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Short Communication

Comparison of Peripheral Neuropathy Induced by Standard and Nanoparticle Albumin–Bound Paclitaxel in Rats

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Abstract. Nanoparticle albumin–bound paclitaxel (nab-paclitaxel) is delivered to tumors and increases antitumor activity compared with solvent-based paclitaxel. However, in a clinical trial, higher and lower rates of peripheral neuropathy and neutropenia were observed. In this study, we compared the effects of nab-paclitaxel and standard paclitaxel on pain behaviors in rats. Repeated administration of nab-paclitaxel dose-dependently induced both mechanical and cold allodynia, and the effects of nab-paclitaxel on pain behaviors tended to be stronger than that of standard paclitaxel at the doses used clinically. These results suggest that closer attention must be paid to the neuropathy when administering nab-paclitaxel in clinical settings.

Keywords: nanoparticle albumin–bound paclitaxel, paclitaxel, peripheral neuropathy

Paclitaxel (Taxol®), an anticancer agent with a tubulin-stabilizing action, is one of the key drugs in the treatment of breast cancer, ovarian cancer, and other solid tumors. Since standard paclitaxel is a highly hydrophobic agent, available products are dissolved in polyethylated castor oil (Cremophor EL®) and ethanol. Recently, nanoparticle albumin–bound paclitaxel (nab-paclitaxel, Abraxane®), a novel solvent-free formulation of paclitaxel, has been approved in many countries. Nab-paclitaxel has several practical advantages over solvent-based paclitaxel, including a higher administration dose of paclitaxel (175 – 210 to 260 mg/m^2), better transportability to tumor, shorter infusion time (3 h to 30 min) and no need for premedications for hypersensitivity reactions. In a Phase III trial, nab-paclitaxel exhibited a higher response and longer time to tumor progression compared with solvent-based paclitaxel in patients with metastatic breast cancer (1). On the other hand, in this trial, patients treated with nab-paclitaxel had a lower rate of neutropenia but a higher rate of sensory neuropathy (1). We previously reported that repeated administration of paclitaxel induced pain behaviors in rats (2, 3). However, the effects of nab-paclitaxel on the pain behaviors have not been studied. In this study, therefore, we compared the effects of nab-paclitaxel and standard paclitaxel on pain behaviors in rats.

Male Sprague-Dawley rats (Kyudo Co., Tosu) were used for the peripheral neuropathy model. Rats were housed in groups of four to five per cage, with lights on from 7:00 AM to 7:00 PM. Animals had free access to food and water in their home cages. The experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals at the Faculty of Medicine, Kyushu University.

Paclitaxel (Taxol®, 6 mg/mL in cremophor EL / ethanol 1:1) and nab-paclitaxel (Abraxane®) were obtained from Bristol-Myers Squibb Co. (Tokyo) and Taiho Pharmaceutical Co., Ltd. (Tokyo), respectively. Nab-paclitaxel was dissolved in saline. Paclitaxel (6 mg/kg), nab-paclitaxel (6 or 7.4 mg/kg), or saline was injected intravenously (i.v.) once a week for 4 weeks (on days 1, 8, 15, and 22). The dose of paclitaxel was chosen according to the previous reports (2, 3). Since the clinical dose of nab-paclitaxel is 23.8% higher than that of standard paclitaxel [nab-paclitaxel: 260 mg/m^2 (body surface area) and paclitaxel: 210 mg/m^2 (body surface area)], the doses of nab-paclitaxel were chosen to be 7.4 mg/kg (the 23.8% higher dose).

We investigated the effects of nab-paclitaxel and standard paclitaxel on mechanical and cold allodynia in the von Frey and acetone tests. These tests were performed before the first drug administration (day 0) and

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on days 5, 7, 11, 14, 18, 21, 25, 28, 35, 42, and 49. Rats were placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. The mechanical allodynia was assessed by the von Frey test. Filaments (The Touch Test Sensory Evaluator Set; Linton Instrumentation, Norfolk, UK) ranging from 2 to 15 g bending force were applied to the mid-plantar skin of each hind paw six times, with each application held for 6 s. The paw withdrawal threshold was determined by a modified up-down method (4). The cold allodynia was assessed by the acetone test according to the method described by Flatters and Bennett (5). A 50-μL aliquot of acetone (Wako Pure Chemical, Ltd., Osaka) was sprayed onto the plantar skin of each hind paw three times with a Micro Sprayer® (Penn Century Inc., Philadelphia, PA, USA), and the number of withdrawal responses was counted for 40 s from the start of the acetone spray.

The rota rod and grip strength tests were performed to investigate the change of motor coordination and motor strength, respectively. The rota rod test was performed on days 12 and 26. Rats were placed on a rotating rod (Muromachi Kikai Co., Ltd., Tokyo) and the latency to falling was measured for up to 2 min according to the method described previously (6). The test was performed three times, and the rotating speed was 10 rpm. The grip strength test was performed on days 12 and 26. This test was performed using a tension gauge (Oba Keiki Co., Ltd., Tokyo) according to the method described by Authier and colleagues (7). Each rat was placed with both forepaws inside the front grip. When the rat gripped the grid, it was steadily pulled backwards by the tail until its grip was broken. The reading on the strain gauge was recorded. The test was performed four times. The body weights of animals was measured before the first drug administration (on day 0) and on days 5, 8, 12, 15, 19, 22, and 26 using the KN type animal scale (Natsume Seisakusho Co., Ltd., Tokyo).

Values were expressed as the mean ± S.E.M. The area under the 'paw withdrawal threshold – day' and 'number of withdrawal responses – day' curve (AUC) was estimated by the trapezoidal method. The values were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer's post-hoc test (StatView; Abacus Concepts, Berkeley, CA, USA) to determine differences among the groups. A probability level of $P < 0.05$ was accepted as statistically significant.

Before the first drug administration, each group had equivalent body weight, paw withdrawal threshold in the von Frey test, and number of withdrawal responses in the acetone test. Two of 13 rats treated with nab-paclitaxel at the higher dose (7.4 mg/kg) died, and no rats in the other groups died during the course of the experiment. In the standard paclitaxel group, a significant difference in weight gain compared with the saline group was observed on day 26 ($P < 0.05$, Fig. 1A). On the other hand, the group treated with nab-paclitaxel at 7.4 mg/kg consistently showed less weight from day 19 to the end of the test ($P < 0.01$, Fig. 1A). No significant difference in motor coordination and motor strength compared with the saline group was observed in all groups on days 12 and 26 (Fig. 1: B and C).

Nab-paclitaxel (6 mg/kg) significantly reduced the paw withdrawal threshold compared with saline on day 18 and from days 25 to 35 at the same level as standard paclitaxel in the von Frey test ($P < 0.05$ or $P < 0.01$, Fig. 2A). Nab-paclitaxel at the higher dose (7.4 mg/kg) showed the reduction of paw withdrawal threshold from days 18 to 35 ($P < 0.01$, Fig. 2A). Moreover, nab-paclitaxel (7.4 mg/kg) significantly reduced the AUC of 'paw withdrawal threshold-day' compared with saline ($P < 0.05$), and this effect tended to be stronger than those of standard paclitaxel and nab-paclitaxel (6 mg/kg) (Fig. 2B). In the acetone test, nab-paclitaxel (6 mg/kg) significantly increased the number of withdrawal responses compared with saline on day 11 ($P < 0.05$, Fig. 3A). Nab-paclitaxel (7.4 mg/kg) increased the number of withdrawal responses on days 5, 11, 25, and 28 ($P < 0.05$, Fig. 3A). Moreover, nab-paclitaxel at the higher dose significantly increased the AUC of 'number of withdrawal responses-day' compared with saline ($P < 0.05$), and this effect tended to be stronger than those of standard paclitaxel and nab-paclitaxel (6 mg/kg) (Fig. 3B). In addition, there was no difference between standard paclitaxel and nab-paclitaxel in the recovery period of both reduced paw withdrawal threshold and increased paw withdrawal responses (Figs. 2A and 3A).

The paclitaxel-induced peripheral neuropathies are characterized by frequently occurring sensory neuropathies, such as dysesthesia, numbness, pain, and thermohyperesthesia in the feet and hands, and sometimes limit the clinical use of this drug (8 – 10). The data obtained in this study revealed that repeated administration of nab-paclitaxel dose-dependently induced both mechanical and cold allodynia in rats, and the effect of nab-paclitaxel at the higher dose tended to be stronger than that of standard paclitaxel. This supports the data obtained in a Phase III clinical trial (1). Although nab-paclitaxel may be very useful for cancer treatment, it should be used with great attention to the neuropathy that may arise during its use. In addition, the neuropathy induced by nab-paclitaxel was thought to improve rapidly with interruption of treatment (1). However, we observed that there was no difference between standard paclitaxel and nab-paclitaxel in the recovery period of mechanical and cold allodynia. These findings could provide important infor-

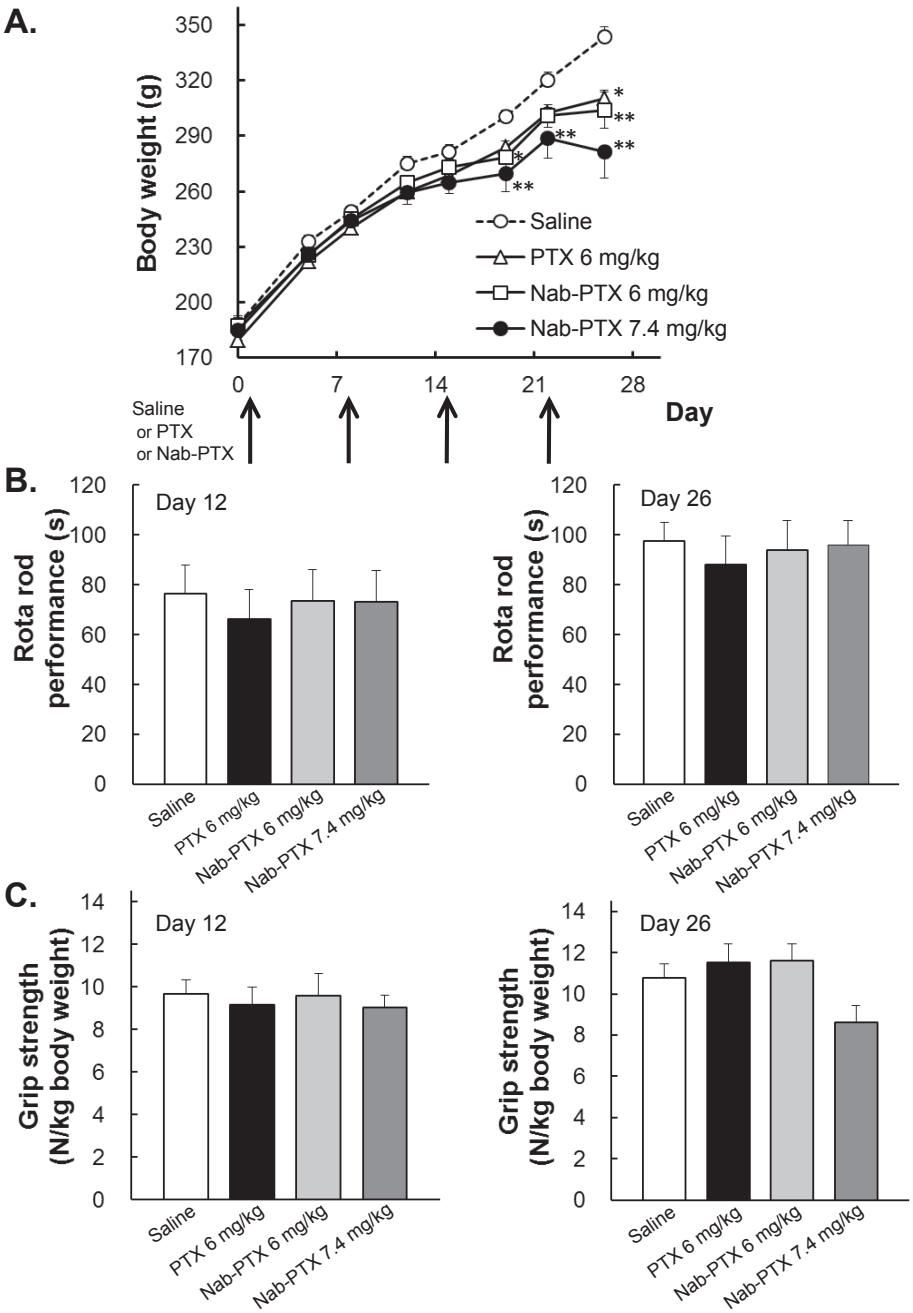

Fig. 1. Effects of paclitaxel (PTX) and nanoparticle albumin–bound paclitaxel (Nab-PTX) on body weight (A), motor coordination and motor strength in the rota rod (B), and grip strength (C) tests. Rats were treated with saline, PTX (6 mg/kg), or Nab-PTX (6 or 7.4 mg/kg) once a week for 4 weeks. Results are expressed as the mean ± S.E.M. of 10 – 11 animals. *$P < 0.05$, **$P < 0.01$: compared with saline.

mation for the clinical use of nab-paclitaxel.

Our results also indicated that repeated administration of nab-paclitaxel had no effect on motor coordination and motor strength. Therefore, it is unlikely that the nab-paclitaxel–induced pain behavior is due to impairment of motor functions.

Nab-paclitaxel is delivered to tumors via transcytosis mediated by the albumin receptor and caveolar transport on endothelial cells and this increases its antitumor activity compared with solvent-based paclitaxel (11). In the present study, nab-paclitaxel at the dose of 6 mg/kg induced pain behavior at the same degree as standard

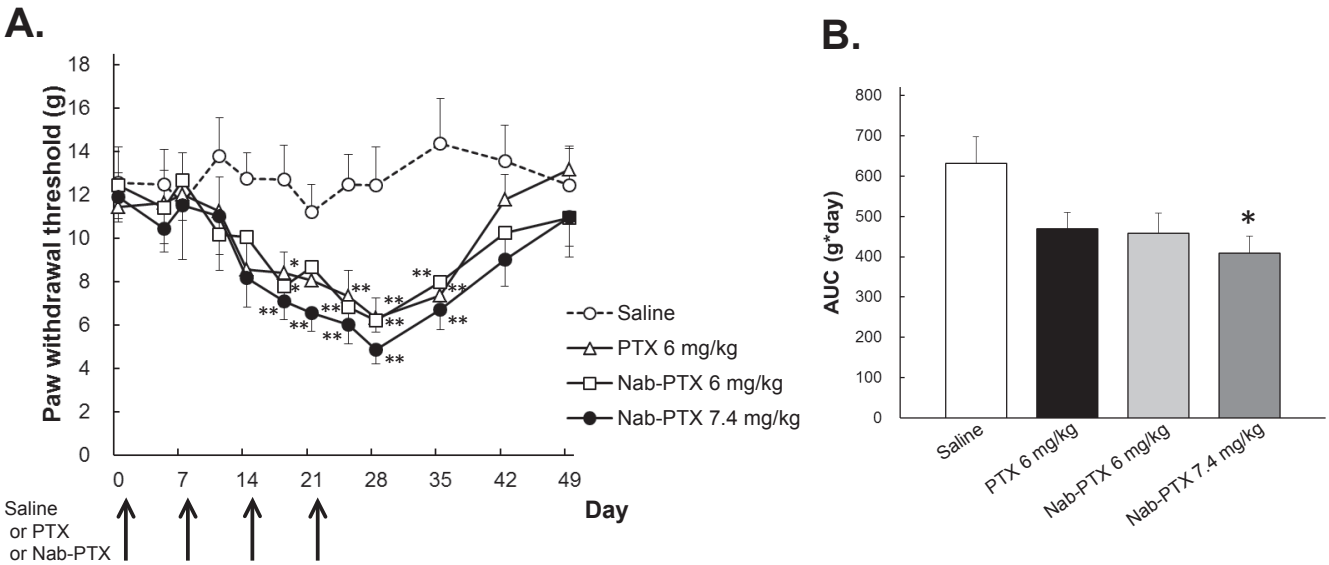

Fig. 2. Effects of paclitaxel (PTX) and nanoparticle albumin–bound paclitaxel (Nab-PTX) on mechanical allodynia in the von Frey test. Rats were treated with saline, PTX (6 mg/kg), or Nab-PTX (6 or 7.4 mg/kg) once a week for 4 weeks. Paw withdrawal threshold (A) and area under the 'paw withdrawal threshold – day' curve (B) are expressed as the mean ± S.E.M. of 10 – 11 animals. *$P < 0.05$, **$P < 0.01$: compared with saline.

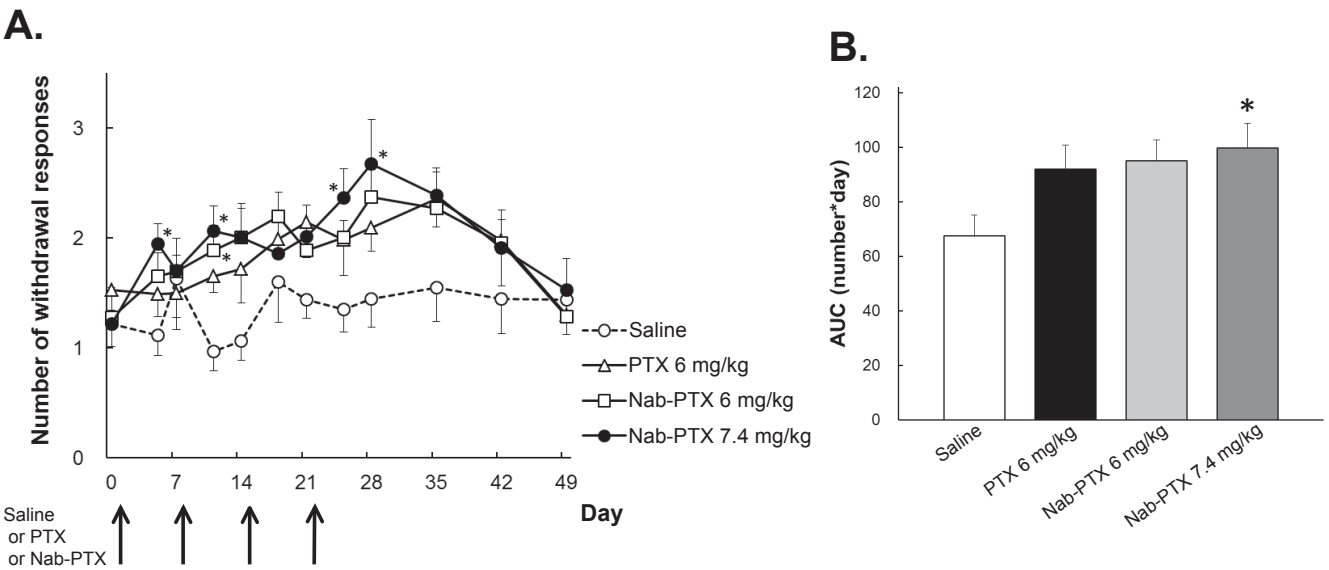

Fig. 3. Effects of paclitaxel (PTX) and nanoparticle albumin–bound paclitaxel (Nab-PTX) on cold allodynia in the acetone test. Rats were treated with saline, PTX (6 mg/kg), or Nab-PTX (6 or 7.4 mg/kg) once a week for 4 weeks. Number of withdrawal responses (A) and area under the 'number of withdrawal responses – day' curve (B) are expressed as the mean ± S.E.M. of 10 – 11 animals. *$P < 0.05$: compared with saline.

paclitaxel, suggesting that the transcytosis pathway is unlikely to have a significant impact on the nab-paclitaxel–induced pain behavior.

In this study, nab-paclitaxel (6 mg/kg) induced pain behaviors as much as standard paclitaxel in von Frey and acetone tests. We previously observed the pain behaviors of rats treated with a cremophor/ethanol mixture were normal in both tests (2, 3). Therefore, these pain behaviors may be due to neurological damage including axon degeneration caused by paclitaxel itself.

In addition, Authier et al. (7) reported that a significant reduction in the withdrawal threshold to von Frey fila-

ments was observed in two rats repeatedly treated with Taxol from the fourth injection to the end of the experiment. However, no significant difference in mean withdrawal threshold was observed in treated rats compared to control groups. In this study and our previous studies (2, 3), we observed that standard paclitaxel induced mechanical allodynia in the von Frey test. This discrepancy may be due to the difference of experimental methods such as dose, volume, and schedule of paclitaxel and behavioral evaluation.

In conclusion, nab-paclitaxel dose-dependently induces mechanical and cold allodynia in rats, and this effect tends to be stronger than that of standard paclitaxel at the doses used clinically. Therefore great attention must be paid to the neuropathy accompanying the use of this drug in clinical practice.

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