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The Role of Ginseng Total Saponins in the Inhibition of the Development of Analgesic Tolerance to Morphine

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Abstract□The relationship between the brain monoamines and morphine tolerance was examined in ginseng total saponins treated mice. Ginseng total saponins (100 mg/kg, i.p.) did not antagonize morphine (10 mg/kg, s.c.) analgesia in mice. Daily treatment with ginseng total saponins (100 mg/kg) did not affect the brain levels of noradrenaline, dopamine and serotonin for 5 days but inhibited the development of morphine tolerance. This inhibition of the development of morphine tolerance was not attributed to the reductions of brain noradrenaline, dopamine and serotonin in mice treated with ginseng total saponins (100 mg/kg) daily. This result suggest that a newly equilibrated state of neurologic function may involve an underlying mechanism in mice treated with ginseng total saponins.

Keywords□ginseng total saponins, morphine, analgesia, tolerance, biogenic monoamines.

Introduction

It is well known that reserpine and tetrabenazine antagonize the analgesic effects of morphine and inhibit the development of morphine tolerance. These results are attributed to the reductions of brain biogenic monoamines by reserpine and tetrabenazine.[1,2] However, treatment with a small dose of reserpine (0.1 mg/kg) which does not reduce catecholamines and serotonin contents in the whole brain, does not antagonize morphine (10 mg/kg) analgesia, but inhibits the development of morphine tolerance without the reductions of catecholamines by this dose of reserpine for 10 days.[3]

Kim *et al.* reported that ginseng total saponins inhibited the development of morphine tolerance.[4]. The present experiments were performed to investigate the possible involvement of brain biogenic monoamines on the inhibition of the development of morphine tolerance by daily administration of ginseng total saponins.

Materials and Methods

Male mice of the ICR strain weighing 12-15 g 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± 1C and given a normal laboratory diet and tap water *ad libitum*. After their weights increased to 18-22 g, they were employed for the experiments. Morphine hydrochloride (Dae-Won Pharm. Co.) and ginseng total saponins (Korea Ginseng & Tobacco Research Institute) were dissolved in saline just before the use.

1. Measurement of analgesic effect

The analgesic effects were measured by a modified Haffner's method,[7] a tail pinch (T.P.) test using a 1.0± 0.1 sec pre-drug time and a 6 sec cut-off time, and a D'Amour and Smith method,[8] a tail flick (T.F.) test using a 2.0± 0.2 sec pre-drug time and a 10 sec cut-off time, every 30 min for 120 min, after morphine (10 mg/kg) was administrered subcutaneously (s.c.). In the prelimainary experiment, the inhibition of the development of morphine tolerance by ginseng total saponins was investigated at various pre-treatment time intervals. When ginseng total saponins was injected i.p. 3 hours in the T.P. test and 4 hours in the T.F. test prior to the injections of morphine, the maximal

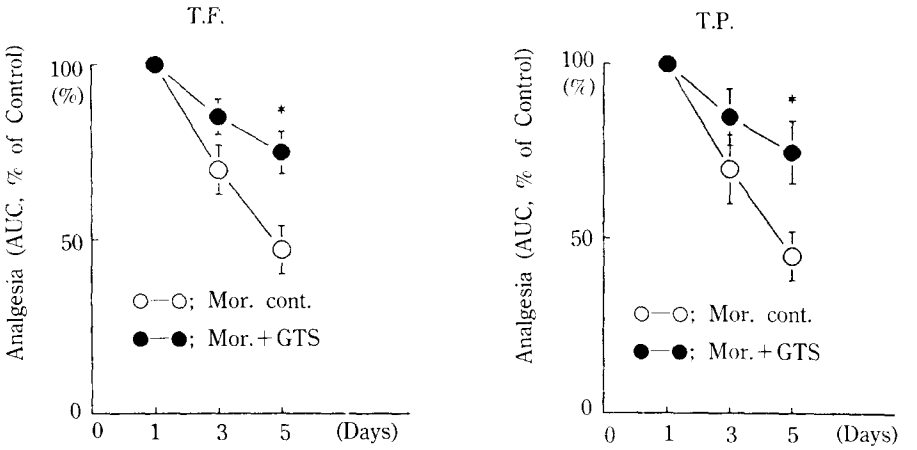

Fig. 1. Effect of ginseng total saponins on the development of morphine 10 mg/kg tolerance.
Mice were pretreated with ginseng total saponins 100 mg/kg i.p. 3 hr in the T.P. test (left) or 4 hr in the T.F. test (right) prior to the administration of morphine 10 mg/kg s.c. for 5 days. Control group received saline instead of ginseng total saponins. *$p<0.05$, compared with that of Sal.+Mor.

inbibitory effect of morphine tolerance developed was observed. So, in these experiments, ginseng total saponins (100 mg/kg) was administered intraperitoneally (i.p) 3 hours in the T.P. test and 4 hours in the T.F. test prior to administration of morphine. The analgesic effects measured by both tests were calculated as area under the curve (A.U.C.) by plotting the changes in response time (sec) on the ordinate and the intervals (min) on the abscissa.

2. Assessment of analgesic tolerance

The analgesic effect of morphine (10 mg/kg) was determined for 5 days every the other day and the degree of the development of tolerance to morphine was expressed as percent of the effect obtained in the control animals.

3. Determination of brain biogenic monoamines in mice pretreated with ginseng total saponins

To test the effects of ginseng total saponins on the contents of brain biogenic monoamines in mice, the brain monoamines contents were determined in mice treated with 100 mg/kg of ginseng total saponins daily on the 1st, 3rd and 5th days respectively, 24 hours after the final i.p. administration of ginseng total saponins (100 mg/kg) 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 method of Maruyama *et al.*[5] and Sperk[6] 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, 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. The brain levels of monoamines were expressed as percent of the control.

Results and Discussion

Daily injection of morphine (10 mg/kg) for 5 days, rapidly caused the development of tolerance. The development of tolerance to morphine (10 mg/kg) in mice pretreated with ginseng total saponins was inhibited in both T.P. and T.F. tests (Fig. 1). Daily levels of noradrenaline, dopamine and serotonin were not modified by ginseng total saponins (100 mg/kg) when estimated at various day intervals for 5 days (Fig. 2).

Accumulating evidence suggests the important roles of brain biogenic monoamines in morphine analgesia and the development of morphine tolerance to the effect. The antagonistic effect of reserpine on the morphine analgesia has been widely

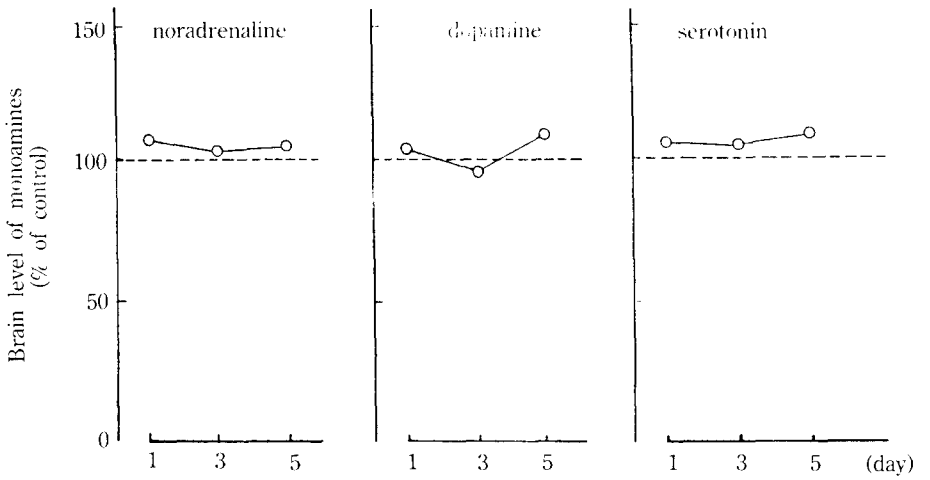

Fig. 2. Changes of brain levels of noradrenaline, dopamine and serotonin during daily administration of ginseng total saponins.
Ginseng total saponins 100 mg/kg was injected i.p. daily for 5 days. Brain biogenic monoamines were measured 24 hr after the final injection. Five mice were used at least.

accepted, and it is suggested that brain catecholamine, particular noradrenaline, play a role in the mechanism. Among the reports, Takagi *et al.* have described that tetrabenazine antagonized the analgesic effect of morphine in mice, and the effect of tetrabenazine may be attributed to the reduction of brain dopamine and noradrenaline. They have also reported that tetrabenazine given 2 hours before daily injection of morphine markedly suppressed the development of tolerance, and the suppressive effect was antagonized by repeated administration of DOPA.[9]

However, Kaneto *et al.* reported that daily treatment with small dose of reserpine (0.1 mg/kg) which did not affect any appreciable reductions in brain catecholamines contents could effectively block the development of morphine tolerance. They demonstrated that the inhibition of the development of morphine tolerance was not attributed to the reductions of brain noradrenaline and dopamine by reserpine.[10] Accordingly, they provides evidence that morphine analgesia and tolerance to the effect are separable from each other by underlying mechanism.

Similarly, a single or daily pretreatment with ginseng total saponins did not antagonize the analgesic effect of morphine (10 mg/kg) but inhibited the development of morphine tolerance without any appreciable reductions in monoamines levels of the whole brain in this experiment.

In addition, it was also reported that alpha- and beta- adrenergic blockers inhibited the development of morphine tolerance.[11-13] Kihara *et al.* supposed that the inhibition of the development of morphine tolerance by phentolamine and propranolol might be related to the equilibrated state of adrenergic function rather than to brain monoamines levels in the mechanism.[14] These results provided further evidence that reductions in brain biogenic monoamines contents were not essential for the development of morphine tolerance.

Brekhman and his coworkers reported that ginseng have the capacity for enabling living organism to adapt to various actions coming from and maintaining a constant equilibrium.[15] Therefore, these results indicate that a newly equilibrated state of neurologic function in mice can be an underlying common mechanism in the group pretreated with ginseng total saponins, and a small dose of reserpine or adrenergic blockers as Kaneto *et al.* reported.[2]

References

1. Friedler, G., Bhargava, H.N., Guock, R. and Way,

E.L.: *J. Pharm. Exp. Ther.* **183**, 49 (1972).
2. Nakamura, K., Kunzman, R., Maggio, A. and Conney, A.H.: *J. Pharm. Pharmacol.* **24**, 484 (1972).
3. Kaneto, H. and Kihara, T.: *Jap. J. Pharmacol.* **42**, 169 (1986).
4. Kim, H.S., Oh, K.W., Park, W.K., Shigeru, Y. and Satoshi, T.: *Kor. J. Ginseng Sci.* **11**(2), 182 (1987).
5. Maruyama, Y., Oshima, T. and Nakajima, E.: Life Sci. **26**, 1115 (1980).
6. Sperk, G.: *J. Neurochem.* **38**, 840 (1982).
7. Takagi, H., Inukai, T. and Nakama, M.: *Jap. J. Pharmacol.* **16**, 287 (1966).
8. D'Amour, F.E. and Smith, D.L.: *J. Pharmacol. Exp. Ther.* **72**, 74 (1941).
9. Takagi, H., Takashima, T. and Kimura, K.: *Pharmacodyn. Ther.* **149**, 484 (1964).
10. Kaneto, H., Hirota, N. and Yamazaki, A.: *Life Sci.* **33**, 353 (1983).
11. Bhargava, H.N., Chan, S.L. and Way, E.L.: *Proc. West. Pharmacol. Soc.* **15**, 4 (1972).
12. Heller, B., Saavedra, J.M. and Fischer, E.: *Experientia.* **24**, 804 (1968).
13. Vedernikov, Y.P. and Afrikanov, I.I.: *J. Pharm. Pharmacol.* **21**, 845 (1969).
14. Kihara, T. and Kaneto, H.: *Jap. J. Pharmacol.* **42**, 419 (1986).
15. Brekhman, I.I.: *Annual Review of Pharmacology* **6**, 419 (1969).