Suppression by Gabapentin of Pain-Related Mechano-Responses in Mice Given Orthotopic Tumor Inoculation

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In this study, we examined whether several types of non-opioid agents would inhibit the pain-related responses of melanoma-bearing mice. Orthotopic inoculation with melanoma into the hind paw induced marked tactile allodynia and mechanical hyperalgesia. A peroral injection (p.o.) of gabapentin (100—300 mg/kg) inhibited the alldynia and hyperalgesia, without effects on gross behaviors. An intraperitoneal injection (i.p.) of ketamine hydrochloride (30 mg/kg) produced partial inhibition in alldynia and hyperalgesia and prostate posture at 15 min after injection. Diclofenac sodium (10 and 30 mg/kg, i.p.), mecloxetine hydrochloride (20 mg/kg, i.p.), clonidine hydrochloride (0.1 mg/kg, i.p.) and suramin (100 mg/kg, i.p.) were without effects on alldynia and hyperalgesia. Subcutaneous injections of baclofen (3 mg/kg) and N^6-nitro-L-arginine methyl ester (100 mg/kg) were also without effects. Repeated administration of gabapentin (150 mg/kg, p.o.) produced constant inhibitions, suggesting no analgesic tolerance. Gabapentin may be useful for the management of cancer pain.

Key words cancer pain; gabapentin; analgesia; acute tolerance; morphine; analgesic adjuvants

Pain at the end stage of cancer is a severe problem for patients. Although non-opioid analgesics are used for patients with mild-to-moderate cancer pain, morphine is the mainstream of the management of pains of patients with terminal cancer.1) Although morphine relieves cancer pain in many patients, some cancer pains are resistant to it or need high doses, which induce adverse effects.1) Thus, potent analgesics with few adverse effects and adjuvant drugs enhancing the analgesic efficacy of opioids are desired.

Orthotopic inoculation with melanoma into the mouse hind paw induces two phases of thermal hyperalgesia.2) Although the aspirin-like drug inhibits early-phase hyperalgesia, it is without effects at the late phase.2) Morphine (1 mg/kg) completely inhibits early-phase hyperalgesia but higher doses are needed to inhibit late-phase hyperalgesia.3) In the present experiments, we investigated whether several non-opioid agents which had been shown to inhibit neuropathic or inflammatory pain would inhibit the tactile alldynia and mechano-hyperalgesia of melanoma-bearing mice.

MATERIALS AND METHODS

Materials  Gabapentin, synthesized by H.T. and H.O., was dissolved in water and administered perorally (p.o.). Diclofenac sodium (Research Biochemical International, Natrick, MA, U.S.A.), ketamine hydrochloride (Sigma, St. Louis, MO, U.S.A.), mecloxetine hydrochloride (Sigma), clonidine hydrochloride (Sigma) and suramin (Sigma) were dissolved in saline and administered intraperitoneally (i.p.). Baclofen (Sigma) and N^6-nitro-L-arginine methyl ester (Research Biochemical International) were also dissolved in saline and administered subcutaneously (s.c.).

Animals  Male C57BL/6 mice (6 weeks of age at the melanoma inoculation; Japan SLC Ltd., Shizuoka) were used. They were kept in a room under controlled temperature (22±1°C), humidity (55±10%) and light (lights on 0700—1900 h). Food and water were available ad libitum. The study was approved by the Committee for Animal Experiments at Toyama Medical and Pharmaceutical University.

Tumor Inoculation  B16-BL6 cells (2×10^5 cells), melanoma derived from melanocyte of the C57BL/6 mouse, were inoculated into the planter region of unilateral hind paw.2)

Behavioral Test  Von Frey filaments with a bending force of 0.07 or 1.2 g were pressed against the plantar skin with it slightly buckled and responses were scored as follows: 0=no response; 1=immediate flinching or licking of the hind paw; 2=immediate flinching or licking of the hind paw followed by moving away from the filament; 3=immediate flinching or licking of the hind paw and moving away from the filament.4) The stimulation of the same intensity was applied six times to each test site at intervals of several seconds and the average served as pain-related score. After melanoma growth, the periphery of tumor mass was stimulated with the filaments.3)

Statistical Analysis  Data were presented as the mean±S.E.M. Results were analyzed with Mann-Whitney U-test or Kruskal-Wallis analysis of variance on ranks and post hoc Dunnett’s test; p<0.05 was considered significant.

RESULTS

Tactile allodynia (response to the filament of 0.07 g strength) and mechano-hyperalgesia (response to the filament of 1.2 g strength) abruptly increased in the tumor region on days 11—14 post-inoculation and thereafter relatively con-
GABAergic excitatory postsynaptic currents mediated by glutamic acid. Therefore, the inhibition of pain-related responses to the same dose does not affect spontaneous locomotor activity in sedative after the highest dose (300 mg/kg) tested, but the effective dose was 10—30 mg/kg. Mice appeared to be shown).

6) did not affect the allodynia and hyperalgesia (data not shown).

Among seven agents examined, only gabapentin (100—300 mg/kg) suppressed allodynia and hyperalgesia without effects on gross behaviors. The effective doses (≥100 mg/kg) were higher than those which inhibit allodynia and hyperalgesia in rodents with neuropathic pain, in which minimum effective dose was 10—30 mg/kg. Mice appeared to be sedative after the highest dose (300 mg/kg) tested, but the same dose does not affect spontaneous locomotor activity in mice. Therefore, the inhibition of pain-related responses may not be due to the suppression of locomotor activity. In human subjects, gabapentin is effective against neuropathic pain with few side effects. It was reported to be useful for the treatment of neuropathic cancer pain as an adjuvant to opioid analgesics. The present results raise the possibility that gabapentin alone relieves severe cancer pain in patients. Daily administration of gabapentin produced similar antiallodynic and antihyperalgesic effects for at least 7 d, suggesting the absence of acute tolerance. Gabapentin may be useful for long-term treatment of cancer pain.

GABAergic may inhibit pain primarily through the action on the spinal cord. It binds with a high affinity to the α2δ-subunit of voltage-dependent Ca2+ channel. It suppresses evoked excitatory postsynaptic currents mediated by glutamate receptors in the spinal dorsal horn. D-serine, an antagonist at the strychnine-insensitive glycine site on the NMDA glutamate receptor, inhibits antihyperalgesic effects of intrathecal gabapentin. In this context, N- and P-type Ca2+ channels localized at synaptic sites are involved in the release of transmitters, such as glutamate. In addition, blockade of N- and P-type Ca2+ channels in the spinal cord inhibits pain-related responses. Thus, blockade of voltage-dependent Ca2+ channels may be partly involved in gabapentin analgesia. The result that ketamine, non-competitive NMDA receptor antagonist, did not produce selective antiallodynic and antihyperalgesic effects suggest that inhibition of glutamate release is not exclusively involved in gabapentin analgesia.

The aspirin-like drug diclofenac did not affect allodynia and hyperalgesia. The results support the idea that prostaglandins do not play an important role in late-phase pain in mice with melanoma inoculation.
inhibitor N<sup>G</sup>-nitro-L-arginine methyl ester inhibits inflammation-induced hyperalgesia.<sup>18</sup> However, it did not affect allodynia and hyperalgesia in this experiment, suggesting that NO may not be involved in this pain state. The non-specific P<sub>2X</sub> and P<sub>2Y</sub> receptor antagonist suramin (100 mg/kg) inhibits mechanical allodynia induced by nerve injury.<sup>19</sup> However, suramin at the same dose did not affect tactile allodynia and mechano-hyperalgesia in melanoma-bearing mice.

The Na<sup>+</sup> channel blocker mexiletine, the α<sub>2</sub>-adrenoceptor agonist clonidine, and the GABA<sub>B</sub> receptor agonist baclofen are used for the management of cancer pain as analgesic adjuvants.<sup>1</sup> However, these agents did not affect tactile allodynia and mechanical hyperalgesia in melanoma-bearing mice.

In summary, although relatively high doses were needed, gabapentin markedly inhibited allodynia and hyperalgesia in a cancer pain model without apparent adverse effects. No acute tolerance was developed to the antiallodynic and anti-hyperalgesic effect. It may be worth testing the potency of gabapentin to inhibit cancer pains in human subjects.

REFERENCES