Studies on Bioresponses of Sophorae Radix(I)

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INTRODUCTION

From ancient period, it is well known that Sophorae Radix(Sophora flavescens) drains Damp Heat, disperses Wind, and clears Heat. Accordingly, this herb has been used for dysentery, skin lesion with chronic itching, jaundice, urinary dysfunction, and edema in the oriental medicine(1). In modern studies, it has been reported that extract of Sophorae Radix showed the depressive effects on blood pressure, heart rate(2), and protective effect on HCL/ethanol-induced ulcer(3).

An major components of Sophora flavescens consist of a various alkaloids matrine, oxymatrine, anagyrine, baptifoline, lupine, and flavoids isoanhydrocariin, N-anhydrocariin etc(4). Many researchers have been paid attention to obtain the biologically effective principles from natural herb sources. In the course of these studies, sofranol, kararinone isolated from Sophorae Radix has been known that posses sedative, hypotonic, anticonvulsive activities(5), and aorta relaxative activity(6). Matrine, one of main component s in many plants as well as in Sophorae Radix(7), has been shown to inhibit some biological activities such as contraction of smooth muscle(8), heart arrhythmia(9), CNS transmission(10), cell proliferation(11), and to stimulate plant growth and development(12). Additionally, this substance has been demonstrated to have antipyretic, antiinflammatory properties(13, 14). Another reports suggested that antiinflammatory agents may influence a variety of immune responses, in part, by modulating synthesis of prostaglandins and leukotrienes(15). Therefore, we supposed that Sophorae Radix containing matrine may also affect immune

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functions, and attempted to examine the immune responses of this herb in mice.

〈Abbreviation〉 SRE; Sophorae Radix Extract,
PFC; plaque forming cells
DH; delayed hypersensitivity

EXPERIMENTAL METHODS

Animals

Male Balb/c and male ICR mice were purchased from Laboratory Animal Center of Seoul National University, 6 to 8 weeks of age, weighing 18~22 grams, were used. The animals were housed 5 per cage in polypropylene cages on hard wood chips and acclimatized for at least 5 days prior to use. The animal room was mechanically maintained on a diurnal cycle of 12 hours interval. The room temperature was maintained at 20~24°C and relative humidity at 50~60%. The defined laboratory rodent chow and municipal tap water were provided **ad libitum**.

Sample preparation and treatment

Sophorae Radix were purchased from Kyung-dong Korean market in Seoul. The 0.5kg of this Radix was disintegrated and extracted in boiling water for 8 hours, and then concentrated to the volume of 500ml using evaporator. Decoction extracted from Sophorae Radix was administered orally for 10 days at the dose 10ml/kg and 25ml/kg. All treatments and sampling were made at 9 : 00~10 : 00 AM, taking into consideration of the aspect of immune responses(16).

Circulating leukocytes and relative immunorgan weights

Blood samples were collected via retroorbital plexus with heparinized capillary and the number of leukocytes were determined on hemacytometer. Body, spleen, liver, thymus were weighed and relative weight were calculated.

Delayed Hypersensitivity and Arthus reaction

Male ICR mice were sensitized by subcutaneously injecting 100g of bovine serum albumin (Sigma) esmulsified in complete Freund's adjuvants(Sigma) at the base of tail. Seven days later delayed hypersensitivity and arthus reation were elicited by challenging mice in the footed with 30ul of 2% heat aggregated BSA in saline, as previously described(17, 18). At 3 and 24hours post-challenge, footpad swelling thickness was measured with micrometer (Mitsutoyo, Japan) and the extent of swelling was calculated by substracting the thickness of the negative footpad from that of the antigen injected footpad.

in vivo phagocytosis

Intravascular phagocytosis by reticuloendothelial system, particulary kupffer cell and splenic macrophage, was evaluated in ICR mice. Colloidal carbon(80mg carbon/ml, 2~2.5 um in diameter, pelican drawing ink, D-3000 Hannover 1, 17 Black India, Germany) suspended in 1% gelatin-saline was injected intravenously at a dose of 16mg carbon per 100 grams of body weight, as previously described (19, 20). Blood was collected from retroorbital plexus at 5 minutes interval after carbon administeration. Aliquot of blood(20ul) was lysed in 2ml of 0.1% sod. carbonate and carbon absorbances were determined using a UV spectrophotometer at 600nm. Phagocytosis was expressed as the phagocytic index and corrected phagocytic index.

Plaque forming cell assay

Splennic IgM antibody forming cells to thymic dependent antigen, sheep RBC were quantified by the modified Cunningham's liquid monolayer slide method, 4 days following a single intraperitioneal injection with 0.2ml of a 2% suspension of sheep RBC(8×10^7 cells) in male CBA/J inbred micel(21, 22). Spleen single cell suspension was prepared in BSS(PH 7.2) using a stainless #200 sieve and adjusted to a concentration of 2×10^7/ml. Reaction mixture was composed of spleen cell suspension(50ul), 12.5% sheep RBC(100ul), 1/5 diluted complement(100ul, Gibco) and BSS(250ul). The capacity of microchamber was slightly more than 50ul. Reaction mixture(50ul) was pipetted into each chamber in quadraplicate

per sample.

Stastical analysis

Student's t-test was employed to assess the statistical significance. Values which differ from control over $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

The immune network in animal involves a complex series of interrelated and mutually regulatory events. Under normal conditions, this scheme functions efficiently to get rid of the foreign materials and organisms(23). If the immune regulatory system is disordered, the host may be lack in immune response sufficient to eliminate detrimental antigen, resulting in widespread infection or malignancy. Conversely, if the immune responses become aberrantly excess and percieve self-antigen as being foreign, autoimmune disease may be developed(24). Thus, the potentiation or suppression of immune responses induced by exogenous influences may be beneficial and achievable goal in immunotherapy. Additionally, a lot of study have been made to elucidate the mechanism of potentiator and suppressor activities, and to isolate the more effective biological response modifier(25, 26).

In this study, we performed some preliminary test, to examine the possibilities of SRE as biological response modifier, including circulating leukocytes counts, immunoorgan weights, Arthus reaction, delayed hypersensitivity, carbon clearance test, and plaque forming cells.

Circulating leukocytes counts of control group in normal male ICR mice were about 7,000/ul(Table I). This result was consistent with the previous reports(27). Treatment with high and low dose of SRE showed no significant difference compared with control, but slightly increased in the case of 25ml/kg administeration. To elucidate the precise immune cell composition, further investigation on PMN, B-lymphocytes, plasma cell, monocytes, and macrophases should be performed. Immunoorgan weights including spleen, liver were not changed in experimental animals(Table I).

In order to examine the humoral immunity, the effect of SRE on Arthus reaction in normal male ICR mice and thymus dependent antigen, SRBC, in normal Balb/c mice were

〈Table I〉 **Effects of SRE on the circulating leukocytes and immunoorgans**(spleen, liver) **in normal young male ICR mice.**

Group	Number of mice	Circulating leukocytes/ul	Spleen/body (10^{-3})	Liver/body (10^{-3})
Control	6	7013±238	5.23±0.58	5.74±0.20
SRE(10ml/kg)	6	7950±338	5.30±0.75	5.89±0.33
SRE(25ml/kg)	6	7050±941	5.29±0.42	5.45±0.29

SRE; were administered per orally for 10 days. Each value represents mean+SE

〈Table II〉 **Effects of SRE on the IgM plaque forming cells in normal male Balb/c mice*,**

Group	(N)	IgM PFCs/10^6 spleen cells	IgM PFCs/spleen
Control	5	641± 85	55,800± 9,100
SRE(10ml/kg)	5	610±274	50,700±27,100
SRE(25ml/kg)	5	731±175	66,800±19,600

* Mice were immunized with 0.2ml of 2% SRBC(4×10^8cells/ml) intraperitoneally. Four days after the immunization, assay was made. The values represent the arithmetic mean+standard error from 5 mice.

measured. AMH was induced by challenging ICR mice with 2% HA-BSA. Mice presentized with Bovine Serum Albumin(BSA) in Freund's Complete Adjuvant(FCA) have the high contents of circulating antibodies. Aggregates of precipitating IgG and IgA class stimulate the release of lysosomal enzymes in neutrophil. Challenged antigen-antibody complexes in blood vessels fix and activate complement. Neutrophil chemotaxis by Csa, C, and phagocytosis by those complexes are followed by the secretion of injurious inflammatory mediators from neutrophil. As described in Table Ⅳ, AMH was not altered by SRE. And, SRE showed no significant effect on the IgM PFCs in high and low dose(Table Ⅱ). This result implicate that SRE may not involved the synthesis or secretion of precipitating IgG antibody, complement fixation, and neutrophil chemotaxis.

〈Table III〉 **Effects of SRE on the** *in vivo* **phagocytosis of carbon as macrophage function in male ICR mice.**

Group	N	Phagocytic index(P.I.) (10^{-3})	corrected P.I.*
Control	6	2.73±0.29	5.62±0.26
SRE(10ml/kg)	6	2.15±0.39	5.08±0.36
SRE(25ml/kg)	6	2.35±0.32	5.33±0.23

* Phagocytic index and corrected phagocytic index was calculated according to Stuart's methods. The data represent the arithmetic mean+standard error.

〈Table IV〉 **Effects of SRE on the arthus and delayed hypersensitivity(DH) reaction in male ICR mice*.**

Group	Number of mice	Footpad Arthus	swelling thickness(10^{-1}mm) delayed hypersensitivity
Control	6	7.85±0.83	8.40±0.70
SRE(10ml/kg)	6	8.90±0.48	5.35±0.92
SRE(25ml/kg)	6	7.74±0.71	7.92±1.11

* Groups of ICR mice were sensitized to 100 ug BSA in CFA subcutaneously at the base of the tail. Seven days following sensitization, the mice were challenged with saline or 30 ul of 2% HA-BSA in the footpad. At 3 and 24 hours after challenge the increase in thickness of the footpad was determined. The value represent the arithmetic mean±standard error of the swelling response.

In delayed Hypersensitive Reaction, SRE exhibited significant decrease in low dose(Table Ⅳ). DH test has been considered as a typical in vitro model for assessing immunomodulation of cell-mediated immunity. Measurement of the thickness of swelling foot pad in mice or rats has been known to be quantitative, sensitive, and simple method to perform accuratly immunomodulation induced by chemicals. DH reaction involves the sequential recognition of challenged antigen by the specific presensitized T cells, immobilization of T cells at the site of challenge followed by production and release of lymphokines which mediate vasodilation, accumulation of macrophage and destruction of challenged antigen.

The involvement of macrophages in immune responses have been well known for many years. The basic functional property of a macrophage is its ability to engulf and remove foreign and effete materials. A second function attributed to the macrophages is antigen presentation(28). In normal mice, SRE slightly decreased phagocytic index and corrected phagocytic index. This result might be due to its direct cytotoxicity, and it is consistent with the previous report(11) in which matrine, one of main component, inhibited immune cell function.

In summary, SRE might posses a suppressive activity in cell-mediated immunity, but not in humoral immunity. SRE didn't affect the phagocytic function of macrophages.

REFERENCES

1. 中藥大辭典, 江蘇新醫學院(1979), 1283.
2. Dai, S., Chan, M.-Y., Lee, S.-S., Ogle, C.W. (1987) ; Effects of Sophora-flavescens ait. on hemodynamics and ventricular fibrillation threshold in anesthetized dogs., Am J. Chin. Med.

15 : 53

3. Yamahara, J., Mochizuki, M., Fujimura, H., Takahisi, Y., Yoshida, M., Tomimatsu, T., Tamai, Y. (1990) ; Antiulcer action of Sophora flavescens rootand an active constituent I., J. Ethnopharmacol. 29 : 173.
4. Cui, J., Zhang, G. (1986) ; Analysis of alkaloid constituent in four Sophora species., Zhongyao Tongbao 11 : 102.
5. Abdel-Raheem, M.H., Abbel-Baky, A.M. (1987) ; Preliminary pharmacological properties of sofranol., Fitoterapia 58 : 295
6. Yamahara, J., Kobayasi, G., et al. (1990) ; Vasodilatory active principles of Sophora flavescens root., J. Ethnopharmacol 29 : 79
7. Wei, H., Li, Z., Wang, D., Hao, C., Fan, G. (1988) ; Distribution and determination of alkaloids in the aerial parts of Sophora flavescens Alit., Hebei Yixueyuan Xuebao 9 : 241
8. Yamazaki, M., Arai, A. (1985) ; On the contractile response of fundus strip from rats to matrine, An alkaloidal response of Sophora flavescens., J. Pharmacobio-Dyn. 8 : 513
9. Zhang, B., Wang, N., Li, X., Kong, X., Cai, Y. (1990) ; Antiarrythmic effects of matrine., Zhongguo Yaoli Xuebao 11 : 253
10. Ishida, M., Shinozaki, H. (1984) ; Glutamine inhibitory action of matrine at the crayfish neuromuscular junction., Br.J. Pharmacol 82 : 523
11. Chen, W., Xie, D., Wu, Q., Zhang, H. (1985) ; d-Matrine-a potent immunosuppressant., Soshiran 2 : 207
12. Zhao, Z., Li, G., Zhen, Q., Shi, Y., Chen, R. (1988) ; Study ot the plant growth-stimulating activities of matrine., Zhiwu Xuebao 30 : 614
13. Cho, C.H., Chung, C,Y., Chen, C.F. (1986) ; Study of the antipyretic activity of matrine, A lupine alkaloid isolated from Sophora subprostrata., Planta Med. 5 : 343
14. Cho, C.H., Chung, C.Y. (1986) ; Study of the anti-inflammatory action of matrine, An aslkaloid isolated from Sophora subprostrata., ICRS Med. SCI. 14 : 441
15. Ball, S.S., Weindruch, R., Walford, R.C. (1986) ; Antioxidants and the immune response., Modern Aging Res. 8 : 427
16. Ferandes, 6., Halbrg, F., Yunis, E.J., Good, R.A. (177) ; Circadian rhythmic plaque forming cell response fspllen from mice immunized with SRBC., J. Immunol. 117 : 962
17. Austan, K.F. (1974) ; Reaction mechanisms in the release of mediators of immediate Hyper sensitivity from Human lung tisse., Fed. Pro. 33 : 2256
18. Henningsten, G.M., Koller, L.D., Exon, J.H., Talcott, P.A., Osborne, C.A. (1984) ; A sensitive delayed "type of hypersensitivity "model in the rats for assesing in vivo cell mediated immunity., J. Immunol, Method 70 : 153
19. Biozzi, G., Benacerraf, B., Halpern, B.N. (1954) ; Quantitative study of the granulopectic activity of the reticuloendothelial system., Brit. J. Exp. Pathol. 34 : 441
20. Stuart, A.E., Habesham, J.A., Davison, A.E. (1978) ; The carbon clearance test for in vivo phagocytosis, in "Handbook of experimental immunology(vol. 2)", Weir, "D.M., Blackwell Sci., 28 : 1
21. Taylor, G.M., Bennett, J. (1973) ; A modified plaque test for the detection of cells forming alloantibody to alloantigens., J. Immunol. Meth. 2 : 213

22. Kissinger, R., David, M.A. (1984) ; Improvements to the plaque assay for antibody secreting cells., J. Immunol. Meth. 66 : 377.
23. Benacerraf, B., Unanue, E.R. (1979) ; Text book of immunology, Williams & Wilkins, Baltimore.
24. Immunopotentiation. Ciba foundation symposium 18, Amsterdam, Associated Scientific Publishers. (1973)
25. Strack, M., Walder, S., Lyver, A., et al. (1990) ; Phase Ⅱ trial of fluorouracil and recombinant alpha 2a interferon in patients with advanced carcinoma of the esophagus., Proc. Am. Soc. Clin. Oncol. 9 : 119.
26. Stockman, G.D., Heim, L.R., South, M.A., Trentin, J.J. (1973) ; Differential effects of cyclophosphamide on the B and T cell compartments of adult mice., J. Immunol 110 : 277
27. Melby, E.C. Jr., Altman, N.H. (1976) ; Handbook of laboratory animal science., CRC Press, Cleveland.
28. Unanue, E.R. (1972) ; The regulatory role of macrophages in antigenic stimulation., Adv. Immunol. 15 : 95

=국문 초록=

苦蔘(Sophorae Radix)의 생체 반응에 대한 연구(Ⅰ)

강 탁 림·황 귀 서*

淸熱燥濕, 祛風殺蟲의 효능이 있는 苦蔘의 체내 면역활성에 미치는 영향에 대해 실험한 결과 아래와 같은 지견을 얻었다. ICR 생쥐에 苦蔘 수침액을 투여한 결과 백혈구수 및 면역장기 변화는 없었다. in vitro실험에서 IgM PFCs에 유의성 있는 영향을 주지 못했고, BSA를 주사한 생쥐에서의 Arthus 반응에도 영향이 없었다. 그러나 지연형 과민반응을 억제하였으며, 비특이적 면역에 관여하는 macrophage의 탐식능을 약간 저하시켰다. 이상의 결과 苦蔘 水浸液은 세포성 면역을 억제하는 것으로 사료된다.

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