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Fucoidan Increases Phagocytic Capacity and Oxidative Burst Activity of Canine Peripheral Blood Polymorphonuclear Cells Through TNF-α from Peripheral Blood Mononulear Cells

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Abstract : The objective of this study was to examine the effect of fucoidan on the phagocytic capapcity and oxidative burst activity (OBA) of canine peripheral blood polymorphonuclear cells (PMNs). The phagocytic capacity and OBA of PMNs were evaluated simultaneously by using a flow cytometer. Fucoidan itself did not cause any direct effect on the phagocytic capacity and OBA of PMNs. However, the phagocytic capacity and OBA of PMNs were enhanced by the culture supernatant from PBMCs treated with fucoidan. The phagocytic capacity and OBA of PMNs were also increased by treatment with recombinant canine (rc) tumor necrosis factor (TNF)-α. The ability of the culture supernatant from fucoidan-treated PBMCs to stimulate the phagocytic capacity and OBA of PMNs was inhibited by addition of anti-rc TNF-α polyclonal antibody (pAb) prior to the culture. The amount of TNF-α in the culture supernatant from PBMCs was shown to increase upon treatment of fucoidan as compared with that of vehicle-treated PBMCs culture supernatant. The level of TNF-α mRNA expression in PBMCs was also up-regulated by the fucoidan treatment. These results suggest that fucoidan has an immunoenhancing effect on the phagocytic capacity and OBA of canine PMNs, which is mainly mediated by TNF-α released from fucoidan-stimulated PBMCs.

Key words **:** fucoidan, tumor necrosis factor-α, phagocytic capacity, oxidative burst activity, dog.

Introduction

Fucoidan is sulfated polysaccharides found in the extracellular matrix of various brown algae (2,9). It has many potent biological activities due to their ability to imitate patterns of sulfate substitution on glycosaminoglycans and other sulfated glucans (9). It has been also reported that fucoidan has anti-thrombotic (5), anti-viral (9,13), anti-angiogenic (17), anti-tumor effects (1,11), anti-proliferative and anti-adhesive activities (20). In addition, it inhibits adipogenesis (30), and can induce dendritic cells (DCs) maturation (29). Recently, fucoidan has been shown to modulate inflammatory reaction (8) and to stimulate both humoral and cell-mediated immune responses under *in vitro* and *in vivo* conditions (13). The secretion of interleukin (IL)-6 and tumor necrosis factor (TNF)-α were also enhanced by fucoidan in human T and B cell (12). The phagocytic activities of macrophages were enhanced by fucoidan, and these activated cells exhibited significantly increased production of nitric oxide (NO) and TNF-α (7). Although various immunomodulatory activities of fucoidan have been previously demonstrated, there are no studies for the effect of fucoidan on the phagocytic capacity and OBA of polymorphonuclear cells (PMNs), which may be an important mechanism for the enhancement of the innate immune response.

Therefore, the aim of this study is to examine the effect of fucoidan on the phagocytic capacity and OBA of PMNs. we examined the phagocytic capacity and OBA of PMNs treated with culture supernatant of peripheral blood mononuclear cells (PBMCs) with fucoidan. We also examined the effect of fucoidan treatment on TNF-α production of PBMCs.

Materials and Methods

Chemicals and reagents

Fucoidan purified from *Focus vesiculosus*, a kind of brown algae, 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 (10 mg/ml) until before use. Bovine serum albumin (Sigma-Aldrich), recombinant canine (rc) TNF-α, goat anti-rcTNF-α polyclonal antibody (pAb; IgG) (R&D Systems Inc., Minneapolis, MN, USA) and rabbit anti-recombinant mouse (rm) IL-6 pAb (IgG) (Sigma-Aldrich) were also commercially purchased.

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Animals

For the blood donor, we used two mixed breed and six Beagle dogs (two females and six males) with their age of 3.63 years old. The mean weigh of the dogs were 7.2 kg and all were healthy. 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 as described previously (6). 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 3×10^6 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 of 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 required.

Viability assay

Canine PBMCs and PMNs of 2×10^6 cells/ml, respectively, were incubated with or without fucoidan (0-500 μg/ml) in 24-well plastic culture plates for 24 h at 37°C in a 5% CO_2-humidified atmosphere. Cell viability was determined by trypan blue exclusion.

Simultaneous measurement of phagocytic capacity and OBA

The phagocytic capacity and OBA were evaluated simultaneously as described previously (6). All steps after beginning cultivation were conducted in the dark. The cells were analyzed within 30 min by using a flowcytometer (FACS calibur; Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) with an argon laser set at 488 nm and by using an analysis software (CELLQuest). The FL1 channel was set to 525 nm to detect green fluorescent rhodamine123, and the FL3 channel was set to 667 nm to detect red fluorescent microspheres on 10,000 cells per sample. Phagocytic capacity and OBA were expressed as percentages and mean fluorescence intensity (MFI), respectively.

Neutralization test

Freshly isolated PMNs (2×10^6 cells/ml) were incubated for 24 h with rcTNF-α (0, 10, 50 and 100 pg/ml) the presence or absence of different concentration of anti-rcTNF-α pAb at 37°C in a 5% CO_2-humidified atmosphere. And various concentrations of anti-rcTNF-α pAb were mixed with the PBMCs culture supernatant (75%) for 30 min at room temperature. The effect of this mixture to stimulate the phagocytic capacity and OBA of the PMNs was evaluated as described above.

Measurement of TNF-α in the culture supernatant of fucoidan-treated PBMCs

The culture supernatants of PBMCs treated with or without fucoidan (200 μg/ml) were collected after 24 h incubation. The TNF-α level in the culture supernatants from PBMCs were determined by the direct sandwich enzyme-linked immunosorbent assay (ELISA) in the DuoSet ELISA deveolpment TNF-α kit (R&d System Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. All samples, standard sand controls were assayed in triplicate. The optical density was determined using an automated microplate reader (ELx808, Bio-Tek Instruments Inc., Winooski, Vermont, USA) at 450 nm. The TNF-α levels in the samples were quantified from standard curves generated with purified canine TNF-α tested at eight titration points. The lower and upper detection limits were 31.25 and 2000 pg/ml, respectively.

Ribonucleic acid preparation and reverse transcription polymerase chain reaction

Total RNA was extracted using the Trizol reagent (Invitrogen Co.) according to the methods outlined in the protocol. The concentration of total RNA was determined by measuring the absorbance at 260 nm. First strand complementary DNA (cDNA) was prepared by subjecting total RNA (1 μg) to reverse transcription using Moloney Murine Leukemia Virus (mMLV) reverse transcriptase (Invitrogen Co.) and random primers (9-mers; Takara Bio Inc., Otsu, Shiga, Japan). The GAPDH gene was PCR-amplified to rule out the possibility of RNA degradation and was used to control for variations in mRNA concentration in the reverse transcription (RT) reaction. A linear relationship between PCR product band visibility and number of amplification cycles was observed for target mRNAs. The GAPDH and target genes were quantified using 25 cycles, respectively. The cDNA was amplified in a 20 μl PCR reaction containing 1 U *Taq* polymerase (iNtRON Biotechnology Inc., Sungnam, South Korea), 2 mM dNTP, and 10 pmol specific primers. PCR reactions were denatured at 95°C for 30 sec, annealed at 60°C for 30 sec, and extended at 72°C for 30 sec. The oligonucleotides for TNF-α were based on the cDNA sequence (GenBank accession number Z70046) 5'-CTC CCA GGT CCT CCT CAA GG-3' (sense) and 5'-AGA AAC TTG AAG AAT ACC TT-3' (anti-sense). The primer for GAPDH gene (NCBI NO. NM_001003142) was 5'-AGA ACA TCA TCC CTG CTT C-3' (sense) and 5'-TTG AAG TCA CAG GAG ACC AC-3' (anti-sense). PCR products (8 μl) were fractionated on a 2.3% agarose gel, stained with ethidium bromide, and photographed under UV illumination. The photograph was

Table 1. Cell viability (%) of PBMCs and PMNs treated with fucoidan for 24 h

	fucoidan concentration (μg/ml)						
cell type	0	50	100	150	200	250	500
PBMCs	99.62 ± 0.14	99.31 ± 0.21	99.40 ± 0.11	99.98 ± 0.03	99.81 ± 0.20	97.41 ± 0.27*	-
PMNs	99.85 ± 0.04	99.03 ± 0.02	99.51 ± 0.01	99.01 ± 0.06	99.88 ± 0.01	99.82 ± 0.21	81.81 ± 0.32

The values represent men ± SD (n=3). *$p < 0.05$ *versus* untreated control (0 μg/ml fucoidan).

scanned using a Gel Doc EQ system (Bio-Rad Laboratories., Hercules, CA, USA).

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

Effect of fucoidan on PBMCs and PMNs cell viability

PBMCs and PMNs preparations with > 98% viability were cultured for 24 h with fucoidan at concentrations ranging from 0 to 500 μg/ml, after which their viability was assessed by trypan blue dye exclusion methods. The viability of PMNs was significantly reduced by fucoidan at concentrations of 500 μg/ml, ($p = 0.012$) when compared with the untreated control (0 μg/ml fucoidan). PBMCs at the concentration of 250 μg/ml fucoidan revealed the lower viability ($p = 0.049$) as compared to that of the untreated control cells (Table 1). Consequently, fucoidan was used in subsequent experiments at a concentration of 200 μg/ml.

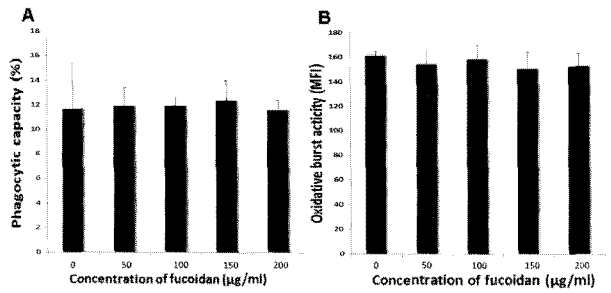

Fig 1. Effect of fucoidan treatment on the phagocytic capacity (A) and OBA (B) of PMNs. Freshly isolated PMNs (2×10^6 cells/ml/well) were incubated for 24 h with fucoidan (0-200 μg/ml). Trans-FluoSpheres® carboxylate-modified microspheres were added to the cultures for the final 1 h and DHR123 was added cultures for the final 15 min for the simultaneous measurement of phagocytic capacity (A) and OBA (B). The phagocytic capacity and OBA were measured by flowcytometer. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. The values indicate means ± SD (n = 3). MFI, mean fluorescence intensity.

Direct effect of fucoidan treatment on the phagocytic capacity and oxidative burst activity (OBA) of PMNs

To examine the direct effect of fucoidan on the phagocytic capacity and OBA of PMNs, freshly isolated PMNs were cultured for 24 h with fucoidan at concentrations ranging from 0 to 200 μg/ml. Direct treatment of fucoidan did not show any effect on the phagocytic capacity (Fig 1A) and OBA (Fig 1B) of PMNs.

Effect of the culture supernatant from fucoidan-treated PBMCs on the phagocytic capacity and OBA of PMNs

We examined whether the culture supernatant from fucoidan-treated PBMCs has any effect on the phagocytic capacity and OBA of PMNs. Freshly prepared PMNs were incubated for 24 h with culture supernatant (0-100%) from PBMCs (3×10^6 cells/ml) treated with 200 μg/ml of fucoidan for 24 h. The phagocytic capacity (12.5%; $p = 0.023$, 25-75%; $p < 0.001$) (Fig 2A) and OBA (6.25%; $p = 0.016$, 12.5-75%; $p < 0.001$) (Fig 2B) of PMNs were significantly increased in a dose-dependent

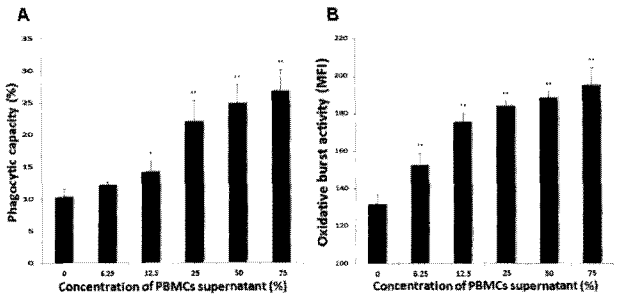

Fig 2. Effect of the culture supernatant of fucoidan-treated PBMCs on the phagocytic capacity (A) and OBA (B) of PMNs. Freshly isolated PMNs (2×10^6 cells/ml) were incubated for 24 h with culture supernatant (0-75%) from PBMCs (3×10^6 cells/ml) that had been treated with fucoidan (200 μg/ml) for 24 h. Trans-FluoSpheres® carboxylate-modified microspheres were added to the cultures for the final 1 h and DHR123 was added cultures for the final 15 min for the simultaneous measurement of phagocytic capacity (A) and OBA (B). The phagocytic capacity and OBA were measured by flowcytometer. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. The values indicate means ± SD (n = 7). *$p < 0.05$, **$p < 0.01$, compared with those of 0% culture supernatant groups. MFI, mean fluorescence intensity.

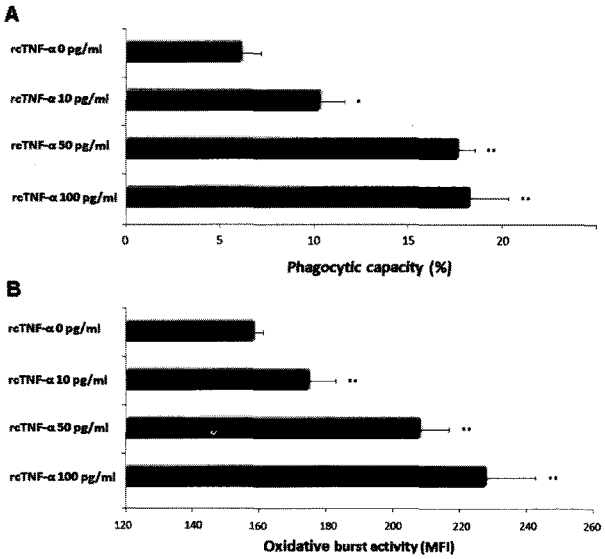

Fig 3. Effect of rcTNF-α on the phagocytic capacity (A) and OBA (B) of PMNs. Freshly isolated PMNs (2×10^6 cells/ml) were incubated for 24 h with the indicated concentrations of rcTNF-α. TransFluoSpheres® carboxylate-modified microspheres were added to the cultures for the final 1 h and DHR123 was added cultures for the final 15 min for the simultaneous measurement of phagocytic capacity (A) and OBA (B). The phagocytic capacity and OBA were measured by flowcytometer. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. The values indicate means ± SD (n = 3). *p < 0.05, **p < 0.01, compared with rcTNF-α (0 pg/ml) treated groups. MFI, mean fluorescence intensity.

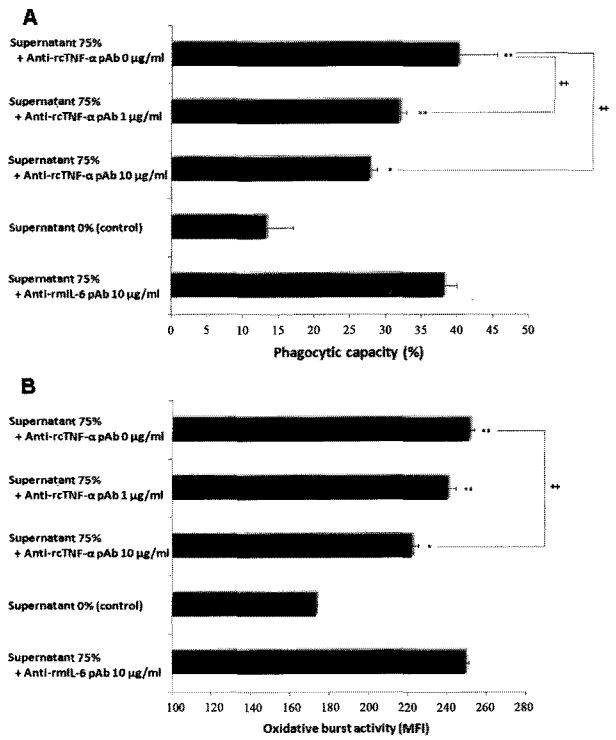

Fig 4. Neutralizing effect of anti-rcTNF-α pAb on the phagocytic capacity (A) and OBA (B) of PMNs mixed with fucoidan-treated PBMCs culture supernatant. Anti-rcTNF-α pAb (0, 1, and 10 μg/ml) and anti-rmIL-6 pAb (10 μg/ml) were mixed with the fucoidan-treated PBMCs culture supernatant (75%) for 30 min at room temperature. Freshly isolated PMNs (2×10^6 cells/ml) were incubated for 24 h with anti-rcTNF-α pAb-treated culture supernatant. TransFluoSpheres® carboxylate-modified microspheres were added to the cultures for the final 1 h and DHR123 was added cultures for the final 15 min for the simultaneous measurement of phagocytic capacity (A) and OBA (B). The phagocytic capacity and OBA were measured by flowcytometer. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. The values indicate means ± SD (n=3). $^{*}p < 0.05$, $^{**}p < 0.01$, compared with supernatant (0%) groups, $^{+}p < 0.05$, $^{++}p < 0.01$, compared with supernatant (75%) without anti-rcTNF-α pAb groups. MFI, mean fluorescence intensity.

manner by exposure to the culture supernatant of fucoidan-treated PBMCs, respectively, when compared with those exposed to untreated control; the greatest enhancement on the phagocytic capacity and OBA was observed in cells exposed to 75% of fucoidan-treated PBMCs culture supernatant.

Effect of rcTNF-α on the phagocytic capacity and OBA of PMNs

The effect of rcTNF-α on the phagocytic capacity and OBA of PMNs was also tested. The phagocytic capacity (50 pg/ml; $p = 0.003$, 100 pg/ml; $p < 0.001$) (Fig 3A) and OBA (10 pg/ml; $p = 0.023$, 50 and 100 pg/ml; $p < 0.001$) (Fig 3B) of PMNs were augmented in a dose dependent manner by addition of rcTNF-α, compared to those of untreated control, respectively. The effect of rcTNF-α on the phagocytic capacity and OBA peaked at 100 pg/ml.

Neutralization effect of anti-rcTNF-α pAb on the phagocytic capacity and OBA of PMNs treated with either the culture supernatants from PBMCs or rcTNF-α

To examine whether the enhancement of the phagocytic capacity and OBA of PMNs to culture supernatant from PBMCs treated with fucoidan is due to TNF-α or not, the neutralization test using the anti-rcTNF-α pAb was performed. The phagocytic capacity and OBA of PMNs in response to either culture supernatant (1 μg/ml; $p = 0.008$, 10 μg/ml; $p < 0.001$; Fig 4A) (10 μg/ml; $p = 0.026$; Fig 4B) or rcTNF-α (0.1 μg/ml; $p = 0.013$, 1 ug/ml; $p < 0.001$, 10 ug/ml; $p < 0.001$; Fig 5A) (0.1 μg/ml; $p = 0.037$, 1 ug/ml; $p = 0.007$, 10 ug/ml; $p < 0.001$; Fig 5B) were inhibited by the addition of anti-rcTNF-α pAb when compared with those of positive controls (75% culture supernatant and 100 pg/ml rcTNF-α, respectively). However, in the examination of the possibility of nonspecific inhibition for immunoglobulin isotype, IgG, of anti-rcTNF-α pAb, the enhancement of phagocytic capacity and OBA of PMNs to either culture supernatant (75%) or rcTNF-α (100 pg/ml) was not inhibited by the addition of high concentration of 10 μg/ml of anti-rmIL-6 pAb (control IgG) instead of anti-rcTNF-α pAb.

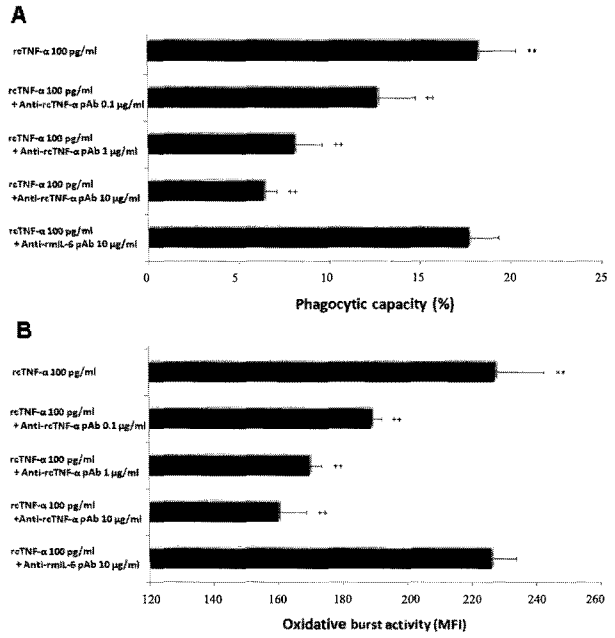

Fig 5. Neutralizing effect of anti-rcTNF-α pAb on the phagocytic capacity (A) and OBA (B) of the rcTNF-α treated PMNs. Anti-rcTNF-α pAb (0, 0.1, 1, and 10 μg/ml) and anti-rmIL-6 pAb (10 μg/ml) were mixed with the rcTNF-α (100 pg/ml) for 30 min at room temperature. Freshly isolated PMNs (2×10^6 cells/ml) were incubated for 24 h with these mixtures. TransFluoSpheres® carboxylate-modified microspheres were added to the cultures for the final 1 h and DHR123 was added cultures for the final 15 min for the simultaneous measurement of phagocytic capacity (A) and OBA (B). The phagocytic capacity and OBA were measured by flowcytometer. One-way ANOVA was used to investigate differences between control and treatments, followed by a Dunnett's test. The values indicate means ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$, compared with rcTNF-α (100 pg/ml) groups. MFI, mean fluorescence intensity.

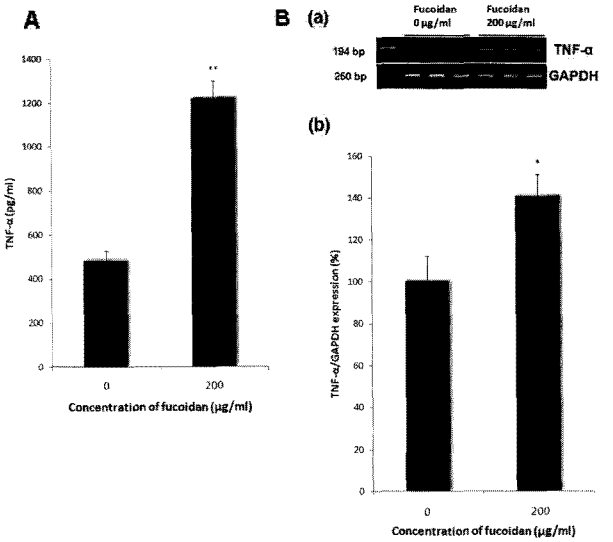

Fig 6. The effect of fucoidan on TNF-α production (A) and expression (B) in PBMCs. PBMCs (3×10^6 cells/ml) were incubated with fucoidan (200 ug/ml) for 24 h. The concentration of TNF-α in the culture supernatant from PBMCs was measured by ELISA (Quantikine® canine TNF-α immunoassay kit; R&D Systems) (A). RT-PCR analysis was performed on TNF-α mRNA in PBMCs (3×10^6 cells/ml) treated with fucoidan (200 μg/ml) for 24 h. Normalization of the TNF-α mRNA expression with GAPDH (B-(a)). The expected product sizes of TNF-α and GAPDH mRNA are 194 and 250 bp, respectively. Signals were quantified by a digital analysis program and expressed as a percent of the maximum values (B-(b)). Comparison of two groups was made by *t*-test. The data represent mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$.

Amount of TNF-α in culture supernatant from PBMCs treated with fucoidan

The amount of TNF-α in the culture supernatant (100%) from PBMCs treated with fucoidan (200 μg/ml) for 24 h was quantified. The level of TNF-α production in the fucoidan-treated PBMCs culture supernatant was significantly higher ($p = 0.001$), compared to the culture supernatant (100%) from fucoidan-untreated PBMCs (Fig 6A).

TNF-α mRNA expression in PBMCs

To examine whether fucoidan induces the expression of TNF-α mRNA in PBMCs in response to fucoidan, PBMCs (3×10^6 cells/ml) were incubated with fucoidan (200 μg/ml) for 24 h and harvested for RNA isolation. Fucoidan significantly increased ($p = 0.012$) TNF-α mRNA expression in PBMCs compared to vehicle-treated control group (Fig 6B).

Discussion

In the present study, fucoidan at concentration around 500 μg/ml reduced the cell viability of PBMCs and PMNs, and 200 μg/ml had no apparent effects on their viability. Similar to this results, it has been reported that fucoidan at concentrations of 50 to 200 μg/ml had no effects on the viability of monocytic cell line U973 (28). Therefore, in the present study fucoidan was used at concentration of 200 μg/ml for the most effective dose.

In our study, the culture supernatant of fucoidan-treated PBMCs enhanced the phagocytic capacity and OBA of PMNs. This suggests that there are soluble factor(s) in the culture supernatant of fucoidan-treated PBMCs that up-regulates the phagocytic capacity and OBA of PMNs. Phagocytosis and OBA can be modulated by inflammatory cytokines (3). PBMCs generate in a variety of cytokines, such as IL-1, IL-6 and TNF-α (23). Among of these cytokines, TNF-α is a powerful PMNs priming agent that increase the phagocytosis of neutrophils (22) and enhance the NADPH oxidase dependent superoxide anion production by PMNs (10). Fucoidan can increase various cytokine productions in many situations (12,16). Specially, fucoidan increased the production of TNF-α and IL-6, but not IL-1β from macrophages (7). Consequently, we hypothesized that the soluble factor in the culture supernatant of fucoidan-treated PBMCs may be related with TNF-α. We tested the effect of rcTNF-α on the phagocytic capacity and OBA of PMNs. Similar to the effects of fucoidan-treated culture supernatant to the phagocytic

response of PMNs, rcTNF-α could increase the phagocytic capacity and OBA of canine PMNs. We found that the TNF-α level of culture supernatants of PBMCs were increased by the fucoidan treatment. Moreover, mRNA expression levels of TNF-α in PBMCs was enhanced by the fucoidan treatment, as expected. Next, we examined whether an anti-rcTNF-α pAb neutralizes the enhancement of phagocytic capacity and OBA induced by fucoidan-treated PBMCs culture supernatant. The anti-rcTNF-α pAb completely neutralized the ability of the culture supernatant from fucoidan-treated PBMCs to enhance the phagocytic capacity and OBA of PMNs. These findings that the culture supernatant from PBMCs treated with fucoidan is able to enhance the phagocytic capacity and OBA of PMNs might suggest that the immunoenhancing effect of fucoidan on the phagocytic response of PMNs is mediated by TNF-α produced by fucoidan-stimulated PBMCs.

In this study, direct treatment of fucoidan did not enhance the phagocytic capacity and OBA of PMNs at the condition of 24 h incubation. However, it has been recently reported that the phagocytosis significantly increased in the macrophage *in vitro* treatment with fucoidan for 48 h (7,13). This effect may be also related to TNF-α from macrophages. Generally, cytokines act both as paracrine and/or autocrine signals (27). Fucoidan may stimulate macrophages to produce TNF-α, which then acts in an autocrine manner to promote phagocytosis of macrophages. In addition, obvious differences are noted between neutrophils and macrophages, which not all ligand and receptor pairs trigger comparable modes of phagosome formation, and the rate and extent of maturation also seem to differ between neutrophils and macrophages (18). The difference of incubation time might result in the different effect on phagocytosis between PMNs and macrophages. Further investigations are needed to elucidate the effects of fucoidan on the phagocytic capacity and OBA of PBMCs.

In our study, fucoidan had immunostimulating effects on the phagocytic responses of peripheral blood phagocytes. Fucoidan could be produced in large quantities, since various brown algae contain fucoidan in their extracellular matrix. Thus, it may indicate the possible utility of fucoidan *in vivo*. Fucoidan has great potential as a nutritional supplement, and it could be used as a feed additive for the immunoenhancing effect.

In summary, fucoidan increases phagocytic capacity and OBA of canine PMNs through TNF-α from PBMCs.

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Fucoidan에 의한 개 말초혈액 단핵구세포에서 생산된 TNF-α의 다형핵백혈구에 대한 탐식능과 순간산소과소비력의 증가효과

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요 약 : 개 말초혈액 다형핵 백혈구(PMNs)의 탐식능력과 순간산소과소비력(OBA) 에 대한 fucoidan의 효과를 검토하였다. Fucoidan 그 자체는 PMNs의 탐식능력과 OBA에 직접적인 효과를 보이지 않았다. 그러나 fucoidan으로 배양한 PBMCs의 배양상층액은 PMNs의 탐식능력과 OBA를 농도의존적으로 증가시켰다. 또한, TNF-α에 의한 PMNs의 탐식능력과 OBA의 측정 결과도 fucoidan으로 배양한 PBMCs 배양상층액의 그것과 유사하였다. 이러한 탐식능력과OBA의 활성은 anti-TNF-α pAb를 처리했을 때 억제되었다. PBMCs 배양 상층액 속의 TNF-α의 양을 정량한 결과 대조군에 비해 증가되었으며, PBMCs의 TNF-α mRNA 발현 정도도 fucoidan을 첨가한 경우 증가되었다. 이상의 결과로부터, fucoidan은 개 말초혈액 PMNs의 탐식능력과 OBA에 대하여 면역자극 작용을 가지고 있으며, 이것은 fucoidan의 자극에 의해 PBMCs에서 생산되어 분비되는 가용성 물질인 TNF-α에 의해 나타나는 것으로 사료되었다.

주요어 : fucoidan, TNF-α, 탐식능, 순간산소과소비력, 개