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Effect of Spinally Administered Ginseng Total Saponin on Capsaicin-Induced Pain and Excitatory Amino Acids-Induced Nociceptive Responses

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Abstract : Ginseng total saponins (ginsenosides) are biologically active main ingredients of *Panax ginseng*. In present study, we have investigated whether pretreatment of ginsenosides inhibited capsaicin-induced pain at the spinal level, in the view that capsaicin causes substance P (SP) release from primary afferents. Ginsenosides relieved capsaicin-induced pain in a dose-dependent manner. The ED_{50} of the effect was 43 (20-93, 95% C.I.) μg/mouse. We investigated excitatory amino acids-induced nociceptive responses in mice, because these agents are also involved in nociceptive transmission in the spinal cord. Coadministration of ginsenosides with N-methyl-D-aspartate (NMDA) or kainate via i.t. inhibited NMDA- but not kainate-induced pain behaviors. The ED_{50} for the inhibition of NMDA-induced pain by ginsenosides was 37 (21-66, 95% C.I.) μg/mouse. These results suggest that the ginsenosides-induced antinociception results from blocking of pain transmitter-induced nociceptive information at the spinal level.

Key words : Ginsenosides, NMDA, Substance P, Nociceptive response, Antinociception, Postsynatic site.

Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer, is an oriental folk medicine that has been shown to produce many medicinal effects. Recent studies showed that ginsenosides are the main components responsible for the beneficial actions of ginseng.[1] Much evidences also showed that ginsenosides act at the level of peripheral nervous system as well as central nervous system in association with pain modulation. For example, they inhibit voltage-dependent Ca^{2+} channels of sensory neurons.[2,3] They also relieve pain induced by dilute formalin[4,5] and inhibit the development of tolerance and dependence of morphine in mice.[6]

Excitatory amino acids such as glutamate, aspartate, and substance P (SP) exist in the cell body of primary sensory neurons and in dorsal horn of the spinal cord where these neurons terminate at relatively high levels.[7] These neurotransmitters are released from primary afferent fibers into dorsal horn of the spinal cord by stimuli such as formalin or noxious heat.[8,9] It is believed that these transmitters are the main mediators for nociception, given the view that they stimulate dorsal horn neurons in electrophysiological experiments and induce nociceptive response *in vivo* experimental animal studies. Thus, the blocking of transmission of nociceptive information using ne-

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urokinin or NMDA antagonists induces spinal antinociception.[10]

We demonstrated in previous study that i.t. administration of ginsenosides induces antinociception in formalin test.[5] The aim of this study was to further investigate the inhibition of capsaicin-induced pain by ginsenosides, since capsaicin may stimulate the pathways for transmission of pain in a more physiological way than the direct intrathecal application of SP. We also investigated the effects of ginsenosides on excitatory amino acids-induced nociceptive responses at the spinal level, since it is not yet known whether ginsenosides antinociception is achieved by regulation of excitatory amino acids-induced nociceptive responses.

Materials and Methods

1. Animals

ICR mice (20~25 g, 9-10 mice per group) were intrathecally administered with 5 μl of NMDA (0.2 nmol) or kainate (0.14 nmol) alone or with various concentrations of ginsenosides dissolved in 0.9% saline using the method of Hylden and Wilcox.[11] Injection procedure involves direct inserting a 30-gauge needle, matched to a 50 μl microsyringe, into an intervertebral space at approximately the level of the 5th or 6th lumber vertebrae. The success rate for the injection was consistently over 95% as detected by application of 1% methylene blue solution before the experiments carried out. Capsaicin (2 μg/40 μl/mouse) dissolved in DMSO (5% in final concentration) was injected just under the skin of the plantar surface of the left hind paw. Control group was only injected with 5% DMSO. The total time that was spent bitings and licking the left hind paw over the next 10 min was measured with a stopwatch and recorded to the nearest second. Ginsenosides used in this experiment were ginseng total saponins. These ginsenosides were isolated according to the method of Ando *et al.*[12] and were obtained form Korea Ginseng and Tobacco Research Institute. Glutamate, kainate, NMDA, and MK801 were purchased from RBI.

Excitatory amino acid-induced nociceptive response in mice: Either NMDA (0.2 nmol) or kainate (0.14 nmol) was i.t. injected and the time spent for caudally directed biting, licking or scratching was counted for 2 min after injection. Co-administration of test substance with 0.2 nmol NMDA was chosen as a convenient way to minimize i.t. injection volume and to ensure similar localization of test substance.[5]

2. Data analysis

The results were expressed as percent inhibition of the response to 0.2 nmol of NMDA or 0.14 nmol of kainate alone. ED_{50} values were determined according to the method of Lichfield and Wilcoxon.[13] Other statistical analysis was performed using Dunnett's test for multiple comparisons after analysis of variance (ANOVA). A difference was considered statistically significant at $P<0.05$. All results were given as means±S.E.M.

Results

Subcutaneous (s.c.) administration of capsaicin induced intense burning pain and neurogenic hyperalgesia.[14] The accumulated evidence showed that SP released from primary afferents after capsaicin treatment caused pain sensation.[15] In present study we tested whether intrathecal (i.t.) administration of ginsenosides relieved capsaicin-induced pain. As shown in Fig. 1, administration of capsaicin into intraplantar surface of hind paw induced typical pattern of pain responses such as licking and biting of affected hind paw. These behaviors were similar to those after treatment with dilute formalin but the duration of pain response was shorter (5~8 min) than that. Tonic phase of pain was not observed. Pretreatment with ginsenosides for 20 min via i.t. route with 15 μg/mouse did not inhibit capsaicin-induced pain but doses of over 30 μg/mouse relieved capsaicin-induced pain. The ED_{50} induced via i.t. administration of ginsenosides was 43 (20~93, 95% C.I.) μg/mouse. In a separate experiment designed to study whether the observed ginsenosides antino-

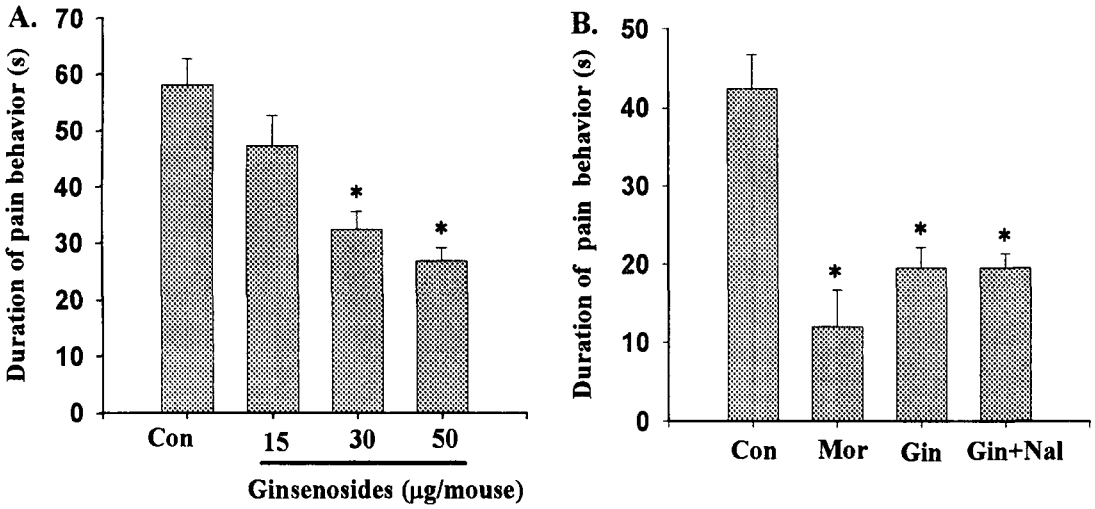

Fig. 1. (A) Effect of spinally administered ginsenosides on capsaicin-induced pain. Ginsenosides dissolved in saline was pretreated for 20 min via i.t. route with indicated doses before capsaicin. Control (Con) group was only treated with saline. Capsaicin (2 μg/40 μl/mouse) then was injected to all group just under the skin of the plantar surface of the left hind paw. The total time that was spent biting and licking the left hind paw over the next 10 min was measured. (B) Morphine (2 μg/mouse) was pretreated via i.t. route for 20 min before capsaicin. Naloxone (5 mg/kg) was pretreated via intraperitoneal route for 20 min before ginsenosides treatment. Then, capsaicin was treated as described in section (A). The results were expressed with means ±S.E.M (n=9-10). * $p<0.05$ when compared to capsaicin-injected controls (by one way ANOVA with Dunnett's test).

ciception against capsaicin-induced pain was mediated via opioid receptor activation, we examined the effect of 5 mg/kg naloxone (an opioid antagonist) pretreatment on ginsenosides antinociception. As shown in Fig. 1B, naloxone had no significant effect on ginsenosides antinociception on capsaicin-induced pain, indicating that ginsenosides-induced antinociception may not be the activation of the spinal opioid system. Intrathecal administration of morphine (2 μg/mouse) also relieved capsaicin-induced pain (Fig. 1B). These results were well consistent with those of the previous report that ginsenosides induce antinociception in formalin test,[4] since s.c. injection of dilute formalin also releases substance P from central terminals of primary afferent.[15]

In addition to SP, excitatory amino acids such as glutamate and aspartate is also released from primary afferents after s.c. formalin treatment.[9] As more direct evidences for their roles in pain sensation, i.t. administration of NMDA or kainate induces intense nociceptive behaviors as shown in i.t. SP treatment. Intrathecal NMDA treatment causes noiciceptive behavior as well as hyperalgesia but kainate only induces nociceptive behavior. Interestingly, opioids usually attenuated SP-induced nociceptive behaviors as mentioned above. Opioids also attenuated NMDA-induced nociceptive behavior and hyperalgesia but not kainate-induced nociceptive behaviors, suggesting that NMDA is mainly involved in pain transmission at the spinal level.[16]

It is not yet known whether ginsenosides antinociception in formalin test is achieved by inhibiting the action of excitatory amino acids at the spinal level, although excitatory amino acids and SP coexist in small dorsal root ganglion cells[8] and to corelease from spinal primary afferents following painful stimuli.[9] To test the possible involvement of excitatory amino acids in ginsenosides antinociception, we coadministered ginsenosides with NMDA or kainate via i.t. route. As shown in Fig. 2, when NMDA or kainate was i.t. administered to mice, these two agents induced

nociceptive responses as shown in i.t. SP administration.[16] These characteristic and reproducible behaviors peaked at 1~2 min and almost disappeared at 5 min post-injection. When NMDA was administered together with ginsenosides, 15 μg/mouse of ginsenosides did not attenuate NMDA-induced pain responses but at dose over 15 μg/mouse of ginsenosides inhibited pain responses. The ED_{50} of ginsenosides inhibiting NMDA-induced pain response was 37 (21-66, 95% C.I.) μg/mouse. However, kainate-induced pain response was not attenuated by ginsenosides coadministration at the dose even inducing attenuation of NMDA-induced pain response.

Discussion

We have demonstrated in previous study that systemic or i.t. administration of ginsenosides induces antinociception in the formalin test.[4,5] In this study we further confirmed that i.t. administration of ginsenosides relieved capsaicin-induced pain. Moreover, i.t. administration of ginsenosides also attenuated NMDA- but not kainate-induced pain responses. The mechanism that ginsenosides attenuated capsaicin-induced pain and NMDA- but not kainate-induced pain responses is not yet understood. Recent studies showed some evidences that ginsenosides could exert their analgesic efficacy by acting on presynaptic site(s). At cellular level ginseng root extracts and ginsenoside Rf inhibits voltage-dependent Ca^{2+} channels in sensory neurons as well as nociceptors as opioids do.[2,3] This inhibitory effect on voltage-dependent Ca^{2+} channels by ginsenosides may provide possible explanation for the analgesic activity of ginsenosides, since sensory neurons are involved in sensory transmission such as pain from peripheral nervous system to central nervous system. The voltage dependent Ca^{2+} channels in sensory neurons are involved in release of pain transmitters from primary afferent neurons following noxious peripheral stimulations such as capsaicin or formalin.[8]

However, the presynaptic regulation of ginsenosides on voltage-dependent Ca^{2+} channels probably are not enough for all explanation of ginsenosides antinociception and ginsenosides attenuation of pain response induced by SP or NMDA, since these pain transmitters also bind postsynaptic site(s).[16] The activation of SP receptor opens cation channels for the induction of depolarization and induces inward currents in dorsal horn neurons and increases intracellular free Ca^{2+} concentration.[17] Dorsal horn neurons of spinal cord contain both NMDA and non-NMDA receptors. Glutamate released from primary afferents following peripheral noxious stimulation binds NMDA and non-NMDA receptors.[18]

At the cellular level, it was not yet reported that ginsenosides act on postsynaptic site(s) at the spinal level. We demonstrated that ginsenosides could interact postsynaptic site(s) in the present and previous studies using experimental animals, since ginsenosides attenuated NMDA- and SP-induced pain responses. Similarly, opioids inhibit NMDA-induced but not kainate-induced pain responses.[16] The one possible explanation on these results is that pretreatment of ginsenosides might exert inhibitory effect on spinal neurons by inhibiting Ca^{2+} influx through Ca^{2+} channels that might be stimulated by NMDA or SP treatment. However, other possible pathway(s) could also be possible. For example, protopanaxatriol ginsenosides such as ginsenoside Rf and Rg_2 mainly inhibit acetylcholine-stimulated catecholamine releases from bovine chromaffin cells. The inhibition of catecholamine secretion by ginsenoside Rg_2 is achieved via inhibition of Na^+ influx through nicotinic receptor-gated cation channels but not via inhibition of Ca^{2+} influx through voltage dependent Ca^{2+} channels.[19] These results show the possibility that ginsenosides may regulate ligand-gated cation channel(s) and inhibit NMDA- or SP-induced nociceptive responses by inhibiting cation influx into neurons.

These results suggest the possibility that ginsenosides for antinociception induction modulate neuronal cells by more than one way. One is the regulation of voltage-dependent Ca^{2+} channels. The

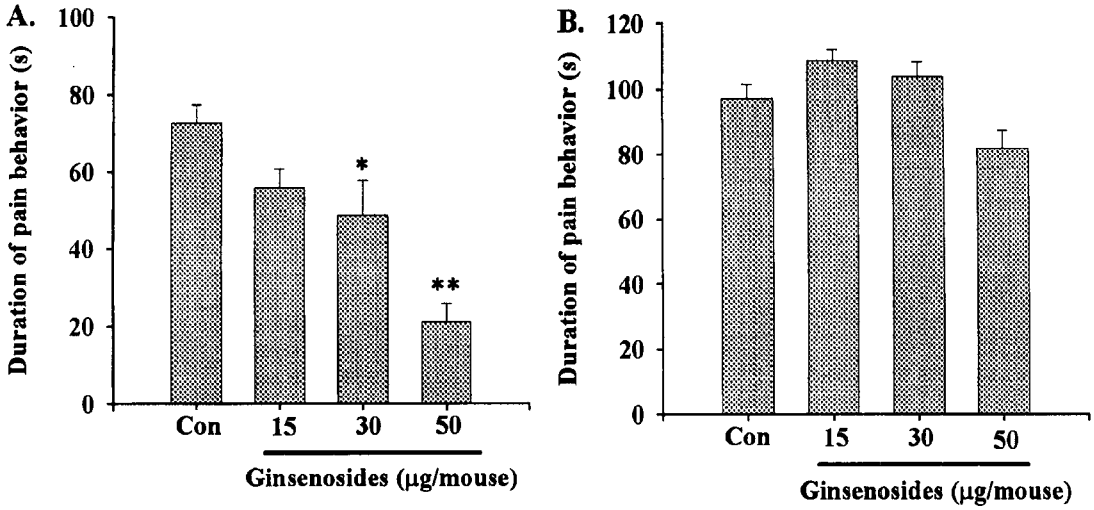

Fig. 2. Effect of spinally administered ginsenosides on NMDA- or kainate-induced nociceptive responses. NMDA (0.2 nmol/mouse) (A) or kainate (0.14 nmol/mouse) (B) was administered alone (con) or together with indicated doses of ginsenosides. This procedure was convenient to minimize i.t. injection volume and to ensure similar localization of the test substances. The spent time for biting, licking or scratching of caudal portion of mouse was counted for 2 min after injection. The results were expressed with means±S.E.M (n=9-10). * $p<0.05$ when compared to NMDA-injected controls (by one way ANOVA with Dunnett's test). ** $p<0.01$ when compared to NMDA-injected controls (by one way ANOVA with Dunnett's test).

other is probably selective regulation of ligand-gated cation channels, since ginsenosides had no effect on kainate-induced pain responses as shown in Fig. 2B.

요 약

진세노사이드(ginseng total saponin)는 인삼의 주요 약리학적 성분이다. 본 연구는 척수강내로 투여된 진세노사이드가 캡사이신에 의하여 유도된 통증을 억제하는가를 연구하였다. 진세노사이드의 척수강내 전투여는 캡사이신에 의하여 유도되는 통증을 투여용량에 의존적으로 억제하였다. 통증 억제 효과를 나타내는 ED_{50}은 43 μg/mouse 이었다. 흥분성 아미노산들도 척수 수준에서 통증전달에 포함되기 때문에 본 연구에서는 또한 진세노사이드가 흥분성 아미노산에 의하여 유도되는 아픈 행동(nociceptive behaviors)을 억제하는 가를 연구하였다. 진세노사이드와 NMDA를 같이 투여할 경우 NMDA를 단독 투여할 때 나타나는 아픈 행동을 억제하는 것으로 나타났다. 진세노사이드가 NMDA에 의하여 나타나는 아픈 행동을 억제하는 ED_{50}은 37 μg/mouse 이었다. 그러나 진세노사이드는 kainate 투여에 의하여 나타나는 아픈 행동을 억제하지 않은 것으로 나타났다. 이러한 연구 결과들은 진세노사이드에 의한 항통증 효능중의 하나는 척수 수준에서 통증 전달 물질에 의하여 유도되는 통증 전달 정보의 선택적 억제에 의하여 이루어진다는 것을 보여준다.

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