Pharmacological Evaluation of Morphine and Non-opioid Analgesic Adjuvants in a Mouse Model of Skin Cancer Pain

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Using a mouse model of advanced skin cancer which has mixed noxious-neuropathic pain, we evaluated the analgesic effects of morphine and analgesic adjuvants. Morphine hydrochloride (10–30 mg/kg, oral) and mexiletine hydrochloride (10–30 mg/kg, intraperitoneal) dose-dependently inhibited thermal hyperalgesia. Baclofen (10 mg/kg, subcutaneous) suppressed thermal hyperalgesia, without effects at lower doses of 1 and 5 mg/kg. Ketamine hydrochloride (50 mg/kg, oral) was without effect. Analgesic tolerance was observed after 6th administration of morphine, and it was not developed until at least 7th administration of mexiletine and baclofen. This mouse model of skin cancer may be useful for the pharmacological evaluation of the effects of opioids and analgesic adjuvants on mixed noxious-neuropathic pain of advanced cancer.

Key words skin cancer pain; thermal hyperalgesia; melanoma; morphine; analgesic adjuvant

Pain at the end stage of cancer is a serious problem for patients. Since severe pain enhances the growth and metastasis of tumors,1,2) pain relief is important for not only the quality of life of patients but also cancer therapy. The three-step analgesic ladder advocated by the World Health Organization is effective in cancer pain treatment.3) Strong opioid analgesics such as morphine are used to relieve severe pain of patients with advanced cancer. However, neuropathic cancer pain has been claimed to be resistant to opioid analgesics and the efficacy of opioid analgesics and analgesic adjuvants are controversial.4–6) The proportion of patients with pure neuropathic cancer pain is relatively low and many patients experience cancer pain that is driven by a mix of both nociception and neuropathy.7)

We have developed a mouse model of skin cancer pain,3) which has mixed noxious-neuropathic pain.8,9) Parenteral administration of morphine inhibits thermal hyperalgesia at relatively high doses; but the effects almost disappeared after the sixth administration.3) In the present experiments, we examined analgesic potency and tolerance of oral administration of morphine. Regarding analgesic adjuvant such as ketamine, mexiletine and baclofen which are used in patients with refractory pain including neuropathic pain,10–12) allodynia of mice with skin cancer is partially inhibited by ketamine, but not mexiletine and baclofen.13) Thus, we also examined the effects of analgesic adjuvants on the thermal hyperalgesia in the skin cancer pain model.

MATERIALS AND METHODS

Animals Male C57BL/6 mice (6 weeks of age at the melanoma inoculation; Japan SLC, Ltd., Shizuoka, Japan) were used. They were housed six per cage under controlled temperature (22 ± 1 °C) and humidity (55 ± 10%). The room was lighted from 7:00 a.m. to 7:00 p.m. 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.

Drugs Morphine hydrochloride (Sankyo, Tokyo, Japan) and ketamine (Sigma, St. Louis, MO, U.S.A.) were dissolved in tap water. Mexiletine hydrochloride and baclofen, purchased from Sigma, were dissolved in physiological saline.

Tumor Inoculation B16-BL6 cells, a highly invasive variant of B16 melanoma derived from the C57BL/6 mouse,14) were kindly provided by Dr. I. J. Fidler, MD Anderson Cancer Center, Houston, TX, U.S.A. B16-BL6 cells were cultured in Eagle’s minimum essential medium containing 10% fetal bovine serum. The melanoma cells (2×10^5 cells/20 µl) suspended in the medium was subcutaneously injected into the plantar region of the unilateral hind paw. To assess the growth of melanoma in situ, the volume of glabrous region of the hind paw was plethysmographically determined.

Behavioral Test For the test of thermal hyperalgesia, radiant heat was applied to the plantar region of the hind paw and the latency of its withdrawal response was determined, using a tail-flick apparatus (Ugo Basile, Milan, Italy). The intensity of radiant heat was adjusted to elicit a response of around 13 s in normal mice. The cut off time was 21 s. Nociceptive tests were performed according to the guidelines published in a guest editorial in Pain on ethical standards for investigations of experimental pain in animals.15)

Statistical Analysis All data are presented as mean ± S.E.M. Statistical significance was analyzed using paired t-test, one-way analysis of variance (ANOVA) or two-way repeated measures (RM)-ANOVA followed by Dunnott’s multiple comparisons; p<0.05 was considered significant.

RESULTS

Nociceptive Responses after Melanoma Inoculation Melanoma cell inoculation did not affect the size and pain-related response of the inoculated hind paw until day 7 post-inoculation. The paw volume increased exponentially as a function of time from day 8 post-inoculation (n=9). The latency of withdrawal response to heat stimulation was moderately shortened on days 8–9 post-inoculation and markedly shortened from day 12 to at least until day 19 (n=9).
Melanoma growth did not affect pain-related response of the contralateral hind paw. These results were consistent with those reported previously.\(^3\)

**Single Administration** In this series of experiments, agents were administered on day 16 post-inoculation. Morphine (10—30 mg/kg, oral) dose-dependently inhibited thermal hyperalgesia; the effects peaked 30 min after administration and subsided by 90 or 180 min after doses of 20 or 30 mg/kg, respectively (Fig. 1A). Mexiletine hydrochloride (10—30 mg/kg, intraperitoneal) dose-dependently inhibited thermal hyperalgesia; the effects peaked 15 min after administration and almost subsided by 60 min (Fig. 1B). Baclofen (10—30 mg/kg, subcutaneous) dose-dependently inhibited thermal hyperalgesia at an oral dose of 20 mg/kg in mice with advanced skin cancer. A higher dose of 30 mg/kg produced almost complete inhibition of thermal hyperalgesia.\(^3\) Therefore, the potency of oral dose is a quarter of that of subcutaneous dose. The inhibition of thermal hyperalgesia was significantly reduced after the 6th administration of oral dose of 20 mg/kg, but clear anti-hyperalgesic action was still observed after the 7th administration. On the other hand, the inhibitory action of a subcutaneous dose of 5 mg/kg is markedly reduced after the 6th administration.\(^3\) Thus, analgesic tolerance may develop more slowly to oral than to subcutaneous morphine.

**Repeated Administration** In this series of experiments, agents were administered three times per day from day 14 post-inoculation. Morphine hydrochloride (10, 20, 30 mg/kg, oral). Two-way RM-ANOVA: main effect, \(F(2, 8) = 10.48, p<0.0019;\) treatment \times time interaction, \(F(8, 52) = 7.02, p<0.0001.\) (B) Mexiletine hydrochloride (10, 20, 30 mg/kg, intraperitoneal). Two-way RM-ANOVA: main effect, \(F(2, 8) = 10.48, p<0.0019;\) treatment \times time interaction, \(F(8, 52) = 7.02, p<0.0001.\) (C) Baclofen (1, 5, 10 mg/kg, subcutaneous). Two-way RM-ANOVA: main effect, \(F(2, 12) = 9.08, p=0.0026;\) treatment \times time interaction, \(F(12, 90) = 3.63, p=0.0002.\) Closed and open circles indicate the inoculated and contralateral hind paws, respectively. The data are represented means and S.E.M. (n=6). *p<0.05 as compared with pre-administration (Dunnett’s test).

**DISCUSSION**

Morphine almost completely inhibited thermal hyperalgesia at an oral dose of 20 mg/kg in mice with advanced skin cancer. A higher dose of 30 mg/kg produced similar maximum inhibition, but the duration of action was longer. A subcutaneous dose of 5 mg/kg produces almost complete inhibition of thermal hyperalgesia.\(^3\) Therefore, the potency of oral dose is a quarter of that of subcutaneous dose. The inhibition of thermal hyperalgesia was significantly reduced after the 6th administration of oral dose of 20 mg/kg, but clear anti-hyperalgesic action was still observed after the 7th administration. On the other hand, the inhibitory action of a subcutaneous dose of 5 mg/kg is markedly reduced after the 6th administration.\(^3\) Thus, analgesic tolerance may develop more slowly to oral than to subcutaneous morphine.

The Na\(^+\) channel blocker mexiletine is used as an analgesic adjuvant in the treatment of neuropathic pain.\(^9\) Mexiletine (10—30 mg/kg) produced a dose-dependent inhibition of thermal hyperalgesia. A submaximal inhibition was obtained at a dose of 20 mg/kg, no tolerance was observed after repeated administration. Mexiletine (20 mg/kg) has been shown not to affect mechanical allodynia in mice with advanced skin cancer.\(^3\) It is suggested that the analgesic efficacy of mexiletine depends on the type of cancer pain. Mechanical allodynia and thermal hyperalgesia is mainly mediated by A\(\beta\) and C primary afferents, respectively,\(^6\) and tumor bearing may results in more frequent activity in C primary afferents than in A\(\beta\) afferents. Hence, the use-dependent action of Na\(^+\) channel blockers may be a cause of the pain type-dependent action.

The \(\gamma\)-aminobutyric acid (GABA) receptor B agonist baclofen is used as an analgesic adjuvant in the treatment of cancer pain.\(^1\) Baclofen inhibited thermal hyperalgesia at a dose of 10 mg/kg, without tolerance after repeated administration, although lower doses were without effects on thermal hyperalgesia. However, the effects peaked around 90 min and subsided by 180 min. The Na\(^+\) channel blocker baclofen was administered day 16 post-inoculation. A two-way RM-ANOVA: main effect, \(F(2, 8) = 10.48, p<0.0019;\) treatment \times time interaction, \(F(8, 52) = 7.02, p<0.0001.\) The data are represented means and S.E.M. (n=6). *p<0.05 as compared with the first injection (Dunnett’s test).
hyperalgesia (present experiments) and mechanical allodynia. The results support its significance in cancer pain therapy. Baclofen has been shown to suppress carcinogenesis and tumor growth. Conversely, it was also shown to increase the invasiveness of tumor cells in vitro, probably through increase in the production of matrix metalloprotease, which should be considered as used for cancer pain.

The non-competitive NMDA receptor antagonist ketamine is used as an analgesic adjuvant in the treatment of neuropathic and cancer pain. Ketamine did not affect thermal hyperalgesia at an oral dose of 50 mg/kg. Ketamine produces the partial inhibition of mechanical allodynia of skin cancer at an intraperitoneal dose of 30 mg/kg and the complete inhibition of pain-related behaviors of mice with bone cancer. Thus, the analgesic efficacy of ketamine may also depend on the types of cancer and pain.

This mouse model of skin cancer pain may be useful for the pharmacological evaluation of the effects of opioids and analgesic adjuvants on mixed nociceptive and neuropathic pain of advanced cancer.

REFERENCES AND NOTES

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