Mechanical Alldynia Induced by Paclitaxel, Oxaliplatin and Vincristine: Different Effectiveness of Gabapentin and Different Expression of Voltage-Dependent Calcium Channel α2δ-1 Subunit

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We compared the inhibitory action of gabapentin, which is used to treat neuropathic pain, on mechanical alldynia induced by chemotherapeutic agents, paclitaxel, oxaliplatin, and vincristine, in mice. Single injections of paclitaxel, oxaliplatin, and vincristine at the doses corresponding to doses clinically used caused mechanical alldynia of similar intensity. Oral administration of gabapentin (30, 100 mg/kg) produced a dose-dependent inhibition of alldynia caused by paclitaxel and oxaliplatin, but not vincristine. Intrathecal injection of gabapentin (30, 100 μg/site) significantly inhibited alldynia induced by paclitaxel, but not oxaliplatin and vincristine. Intraplantar injection of gabapentin (30, 100 μg/site) did not significantly inhibit alldynia induced by paclitaxel and oxaliplatin. Paclitaxel increased the expression of mRNA of voltage-dependent calcium channel α2δ-1 subunit, an action site of gabapentin, in the dorsal spinal cord, and oxaliplatin increased it in the dorsal root ganglia. Vincristine was without effects on α2δ-1 subunit mRNA in these regions. These results suggest that the efficacy of gabapentin in the treatment of mechanical alldynia is dependent on chemotherapy agent used. It may be partly due to the distinct effects of chemotherapy agents on the expression of α2δ-1 subunit of voltage-dependent calcium channel.

Key words paclitaxel; oxaliplatin; vincristine; gabapentin; α2δ-1 subunit

Peripheral neuropathy is a common adverse effect of several classes of anti-cancer drugs, including the vinca alkaloid vincristine, the taxane paclitaxel, and the platinum-based drug oxaliplatin. Chemotherapy-induced peripheral neuropathy can be extremely painful.1) These three agents exert direct and indirect effects on sensory nerves to reduce the amplitude of action potential and to slow conduction velocity, and pain is common as a chronic consequence for many patients, especially those who experienced nociceptive sensory loss during treatment.2) Thus, the association with deafferentation pain is suggested, but mechanisms of pain, including relationship between peripheral neuropathy and pain, remain unclear. There are few effective pharmacological options to treat pain due to chemotherapy-induced peripheral neuropathy.3) Gabapentin, an anticonvulsant, has proven to be effective in the treatment of painful diabetic neuropathy, postherpetic neuralgia, and other neuropathic pain syndromes.3) A recent clinical trial failed to demonstrate the effectiveness of gabapentin against neuropathic pain of patients who were treated with vinca alkaloids, taxanes, platinum compounds, or their combination.4) However, that trial did not analyze its efficacy against neuropathic pain induced by each chemotherapy agent. In animal experiments, the effectiveness of gabapentin has been examined on neuropathic pain induced by these chemotherapeutic agents, and the efficacy is different between chemotherapeutic agents5—9) Gabapentin binds to α2δ-1 subunit of voltage-dependent calcium channel, which plays a key role in its inhibitory action on neuropathic pain.10) The increased expression of α2δ-1 subunit in neurons, especially in the primary sensory neurons, was claimed to be involved in the increase of analgesic potency of gabapentin.10,11) The spinal cord is thought to be an important site of analgesic action of gabapentin,12,13) although the brain may also be involved.14) Here we report that the analgesic efficacy of oral and intrathecal administration of gabapentin is different between paclitaxel, oxaliplatin, and vincristine, and that these three agents exert distinct effects on the expression level of α2δ-1 subunit mRNA in the dorsal spinal cord and dorsal root ganglion.

MATERIALS AND METHODS

Animals Male C57BL/6 mice (Japan SLC, Ltd., Shizuoka, Japan), 6 weeks of age at the start of experiments, were used. They were housed 4—6 per cage under controlled temperature (22±1°C) and humidity (55±10%). The room was lighted from 7:00 am to 7:00 pm and during the behavioral test. Food and water were available ad libitum. The study was approved by the Committee for Animal Experiments at University of Toyama.

Agents Paclitaxel, oxaliplatin, and vincristine sulfate, purchased from Sigma (St. Louis, MO, U.S.A.), were dissolved in Cremophor® EL (Sigma), saline and 5% glucose, respectively. Gabapentin (Sigma), dissolved in normal saline, was administered intracereally and intraplantarly in volumes of 5 and 20 μl, respectively, as described.13) For oral administration, gabapentin was dissolved in tap water.

Neuropathic Pain To induce neuropathic pain, paclitaxel (5 mg/kg), oxaliplatin (3 mg/kg), and vincristine sulfate (0.1 mg/kg) were administered intraperitoneally; the doses were calculated from doses clinically used. In our preliminary experiments, these chemotherapeutic agents gradually increased mechanical allodynia and the effects peaked around 14, 10, and 14 d after administration of paclitaxel, ox-
aliplatin, and vincristine. Therefore, experiments were done 14 d after paclitaxel and vincristine and 10 d after oxaliplatin administration.

**Behavioral Test**  Mechanical allodynia of the hind paw was assessed using von Frey filament as described. After an acclimation period of at least 30 min, von Frey filament with the bending force of 0.69 mN was pressed perpendicularly against the plantar skin and was held for 1—3 s with it slightly buckled. Responses to the stimulus were ranked as follows: 0, no response; 1, lifting of the hind paw; and 2, flinching or licking of the hind paw. The stimulation of the same intensity was applied six times to each hind paw at intervals of several seconds and the average served as a response score.

**Reverse Transcription and Polymerase Chain Reaction (RT-PCR)**  The dorsal spinal cord at the level of lumbar enlargement and the dorsal root ganglia at the level of L4 and 5 were isolated from the animals. Total RNA was extracted by using GenElute™ (Sigma) and RT-PCR assay of gabapentin (30, 100 mg/kg) and β-actin mRNA was done as described. The sequences of primers were as follows: αδ-1 subunit, 5’-gtgctgccgttggagtgaa-3’ (sense) and 5’-actggttctgtcgctgtc-3’ (antisense); β-actin, 5’-tcgacgactctatgtgg-3’ (sense) and 5’-tcctggtgcatgca-3’ (antisense). After the separation of PCR product and ethidium bromide staining, the density of band of predicted size was analyzed with NIH Image software (National Institute of Health, Bethesda, Maryland, U.S.A.).

**Statistical Analysis**  Data are presented as means and S.E.M. Results were analyzed with Student’s t-test or Student–Newman–Keuls multiple comparisons; p<0.05 was considered significant.

**RESULTS**

**Effects of Gabapentin on Mechanical Alldynia**  Single intraperitoneal injections of paclitaxel (5 mg/kg), oxaliplatin (3 mg/kg), and vincristine (0.1 mg/kg) induced mechanical allodynia of similar intensity (Fig. 1). Oral administration of gabapentin (30, 100 mg/kg) produced a significant and dose-dependent inhibition of mechanical allodynia induced by paclitaxel and oxaliplatin, without effects on vincristine-induced allodynia (Fig. 1A). The inhibition peaked 1 h after administration and subsided by 6 h. Intrathecal injection of gabapentin (30, 100 μg/site) produced a significant inhibition of mechanical allodynia induced by paclitaxel; the effect peaked 0.5—1 h after injection and almost subsided by 1.5 h (Fig. 1B). There was an inhibited tendency of oxaliplatin-induced mechanical allodynia after intrathecal injection of gabapentin (30, 100 μg/site), and vincristine-induced allodynia was not affected by gabapentin at the same intrathecal doses (Fig. 1B). There was an inhibited tendency of oxaliplatin-induced mechanical allodynia after intraplantar injections of gabapentin (30, 100 μg/site); pain-related scores 30 min after injection were 0.86±0.19 (n=6), 0.90±0.12 (n=5) and 1.17±0.11 (n=5) in 100 and 30 μg/site gabapentin and vehicle groups, respectively. Paclitaxel-induced mechanical allodynia was not affected by intraplantar injections of gabapentin (30, 100 μg/site); pain-related scores 30 min after injection were 1.42±0.04 (n=6), 1.43±0.04 (n=5) and 1.45±0.04 (n=5) in 100 and 30 μg/site gabapentin and vehicle groups, respectively.

**DISCUSSION**

Oral administration of gabapentin (30, 100 mg/kg) significantly alleviated mechanical allodynia induced by paclitaxel.
and oxaliplatin. The effective doses are similar to the reports of other groups.7—9) The same oral doses of gabapentin did not affect vincristine-induced allodynia. There are controversial reports about the effect of gabapentin on vincristine-induced allodynia. Single oral (about 70 mg/kg) and repeated intraperitoneal dose (100 mg/kg) of gabapentin was reported to reverse vincristine-induced allodynia,6,9) whereas single intraperitoneal dose of 50—300 mg/kg was reported to be without effects.5) This discrepancy may be due to differences in gabapentin dosage (single or repeated injection) and species and strain of animals. The present results clearly show that gabapentin is more effective against paclitaxel- and oxaliplatin-induced allodynia than vincristine-induced allodynia.

Intrathecal injection of gabapentin significantly inhibited mechanical allodynia induced by paclitaxel, whereas intraplantar injection of the same dose was without effect. If intrathecal gabapentin acted systemically, intrathecal dose of 100 μg/site would be as low as 3.3 mg/kg in the mouse of 30 g body weight. Effectiveness of low intrathecal dose suggests that the spinal action plays an important role in the effect of systemic administration of gabapentin at doses of 30 and 100 mg/kg. Paclitaxel increased the expression level of α2δ-1 mRNA in the dorsal spinal cord, but not in the dorsal root ganglia. These results are consistent with those reported by others, in which paclitaxel increased α2δ-1 protein in the dorsal spinal cord, but not in the dorsal root ganglia5) and raise the possibility that α2δ-1 subunit increased in the spinal dorsal horn is a main site of inhibitory action of gabapentin on paclitaxel-induced allodynia. In the spinal dorsal horn, α2δ-1 mRNA is expressed in the superficial layers.17) Paclitaxel causes the hypersensitivity of Aδ- and Aβ-fiber, but not C-fiber, afferents, which are reversed by gabapentin.7) Static (punctuate-evoked) and dynamic (brush-evoked) allodynia of neuropathy may mediated by C- and A-fiber afferents, respectively,18) and gabapentin more potently inhibits static allodynia than dynamic allodynia.19) With these findings taken into account, the result that intraplantar injection of gabapentin did not inhibit mechanical allodynia (probably mediated by C-fiber afferents) induced by paclitaxel suggests that central terminals of primary afferents are not primary site of inhibitory action of intrathecal gabapentin.

Intrathecal injection of gabapentin produced the slight but not significant inhibition of oxaliplatin-induced allodynia although oral gabapentin exerted marked inhibitory effects. These results suggest that spinal cord is not the primary site of inhibitory action of gabapentin on oxaliplatin-induced allodynia. Since the effects of intrathecal and intraplantar injections of gabapentin were dose-dependent, we do not deny the possibilities that higher doses produce significant inhibition and that the actions on central and peripheral terminals of primary afferents play a role in the effect of systemic gabapentin. The slight inhibitory effect of intrathecal gabapentin is similar to the effects observed in rats which underwent partial sciatric nerve ligation or peripheral inflammation.20) Oxaliplatin significantly increased mRNA of Ca2+ channel α2δ-1 subunit in the dorsal root ganglia, but not in the dorsal spinal cord. Although it remains unrevealed that oxaliplatin increases α2δ-1 subunit protein, it is suggested that α2δ-1 subunit increased in the primary sensory neuron is a site of the inhibitory action of gabapentin on oxaliplatin-induced allodynia. It should be examined which types of sensory neurons increase the expression of α2δ-1 subunit after oxaliplatin administration.

Vincristine did not affect the expression level of α2δ-1 mRNA in the spinal dorsal horn and dorsal root ganglia at the dose that induced mechanical allodynia. The result is consistent with the report of other group, in which 2-week intravenous infusion of vincristine did not increase the expression level of α2δ-1 in the dorsal horn and dorsal root ganglia.5) Ineffectiveness of oral and intrathecal injections of gabapentin against vincristine-induced mechanical allodynia may be partly due to unchange in the expression level of α2δ-1 in these regions.

In conclusion, paclitaxel, oxaliplatin and vincristine at doses corresponding to clinical doses induced mechanical allodynia of similar intensity, but the effectiveness and change in expression of the candidate molecule of gabapentin action were different between the chemotherapeutic agents. Thus, effective pharmacological treatment of chemotherapy-induced pain should be investigated and selected for each chemotherapy agent.

REFERENCES