J Vet Clin 29(3) : 207-212 (2012)

Journal of Veterinary Clinics
www.ksvc.or.kr

Fucoidan Upregulates Chemotactic Activity of Canine Peripheral Blood Polymorphonuclear Cells Through Interleukin-8 from Peripheral Blood Mononuclear Cells *in vitro*

Chun-Jin Jeon, Soo-Hyun Kim, Sung-Soo Kim, Ji-Houn Kang and Mhan-Pyo Yang[1]

Laboratory of Veterinary Internal Medicine, Department of Veterinary Medicine, College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea

(Accepted: June 11, 2012)

Abstract : Fucoidan has been shown to enhance immune function. The objective of this study was to examine the *in vitro* effect of fucoidan on the chamotactic activity of canine peripheral blood polymorphonuclear cells (PMNs). The chemotactic activity of PMNs was evaluated by method of a modified Boyden chamber assay. The amount of interleukin (IL)-8 in the culture supernatants from peripheral blood mononuclear cells (PBMCs) treated with fucoidan was determined by means of ELISA. Fucoidan itself could not have chemoattract effects for PMNs. However, the chemotaxis of PMNs was remarkably enhanced by culture supernatant from PBMCs treated with fucoidan. Similarly, it was also increased by recombinant canine (rc) IL-8. These chemotactic activities of PMNs were inhibited by addition of anti-rcIL-8 polyclonal antibody (pAb). The amount of IL-8 in the culture supernatant from PBMCs was shown to increase upon treatment of fucoidan as compared with that of untreated PBMCs culture supernatant. These results suggest that fucoidan upregulates the chemotaxis of PMNs, which is mainly mediated by IL-8 released from fucoidan-stimulated PBMCs.

Key words **:** fucoidan, interleukin-8, chemotaxis, dog.

Introduction

Peripheral blood polymorphonuclear cells (PMNs) play an important role in the host defense mechanism. Specially, neutrophils are the main effecter cells which are involved in the elimination of microorganisms. Neutrophils is mediated by chemoattractants such as bacterially produced n-formyl-methionine-leucine-phenylalanine (fMLP), complement-derived C5a, leukotriene (LT) B_4 and interleukin (IL)-8 (16,21). Neutrophils adhere to the endothelium via cell-surface receptors for the transmigration (4). The soluble products from activated monocytes and lymphocytes have been considered to cause a cellular infiltration into inflamed sites (2,24) and shown to directly induce chemotactic response for phagocytes (5). Chemotactic cytokines derived from activated monocytes or tissue macrophages typically include interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α (19,29). These are also released by peripheral blood mononuclear cells (PBMCs) (27,32).

Fucoidan is sulfated polysaccharides containing large proportions of l-fucose and sulfate (10,14,20). Many papers reported that fucoidan has various biological activity, such as anti-coagulant (6), anti-angiogenic (20), anti-tumor (1,23), anti-thrombotic (13), and anti-viral (10,14) effects. Fucoidan also has inflammatory modulatory effects (9) and stimulates both humoral and cell-mediated immune responses under *in vitro* and *in vivo* conditions (14). Another study suggested that fucoidan has an immunoenhancing effect on the phagocytic capacity and oxidative burst activity of canine PMNs, which is mainly mediated by TNF-α released from fucoidan-stimulated PBMCs (19). Although these biological activities of fucoidan have been previously demonstrated, there are no researches of the chemotaxis of PMNs.

The aim of this study is to examine the *in vitro* effect of fucoidan on the chemotaxis of PMNs. We examined the migration distances of PMNs chemoattracted by culture supernatant of PBMCs treated with fucoidan. We also examined the level of IL-8 in the culture supernatant of PBMCs.

Materials and Methods

Reagents

Fucoidan purified from *Focus vesiculosus* was purchased commercially from Sigma-Aldrich (St. Louis, MO, USA). The stock solution was prepared by dissolving fucoidan in phosphate buffered saline (PBS) to a final concentration of 10 mg/ml and passing it through a 0.2 μm membrane filter (Millipore Co., Bedford, MA, USA). And it aliquoted until before

[1]Corresponding author.
E-mail : mpyang@chungbuk.ac.kr

use. Bovine serum albumin (Sigma-Aldrich), recombinant canine (rc) IL-8, goat anti-rcIL-8 polyclonal antibody (pAb; IgG) and rabbit anti-HRV G4 (ST-3) pAb (IgG) (R&D Systems Inc., Minneapolis, MN, USA) were also commercially purchased.

Animals

For the blood donor, we used clinically healthy five male Beagle dogs. Their mean age was 10 months old. All dogs were housed separately in cages with a 12 hours light and 12 hours dark cycle and were fed a commercial diet (ProPlan; Purina Korea, Seoul, Korea) and provided tap water. All experimental procedures were approved by the ethics committee of the Chungbuk National University.

PBMCs and PMNs isolation

Blood samples were collected by heparinized syringe at jugular vein. The PBMCs and PMNs were isolated by double density-gradient centrifugation immediately after collecting the blood samples. Briefly, Percoll™ solution (GE Healthcare Bio-sciences AB, Uppsala, Sweden) of specific gravity 1.077 was layered carefully onto the same volume of the Percoll™ solution of specific gravity 1.119 as described previously (17). The purity of neutrophils in the final PMNs suspension was routinely greater than 97%, as determined by cytospin smear and Diff-Quik staining analyses. The viability of PBMCs and PMNs, as determined by trypan blue dye exclusion, always exceeded 98%. Both PBMCs and PMNs were resuspended in RPMI 1640 medium (Sigma-Aldrich) supplemented with 5% heat-inactivated fetal bovine serum (Gibco Co., Grand Island, NY, USA), and 0.02 mg/ml gentamicin.

Culture supernatants

PBMCs were incubated at a density of 2×10^6 cells/ml in 24-multiwell plates (Nunc Co., Naperville, IL, USA) with fucoidan (200 μg/ml) preparation. Control cells were treated with the same amount PBS as vehicle. After incubation for 24 h at 37°C under 5% CO_2-humidified atmosphere, the supernatants were centrifuged at 14,000 × g for 5 min, filtered through a 0.2 μm-pore size membrane filter, and stored at −78°C until used.

Chemotaxis assay

Chemotactic activities for PMNs were determined as migration distance in Millipore membrane filters by modified Boyden chamber method, as previously described (27). Briefly, the chemotaxis chamber (Neuro probe, Gaithersburg, MD, USA), RPMI 1640 medium and culture superantant of PBMCs treated with fucoidan were pre-warmed for 2 h at 37°C. The lower chamber was filled with 200 μl of culture supernatant (0-100%) or rcIL-8. A nitrocellulose filter (120 μm thick and 3.0 μm pore size; Millipore Corporation, Bedford, MA, UK) were placed on top of the well of the lower compartment. And then, 200 μl of PMNs suspension (1×10^6 cells/ml) was put into the upper compartment. The chambers were incubated for 45 min at 37°C in a 5% CO_2-humidified atmosphere. After incubation, the membrane filters were immediately removed, fixed in ethyl alcohol, dried, stained with hematoxylin, decolorized in ethyl alcohol, and mounted on a slide glass. The migration distance of cells through the nitrocellulose filter towards the other side was measured using bright field microscopy at 400 × magnification. Five fields per filter were selected randomly in triplicate assay. The chemotactic responses of input cells were evaluated as the absolute distance (μm/45 min) in the directional migration of PMNs in response to chemoattractant.

Neutralization test

Anti-rcIL-8 pAb diluted with various concentrations (0-100 μg/ml) were added to the culture supernatant from fucoidan-treated PBMCs. As a control isotype IgG, anti-HRV G4 pAb instead of anti-rcIL-8 pAb was added to the well. The mixed samples were placed for 30 min at room temperature. The effect of this mixture to stimulate the chemotactic activity of PMNs was also evaluated as described above.

Measurement of IL-8 in the culture supernatant from PBMCs treated with fucoidan

The culture supernatants of PBMCs treated with or without fucoidan (200 μg/ml) were collected after 24 h incubation. The IL-8 levels in the culture supernatants of PBMCs were determined by the direct sandwich enzyme-linked immunosorbent assay (ELISA) in the Quantikine® Canine CXCL8/IL-8 Immunoassay kit (R&D System Inc.) according to the manufacturer's protocol. All samples, standard and controls were assayed in triplicate. The optical density was determined using an automated microplate reader (Bio-Tek Instruments Inc., Winooski, Vermont, USA) at 450 nm. The IL-8 levels in the samples were quantified from standard curves generated with purified canine IL-8 tested at eight titration points. The inter- and intra-assay coefficients of variation for this assay are less than 10%, and the lower limit of detection for this assay is 15.6 pg/ml.

Statistical Analyses

All statistical analyses were carried out by using SPSS V12.0 for Windows (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to investigate differences between the control and treatment groups, followed by Dunnett's *post hoc* test. Comparison of two groups was made by *t*-test. *P* value of under 0.05 was considered statistically significant. Results are expressed as means plus or minus standard deviation (± SD).

Results

Direct effect of fucoidan on chemotaxis of PMNs

To examine the direct effect of fucoidan on chemotactic activity of PMNs, the migrated distance of PMNs to fucoidan was measured. The direct treatments of fuocidan at concentra-

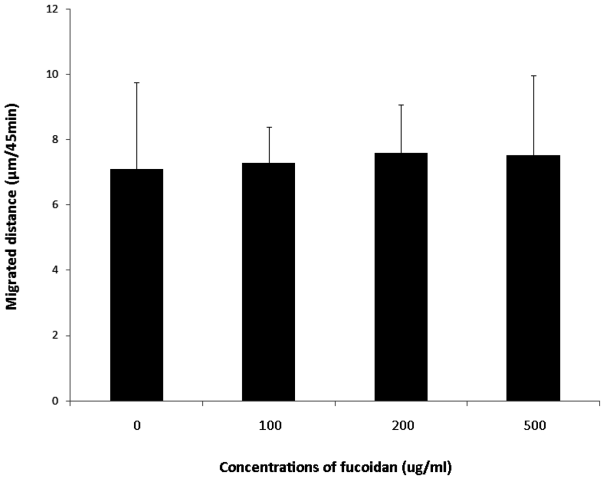

Fig 1. Effect of fucoidan treatment on the chemotaxis of PMNs. Freshly isolated PMNs (1×10^6 cells/ml) were placed in the upper chamber. Fucoidan (200 μg/ml) were placed in the lower chamber, and incubation was allowed for 45 min. The migration distance of cells through the filter towards the other side was then measured. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. Data represent the means ± SD (n = 3).

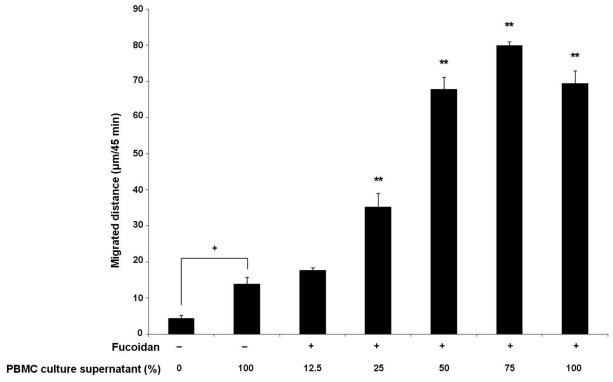

Fig 2. Chemotactic response of PMNs in response to culture supernatant from PBMCs treated with fucoidan. The culture supernatants (0-100%) from PBMCs treated with (+) or without (–) fucoidan for 24 h were placed in the lower chamber. Control cells were treated with the same amount RPMI 1640 medium alone as vehicle. Freshly isolated PMNs (1×10^6 cells/ml) were placed in the upper chamber. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. And, comparison of two groups was made by *t*-test. Data represent the means ± SD (n = 3). **P < 0.01 vs. untreated PBMCs culture supernatant (100%), +p < 0.01 untreated PBMCs culture supernatant (100%) vs. vehicle control.

tion of 0 to 500 μg/ml showed no effects on chemotaxis of PMNs as compared with controls treated without fucoidan (Fig 1).

Effect of culture supernatant from PBMCs treated with fucoidan on chemotaxis of PMNs

To examine the effect of culture supernatant from PBMCs treated with fucoidan (200 μg/ml) on chemotaxis of PMNs, the migrated distance of PMNs to culture supernatant was measured. The culture supernatant (100%) from PBMCs without fucoidan significantly increased the migrated distance of PMNs ($p = 0.002$) when compared to the vehicle control (RPMI 1640 medium alone) (Fig 2). The culture supernatant (25-100%) from PBMCs treated with fucoidan (200 μg/ml) also significantly enhanced the migrated distance of PMNs in a dose-dependent fashion when compared to the culture supernatant (100%) of PBMCs without fucoidan ($p < 0.001$). This chemotactic activity was peaked at 75% of culture supernatant from PBMCs treated with fucoidan. But the migrated distance of PMNs treated with 100% culture supernatant was mildly reduced when compared to that of 75% culture supernatant ($p = 0.027$).

Effects of rcIL-8 on chemotactic activity of PMNs.

The chemotactic activity of PMNs to rcIL-8 was also examined. Indeed, rcIL-8 enhanced the chemotactic activity of PMNs at concentrations of 1 to 50 nM in a dose-dependent manner ($p < 0.001$) when compared to vehicle-treated control (Fig 3). This activity of PMNs was peaked at 10 nM of rcIL-8.

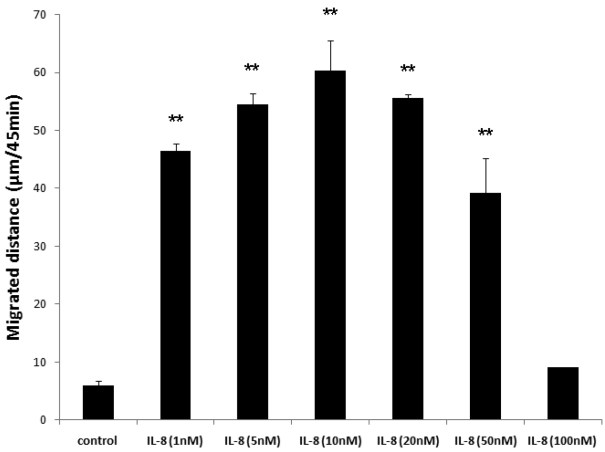

Fig 3. Chemotactic response of PMNs in response to rIL-8. Recombinant IL-8 at the indicated concentrations (0-100 nM) was added in the lower chamber. One-way ANOVA was used to investigate differences between rIL-8 treated groups and vehicle control, followed by a Dunnett's test. Data represent the means ± SD (n = 3). **P < 0.01 vs. control.

Neutralization effect of anti-rcIL-8 pAb on chemotaxis of PMNs.

To examine whether the enhanced chemotactic acitvity of PMNs to the culture supernatant from PBMCs treated with fucoidan is due to IL-8, the neutralization effect of anti-rcIL-8 pAb on chemotactic activity of PMNs in response to culture supernatant of PBMCs was examined. The enhanced chemotactic activity of PMNs to culture supernatant from PBMCs treated with fucoidan was inhibited by addition of anti-rcIL-8

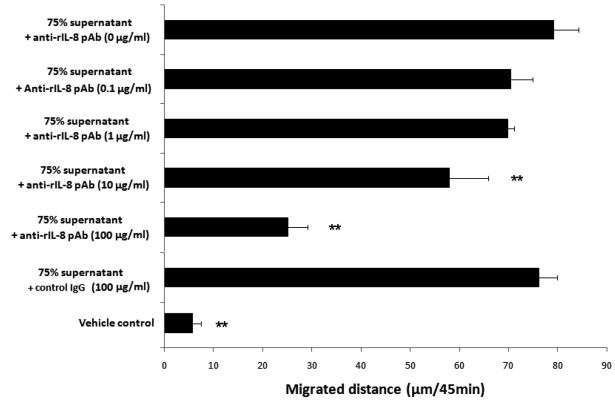

Fig 4. Neutralization effect of anti-rIL-8 pAb on chemotactic activity of PMNs to culture supernatant PBMCs treated with fucoidan. Anti-rIL-8 pAb at the indicated concentrations (0-100 μg/ml) and rabbit anti-HRV G4 pAb (100 μg/ml) as a contrl isotype IgG were added to the culture supernatant (75%) from PBMCs treated with fucoidan. The mixtures were placed in the lower chamber. One-way ANOVA was used to investigate differences between fucoidan-treated PBMCs culture supernatant (75%) alone and other treatments, followed by a Dunnett's test. Data represent the means ± SD (n = 3). **P < 0.01 vs. fucoidan-treated PBMCs culture supernatant (75%).

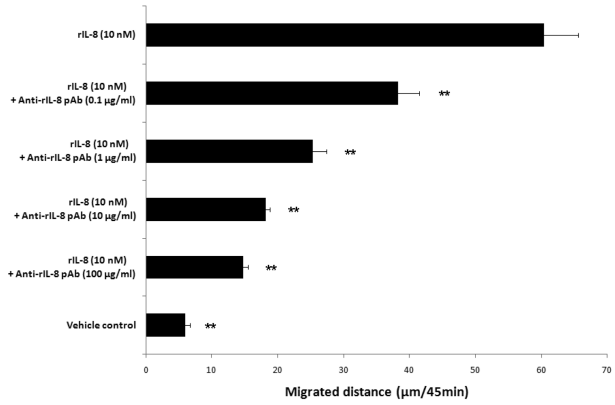

Fig 5. Effect of anti-rIL-8 pAb on chemotactic activity of PMNs to rIL-8. Anti-rIL-8 pAb at the indicated concentrations (0-100 μg/ml) was added to rIL-8 (10 nM). The mixtures were placed in the lower chamber. One-way ANOVA was used to investigate differences between rIL-8 (10 nM) treated group and other treatments, followed by a Dunnett's test. Data represent the means ± SD (n = 3). **P < 0.01 vs. rIL-8 (10 nM) alone.

pAb at concentration of 10 and 100 μg/ml in a dose-dependent manner when compared with that of culture supernatant (75%) from PBMCs treated with fucoidan ($p < 0.001$) (Fig 4). However, in the examination of the possibility of nonspecific inhibition for immunoglobulin isotype, IgG, any chemotactic activity of PMNs to culture supernatant (75%) from PBMCs treated with fucoidan was not inhibited by addition of high concentration (100 μg/ml) of control IgG, anti-HRV G4 pAb instead of anti-rcIL-8 pAb. Similarly, the enhanced chemotactic activity of PMNs to rcIL-8 at 10 nM was also

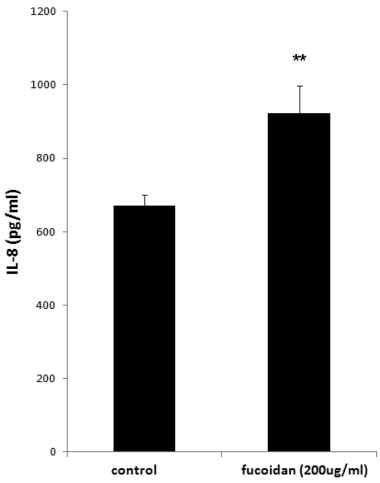

Fig 6. The effect of fucoidan on IL-8 production from PBMCs. PBMCs (2×10^6 cells/ml) were incubated with fucoidan (200 μg/ml) for 24 h. The concentration of IL-8 in the culture supernatant from PBMCs was measured by ELISA. Comparison of two groups was made by *t*-test. The data represent mean ± SD (n = 3). $^{**}P < 0.01$.

abrogated by addition of anti-rcIL-8 pAb (0.1-100 μg/ml) in a dose-dependent manner (Fig 5).

Amount of IL-8 in the culture supernatant from PBMCs treated with fucoidan.

The amount of IL-8 in the culture supernatant (100%) from PBMCs treated with fucoidan (200 μg/ml) for 24 h was quantified by ELISA. The level of IL-8 in culture supernatant from PBMCs treated with fucoidan was significantly higher than that of culture supernatant from PBMCs without fucoidan ($p = 0.006$) (Fig 6).

Discussion

In the present study, we examined the effect of fucoidan on the chemotactic activity of PMNs. Fucoidan showed directly no effects on the migration of PMNs. It was assumed that fucoidan itself do not cause any effect on the chemotaxis of PMNs. However, the chemotaxtic activity of PMNs was enhanced by either culture supernatant from fucoidan-treated PBMCs or rcIL-8. This enhancing effect of the culture supernatant of fucodian-treated PBMCs on the chemotaxis of PMNs may be supported that the chemotactic factor(s) were existed in soluble products from PBMCs treated with fucoidan. This also suggested that fucoidan is capable of releasing the chemoattractant(s) from PBMCs.

IL-8 is a member of a family of related chemotactic and proinflammatory cytokines which contain conserved positions of four cysteines that from two disulfide bridge (8). This cytokine has been shown to be more selective for neutrophils than other chemotactic factors (30) and was a specific neutrophil chemattractant with only minor effects on eosinophils,

monocytes, and basophils (3,31). It induces lysosomal enzyme release from neutrophils, expression of adhesion molecules on neutrophils (11). Consequently, we hypothesized that the soluble factor(s) in the culture supernatant of fucoidan-treated PBMCs may be associated with IL-8-like chemotactic factor. We tested the effect of IL-8 on the chemotactic activity of canine PMNs. Similar to the effect of rcIL-8 to PMNs, the chemotactic activity of PMNs was also increased by culture supernatant from PBMCs treated with fucoidan. We also found that the IL-8 level of culture supernatant of PBMCs were increased by the fucoidan treatment, as expected. These results indicated that fucoidan stimulates canine PBMCs to produce IL-8, which enhances the chemotactic activity of PMNs.

Next, we examined whether an anti-rcIL-8 pAb negates the enhancement of canine PMNs chemotaxis induced by the fucoidan-treated PBMCs culture supernatant. The anti-rcIL-8 pAb completely negated the ability of the culture supernatant from fucoidan-treated PBMCs to enhance the chemotaxis of PMNs. These findings might suggest that the immunoenhancing effect of fucoidan on the chemotaxis of PMNs is mediated by IL-8 produced by fucoidan-stimulated PBMCs.

We found that the culture supernatant from untreated PBMCs also increased the migrated distance of PMNs compared with vehicle control. This is consistent with several studies showing that unstimulated blood PBMCs expressed low levels of cytokine mRNA such as granulocyte macrophage colony stimulating factor, IL-6, IL-8 and TNF-α (7,22,32).

It was known that IL-8 is associated with rheumatoid arthritis (12,25), a systemic fungal infection (28), some bacterial infections (15,26), and hypoxia (18). Therefore, IL-8 will be used as a therapeutic target in various inflammatory diseases. To the clinical application, the co-administration of fucoidan will be able to augment the host defense in animals with diseases. In conclusion, this study suggested that fucoidan-treated PBMCs culture supernatant enhances the chemotactic activity of canine PMNs, potentially through the increased IL-8.

Acknowledgment

This work was supported by the research grant of the Chungbuk National University in 2011.

References

1. Alekseyenko TV, Zhanayeva SY, Venediktova AA, Zvyagintseva TN, Kuznetsova TA, Besednova NN, Korolenko TA. Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk Sea Fucus evanescens brown alga. Bull Exp Biol Med 2007; 143: 730-732.
2. Aloisi F, Carè A, Borsellino G, Gallo P, Rosa S, Bassani A, Cabibbo A, Testa U, Levi G, Peschle C. Production of hemolymphopoietic cytokines (IL-6, IL-8, colony-stimulating factors) by normal human astrocytes in response to IL-1 beta and tumor necrosis factor-alpha. J Immunol 1992; 149: 2358-2366.
3. Baggiolini M, Imboden P, Detmers P. Neutrophil activation and the effects of interleukin-8/neutrophil-activating peptide 1 (IL-8/NAP-l). Cytokine 1992; 4: 1-17.
4. Bashir S, Cardigan R. Granulocyte concentrates: how can we assess their quality? Transfus Med 2003; 13: 245-257.
5. Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, Baggiolini M. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. J Exp Med 1991; 173: 771-774.
6. Chandia NP, Matsuhiro B. Characterization of a fucoidan from Lessonia vadosa (Phaeophyta) and its anticoagulant and elicitor properties. Int J Biol Macromol 2008; 42: 235-240.
7. Cromwell O, Hamid Q, Corrigan CJ, Barkans J, Meng Q, Collins PD, Kay AB. Expression and generation of interleukin-8, IL-6 and granulocyte-macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 beta and tumour necrosis factor-alpha. Immunology 1992; 77: 330-337.
8. Cross AK, Richardson V, Ali SA, Palmer I, Taub DD, Rees RC. Migration responses of human monocytic cell lines to alpha- and beta-chemokines. Cytokine 1997; 9: 521-528.
9. Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L, Tinari N, Morozevich GE, Berman AE, Bilan MI, Usov AI, Ustyuzhanina NE, Grachev AA, Sanderson CJ, Kelly M, Rabinovich GA, Iacobelli S, Nifantiev NE. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology 2007; 17: 541-552.
10. Damonte EB, Matulewicz MC, Cerezo AS. Sulfated seaweed polysaccharides as antiviral agents. Curr Med Chem 2004; 11: 2399-2419.
11. Detmers PA, Lo SK, Olsen-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. J Exp Med 1990; 171: 1155-1162.
12. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell 1996; 85: 307-310.
13. Grauffel V, Kloareg B, Mabeau S, Durand P, Jozefonvicz J. New natural polysaccharides with potent antithrombic activity: fucans from brown algae. Biomaterials 1989; 10: 363-368.
14. Hayashi K, Nakano T, Hashimoto M, Kanekiyo K, Hayashi T. Defensive effects of a fucoidan from brown alga Undaria pinnatifida against herpes simplex virus infection. Int Immunopharmacol 2008; 8: 109-116.
15. Hinode D, Yoshioka M, Tanabe S, Miki O, Masuda K, Nakamura R. The GroEL-like protein from Campylobacter rectus: immunological characterization and interleukin-6 and -8 induction in human gingival fibroblast. FEMS Microbiol Lett 1998; 167: 1-6.
16. Jagels MA, Hugli TE. Neutrophil chemotactic factors promote leukocytosis. A common mechanism for cellular recruitment from bone marrow. J Immunol 1992; 48: 1119-1128.
17. Kang JH, Lee GS, Jeung EB, Yang MP. Trans-10, cis-12-conjugated linoleic acid increases phagocytosis of porcine peripheral blood polymorphonuclear cells in vitro. Br J Nutr 2007; 97: 117-125.
18. Karakurum M, Shreeniwas R, Chen J, Pinsky D, Yan SD, Anderson M, Sunouchi K, Major J, Hamilton T, Kuwabara K, Rot A, Nowygrod R, Stern D. Hypoxic induction of

interleukin-8 gene expression in human endothelial cells. Clin Invest 1994; 93: 1564-1570.
19. Kim SH, Kang JH, Yang MP. Fucoidan increases phagocytic capacity and oxidative burst activity of canine peripheral blood polymorphonuclear cells through TNF-α from peripheral blood mononuclear cells. J Vet Clin 2011; 28: 183-189.
20. Koyanagi S, Tanigawa N, Nakagawa H, Soeda S, Shimeno H. Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. Biochem Pharmacol 2003; 65: 173-179.
21. Kreisle RA, Parker CW, Griffin GL, Senior RM, Stenson WF. Studies of leukotriene B_4-specific binding and function in rat polymorphonuclear leukocytes: absence of a chemotactic response. J Immunol 1985; 134: 3356-3363.
22. Lardot C, Dubois V, Lison D. Sulfur mustard upregulates the expression of interleukin-8 in cultured human keratinocytes. Toxicol Lett 1999; 110: 29-33.
23. Riou D, Colliec-Jouault S, Pinczon du Sel D, Bosch S, Siavoshian S, Le Bert V, Tomasoni C, Sinquin C, Durand P, Roussakis C. Antitumor and antiproliferative effects of a fucan extracted from ascophyllum nodosum against a non-small-cell bronchopulmonary carcinoma line. Anticancer Res 1996; 16: 1213-1218.
24. Schratzberger P, Reinisch N, Prodinger WM, Kähler CM, Sitte BA, Bellmann R, Fischer-Colbrie R, Winkler H, Wiedermann CJ. Differential chemotactic activities of sensory neuropeptides for human peripheral blood mononuclear cells. J Immunol 1997;158: 3895-3901.
25. Seitz M, Dewald B, Gerber N, Baggiolini M. Enhanced production of neutrophil-activating peptide-1/interleukin-8 in rheumatoid arthritis. J Clin Invest 1991; 87: 463-469.
26. Shaio MF, Lin PR, Liu JY, Yang KD. Generation of interleukin-8 from human monocytes in response to Trichomonas vaginalis stimulation. Infect Immun 1995; 63: 3864-3870.
27. Son SM, Kang JH, Lee GS, Jeung EB, Yang MP. Induction of interleukin-8 expression in porcine peripheral blood mononuclear cells by trans10?cis12 conjugated linoleic acid. Vet Immunol Immunopathol 2006;112:284-289.
28. Suzuki T, Tsuzuki A, Ohno N, Ohshima Y, Yadomae T. Enhancement of IL-8 production from human monocytic and granulocytic cell lines, THP-1 and HL-60, stimulated with Malassezia furfur. FEMS Immunol Med Microbiol 2000; 28: 157-162.
29. Thornton AJ, Strieter RM, Lindley I, Baggiolini M, Kunkel SL. Cytokine-induced gene expression of a neutrophil chemotactic factor/IL-8 in human hepatocytes. J Immunol 1990; 144: 2609-2613.
30. Walz A, Peveri P, Aschauer H, Baggiolini M. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem Biophys Res Commun 1987; 149: 755-761.
31. White MV, Yoshimura T, Hook W, Kaliner MA, Leonard EJ. Neutrophil attractant/activation protein-1 (NAP-1) causes human basophil histamine release. Immunol Lett 1989; 22: 151-154.
32. Yang MP, Lee KJ, Yun SM, Kim JH, Ko IK, Jeung EB. Feline interleukin-8 expression in peripheral blood mononuclear cells induced by egg white derivatives. Vet Immunol Immunopathol 2002; 86: 43-53.

개 말초혈액 다형핵백혈구의 유주활성에 있어 fucoidan의 효과

전춘진 · 김수현 · 김성수 · 강지훈 · 양만표[1]

충북대학교 수의과대학

요 약 : 개 말초혈액 다형핵백혈구(PMNs)의 유주활성에 대한 fucoidan의 면역증효과를 검토하였다. Fucoidan 그 자체는 PMNs에 대해 직접적인 유주활성 효과를 보이지 않았다. 그러나 recombinant IL-8에 대한 PMNs의 유주활성과 유사하게 fucoidan으로 배양한 말초혈액 단핵구세포(PBMCs)의 배양상층액은 PMNs에 대해 유주활성을 증가시켰다. 또한, fucoidan으로 배양한 PBMCs의 배양상층액에 대한 PMNs의 유주활성 증가효과는 anti-IL-8 pAb를 처리했을 때 억제되었다. PBMCs 배양 상층액 속의 IL-8의 양을 정량한 결과 fucoidan무처치 대조군에 비해 증가하였다. 이상의 결과로부터, fucoidan은 개 PMNs의 유주능에 대하여 면역증가 작용을 가지고 있으며, 이것은 fucoidan의 자극에 의해 PBMCs에서 생산되어 분비되는 IL-8에 의해 나타나는 것으로 사료되었다.

주요어 : fucoidan, interleukin-8, 유주능, 개