Suppressive Effect of Imipramine on Vincristine-Induced Mechanical Allodynia in Mice

Fumihiro SAIKA, Masanobu OZAKI, Fumihiro SAIKA, Yuka KOBAYASHI, Yohji FUKAZAWA, Takehiko MAEDA, Masanobu OZAKI, and Shiroh KISHIOKA

a Department of Pharmacology, Wakayama Medical University; 811–1 Kimiidera, Wakayama, Wakayama 641–0012, Japan; and b Department of Toxicology, Niigata College of Pharmacy and Applied Life Sciences; 265–1 Higashijima, Niigata, Niigata 956–8603, Japan. Received March 10, 2009; accepted March 25, 2009; published online April 1, 2009

Because chronic vincristine (VCR) treatment causes neuropathic pain, as demonstrated by mechanical allodynia, effective therapeutic strategy is required. In this study, we investigated a suppressive effect of imipramine (IMI) on VCR-induced mechanical allodynia in mice. VCR (0.1 mg/kg, intraperitoneally (i.p.)) was administered once per day for 7 d in ICR male mice. Mechanical allodynia was evaluated by withdrawal response using von Frey filaments. In VCR-treated mice, mechanical allodynia was observed on day 3, 7, and 14. On day 14, morphine (3 mg/kg, subcutaneously) slightly but significantly suppressed VCR-induced mechanical allodynia. The percent inhibition by morphine of VCR-induced mechanical allodynia was less than that of the λ-carrageenan-induced inflammatory pain and was similar to that of nerve injury-induced neuropathic pain. Although single administration of IMI (30 mg/kg, i.p.) had no effect on VCR-induced mechanical allodynia, repeated administration of IMI (30 mg/kg, i.p.) for 7 d significantly suppressed VCR-induced mechanical allodynia. Suppressive effects by repeated IMI administration were observed in both early phase (day 0—6) and late phase (day 7—13) of VCR-induced mechanical allodynia. These results suggest that chronic VCR administration induces opioid analgescis-resistant mechanical allodynia, and repeated IMI administration may be an effective therapeutic approach for the treatment of VCR-induced mechanical allodynia.

Key words  vincristine; allodynia; imipramine; morphine; neuropathic pain

The vinca-alkaloid vincristine (VCR) is widely used as an anti-cancer agent. However, the clinical use of VCR is limited by VCR-induced neuropathic pain.1—3) Generally, neuropathic pain consists of hyperalgesia and allodynia as typical symptoms, and these symptoms are resistant to both opioid analgesics and non-steroidal anti-inflammatory drugs.4,5) Therefore, an effective therapeutic strategy against VCR-induced neuropathic pain is required to make VCR more acceptable for the treatment of cancer.

Recently, some investigators established a rat model of VCR-induced neuropathic pain and studied some effective therapeutic analgesics.6,7) We also observed VCR-induced mechanical allodynia in mice, and demonstrated that pro-inflammatory cytokines, i.e., interleukin-6 and tumor necrosis factor-alpha (TNF-α), played an important role in the regulation of VCR-induced mechanical allodynia. Indeed, administration of the neutralizing antibodies of these cytokines could prevent VCR-induced mechanical allodynia.8,9) However, there is no effective agent in clinical use for the acceptable treatment of VCR-induced neuropathic pain.

Supplementary analgesics (e.g., anticonvulsants and antidepressants) have suppressive effects on opioid-resistant neuropathic pain.10,11) Antidepressants, such as tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI), have been studied and found to be among the most effective agents for the treatment of neuropathic pain.12,13) However, the effects of antidepressants on VCR-induced mechanical allodynia are poorly understood. There is only one report, indicating acute analgesic effect of venlafaxine (serotonin-noradrenaline reuptake inhibitors) on VCR-induced allodynia in rats in the past.14) In this study, we investigated a suppressive effects and validity period of the TCA imipramine (IMI) on VCR-induced mechanical allodynia in mice.

MATERIALS AND METHODS

Animals  Male ICR mice (SLC, Osaka, Japan) weighting 20—22 g were used. They were housed in plastic cages with water and food available ad libitum. Mice were maintained in an air-conditioned (23—24°C, 60—70% relative humidity) vivarium with a 12-h dark/light cycle (light on from 8:00 a.m. to 8:00 p.m.). All experimental procedures were approved by the Animal Research Committee of Wakayama Medical University and complied with the ethical guidelines of the International Association for the study of Pain.15)

Drug Administration  Vincristine sulfate (Oncovin; Nippon Kayaku, Tokyo, Japan) was administered intraperitoneally (i.p.) in a dose of 0.1 mg/kg, once per day for 7 consecutive days in mice as described in our previous report.9) Morphine hydrochloride (Takeda, Osaka, Japan) and imipramine hydrochloride (Sigma, St. Louis, U.S.A.) were administered subcutaneously (s.c.) and i.p., respectively. All drugs were dissolved in physiological saline, and injection volume was set to 0.1 ml/10 g.

Pain Models  To induce inflammatory pain, mice were given 1% λ-carrageenan, which was dissolved in physiological saline, into the intraplantar (i.pl.) surface of right hind paw. The i.pl. injection was given using a Hamilton microsyringe fitted with a 30-gauge needle and injection volume was set to 20 μl. To produce peripheral nerve injury-induced neuropathic pain, mice were anesthetized with sodium pentobarbital (75 mg/kg, i.p.) and partial sciatic nerve ligation was performed following the modified method described in a previous report.16) Briefly, sciatic nerve of right hind limb was exposed at high thigh level and 1/2 of the nerve thickness was tightly ligated with a silk suture. The incision site was closed with a silk suture.

Mechanical Paw Withdrawal Test  Mechanical allody-
nia was evaluated by withdrawal responses, using von Frey filaments (Neuroscience, Tokyo, Japan) as described in our previous report. Briefly, mice were placed on a 10×10-mm wire mesh grid floor, and covered with an opaque cup to avoid visual stimulation. Mice were allowed to adapt for 3 h. The von Frey filaments were inserted through the mesh floor bottom and were applied to the middle of plantar surface of hind paw 10 times with the weight of 0.4 g (day 0—7) or 0.6 g (day 14). Because the withdrawal response is different according to growth of mice, that is, response to the stimulation by filament of 0.4 g on day 14 was extremely lower than that on day 7 in control mice. Mechanical allodynia was designated as the percentage of withdrawal responses (% response) to stimulation of the hind paws. An inhibition ratio (% inhibition) produced by morphine in each mouse was calculated by the following formula:

\[ \text{\% inhibition} = 100 \times \left( \frac{\text{value of \% response before morphine administration}}{\text{value of \% response at 30 min after morphine administration}} \right) \]

**Thermal Paw Withdrawal Test**

Thermal hyperalgesia was evaluated by withdrawal latency using the IITC 390 Plantar Test Analgesia Meter (Neuroscience, Tokyo, Japan). Mice were placed on top of a glass sheet, and covered with clear cage. Mice were allowed to adapt for 3 h. The radiant heat source was positioned under the glass sheet and applied to the plantar surface of hind paw. Withdrawal latencies were measured 5 times for the hind paws. Data are presented as the mean latency of 5 times stimulations. The heat intensity was calibrated to give a control latency of approximately 8 s. A cut-off latency of 15 s was set in each measurement to avoid tissue damage and unnecessary suffering to mice.

**Statistical Analysis**

Data are presented as the mean±S.E.M. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test, a two-way ANOVA followed by Bonferroni multiple comparisons test or Student’s t test. Significance was established at \( p<0.05 \).

**RESULTS**

**Analgesic Effect of Morphine on Vincristine-Induced Mechanical Allodynia**

VCR or Veh was administered to mice once per day for 7 d (day 0—6). We then examined thermal withdrawal latency and withdrawal responses to mechanical stimulation in both groups of mice. There was no significant difference in thermal withdrawal latency between VCR-treated mice and Veh-treated mice on day 14 (Fig. 1A). In Veh-treated mice, the percent response to mechanical stimulation was constant on day 0—14. On the other hand, in VCR-treated mice, the percent response to mechanical stimulation was significantly increased on day 3 and 7, indicating that VCR treatment elicited mechanical allodynia. In addition, VCR-induced mechanical allodynia persisted until at least day 14 (8 d after cessation of VCR treatment) (Fig. 1B).

We examined the effect of morphine on VCR-induced mechanical allodynia on day 14. Morphine (3 mg/kg, s.c.) produced a slight but significant suppression of VCR-induced mechanical allodynia at 30 min after administration (Fig. 2). However, neither 0.3 nor 1 mg/kg of morphine had a significant effect. In this behavioral test, we cannot use the higher doses of morphine (more than 3 mg/kg), because of hyperlocomotion caused by morphine. The analgesic effect of morphine on VCR-induced mechanical allodynia was compared with the effect of morphine on the carrageenan (CAR)-induced inflammatory pain and partial sciatic nerve ligation (PSL)-induced neuropathic pain. In CAR-treated mice, pain response to mechanical stimulation was observed at 3 h after CAR injection. In PSL-treated mice, mechanical allodynia was observed at 7 d after the operation. Percent response before morphine administration in VCR-treated mice was similar to that in CAR-injected mice and was less than that in PSL-treated mice (Table 1). Percent inhibition by morphine (0.3—3 mg/kg, s.c.) in VCR-induced mechanical allodynia
was less than that in CAR-induced inflammatory pain and was similar to that in PSL-induced neuropathic pain (Table 1), indicating that VCR-induced mechanical allodynia, like PSL-induced neuropathic pain, was resistant to morphine analgesia.

**Suppressive Effects of Chronic Imipramine Treatment on VCR-Induced Mechanical Allodynia**  A single administration of IMI (30 mg/kg, i.p.) had no effect on VCR-induced mechanical allodynia on day 14 (Fig. 3). However, repeated IMI administration (3—30 mg/kg, once per day, i.p.) for 7 d (day 0—6), in combination with VCR, suppressed VCR-induced mechanical allodynia on day 7 in a dose-dependent manner (Fig. 4). Moreover, repeated IMI administration (3—30 mg/kg, once a day, i.p.) on day 7—13 (after cessation of VCR treatment) also suppressed VCR-induced mechanical allodynia on day 14 (Fig. 5). However, suppressive effects of IMI on VCR-induced mechanical allodynia were not observed 7 d after cessation of repeated IMI administrations in either experiment, indicating that the suppressive effects of IMI waned one week after the cessation of repeated IMI administration (Figs. 4, 5).

**DISCUSSION**

We did not observe mechanical allodynia after a single administration of VCR (0.1 mg/kg) (data not shown). However, mechanical allodynia was observed on day 3 and 7 following VCR administration once per day for 7 d, and continued until at least day 14. These results are consistent with other reports, demonstrating that long-lasting mechanical allodynia was induced by the chronic VCR administration.17,18) In general, thermal hyperalgesia and allodynia are present in nerve injury-induced neuropathic pain. However, in this experiment, thermal hyperalgesia was not observed in VCR-treated mice, as others have also shown.19) This indicates that VCR-induced neuropathic pain may have a different mechanism from the nerve injury-induced neuropathic pain.

Except for neuropathic pain, morphine is one of most effective analgesics in clinical and experimental models.20,21) We compared the analgesic effects of morphine on VCR-induced mechanical allodynia with those on CAR-induced inflammatory pain or PSL-induced neuropathic pain. Morphine was less effective in reducing VCR-induced mechanical allodynia than in reducing CAR-induced inflammatory pain, and was similar in effectiveness against PSL-induced neuropathic pain. In a previous report, it was demonstrated that resistance to the analgesic effects of morphine in PSL-induced neuropathic pain is accompanied by down-regulation of the peripheral μ-opioid receptor.22) Therefore, dysfunction of this receptor may also participate in VCR-induced neuropathic pain.

Antidepressants are used for the treatment of analgesic-resistant neuropathic pain as supplementary analgesics.23,24) Recently, there were several reports indicating that antidepressants such as TCAs and SSRIs have significant antinociceptive effects against nociceptive and inflammatory pain.12,25) Moreover, antidepressants also suppressed neuropathic pain, and TCAs especially were more effective than SSRIs on neuropathic pain.26) In most cases, suppressive effects of TCAs on neuropathic pain were produced by repeated administration. Indeed, repeated TCA (e.g., amitriptyline, nortriptyline and clomipramine) administration suppressed neuropathic pain induced by peripheral nerve injury.26,27) However, the suppressive effects and validity periods of TCAs on VCR-induced mechanical allodynia are poorly understood. In this study, repeated administration, but not acute administration, of IMI significantly suppressed VCR-induced mechanical allodynia. Moreover, repeated IMI
administration was effective on both the developmental stage (day 0—6) and the maintenance stage (day 7—13) of VCR-induced mechanical allodynia. We also observed that repeated IMI administration suppressed not only VCR- but also PSL-induced mechanical allodynia (unpublished data).

There are various reports studying the mechanisms of the suppressive effect of antidepressants on nociceptive, inflammatory and neuropathic pain. \(^{10,28,29}\) It is well known that endogenous pain-control systems are mediated by noradrenergic and serotonergic neurons projecting from brain to spinal cord, and antidepressants including IMI increase monoamines such as noradrenaline (NA) and serotonin (5-HT), in the synaptic clefts in the central nervous system. The antinociceptive effects of antidepressants were inhibited by the administration of NA and 5-HT receptors antagonists, \(^{30,31}\) while injection of a monoamine into the subarachnoidal space of spinal cord produced an antinociceptive effect. \(^{32}\) The antinociceptive effect of a TCA was attenuated in animals that lacked serotonergic neurons. \(^{33}\) Therefore, it is possible that endogenous monoamines system might play a critical role in the effects of IMI.

Generally, activation of spinal glial cell such as microglia and astrocytes elicits neuropathic pain through the production and release of inflammatory cytokine following peripheral nerve injury. \(^{34}\) As the envisioned mechanisms of neuropathic pain, microglia was rapidly activated after nerve injury, and then astrocytes were activated. Therefore, it is considered that microglia and astrocytes play critical roles in the development and the maintenance of neuropathic pain, respectively. We previously reported that TNF-\(\alpha\) derived from microglia and astrocytes, which were activated by chronic VCR administration, contributed to VCR-induced mechanical allodynia. \(^{8}\) There were previous reports indicating that antidepressants including TCAs inhibited the production and release of pro-inflammatory cytokines from microglia and astrocyte cultures. \(^{35,36}\) These evidences suggest that repeated, but not a single, IMI treatment might suppress VCR-induced mechanical allodynia through the inhibition of microglia activation at the developmental stage, and astrocytes activation at the maintenance stage.

In conclusion, we demonstrated a significant suppressive effect of repeated IMI treatments on VCR-induced mechanical allodynia, which was resistant to morphine analogues. Moreover, this effect was observed not only at the developmental stage (day 0—6) but also at the maintenance stage (day 7—13) of VCR-induced mechanical allodynia. These results suggest that IMI could use for the treatment of neuropathic pain, including VCR-induced mechanical allodynia.

Acknowledgments We appreciate Dr. James H. Woods and Dr. Gail Winger (Department of Pharmacology, University of Michigan Medical School) for review of this manuscript. This work was supported by the Research Grant on Priority Areas from Wakayama Medical University.

REFERENCES