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# Antagonism of Analgesic Effect of Morphine in Mice by Ginseng Saponins

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# 인삼 사포닌의 몰핀 길항작용

**김학성 · 오기완 · 오세관**
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Ginseng total saponins (GS), protopanaxadiol saponins (PD) and protopanaxatriol saponins (PT) antagonized the analgesia in mice induced by morphine. The administrations of 2, 4-dihydroxyphenylalanine, and 5-hydroxytryptophan reduced the GS, PD and PT antagonisms of morphine analgesia. Possible mechanisms involved in the antagonistic actions of GS, PD and PT on morphine analgesia were described.

It has been demonstrated that there were analgesic and hypothermic effects in ginseng extract and saponins[1,2], inhibition of development of morphine induced tolerance and dependence in ginseng total saponins, protopanaxadiol saponins and protopanaxatriol saponins fractions[3,4] and inhibition of the development of morphine induced dopamine receptor supersensitivity[5]. But ginseng saponins have not been reported to antagonize the analgesic effect of morphine.

The present experiments were performed to test whether ginseng saponins treated in acute and chronic antagonize the analgesic effect of morphine in mice and also whether ginseng saponins antagonism of morphine analgesia is related to the changes of catecholamine and/or serotonin levels in the central nervous system.

## EXPERIMENTAL

### Animals

White ICR mice weighing 18-22g in a group of 10-15 mice were used in all experiments.

### Testing Procedure

L-dihydroxyphenylalanine hydrochloride (L-DOPA, Sigma) and 5-hydroxytryptophan hydrochloride (5-HTP, Sigma) in 0.5% CMC suspension, ginseng total saponins (GS), protopanaxadiol saponins (PD) and protopanaxatriol saponins (PT) (Korea Ginseng & Tobacco Research Institute) dissolved in distilled water were administered intraperitoneally to mice except morphine hydrochloride (Dae-Won Pharm. Co.) which was given subcutaneously.

The analgesic action of morphine 5 mg/kg (s.c.) was tested by the tail flick method de-

scribed by the Damour and Smith[6]. The measurement was made every 30 min for 90 min by the tail flick method. The tail flick latencies to thermal stimulation were determined in seconds prior to and at 30, 60 and 90 min after the injection of morphine. A value of 10 sec was used as the cut-off point to avoid damage to the tail. The analgesic response for each mouse was calculated by the following formula:

$$\text{Percent analgesia} = \frac{T_t - T_o}{T_c - T_o} \times 100$$

Where $T_o$ is base line or pre-morphine tail flick reaction time; $T_t$ is the reaction time at t min after morphine injection, and $T_c$ is cut-off time. The base line of tail flick latencies in different groups were around $2 \pm 0.2$ seconds. The effect was calculated as area under the curve (AUC) that was obtained by plotting the analgesic percent on the ordinate and the time intervals (min) on the abscissa, and expressed as percent of the effect obtained in morphine alone treated control animals[7].

A single dose of ginseng saponins 100 mg/kg (i.p.) was administered at the time intervals of 0, 2, 4, 8, and 16 hours prior to morphine 5 mg/kg(s.c.) injection to test the antagonized and restored degrees of morphine analgesia in mice. Meanwhile, mice daily pretreated with ginseng saponins 100 mg/kg (i.p.) for a period of 6 days and after 24 hours were used to test the influences of L-DOPA or 5-HTP (50 mg/kg and 100 mg/kg, i.p.) on the antagonisms of morphine analgesia by ginseng saponins.

**Statistics**

The differences in the means for different responses in different groups were analyzed by student's test.

## RESULTS

In a preliminary experiment we studied the analgesic effect of each saponin 100 mg/kg in mice. GS, PD and PT in a dose of 100 mg/kg showed their own weak analgesic effects (Fig. 1). The effect of morphine alone and the

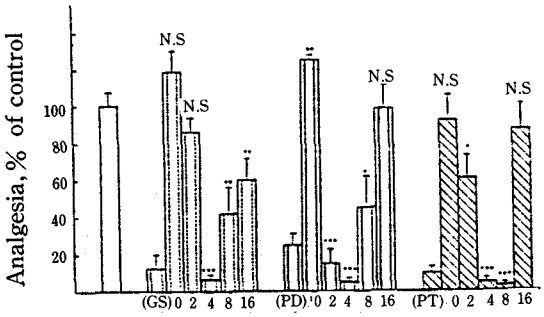


**Figure 1** —Antagonism of morphine at time intervals after the administrations of GS, PD and PT.

Key: □, MOR 5 mg/kg; ▨, GS+MOR; ▩, PD+MOR; ▧, PT+MOR

MOR, morphine hydrochloride 5 mg/kg(s.c.); GS, ginseng total saponins 100 mg/kg(i.p.); PD, protopanaxadiol saponins 100 mg/kg(i.p.); PT, protopanaxatriol saponins 100 mg/kg(i.p.)

0, 2, 4, 8 and 16 : time intervals of morphine injection after ginseng saponin injection. The analgesic measurement was described in the text. The values are the means±S.E. of 10 experiments.

$* p<0.05$, $** p<0.01$, $*** p<0.001$

combined effects of morphine and GS, or PD and PT were investigated at various time intervals. GS, PD and PT in a dose of 100 mg/kg antagonized morphine analgesia and their maximal effects were observed when injected 4 hours prior to the morphine administration. Fig.1 illustrates the results.

A single dose treatment of each saponin 100 mg/kg 4 hours prior to morphine administration and daily pretreatment of each saponin 100 mg/kg for 6 days showed no significant differences among their maximum antagonized effects of morphine analgesia (Fig.1, 2, 3, and 4). The effect of morphine in a dose of 5 mg/kg was reduced to about one tenth of its analgesic activity when pretreated with a single or repeated administrations of each saponin.

In our laboratory, a single injection of L-DOPA or 5-HTP up to a dose of 200 mg/kg had no significant effects on the analgesic effect of morphine as Takagi and his co-workers described[8]. When L-DOPA and 5-HTP were injected 30 minutes prior to the morphine administration to mice pretreated with each of

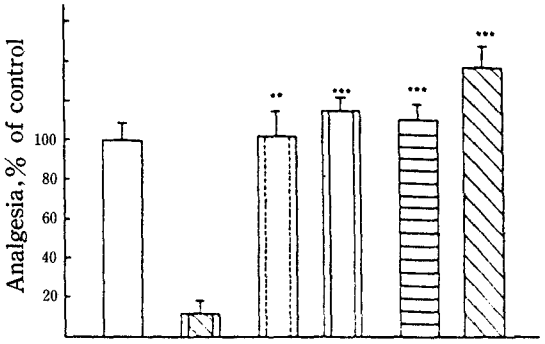


**Figure 2** —Analgesic activity of morphine alone and after pretreatment with GS, L-DOPA and 5-HTP.

Key: □, MOR 5 mg/kg; ▨, GS+MOR; ▦, GS+L-DOPA 50 mg/kg+MOR; ▩, GS+L-DOPA 100 mg/kg+MOR; ▤, GS+5-HTP 50 mg/kg+MOR; ▧, GS+5-HTP 100 mg/kg+MOR

GS, ginseng total saponins pretreated with a dose of 100 mg/kg(i.p.) once a day for 6 days and 24 hr after the test was measured.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

GS, PD and PT once a day for 6 days, L-DOPA and 5-HTP on both doses of 50 mg/kg and 100 mg/kg enhanced the reduced

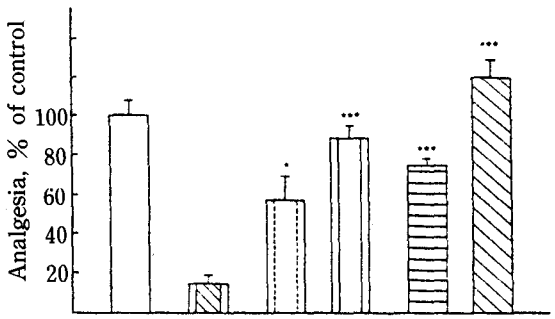


**Figure 3** —Analgesic activity of morphine alone and after pretreatment with PD, L-DOPA and 5-HTP.

Key: □, MOR 5 mg/kg; ▨, PD+MOR; ▦, PD+L-DOPA 50 mg/kg+MOR; ▩, PD+L-DOPA 100 mg/kg+MOR; ▤, PD+5-HTP 50 mg/kg+MOR; ▧, PD+5-HTP 100 mg/kg+MOR

PD, protopanaxadiol saponins pretreated with a dose of 100 mg/kg(i.p.) once a day for 6 days and 24 hr after the test was measured.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

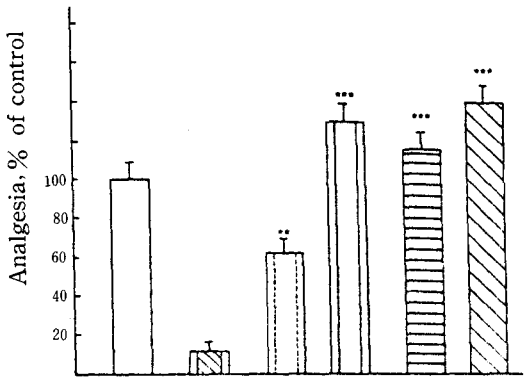


**Figure 4** —Analgesic activity of morphine alone and after pretreatment with PT, L-DOPA and 5-HTP.

Key: □, MOR 5 mg/kg; ▨, PT+MOR; ▦, PT+L-DOPA 50 mg/kg+MOR; ▤, PT+L-DOPA 100 mg/kg+MOR; PT+5-HTP 50 mg/kg+MOR; ▧, PT+5-HTP 100 mg/kg+MOR.

PT, protopanaxatriol saponins pretreated with a dose of 100 mg/kg(i.p.) once a day for 6 days and 24 hr after the test was measured.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

effects of morphine to over the degrees of 60-100% of the activity of morphine alone (Fig.2, 3 and 4).

L-DOPA and 5-HTP produced suppressive effects on GS, PD and PT antagonisms of morphine analgesia.

## DISCUSSION

The present results showed that GS, PD and PT antagonized the analgesic effect of morphine. These findings suggest that the antagonisms to morphine analgesia produced by ginseng saponins might be primarily due to their central action. The ginseng saponins antagonisms of morphine analgesia observed by tail flick method varied depending on the time intervals of morphine and ginseng saponins administrations in this experiment. In order to investigate the possibility that ginseng saponins induced decreases in brain levels of noradrenaline or serotonin are responsible for their antagonistic actions, the influence of administration of each amine precursor was deter-

mined. The marked suppressive effects of L-DOPA and 5-HTP were observed upon ginseng saponins antagonisms of morphine analgesia. In this connection, it is interesting to note that morphine has been shown to modify the content of brain noradrenaline, but not that of brain serotonin[8]. When both precursors, L-DOPA and 5-HTP in subeffective doses were injected to ginseng saponins treated mice, morphine showed nearly the same analgesic effect as or over that of morphine alone.

Considering these findings, ginseng saponins antagonisms of morphine analgesia may be attributed to several factors: a decrease of catecholamines and or serotonin content, a change in the concentration ratio of both amines in the brain and direct action of ginseng saponins and or their metabolites.

Tetrabenazine or reserpine has been known as the similiar antagonist of morphine analgesia[8] as shown in the present results of ginseng saponins.

## ACKNOWLEDGEMENTS

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## REFERENCES

1) H. Nabata, H. Saito and K. Takagi, Pharmacological studies of neutral saponins of panax ginseng root, *Japan. J. Pharmacol.*, **23**, 29 (1973)
2) T. Namba, M. Yoshizaki, T. Yomimori, K. Kobashi, K. Mitsui and J. Hase, Chemical and biochemical evaluations of ginseng and related crude drugs, *Yakugaku Zasshi*, **94**, 252 (1974)
3) H.S. Kim and S.K. Oh, Effect of panax ginseng on the development of morphine induced tolerance and dependence (I), *Yakhak Hoeji*, **29**(1), 27 (1985)
4) H.S. Kim, S.K. Oh, K.J. Choi and H.B. Lee, Effects of panax ginseng on the development of morphine induced tolerance and dependence (III), *Yakhak Hoeji*, **29**(4), 188 (1985)
5) H.S. Kim, S.K. Oh and G.C. Kim, Effects of panax ginseng on the development of morphine induced tolerance and dependence (II), *Kor. J. Pharmacogn.* **16**(1), 31 (1985)
6) F.E. Damour and D.L. Smith, A method for determining loss of pain sensation, *J. Pharmacol. Exp. Ther.*, **72**, 74 (1941)
7) H. Kaneto, N. Kosaka and N. Hirota, Timing of cycloheximide administration of the development of analgesic tolerance to morphine, *Life Sci.*, **31**, 2351 (1982)
8) H. Takagi, Takashima and K. Kimura, Antagonism of the analgesic effect of morphine in mice by tetrabenazine and reserpine, *Arch. Int. Pharmacodyn.*, **149**, 484 (1964)