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Effect of *Panax ginseng* on the Development of Morphine Induced Tolerance and Dependence (VI). On the oral administration of ginseng ether fraction and saponins

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Abstract□The present study was undertaken to determine the inhibitory effects of orally administered ginseng saponins(GS), protopanaxadiol saponins(PD), protopanaxatriol saponins(PT) and ginseng ether fraction(GE) on the development of morphine induced tolerance and physical dependence in mice and also to determine the hepatic glutathione contents. GS, PD and PT inhibited significantly the development of morphine induced tolerance and physical dependence, but GE was effective only on the inhibition of the development of morphine induced physical dependence. GS, PD, PT and GE also inhibited the hepatic glutathione level decrease induced by morphine multiple injections.

Keywords□Morphine, Panax ginseng, Glutathione, Tolerance, Dependence.

The analgesic action of morphine is very remarkable, but repeated treatment with morphine produces physical dependence, characterized by withdrawal symptoms and a tolerance to most of its effects develops. Thus, there must be a continueing search for morphine-type compounds which are devoid of addiction liability and are orally effective narcotic antagonists with minimum secondary effects.

A folk medicine composed of seven herbal drugs including *Panax ginseng* has been used as antidote in the treatment of morphine tolerant-dependent patients. It was reported that its effective component was keratin of *Manis squama.*[1] And researchers have reported the inhibition of the development of morphine induced tolerance and dependence in ginseng butanol fraction,[2] protopanaxadiol fraction and protopanaxatriol fraction administered intraperitoneally.[3] It has been also reported the inhibition of the development of morphine induced dopamine receptor supersensitivity.[4] But there has been no report that discussed the effects of ginseng saponins and ginseng ether fraction administered orally to morphine treated mice.

The present study was undertaken to determine the inhibitory effects of orally administered ginseng saponins(GS), protopanaxadiol saponins(PD), protopanaxatriol saponins(PT) and ginseng ether fraction(GE) on the development of morphine induced tolerance and physical dependence in mice and also to determine the hepatic glutathione contents which are closely related to the degree of detoxication of morphinone, a novel metabolite of morphine.

EXPERIMENTAL METHODS

White ICR male mice weighing 18-22 g, in a group of 10-15, were used in all experiments. GS, PD and PT (kindly supplied from the Korea Ginseng & Tobacco Research Institute) dissolved in saline and GE suspended in 0.5% CMC solution were administered to mice orally once a day 30 minutes prior to the injection of morphine. To induce morphine tolerance and dependence in mice, morphine hydrochloride (Dae-Won Pharm. Co.) 10 mg/kg was administered subcutaneously to mice every 24 hours for a period of 6 days by Kaneto's method.[5]

Measurement of analgesic tolerance

The inhibitory degree of morphine tolerance development of the test drugs by oral administration was evidenced by the increase in analgesic response to morphine hydrochloride (5 mg/kg, *s.c.*), estimated at 0, 30, 60 and 90 minutes by the tail flick method[6] 24 hours after the final injection of morphine and calculated as area under the curve by Kaneto and his co-worker's method.[5]

The tail flick latencies to thermal stimulation

were determined in seconds prior to and at 0, 30, 60 and 90 minutes after the morphine injection. A value of 10 seconds was used as a cut-off point to avoid damage to the tail. The analgesic response for each mouse was calculated by the following formula;

$$\text{Percent Analgesia (\%)} = \frac{\text{Tt-To}}{\text{Tc-To}} \times 100$$

where To is the base line or pre-morphine tail flick reaction time, Tt is the reaction time at t minutes after morphine injection, Tc is cut-off time. The base line of tail flick latencies in different groups were around 2 ± 0.2 seconds. The effect was calculated as area under the curve (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 only with morphine (5 mg/kg).

Measurement of inhibition of naloxone induced withdrawal

The inhibition of naloxone induced withdrawal syndrome in mice treated with morphine alone and in morphine treated mice with test drugs was estimated by the decreased scores of the withdrawal induced by naloxone (1 mg/kg, *i.p.*) for 30 minutes, 24 hours after the final injection of morphine on the seventh day. The abstinence syndrome was quantified by placing animals on a diaphanous circular cylinder 35 cm in diameter and 70 cm in height and by scoring the withdrawal induced by naloxone as follows; jumping and diarrhea 2 point, ptosis, defecation, wetdog shake, writhing syndrome, rearing and grooming 1 point by all or none response by the modified Tagashira and Dewey's method.[7]

Measurement of the hepatic glutathione contents in mice

The mice treated with morphine only and those treated with the test drugs regularly for 6 days were killed by decapitation on the seventh day, 24 hours after the final injection of morphine. Their livers were removed immediately and the glutathione concentration was determined by the method of Ellman as follows;[8] The removed livers were homogenized in 4 volumes of ice-cold phosphate buffer, pH 7.4, to give a suspension equivalent to 250 mg/ml of wet liver. For estimation of reduced glutathione, an aliquot was deproteinized by addition of an equal volume of 4% trichloroacetic acid containing 1 mM Na-EDTA and after centrifugation (2000 x *g*, 5 min.), 0.5 ml of the supernatent was added to 4.5 ml of 5.5 '-dithiobis-2-nitrobenzoic acid. After mixing, absorbance at 412 nm was recorded against a reagent blank to determine the glutathione concentration. All the operations were carried out at 0-4 °C.

Statistics

The data were expressed as mean of changes ± S.E. The differences in the means for different responses in different treatment groups were analyzed by the student's *t*-test.

RESULTS

The base line of each group in analgesia changes was determined to check the residual effects of ginseng saponins and morphine 30 min prior to the tolerance tests. The pre-morphine treatment base line tail flick latencies in the different groups were shown in Table I. There were no differences in the base line tail flick latencies in the different groups.

Inhibition of analgesic tolerance development

The analgesia of each group calculated as the AUC to morphine 5 mg/kg showed 3.1 in GS 50 mg/kg, 8.5 in GS 200 mg/kg, 4.9 in PD 200 mg/kg, 6.2 in PT 50 mg/kg and 8 times in PT 200 mg/kg, compared with that of the morphine control group, but no significant differences were observed in PD 50 mg/kg and in both doses of GE (Fig. 1).

Table I. The base line of analgesia 30 minutes prior to the tolerance test on the 7 th day, 24 hours after the daily injection of 10 mg/kg of morphine (*s.c.*) for a period of 6 days. All values indicate mean ± S.E. of 10-15 mice. SAL: Saline 0.2 ml/20 g

Dose (mg/kg)		Analgesia (second)
Mor + SAL		2.17 ± 0.04
SAL + SAL		1.99 ± 0.06
Mor + GS	50	2.03 ± 0.04
	200	2.12 ± 0.06
Mor + PD	50	2.15 ± 0.08
	200	1.99 ± 0.05
Mor + PT	50	2.14 ± 0.06
	200	2.11 ± 0.05
Mor + GE	50	2.09 ± 0.03
	200	2.12 ± 0.05

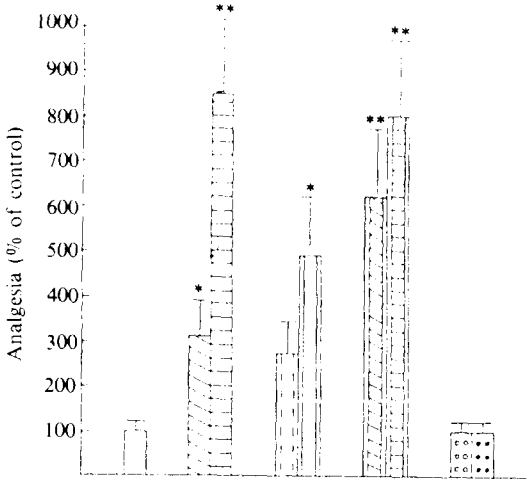

Fig. 1. Effects of GS, PD, PT and GE administered orally on tolerance to the analgesic action of morphine in mice.

Morphine 10 mg/kg was injected into the mice every 24 hours for 6 days and saline or daily doses, 50 and 200 mg/kg of GS, PD, PT and GE were administered to the respective group. The inhibitory degree of tolerance development by GS, PD, PT and GE was evidenced by the increase in analgesic response to morphine hydrochloride 5 mg/kg *s.c.*

* $P<0.05$ ** $P<0.01$

Mor + Saline
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 + GE 50 mg/kg
Mor + GE 200 mg/kg

Inhibition of withdrawal induced by naloxone

The inhibitory degrees of naloxone induced withdrawal scores were 30% in GS 50 mg/kg, 40% in GS 200 mg/kg, 23% in PD 50 mg/kg, 32% in PD 200 mg/kg, 35% in PT 50 mg/kg, 40% in PT 200 mg/kg, 30% in GE 50 mg/kg and 35% in GE 200 mg/kg group, compared with that of the morphine control group (Fig. 2).

Inhibition of the hepatic glutathione concentration decrease

The hepatic glutathione levels in the mice groups treated with ginseng saponins or GE alone were slightly increased from 0.03 (PD 50 mg/kg) to 0.55 u mol/g tissue (GE 200 mg/kg), compared with 3.39 ± 0.15 u mol/g tissue of the saline group. However, the glutathione level in the morphine control group was decreased to 2.41 ± 0.12 u mol/g tissue.

The glutathione levels of the groups treated with morphine and test drugs were observed from 2.77 ± 0.09 (PD 200 mg/kg) to 3.52 ± 0.12 u mol/g tissue (GE 200 mg/kg), showing the significant inhibitory effects in the hepatic glutathione level decreases, compared with that of the morphine control group. However no significant difference was shown in the group treated with GS 50 mg/kg or PD 50 mg/kg (Fig. 3).

DISCUSSION

Kim and his co-workers reported that GS, PD and PT administered intraperitoneally inhibited the development of morphine induced tolerance and physical dependence[9] and their active components were Rb_1 and Rg_1.[10] In this experiment, GS, PD,

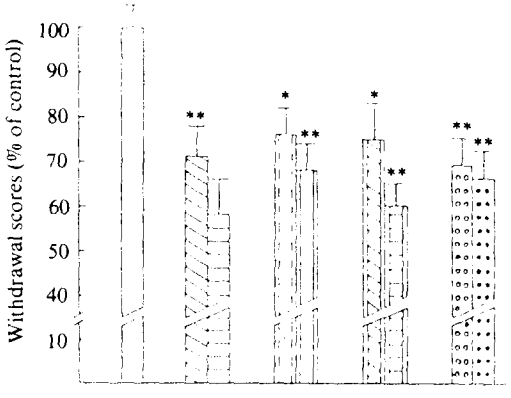

Fig. 2. Effects of GS, PD, PT and GE on the development of morphine dependence in mice by the naloxone induced withdrawal syndrome.

Each group of mice was injected with morphine hydrochloride 10 mg/kg *s.c.* at 24 hours intervals and administered orally with 50 and 200 mg/kg of GS, PD, PT and GE for the respective group at 24 hours intervals for 6 days. The withdrawal test was made 24 hours after the final injection, by challanging with naloxone 1 mg/kg *i.p.*

* $P<0.05$ ** $P<0.01$

Mor + Saline
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 + GE 50 mg/kg
Mor + GE 200 mg/kg

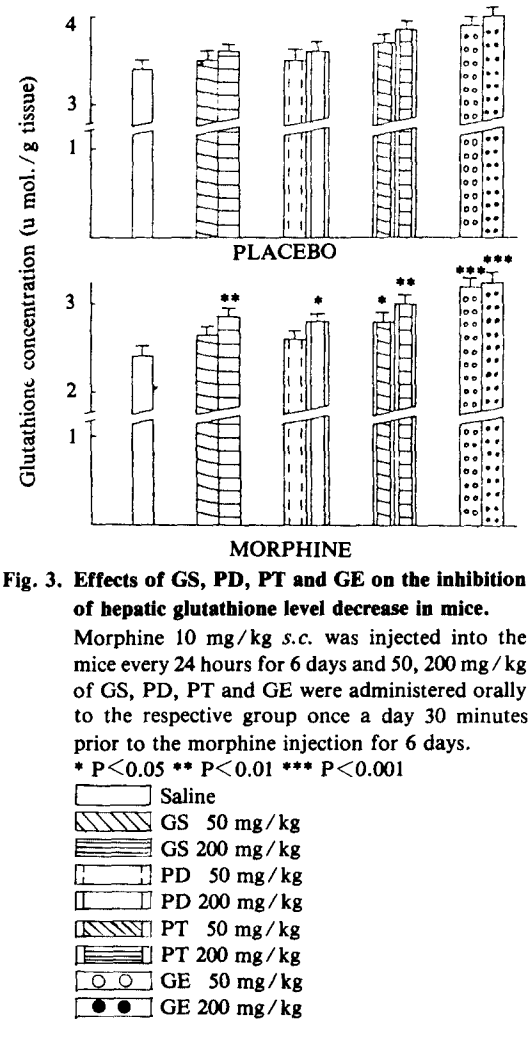

Fig. 3. Effects of GS, PD, PT and GE on the inhibition of hepatic glutathione level decrease in mice.
Morphine 10 mg/kg *s.c.* was injected into the mice every 24 hours for 6 days and 50, 200 mg/kg of GS, PD, PT and GE were administered orally to the respective group once a day 30 minutes prior to the morphine injection for 6 days.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

PT and GE administered orally were also found to inhibit the development of morphine induced tolerance and physical dependence. In the liver of the mice, a portion of morphine was metabolized into morphinone which was a novel metabolite of morphine and had 9 times the toxicity and a half the analgesic activity of morphine based on LD_{50} and ED_{50} value each in mouse (*s.c.*). Kim and Toki's joint research showed that morphine 6-dehydrogenase which catalyzed morphinone production from morphine was inhibited by ginseng saponins, especially PT, in vitro.[12]

An aliquot of morphinone conjugated with glutathione was closely related to the detoxication process and the other aliquot of morphinone was metabolized into morphinone-protein SH conjugate concerned with the development of morphine induced tolerance and physical dependence by covalent binding to the sulfhydryl group of opiate receptor.[13] Schole *et al.* reported that the standardized ginseng extract G115 significantly increased the glutathione level of rat's liver within minutes,[14] as observed by similar increase of glutathione level in ginseng saponins treated mice (Fig. 3). And the inhibition of the hepatic glutathione level decrease in morphine treated mice with GS, PD, PT and GE showed the inhibitory tendency of the development of morphine induced tolerance and physical dependence in this experiment. Thus, we hypothesized that these results were due to the dual action of the test drugs, the inhibition of morphinone production and the activation in morphinone-glutathione conjugation due to the increased glutathione level for detoxication. In addition, the newly equilibrated state of neurogic function rather than the changed brain levels of neurotransmitters on the inhibition of morphine tolerance and dependence development by ginseng saponins can also be considered as an acting point, as Takahashi and Kaneto discovered the newly equilibrated state of adrenergic function as weil as the inhibition of the development of morphine tolerance in mice treated daily with a small dose of reserpine.[15]

The mechanism of inhibition of the abstinence syndrome in morphine dependent-tolerant animals by ginseng saponins remains unclear. Several neurotransmitters, Ach, dopamine and *c*-AMP, have been implicated in the abstinence syndrome. The expression of the abstinence syndrome is associated with an increase in brain dopamine level,[16] an increase in *c*-AMP level[17] and a decrease in brain Ach level.[18] The studies involving the effects of ginseng saponins on whole brain neurotransmitter levels and on the neurotransmitters turn-over rates in the total brain and in the various region of brain have yielded conflicting data. Most of the studies showed increases in noradrenaline, dopamine, serotonin and *c*-AMP in ginseng saponins treated animals[19,20] while there was no report on Ach level. Norepinephrinergic neurons in the brain of the mice were more influenced by oral chronic treatment of panax ginseng than dopaminergic neurons.[21] We hypothesized that the inhibitory effects of morphine induced physical dependence by GS, PD, PT and GE were closely related to the changed ratios of EP, NE, dopamine and serotonin as well as the newly equilibrated state of neurogic function in brain.

The results of the present study showed that GS,PD, PT and GE administered orally inhibited the morphine induced tolerance and physical dependence, and also the reduction of hepatic glutathione concentration in mice treated chronically

with morphine.

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