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Effects of Ginsenosides on the Development of Morphine-induced Tolerance and Physical Dependence in Mice

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Abstract—The effects of ginsenosides on the development of morphine induced tolerance and physical dependence were investigated. Rb_1, Rb_2, Rg_1 and Re inhibited significantly the development of morphine induced tolerance but Rb_1 and Rg_1 showed significant inhibitory effect on the naloxone induced withdrawal jumping response. Ginsenosides inhibited the body weight decrease in physically dependent mice during multiple injection of morphine.

Keywords—Ginsenosides Rb_1, Rb_2, Rg_1 and Re • morphine • tolerance • dependence • naloxone • withdrawal

The analgesic action of morphine is very remarkable. But the repetitive treatment of morphine produces physical dependence, characterized by withdrawal symptoms and a tolerance. The continuing search for morphine type compounds has failed to produce an analog that exhibits most of the useful properties of morphine, but which is devoid of addiction liability. Similarlly, long action and orally effective narcotic antagonists with minimum secondary effects are being sought to treat narcotic addicts.

A folk medicine prescribed seven herbal drugs including *Panax ginseng* has been used as antidote in the treatment of morphine tolerant-dependent patients. Its effective component is keratin of Manis squama but the researches didn't discuss any effects of panax ginseng on morphine tolerant-dependent patients[1].

Researches have reported the analgesic and hypothermic effects in ginseng extract and saponins(GS)[2,3], and the development of analgesic and hypothermic tolerance[4], and the inhibition of the development of morphine induced tolerance and dependence in ginseng butanol fraction[5], protopanaxadiol fraction(PD) and protopanaxatriol fraction(PT)[6], and the inhibition of the development of morphine induced dopamine receptor supersensitivity[7].

The present study was undertaken to determine the inhibitory effects of ginsenosides(Rb_1, Rb_2, Rg_1 and Re) on the development of morphine induced tolerance and physical dependence in mice for the development of a narcotic antidote.

Materials and Methods

White ICR mice weighing 18~22 g in a group of 10~15 mice were used in all experiments. Rb_1, Rb_2 and Rg_1(Korea Ginseng and Tobacco Research Institute) were dissolved in distilled water and Re(Korea Ginseng and Tobacco Re-

search Institute) was suspended in 0.5% CMC solution. The drugs were administered to mice intraperitoneally(i.p.) once a day 1 hr prior to the last administration of morphine.

To induce morphine tolerance and dependence, morphine hydrochloride (Dae-won Pharm. Co.) 40 mg/kg was administered subcutaneously (s.c.) to mice every 8 hrs for a period of 6 days by Way and his coworker's method[8].

Measurement of analgesic tolerance

The inhibition degree of morphine tolerance development by the administration of each saponin was evidenced by the increase in analgesic response to morphine hydrochloride(10 mg/kg, s.c.) as an analgesic percent(at 30, 60 and 90 min.) estimated by the tail flick method[9] 8 hrs after the final injection of morphine and calculated as an area under the curve(A.U.C.) by Kaneto and his coworker's method[10].

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;

$$\text{Percent analgesia} = \frac{T_t - T_0}{T_c - T_0} \times 100$$

Where T_0 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±0.2 sec. The effect was calculated as an A.U.C. that was obtained by plotting the analgesic percent on the ordinate and the time intervals(min) on the abscissa, and expressed as a percent of the effect obtained in control animals treated morphine alone.

Measurement of naloxone induced jumping response

The inhibition of naloxone induced withdrawal jumping in morphine alone treated mice and in morphine ginsenosides coadministered mice was estimated by the decreased number of the naloxone(4 mg/kg, i.p.) induced withdrawal jumping mice for 30 min 8 hrs after the final injection of morphine on the 6th day. The abstinence syndrom was quantified by placing the animal in a circular plateform 35 cm in diameter and 70 cm in height and counting the number of jumping animal for 30 min[8].

Measurement of body weight change

The effects of ginsenosides on the body weight of mice with multiple injection of morphine were estimated by comparing the body weight between non-drug treated mice on the 1st day and morphine induced tolerant-dependent mice on the 6th day.

Statistics

The differences in the means for different responses in different treatment groups were analyzed by Student's t-test except Fisher's probability test in naloxone induced jumping response.

Results

The base line of each group in analgesia changes was determined to check the residual effects of ginsenosides and morphine 30 min prior to the tolerance test.

Pre-morphine treatment base line of tail flick latencies in different groups were as follows; saline(1.96±0.04 sec), Rb_1(1.94±0.06 sec), Rb_2(1.96±0.06 sec), Rg_1(2.10±0.06 sec) and Re(1.87±0.05 sec) (Table I). There was no difference in base line of tail flick latencies in different groups.

Inibition of analgesic tolerance development

The analgesia of each group calculated as the A.U.C. to morphine 10 mg/kg was observed

Table I—The base line of analgesia 8 hrs after the final injection of morphine in mice

Treatment	Analgesia(sec)
Morphine+saline	1.96±0.04
Morphine+Rb_1 100 mg/kg	1.94±0.06
Morphine+Rb_2 100 mg/kg	1.96±0.06
Morphine+Rg_1 100 mg/kg	2.10±0.06
Morphine+Re 100 mg/kg	1.87±0.05

Results are given as the mean±S.E.

by 7.7 times in Rb_1 100 mg/kg, 3.8 times in Rb_2 100 mg/kg, 13.3 times in Rg_1 100 mg/kg and 3.2 times in Re 100 mg/kg as compared with that of the morphine control group (Fig. 1).

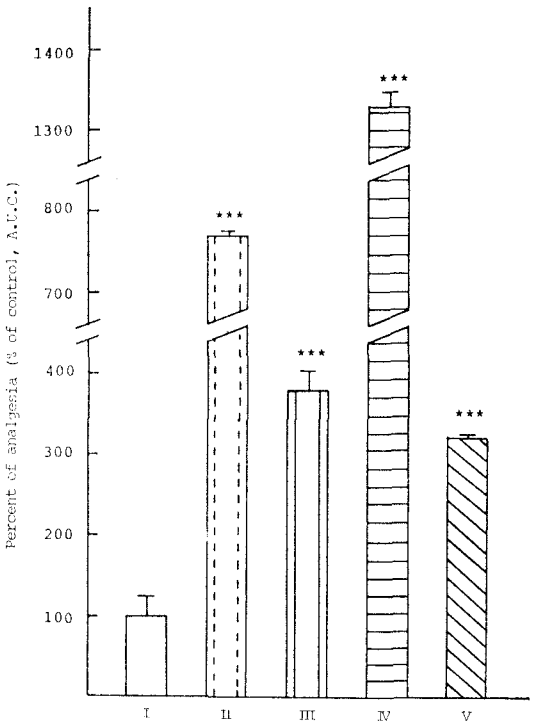

Fig. 1—Effects of Rb_1, Rb_2, Rg_1 and Re on tolerance to the analgesic action of morphine in mice.
***: $p<0.001$
I: morphine+saline
II: morphine+Rb_1 100 mg/kg
III: morphine+Rb_2 100 mg/kg
IV: morphine+Rg_1 100 mg/kg
V: morphine+Re 100 mg/kg

Inhibition of naloxone induced jumping response

Rb_1 100 mg/kg and Rg_1 100 mg/kg produced significant inhibitions of naloxone induced jumping response by 70% and 50%, but not significant 30% in Rb_2 100 mg/kg, 20% in Re 100 mg/kg and 0% inhibition in morphine control group (Fig. 2).

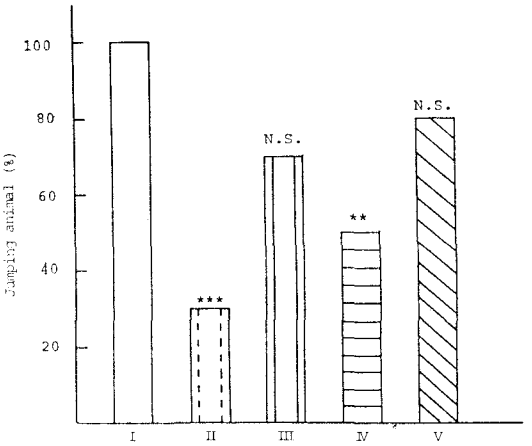

Fig. 2—Effects of Rb_1, Rb_2, Rg_1 and Re on the development of morphine dependence in mice by the naloxone induced jumping response.
**: $p<0.01$, ***: $p<0.001$, N.S.: non-significant
Columns: see Fig. 1.

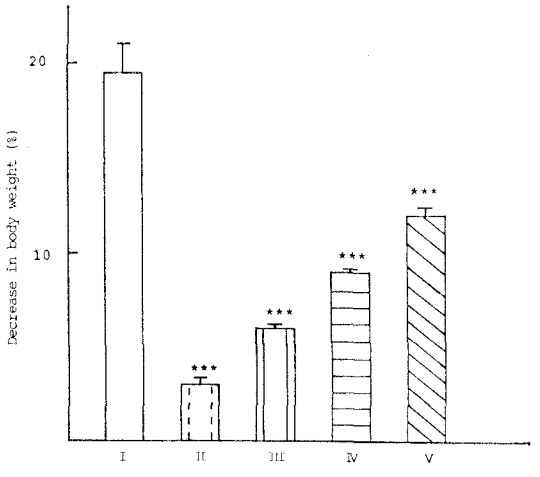

Fig. 3—Effects of Rb_1, Rb_2, Rg_1 and Re on the body weights of mice with multiple injection of morphines.
***: $p<0.001$
Columns: see Fig. 1.

Effect of body weight change

Ginsenosides(Rb_1, Rb_2, Rg_1 and Re) produced significant inhibitions of body weight decrease by 3% in Rb_1 100 mg/kg, 6% in Rb_2 100 mg/kg, 9% in Rg_1 100 mg/kg, 12% in Re 100 mg/kg but 19.5% in the morphine control group (Fig. 3).

Discussion

Kim et al.[11] reported that GS and PT inhibited both the development of morphine induced tolerance and dependence but PD inhibited the development of morphine induced dependence only. They presumed that the inhibition of GS, PD and PT on the development of morphine induced tolerance and dependence might be mainly due to their reserpine or tetrabenazine like action[12,13] and the inhibition of dopamine receptor supersensitivity.[7]

In this experiment, it is interesting to note that ginsenosides showed the inhibition of morphine induced tolerance but in the inhibition of the development of morphine induced dependence, Rb_1, a series of PD, is more effective than that of Rg_1 and Re, a series of PT. In some degree, this result conflicts with the previous report that PT was more powerful than PD in the inhibition of the development of morphine induced dependence.[11]

Kim and Toki's joint research[14] showed that morphine 6-dehydrogenase which catalyzed morphinone production from morphine was inhibited by GS, especially PT *in vitro*. In mouse liver, a portion of morphinone was metabolized into morphinone-glutathione conjugate concerned with detoxification and the other portion of morphinone was metabolized into morphinone-protein SH conjugate concerned with the development of morphine induced tolerance and dependence by covalent binding to sulfhydryl group of opiate receptor.[15]

Our hypothesis was that the mechanism of the inhibition of morphine induced tolerance by ginsenosides might be due to the inhibition of morphinone production which was a metabolite of morphine and had about 9 times toxicity of morphine based on LD_{50} value in mouse when they were given s.c.. In addition, the importance of the equilibrated state of neurogic function rather than the brain levels of neurotransmitters on the development and the loss of morphine tolerance and dependence by GS could be shown in daily treatment with a small dose of reserpine.[16]

The inhibitory mechanism of the abstinence syndrome in morphine dependent-tolerant animals by ginseng saponins remains unclear. Several neurotransmitters, acetylcholine, dopamine and c-AMP have been implicated in the abstinence syndrome. The expression of an abstinence syndrome is associated with an increase in brain dopamine level,[17] an increase in c-AMP level[18] and a decrease in brain acetylcholine level.[19]

The earlier reports involving the effects of GS on whole brain neurotransmitter levels and on the neurotransmitter turnover rates in whole brain and various regions of brain, have yielded conflicting data. Most of reports showed increases in noradrenaline, dopamine, serotonin and c-AMP in ginseng saponin treated animals[20,21] while there was no report on acetylcholine levels.

We hypothesized that the inhibition of an abstinence syndrome in morphine tolerant-dependent mice by ginsenosides might be due to decreases in dopamine and/or serotonin levels in the brains of ginsenosides treated mice such as Kim and Oh's suggestion.[22]

The possible differences in the above reported effects in brain neurotransmitters levels could be originated from the different experimental methods such as administration routes, dosage

size and tenures, animals, materials and so on.

In the present study, we suggest that Rb_1 and Rg_1 are active components of ginseng saponins on the inhibition of development of morphine induced tolerance and dependence.

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Literature Cited

1. Kim, Y.E., Such, H.S., Kim, W.K. and Kim, K.S.: *Seoul Univ. J.* **18**, 83 (1967).
2. Nabata, H., Saito, H. and Takagi, K.: *Japan J. Pharmacol.* **23**, 29 (1973).
3. Namba, T., Yoshizaki, M., Yomimori, T., Kobashi, K., Mitsui, K. and Hase, J.: *Yakugaku Zasshi* **94**, 252 (1974).
4. Kim, H.S. and Oh, S.K.: *Res. Rev. Chungbuk National Univ.* **28**, 423 (1984).
5. Kim, H.S. and Oh, S.K.: *Yakhak Hoeji* **29**, 27 (1985).
6. Kim, H.S., Oh, S.K., Choi, K.J. and Lee, H.B.: *Yakhak Hoeji* **29**, 188 (1985).
7. Kim, H.S., Oh, S.K. and Kim, G.C.: *Kor. J. Pharmacogn.* **16**, 31 (1985).
8. Way, E.L., Loh, H.H. and Schen, F.: *J. Pharmacol. Exp. Ther.* **167**, 1 (1969).
9. D'Amour, F.E. and Smith, D.L.: *J. Pharmacol. Exp. Ther.* 72, 74 (1941).
10. Kaneto, H., Kosaka, N. and Hirota, N.: *Life Sci.* **31**, 2351 (1982).
11. Kim, H.S., Park, J.S., Oh, K.W. and Kim, M. W.: *Res. Rev. Chungbuk National Univ.* **32**, 473 (1986).
12. Takagi, H., Takashima, T. and Kimura, K.: *Arch. Int. Pharmacodyn.* **149**, 484 (1964).
13. Takagi, H. and Kuriki, H.: *Int. J. Neuropharmac.* 8, 195 (1969).
14. Kim, H.S. and Toki, S.: Unpublished data (1986).
15. Nagamatsu, K., Kido, Y., Terao, T., Ishida, T. and Toki, S.: *Drug meta. dispos.* **11**, 190 (1983).
16. Takahashi, M. and Kaneto, H.: Presented at the 5th Korea-Japan Joint Seminar on Pharmacology on 22th August, 1986, in Cheju, Korea.
17. Iwamoto, E.T., Ho, I.K. and Way, E.L.: *J. Pharmacol. Exp. Ther.* **187**, 558 (1973).
18. Collier, H.O.J. and Francis, D.L.: *Nature*(London) **255**, 159 (1975).
19. Bhargava, H.N. and Way, E.L.: *J. Pharmacol. Exp. Ther.* **194**, 65 (1975).
20. Joo, C.N.: *Kor. Biochem. News* **4**, 5 (1985).
21. Park, C.W.: *Kor. Biochem.* **4**, 37 (1985).
22. Kim, H.S.: Unpublished data (1985).