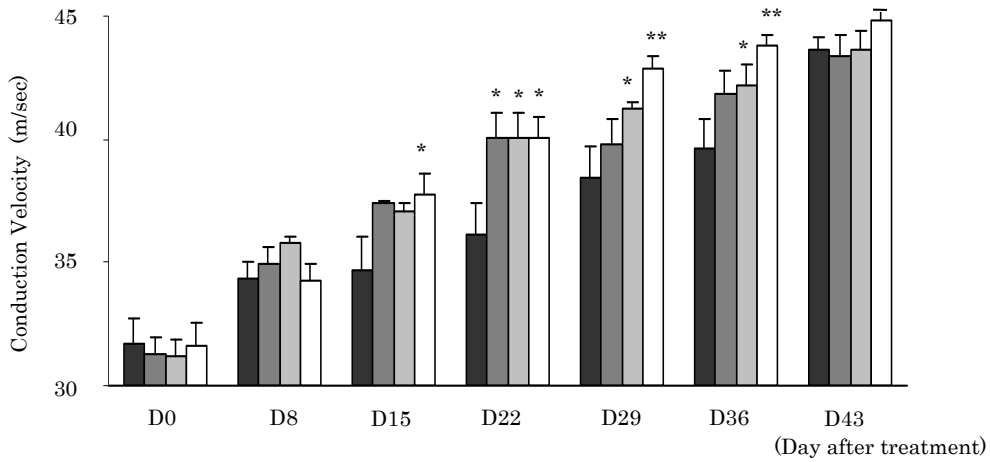
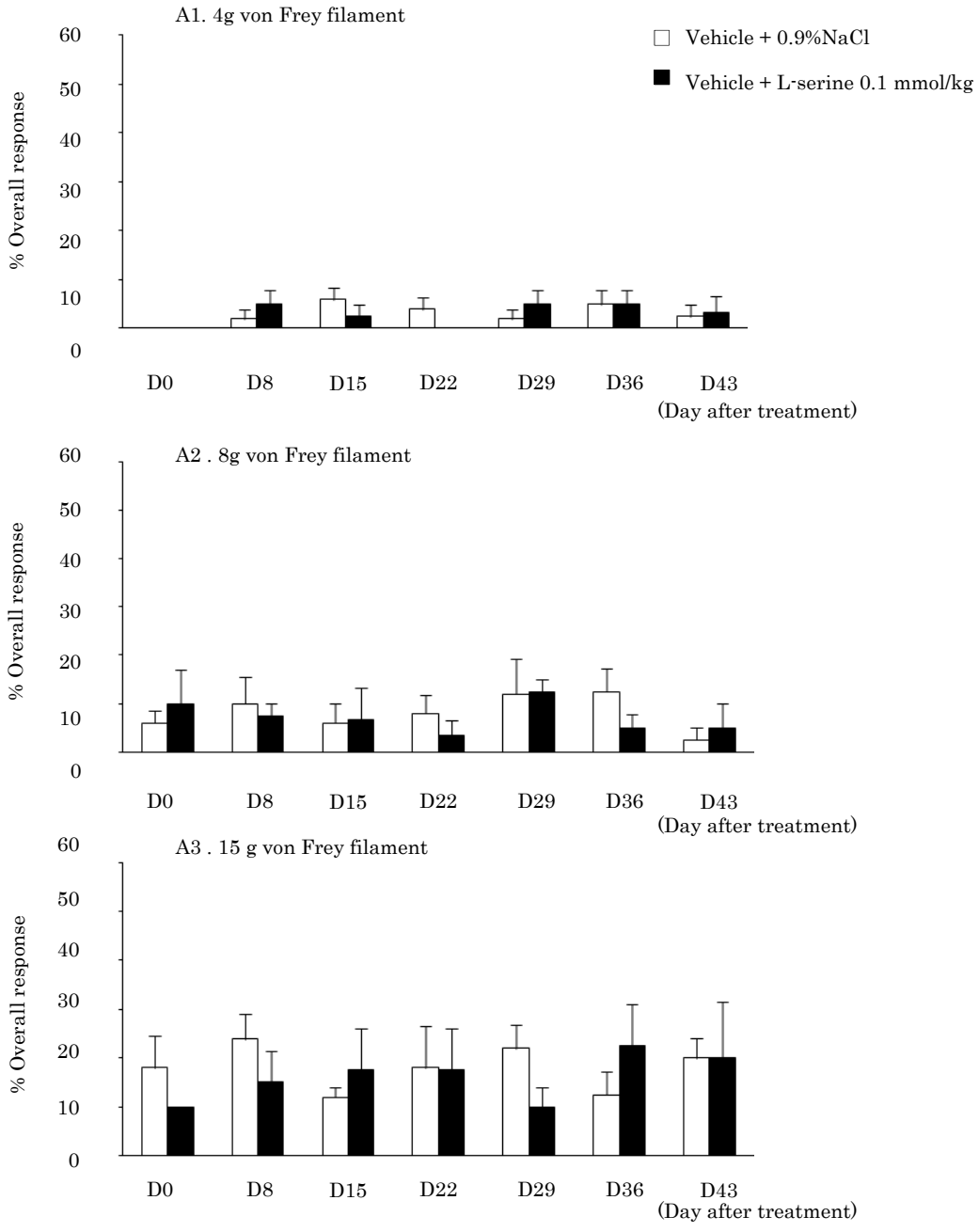


Figure 5

A. Mechanical sensitivity



B.SNCV

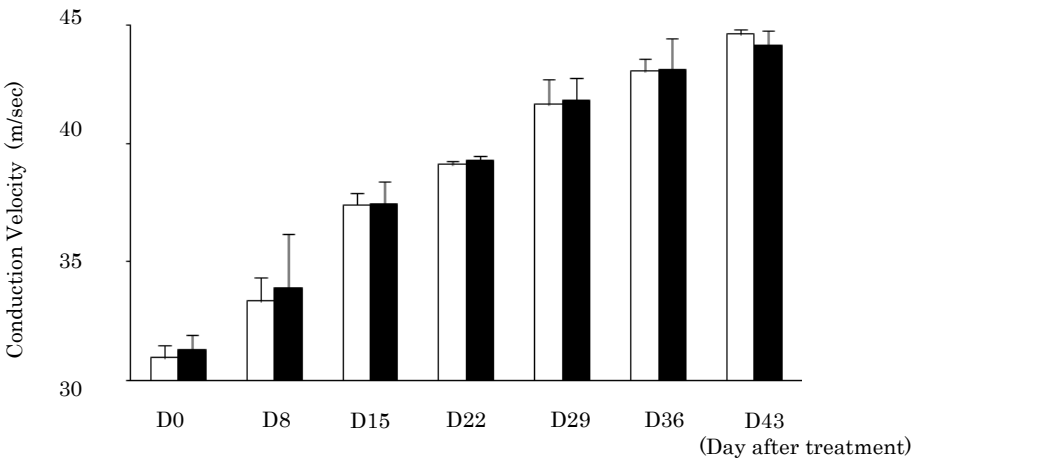


Figure 6