▸원 저◂

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Radiation-Induced IL(interleukin)-6 in Mice with Algin-Oligosaccharide Treatment

— 알긴산올리고당 처치 마우스의 방사선 유도 IL-6 —

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— Abstract —

To examine the radioprotective effect of algin-oligosaccharide(AOS), radiation-induced IL(interleukin)-6 in mice treated with 3 Gy whole body irradiation once were examined.

In the measurement of irradiation-induced IL-6, in comparison with the irradiation control group, in both small intestine and liver tissues of the group treated with algin-oligosaccharide for 7 days prior to irradiation, was suppressed IL-6 synthesis($p < 0.001$). It is considered that the protection against radiation hazard by antioxydative reaction of algin-oligosaccharide results in down control of IL-6 value in experimental groups treated with algin-oligosaccharide.

In conclusion, through our study, the fact that algin-oligosaccharide has irradiation protection effects was elucidated, and simultaneously, the possibility of the use of a natural product without chemical toxicity as an irradiation protection agent was confirmed.

Key Words : Radiation-induced IL-6, Radiation-induced CYTOKINE, algin-oligosaccharide

Ⅰ. Introduction

Recently, as chemical protective agents that reduce radiation damage, studies on Macro Glucan, TMG(vitamin E derivatives), Guarana, propolis, extracts of edible mushrooms(EEM), green tea, thio substances, melatonin, vitamin C, ginseng, etc. are ongoing[1,2]. However, regarding most protection agents except vitamin agents, green tea, ginseng, etc., due to accompanying potent toxicity at effective doses, their use is limited, and particularly, it has a shortcoming that it must be administered prior to irradiation exposure[1,2]. Therefore, recently, natural products became the focus of the interest of studies on the change of reaction to irradiation in vivo[1-3].

Studies on the reaction factors or regulation materials associated with radiation protection are also actively ongoing. Human IL-1β nonapeptide is a palmitoyl residue mediating immunostimulatory activities of the native molecule without inflammatory or febrile characteristic. This IL-1β nonapeptide significantly protects mice exposed to the potentially lethal dose of ionized radiation. Such results suggest not only the importance of the derivation of small

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peptidesin radioprotection, but also toxic cytokines are produced for the purpose of increasing radio-protective actions[4].

In mice irradiated with either a sublethal dose or s lethal dose, if the bacteria lysate IRS-19 were administered for a certain period prior to radiation, the proliferation of hematopoietic cells was accelerated. In other words, administered IRS-19, the substantiality of hematopoietic cells in the bone marrow and the spleen was increased to a substantial level, and the regeneration of hematopoietic cells in the bone marrow and the spleen was accelerated. The radioprotective characteristic of IRS-19 is to greatly induce hemaepoetic colony-stimulating activities and sequential reactions of cytokines[5]. In addition, manganous superoxide dismutase(MnSOD) that is an enzyme produced in mitochondria with excellent function of removing oxygen free radicals is effective on radioprotection[6]. Simultaneously, among radiation-induced cytokines, cytokines contributing to radioprotection and cytokines inducing the opposite effects are present, and thus if such facts were elucidated, it would be possible to apply a new cytokine approach from the clinical aspect for radioprotection[7].

Alginic acid is a component comprising cell wall in brown algae, such as brown seaweeds and tangle weeds and called also seaweed acid. Algin-oligosaccharide derived from alginic acid is known to accomplish a biological protective function through the mechanism eliminating free radicals or active oxygen by its excellent antioxydative effect[1,2,8]. Particularly, this mechanism prevents free radicals, active ions, or excited molecules to be expressed in biological macromolecule (e.g. DNA) by eliminating ions or excited molecules generated rapidly from physical and chemical actions following irradiation[1,2].

To prevent irradiation damage, treatments with appropriate radiation protective agents either prior to or after irradiation are required. Therefore, studies on the radioprotective effect of natural products that are not harmful to human body and could be taken by everybody readily are required more than any other times. Particularly, regarding radioprotective effect of natural products for in vivo tissues, it is determined that to characterize definite influential factors or action mechanisms are most important than anything else.

In our study, in irradiated mice administered algin-oligosaccharide(AOS), by examining the radiation-induced synthesis of IL(interleukin)-6, the level of radioprotective effect of algin-oligosaccharide was assessed. For this, algin-oligosaccharide was administered to mice treated with 3 Gy whole body irradiated once, their small intestine tissue and the liver tissue were dissolved, and IL-6 were measured.

Ⅱ. Materials and methods

1. Experiment materials

1) Reagents

Reagents used in our study were a Bicincchoninic acid protein assay kit(Pierce U.S.A), Tris(Bio Basic, USA), Ethylenediaminetetra acetic acid(EDTA, Sigma, USA), sodium chloride(NaCl, Junsei, Japan), Triton-X 100(Junsei, Japan), sodium nitrate(Sigma, USA), etc., and as monoclonal anti-IL-6 antibody, ELISA kits (R&D system, MIN, USA) were used. In addition, as algin-oligosaccharide, the product(Sigma, USA) imported and distributed by EcoBio was used.

2) Irradiation equipment

For irradiation, 10 MeV level Linac X-ray therapeutic equipment(Clinac 21Ex, Varian, 2004) was used.

3) Experimental animals

As experimental animals, C57BL/6 mice(body weight, 25-35 g) bred and distributed by the Damul Laboratory Animal Center, Korea, were used, and at the time of actual measurements, the small intestine and the liver tissues of mice were isolated and used. Mice were maintained in an animal room of which

temperature was maintained as 23±2℃ humidity as 45±5%, and cages made of polycarbonate(40×25×17 cm) were used, and animal feed(Cheil Jedang, Korea) and drinking water were supplied freely. As experimental mice, 7 animals per group, and total 42 animals were used. The experimental animals were used according to the *Helsinki Declaration*.

2. Assignment of experiment groups

Experiment groups were designated as normal, irradiation control, treatment group of before irradiation, and treatment group of after irradiation, the treatment group of before irradiation was subdivided to the AOS treatment for 7 days + irradiation group and the AOS treatment for 3 days + irradiation group, and the treatment group of after irradiation was subdivided to the irradiation + AOS treatment for 3 days group and the irradiation + AOS treatment for 7 days group(Table 1).

Table 1. Classification of experimental groups

Experimental groups	
Group 1	normal
Group 2	irradiation control
Group 3	AOS for 7days + irradiation
Group 4	AOS for 3days + irradiation
Group 5	irradiation + AOS for 3days
Group 6	irradiation + AOS for 7days

3. Experiment methods

1) Irradiation

For this experiment, using a 10 MeV level Linac X-ray therapeutic equipment, total 3 Gy dose was irradiated once at 300 cGy/min irradiation rate.

2) Administration of reagents

The reagent of our experiment algin oligosaccharide(AOS) was administered per oral based on 5 mg/kg/day standard using an oral injector. For the AOS treatment for 7 days + irradiation group cases, the reagent was administered for 7 days prior to irradiation, for the AOS treatment for 3 days + irradiation group cases, for 3 days prior to irradiation, for the irradiation + AOS treatment for 3 days group, for 3 days after irradiation, and for the irradiation + AOS treatment for 7 days group cases, it was administered for 7 days after irradiation.

3) Measurement

As the method to measure the IL-6, the manufacturer's instruction was used. First, 50 μL assay diluent was added to the provided wells, 50 μL each cytokine standard solution or the experiment solution was added to the center area of the wells, the bottom of plates was tapped to mix well, the plates were covered with the provided sealing tape, and reacted at room temperature for 2 hours. The sealing tape was removed, and the wells were washed with the provided washing buffer 5 times repeatedly. 100 μL conjugated solution of the cytokine to be measured was added to each well, covered with the sealing tape, reacted for 2 hours, and washed with washing buffer 5 times repeatedly. 100 μL substrate solution was added to each well, reacted for 30 minutes at room temperature in dark, 100 μL stop solution was added to each well, and measured within 30 minutes(micro-reader: 450 nm, wavelength correction: 570 nm, USA).

Protein concentration was measured using a BCA protein assay kit. BCA was used as the standard substance, 25 μL each protein was aliquoted to 96 well plates, the BCA agent consisted of the reagent A and the reagent B (50 : 1) (200 μL) was added, and incubated at 37℃ for 1 hour. After incubation, the optical density(O.D.) at 540 nm was measured using a microplate reader(Molecular Devices, Sunnyvale, CA, USA).

4. Statistical analysis

The result of each experiment was presented as a

mean and standard deviation (Mean±S.D.), as statistical analysis, ANOVA was performed using the SPSS 10.1 statistic program, and for the multiple comparison, tukey test was performed. $p < 0.05$ was considered to be significant.

Ⅲ. Results

1. IL-6 measurement of the small intestine tissue

Algin-oligosaccharide was administered to mice treated with 3 Gy whole body irradiation once, small intestine tissues were dissolved and IL-6 was examined(Table 2). In comparison with the IL-6 value of the irradiation control group, 0.040±0.0081, the AOS for 7days + irradiation group was shown to be 0.022±0.0034, and it was found that in the cases treated with algin-oligosaccharide for 7 days prior to irradiation, IL-6 immune activity was elevated($p < 0.001$).

Table 2. IL-6 in the small intestine tissue (unit : pg/mG)

Groups	IL-6 (mean±S.D.)	
Normal	0.005±0.0030	
Irradiation control	0.040±0.0081	
AOS for 7days + irradiation	0.022±0.0034	***
AOS for 3days + irradiation	0.036±0.0026	
Irradiation + AOS for 3days	0.036±0.0020	
Irradiation + AOS for 7days	0.043±0.0050	

*** $p < 0.001$ as compared with irradiation control group.

2. IL-6 measurement of the liver tissue

Algin-oligosaccharide was administered to mice treated with 3 Gy whole body irradiation once, liver tissues were dissolved and IL-6 was examined(Table 3). In comparison with the IL-6 value of the irradiation control group, 0.030±0.0036, the AOS for 7days + irradiation group was shown to be 0.013±0.0038, and thus it was found that in the cases treated with algin-oligosaccharide for 7 days prior to irradiation, IL-6 immune activity was elevated($p < 0.001$).

Table 3. IL-6 in the liver tissue (unit : pg/mG)

Groups	IL-6 (mean±S.D.)	
Normal	0.009±0.0017	
Irradiation control	0.030±0.0036	
AOS for 7days + irradiation	0.013±0.0038	***
AOS for 3days + irradiation	0.028±0.0036	
Irradiation + AOS for 3days	0.026±0.0026	
Irradiation + AOS for 7days	0.032±0.0034	

*** $p < 0.001$ as compared with irradiation control group.

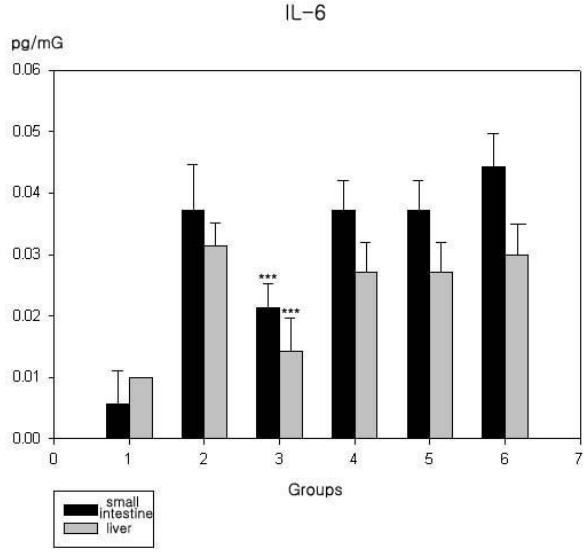

Fig. 1. IL-6 in small intestine and liver tissue of 3 Gy irradiated mice with algin-oligosaccharide treatment.

*** $p < 0.001$ as compared with irradiation control group.
Group 1 : normal
Group 2 : irradiation control
Group 3 : AOS for 7days + irradiation
Group 4 : AOS for 3days + irradiation
Group 5 : irradiation + AOS for 3days
Group 6 : irradiation + AOS for 7days

Ⅳ. Discussion

In our study, to examine the radioprotective effect of algin-oligosaccharide, the small intestine and liver tissues of mice treated with 3 Gy whole body ir-

radiation once were dissolution, and the cytokines IL-6 were examined. The result confirmed that for the prevention of radiation damage, algin-oligo-saccharide had great effects.

S.K. Choi et al.(2007) have reported that cas-pase-3 and caspase-9 in cell signaling pathway leading to cell death after irradiation were observed. While about both caspase-3 and caspase-9, the OD values of irradiation control group displayed high increase, the OD values of all experimental groups treated with AOS before or after irradiation appeared significantly lower than that of irradiation control group. Moreover, the largest difference appeared in AOS treatment group for 7 days before irradiation in particular[1].

W.Y. Jang et al.(2009) have reported that NO(ni-tric oxide) showed decreased in the irradiation con-trol group, while 3 day's treatment group with al-gin-oligosaccharide before or after irradiation in-dicated higher than the irradiation control group, especially showed big difference in 3 day's treatment group before irradiation[2].

Among irradiation-induced cytokines, regarding interleukins(IL), Hidetoshi et al.(1995) have reported that including IL-2, IL-4 and IL-7 immune factors rescued both CD^+4 T cells and CD^+8 T cells from ir-radiation-induced apoptosis[9], Legue et al.(2001) have reported that injected cytokines such as IL-6 prior to whole body irradiation, cell survival rate was increased and thus radioprotective effect was shown, and simultaneously, radioprotective effects on testis cells such as sertoli cells were observed[10], Frasca et al.(2000) have reported that irradiated pe-ripheral mononuclear cells obtained from young and middle aged groups, the effect of cytokines gen-erated during the DNA binding process of eukaryotic cell ku was examined, and it was found that in the young age group, the DNA binding activity of ku cells was increased substantially, and thus the IL-6 modified cytokine K-7/D-6 was produced, and thus DNA repair was induced[11]. In addition, Meeren et al.(1999) have reported that irradiated human endo-thelial cells, IL-6 synthesis was increased[12], Ross et al.(1997) have reported than in a human glioblastoma cell line, IL-6 mRNA synthesis was stimulated in response to low or very low radiation dose[13]. In our study, algin-oligosaccharide was administered to mice treated with 3 Gy whole body irradiation once, and IL-6 was examined, and it was found that in comparison with the irradiation control group, in both small intestine and liver tissues, in the group treated with algin-oligosaccharide for 7 days prior to irradiation, IL-6 synthesis was suppressed($p < 0.001$).

Ⅴ. Conclusion

In order to find out the Radioprotective effect of algin-oligosaccharide(AOS), this study, with a mouse of which whole frame irradiated by 3 Gy radiation once, measured IL-6 amid cell signaling connected to apoptosis in order to observe cell activation.

In the measurement of irradiation-induced IL-6, in comparison with the irradiation control group, in both small intestine and liver tissues of the group treated with algin-oligosaccharide for 7 days prior to irradiation, was suppressed IL-6 synthesis($p < 0.001$).

In conclusion, through our study, the fact that algin-oligosaccharide has irradiation protection ef-fects was elucidated, and simultaneously, the possi-bility of the use of a natural product without chem-ical toxicity as an irradiation protection agent was confirmed.

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• 국문초록

알긴산올리고당 처치 마우스의 방사선 유도 IL-6

최성관 · 지연상

광주보건대학 방사선과

본 연구에서는 미역이나 다시마에 많이 분포하면서 항산화작용이 탁월한 알긴산올리고당의 방사선 방어효과를 알아보기 위해 3 Gy 방사선이 전신 1회 조사된 마우스를 가지고 IL-6을 측정하였다.

측정 결과 방사선조사대조군과 비교하여 볼 때 소장과 간 조직 모두 방사선조사 전 7일간 알긴산올리고당의 처치를 시행한 그룹에서 IL-6 생성이 억제됨을 관찰하였다($p < 0.001$). 이는 알긴산올리고당이 항산화작용을 통해 방사선이 피폭된 생체조직을 방어함으로써 IL-6의 생성을 억제한 것으로 사료된다.

결론적으로, 본 연구를 통해 알긴산올리고당의 일부 방사선 방어효과를 규명했고 아울러 화학적 독성이 없는 자연산생물이 방사선 방어제로 활용될 수 있을 가능성을 확인하였다.

중심 단어: 방사선유도 IL-6, 방사선유도 사이토카인, 알긴산올리고당