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Effect of *Panax ginseng* on the Development and Loss of Morphine Tolerance and Dependence

Hack Seang Kim, and Ki Wan Oh

Department of Pharmacology, College of Pharmacy, Chungbuk National University, Cheong-ju, Korea

Abstract

The present study was undertaken to determine the inhibitory effects of orally administered ginseng saponins (SP), protopanaxadiol saponins (PD), and protopanaxatriol saponins (PT) on the development of morphine-induced tolerance and physical dependence in mice. The study also sought to determine the hepatic glutathione contents, which are closely related to the degree of detoxification of morphinone, a novel metabolite of morphine, and to determine the effects of GS on morphine 6-dehydrogenase, which catalyzes the production of morphinone from morphine, and the roles of spinal descending inhibitory systems in the production of antagonism. The results of the present study showed that GS, PD and PT administered orally inhibited the development of morphine induced tolerance and dependence. GS, PD and PT inhibited the reduction of hepatic glutathione concentration in mice treated chronically with morphine and the activity of morphine 6-dehydrogenase, and the activation of spinal descending inhibitory systems was inhibited by GS. So we hypothesized that the results were partially due to the dual action of the test drugs, the inhibition of morphinone production and the activated formation of morphinone-glutathinone conjugation, and the inhibition of the activatin of spinal descending inhibitory systems and the others.

It is generally known that *Panax ginseng* has been used as tonics for thousand years. But Kim and his coworkers[1] (1967) reported that a folk medicine prescribed by seven herbal drugs including *Panax ginseng* has been used as antinarcotics in the treatment of mophine tolerant-depedent patients, and its effective component was keratin of Manis squama. But there were no reports that discussed the effects of *Panax ginseng* in the treatment of morphine tolerant-dependent patients.

For this reason, we started to work with *Panax ginseng* in the antinarcotic aspects[2,3,4].

The 1st table shows the traditional prescription including *Panax ginseng* which has been used as antinarcotic drug.

Table 1. Traditional prescription for the treatment of morphine tolerant-dependent patient

Ginseng Radix
Euphobiae Pekinensis
Manis squama
Zizyphi Spinosi Semem
Angelicase Gigantis Radix
Cnidii Rhizoma
Paeoniae Radix

The topics about the antinarcotic actions of *Panax ginseng* which I am going to talk about mainly today as follows :

1. Inhibition of the development of morphine tolereance and dependence.
2. Increase in the hepatic glutathione contents which are closely related with degree of detoxication of morphinone, a novel and active metabolite of morphine during chronic morphinization.
3. Inhibition of morphine 6-dehydrogenase which catalyzes the production of morphinone from morphine.
4. Antagonism of morphine analgesia on mechanical and thermal nociception by systemic, intracerebral and intrathecal pretreatments of ginseng saponin.

Inhibition of the development of morphine tolerance and dependence : The 1st figure tells the inhibitory effect of ginseng saponin (GS), protopanaxadiol saponin (PD) and protopanaxatriol saponin (PT) on the development of morphine tolerance.

To induce morphine tolerance in mice, 10mg/kg of morphine was administered (s.c.) to mice once a day for a period of 6 days. Meanwhile each of the ginseng saponins was administered to the mice 3 hrs in advance to morphine injection daily. A tolerance test to 5mg/kg of morphine was determined every 30 min for 2 hours by the tail flick method on day 7 and calculated as % of the control by A.U.C. method. 24 hrs after the final injection of morphine. The AUC value was expressed as % of the control on the

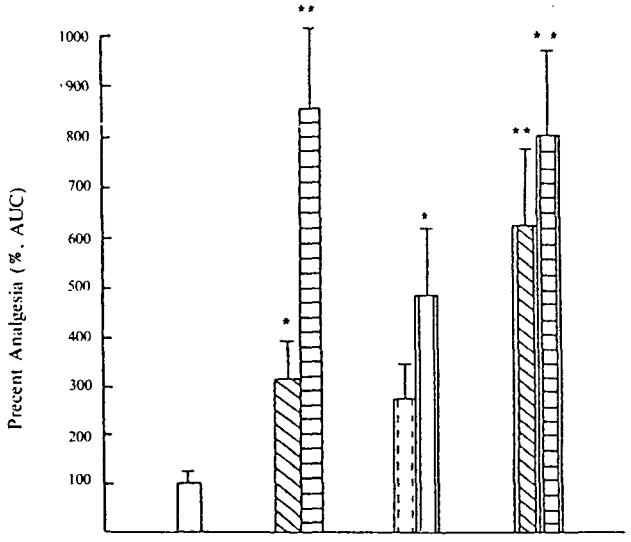

Fig. 1. Effects of GS, PD and PT administered orally on tolerance to the analgesic action of morphine in mice.

*p<0.05 **p<0.01

Ⅰ Mor + GS 50 mg/kg
Ⅱ Mor + GS 200 mg/kg
Ⅲ Mor + PD 50 mg/kg
Ⅳ Mor + PD 200 mg/kg
Ⅴ Mor + PT 50 mg/kg
Ⅵ Mor + PT 200 mg/kg
Ⅶ Mor + Saline

ordinate. The inset white column represents the morphine control group as 100%, GS 50 mg/kg treated group, inset sliding bars represents 3 times, GS 200mg/kg treated group, inset horizontal bars 8.2 times. PT 50 mg/kg treated group inset sliding bars, 6 times and PT 200 mg/kg treated group, inset horizontal bars 8 times compared with that of the morphine control group while both of the PD treated groups are shown to be not so effective as the others.

The 2nd figure shows the inhibitory effects of GS, PD and PT on the development of morphine dependence by naloxone 1 mg/kg induced withdrawal scores. Additional groups of mice that had received the same morphine and ginseng saponins as described before were used to determine naloxone 1 mg/kg induced withdrawal scores 24 hrs after the final injection of morphine. The scoring was made for 30 min immediately after the injection of naloxone. Scoring is as follows : jumping, diarrhea 2, rearing, grooming, defecation, wet dog shake, writhing and ptosis 1. The morphine control group, the inset white column, represents 100% of withdrawal scores. Most of the 200 mg/kg of GS, PD and PT treated groups show about 70% of withdrawal scores. The inhibition degree of dependence tells the decrease in the scores of withdrawal signs.

Increase in the hepatic glutathione contents : The 3rd figure tells the inhibitory effects of GS, PD and PT on the hepatic glutathione level decrease in mice by daily injection of morphine for 6 days. Other additional groups of mice that had received the same morphine and ginseng saponin as described before were used to determine the hepatic glutathione levels by the modified Ellman's method.[5]

The glutathione contents in mice treated with only GS, PD and PT are shown on the upper panel. Those values are generally a little bit higher than that of the saline control group.

The lower panel tells the values of ginseng saponins and morphine treated groups, but most of the values of the glutathione contents in ginseng treated groups show higher than that of the morphine control group.

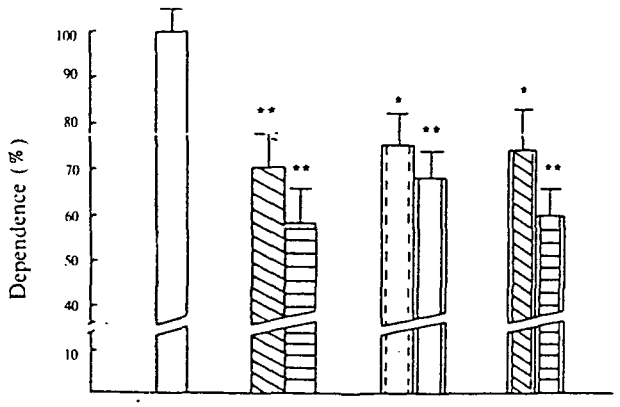

Fig. 2. Effects of GS, PD and PT on the development of morphine dependence in mice by the naloxone induced withdrawal syndrome.

*p<0.05 **p<0.01

- Mor + GS 50 mg/kg
- Mor + GS 200 mg/kg
- Mor + PD 50 mg/kg
- Mor + PD 200 mg/kg
- Mor + PT 50 mg/kg
- Mor + PT 200 mg/kg
- Mor + Saline

Schole et al.[6] (1978) reported that ginseng extract increased the hepatic glutathione levels in rats as we observed by the similar increased in ginseng saponins treated mice. The present results show that ginseng saponins inhibited the reduction of hepatic glutathione levels by daily injection of morphine for 6 days.

Inhibition of morphine 6-dehydrogenase : The 2nd table shows the inhibitory effects of GS, PD and PT on guinea pig liver morphine 6-dehydrogenase. Morphine 6-dehydrogenase was prepared and assayed by Yamano's method.[7] PT functioned as effective inhibitor. We found that, PT is a little more effective than naloxone. The enzyme was inhibited about 50% by 0.01% PT (corresponding to approximately 0.125 mM based on an average M.W. 800) at the physiological condition (pH 7.4).

The 4th figure tells the metabolism of morphine to morphine and its SH conjugate.

Morphine 6-dehydrogenase catalyzes the production of morphine from morphine and a part of the produced morphinone is nonenzymatically conjugated with non protein-SH at the opiate receptor to produce tolerance. The rest goes to form morphinone-glutathione conjugate for detoxication and excretion. Ginseng saponin increased the production of hepatic non protein-SH levels, the formation of morphinone-glutathione conjugate and its excretion while it inhibited morphine 6-dehydrogenase which catalyzed the production of morphinone from morphine. This result suggests that the dual action of the above plays an important role in the inhibition of the development of morphine tolerance and dependence.

The 5th figure shows the binding mechanism of morphine and morphinone with opiate receptors. Morphinone can not produce normal analgesic effects after morphinone is conjugated with non protein-SH at the opiate receptor

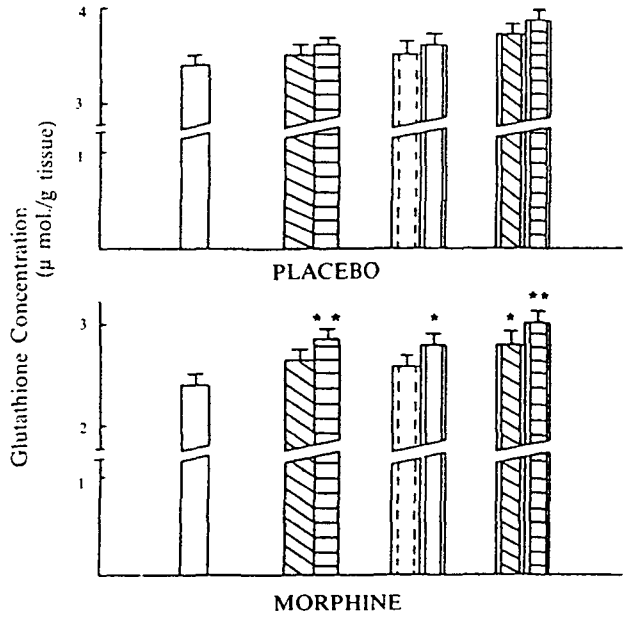

Fig. 3. Effects of GS, PD and PT on the inhibition of hepatic glutathione level decrease in mice.

*p<0.05 **p<0.01

- GS 50 mg/kg
- GS 200 mg/kg
- PD 50 mg/kg
- PD 200 mg/kg
- PT 50 mg/kg
- PT 200 mg/kg
- Saline

Table 2. Effects of ginseng total saponins, protopanaxadiol and protopanaxatriol on guinea pig liver morphine 6-hydrogenase

Saponin fraction	Concentration (%)	Inhibition (%)			
		with NADP		with NAD	
		at optimum pH	at pH 7.4	at optimum pH	at pH 7.4
Ginseng total saponins	1.0	36.6	—	—	—
	0.1	7.9	18.1	9.5	9.1
	0.01	0	3.6	0	0
	0.001	0	0	0	
Protopanaxadiol	1.0	44.9	—	—	—
	1.0	7.3	15.5	6.9	9.1
	0.01	0	0	0	0
	0.001	0	0	0	0
Protopanaxatriol	1.0	75.1	—	—	—
	0.1	53.1	67.8	44.4	67.3
	0.01	34.5	51.1	28.6	45.4
	0.001	4.0	11.9	4.8	—
	0.0001	0	3.9	0	—

—, not determined

site like this, but at the normal opiate receptors morphine can produce analgesic effects this way.

Antagonism of morphine analgesia on mechanical and thermal nociception : The 6th figure shows the pain transmission and spinal decending inhibitory systems. The analgesic action of morphine is the result of direct or indirect inhibition pain transmission in the spinal dosal horn. The analgesic action of systemic morphine in analgesic doses is primarily mediated by the activation of the descending inhibitory systems. Recent studies (Kuraishi et al.[8](1983)) have shown that descending inhibitory systems consist in part of the noradrenergic and serotonergic systems. The noradrenergic descending inhibitory system in mechanical nociception (tail pinch test) and the serotonergic system in thermal nociception (tail flick test) play more important roles respectively in the production of morphine analgesia. For this reason I am going to tell whether the analgesic action of morphine in analgesic doses on mechanical (tail pinch test) and thermal (tail flick test) nociception is antagonized by 1) systemic (100 mg/kg of GS i.p.) 2) intracerebral[9] (40 ug/body i.c.) and 3) intrathecal[10] (40 ug/body i.t.)

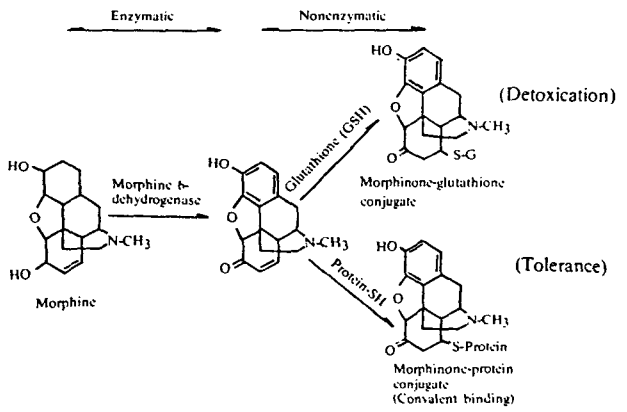

Fig. 4. Metabolism of morphine to morphine and its conjugates.

pretreatments of ginseng saponins and its reversal by L-DOPA or 5-HPT to check the acting sites of antagonism and the roles of spinal descending inhibitory systems in the production of antagonism.

The analgesic action or morphine 5 mg/kg (s.c.) was determined by the tail pinch or tail flick method as I described before and calculated as % of the control by the AUC method and compared with that of the mice pretreated with ginseng saponin.

The 7th figure shows antagonism of morphine analgesia in the tail pinch test by ginseng saponin and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HPT.

The inset white column represents the analgesic activity of morphine 5 mg/kg as 100%, the parallel cut out lines column the antagonized activity of morphine 5 mg/kg by the pretreatment of ginseng saponins 100 mg/kg, and the others, the suppression of antaginism by 10, 30 and 50 mg/kg of L-DOPA or 5-HTP.

In the tail pinch test (Fig. 7), analgesic action of morphine 5 mg/kg administered subcutaneously was antagonized by 50% with the i.p. pretreatment of ginseng saponin 100 mg/kg, but the suppression of morphine antagonism by L-DOPA was more predominant than that of 5-HTP.

In the tail flick test (Fig. 8), analgesic action of morphine was antagonized by about 20% with the i.p. pretreatment of ginseng saponin 100 mg/kg, and its

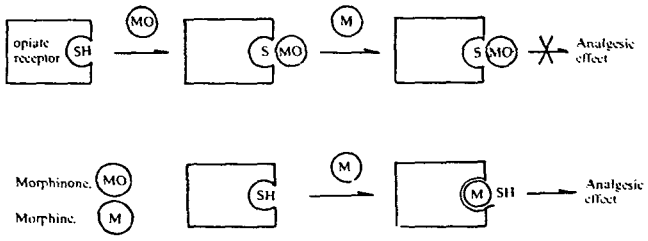

Fig. 5. Binding mechanism of morphine and morphinone with opiate receptor.

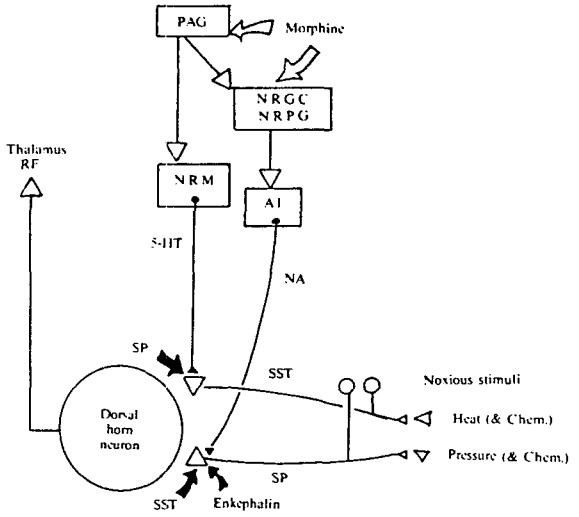

Fig. 6. Pain transmission and inhibition of peptides

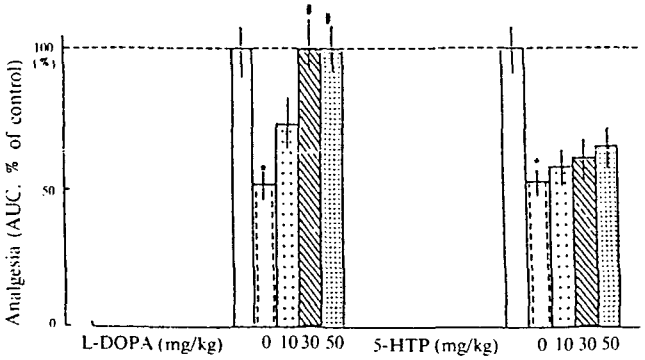

Fig. 7. Antagonism of morphine analgesia in the tail pinch test by ginseng saponin (GS) and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HPT. 100mg/kg of GS(i.p.) was injected 3hrs before 5mg/kg of morphine(s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HPT (i.p.) were given 30 min before morphine injection.

*p<0.05, **p<0.01, compared with that of morphine (Mor).
#p<0.05, ##p<0.01, compared with that of GS+Mor.
□ Morphine control ▦ G.S + Mor
▤ GS + Mor + 10 mg/kg of L-DOPA or 5-HTP
▨ GS + Mor + 30 mg/kg of L-DOPA or 5-HTP
▩ GS + Mor + 50 mg/kg of L-DOPA or 5-HTP

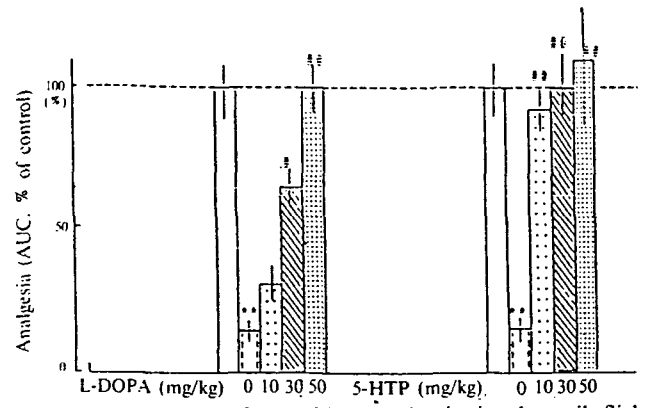

Fig. 8. Antagonism of morphine analgesia in the tail flick test by GS and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HTP. 100mg/kg of GS (i.p.) was injected 4 hrs before 5 mg/kg of morphine (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min before morphine injection.

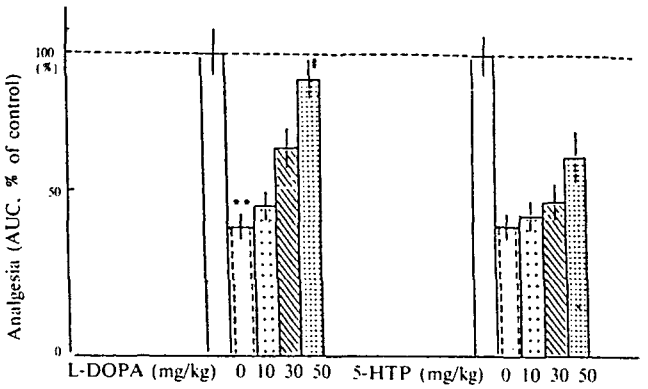

Fig. 9. Antagonism of morphine analgesia in the tail pinch test by GS and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HTP. 40 ug/kg of GS (i.p.) was injected 2 hrs before 5 mg/kg of morphine (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min before morphine injection.

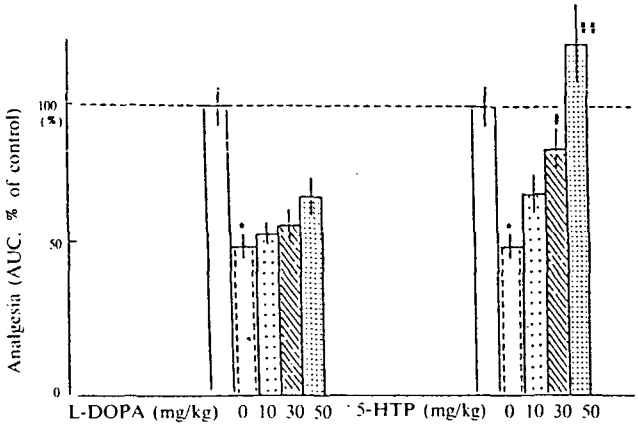

Fig. 10. Antagonism of morphine analgesia in the tail flick test by GS (i.c.) and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HTP. 40 ug/kg of GS was injected 3 hrs before 5 mg/kg of morphine injection (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min before morphine injection.

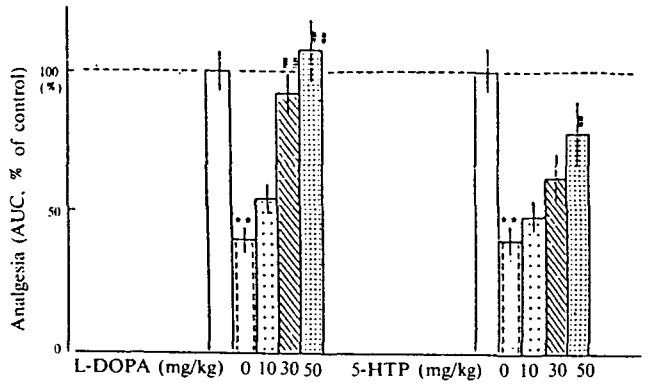

Fig. 11. Antagonism of morphine analgesia in the tail pinch test by GS (i.t.) and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HTP. 40 ug/kg of GS was injected 2 hrs before 5 mg/kg of morphine injection (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min before morphine injection.

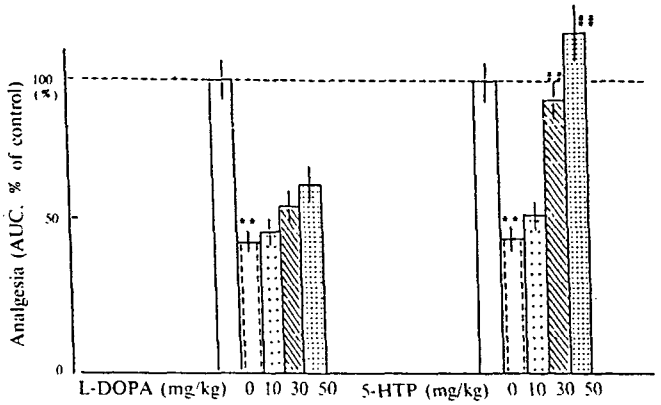

Fig. 12. Antagonism of morphine analgesia in the tail flick test by GS (i.t.) and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HTP. 40 ug/kg of GS (i.p.) was injected 3 hrs before 5 mg/kg of morphine (s.c.). 10, 30 and 50 mg/kg of L-DOPA or 5-HTP (i.p.) were given 30 min before morphine injection.

reversal by 5-HTP was more predominant than that of L-DOPA

In the tail pinck test (Fig. 9), analgesic action of morphine 5 mg/kg administered subcutaneously was antagonized by 45% with the intracerebral pretreatment of ginseng saponin (40 ug/body) and its reversal by L-DOPA was more predominant than that of 5-HTP.

In the tail flick test (Fig. 10.), analgesic action of morphine 5 mg/kg administered subcutaneously was antagonized by 50% with the intracerebral pretreatment of ginseng saponin 40 ug/body and its reversal by 5-HTP was more predominant than that of L-DOPA.

In the tail pinch test (Fig. 11.), analgesic action of morphine 5 mg/kg administered subcutaneously was antagonized by 45% with the intrathecal pretreatment of ginseng saponin 40 ug/body and its reversal by L-DOPA was more predominant than that of 5-HTP.

In the tail flick test (Fig. 12.), analgesic action of morphine 5 mg/kg administered subcutaneously was antagonized by 45% with the intrathecal pretreatment of ginseng saponin 40 ug/body and its reversal by 5-HTP was more predominant than that of L-DOPA.

The summary of morphine antagonism by ginseng saponin as follows :

1. The analgesic action of systemic morphine in analgesic doses on mechanical and thermal nociception was antagonized by systemic, intracerebral and intrathecal pretreatment of ginseng saponin.
2. L-DOPA in the tail pinch test and 5-HPT in the tail flick played respectively more important roles in the supression of morphine antagonism by ginseng saponin.
3. The above results suggest that ginseng saponins inhibit the activation of the spinal descending inhibitory noradrenergic and serotonergic systems as well as the activation of the inhibitory cerebreal-PAG system including thalamus.

Reference

1. Kim, Y.E., Such, H.S., Kim, W.K. and Kim, K.S. : Studies on the animal hard tissue extract as an agent against narcotism. Seoul Univ. K.18,83 (1967)
2. Ellman, G.L. : Tissue sulfhydryl groups. Arch. Biochem. Biophus. 82, 70-77
3. Schole, J. : Influence of *Panax ginseng* on the glutathione system of rat liver. Belastung. Ernahrug und Resistenz-Froschritte in der Tier-Physiologie und Tier-Ernahrug 9. Ed. Paul Prey, Hambrug/Berilin. 35 (1978)
4. Yamano, S., Kageura, E., Ishida, T. and Toki, S. : Purfication and characterization of gulinea pig liver morphine 6-hydrogenase. J. Biol. Chem. *260*, 5259-5264 (1985)
5. Y. Kuraishi, Y. Harada, S. Aratani, M. Satoch and H. Takagi : Separated involvement of the spinal noradrengic and serotonergic systems in morphine analgesia. Brain Research. 273, 245-252 (1983)
6. T.J. Haley and W.G. McCormic : Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse . Brit. J. Pharmacol., 12, 12(1957)
7. Janice L.K. Hylden and George L. Wilcox : Intrathecal morphine in mice. Eurpean Journal of Pharmacology. 67, 313-316 (1980)

F. Soldati : You have used ginsenosides concentrations in the range of 100 mg/kg i.p. in mice. Would you inject such quantities into man? Do you expect any side effects such as hemolysis?

H.S.Kim : No, I didn't inject that dosage in man and I didn's expect any side effects.

T.Y.Lee : May I comment on the previous question for the possibility of causing hemolysis by the dosage of ginsenosides? It has been well recognized that ginseng saponins do not show hemolysis. Recently, we confirmed that ginsenosides do not exhibit photohemolysis induced by singlet oxygen rather than causing hemolysis. Also I would like to point out that some of the polyacetylene components exhibit protective activity against photohemolysis.

인삼이 몰핀의 내성 및 의존성 형성에 미치는 영향

김 학 성　　오 기 완

충북대학교 약학대학
충북 청주시 개신동 산 48

인삼사포닌, 푸로토파낙사다이옥 사포닌 및 푸로토파낙사트리올 사포닌의 몰핀내성 및 의존성 형성억제 작용을 연구하였다. 인삼사포닌은 독성이 10배, 진통력은 1/2인 morphinone으로 대사시키는 morphine 6-hydrogenase의 작용을 억제시켜 morphinone의 생성을 증가시켜 morphine glutathione conjugation을 촉진시키므로 morphinone의 해독작용이 증가된다. 또한 인삼은 척수하행성 억제계의 활성을 억제하여 몰핀의 진통력을 감항하므로 신경계 기능상의 변화도 몰핀의 내성 및 의존성형성 억제작용에 관여하는 것으로 추정된다.