Studies on Bioresponses of Galangae Rhizoma(I)

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INTRODUCTION

In spite of the tremendous efforts made by numerous researchers throughout the world, no decisive cancer chemotherapeutic agent has appeared except for a few that have been found effective for some leukemias and for the tumors having special characteristics. Anticancer agents that attack cancer cells directly may exhibit the toxicity to the normal host cells, in part, by destroying immune cells which play an important role in the mechanism of a specific protective response to a noxious agent of organisms(1, 2). This seems to be some limitation in application of the chemotherapeutic agents. Therefore, development of a substance which attacks cancer without damaging host cells, or potentiates host immune function may be one of the most useful means of opening a new way for the arrest of cancer.

Galangae Rhizoma(Alpinia officinarum) which warms the Middle and alleviates pain, has been previously used as a decoction for epigastric pain, abdominal pain, vomitting hiccough or diarrhea from Cold in the Middle Burner in the oriental medicine(3). In modern medicinal studies, extract of this herb has been reported to show a stimulatory effect on intestinal smooth muscle(4). It has also been reported to possess antimicrobial activities against many pathogenic bacteria(5), and be effective preservative for fruit and vegetables (6). But the information is lack about the effect of Galangae Rhizoma on immune functions.

From ancient period, a number of medicinal herbs have been used for treatment of various types of diseases. Recently, some of them has been reported to stimulate immune functions

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(7,8,9,10). These suggests that biological response modifiers may be isolated from natural sources, and these herbs extract may be use as therapeutic agents of cancer in the rational basis(11,23). In these points of view, we started to study the immune responses of extract from herbs. In present study, Galangae Rhizoma were examined for its immune response.
〈Abbreviation〉 GRE; galangae rhizoma extract.

PFC; plaque forming cell.

DH; delayed hypersensitivity.

MATERIALS AND 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

Galangae Rhizoma, Ginseng Radix were purchased from Kyung-dong Korean market in Seoul. The 0.5kg of these herbs were disintegrated and extracted in boiling water for 8 hours, and then concentrated to the volume of 500ml using evaporator. Decoction extracted from Galangae Rhizoma and Ginseng Radix were administered orally for 10 days at the dose of 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(12,13).

Antitumor activity

ICR mice were inoculated intraperitoneal with 0.1ml of Sarcoma-180 tumor celll suspension

(1×10^7 cells/ml). Test samples were injected i.p. for consecutive 10 days, starting on the first day after tumor implantation, The life span of the mice was obserbed for one month.

Circulating leukocytes, and relative immunoorgan weights.

Blood samples were collected via retroorbital plexus with heparinized capillary and the number of leukocytes were determined on hemacytometer stained with Turk's solution(14). 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) emulsified in complete Freund's adjuvants(Sigma) at the base of tail. Seven days later delayed hypersenitivity and arthus reaction were elicited by challenging mice in the footpad with 30 ul of 2% heat aggregated BSA in saline, as previously described(15, 16, 17). At 3 and 24 hours 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(18, 19). Blood was collected from retro orbital 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 cells assay

Splenic 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 intraperitoneal injection with 0.2ml of a 2% suspension of sheep RBC(8×10^7) in male Balb/c mice(20, 21). Spleen single cell suspension was prepared in BSS(PH 7.2) using a stainless #200 sieve and adjusted to a cell 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.

Statiscal 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 essential role of the immune system in animal is a specific protective response to the foreign materials and organisms(1). Lower animals posses so-called innate or nonspecific immune system such as phagocytosis of bacteria by specialized cells. And higher animals have an evolved system which provide a flexible, specific and effective immune responses to different organisms. In host tissue carrying tumor, in a part, a certain amount of immunological response may be modified, and it is commonly recognized as the cause of the promotion of tumor. Therefore, myriad attention have been paid to immunomodulators for the purpose of enhancement of immune response against tumor antigen(22, 24), and a number of biological response modifiers have been reported to be effective enhancer.(28, 29).

In our study, we examine the effect of Galangae Rhizoma extract(GRE), Ginseng extract as reference, on solid tumor and immune functions in mice, and evaluate its possibilities

〈Table I〉 **Effects of GRE on survival of ICR mice transplanted I.P. with sarcoma-180 ascites.**

Group	Dose (ml/kg xdays)	Number of mice (N)	Number of tumor cells	Average survival days
Control	—	8	1×10^6	14.1
GRE	10×10	10	1×10^6	15.4
GRE	25×10	10	1×10^6	16.1
Ginseng	10×10	8	1×10^6	17.2
Ginseng	25×10	8	1×10^6	17.4

GRE; Galangae Rhizoma extract

as biological response modifier(BRM). In order to examine the antitumor activity, the life span of ICR mice transplanted with sarcoma-180 acites was measured. As shown in Table I, GRE and Ginseng extract prolonged survival time of ICR mice. This result suggest that GRE and Ginseng extract would affect the immune response in mice.

In order to evaluate the effects of them on immune function, numbers of the circulating leukocytes and weights of immunoorgans were investigated. Circulating leukocytes counts of countrol of control group in nomal male ICR mice were about 7,000(Table II). This result was consistent with the previous reports(27). Leukocytes were slightly decreased by treatment of GRE(25ml/kg, 10ml/kg), while significantly increased by treatment of Ginseng extract. Treatment with high and low dose of GRE showed no significant difference in leukocyte counts compared with control. Though precise reason of this leukocytopenialike is not clear, it suggest that direct inhibition of leukocytopoiesis or redistribution of lymphocytes to the other tissues may be initiated by GRE and Ginseng extract. Additionally, futher investgation on composition of PMN, lymphocytes, macrophages in blood and eryth-

〈Table II〉 **Effects of GRE and Ginseng Radix 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
GRE(10ml/kg)	6	6850±498	4.75±0.45	5.89±0.18
GRE(25ml/kg)	6	6250±441	4.76±0.43	5.52±0.25
Ginseng(10ml/kg)	6	9500±765	5.19±0.64	5.72±0.21
Ginseng(25ml/kg)	6	8748±640	5.22±0.52	5.80±0.27

GRE; and Ginseng Radix extract were administered per orally for 10 days. Each value represents mean+SE

ropoiesis in bone marrow should be performed to elucidate the precise cause. Immunoorgan weights were not altered by treatment of GRE and Ginseng extract(Table Ⅱ).

The effects on the capacity of B cell, T cell and macrophage to cooperate in the production of antibody to thymic dependent antigen, sheep RBC(SRBC) as humoral immunity, were evaluate in normal male Balb/c mice. Treatment of GRE increased significantly the number of IgM plaque forming cells(PFC) as shown in Table Ⅲ. Ginseng also increased significantly the number of PFC(Table Ⅲ). Although the reason of increased IgM synthesis is not clear, it implicate that GRE and Ginseng extract might activate the synthesis or secretion of antibody against SRBC in assay of hemagglutinin titration.

〈Table III〉 Effects of GRE and Ginseng radix 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
GRE(10ml/kg)	5	1,129±158	70,500±19,600
GRE(25ml/kg)	5	1,239±112	102,100±16,200
Ginseng(25ml/kg)	5	1,227±307	112,200±27,100

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

In order to evaluate the humoral immunity, the affects of GRE on arthus reaction were examined in nomal male ICR mice. GRE increased arthus reaction. Previous datas (Table Ⅲ, Ⅳ) suggest that GRE activate B cell and/or helper T cell result in potentiation of IgM PFC and Arthus reaction.

Delayed hypersensitivity(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 increasing vasodilation, accumulation of macrophage and ultimate destruction of challenged antigen. GRE did not any significant difference in delayed hypersensitivity(Table Ⅳ).

The involvement of macrophages in immune responses have been well known for many years(25, 26). The effects of GRE on nonspecific phagocytic function of splenic macrophages and kupffer cells were presented in Table V. GRE did not show any significant changes in phagocytic index and corrected phagocytic index.

There are many reports that polysacchrides from a various natural sources(30, 31, 32) including plants, fungi, lichen, yeast, and bacteria showed the antitumor activity against

<Table IV> Effects of GRE 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
GRE (10ml/kg)	6	9.88+0.48	7.92+0.92
GRE (25ml/kg)	6	10.44+0.67	8.78+1.26

* 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.

<Table V> Effects of GRE 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
GRE (10ml/kg)	6	2.85+0.32	5.32+0.17
GRE (25ml/kg)	6	2.17+0.26	5.14+0.27

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

experimental tumors. Their augumentation of antitumor activity should be achieved by modulating the cellular components of immune system, and restoring the effector cells-subsets of lymphocytes, monocytes/macrophage, and natural killer cells, etc. (22, 24). GRE did not show any significant effects on nonspecific macrophage function and cell mediated immunity, but potentiated humoral immunity. Taking into consideration that these extract were crude samples, the potent effect on immune functions should be expected if the active principle of this rhizoma are used. From this point of view, further investigations such as T cell function, B cell proliferation, release of lymphokines may be worth while.

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=국문 초록=

高良薑(Galangae Rhizoma)의 생체 반응에 대한 연구(Ⅰ)

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散寒止痛의 效能이 있는 高良薑이 체내 면역활성에 미치는 영향을 검토하기 위하여 인삼과 함께 실험을 행하여 아래와 같은 결과를 얻었다. 高良薑 및 인삼 수침액을 Sarcoma-180 고형 종양 세포를 이식한 생쥐에 투여하여 생존기간을 연장시켰다. 이를 바탕으로 면역에 대한 효과를 검토한 결과, 정상 ICR 생쥐에서 高良薑 수침액은 백혈구수, 체중에 대한 상대적 비장의 무게를 약간 감소시켰으나 통계적으로는 유의성이 없었고, 간에는 영향을 주지 않았다. in vitro 실험에서, splenic IgM PFCs을 유의성있게 증가시켰으며, BSA를 주사한 생쥐에서의 Arthus반응도 증가시켰다. 그러나, 지연형 과민반응에는 영향을 주지 않았으며, 비특이성 면역에 관여하는 Macrophage 등의 탐식 세포의 기능에도 영향을 주지 않았다. 이상의 결과, 고양강은 체액성 면역을 강화할 것으로 보여진다.

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