한국임상수의학회지 15(1) : 104-109 (1998)
Korean J. Vet Clin Med. 15(1) : 104-109 (1998)

Effect of Ginseng Saponins on Chemotaxis of Feline Peripheral Blood Polymorphonuclear Cells

Se-hyung Park, Young-won Yun and Mhan-pyo Yang[1]
College of Veterinary Medicine, Chungbuk National University, Cheongju, 361-763, Korea

고양이 말초혈액 다형핵백혈구의 유주성에 있어서 인삼 사포닌의 효과

박세형 · 윤영원 · 양만표[1]
충북대학교 수의과대학

초 록 : 고양이 말초혈액 다형핵백혈구(PMNC)의 유주성에 있어서 인삼 saponins(ginseng total saponin(GTS), ginseng PT saponin(GPT) 및 ginseng PD saponin(GPD))의 면역증강 효과를 검토하였다. PMNC에 대한 유주성을 Boyden chamber 변법으로 측정한 결과, GTS, GPT, GPD는 PMNC에 대해 직접적인 유주활성을 나타내지 않았다. 또한 인삼 saponins을 첨가하여 배양한 PMNC 배양상층액의 경우에는 미약한 유주활성을 나타내었다. 그러나 GPT 및 GPD를 첨가하여 배양한 말초혈액 단핵구세포(MNC) 배양상층액에서는 PMNC의 유주활성이 현저하게 증가하였다. PMNC에 대해 유주활성이 인정된 GPD로 배양한 MNC 배양상층액을 이용하여 checkerboard assay를 실시한 결과 배양상층액의 농도차에 의존하는 眞의 유주활성임을 알 수 있었다. 이상의 결과로부터 인삼 saponins 중 GPT 및 GPD가 고양이 말초혈액 다형핵백혈구의 유주성을 증강시키는 효과가 있으며, 이것은 이들 saponins에 의해 활성화된 단핵구세포에서 분비되는 유주성인자에 의해 다형핵백혈구의 유주활성이 증강되는 것으로 사료되었다.

Key words : cat, chemotaxis, ginseng saponins, mononuclear cells, polymorphonuclear cells

Introduction

Many acute inflammatory responses are generally characterized first by neutrophil infiltration and later by accumulation of monocytes. These neutrophils and monocytes are vital in host defense against microorganisms and regulation of the inflammatory process. The passage of PMNC from blood vessel into inflammatory sites involves a number of discrete steps including rolling, adhesion and transmigration[8]. Transmigration is also known to be mediated by several chemoattractants such as fMLP (n-formyl-methionine-leucine-phenylalanine) of bacterial origin, complement-derived C5a and leukotriene B_4[1,9,15].

Ginseng has been used as a tonic and restorative medicine in the Orient for more than two thousand years. Ginseng has a variety of effects such as promotion of immunity and metabolism function, biomodulation action, anti-stress and anti-aging activities, etc[11]. In regulation of immune function, ginseng saponins enhance the resistance to parasitic infection[21]. They also induce the production of cytokines including interferons[2], the increase of numbers of antigen-reactive T-cells and helper T cells, the activation of NK cell, and the cell-mediated immune responses[6,14]. They also increase the synthesis of serum protein, showing anti-tumor activity in the autochthonous lung tumor system[7,10,12,18]. These findings indicated that ginseng saponins have an enhancing effect

This work was supported by Grant-in-Aid from Korea Science and Engineering Fundation (KOSEF; 971-0605-042-1).

[1]Corresponding author.

of non-specific or specific immune responses. In clinics, cats who are sensitive to stresses show many immunodeficiency diseases than other animals. To examine the enhancing effects of ginseng saponin as an immunopotentiator in the non-specific immunity, adult cats as laboratory animals are used in this study based on similarity of clinical signs and molecular biological characteristics between immune-related diseases of cats and human. Molecular biological characteristics of feline immunodeficiency virus (FIV) are similar to those of human immunodeficiency virus (HIV) which induces acquired immune deficiency syndrome (AIDS) in human[5]. Therefore, the aim of current study is to examine the immunostimulating effects of ginseng saponins on chemotaxis of feline peripheral blood polymorphonuclear cells (PMNC).

Materials and Methods

Animals

Fifteen healthy cats of average 1.5-year-old were housed at animal cage. All cats utilized in this study were free from FeLV infection as examined by an enzyme-linked immunosorbent assay using a Leukassay F kit (Pitman Moore, NJ, USA).

Reagents

Ginseng total saponin (GTS), PT saponin (GPT) and PD saponin (GPD) were kindly donated by the Korea Ginseng and Tobacco Research Institute (Taejeon, Korea).

MNC and PMNC isolation

Blood was collected in heparinized tubes by jugular venipuncture. Blood diluted 1:1 in phosphate-buffered saline (PBS) at pH 7.6 was layed on the equal volume of Lymphoprep (specific gravity, 1.077; Nycomed Pharma As, Oslo, Norway) and centrifuged at 400×g for 40 min at room temperature. The cells in interface between PBS plus plasma and Lymphoprep were obtained and subjected to 0.83% NH_4Cl in Tris-base buffer (pH 7.6) containing 1% bovine serum albumin (BSA; Sigma, MO, USA) for 5 min at 37°C. The resulting peripheral blood mononuclear cells (MNC) were washed 3 times with PBS. PMNC was obtained from layer of erythrocyte sediment containing PMNC after removal of MNC layer. One ml of pellet of erythrocyte sediment was mixed with 10 ml of 1.5% dextran (molecular weight, 200,000; Wako, Osaka, Japan) in PBS and allowed to sediment for 60 min. The floating cells of upper compartment were collected and centrifuged at 400×g for 5 min. The residual erythrocytes were removed by treatment with 0.83% NH_4Cl solution for 5 min at 37°C and washed 3 times with PBS. The purity of neutrophils in final PMNC suspension was 96% when determined by cytospin smear and Giemsa stain. Cell viabilities determined by trypan blue dye exclusion always exceeded 98%. All cells were suspended in RPMI 1640 (Sigma, MO, USA) supplemented with 2 mM L-glutamine, 0.02 mg/ml of gentamicin, and 5% fetal bovine serum (FBS; Gibco, MD, USA) and finally adjusted to 2×10^6 cells/ml.

Culture supernatants

The PMNC and MNC at a density of 2×10^6 cells/ml in a well of a 24-multiwell plate (Falcon 3047, Becton Dickinson Labware, NJ, USA) were incubated with concentration of 200 μg/ml of GTS, GPT and GPD for 24 hr at 37°C under 5% CO_2-humidified atmosphere, respectively. All supernatants were collected by centrifugation (5,000×g for 30 min), filtered with 0.45 μm-pore size membrane filter (Millipore, MA, USA) and stored below -70°C until use for assay.

Chemotaxis assay

A modified Boyden chamber assay was used to measure chemotaxis[16]. The chemotaxis chamber (Neuro Probe, MD, USA) and cell suspension solution, RPMI 1640 medium containing 1% BSA were prewarmed for 2 hr at 37°C. A nitrocellulose filter with 120-μm thick and 3.0-μm pore size (Nihon Millipore, Yonezawa, Japan) was placed on the top of well of the lower chamber that previously filled with 200 μl of either ginseng saponins at various concentration or culture supernatants at various dilution. The controls received either medium alone or culture supernatants from untreated cells in the lower compartment. Then, 200 μl of PMNC suspension (2×10^6 cells/ml) was placed in the upper compartment of the chamber. The chambers were incubated for 45 min at 37°C

under 5%-CO_2 in humidified atmosphere. After incubation, the membrane filters were immediately taken out, fixed in ethyl alcohol, dried, stained with hematoxylin, and mounted on glass slide. The migrated distance of PMNC through the filter toward the other side was measured by microscopy at 400× magnification. Five fields for a filter were selected randomly in triplicate assay. The chemotactic responsiveness of input PMNC was evaluated as absolute distances (μm/45 min±SEM) in the directional migration of PMNC in response to chemoattractants.

Checkerboard assay

Checkerboard assay was carried out according to the method of Zigmond and Hirsh[19].

Data analysis

The Student's *t* test was used for statistical significance determinations. All data expressed mean± SEM.

Results

Effect of GTS on feline PMNC chemotaxis

Feline PMNC in response to GTS was migrated slightly when compared with control cells (Fig 1A). PMNC did not show any migration in response to culture supernatant from PMNC exposed to GTS (Fig 1B). But there was a tendency to migrate in all dilutions of culture supernatant from MNC exposed to with GTS (Fig 1C).

Effect of GPT on feline PMNC chemotaxis

GPT reduced PMNC chemotaxis as shown in Fig 2A. This reduction is a weak dose-dependent fashion. Culture supernatant from PMNC exposed to GPT increased slightly PMNC chemotaxis (Fig 2B). However, culture supernatant from MNC exposed to GPT showed a significant enhancement of PMNC chemotaxis as compared to that of untreated control ($p<0.01$). This enhancement is a dose-dependent manner (Fig 2C).

Effect of GPD on feline PMNC chemotaxis

GPD at concentrations tested did not enhance the chemotactic activity of feline PMNC (Fig 3A). Culture supernatant from PMNC exposed to GPD enhanced slightly feline chemotaxis (Fig 3B). Culture supernatant from MNC exposed to GPD, however, enhanced remarkably PMNC chemotaxis ($p<0.01$). This chemotactic activity is peaked at 1/4 dilution of culture supernatant in a dose-response curve (Fig 3C).

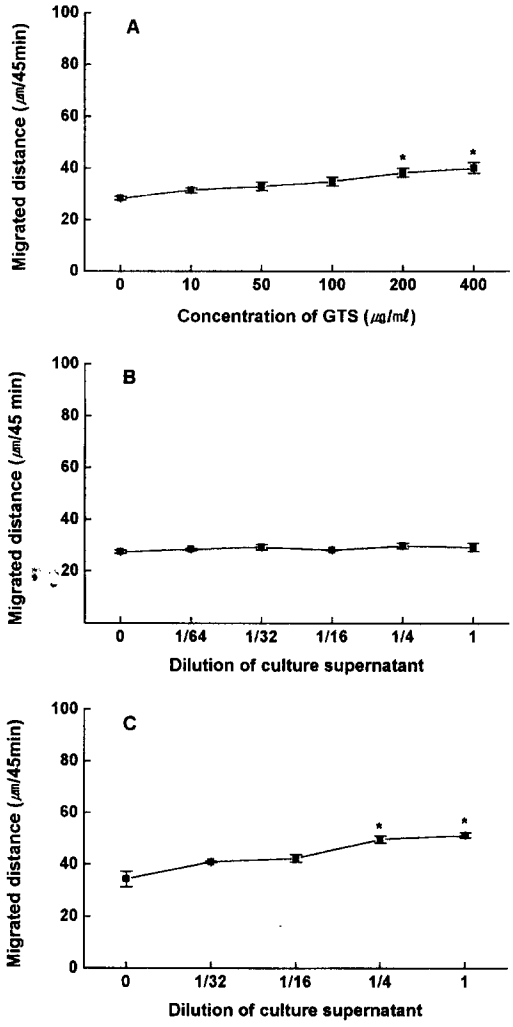

Fig 1. Effect of GTS(A) and diluted culture supernatant of PMNC(B) and MNC(C) exposed to GTS on PMNC chemotactic activity. The values represent mean±SEM (n=3). *$p<0.05$, compared to control (medium alone)

Checkerboard assay

In order to determine whether PMNC migration

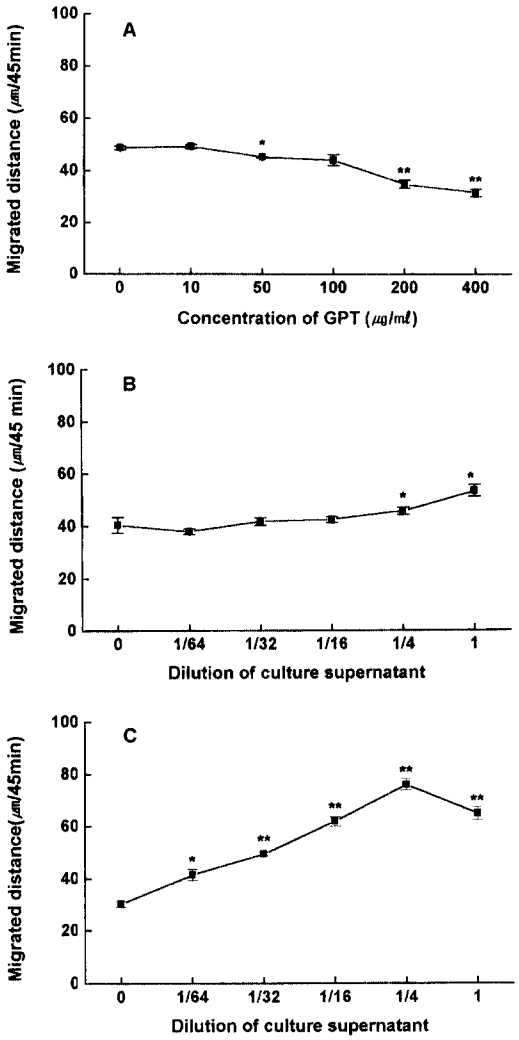

Fig 2. Effect of GPT(A) and diluted culture supernatant of PMNC(B) and MNC(C) exposed to GPT on PMNC chemotactic activity. The values represent mean±SEM (n=3). *p<0.05, **p<0.01, compared to control (medium alone)

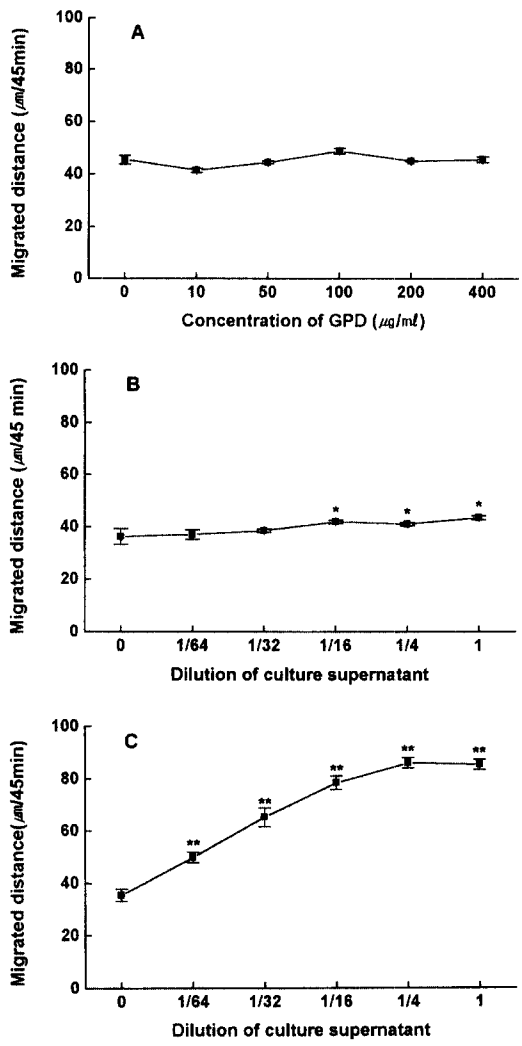

Fig 3. Effect of GPD(A) and diluted culture supernatant of PMNC(B) and MNC(C) exposed to GPD on PMNC chemotactic activity. The values represent mean±SEM (n=3). *p<0.05, **p<0.01, compared to control (medium alone)

by culture supernatants of MNC exposed to GPT and GPD is a true chemotaxis, the checkerboard assay using the culture supernantant of GPD-exposed MNC was performed. As shown in Table 1, PMNC migration strongly depended on both the increase of a concentration gradient of culture supernatant in lower chamber and the decrease of a concentration gradient of culture supernatant in upper chamber. This indicated that chemotactic activity of PMNC by culture supernatant exposed to GPD was true chemotaxis but not random migration.

Discussion

Direct responses of GTS, GPT and GPD in the migration of freshly prepared PMNC were not effective. It is conceivable that antigenic components of ginseng saponins themselves may be less respon-

Table 1. Checkerboard assay of feline PMNC migration to culture supernatant from MNC exposed to GPD

Dilution of culture supernatant on upper chamber	Dilution of culture supernatant on lower chamber			
	0	1/128	1/32	1/4
0	33.6±1.5	34.2±2.9	41.8±3.4	55.8±0.8
1/128	27.4±1.9	29.0±2.9	34.8±0.8	50.0±3.6
1/32	22.8±2.5	24.8±1.6	27.4±5.1	42.2±2.1
1/4	18.8±2.7	24.8±1.8	28.8±3.5	35.4±1.8

Culture supernatant from MNC exposed to GPD (200 μg/ml) for 24 hr and diluted with RPMI 1640 medium. Values of migrated distance (μm/ 45 min) represent as mean±SEM of three determinations.

sible for feline PMNC chemotaxis. Especially GPT failed to induce direct PMNC migration, with reduced responses at higher concentrations of 200 to 400 μg/ml. At these higher concentrations, receptors for chemotactic factor(s) on PMNC may become desensitized, as already observed with other chemotactic ligands[13,17]. Another possibility is due to the cytotoxic effect of components of GPT, since it reduced the cell viability of PMNC and MNC (data not shown). Culture supernatants of PMNC exposed to GTS, GPT and GPD were also not active or slight chemtactic for PMNC. These results indicated that PMNC did not release chemotactic factors by any antigenic or mitogenic stimulation. This finding was consistent with that of prevoius studies on chemotaxis and phagocytosis for canine PMNC[3,4].

The results demonstrated that culture supernatants of MNC exposed to GPT and GPD were able to enhance high chemotactic activity for PMNC and that these saponins were capable of releasing the chemoattractants from MNC containing both monocytes and lymphocytes. It was, however, interesting that chemotactic activity of PMNC in culture supernatant of MNC exposed to GTS was very weak. Although it is difficult to explain the reason for low activity in GTS because it has all components of GPT and GPD, it would be thought that some pharmacologigcal activities of ginsenosides were contrary to each other. For examples, ginsenoside Rg_1 increased the blood pressure whereas ginsenoside Rb decreased it. Ginsenoside Rg_1 stimulated the central nervous system whereas ginsenoside Re inhibited it[20]. The pharmacological activity of a ginsenoside might be also counterbalanced by other ginsenosides.

The migration of PMNC to culture supernatant of MNC exposed to GPD was true chemotaxis by concentration gradients of chemoattractants rather than random migration, called chemokinesis. However, this activity was very slight in culture supernatant of PMNC similarly exposed to GPT and GPD when compared with that of MNC. Therefore, these results suggested that mostly enhancing effect of GPT and GPD on chemotactic response of feline PMNC is mediated by humoral factors produced by MNC exposed to GPT and GPD. The elucidation of soluble products produced from MNC exposed to ginseng saponins will be considerably important data in the study of animal cytokines as well as immunostimulators. For the clinical application, the co-administration of ginseng saponins will be able to augment the host defense in animals with immunodeficiency or no response to antibiotic treatment.

Conclusion

The immunostimulating effects of GTS, GPT and GPD on the chemotactic activity of feline PMNC were examined. The chemotactic activity of PMNC was evaluated by a modified Boyden chamber assay. Ginseng saponins themselves and culture supernatant from PMNC exposed to one of the overall ginseng saponins were not active or slight chemotactic for PMNC. But culture supernatant from MNC exposed to GPT and GPD except for GTS remarkably enhanced feline PMNC chemotaxis. This migration of PMNC by culture supernatant from MNC exposed to GPD was found to be true chemotaxis by checkerboard assay. It is, therefore, suggested that active components of GPT and GPD activate MNC to elaborate chemotactic factor(s), which may be an important

mechanism for the enhancement of chemotactic activity of feline PMNC.

References

1. Fernandez HN, Henson PM, Otani A, Hugli TE. Chemotactic response to human C3a and C5a anaphylatoxin. I. Evaluation of C3a and C5a leukotaxis *in vitro* and under stimulated *in vivo* conditions. J Immunol 1978; 120: 109-115.
2. Gupta S, Agarwal SS, Epstein LB, Fernandes G, Good RA. A new mitogen and interferon inducer. Clin Res 1980; 28: 504A.
3. Hirota, Y, Yang MP. Enhancing effects of chicken egg white derivatives on the phagocytic response in the dog. J Vet Med Sci 1995; 57: 825-829.
4. Hirota Y, Yang MP, Araki S. Immunostimulating activities of chicken egg white derivatives(EWD) and recombinant bovine interferon alpha 1(rBoIFNα1) in dogs and cattle. Bull Soc Fr-Jpn Sci Vet 1995; 6: 58-67.
5. Hosie MJ. The development of a vaccine against feline immunodeficiency virus. British Veterinary Journal 1994; 150: 25-39.
6. Kenarova B, Neychev H, Hadjiivanova C, Petokov VD. Immunomodulating activity of ginsenoside Rg_1 from *Panax ginseng*. Japan J Pharmacol 1990; 54: 447-454.
7. Kim MJ, Jung NP. The effect of ginseng saponin on mouse immune system. Korean J Ginseng Sci 1987; 11: 130-135.
8. Klaus L, Joffre BB, Myron IC, Michael AG jr., Francis WL. Intravenous interleukin-8 inhibits granulocyte emigration from rabbit mesenteric venules without altering L-selectin expression or leukocyte rolling. J Immunol 1993; 151: 6347-6357.
9. Kreisle RA, Parker CW, Griffin GL, Senior RM, Stenson WF. Studies of leukotrien B_4-specific binding and function in rat polymorphonuclear leukocytes: Absence of a chemotactic response. J Immunol 1985; 134: 3356-3363.
10. Lee JW, Park KM, Park KH. Serum IgE immune response after the exposure to korean ginseng. J Ginseng Sci 1994; 18: 113-117.
11. Liu CX, Xiao PG. Recent advances on ginseng reserch in China. J Ethnopharmacol 1992; 36: 27-38.
12. Park HW, Kim SC, Jung NP. The effect of ginseng saponin fractions on humoral immunity of mouse. Korean J Ginseng Sci 1988; 12: 63-67.
13. Samata AK, Oppenheim JJ, Masushima K. Interleukin-8(monocyte derived neutrophil chemotactic factor) dynamically regulates its own receptor expression in human neutrophils. J Biol chem 1990; 265: 183-189.
14. Scaglione F, Ferrara F, Duanani S, Falchi M, Santoro G, Fraschini F. Immunomodulatory effects of two extracts of *Panax ginseng* C.A. Meyer. Drugs Exptl Clin Res 1990; XVI: 537-542.
15. Smith WB, Leanne N, Yeesim KG, Sandra I, Mathew AV, Jennifer RG. Transforming growth factor-β1 inhibits the production of IL-8 and the transmigration of neutrophils through activated endothelium. J Immunol 1996; 157: 360-368.
16. Watanabe K., Nakagawa H, Tsurufuji S. A new simple plastic chemotaxis device of the boyden chamber type utilizing an immunoassay plate. Jan J Pharmacol 1985; 39: 102-104.
17. Wiedermann CJ, Reinisch N, Brausteiner H. Stimulation of monocyte chemotaxis by human growth hormone and its deactivation by somatostatin. Blood 1993; 82: 954-960.
18. Yun YS, Lee YS, Jo SK, Jung IS. Inhibition of autochthonous tumor by ethanol insoluble fraction from *Panax ginseng* as an immunomodulator. Planta Med 1993; 56: 521-524.
19. Zigmond SH, Hirsch JG. New method for evaluation and demonstration of a cell-derived chemotactic factor. J Exp Med 1973; 137: 387-410.
20. 박명규. 인삼효능 · 제품연구결과요약집 제1집. 대전: 한국인삼연초연구원. 1993.
21. 소진탁, 이건수, 김상준. 인삼이 기생충 감염에 미치는 영향에 관한 연구. 연세의대논문집, 1976; 9: 119-128.