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Inhibitory Effect of *Scutellaria baicalensis* Root Extract on Chemical Mediator Release and Immune Response

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ABSTRACT : Inhibitory effect of *Scutellaria baicalensis* ethanol extracts (SR) on chemical mediator release and immunoglobulin (Ig) production from Sprague-Dawley rats originated cells as type I allergic reaction was examined. SR showed concentration-dependent inhibition on basal and concanavalin A (ConA)-stimulated Ig production. In the mesenteric lymph node lymphocytes, the inhibitory effect of SR on the IgE production in the presence of Con A was stronger than these on IgA and IgG production. Moreover, tumor necrosis factor-alpha (TNF-α) production-inhibiting effect of SR in the presence ConA was observed. However, SR did not affect the production of interferon-γ. SR also inhibited histamine release from the peritoneal exudate cells stimulated with a calcium ionophore A23187. In the case of leukotriene B4, SR markedly inhibited it at the concentration of 100 ㎍/㎖. From these results, ethanol extracts obtained from *Scutellaria baicalensis* may have an anti-allergic effect on the intestinal system of rats.

Key words **:** *Scutellaria baicalensis* ethanol extract (SR), chemical mediators, Ig production, TNF-α, allergy

INTRODUCTION

Scutellaria baicalensis has long been used as the chinese medicine or as medicinal plant in oriental countries for various purposes. It has recently been reported that *Scutellaria baicalensis* had diverse pharmacological effects (Kimura *et al.*, 1982). *Scutellaria baicalensis* ethanol extracts (SR) have very potent antioxidative action in vivo as well as in vitro (Lim *et al.*, 1998 and 1999; Choi *et al.*, 2000), and much more potent than β-carotene (Kim *et al.*, 1996). However, the allergic background of this has not been totally appreciated. We therefore examined the effect of SR on allergic activity by using peritoneal exudates cell (PEC) and mesenteric lymph node (MLN) lymphocyte.

Immunoglobulin (Ig) production by lymphocytes is class-specifically regulated by cytokines (Pene *et al.*, 1988; Gauchat *et al.*, 1991; Elson and Beagley, 1994), and some bioproducts can modify the process (Yamada *et al.*, 1996). For example, bile acids and unsaturated fatty acids inhibit the production of these antibodies in spleen or MLN lymphocytes (Lim *et al.*, 1995). In addition, concanavalin A (ConA) modulates IgE production in rat MLN lymphocytes (Lim *et al.*, 2000). Lectins are plant glycoproteins that regulate proliferation and Ig production of lymphocytes (Gianni *et al.*, 1980). Yamada *et al.* (1996) reported that antioxidants are partly responsible for the enhancement of IgE level induced by food components. The modification of IgE productivity may affect the incidence of various diseases through induction of hypersensitivity (Vollenweider *et al.*, 1991) and immunodepression (Newble *et al.*, 1975). Therefore, we examined the effect of SR on Ig and cytokine production in MLN lymphocytes induced by ConA.

The type I allergic reaction includes a series of events: production of antigen specific IgE, followed by its binding to the FceRI receptor on mast cells or basophils, cross-linking of newly absorbed allergens with IgE, and release of chemical mediators such as histamine and leukotriene from mast cell (Plaut *et al.*, 1993). The release of histamine and other chemical mediators from mast cells is involved in pathogenesis of acute allergic reactions and inflammatory responses. The research into the inhibition of histamine secretion from mast cells and basophils by flavonoids (Pearce *et al.*, 1984) is special interest. Some biomaterial components have been reported to affect the above reaction (Yamada *et al.*, 1990; Watanade *et al.*, 1994).

Since SR exerts its effect entirely, but not exclusively, we focused this study on the response of chemical mediators,

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immunoglobulin and cytokine production. This is the report describing the significant effect of SR on allergic parameters in MLN lymphocytes and PEC cells from Sprague-Dawley rats.

MATERIALS AND METHODS

Materials

Concanavalin A was purchased from List Biological Lab. (Campbell, CA). SR was dissolved in phosphate buffered saline (PBS, pH 7.4) and used for cell culture experiments. For enzyme-linked immunosorbent assay (ELISA) of rat Igs, 0.05% Tween 20 in PBS (TPBS) for rinsing and Block Ace (Dainihon Pharmaceutical Co., Osaka) was used for blocking and dilution of antibodies as described previously (Lim *et al.*, 1995 & 1996).

A calcium ionophore A23187, prostaglandin B_2 (PGB_2) and leukotrienes B_4 (LTB4) were purchased from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (BSA) was purchased from Boehringer Mannheim GmbH (Germany). Tyrode buffer, containing 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 5.6 mM glucose (pH 7.2) was used for histamine release assay.

ELISA kits for determination of interferon-gamma (IFN-γ) and tumor necrosis factor (TNF-α) were purchased from Biosource International Co. (Camarillo, CA).

Preparation of SR extracts

Scutellaria baicalensis was ground to a fine powder with a grinder. Powder (3 kg) was extracted with 70% ethanol for 3 hr at room temperature. The extract was filtered, and the filtrate was concentrated under reduced pressure.

Preparation of MLN and PEC

All animal-care techniques were performed within the guidelines approved by the Institutional Animal Care and Use Committee. MLN was excised from Sprague-Dawley rats (9 weeks-old) under diethyl ether anesthesia and lymphocytes were squeezed out into the RPMI 1640 medium. After incubating the cells at 37℃ for 30 min to remove fibroblasts, 5 ㎖ of the cell suspension was layered on 4 ㎖ of Lympholyte-Rat (Cedarlane, Hornby, Canada) and centrifuged at 1,500 ×g for 30 min. The lymphocyte band at the interface was recovered and the cells were rinsed three times with the RPMI 1640 medium. The lymphocytes were cultured in a 10% FBS/RPMI 1640 medium and the Ig content of the culture supernatant was measured by enzyme linked immunosorbent assay (ELISA) (Enagvall and Perlman, 1959).

Tyrode buffer containing 0.1% (wt/vol) BSA was injected into the peritoneal cavity and the abdomen was gently massaged for 2 min. The peritoneal cavity was then opened, and the fluid containing the PEC was collected with a Pasteur pipette. Cells were gently washed with Tyrode buffer and centrifuged at 200 ×g for 10 min at 4℃. The cell pellets were resuspended in a modified-ammonium chloride buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 10 mM EDTA-2Na, pH 7.4) and then incubated for 5 min at 4℃. The cell suspension was centrifuged at 200 ×g for 5 min at 4℃ and the cells were resuspended in the Tyrode buffer. Cell viability was measured by trypan blue staining and toluidine blue staining identified mast cells. Viability of this preparation was more than 95% and mast cells occupied 5-10% of the total cells. The supernatant from each 72 hr ConA-activated MLN lymphocytes culture obtained. Contents of IFN-γ and TNF-α were measured using ELISA kits (ASY-18 and KRC 3012, Biosource International Products, Camarillo, CA for IFN-γ and TNF-α, respectively).

Enzyme-linked immunosorbent assay of rat antibodies

Measurements of Igs were executed using sandwich ELISA methods, as reported previously (Lim *et al.*, 1995 & 1996).

Measurement of histamine and leukotriene B4

PEC (1×10^6 cells) were suspended in 2 ㎖ of Tyrode buffer containing 0.9 mM $CaCl_2$ in polypropylene tubes at 4℃. The cell suspension was mixed with 0.25 ㎖ of various concentrations of the SR solution, and 0.25 ㎖ of 50 μM A23187 solutions, and incubated for 20 min at 37℃. The reaction was performed by incubating for 15 min at 4. The cell suspension was then centrifuged at 300 ×g for 10 min and the content of histamine in the supernatants was measured. The histamine content was measured by the fluorometric assay (Shore. 1959). PEC (1×10^6 cell) were suspended in 40 ㎕ of Tyrode buffer containing 0.9 mM $CaCl_2$ in polypropylene tubes at 4℃. The cell suspension was mixed with 5 ㎖ of various concentrations of SR solution and 5 ㎕ of 50 μM A23187, and incubated for 20 min at 37℃. The reaction was terminated by adding 50 ㎕ of the mixture of acetonitrile: methanol (30 : 25, v/v) and kept at −30℃ for 15 min. To measure LTB4, the internal standard, 50 ng of PGB_2, was added to the cell suspensions and centrifuged at 300 ×g for 10 min. The supernatant was filtrated through 0.22 ㎛ filter (Millipore, Tokyo), and LTB4 was measured by reversed-phased HPLC (SCL-10A, Shimadz Co., Kyoto) as described previously (Powell, 1987).

RESULTS

Effect of SR on immunoglobulin production in MLN lymphocytes

The effect of SR on Ig production by MLN lymphocytes

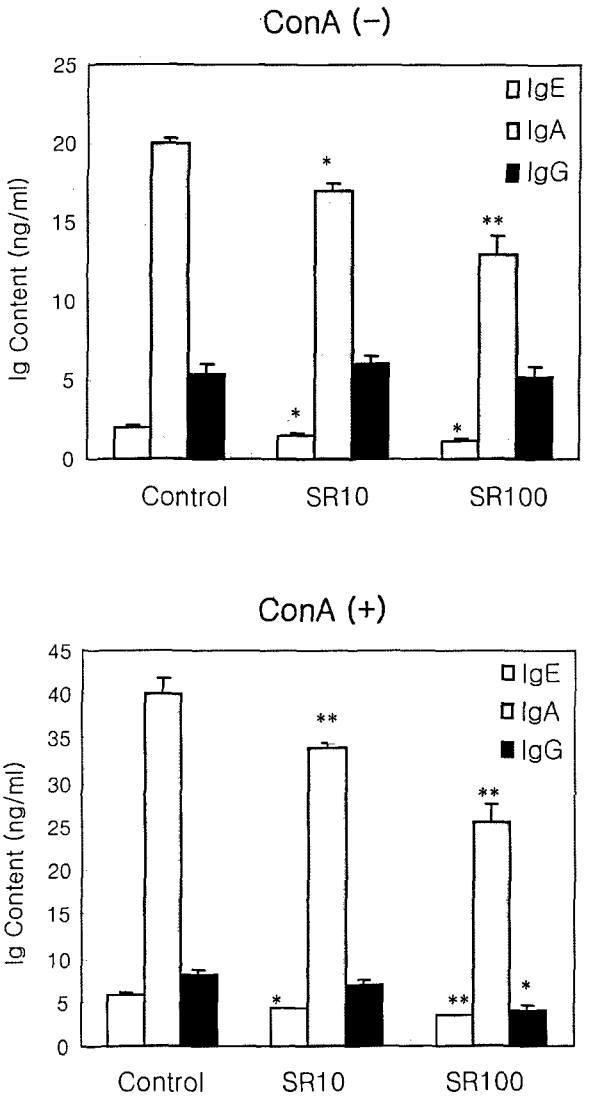

Fig. 1. Effect of SR on IgE, IgA and IgG production in rat MLN lymphocytes. Rat MLN lymphocytes (2×10^6 cells/mℓ) were cultured for 3 days with SR in the absence or in the presence 25 μg/mℓ of ConA, and the Ig contents in the culture supernatants were measured by ELISA. Results are the mean ± SE (n = 3). Significantly different from Control at $^*P < 0.05$ and $^{**}P < 0.01$.

was examined to estimate their activity against the gut immune system. As shown in Fig. 1, in the absence of ConA, IgE and IgA production in MLN lymphocytes from rat treated by 100 mg/mℓ of SR was lower than control group. However, SR did not affect IgG production in MLN lymphocytes. When the cells were cultured with ConA, it significantly enhanced Ig production. In this case, SR showed a tendency to inhibit IgA and IgG production at 10 mg/mℓ. The inhibition on IgE, IgA and IgG production was obvious at 100 mg/mℓ of SR. As a result, the inhibitory effect on the IgE production of SR in the presence of ConA was stronger than on IgA and IgG production at 10 mg/mℓ.

Table 1. Cytokine concentrations in rat MLN lymphocytes

Additives (mg/mℓ)	IFN-γ (pg/mℓ)	TNF-α (pg/mℓ)
ConA (-)	<10.9	< 7.8
SR10	<10.9	< 7.8
SR100	<10.9	< 7.8.
ConA (+)	1096.5 ± 14.1	1200.6 ± 17.4
SR 10	1067.7 ± 17.8	997.5 ± 7.8*
SR100	1068.2 ± 11.3	785.4 ± 6.9*

Effect of SR on cytokine production in MLN lymphocytes

Various types of lymphokines specifically regulate Ig production by class. To examine how cytokines are involved in observed effect of SR on Ig production, the levels of IFN-γ and TNF-α in the culture supernatant were measured. When lymphocytes were cultivated for 72 hr without ConA, the concentration of IFN-γ and TNF-α was below the detection limit. In the presence of ConA, the cells produced fairly high levels of IFN-γ and TNF-α production. In the presence of ConA, SR significantly inhibited TNF-α production at both 10 and 100 mg/mℓ. On the other hand, the effect of SR on the production of IFN-γ was not significant (Table 1).

Effect of SR on histamine release by A23187

When PEC was stimulated with calcium ionophore A23187, histamine concentrations were significantly higher in PEC than that in cells without A23187 (Fig. 2A). However, the increase of histamine release was significantly inhibited by SR at the concentrations of 10 and 100 mg/mℓ.

Effect of SR on LTB_4 release from peritoneal exudate cells

In addition to the effect of SR on the release of histamine, we examined its effect on the production of LTB_4, a mediator produced by the stimulation of mast cells (Fig. 2B). SR showed a significant inhibition on A23187-induced LTB_4 production at a concentration of 100 mg/mℓ.

DISCUSSION

Scutellaria baicalensis well known as antioxidant roles are publicized for their putative efficacy against the incidence of major diseases such as arteriosclerosis, cancer and diabetes

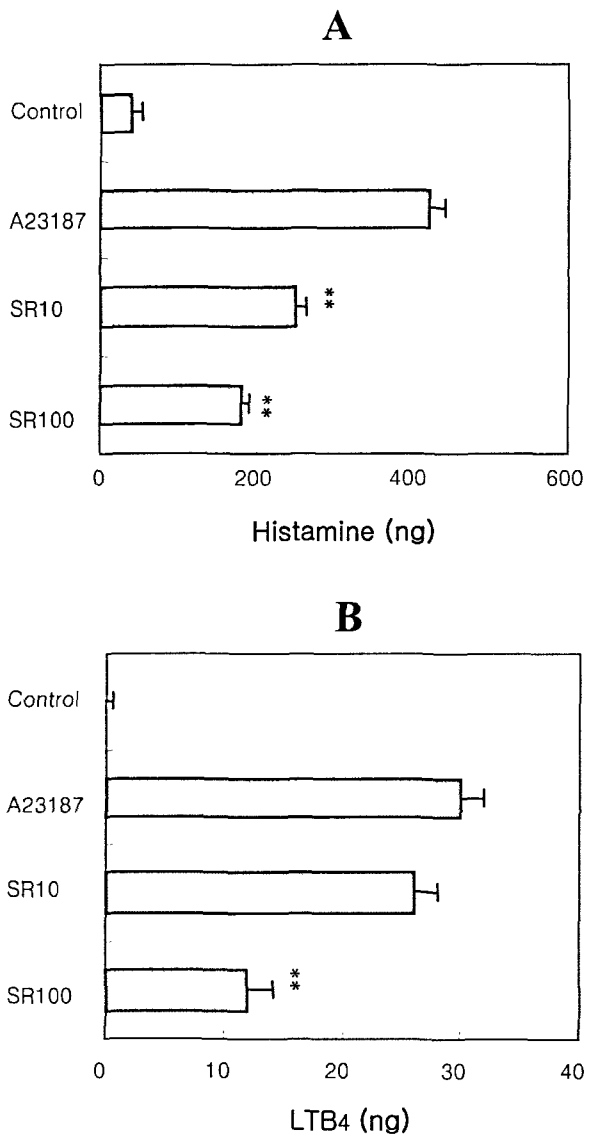

Fig. 2. Dose-dependent effect of SR on histamine and LTB_4 release from PEC stimulated with A23187. Cells (1×10^6 cells/㎖) were incubated for 20 min in the presence of 10 and 100 ㎎/㎖ of SR. Histamine content of the supernatant was measured by a fluorometric assay. The cells were stimulated with A23187. Results are the mean ± SE (n = 3). Significantly different from Control at $*P < 0.05$ and $**P < 0.01$.

(Huang *et al.*, 2006; Kowalczyk *et al.*, 2006). *Scutellaria baicalensi* contains a group of polyphenol that is ubiquitous in plant. We studied SR because of our previous finding of their potent antioxidative effect on the peroxidation of phospholipid bilayers from free radical attack (Lim *et al.*, 1999; Choi *et al.*, 2000; Kim *et al.*, 1996). Also, it has been reported that *Scutellaria baicalensis* (70% methanol extract) and its components, baicalein, baicalin and wogonin possess anti-hyperlipemic action and anti-inflammatory action in rats (Kimura *et al.*, 1982; Kimura *et al.*, 1985). In addition, Pham *et al.* reported antioxidant is responsible for the inhibition of IgE level (1997). Therefore, we attempted to document SR's allergic activity in a biological system.

Lectins are plant glycoprotein that regulates proliferation and Ig production of lymphocytes (Yamada *et al.*, 1993). In addition to ConA, several T cell mitogens such as anti-CD3 antibodies and phytohemmaglutinin have been used in immunological studies. Because some of these mitogens may stimulate selected lymphocytes populations, in this study we used ConA, which stimulates IgE production by MLN lymphocytes in culture and induces the production of those cytokines which enhance IgE production (Lim *et al.*, 2000). SR significantly inhibited the stimulation of IgE, IgA and IgG production by ConA (Fig. 1). The inhibitory effect of SR on the IgE production was stronger than IgG and IgM production. SR inhibited the Ig production nonspecifically. Kakumu *et al.* (1983) reported that cyanidanol suppressed Ig synthesis at high concentration. You *et al.* (1998) reported a *Vitaxcarpin*, a flavonoid from the fruit of vitex rotundiflia, inhibits mouse lymphocyte proliferation and growth of cell lines in vitro. This observation could suggest that suppression of lectin-induced Ig production and mixed lymphocytes culture reaction by SR have some common mechanism. Our results clearly demonstrated that SR inhibited the actions on Ig production by ConA-induced MLN lymphocytes.

Ig production is class specifically regulated by various types of lymphokines (Gauchat *et al.*, 1991; Elson and Beagley, 1994). To examine how cytokines are involved in the observed effects of SR on Ig production in MLN lymphocytes, the levels of IFN-γ and TNF-α in culture supernatants were measured. In the presence of ConA, SR inhibited TNF-α production at 10 and 100 ㎎/㎖ (Table 1). On the other hand, the effect of SR on the production of IFN-γ was marginal. Th1 cells produce IL-2, IFN-γ and lymphotoxin, whereas Th2 cells produce IL-4 and IL-5 (Fiorentino *et al.*, 1989). Both population of T cells produce TNF-α, and TNF has been suggested to have pro-inflammatory role (Bharat and Jordan 1992). Therefore, we suggested that the effect of SR might possibly contribute to the known anti-inflammation.

Histamine is a typical preformed mediator and causes various pathophysiologic events in acute allergic reactions (Kaliner *et al.*, 1982). In the present study, the effect of SR on histamine release from rat PEC was examined. SR significantly inhibited the release of histamine in the concentration 10 and 100 ㎎/㎖ (Fig. 2A). Since histamine not only has a potent bronchoconstricting effect, but also increase vascular permeability, and hence, promotes edema and pain that are

characteristic of inflammation response (Fennesy, 1986). Leukotriene (LT) is newly generated mediators produced from membrane phospholipids after stimulation of mast cells. LTB_4 has inflammatory effects as characterized by neutrophil chemotaxis (Ford-Hutchinson *et al.*, 1980). In addition, LTB_4 also enhances expression and release of soluble CD23 through the activation of B lymphocytes, and induces IgE production (Dugas *et al.*, 1990). SR inhibited A23187, a Ca^{2+} ionophore, induced LTB_4 release in this study, It thus, is suggested that SR may inhibit LTB_4 release via reducing Ca^{2+} uptake into the cell or membrane fluidity (Fig. 2B). LTs are generated by the activation of phospholipase A2, an intracellular Ca^{2+}-dependent enzyme, and by the oxidation of fatty acids with lipoxygenase (Dahlen *et al.*, 1986; Kawabe *et al.*, 1987). It is evident that SR has an ability to protect LTB_4 release by A 23187, and this effect may partly explain LTB_4-protective mechanism of SR against membrane fluidity or intracellular Ca^{2+} uptake.

When one considers the response of IgE, histamine and LTB4, it is likely that SR can alleviate the type I allergic reaction. It is possible that SR may affect the common pathway related to the release of histamine and LTB_4.

Our study suggest the possibility of clinical applications of SR based on it antiallergic effects. Although the mechanism by which the SR modify allergic indices is not apparent at present, the current observations opened a new aspect of the pharmacological role of *Scutellaria baicalensis*.

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