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Effects of *Panax ginseng* on Morphine-induced Immune Suppression

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Abstract—To investigate the possibility of *Panax ginseng* as a therapeutic agent for the immune suppression, ginseng total saponin (GTS) extracted from korean red ginseng was tested on immune functions from morphine-induced immune suppressed mice. To study how immune functions are affected by morphine and also to test whether GTS can be an useful therapeutic agent for morphine toxicity, several parameters were employed, body weight, immune organ weight, B cell functions, and T cell function. Morphine impaired the development of body weight and immune organ weight such as spleen and thymus. Morphine also depressed a B-cell function, antibody production. T-cell functions studied by type IV hypersensitivity test were most markedly affected by morphine treatment. GTS restored most of morphine-induced immune suppression. GTS restored the morphine-induced decrease in spleen weight to body weight ratio in a dose dependent manner, but not the body weight decrease. Also all of the morphine-induced impairments of B cell functions and cell-mediated immunity were fully recovered by GTS. These results suggest that ginseng product could be very helpful for the treatment of immune suppression occurring in morphine abusers.

Keywords □ ginseng, morphine, immune, B cell, T cell

Panax ginseng C. A. Meyer (ginseng) has been widely used as a preventive or therapeutic agent in oriental medicine. There are numerous reports regarding the therapeutic actions of ginseng (Okamura *et al.*, 1994; Kenarova *et al.*, 1990; Kim *et al.*, 1992b). Ginseng seems to affect widespread body functions and body organs. Various actions such as antitumor (Rhee *et al.*, 1990), anti-ulcer (Sun *et al.*, 1992), anti-hepatitic (Matsuda *et al.*, 1991), insulin-like (Takaka *et al.*, 1990), anti-narcotic activity (Kim *et al.*, 1990), and immune enhancing actions (Keanrova *et al.*, 1990) have been reported.

Opioid drugs, a prototype of analgesics, bring in physical and mental side effects in abusers. Opiates also affect wide-spread immune functions; interferon (Lysle *et al.*, 1993) and interleukin-2 production (Bussirer *et al.*, 1993), phagocytic activity (Saini and Sei, 1993; Szabo *et al.*, 1993; Pacifini *et al.*, 1993; Rojavin *et al.*, 1993), natural killer cell activity (Pruett *et al.*, 1992; Bayer *et al.*, 1990), cell proliferation and cell-mediated immunity (Bryant and Roudebush, 1990; Arora *et al.*, 1990).

The facts that central nervous system and immune system are most affected by opiate abuses, and the facts that the representaive action of ginseng is immune enhancement, encouraged us to study ginseng as a therapeutic agent for morphine intoxication. Interestingly, there are also several reports which suggest that ginseng might be effective for antagonizing morphine actions in the brain (Ramarao and Bhargava, 1991; Bhargava Ramarao, 1991; Kim *et al.*, 1992a).

With these backgrounds, we studied the effects of morphine on immune functions such as B cell functions and T cell functions. We also tested whether *Panax ginseng* restores morphine-induced immunosuppression.

Material and Methods

Animal Treatments

Male ICR mice weighing 20~25 g were used. Animals were maintained at 12 hour light/dark cycle (8 : 00 a.m.~8 : 00 p.m.), and were freely accessible to food and water. Immune functions were suppressed by morphine·HCl (MOR; Samsung pharmaceutical Co., 10

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mg/kg, for 2 weeks, once or twice per day, s.c.). Control mice received saline only. Ginseng total saponin was orally administered one hour prior to morphine injection once a day at doses of 100, 200, and 400 mg/kg.

Measurement of Body Weight and Lymphoid Organ Weight

Body weight changes were calculated from the body weight measured on day-0 and day-14. Only water was provided to animals for 16 hours before weighing. Thymus, spleen, and liver were also weighed, and the body weight to organ weight ratio was calculated.

Hemagglutinin (HA) and Complement-dependent Hemolysis (HL)

Hemagglutinin and complement-dependent hemolysis assay were prepared according to the method described by Ha and Rhee (1981) with a slight modification.

Hemagglutinin Reaction

Sheep red blood cells (SRBC; Korea Media Co.) were prepared by washing three times with PBS (400 ×g, 5 min, 4℃) and were used as antigen (2×10^9 cells/ml in PBS, 0.2 ml per mouse, i.p.). Animals were sensitized on day-10, and blood was collected on day-14 from orbital sinus. The serum was heat-inactivated at 56℃ for 30 min, and was added to microtitration tray wells together with SRBC. Agglutination was measured after 2 hours incubation at 37℃, and HA titers were calculated.

Complement-dependent Hemolysis

Almost the same procedure as HA was employed for the HL assay. Serum and SRBC were added to microtitration tray wells, followed by guinea pig serum (Sigma) as a complement. After incubation at 37℃ for 2 hours, lysis was measured, and HL titers were calculated.

Delayed-type Hypersensitivity in Mice Ear Skin

Mice were sensitized by epicutaneous application of 100 μl of 7% tetranitrochlorobenzene (TNCB; Kishida Chemical Co., Japan) solution onto the shaved abdomen. At 6th day, the baseline ear thickness was measured using thickness gauge (Mitutoyo 111-115, SPM-25). Immediately after measuring ear thickness, the ear was treated epicutaneously with 10 μl of 1% TNCB solution (20 μl total). The naive mice were served as negative control for nonspecific ear swelling induced by irritating chemical only. After 24 hours, the ear thickness was measured both in sensitized and naive animals.

Measurement of Antibody Production

Antibody production function was determined using ELISA according to the method described by Yoon *et al.* (1994) with a slight modification. Mice were sensitized with antigen (hen egg lysozyme; Sigma, 2 mg /mouse, i.p. 2 times at day-0 and day-7). Blood was collected at day-14, and was tested for antibody production. The flat-bottom 96-well plates (Corning) were coated with hen egg lysozyme (100 μl, 50 μg/ml) overnight at 4℃. To reduce background noise, each well was blocked with 5% normal goat serum. One hundred microliters of serum (diluted 50x in 5% normal goat serum) was added into each well. After incubating 90 min at 37℃, 100 μl goat anti-mouse IgG-peroxidase conjugate (CalBiochem, 1 : 500 diluted) was added and incubated 90 min at 37℃. After washing, 100 μl substrate solution was added and incubated 15 min at room temperature in dark place. Fifty microliter of 2.5 N sulfuric acid was added to each well for stopping the reaction and the absorbance was measured at 492 nm.

Statistics

All of the statistical significance were determined using Student's t test.

Results

Effects of GTS and Morphine on Body Weight and Organ Weight

To investigate overall effects of GTS and morphine on immune system, body weight and lymphoid organ weight were measured. As shown in Table I, morphine significantly reduced body weight and it was not recovered by GTS at doses of 100, 200, and 400 mg/kg. Morphine also significantly decreased spleen weight to body weight ratio, and this effect was restored by GTS in a dose dependent manner at doses of 100, 200, and 400 mg/kg. Liver and thymus weight to body weight ratio were not affected by morphine or GTS.

Effects of GTS and Morphine on Hemagglutinin and Complement-dependent Hemolysis

To investigate the effects of morphine and GTS on humoral immunity, antibody production was tested using hemagglutinin reaction and complement-dependent hemolysis assay. These assays are widely used technique for antibody detection in sample (Basso *et al.*, 1994; Obminska-Domoradzka, 1994; Woodward *et al.*, 1992).

The effects of morphine and GTS on hemagglutinin and complement-dependent hemolysis titer are shown in Table II. Morphine, though statistically insignificant, showed a tendency to reduce both hemagglutinin and complement-dependent hemolysis titer. This effect was fully recovered by GTS at the dose of 400 mg/kg.

Effects of GTS on Antibody Production

Table I. Effects of morphine and GTS on body and organ weight

Drug	Dose (mg/kg)		Increasing rate for 14 days (%)	Spleen (w/w %)	Liver (w/w %)	Thymus (w/w ‰)
	MOR	GTS				
Control			27.80± 5.14	0.91± 0.15	4.64± 0.32	1.19± 0.15
MOR	10		16.50± 4.71*	0.56± 0.00*	5.13± 0.20	1.32± 0.35
MOR+GTS	10	100	19.97± 4.27	0.81± 0.09°	5.05± 0.09	1.33± 0.12
	10	200	8.94± 7.80**	0.91± 0.16	6.22± 0.79*	1.20± 0.15
	10	400	15.20± 3.29**	0.95± 0.11°	5.39± 0.24*	1.41± 0.16

GTS was orally administered to mice once per day for 14 consecutive days. Morphine was injected subcutaneously twice per day for 14 consecutive days. The control mice were treated with saline instead of morphine and GTS. Each values represents the mean± S.E. from 4 to 7 mice. Significances of difference compared with control group; *$p<0.1$, **$p<0.05$ Significances of difference from morphine-treated group; °$p<0.1$, °°$p<0.05$.

Table II. Effects of morphine and GTS on hemagglutinin and complement-dependent hemolysis

Drug	Dose (mg/kg)		HA Titer	HL Titer
	MOR	GTS		
Control			4.25± 0.25	3.63± 0.26
MOR	10		4.00± 0.58	3.00± 0.58
MOR+GTS	10	400	4.17± 0.17	3.83± 0.40

GTS was orally administered to mice once per day for 14 consecutive days. Morphine was injected subcutaneously to mice once per day for 14 consecutive days. Each values represents the mean± S.E. from 4 to 8 mice.

Table III. Effects of morphine and GTS on antibody production

Drug	Dose (mg/kg)		Absorbance (492 nm)
	MOR	GTS	
Control			0.306± 0.04°°°
MOR	10		0.204± 0.02***
MOR+GTS	10	400	0.334± 0.03°°°°

GTS was orally administered to mice once per day for 14 consecutive days. Morphine was injected subcutaneously to mice twice per day for 14 consecutive days. Each values represents the mean± S.E. from 3 to 5 mice. Significances of difference compared with control group; ***$p<0.025$, Significances of difference from morphine-treated group; °°°$p<0.025$, °°°°$p<0.01$.

Antibody, a flexible adaptor, plays an important role in humoral immunity. In this experiment, sandwich ELISA technique was utilized to detect antibody produced against HEL, which is known to be T cell dependent antigen (Allen *et al.*, 1992).

In Table III, the relative degree of antibody production is expressed by absorbance value. Absorbance, which results from interaction between enzyme-linked antibody and substrate, represents the amount of antibody produced. Morphine significantly inhibited antibody production and this effect was fully recovered by GTS (400 mg/kg).

Table IV. Effects of morphine and GTS on type IV hypersensitivity

Drug	Dose (mg/kg)		Thickness ($\times 10^{-2}$ mm)
	MOR	GTS	
NC			1.58± 1.07
Control			8.52± 1.80°°
MOR	10		3.78± 1.52**
MOR+GTS	10	400	8.29± 2.00°

GTS was orally administered to mice once per day for 14 consecutive days. Morphine was injected subcutaneously to mice once per day for 14 consecutive days. The control mice were received saline instead of morphine and GTS. The negative control (NC) mice were treated with 1% TNCB on right ear both side epicutaneously without any prior sensitization. Each values represents the mean± S.E. from 6 mice. Significances of the difference compared with control group; **$p<0.05$, Significances of difference from morphine-treated group; °$p<0.1$, °°$p<0.05$

Effects of GTS on Delayed-Type Hypersensitivity

Delayed-type hypersensitivity (DTH) is a simple *in vivo* assay for cell-mediated immune function. Exposure of epidermal cells to exogeneous haptens results in a delayed-type hypersensitive reaction that can be measured and quantified. The change in ear thickness after allergen treatment can be used to calculate the percent suppression of contact hypersensitivity. Contact hypersensitivity test in mice ear was performed according to the method described by Gaspari and Katz (1991) with a slight modification.

Effect of GTS and morphine on cell-mediated immune function is shown in Table IV as ear thickness value. Morphine treated group showed remarkable reduction of ear thickness compared with control group. Combined administration with GTS (400 mg/kg) dramatically recover the reduction of cell-mediated immune function. These results suggest that morphine decreases the function of cells such as helper T cell and cytotoxic T cells. Suppression effects of morphine on DTH reaction is also reported by Bryant and Rou-

debush (1990). Also our results, ginseng restored suppressed cell-mediated immune function, are in agreement with other reports. Ahn *et al.* (1987) reported that ethanol and petroleum ether extract of ginseng have restoration effect on the immunotoxicity of mitomycin C which suppresses T and B lymphocyte function.

Discussion

Opioid drugs, in spite of their serious side effects, are excellent analgesics in that they greatly reduce the distress associated with pain by changing the affective component of the nociception. Along with mental and physical dependence, chronic uses of morphine result in serious immune suppression.

Recently several studies suggested that morphine-induced immune suppression is mediated through central nervous system but not through its direct actions on immune system. This is strongly supported by the facts that among various morphine derivatives, only those which can enter the central nervous system produce immune suppression (Fuchs and Pruett, 1993; Hernandez *et al.*, 1993). Morphine acts on opioid receptors located in hypothalamus (Buckingham and Cooper, 1986; Stojikovic *et al.*, 1987; Yang *et al.*, 1989) to release corticotropin releasing factor. Therefore, it is likely to be released corticosterone rather than morphine itself that suppresses immune system. Corticosterone destroys immune organs by evoking apoptosis (Baughman *et al.*, 1991; Compton and Cidlowski, 1987; Compton *et al.*, 1991; Cohen, 1992; Garvy *et al.*, 1993; Iwata *et al.*, 1991).

On the bases of these results, we can speculate several possibilities underlying cellular mechanism of ginseng action on immune system. First, it is possible that ginseng blocks the morphine-induced release of CRH to lower the plasma corticosterone level to the normal value. This is a quite plausible hypothesis considering the effects of ginseng on stress. It was shown that ginseng blocked cold water swim stress-induced increase inplasma corticosterone level (Luo *et al.*, 19 93). Second, ginseng might act on immune organs to block the corticosterone-induced apoptosis. Apoptosis is a positive process involving the protein synthesis. Even though this hypothesis is not tested yet, considering mitotic effects of ginseng on cell proliferation, it is likely that ginseng functionally antagonize glucocorticoid-induced proteins. In this case, it is unlikey that ginseng blocks the apoptosis process directly, probably it will activate other cellular processes to combat the actions of corticosterone-induced proteins.

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