Attenuating Effect of Artemin on Herpes-related Pain Responses in Mice Infected with Herpes Simplex

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Abstract. The influence of artemin (AR) on herpes-related pain responses was examined using mice infected with herpes simplex virus (HSV). BALB/c mice were inoculated with HSV (1x10⁶ plaque-forming units) on the right hind paw, while the contralateral hind paw was without inoculation. The changes in nociceptive threshold were examined using an electric Von Frey meter. Intraperitoneal administration of AR prevented a decrease in nociceptive threshold dose-dependently in HSV-inoculated mice, which was first observed at a dose of 1.0 mg/kg and peaked at doses higher than 1.5 mg/kg. This antinociceptive effect of AR attained peaks at 120 min after administration and declined gradually to non-treated levels by 270 min. Intraperitoneal administration of AR at a dose of 1.5 mg/kg scarcely affected β-endorphin and noradrenaline levels in the central nervous system of HSV-inoculated mice. However, AR caused a significant decrease of the dynorphin levels in spinal cord. These results strongly suggest that AR exerts antinociceptive effects on herpes-related pain through changes of the dynorphin levels in the central nervous system of HSV-inoculated mice. It is also suggested that AR will be a good candidate as an antinociceptive drug for the treatment of acute herpetic pain in humans.

Shingles or herpes zoster (HZ) is well known to occur at any stage in human life. Herpes zoster is the clinical manifestation of the reactivation of a lifelong latent infection with varicella zoster virus (VZV), usually contracted after an episode of varicella (chicken pox) in early life (1). Although the presentation of VZV is variable, a prodrome of derma pain typically precedes the appearance of the rash (2, 3). In almost all cases, this prodrome, so-called acute pain, disappears with healing acute eruptions (2, 3), but in unfortunate patients who get the shingles, the pain does not subside and persists long even after healing rash. This type of pain is called a post-herpetic neuralgia (PHN), meaning nerve pain and is characterized by a continuous burning and aching pain, a periodic piercing pain and allodynia elicited by tactile stimulation (2, 4). The PHN pain is often so severe that it significantly compromise the patient’s quality of life (5). Several studies have demonstrated that HZ patients with severe acute pain have an increased risk of developing PHN, indicating that relief of acute pain is the most important in preventing PHN development (2-4). Although the treatments currently recommended and most frequently prescribed for acute pain are antiviral agents and nerve stabilizing tricyclic antidepressants, a substantial number of patients still have chronic pain despite the adequate therapy (2, 6). Therefore, there exists a need for more effective and better-tolerated therapies.

Intradermal inoculation of mice with herpes simplex virus type I (HSV) caused extensive infection of primary sensory neurons and produced unilateral HZ-like skin lesions in the same dermatome (7-10). In addition, they showed aversive responses to innocuous tactile and noxious mechanical stimulation (7, 8).

Artemin (AR), a glial cell line-derived neurotropic factor (GDNF), was reported to be effective in the treatment of neuropathic pain induced by a partial ligation of one sciatic nerve (11) and spinal nerve ligation in rats (12). In the present study, the influence of AR on herpes-related pain responses was examined in a mouse/HSV model.

Materials and Methods

Chemicals. Recombinant mouse AR was purchased from R & D Co. Ltd. (Minneapolis, MN, USA) as a preservative free powder. It was dissolved in sterile phosphate buffered saline (PBS) just before use. Pentobarbital was supplied by Abbott Laboratories (North Chicago, IL, USA). A chemical depilatory cream, Hair Remover Milk-Cream, was purchased from Kanebo Co. Ltd. (Tokyo, Japan).
Mice. Specific pathogen-free BALB/c female mice, 6 weeks of age, were purchased from Charles River Japan Inc. (Atsugi, Japan). After arriving at our University, the mice were housed in filter (0.2 µm)-barriered cages and given autoclaved food and water ad libitum to prevent unwanted microbiological infections. All animal experiments were carried out in accordance with the guidelines of the Physiological Society of Japan.

Virus infection. Mice were anesthetized with pentobarbital (50 mg/kg, i.p.), and then depilated with a chemical depilatory cream. After three days, HSV (1x10^6 plaque-forming units in 10.0 µl) was inoculated on the skin of the right hind paw after scarification with 27-gauge needles (7). The contralateral hind paw was not inoculated and served as control.

Treatment of mice with agent. Various doses of AR in a volume of 0.2 ml PBS were administered once intraperitoneally into experimental mice. Control mice received 0.2 ml PBS alone intraperitoneally.

Assessment of pain-related responses. Pain-related responses were assessed by measuring changes in nociceptive threshold in mouse hind paw with Electronic von Fray Anesthesiometer® (Model 2290; IITC INC., Woodland Hills, CA, USA) on day 6 post HSV-inoculation (10). Mice were placed individually in stainless steel cages with a wire mesh bottom. After acclimation of approximately 15 min, the probe was applied perpendicularly against the plantar skin and measured maximum force applied to flinch the hind paw. The probe was applied five times to each hind paw at intervals of several seconds and obtained the average force per mouse. Pain-related responses were expressed as percent of nociceptive threshold calculated by the following formula: % nociceptive threshold = (average force in HSV-inoculated paw/average force in non-inoculated paw) x 100. The results were shown as the mean % of nociceptive threshold ± SE of five mice.

Measurement of β-endorphin (BE) and noradrenaline (NE) in brain. Mid-brain and hypothalamus were removed from mice sacrificed by decapitation and were homogenized in 4 volumes of PBS by glass tissue homogenizer for one min at 4°C. After centrifugation at 15,000 x g for 30 min at 4°C, the supernatants were obtained and used for water soluble brain extracts. BE levels in brain extract were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Peninsula Laboratories, Inc. San Carlos, CA, USA) according to the manufacturer’s recommendation. To prepare samples for NE measurement, the pons and medulla oblongata were removed from mice killed by decapitation, and water soluble extracts were prepared in a similar manner. The NE levels in the extracts were measured by commercially available ELISA test kits (Peninsula Laboratories, Inc.). The minimum detectable levels of these ELISA test kits were 0.04 ng/ml for BE and for NE. The protein concentrations of the supernatants were measured with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). These two factor levels were expressed as mean ng/mg protein ± SE of five mice.

Measurement of dynorphin (DN) in spinal cord. Spinal DN contents were measured in the ipsilateral dorsal quadrant of the spinal cord (relative to the side of HSV injection) as described (12, 13). Spinal tissues were homogenized in 1 N acetic acid and incubated for 30 min at 95°C. After centrifugation at 15,000 x g for 30 min at 4°C, the supernatants were obtained and assayed for DN contents by commercially available DN ELISA test kits (Peninsula Laboratories, Inc.) according to the manufacturer’s recommendation. The minimum detectable level on this ELISA kit was 0.06 ng/ml. The protein concentrations of the supernatants were also measured with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories). DN levels were expressed as mean ng/mg protein ± SE of five mice.

Results

Suppressive activity of AR on pain-related responses induced by HSV inoculation. The first set of experiments was undertaken to examine the influence of AR on pain-related responses induced by HSV inoculation. Mice were injected intraperitoneally with 1.5 mg/kg AR on day 6 post-HSV inoculation and the nociceptive threshold was monitored at 30 min intervals. As shown in Figure 1, AR could exert suppressive effects on pain-related responses, which peaked 120 min after administration and then gradually declined to non-treated levels by 270 min. The dose response profile of AR effects on the responses was subsequently examined. Mice were injected intraperitoneally with either 0.5, 1.0, 1.5, or 2.0 mg/kg AR on day 6 post-HSV inoculation and the nociceptive threshold was measured 60 min after AR administration. AR at a dose of 0.5 mg/kg did not cause the changes in pain-related responses: the nociceptive threshold in treated-mice was nearly identical to that observed in non-treated (0 m/kg), HSV-inoculated mice (Figure 2). However, mice treated with 1.0 mg/kg AR showed a significant suppression of the decrease in nociceptive threshold induced by HSV inoculation (Figure 2). This effect reached a peak when experimental mice were treated with more than 1.5 mg/kg AR (Figure 2).

Influence of AR on the levels of endogenous anti-nociceptive opioids in HSV-inoculated mice. The second set of experiments was designed to examine the possible mechanisms by which AR could suppress development of pain-related responses in HSV-inoculated mice by examining BE and NA contents in brain and DN levels in spinal cord. Mice inoculated with HSV were treated with 1.5 mg/kg AR and the BE contents in brain were measured 60 min after treatment. Treatment of HSV-inoculated mice with 1.5 mg/kg AR scarcely affected brain BE levels; BE levels in brain from 1.5 mg/kg AR-treated, HSV-inoculated mice were nearly identical (not significant) to that from non-treated and HSV-inoculated mice (Figure 3). In the next experiments, NA levels in pons and medulla oblongata were examined. As shown in Figure 3, AR treatment of HSV-inoculated mice did not affect NA levels in brain, even when 1.5 mg/kg of AR was used for treatment.
Finally, the influence of AR on DN levels was examined in spinal cords from HSV-inoculated mice. HSV-inoculated mice were treated with 1.5 mg/kg AR and the DN levels in spinal cords was examined 60 min after treatment. As shown in Figure 4, mice inoculated with HSV showed a significant increase in spinal DN contents over the non-inoculated controls. AR treatment of HSV-inoculated mice completely normalized spinal DN content, which was not significantly different from that in non-inoculated mice (Figure 4).

Discussion

HZ is caused by reactivation of latent VZV in the sensory ganglia and is characterized by neurocutaneous symptoms including dermatic rash and sever pain (23). Although there is circumstantial evidence that greater acute pain severity in HZ patients is associated with a significantly greater risk of developing PHN (4, 6), the medications used for the treatment of HZ patients are reported to be unable to completely prevent the establishment of PHN (5), indicating that development of antinociceptive drugs for acute herpetic pain to prevent PHN is urgently needed.

AR was shown to be effective and extremely well tolerated for the treatment of neuropathic pain syndromes induced by nerve injury in a rat experimental model (11, 12). We examined the influence of AR on herpes-related pain responses, which is classified as neuropathic pain syndrome, using HSV-inoculated mice.

In the present study, we first examined the influence of AR on pain-related responses induced by HSV inoculation in mice. The results clearly indicate that AR exerts an inhibitory effect on pain-related responses, first noted at a dose of 1.0 mg/kg with a maximum at 1.5 mg/kg. It was also observed that this inhibitory action lasted for 180 min after AR administration.

Infection of nerve cells obtained from dorsal root ganglia with HSV was reported to alter the conformation of the NA channels and to reduce the number of available NA channels in plasma membrane, which are responsible for generation of abnormal spontaneous discharge (14). It was also found that HSV infection in peripheral nerves causes demyelination of nociceptive fibers and the formation of neuromas (8, 14). These morphological changes are associated with the production of nerve impulses in the absence of any noxious stimulus (14). Electrophysiological observations revealed that NA channel blockers are able to suppress spontaneous injury and neuroma discharge in Aβ and C fibers and favorably modify the clinical condition of neuropathic pain, such as diabetic neuropathy, postherpetic neuropathy and phantom limb pain (15-17). The mechanisms of the antinociceptive effect of AR on neuropathic pain were analyzed in rats that received a partial ligation of one sciatic nerve; the results revealed that...
AR exerted antinociception by normalizing the NA channel alteration in both peripheral sensory neurons and dorsal root ganglion after nerve ligation (11). It was also reported that administration of GDNF to rats subjected to nerve injury could prevent A-fiber sprouting (18), which is implicated in the generation of some aspects of neuropathic pain through the production of abnormal spontaneous discharge (18, 19). Taken together, the present results suggest that the mechanisms of action of AR may involve modulation of the nociceptive input at the level of central nervous system or suppression of afferent discharges at the site of the peripheral nerve by blocking NA channel in the plasma membrane and sprouting neuron fiber.

Experimental evidence showed the ability of AR to activate the endogenous opioid-mediated antinociceptive systems, resulting in relieving neuropathic pain (12). Therefore, to further examine the possible mechanisms of AR on HSV-induced pain responses, we examined the influence of AR on endogenous opioid levels in brain. Administration of AR to HSV-infected mice did not change the levels of either BE in mid brain and hypothalamus or NE in pons and medulla oblongata, suggesting that the descending adrenergic and β-endorphinergic antinociceptive system plays a minor role in the induction of antinociception by AR in HSV-inoculated mice.

Neuropathic and other chronic pain states, such as inflammation, are associated with increased spinal DN contents (13, 19, 20). Intrathecal DN injection was reported to produce behavioral signs that mimic nerve injury-induced pain, which are blocked by MK-801 treatment (13, 21). It
was also observed that intrathecal administration of an antiserum to DN can reverse neuropathic pain induced by nerve-injured rats (20) and mice (22), suggesting that spinal DN is an essential endogenous opioid for the development and the maintenance of neuropathic pain. We examined the DN contents in spinal cord obtained from HSV-inoculated, AR-treated mice. The present data clearly show that AR normalized spinal DN levels, which was increased by HSV inoculation in mice. Neuropathic pain is often associated with the appearance of pain in regions not related to the injured nerve. One possible mechanism that may underlie neuropathic pain is abnormal, spontaneous afferent drive, which may contribute to NMDA-mediated central sensitization by the non-opioid actions of spinal DN (20, 23). These reports may suggest that AR suppresses central sensitization through normalization of spinal DN levels, which increased by HSV inoculation results in inhibition of the development of herpes-related pain responses in HSV-inoculated mice.

In conclusion, the present results suggest that AR may be a good candidate as an antinociceptive agent in the treatment of acute herpetic pain. The administration of AR in the acute phase of VZV infection may also reduce the risk of developing PHN in humans.

References


17 Tanelian DL and Brose WG: Neuropathic pain can be relieved by drugs that are use-dependent sodium channel blocker: Lidocaine, carbamazepine, and mexiletine. Anesthesiology 74: 949-951, 1991.

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