Mechanical Hypersensitivity and Alterations in Cutaneous Nerve Fibers in a Mouse Model of Skin Cancer Pain

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Abstract. Melanoma inoculation induced marked mechanical allodynia and hyperalgesia in the periphery of the melanoma mass in mice from about day 10 post-inoculation. In the middle of the tumor, there were slight hyperalgesia and response disappearance in the early and late phases, respectively. PGP9.5-like immunoreactivities increased in the epidermis of the periphery of the tumor and disappeared from the dermis of the middle on day 18 post-inoculation, without apparent alterations on day 10. When using this pain model, one should consider the tumor site-dependent responses.

Keywords: skin cancer pain, primary afferent fiber, allodynia and hyperalgesia

Although pain at the end stage of cancer is a severe problem for patients, the mechanisms of cancer pain remain unclear. Recently, several kinds of animal models of cancer pain have been developed. These include bone cancer pain (1, 2), skin cancer pain (3) and neuropathic cancer pain (4). These models have demonstrated the distinct pharmacological and neurochemical aspects of cancer pain, suggesting that cancer pain is not simply a form of pain due to inflammation and/or neuropathy.

When given orthotopic inoculation of melanoma cells into the hind paw, mice show moderate and marked thermal hyperalgesia on days 7 – 10 (early phase) and from day 14 post-inoculation (late phase), respectively (3). An aspirin-like drug inhibits the hyperalgesia at the early, but not late, phase (3). An injection of the extract from the tumor mass at the late phase, but not the early phase, induced thermal hyperalgesia in healthy mice (H.-W. Zhang et al., unpublished observation). The extract injection also induces mechanical hyperalgesia, which is not inhibited by the aspirin-like drug (5). Therefore, the present study was conducted to determine whether melanoma inoculation would induce mechanical hypersensitivity (hyperalgesia and allodynia) in mice.

Experiments were performed in C57BL/6 mice (6 weeks of age at the melanoma inoculation; Japan SLC, Ltd., Shizuoka). 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. B16-BL6 cells (2 × 10⁵ cells), melanoma derived from C57BL/6 mice, were inoculated into the plantar region of unilateral hind paw (3). To assess tumor growth in situ, the volume of the glabrous region of the hind paw was plethysmographically determined (3). In behavior experiments, von Frey filaments with a bending force of 0.07 or 1.20 g were pressed against the plantar skin with it slightly buckled, and pain-related responses were scored as described (5, 6). After melanoma became apparent, the periphery and middle of the melanoma mass were stimulated separately (Fig. 1A). For immunohistochemistry, under chloral hydrate (300 mg/kg) anesthesia, animals were transcardially perfused with saline and then with 4% paraformaldehyde in phosphate-buffered saline. After post-fixation with 10% neutralized formalin, the skin samples of the paw were embedded in paraffin, sectioned at 2 μm (7). They were stained with anti-
protein gene product (PGP) 9.5 antibody (1/400 dilution; Ultraclone, Incstar, UK) using the peroxidase-
diaminobenzidine method and counterstained with hematoxylin. Results were analyzed with Dunnett’s multiple comparisons; $P<0.05$ was considered significant.

The volume of the inoculated paw significantly increased on day 7 post-inoculation and thereafter increased exponentially as a function of time (Fig. 1B). The stimulation of the middle of the melanoma mass and the contralateral hind paw with a thin filament (0.07 g strength) elicited no apparent responses throughout the experiment period (Fig. 1C). On the other hand, although the stimulation of the periphery of the melanoma mass with the same filament elicited almost no pain-related responses until day 10 post-inoculation, mice showed the abrupt increase of responses on days 11 – 14 and almost maximum responses thereafter (Fig. 1C).

The stimulation of the normal hind paw with a thick filament (1.20 g strength) elicited slight pain-related responses. The responses to the stimulation of the middle of the melanoma mass slightly increased on days 5 – 7 post-inoculation, but mice did not respond to the stimulation after day 18 (Fig. 1D). The responses to the stimulation of the periphery of the melanoma mass increased from day 9 and reached the maximum (Fig. 1D).

PGP9.5-immunoreactive nerve fibers were distributed mainly in the dermis of untreated skin (Fig. 2: A and B). Although the distribution in the periphery of the melanoma mass was unchanged on day 10 post-inoculation (Fig. 2C), it was increased especially in the epidermis on day 18 (Fig. 2D). In the middle of the melanoma mass, although the distribution was not altered on day 10 (Fig. 2E), PGP9.5-immunoreactive nerve fibers almost disappeared on day 18 (Fig. 2F). Similar results were obtained from other animals (n = 5 each). Inflammatory cells were not observed in the skin around the tumor on days 10 and 18 post-inoculation (Fig. 2: C – F).

Fig. 1. Tumor growth, mechanical alldynia, and mechanical hyperalgesia of mice inoculated with melanoma cells. Mice were given a subcutaneous injection of B16-BL6 melanoma cells into the plantar region of one hind paw. A: Lateral aspect of the hind paw on day 16 after inoculation and the sites of stimulation with von Frey filaments. B: The volume of the glabrous region of the hind paw. The middle and the periphery of the melanoma mass and the counterpart site of the contralateral paw were stimulated with von Frey filaments of 0.07 g (C) and 1.20 g strength (D). Pain-related responses were scored as follows: 0 = no response, 1 = moving away from the filament, 2 = immediate flinching or licking of the hind paw. The stimulation of the same intensity was applied six times to each test site at intervals of several seconds and the average served as the pain-related score. Each point represents the mean and S.E.M. of 10 animals. *$P<0.05$, when compared with the pre-inoculation value (Dunnett’s test).
There were two phases of mechanical hyperalgesia, the time-course of which was similar to that of the thermal hyperalgesia observed in our previous study (3). Allodynia was apparent only at the late phase. The difference in time-course between mechanical hypersensitivity and melanoma growth suggest that the former is not simply due to the latter. Lung metastasis was apparent at the late, but not early, phase (3), suggesting that tumor cells at the metastatic phase is related to the increase of mechanical hypersensitivity. The extract of late phase melanoma elicits marked mechanical hyperalgesia in healthy mice (5). In addition, the present study showed the increased distribution of nerve fibers in the skin at the late phase. Since melanoma releases nerve growth factor (8), this factor may be at least partly involved in the increase of nerve fibers.

The notable feature of this cancer pain model is the degree of mechanical allodynia. Allodynia was much higher in this cancer model than in the neuropathic model; for example, mice with herpetic pain show allodynia with a score around 1 (9). Since mechanical allodynia exerts a profound influence on the quality-of-life of cancer patients, it may be a good index for the screening of cancer-pain killers.

Although PGP9.5 is a pan-neural fiber marker, topical capsaicin treatment markedly decreases PGP9.5-positive fibers in the skin (10), suggesting that at least a portion of PGP9.5-positive fibers are nociceptors. Mice did not respond to the stimulation with a thick filament after day 18 post-inoculation. On the same day, there were almost no PGP9.5-immunoreactive nerve fibers in the skin at the middle of melanoma mass, which may be a cause for the disappearance of the pain-related response. Melanoma-inoculated mice showed marked thermal hyperalgesia until 3 weeks after inoculation (3). However, in some experiments, pain-related responses to radiant heat, the diameter of which was 10 mm, decreased after day 18 post-inoculation. The disappearance of nerve fiber in the skin may be also responsible for the decrease of thermal hyperalgesia.

In summary, melanoma inoculation produced marked mechanical allodynia and hyperalgesia in mice. When using this skin cancer model, one should consider the tumor site-dependent responses at the late phase.

References

