Anti-Stress Effect of Ginseng on the Inhibition of the Development of Morphine Tolerance in Stressed Mice

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ABSTRACT — We examined how the ginseng extract (GE) acts on the antinociceptive effect induced by footshock (FS)-, psychological (PSY)- and forced swimming (SW)-stress (stress-induced analgesia, SIA), and also on the suppression by FS- and PSY-stress of the development of tolerance to morphine in mice. Neither an acute treatment nor 5 daily pretreatments with GE at 100 mg/kg, p.o. affected each SIA. Pretreatment with GE at 100 mg/kg, p.o. for 5 days followed by the treatment in combination with the exposure to stresses for another 5 days caused no appreciable changes in the development of tolerance to FS- and SW-SIA, but suppressed the development of tolerance to PSY-SIA. When mice were pretreated with GE for 5 days and given GE daily prior to morphine at 10 mg/kg/day, with stress exposure for another 5 days, the inhibitory effect of FS-stress on the development of tolerance to morphine was completely eliminated. The present results suggest that GE, by improving the general metabolism in the body, directs toward normalization of the adaptability which is impaired by stress exposure, while not compromising morphine antinociceptive activity or the SIA, another adaptability produced in confrontation to abnormal environmental stimuli. In addition, the differences in the mechanism underlying the FS- and PSY-stress effect which we have previously demonstrated are also confirmed.

Keywords: Ginseng extract, Stress-induced analgesia (SIA), Anti-stress effect, Morphine tolerance, Tolerance to SIA

Much interest has been focused on the ginseng sapo-nins which have been used medically for thousands of years in Korea, China and Japan because they possess multiple pharmacological actions mediated by the central nervous system, such as suppression of spontaneous movements (1), facilitation of recovery from exhaustion caused by forced running and by swimming (2, 3), psychotropic effects (4, 5) and blockade of tolerance to and dependence on morphine (6, 7). It is also reported that Panax ginseng normalizes physiological disfunction caused by stress exposure, suggesting that ginseng sapo-nin may have a non-specific anti-stress effect (8–10). Meanwhile, we have reported (11–13) that exposure to footshock (FS)-, forced swimming (SW)- and psychological (PSY)-stress produces antinociceptive effect (stress-induced analgesia, SIA) and also that concurrent exposure to FS- and PSY-stress but not SW-stress suppresses the development of morphine tolerance, which is generally considered to be an adaptive response.

In this context, it is of interest to examine how the ginseng extract acts on various SIAs and the suppressive effect of FS- and PSY-stress on the development of morphine tolerance.

MATERIALS AND METHODS

Animals

Male mice of the ddY strain weighing 18–20 g (Otsubo Exp. Animals, Nagasaki) were purchased and housed in groups of 20 animals in plastic cages with free access to food and water. They were kept in a room maintained at an ambient temperature of 22 ± 1°C under a natural day/night regime. After reaching 22–25 g, they were used for the experiments.

Drugs and route of administration

Standardized Panax ginseng extract powder G115 (GE, gift from Dr. H.-S. Kim, Chungbuk National University, Korea) was dissolved in distilled water; and morphine-HCl (Takeda Pharm. Co., Osaka) was dis-
solved in saline, in a volume of 0.1 ml/10 g body weight. GE was given p.o. at a dose of 100 mg/kg, and morphine was injected s.c. at the dose of 10 mg/kg.

Exposure to stresses
1) FS-stress: Animals were exposed to an inescapable and unsignalled FS (2 mA, 0.2 Hz, 1 sec duration) for 15 min. 2) SW-stress: Mice were forced to swim by being put in a water bath (40L X 35W X 20H) with 15 cm deep water at 20 ± 1°C for 5 min. 3) PSY-stress: With a modification of the method of Ogawa and Kuwahara (15), the communication box which consists of 9 compartments was used for PSY-stress. The mice were placed individually into the compartments, and electric shock was delivered through the floor for 5 min. Animals placed in a compartment in which the floor was covered with a plastic plate are prevented from receiving the shock, but they were exposed to PSY-stress by watching and hearing the struggle, jumping and vocalization of the shocked animals. Details of the exposure to each stress have been described elsewhere (12, 14).

Measurement of antinociception
The antinociceptive effect was measured by the tail pinch method (16), using a 6 sec cutoff time to avoid tissue damage. Measurements were made every 5 min from immediately after the termination of stress exposure for 15 min, or every 15 min after the administration of morphine for a period of 90 min. The 1st measurement was performed at 20 min, instead of at 15 min, after the morphine injection in the case of concurrent exposure to stresses. For evaluating tolerance, the antinociceptive effect was expressed as the area under the curve (AUC), which was obtained by plotting the increase in response time (sec) on the ordinate and the time interval (min) on the abscissa. A significant decrease of AUC, compared to that on the 1st day, was considered to indicate the development of tolerance.

Statistical analysis
The results were expressed as the means ± S.E. Following a two-way analysis of variance for repeated measurements with the overall data to assess statistical significance, differences between the individual mean values in various groups were analyzed with Dunnett’s test. A difference was considered significant at P < 0.05.

RESULTS
Effect of GE on FS-, PSY- and SW-SIA
Exposure to FS-, PSY- and SW-stress produced short-lasting antinociception. A single dose of GE, 1 hr before stress exposure, did not affect the antinociceptive effect. Even after 5 daily pretreatments with GE, the effect of GE on each SIA was not altered (Fig. 1).

Effect of GE on the development of tolerance to each SIA
Daily exposure to FS-, PSY- and SW-stress resulted in the gradual loss of the antinociceptive effect, indicat-
ing the development of tolerance to the effect (Fig. 2). The GE-treated group, in which mice were daily pre-treated with GE at 100 mg/kg, p.o., for 5 days and given GE at the same dose 1 hr prior to stress exposure during the test days, did not show any appreciable changes in the development of tolerance to FS- and SW-SIA. On the contrary, the same regimen of GE suppressed the development of tolerance to PSY-SIA.

Effect of GE on the antinociceptive effect of morphine and the development of tolerance to the effect

Daily treatment with morphine at a dose of 10 mg/kg, s.c. resulted in the development of tolerance. Co-administration of GE at 100 mg/kg, following pre-treatment with GE for 5 days, did not influence the antinociceptive effect of morphine or the development of tolerance to morphine (Fig. 3).

Effect of GE on the blockade by FS-stress and PSY-stress of the development of morphine tolerance

Combination of morphine administration with exposure to FS-stress or PSY-stress suppressed the development of antinociceptive tolerance to morphine. The inhibitory effect of FS-stress on the development of tolerance to morphine was completely eliminated by the treatment with 100 mg/kg of GE; however, the inhibitory effect of PSY-stress was not affected by the treatment (Fig. 4).

![Fig. 2](image-url) Effect of GE on the development of antinociceptive tolerance to FS-, SW- and PSY-stress. Daily changes of the analgesic effect induced by FS-, SW- and PSY-stress was expressed as the area under the curve (AUC), by plotting the increase in response time (sec) on the ordinate and the intervals (min) on the abscissa. Mice were pretreated daily with GE at 100 mg/kg, p.o. for 5 days, and from the 6th day, GE was daily given 1 hr before exposure to FS-stress (C), SW-stress (□) and PSY-stress (◆) for another 5 days. Control mice (open symbols) received saline instead of GE (closed symbols). Each point is the mean ± S.E.M. of 8–12 animals. *P < 0.05, **P < 0.01, compared with the corresponding value on the 1st day. ***P < 0.01, compared with the group pretreated with saline (Dunnett's test).

![Fig. 3](image-url) Effect of GE on the development of antinociceptive tolerance to morphine. Mice were pretreated daily with GE at 100 mg/kg, p.o. for 5 days, and from the 6th day, GE was daily given 1 hr before injection of morphine at the dose of 10 mg/kg, s.c., for another 5 days. The antinociceptive effect of morphine on the 6th day (left panel). Daily changes in the antinociceptive effect (AUC) of morphine during 5 days (right panel). The control group was treated with saline (C) instead of GE (○). **P < 0.01, compared with the corresponding value on the 1st day (Dunnett's test). For other details, refer to the legend of Fig. 2.
DISCUSSION

Considerable research evidence has shown that tolerance to a drug, as well as an adaptation to environmental stimuli, is an expression of the adaptability that living organisms possess. Consistent with our earlier findings (13), we confirmed that concurrent exposure to FS- and PSY-stress suppressed the development of antinociceptive tolerance to morphine. In this experiment, we found that daily pretreatment with GE completely abolished the inhibitory effect of FS-stress. This GE antagonism against the effect of FS-stress to inhibit the development of tolerance to morphine may be attributable to the anti-stress properties of GE (8-10). It is reported that GE acts on the hypothalamus, and consequently facilitates the release of ACTH, indicating the activation of the pituitary-adrenal axis by GE (17). We have previously reported (18) that the inhibitory effect of concurrent exposure to FS-stress on the development of morphine tolerance was eliminated by adrenalectomy and restored by supplement of prednisolone, suggesting the participation of adrenal glucocorticoids in the suppressive effect of FS-stress. Our results lend the support to the possibility that GE produces an anti-stress effect through the mediation of such endocrine systems, i.e., peripheral mechanisms. Kim et al. (9) have reported that Panax ginseng accelerates the recovery of the adrenal ascorbic acid content reduced by stress, and such an anti-stress effect of Panax ginseng is due to its peripheral action rather than a central one. On the other hand, it could not be negligible that central adrenergic systems play an important role in the action of GE as Kim et al. have reported (19) and also it has been found that stress exposure activates catecholamine turnover in the brain (20, 21). In addition, Kaneto and Inoue (22) have reported that the development of tolerance to morphine is prevented by an α-blocker, phentolamine, and a β-blocker, propranolol, suggesting an imbalance of central adrenergic systems in the underlying mechanism. Thus, it is suggested that the GE, by improving the general metabolism such as the acceleration of protein, nucleic acid and lipid syntheses (23, 24) and/or by recovering the imbalance of adrenergic systems in the CNS, normalizes the adaptability (8) which is impaired by stress exposure.

Meanwhile, GE did not affect the antinociceptive effect induced by various stresses despite its anti-stress effect. This provides evidence that the mechanisms that underly both the antinociception and the blockade of tolerance development by these stresses are mutually different, and hence the blocking effect of stress on the development of tolerance is not due to the antinociceptive effect of stress. Furthermore, since SIA is one of the essential adaptive mechanisms of animals and humans for relieving pain, based on the activation of the intrinsic pain-inhibitory system in response to noxious or threatening stimuli, the lack of inhibitory effect of GE on these SIAs may, therefore, support the beneficial effect of Panax ginseng.

Kim et al. have reported that the ginseng saponin...
from *Panax ginseng* suppresses the analgesic effect of morphine and the development of tolerance to the effect (6). The discrepancy between their results and those of our present study may be due to the administration regimen as reported by Bhargava and Ramarao who found that in appropriate doses, GE has an inhibitory effect on the development of tolerance to morphine action (7). In the preliminary experiment, we observed behavior such as piloerection and weight loss in some mice given GE at a dose of 200 mg/kg for 5 days, so this dose appears to be somewhat toxic. Thus, we tested one dose of GE, 100 mg/kg, that seems to be one of the adequate doses (7) and also is the maximal dose for producing various pharmacological effects without toxicity by repeated administrations. GE abolished the suppressive effect of FS-stress on the development of morphine tolerance; however, such an anti-stress effect of GE was not observed in the suppression by PSY-stress. The discrepancy may be attributable to differences in the mechanism for suppressing tolerance development since FS-stress is mediated through μ-opioid receptors while PSY-stress involves κ-opioid receptors (13), or a convincing explanation is that GE exerts its anti-stress effect against physical stress and is less effective on the emotional factors that are involved in PSY-stress, since *Panax ginseng* has anti-fatigue effects as shown in various physical exercises (2).

Thus, we conclude that as shown in GE’s ability to abolish stress-induced suppression of morphine tolerance development, it directs toward normalization of the adaptability which is impaired by stress exposure, while not compromising morphine antinociceptive activity or the SIA, another adaptability produced in confrontation to abnormal environmental stimuli.

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