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Synergistic Interaction Between Intrathecal Ginsenosides and Morphine on Formalin-Induced Nociception in Rats

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Abstract: We defined the nature of the pharmacological interaction between ginsenosides and morphine in a nociceptive state and clarified the role of the different types of opioid receptor in the effects of ginsenosides. An intrathecal catheter was placed in male Sprague-Dawley rats. Pain was induced by formalin injection into the hindpaw. Isobolographic analysis was used to evaluate drug interactions. Furthermore, a nonselective opioid receptor antagonist (naloxone), a μ opioid receptor antagonist (CTOP), a δ opioid receptor antagonist (naltrindole), and a κ opioid receptor antagonist (GNTI) were given intrathecally to verify the involvement of the opioid receptors in the antinociceptive effects of ginsenosides. Both ginsenosides and morphine produced antinociceptive effects in the formalin test. Isobolographic analysis revealed a synergistic interaction after intrathecal delivery of the ginsenosides-morphine mix. Intrathecal CTOP, naltrindole, and GNTI reversed the antinociceptive effects of ginsenosides. RT-PCR indicated that opioid receptors' mRNA was detected in spinal cord of naïve rats and the injection of formalin had no effect on the expression of opioid receptors' mRNA. Taken together, our results indicate synergistic antinociception following intrathecal coadministration of a ginsenosides/morphine mix in the formalin test, and that μ, δ, and κ opioid receptors are involved in the antinociceptive mechanism of ginsenosides.

***Perspective:** This article concerns the antinociceptive activity of ginsenosides, which increases antinociception by morphine. Thus, a spinal combination of ginsenosides and morphine may be useful in the management of acute pain as well as facilitated state pain.*

***Key words:** Antinociception, drug interaction, ginsenosides, morphine, opioid receptors, spinal cord.*

Ginseng, the root of *Pannax ginseng* C.A. Meyer, has long been used in Oriental traditional medicine to improve the weakened physical status brought on by stress or disease.[13] Ginsenosides, ginseng saponins, are the major component responsible for the effects of ginseng and more than 20 different ginsenosides have been found todate.[18] Therefore, many different kinds of ginsenosides may be involved in the effects of ginseng. Several lines of evidence suggest that ginsenosides are effective against various nociceptive states. In particular, intrathecal ginsenosides attenuate formalin-induced, substance P-induced, and capsaicin-induced pain behaviors in mice.[3,17,38] These findings suggest that ginseng may play an important role in the modulation of nociception at the level of the spinal cord.

According to several previous reports, Ca^{2+} channels are the pharmacological sites of action of ginsenosides,[19,20,25] and alpha2, muscarinic, opioid, and GABA receptors are not involved in ginsenoside activity.[19,29] However, a recent study has shown that intrathecal ginsenosides are effective in treating postoperative pain simulated by paw incision in rats and that naloxone attenuates the antinociceptive effects of ginsenosides,[28] indicating that opioid receptors may play an important role in the mechanism of action of ginsenosides at the spinal level. Thus, the involvement of opioid receptors in the actions of ginsenosides remains to be determined.

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As is widely known, the antinociceptive effects induced by opiate drugs occur through their action at opioid receptors, located in the central nervous system, including the spinal cord.[6] Thus, spinal opioid receptor agonists are effective and are widely used in the management of various types of pain.[15,21,23,40]

The aims of the present study were to investigate the effect of ginsenosides in rat formalin-induced nociception in the spinal cord, and to determine the characteristics of the drug interaction between ginsenosides and morphine. We also examined the involvement of opioid receptor types on the effect of ginsenosides at the spinal level.

Methods

Animal Preparation

The experimental protocol was approved by The Institutional Animal Care and Use Committee, Medical Science of Chonnam National University.

Adult male Sprague-Dawley rats, weighing 250 to 300 g each, were used. The animals were housed 4 to a cage and kept in a vivarium, maintained at 22°C, with a 12/12-hour alternating light/dark cycle, and were given food and water ad libitum. All test drugs were administered intrathecally. Thus, rats were implanted with an intrathecal catheter under sevoflurane anesthesia, as described previously.[34] An 8.5-cm polyethylene-10 catheter was advanced caudally through an incision in the cisternal membrane to the thoracolumbar level of the spinal cord. The exterior portion of the catheter was secured at the skull by subcutaneous tunneling and closed with a 28-gauge wire. The skin was sutured with 3-0 silk. After catheter implantation, rats were housed individually. Only animals with no evidence of neurological deficit after catheter implantation were included in the study and they were housed individually. The behavioral study occurred 5 days after intrathecal catheterization.

Drugs

The following drugs were used: ginsenosides, morphine sulfate (Sigma, St Louis, MO), CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Yhr-NH_2; Tocris Cookson, Bristol, UK), naloxone [(5α)-4,5-Epoxy-3,14-dihydro-17-(2-prophenyl) morphinan-6-one hydrochloride, Tocris] naltrindole [17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3, 14-dihydroxy-6,7-2′,3′-indolomorphian hydrochloride; Tocris], and GNTI [5′-Guanidinyl-17-(cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy- 6,7-2′,3′-indolomorphian dihydrochloride; Tocris]. Ginsenosides were provided by the Korea Ginseng and Tobacco Research Institute (Daejon, Korea). The ginsenosides were dissolved in dimethylsulfoxide (DMSO), whereas all other drugs were dissolved in distilled water. Intrathecal administration of these agents was performed using a hand-driven, gear-operated syringe pump. All drugs were delivered in a volume of a 10-μL solution.

Nociceptive Test

Antinociception was assessed using the formalin test.[36] Briefly, 50 μL 5% formalin solution was injected subcutaneously into the plantar surface of a hind paw using a 30-gauge needle. The formalin injection produces specific pain behavior, which is readily discriminated and characterized by rapid and brief withdrawal or flexing of the injected paw. This behavior has been called a flinching response. Such pain behavior was thus quantified by periodically counting the number of flinches of the injected paw after injection. The number of flinches was counted for 1-minute periods at 1 and 5 minutes and in 5-minute intervals from 10 to 60 minutes. Formalin-induced flinching behavior is biphasic; the initial acute phase (0–9 minutes) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (10–60 minutes). At the end of the experiment, the rats were sacrificed by sevoflurane overdose.

Experimental Protocol

Five days after intrathecal cannulation, the rats were placed in a restraint cylinder for the experiment. After acclimatization for 15 to 20 minutes, the rats were allocated to receive one of the experimental drugs. The same volume of the vehicle (saline or DMSO) was used as the control. Each animal was tested only once. All experimental tests were made with the investigator blinded as to treatment condition of each animal.

Effects of Ginsenosides and Morphine

Rats received intrathecal saline and increasing doses of either ginsenosides (30, 100, 300 μg, n = 28) or morphine (1, 3, 10, 30 μg, n = 30) 10 min before formalin injection, and the effects of ginsenosides and morphine were examined. Each ED_{50} value (effective dose producing a 50% reduction in control formalin response) for the agents was calculated separately in 2 phases.

Drug Interaction

To define the properties of the pharmacological interaction between ginsenosides and morphine in the formalin test, isobolographic analysis was used (n = 48).[36] This method is based on comparing doses determined to be equally effective. Initially, each ED_{50} value was determined from the dose-response curves of the 2 agents alone. Then, a dose-response curve was obtained with concurrent delivery of the 2 drugs in a constant dose ratio, based on the ED_{50} values of the single agent. Thus, separate groups received: ginsenosides ED_{50} + morphine ED_{50}; (ginsenosides ED_{50} + morphine ED_{50})/2; (ginsenosides ED_{50} + morphine ED_{50})/4; and (ginsenosides ED_{50} + morphine ED_{50})/8. From the dose-response curves of the combined drugs, the ED_{50} values of the mixture were calculated, and the dose combinations were used to plot the isobologram. The isobologram was constructed by plotting the ED_{50} values of the single agents on the x- and y-axes, respectively. The theoretical additive dose combination was calculated. From the variance of the total dose, the individual variances for the combined agents were obtained. In combination, a total fraction value was calculated to describe the magnitude of the interaction.

Total fraction value = (ED_{50} of drug 1 combined with drug 2)/(ED_{50} for drug 1 given alone) + (ED_{50} of drug 2 combined with drug 1)/(ED_{50} for drug 2 given alone).