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# Antagonism of Morphine Analgesia by the Pretreatment Sites with Ginseng Total Saponin

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**Abstract**□The analgesic effect of morphine was antagonized in mice pretreated with ginseng total saponin intraperitoneally, intracerebrally and intrathecally. The antagonized effects of morphine analgesia were reversed predominantly by treatment with L-3,4-dihydroxyphenylalanine in the tail pinch test and 5-hydroxytryptophan in the tail flick test respectively. These indicate that the antagonistic action of ginseng total saponin might be due to their inhibitions of the activation of descending ihibitory systems at the cerebral site as well as spinal. In addition, any appreciable changes of brain biogenic monoamine levels were not observed in mice pretreated with ginseng total saponin at various time intervals. These results obtained suggest that a newly equilibrated state of neurologic function could be found in mice pretreated with ginseng total saponin, and modification of neurologic function in the mechanism for the antagonism of morphine analgesia by ginseng total saponin was more important than the changes of brain biogenic monoamine levels.

**Keywords**□antagonism, morphine, intracerebral, intrathecal, intraperitoneal administration, ginseng total saponin.

## Introduction

It is well known that reserpine and tetrabenazine antagonize the analgesic effect of morphine in mice and these effects are attributed to the reductions of brain biogenic monoamines by them.[1-4]

There have been reports that a ginseng extract in a dose of 50 mg/kg of body weight for 5 consecutive days increases the noradrenaline and dopamine levels, and decreases the serotonin level in the brain stem, and at the same time the serotonin level is decreased in the brain cortex in rat,[5] and ginseng total saponin increases the noradrenaline content in the mouse brain.[6]

Kim *et al.* reported that ginseng total saponin antagonized morphine analgesia and presumed that the mechanism of the antagonistic effect of morphine analgesia might be associated with the reductions of brain biogenic monoamines.[7,8]

On the other hand, the analgesic action of systemic morphine in analgesic dose is primarily mediated by the activation of descending inhibitory systems. Furthermore, recent studies have shown that the descending inhibitoty systems are in part noradrenergic and serotonergic.[9-11] It was reproted that the extents involved in the spinal noradrenergic and serotonergic systems on morphine analgesia differed according to the types of noxious stimuli used, and the noradrenergic system in the tail pinch test and the serotonergic system in the tail flick played respectively more important roles in the production of morphine antinociception.[12,13]

For these reasons, the present studies were undertaken to investigate the possibility that the anta-

gonisms of morphine analgesia by pretreatment with ginseng total saponin were responsible for the changes of brain biogenic monoamine levels, to determine the acting sites and the extents of the antagonisms of morphine analgesia by systemic, intracerebral and intrathecal ginseng total saponin, and to observe the suppressive extents of ginseng total saponin antagonisms by L-3,4-dihydroxyphenylalanine or 5-hydroxytryptophan in the tail pinch and tail flick tests.

## Materials and Methods

Male mice of the ICR strain weighing 12-15g were purchased and housed as a group of 10 animals in plastic cages. They were kept in a room maintained at an ambient temperature of 22± 2℃ and given a normal laboratory diet and tap water *ad libitum*. After their weights increased to 18-22g, they were employed for the experiments. The following compounds were used: morphine-HCl (Dae-Won Pharm. Co.), naloxone-HCl (Sigma, U.S.A.), reserpine (A-Ju Pharm. Co.), L-3,4-dihydroxyphenylalanine (L-DOPA, Sigma) and 5-hydroxytryptophan (5-HTP, Sigma). Ginseng total saponin was supplied from Korea Ginseng & Tobacco Research Institute.

**1. Determinations of brain biogenic monoamines in mice pretreated with ginseng total saponin**

Animals were sacrificed by decapitation and the brain monoamine contents were determined using HPLC with an electrochemical detector (Schmadzu, L-ECD-6A). The column of shimpack CLC-ODS was used. The detector potentials were set at 800 mV *versus* the Ag/AgCl reference electrode. Extractions of catecholamines and serotonin were done according to the methods of Maruyama *et al.*[14] and Spark [15] respectively. Mobile phases consisting of 0.05 M sodium acetate/citric acid buffer (pH 3.9) containing 0.1 mM EDTA, 330 mM heptane sulfonic acid and 5% $CH_3CN$, and 0.1 M sodium acetate/citric acid buffer (pH 4.5) containing 0.1 mM EDTA and 10% methanol were used for analysis of catecholamines and serotonin respectively. A column was operated at a flow rate of 1.0 ml/min.

**2. Intraperitoneal (i.p.) injection**

Ginseng total saponin and morphine were dissolved in saline and injected into mice at a volume of 0.1 ml/10g of body weight. Ginseng total saponin 100 mg/kg was administered i.p.

**3. Intracerebral (i.c.) injection**

Ginseng total saponin was injected with 40 μg/body i.c. as described by Haley and McCormick.[16] The mice were grasped firmly by the loose skin behind the head. The skin was pulled taut. Injection site is 2-3 mm from either side of the midline on a line drawn through the anterior base of ears. The depth of injection is 3 mm. A 3/8 in., 27 gauge hypodermic needle attached to a 100 μl syringe was injected perpendicularly through the skull into the brain. The solution was injected with a volume of 10 μl/body in mice.

**4. Intrathecal (i.t.) injection**

Giseng total saponin was injected i.t. with 40 μg/body as described by Janice and Wilcox.[17] The mice were firmly grasped by the pelvic girdle with one hand while the syringe was held in the other hand. The needle was inserted carefully forward to the intravertebral space. The injection site was chosen to be between L5 and L6 near to where the spinal core ends and the cauda equina begins.[18] The angle of the syringe is decreased to about 10°. The tip of the needle is inserted so that approx. 0.5 cm is within the vertebral column. This site represented a compromise to minimize the spinal damage. The solution was injected in a volume of 10 μl/body in mice.

**5. Measurement of analgesic effect**

The analgesic effects were measured by a modified Haffner's method (mechanical analgesic test),[19] a tail pinch (T.P.) test using a 1 sec pre-drug time and a 6 sec cut-off time, and a modified D'Amour and Smith's method (thermal analgesic test),[20] a tail flick (T.F.) test, using a 2 sec pre-drug time and a 10 sec cut-off time, every 30 min for 120 min, after morphine 5 mg/kg or 10 mg/kg was administered subcutaneously (s.c.).

In preliminary experiments, the analgesic effects of morphine alone, and combined effects of morphine and ginseng total saponin were investigated at various time intervals. When i.p. ginseng total saponin in a dose of 100 mg/kg antagonized the analgesia of morphine, their maximal effects were

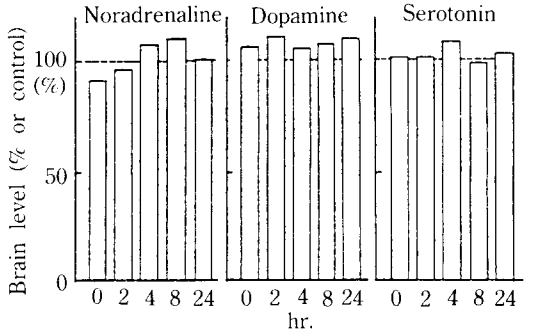


**Fig. 1.** Effects of ginseng total saponin on the brain levels of noradrenaline, dopamine and serotonin in mice. The brain levels of noradrenaline, dopamine and serotonin were estimated at various time intervals, after a single i.p. administration of ginseng total saponin 100 mg/kg. Data were shown as a percent of the content in naive mice (noradrenaline, 0.34± 0.02; dopamine, 1.04± 0.13; serotonin, 0.52± 0.08 μg/g wet tissue). Five mice were used at least.

observed 3 hours in the T.P. test and 4 hours in the T.F. test prior to the administration of morphine, and when ginseng total saponin in a dose of 40 μg/body i.c. and i.t. antagonized them, their maximal antagonistic effects were observed 2 hours in the T.P. test or 3 hours in the T.F. test prior to the administration of morphine. Therefore, the above time intervals of pretreatment were used to determine the antagonistic effects of morphine by ginseng total saponin. Reserpine was administered i.p., 24 hours prior to the administration of morphine. L-DOPA or 5-HTP was given i.p. 30 min prior to the administration of morphine.

The analgesic effect induced by morphine was measured by both tests, calculated as area under the curve (AUC) by plotting the changes in response time (sec) on the ordinate and the intervals (min) on the abscissa and expressed as percent of the effects obtained in control animals.[21]

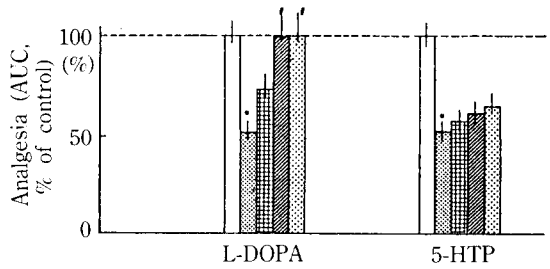


**Fig. 2.** Antagonism of morphine analgesia in the T.P. test by GTS (i.p.) and its reversal by L-DOPA or 5-HTP. GTS 100 mg/kg (i.p.) was injected 3 hours prior to the administration of morphine 5 mg/kg (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min prior to the adimistration of morphine. The analgesia was measured by tail pinch test.
*: $p<0.05$, **: $p<0.01$, compared with that of saline + morphine (mor, #:$p<0.05$, # #:$p<0.01$, compared with that of GTS + Mor., □: Mor. control., ▨: GTS + Mor., ▦: GTS + 10 mg/kg of L-DOPA or 5-HTP + Mor., ▨: GTS + 30 mg/kg of L-DOPA or 5-HTP + Mor., ▨: GTS + 50 mg/kg of L-DOPA or 5-HTP + Mor..

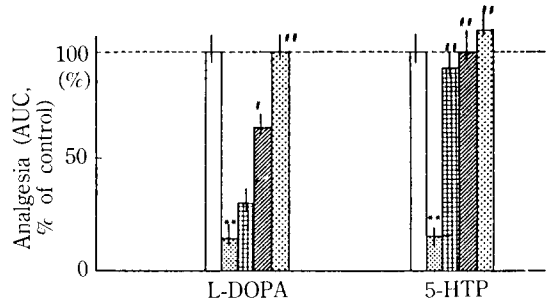


**Fig. 3.** Antagonism of morphine analgesia in the T.F. test by GTS (i.p.) and its reversal by L-DOPA or 5-HTP. GTS 100 mg/kg (i.p.) was injected 4 hours prior to the administration of morphine 5 mg/kg (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min prior to the administration of morphine. The analgesia was measured by tail flick test. For other details, see Fig. 2.

## Results

### 1. Effects of ginseng total saponins on brain biogenic monoamine levels in mice

The brain levels of noradrenaline, dopamine and serotonin were not modified in mice pretreated with a single dose of ginseng total saponin 100 mg/kg when estimated at various time intervals (Fig. 1).

### 2. Antagonism of morphine analgesia by i.p. ginseng total saponin and their reversals by L-DOPA or 5-HTP

Antagonism of morphine analgesia showed 50% in the ginseng total saponin-pretreated group in the

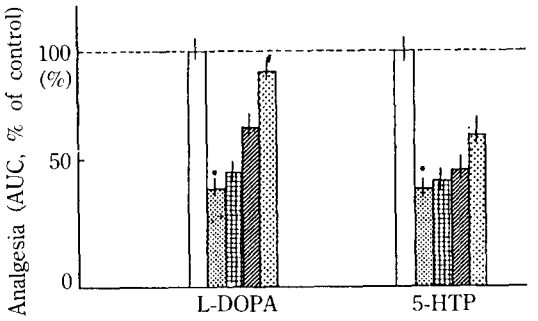


**Fig. 4.** Antagonism of morphine analgesia in the T.P. test by GTS (i.c.) and its reversal by L-DOPA or 5-HTP. GTS 40 μg/body (i.c.) was injected 2 hours prior to the administration of morphine 5 mg/kg (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min prior to the administration of morphine. The analgesia was measured by tail pinch test. For other details, see Fig. 2.

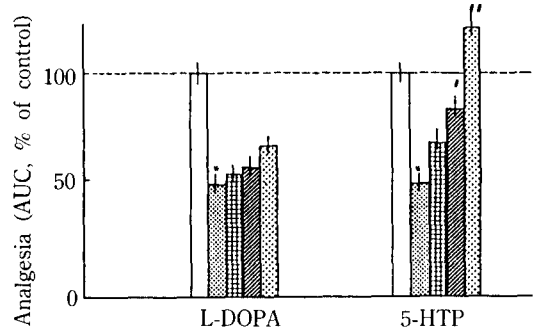


**Fig. 5.** Antagonism of morphine analgesia in the T.F. test by GTS (i.c.) and its reversal by L-DOPA or 5-HTP. GTS 40 μg/body (i.c.) was injected 3 hours prior to the administration of morphine 5 mg/kg (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min prior to the administration of morphine. The analgesia was measured by tail flick test. For other details, see Fig. 2.

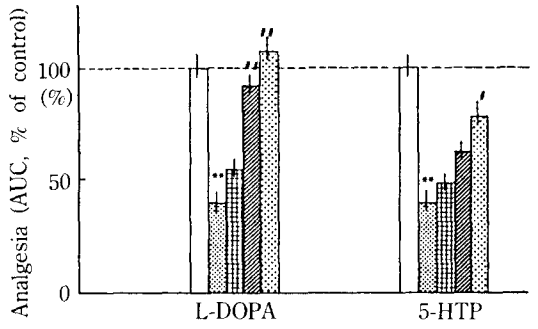


**Fig. 6.** Antagonism of morphine analgesia in the T.P. test by GTS (i.t.) and its reversal by L-DOPA or 5-HTP. GTS 40 μg/body (i.t.) was injected 2 hours prior to the administration of morphine 5 mg/kg (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min prior to the administration of morphine. The analgesia was measured by tail pinch test. For other details, see Fig. 2.

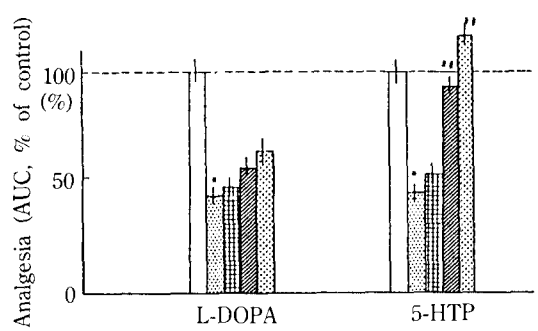


**Fig. 7.** Antagonism of morphine analgesia in the T.F. test by GTS (i.t.) and its reversal by L-DOPA or 5-HTP. GTS 40 μg/body (i.t.) was injected 3 hours prior to the administration of morphine 5 mg/kg (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min prior to the administration of morphine. The analgesia was measured by tail flick test. For other details, see Fig. 2.

T.P. test and 20% in the T.F. test (Fig. 2, 3), compared with morphine controls respectively. The morphine analgesia was more antagonized in the T.F. test than in the T.P. test by pretreatment with ginseng total saponin as compared with those of the control groups respectively. The reduced analgesia of morphine by pretreatment with 100 mg/kg of i.p. ginseng total saponin was restored in mice administered with 30 mg/kg and 50 mg/kg of L-DOPA in the T.P. test (Fig. 2), and was also restored in mice administered with 10 mg/kg, 30 mg/kg and 50 mg/kg of 5-HTP in the T.F. test, up to the normal analgesic effect of morphine control respectively (Fig. 3).

**3. Antagonism of morphine analgesia by i.c. ginseng total saponin and their reversals by L-DOPA or 5-HTP**

Morphine analgesia was antagonized about 40% in the group pretreated with i.c. ginseng total saponin in the T.P. test, and 50% in the T.F. test (Fig. 4, 5), compared with those of the morphine control

**Tabl 1.** Summary of antagonisms of morphine analgesia by the administration routes of GTS and their reversals by L-DOPA or 5-HTP

| Administration routes of GTS | Methods of analgesic tests | Analgesia* | Reversals** by L-DOPA | Reversals** by 5-HTP |
|---|---|---|---|---|
| I.P. | T.P. | ↓ | ↑ | − |
| | T.F. | ↓↓ | ↑ | ↑↑ |
| I.C. | T.P. | ↓↓ | ↑ | |
| | T.F. | ↓ | − | ↑↑ |
| I.T. | T.P. | ↓↓ | ↑↑ | ↑ |
| | T.F. | ↓↓ | − | ↑↑ |

*↓, ↓↓: Moderate or marked reduction, −: No significant change,
**↑, ↑↑: Moderate or marked reversal.

groups respectively. The reduced analgesia of morphine by pretreatment with 40 μg/body of i.c. ginseng total saponin was restored in mice administered with L-DOPA 50 mg/kg in the T.P. test (Fig. 4), and also restored in mice administered with 30 mg/kg and 50 mg/kg of 5-HTP in the T.F. test respectively (Fig. 5).

**4. Antagonism of morphine analgesia by i.t. ginseng total saponin and their reversals by L-DOPA or 5-HTP**

Morphine analgesia was antagonized about 40% in the T.P. test and 45% in the T.F. test in the group pretreated with i.c. ginseng total saponin, compared with those of the morphine control groups respectively (Fig. 6, 7). The reduced analgesia of morphine by pretreatment with 40 μg/body of i.t. ginseng total saponin was restored in mice administered with 30 mg/kg and 50 mg/kg of L-DOPA in the T.P. test (Fig. 6), and was also restored in mice administered with 30 mg/kg and 50 mg/kg of 5-HTP in the T.F. test respectively (Fig. 7). The reduced analgesia of morphine by pretreatment with 100 mg/kg of i.p., 40 μg/body of i.c. and i.t. ginseng total saponin were more predominantly restored in mice administered L-DOPA than 5-HTP in the T.P. test, and 5-HTP than L-DOPA in the T.F. test respectively (Table 1).

## Discussion

In preliminary experiments, it was observed that analgesic effect of morphine 5 mg/kg was antagonized in mice pretreated with 100 mg/kg of ginseng total saponin, however, morphine 10 mg/kg analgesia was not antagonized after repeated injection of ginseng total saponin 100 mg/kg for 5 days.

Ginseng total saponin did not reduce appreciably the brain biogenic monoamines at various time intervals in this experiment. But ginseng total saponin antagonized the morphine analgesia 5 mg/kg. Meanwhile, it was reported that the antagonistic effect of morphine 10 mg/kg was observed in mice pretreated with reserpine 2.5 mg/kg, being accompanied with reductions of brain biogenic monoamines.[1] Kaneto *et al.* reported that the pretreatment with a small dose of reserpine 0.1 mg/kg did not reduced catecholamine and serotonin levels in the whole brain of mice, and the antagonistic effect of morphine 10 mg/kg was not observed in mice pretreated with this small dose of reserpine.[22]

However, it was observed in this experiment that ginseng total saponin antagonized the analgesic effect of low dose of morphine, and even a small dose of reserpine 0.1 mg/kg antagonized the analgesia of morphine when morphine 5 mg/kg was used as a test dose in the test of antagonism, and the analgesia of morphine 10 mg/kg was not antagonized in mice pretreated with a small dose of reserpine 0.1 mg/kg in the T.P. and T.F. tests (Fig. 8). In addition, phentolamine, one of alpha adrenergic blockers attenuated the analgesia induced by morphine 2.0 mg/kg but not by 7.5 mg/kg.[23]

It was found in this study that the dosage size of morphine as a test dose in the antagonism pla-

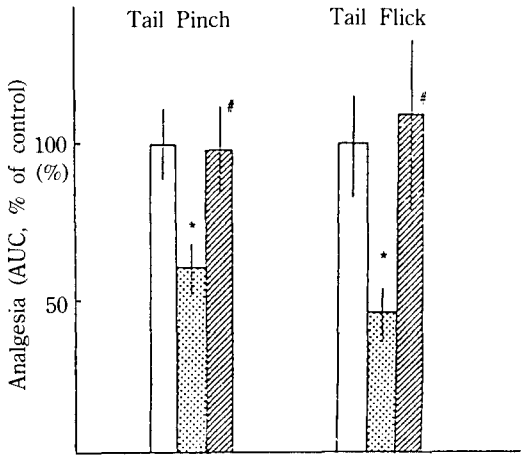


**Fig. 8.** Antagonism of morphine 5 mg/kg analgesia by reserpine and its reversal by L-DOPA or 5-HTP. Reserpine 0.1 mg/kg (i.p.) was given as pretreatment 24 hours in the T.P. and T.F. tests prior to the administration of morphine 5 mg/kg (s.c.). The reduced analgesia by reserpine was restored by 50 mg/kg of L-DOPA in the T.P. test and 50 mg/kg of 5-HTP in the T.F. tests, respectively.
□: Morphine control, ⊡: Reserpine+Mor.
▨: Reserpine+Mor+L-DOPA or 5-HTP.

yed an important role in the antagonism in the measurement of the antagonistic degree of morphine analgesia, Accordingly, the present studies clearly indicate that ginseng total saponin might have a small dose of reserpine or phentolamine like action on the antagonistic action of morphine analgesia.

On the other hand, it was supposed that the antagonism of morphine analgesia was related to the changes of brain biogenic monoamines in any part of the brain which was directly related to the production of morphine analgesia, and/or to the neurotransmitter turnover rates in any part of the brain instead of the changes of biogenic monoamines in the whole brain.[24,25] Satoh *et al.* reported that the action of a low dose of morphine 2-4 mg/kg differed from the action of a high dose of morphine on the production of analgesia.[26] Recently, the nucleus reticularis gigantocellularis including the nucleus reticularis paragigantocellularis of the medulla oblongata in the brain stem was found to be highly sensitive to morphine in the production of analgesia.[27-30] The analgesic action of systemic morphine in analgesic dose was primarily mediated by the activation of descending inhibitoty systems. Tagaki *et al.* reported that morphine analgesia exerted an indirect depressive effect on spinal neurons by the activation of descending inhibitory pathways origined from the brain stem.[31] Further, the fact that the inhibitory effects of morphine on mechanism and thermal nociception were mediated by different descending inhibitory systems was elucidated.[32-34]

It was found that not only the systemic but also i.c. and i.t. administrations of ginseng total saponin inhibited the antinociceptive actions of systemic morphine in a dose of 5 mg/kg in the mechanical and thermal analgesic tests. These results of the present experiments indicate that the antagonistic action of ginseng total saponin might be due to their inhibitions of the activiation of descending inhibitory noradrenergic and serotonergic systems not only in the brain stem but also in the spinal cord.

The antagonism of the analgesic effect of morphine 5 mg/kg and its reversal by L-DOPA or 5-HTP, and no any appreciable changes of brain biogenic monoamine levels were observed commonly in mice pretreated with a small dose of reserpine 0.1 mg/kg or ginseng total saponin 100 mg/kg. The treatment with L-DOPA or 5-HTP restored the reduced analgesic effect of morphine in mice preteated with ginseng total saponin 100 mg/kg or resepine 0.1 mg/kg which did not produce any appreciable reductions of brain biogenic monoamine levels.

These results showed that L-DOPA or 5-HTP could participate in the formation of a newly equilibrated state of neurologic function and this resulted in the reversals of antagonism as Kaneto *et al.*[22] demonstrated that the modification of neurologic function in the production of morphine analgesia was more important than brain biogenic monoamine levels.

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