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Effects of Swainsonine on the Humoral Immune Response of Lipopolysaccharide

Byeong Suk Chae[1], Young Keun Ahn[2] and Joung Hoon Kim[2]

[1]Department of Environmental Engineering, Woosuk Univercity, Samrae-Up 565-800, Korea and [2]Center for Food and Drug Safety, Wonkwang University, Iksan 570-749, Korea

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Effects of swainsonine (SW; 8α,β-indolizidine-1α,2α,8-triol from Locoweed) on the humoral immune responses of lipopolysaccharide (LPS) were studied in ICR mice. Mice were divided into 4 groups (10 mice/group), and LPS was given to each mouse 1 hr after i.p. injection with 3.7 mg/kg of swainsonine, by i.p. injection twice a week for 14 days at a dose of 2 mg/kg. Humoral immune responses were evaluated by hemagglutination (HA) titer and splenic plaque forming cells (PFC). The results of this study were summarized as follows: Mice administrated each of LPS and SW showed significant enhancement of the weight ratios of spleen to body, HA titer, 2-mercaptoethanol-resistant HA (MER-HA) titer and PFC compared with those in controls. However, the LPS plus SW treatment decreased HA titer, MER-HA titer and PFC corresponding to humoral immunity, as compared with those in the mice treated with LPS alone. These findings indicated that LPS significantly enhanced humoral immune responses, but their enhancement effects were lowered somewhat by SW.

Key words : Lipopolysaccharide, Swainsonine, Hemagglutination titer, Splenic plaque forming cell

INTRODUTION

Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, is composed of *O*-antigen polysaccharide side chain, R-core and lipid A (Zarowitz, 1991). LPS is known to stimulate the release and synthesis of several biologically active cytokines and mediators, *e.g.* serum-derived anaphylatoxins, tumor necrosis factor (TNF), interleukines, arachidonic acid metabolites, colony-stimulating factors, platelet activating factors, interferon (Morrison and Ryan, 1987; Dinarello, 1988; Kishimoto, 1989; Rietschel and Brade, 1992). The pathophysiological disorders of these mediators to LPS include high fever, hypotension, acidosis, disseminated intravascula coagulation, hypoglycemia, platelet aggregation and death among others (McGhee *et al.*, 1979; Rothstein and Schreiber, 1988; Marshall and Ziegler, 1991). On the other hand, it was showed that LPS had various immunobiological activities, including specific immunity adjuvant effects, macrophage activity enhancement of resistance to pathogenic infections and antitumor activation (Carswell *et al.*, 1975; Morrison and Ryan, 1979). In addition to the immunological effects mentioned above, LPS was found to be a potent stimulator of B lymphocytes and enhanced B lymphocytes without helper T cells in vivo and *in vitro* (Coutinho *et al.*, 1974). It was found that LPS prevented the induction of T suppressor cells to T cell-independent antigens *in vivo* and *in vitro* (Skidmore *et al.*, 1975). On the other hand, it has been showed that LPS suppresses humoral immune responses to thymus-dependent antigen in mice (Persson, 1977). Mowat *et al.* (1986) reported that LPS prevented the induction of tolerance for serum IgG antibody responses in mice fed ovalbumin. The findings suggest that LPS can be used as T-independent antigen promoting humoral immune responses.

Swainsonine (SW; 8α,β-indolizidine-1α,2α,8β-triol from Locoweed), an indolizidine alkaloid, has been isolated from Astragalus and Swainsona (Molyneux and James, 1982), and also is producted by the fungus *Rhizoctonia leguminicola* (Broquist, 1985). The compound has been synthesized by several different pathways (Bennett *et al.*, 1989). These plants called locoweed showed lesions including neurological damage, habituation, emaciation and reproductive alterations such as abortion and birth defects in livestock (Van Kampen *et al.*, 1978). SW prevented the biosynthesis of complex glycoproteins by inhibiting Golgi mannosidase II activity (Tulsiani *et al.*, 1982; Elbein, 1987). Other investigators have demonstrated enhancement of antitumor activity and immunomodulating

Correspondence to: Dr. Byeong Suk Chae, Department of Environmental Engineering, Woosuk Univercity, Samrae-Up 565-800, Korea

effects of SW (Dennis *et al.*, 1989; Humphries *et al.*, 1990; Olden *et al.*, 1991). Kino *et al.* (1985) showed that SW acted as a potent immunomodulator for the treatment of immunocompromised hosts. Hino *et al.* (1985) reported that SW also enhanced the activities of the mouse immune system *in vitro*. It was found that SW enhanced concanavalin A stimulation of human lymphocyte proliferation (Myc *et al.*, 1989).

The present study was undertaken to investigate the effect of SW on the humoral immune responses of LPS.

MATERIALS AND METHODS

Animals

Male ICR mice (6 weeks of age) weighing 17-21g were used. Animals were housed individually in each cage and acclimatized for at least 7 days prior to the use. The cages were maintained at 23±2°C and 50~60% relative humidity throughout the whole experimental period. Mice were given animal chows (Jeil Ind. Ltd., Korea) and tap water ad libitum but deprived of animal chows for 16 hr prior to sacrifice.

Materials and treatment

Lipopolysaccharide (LPS; *Escherichia coli* Serotype 0127: B8, Sigma Co., Ltd., U.S.A.) or swainsonine (SW; 8α,β-indolizidine-1α,2α,8β-triol from Locoweed, Sigma Co., Ltd., U.S.A.) was dissolved in sterile saline. Mice were divided into 4 groups (10 mice/group), and LPS was given to the mice 1 hr after i.p. injection with 3.7 mg/kg of SW by i.p. injection twice a week for 14 days at a dose of 2 mg/kg. Control mice were treated with the same amount of sterile saline alone.

Lymphoid organ and body weights

Mice were sacrificed by cervical dislocation on the next day after the last LPS or SW treatment, and spleen were removed and weighed. The lymphoid organ weight ratio to body was calculated for each of mice.

Antigen preperation

Sheep red blood cells (SRBC) collected from a single female sheep were kept at 4°C in sterile Alserver's solution (pH 6.1). SRBC were washed three times with phosphate-buffered saline (PBS; Gibco Lab. Co., Grand Island, N.Y., U.S.A., pH 7.4) after centrifugation at 400×g for 10 min and diluted to provide a desired concentration by hemacytometer count.

Immunization

All mice were immunized by intravenous (i.v.) injection of 0.1 ml of SRBC suspension (1×10^8 cells/ml) 4 days prior to each assay as described by Reed *et al.* (1984).

Preparation and inactivation of serum

The blood sample from each mouse was obtained from the carotid artery. The blood was allocated to clot in polyethylene tubes at 4°C for 30~60 min. The serum was withdrawn and heat-inactivation in polyethylene tubes at 56°C for 30 min.

Hemagglutination (HA) titers

HA titer was determined in microtitration trays (Limbro Chemical Co., Inc. New Haven, Connecticut, U.S.A.) using 25 μl volume of diluent by serial dilution of inactivated pooled sera in Hank's balanced salt solution (HBSS; Gibco Lab. Co., Grand Island, N.Y., U.S.A.) in plastic microtiter plate, which was added on to 50 μl volume of 0.5% packed SRBC as described by Yoshikai *et al.* (1979). The specified plate (Flow Lab., U.S.A.) was incubated for 18 hr at 37°C. Each titration was performed in duplicate and the mean titer was expressed as $\log_2$.

2-mercaptoethanol-resistant HA (MER-HA) titer

HA titer assay in the serum treated with 2-mercaptoethanol (Sigma, Co., Ltd., U.S.A.) was the same method mentioned above, excluding the serum is diluted with HBSS containing 0.15N 2-mercaptoethanol instead of HBSS alone. Each titration was performed in duplicate and the mean titer was expressed as $\log_2$.

Preperation of spleen cells

The spleen cells from each group of mice were washed three times by centifugation and finally suspended in cold minimum essential medium (Gibco Co., Grand Island, N.Y., U.S.A.) as described by the modified method of Mishell *et al.* (1980). The cells were counted and the viability was determined by trypan blue exclusion method.

Assay of plaque forming cells (PFC)

In order to examine whether LPS or SW accelerates the antibody production aganist heterologous antigen or not, the slide technique of Cunningham and Szenberg (1968) was utilized. the number of direct PFC was counted 4 days after the immunization by i.v. with 10^7 SRBC. Numbers of PFC were expressed as those per 10^6 viable spleen cells or per spleen.

Statistical analysis

Values are expressed as means±standard error (S.E.). All data were examined for their statistical sig-

nificance of differences with Student's t-test.

RESULTS

The effects of SW on body and selected organ weights of LPS are shown in Table I. The body weight gain was significantly increased in LPS plus SW treatment group (51.70±4.69%) compared with those in LPS alone group (28.28±1.74%). The weight ratios of liver and spleen were significantly increased in all groups compared with those in control group, but there is no significant differances on the weight ratios of liver and spleen in LPS plus SW treatment group compared with those in LPS alone.

Antibody responses were performed to evaluate humoral immunity following i.p. treatment with LPS or SW using the T-dependent antigen, SRBC (Table II). Hemagglutination (HA) titers of serum to SRBC were enhanced in LPS, SW and LPS plus SW treatment groups (4.40±0.20, 4.60±0.41 and 3.60±0.42, respectively) compared with those in controls (2.60±0.20), but deceased in LPS plus SW treatment group compared with those in LPS alone. MER-HA titers were increased LPS, SW and LPS plus SW treatment groups (3.60±0.41, 3.00±0.25 and 2.80±0.16, respectively) compared with those in controls (2.20±0.11), but deceased in LPS plus SW treatment group compared with those in LPS alone.

Direct antibody plaque forming cells (PFC) responses of spleen cells to SRBC after immunization was observed in mice given LPS or SW (Table II). PFC were enhanced in LPS, SW and LPS plus SW treatment groups [292±12 ($\times 10^3$ PFC/ml), 312±17 ($\times 10^3$ PFC/ml) and 268±24 ($\times 10^3$ PFC/ml), respectively] compared with those in controls [201±21 ($\times 10^3$ PFC/ml)], but deceased in LPS plus SW treatment group compared with those in LPS alone. In PFC per 10^6 spleen cells were increased LPS, SW and LPS plus SW treatment groups (1,403±76, 1,433±59 and 1,321±80, respectively) compared with those in controls (1,284±147), but deceased in LPS plus SW treatment group compared with those in LPS alone.

LD_{50} of LPS

LD_{50} of LPS was resulted as follows with i.p. injection in male ICR mice by Litchfield-Wilcoxon method (Litchfield and Wilcoxon, 1949). LD_{50} of LPS was 25.50 mg/kg, 95% confidence limit value was 18.48~35.19 mg/kg with i.p. injection in ICR mice.

Table I. Effects of swainsonine on the liver and spleen weights of lipopolysaccharide in mice

Group	Body weight gain (%)	Liver weight / Body weight ×100 (%)	Spleen weight / Body weight ×100 (%)
Control	59.76±5.77	6.45±0.22	0.46±0.02
LPS	28.28±1.74**	6.68±0.49	0.93±0.10**
SW+LPS	51.70±4.69§§	7.22±0.14*	0.85±0.05**
SW	53.78±4.81	6.72±0.21	0.60±0.03**

LPS: Lipopolysaccharide. SW: Swainsonine. LPS was given to mice 1 hr after i.p. injection with 3.7 mg/kg of SW by i.p. injection twice a week for 14 days at a dose of 2 mg/kg. Each value represents the mean ± S.E. of 10 mice. Asterisks denote a significant difference compared with control group (*P<0.05 and **P<0.01). Section marks denote a significant between LPS and LPS plus SW groups (§§P<0.01).

Table II. Effects of swainsonine on the antibody production of lipopolysaccharide in mice

Group	HA titer (log_2)	MER-HA titer (log_2)	PFC/spleen ($\times 10^3$)	PFC/10^6 spleen cells
Control	2.60±0.20	2.20±0.11	201±21	1,284±147
LPS	4.40±0.20**	3.60±0.4	292±12**	1,403±761**
SW+LPS	3.60±0.42	2.80±0.16	268±24	1,321±80*
SW	4.60±0.41**	3.00±0.25*	312±17**	1,433±59

HA: Hemagglutination. MER-HA: Mercaptoethanol-resistant HA. PFC: Plaque forming cells. Each value represents the mean±S.E. of 10 mice. Other legends and methods are the same as in Table I (*P<0.05 and **P<0.01).

Table III. Data of LD_{50} of lipopolysaccharide in ICR mice

Dose (mg/kg)	Mortality	Observed	Expected	Observed Expected	X^2 (ci)
10.125	0/10	0 (2.6)	8	5.4	0.040
15.188	3/10	30	21	9.0	0.045
22.780	3/10	30	41	11.0	0.048
34.172	7/10	70	70	0.0	0.000
51.258	10/10	100 (95.6)	86	9.6	0.074

($\sum$ci)=0.207

DISCUSSION

In this study, the LD_{50} of LPS intraperitoneally injected in male ICR mice was 25.50 mg/kg (Table III). Engelhardt *et al.* (1990) reported that LPS increased releasing of cytokines including TNF and IL-6 in tumor patients. Carswell *et al.* (1975) found that TNF had tumor hemorrhage necrotic effects *in vivo* or *in vitro*. In addition, Taffet and Russell (1980) described that high level of LPS stimulated antitumor activities of macrophage. Kino *et al.* (1985) reported that SW at 3.7 mg/kg remarkably enhanced immunoactivities in immunosuppressed mice. Based on these reports, we selected that the doses of LPS and SW were 2 and 3.7 mg/kg, respectively.

The body weight gain was significantly increased in LPS plus SW treatment group compared with those in LPS alone (Table I). Shalaby *et al.* (1989) and Semb *et al.* (1987) showed that TNF inhibited lipoprotein lipase and remarkably decreased body weight gain in vivo. These findings indicate that LPS might decrease body weight gain by lowering fat accumulation in ICR mice, and that SW might inhibit these effects of LPS.

The weight ratios of liver and spleen were significantly increased in all groups compared with those in controls, but there are no significant differences on the weight ratios of liver and spleen in LPS plus SW treatment group compared with those in LPS alone (Table I). A report by Skidmore *et al.* (1975) has suggested that LPS acted activator of potent polyclonal B cells. A report by Olden *et al.* (1991) has shown that SW enhanced proliferation of spleen cells. These findings indicated that SW might act as antagonist for proliferation and differentiation of B lymphocytes.

Antibody responses were performed to evaluate humoral immunity following with i.p. treatment with LPS or SW using the T-dependent antigen, SRBC. Hemagglutination (HA) and MER-HA titers of serum to SRBC were enhanced in all groups compared with those in controls, but deceased in LPS plus SW treatment group compared with those in LPS alone (Table II).

Direct antibody plaque forming cells (PFC) responses of spleen cells to SRBC after immunization was observed in mice given LPS or SW. PFC were enhanced in all groups compared with those in controls, but deceased in LPS plus SW treatment group compared with those in LPS alone. In addition, PFC per 10^6 spleen cells were also increased in all groups compared with those in controls, but deceased in LPS plus SW treatment group compared with those in LPS alone (Table II). Hang *et al.* (1983) published that LPS promoted proliferation and differentiation of B lymphocytes *in vitro*. Coutinho *et al.* (1974) found that LPS acted as potent B lymphocyte promotors and induced B lymphocytes without helper T cells *in vivo* or *in vitro*. Persson (1977) showed that LPS suppressed humoral immune responses to thymus-dependent antigen *in vivo* or *in vitro*. Kino *et al.* (1985) reported that SW enhanced immune activities to normal level in immunodeficient mice. On the basis of these findings, we propose that each LPS or SW enhance humoral immune responses by the activating lymphocytes and the increasing immunoglobulins synthesis. However, LPS plus SW treatment have lower the increasing rate of humoral responses than LPS alone. Thus, authors think that SW may act as antagonist on the asntibody production of LPS. Furthermore, numbers of the studies are demanded to elucidate acute mechanism of the action.

In conclusion, humoral immune responses of mouse were less increased by LPS plus SW treatment than LPS alone.

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