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 widespread 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 representative 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
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 x g, 5 min, 4°C) and were used as antigen (2 x 10⁶ 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°C for 30 min, and was added to microtitration tray wells together with SRBC. Agglutination was measured after 2 hours incubation at 37°C, 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°C 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 (TNCS; 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°C. 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°C, 100 µl goat anti-mouse IgG-peroxidase conjugate (CalBiochem. 1: 500 diluted) was added and incubated 90 min at 37°C. 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; Obmiminska-Domardzka, 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.