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마우스에서 스쿠알렌의 방사선방호 효과

朝 鮮 大 學 校 大 學 院

原子力工學科

董 京 來

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Radioprotective Effects of Squalene in Mice

2015年 2月 25日

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마우스에서 스쿠알렌의 방사선방호 효과

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방사선치료 시 방사선피폭으로 인한 정상조직의 장해를 예방하려면 방사선조사 전이나 후에 적절한 방사선 보호제의 처치를 받아야 하므로 본 연구 목적은 심해 상어의 간에 많이 함유된 자연산생물에서 추출한 Squalene(SQ)의 방사선 방호효과를 구명하는데 있다. 이를 위해 3 Gy 방사선이 1회 전신 조사된 마우스에게 SQ을 투여한 후 첫째, 세포 활성능을 관찰하기 위해 세포사(apoptosis)로 이어지는 세포신호경로 중 Caspase-3 및 Caspase-9를 측정하였고, 둘째, 세포 염증반응을 관찰하기 위해 NO(nitric oxide)를 측정하였으며, 셋째, 방사선 유도 사이토카인을 관찰하기 위해 TNF(tumor necrosis factor)-α, IL(interleukin)-6과 IL(interleukin)-10을 측정하였다. 아울러 SQ의 방사선 방호효과에 관한 보조자료 획득 일환으로 동일 마우스의 혈청(serum)에 대한 생화학적 분석도 함께 실시하였다. 실험그룹은 정상(normal), 방사선조사대조군(irradiation control), 방사선조사 전처치군(SQ treatment group of before irradiation), 방사선조사 후처치군(SQ treatment group of after irradiation)으로 설정했고, 다시 방사선조사대조군은 irradiation + Measurement after 3 day과 irradiation + Measurement after 7 day로, 방사선조사 전처치군은 SQ treatment for 7 and 3 days + irradiation + Measurement after 3 day, SQ treatment for 7 and 3 days + irradiation + Measurement after 7 day로, 방사선조사 후처치군은 irradiation + SQ treatment for 3 day + 방사선조사 3일 후에 측정, irradiation + SQ treatment for 7 day + 방사선조사 7일 후의 측정군으로 나누웠다. 방사선장해에 대한 Squalene의 방호효과를 실험하기 위해 마우스에게 10 MeV급 Linac 치료 장치를 사용하여 생체조직의 생화학적 변화가 가장 활발하게 발현되는 선량역인 3 Gy의 선량을 적용하여 300 cGy/min 선량률로 1회 전신 조사하였다. 세포 활성능을 관찰하기 위한 Caspase-3 및 Caspase-9 검사에서 Caspase-3은 소장과 간 모두 7, 3일전 투여, Caspase-9는 소장에서 7일전, 간에서 7, 3일전 투여 시 방사선대조군에 비해 SQ 처치군에서 Caspase 생성이 억제됨을 확인하였다 ($p < 0.05$). 세포 염증반응을 관찰하기 위한 NO 검사에서는 소장과 간 모두 7, 3일전 방사선대조군에 비해 SQ 처치군에서 NO 생성이 증가됨을 확인하였다 ($p <$

0.05). 방사선 유도 사이토카인 중 TNF-α, IL-6, IL-10 검사에서 소장과 간 모두 SQ을 7일전 투여 시 방사선대조군에 비해 SQ 처치군에서 TNF-α, IL-6, IL-10 생성이 억제됨을 확인하였다 ($p < 0.05$). 혈청검사에서 SQ의 투여로 인해 회복을 나타낸 경우로는 TP (total protein)에서 SQ을 방사선 조사 7일전에 투여 후 방사선 조사를 마친 3일 후에서 증가, Albumin에서 SQ을 방사선 조사 7일전에 투여 후 방사선 조사를 마친 3일 후에서 증가, LDH에서 SQ을 방사선 조사 7일전에 투여 후 방사선 조사를 마친 3일 후에서 증가, HDLC에서 SQ을 방사선 조사 7일전과 3일전에 투여 후 방사선 조사를 마친 3일 후에서 감소, LDHC에서 SQ을 방사선 조사 7일전에 투여 후 방사선 조사를 마친 3일 후에서 증가, UA에서 SQ을 방사선 조사 7일전에 투여 후 방사선 조사를 마친 3일 후에서 증가하였다. 결론적으로, 본 연구에서 항산화효과가 탁월한 Squalene은 Caspase 생성 억제, Nitric Oxide 생성 증가, TNF-α 생성 억제, IL-6 생성 억제, IL-6 생성 억제 등을 유도함으로써 방사선 방호작용을 수행한다는 사실을 규명하였고, 화학적 독성이 적은 방사선방호제로 활용될 수 있음을 확인하였다.

중심어: 방사선치료, 방사선방호제, Squalene, 마우스, Caspase-3, Caspase-9, NO, TNF-α, IL-6, IL-10

I. Introduction

Since X-ray was discovered by W. C. Rõntgen in 1895, radiation has rapidly become valuable and has widely been used in such fields as medicine, engineering, and science and, in particular, the area of radiation is also applied to diagnosis and therapy in the medical field due to the increase of medical check-ups and the development of radiation diagnostic equipment; however, how helpful or harmful it is to a human life is very controversial[1]. In this respect, the recently increased use of atomic energy facilities, the potential of regions neighboring nuclear waste sites to be contaminated by radioactive substances, and the increased chances for a human body to be exposed to radiation for the purpose of diagnosis and therapy are increasing the need of radioprotection. A radiation dose above a certain level may cause three types of radiation injury: sublethal damage from which one can recover within several hours after stimulation, potentially lethal damage which is usually lethal but from which one can recover if proper environmental changes are made, and lethal damage from which one can never recover[2]. In particular, a session of high-dose whole-body irradiation can result in more serious radiation injury. Ordinary people can be exposed to radiation through natural radiation exposure, medical exposure, and accidental exposure. Natural radiation exposure refers to exposure to radiation in the air, from underground, and from the space and is made in a lower dose than permissible one. Medical radiation exposure during radiodiagnosis or radiotherapy for cancer is unavoidable to preserve health but can possibly cause serious radiation injury, thereby ruining health. In addition, sudden, accidental exposure, for example, due to radioactivity leakage from nuclear power plants in Chernobyl, the former Soviet Union in 1986 and nuclear test in North Korea in 2006 is a threat that can bring a tremendous disaster to human beings. Materials which are used to reduce radiation injury through proper treatment of an organism before or after irradiation are called radioprotectors. Such materials are classified into two functional categories: protection, which is a requirement of treatment before irradiation, and repair, which is effective as

treatment after irradiation. Radioprotectors include such chemicals as cyanide, carbon monoxide, epinephrine, histamine, serotonin, cysteine, cysteamine, AET glutathione, WR-series, and polymer compounds (e.g. dextran sulfate, Lipopolysaccharide, carbon particle, polyacrylamide beads, etc.)[3-6]. Some of the other radioprotectors are natural protective agents, which can usually be taken thanks to lower levels of chemical toxicity, including ginseng extracts, vitamin C, and vitamin E. Ginseng extracts had the repairability of cells and the survival rate significantly affected by the administration time: the greatest protective effects were found in case of administration 24 hours before irradiation. Polymer compounds gave the greatest protective effects in case of administration one to three days before irradiation[7]. South Korean researchers have recently examined such chemical protective agents to reduce radiation injury as Macro Glucan, TMG (vitamin E derivative), Guarana, propolis, extracts of edible mushrooms (EEM), green tea, thio agents, melatonin, vitamin C, and ginseng[8,9]. However, most of the other protective agents than vitamin agents, green tea, and ginseng have limitations in usage due to high levels of toxicity in the effective dose (ED); in particular, they need to be treated before radiation exposure[10]. So recent research has paid attention to bio-response changes with radiation of natural products, such as epigallocathechin gallote (EGCG), a core catechin component of green tea, ginseng, and red ginseng[11-13]. Research on radioprotection is being very positively conducted abroad as well. An acidic fibroblast growth factor (FGF_1) makes radioprotective effects through a mechanism of stimulating proliferation of bone marrow stem cells and has been found to stimulate not simply fibroblasts but also diverse cells, including bone marrow cells and endothelial cells[14]. In-vitro test was performed to see how protective three stable nitroxides—carbamoyl (CM)-, methoxycarbonyl (MC)-, and hydroxymethyl (HM)-PROXYL—were of a lethal X-ray (8Gy) irradiated mouse from having DNA damage; as a result, radioprotective effects were found in the order of HM- > CM- = MC-PROXYL. Radioprotection by these nitroxides may depend not only on the potential for oxidation-reduction reaction and reactivity but also on dynamic actions, such as absorption and excretion of medicine[15]. It has recently been reported that ammonium trichloro-tellurate (AS101)

has significant radioprotective and chemically protective effects on hematopoiesis in a mouse exposed to radiation or treated with various chemicals. Treatment with AS101 has been found to be very effective in recovering hematopoiesis in patients suffering from excessive or accidental exposure to radiation by reducing the amount of semi-acute protein and albumin[16]. IL-1β nonapeptides in human beings are palmitoyl residues, which perform immunostimulation of innate molecules without inflammation or febrility and significantly protect a mouse exposed to ionizing radiation in the potential lethal dose. This result not only suggests the importance of small peptide derivatization in radioprotection but also implies that toxin cytokine is generated to increase radioprotective activities[17]. In a mouse exposed to a sublethal or lethal dose of radiation, IRS-19 administration may promote the regeneration of hematopoietic cells in the spleen and bone marrow. Such hematopoietic colony stimulation may lead to radioprotection in combination with step-by-step reaction of cytokine[18]. Besides, manganous superoxide dismutase (MnSOD), which is enzyme generated from mitochondria and is excellent in antioxidant protective functions, reportedly has radioprotective effects and a cytokine approach method, which can also be applied to radiation injury in clinical practice, has been presented[19,20]. Squalene (SQ; hexamethyltetracosahexane; $C_{30}H_{50}$) is formed by thirty hydrocarbon chains, including six double bonds, has molecular weight of 410.70, and is structurally similar to β-carotene. As an intermediate generated during the process of cholesterol synthesis in vivo, SQ is hydrocarbon synthesized in vertebrates and plants; in particular, a large amount of SQ is contained in the liver of centrophorusatromarginatus[21]. SQ is synthesized through farnesyl pyrophosphatase after mevalonate phosphorylation during the process of cholesterol synthesis; cholesterol is synthesized through SQ epoxidase and lanosterol and hydrocarbon chains are unstable and are vulnerable to oxidation. Olive oil among plants contains the largest amount of SQ, which is principally synthesized in the liver of animals and a large amount of which is contained in diverse regions, including blood plasma, the skin, subcutaneous fatty tissues, abdominal fatty tissues, lymph nodes, arterial walls, adrenal glands, the pancreas, and the myocardium. SQ is not oil because it contains no hydroxycarboxyl group

but is particularly characterized by easy combination with oxygen ions. It acts in a similar way to ozone, is effective in curing a wound, increases cardiac activity, and inhibits vasodilatation and atherosclerosis. SQ has long been used as a health food in Japan and Korea and is reportedly effective against hyperlipemla, atherosclerosis, myocardial infarction, liver diseases, and gastrointestinal diseases[22]. It has been found that SQ synthesis and synthetase are located at the endoplasmic reticulum in liver cells of a white mouse and that SQ synthesis in a microsomal fraction varies with a few low-fat medicines or diet[23,24]. While an accurate mechanism of SQ has not been discovered yet, dietary SQ intake may activate cholesterol synthesis and low density lipoprotein (LDL) apoB metabolism in human beings (Miettinen et al., 1986) and reduce 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity in the liver of a white mouse[25]. An SQ inducer, 2-aza-2-dihydrosqualene, serves as a cholesterol synthesis inhibitor, facilitates cell division in the stratum basale of the skin with a burn[26], and makes skin regeneration effects by removing harmful oxygen caused by a burn[27]. Storm et al. found that SQ not only removed reactive oxygen species, promoted cellular immune responses, facilitated recovery of damaged cells and cell proliferation but also was effective in protecting tissues and extending lifespan by stabilizing reactive oxygen and activating antioxidants[28]. Bennett et al. noted that tissue recovery or regeneration, inflammatory responses, mitosis, angiogenesis, synthesis, and extracellular matrix as well as delay of aging were performed through a complicated biological process[29]. A peptide growth factor, which plays a crucial role in initiating and maintaining regeneration of these tissues, reportedly controls such a process in vitro and induces generation of a peptide growth factor, which controls an important phase in curing a wound, in vivo. Typical factors include a platelet derived growth factor (PDGF), a transforming growth factor-β (TGF-β), an epidermal growth factor (EGF), an insulin like growth factor I (IGF-I), and a fibroblast growth factor (FGF). A mechanism of the growth factors is performing its function in target cells through internal secretion, iso-secretion, autocrine, and receptor coupling; EGF is polypeptide, which is stable with heat, is safe from dialysis, and consists of 53 amino acids with molecular weight of 6,045 and three intramolecular disulfide bonds

necessary for biological activity[30]. In particular, EGF, which is associated with epidermal regeneration, forms TGF-α, amphiregulin, and heparin-binding EGF families, which have similar structural functions. Human EGF has a similar molecular structure, antigens, and biological properties to that of a mouse, has no species specificity because it acts equally on a receptor of cell membranes, and has a lower level of activity than murine EGF[31]. EGF is coupled with an epidermal growth factor receptor (EGFR) to activate tyrosin kinase of the receptor and to facilitate cell proliferation and differentiation and DNA, mRNA, and protein synthesis[32,33]. EGF has metabolic effects: it facilitates protein and RNA synthesis, facilitates protein synthesis in ribosome, induces ornithine decaboxylase, and accumulates polyamine intracellularly[34]. EGF is secreted from kidneys, the lacrimal gland, the submandibular gland, the Brunner gland, and megakeryocyte and excreted through saliva, lachrymal fluid, and urine, and is discharged by platelet activation in the initial stage of tissue regeneration with blood EGF concentration of approximately 130 pmol/L, which is the amount of secretion that can facilitate cell movement and division[35]. Nanney found that EGF played a role in active skin regeneration by increasing the number of fibroblasts, by increasing neovascularization, and by accumulating collagenous fibers in the process of curing a wound in the porcine skin[36]. Neill et al. noted that EGF and its receptors played a crucial role in restoring soft and hard tissues to cure a wound and that treatment with EGF could accelerate recovery of normal tissues and facilitate cure of a wound[29]. To prevent any injury from radiation exposure, it is necessary to receive treatment with a proper radioprotector before or after irradiation. However, most radioprotectors cannot be effective protectors from radiation injury because they are chemicals accompanied by high levels of toxicity, can hardly be taken usually, and are rarely available to ordinary people. It is therefore essential to conduct research on the radioprotective effects of natural products, such as SQ, which do no harm to human health and which anyone can take at any time (Fig. 1).

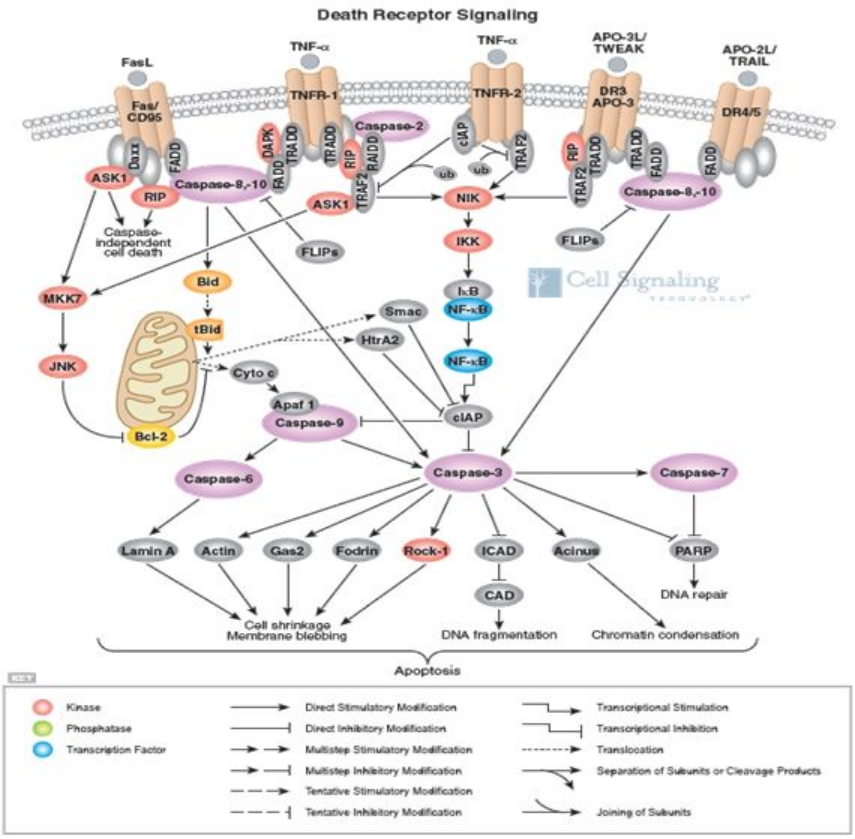

Fig. 1. Cell signaling.

The purpose of this study is to determine the radioprotective effects of SQ extracted from natural products, a large amount of which is contained in the liver of centrophorusatromarginatus. For this purpose, SQ was administered to mice exposed to a session of 3 Gy whole-body irradiation; then, first, Caspase-3 and Caspase-9 in the cell signaling pathways, which led to apoptosis, were measured to examine cell activity; second, nitric oxide (NO) was measured to observe inflammatory responses in cells; and third, a tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interleukin (IL)-10 were measured to observe radiation-induced cytokine. In addition, biochemical analyses of the serum from the same mice were made as part of an attempt to obtain supplementary data about the radioprotective effects of SQ.

II. Materials and Methods

A. Materials

1. Experimental Animals

The experimental animals in this study were C57BL/6 mice (weighing 25-35 g) produced and supplied by Damul Laboratory Animal Center (South Korea). They were raised in a cage made of polycarbonate (40 × 25 × 17 cm) in a breeding room with temperature kept at 23 ± 2℃ and humidity at 45 ± 5% and were allowed to have feed (manufactured by Cheiljedang) and water freely. A total of 49 mice, 7 per experimental group, were used in this study.

The animals were used with the aim of promoting and protecting health of mankind to meet the purport of the Declaration of Helsinki; proper measures were taken to minimize adverse environmental effects; and every effort was made to create the optimum welfare environment for them throughout the period of experiment.

2. Reagents

The reagents in this study included Bicincchoninic acid (BCA) protein assay kit (Pierce, U.S.A.), Sodium nitrate (Sigma, U.S.A.), sulfanilamide (Sigma, U.S.A.), N-(naphthyethylene)diamine (Sigma, U.S.A.), Tris (Bio Basic, U.S.A.), Ethylenediaminetetraaceticacid (EDTA, Sigma, U.S.A.), Sdoium chloride (NaCl, Junsei, Japan), Triton-X 100 (Junsei, Japan), Sodium nitrate (Sigma, U.S.A.), ELISA Kits of monoclonal antibodies for TNF-α, IL-6, and IL-10 (R&D systems, MIN, U.S.A), and Caspase-3/CPP32 & Caspase-9/Mch 6 Colorimetric Assay Kit (Biovision, U.S.A.), and SQ was a product of Semo (South Korea).

3. Serum Analyzer

Serum centrifuged from murine blood was analyzed using an auto analyser (7170, Hitachi, 2004).

4. Irradiator

A 10 MeV Linac X-ray radiation therapy system (Clinac 21Ex, Varian, 2004) was used for irradiation.

B. Grouping for Experiment

The experimental groups were divided into normal, irradiation control, SQ treatment before irradiation, and SQ treatment after irradiation groups; then, the irradiation control group was subdivided into irradiation + measurement after 3 days and irradiation + measurement after 7 days groups, the SQ treatment before irradiation group into SQ treatment for 7 and 3 days + irradiation + measurement after 3 days and SQ treatment for 7 and 3 days + irradiation + measurement after 7 days groups, and the SQ treatment after irradiation group into irradiation + SQ treatment for 3 days + irradiation + measurement after 3 days and irradiation + SQ treatment for 7 days + irradiation + measurement after 7 days groups. There were a total of seven experimental groups; considering the fact that it might take the specimens long to be absorbed into living tissues, seven- and three-day factors were applied to the pretreatment groups and three- and seven-day factors were applied to the posttreatment groups.

C. Methods

1. Irradiation

To determine the radioprotective effects of SQ, a 10 MeV LINAC radiation therapy system was used to expose mice to a session of whole-body irradiation in a dose of 3 Gy, which induces the greatest biochemical changes in living tissues, at the dose rate of 300 cGy/min (Fig. 2).

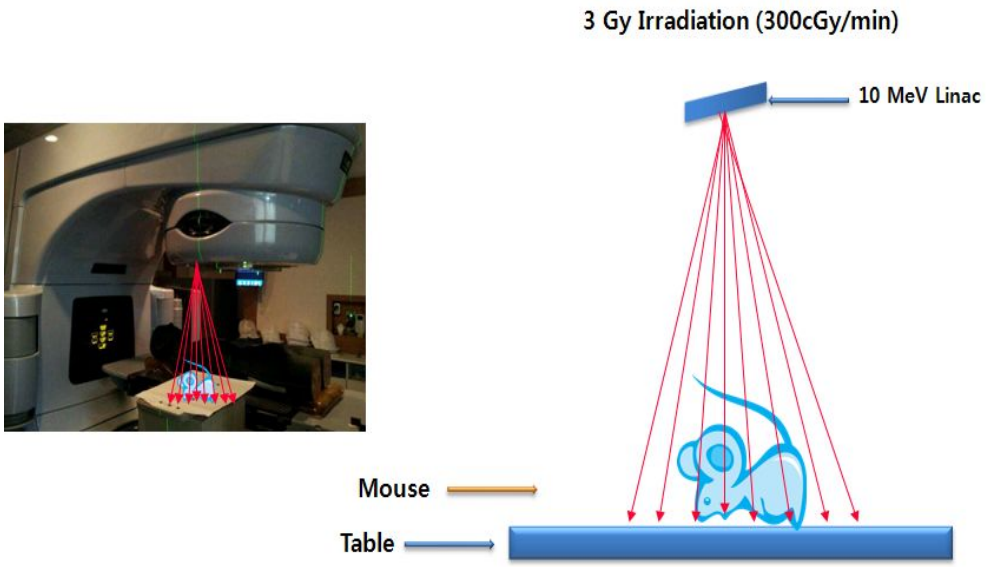

Fig. 2. Irradiation in mice using the 10 MeV Linac X-ray.

2. Specimen Administration

8 mL/kg SQ, which was a specimen in this study, was abdominally injected to C57BL/6 mice. It was administered for seven days before irradiation in the SQ treatment for 7 days + irradiation group, for three days before irradiation in the SQ treatment for 3 days + irradiation group, for three days after irradiation in the irradiation + SQ treatment for 3 days group, and for seven days after irradiation in the irradiation + SQ treatment for 7 days group (Table 1).

Table 1. Classification of experimental groups

Measured after irradiation	Group	N
	Normal	7
3 day	Irradiation control (3 day)	7
	SQ(pre 7 day) + Rad	7
	SQ(pre 3 day) + Rad	7
	Rad + SQ	7
7 day	Irradiation control (7 day)	7
	SQ(pre 7 day) + Rad	7
	SQ(pre 3 day) + Rad	7
	Rad + SQ	7

3. Animal Sacrifice and Tissue Collection

The animals were simultaneously sacrificed three days after irradiation in the normal, irradiation control, and SQ treatment before irradiation groups and three days after the final specimen administration in the SQ treatment after irradiation groups and the experimental tissues from the small intestines and the liver and serum were collected right after the sacrifice. Cervical dislocation was used to sacrifice them.

4. Measurement

a. Caspase-3 and Caspase-9: Cell Activity Measurement

Provide 100 μg of tissues from the liver and 100 μg from the small intestines with cell lysis in a 100 μL cell lysis buffer and incubate it in ice for ten minutes. After one-minute centrifugal in 10,000 g, transfer supernatant to a new tube and keep it in ice. Perform quantitative analysis of protein. Dilute a 50 μL cell lysis buffer to contain 100-200 μg protein and transfer it to 96 well plates. Put a 50 μL 2X Reaction buffer (reaction reagent containing 10

mM DTT) in each tube. Add 5 μL 4mM LEHD-pNA substrate to each tube and incubate it at 37℃ for 1-2 hours. Then, perform measurement in a microplate reader (microreader: 405 nm, U.S.A.).

b. Nitric Oxide (NO): Inflammatory Response Measurement

The tissues from the small intestines and the liver were completely dissolved in a lysis buffer to measure NO. The experiment was carried out as follows: Use a Griess reagent in a form of NO2- to measure the amount of NO generated in the tissues from the small intestines and the liver. Elute the supernatant to 96 well plates and add a 100 μL Griess reagent (0.8% sulfanilamide/0.75% N-(naphthyethylene) diamine in 0.5N HCl, Sigma) to each of them. Leave the plates at room temperature for 15 minutes; then, use a microplate reader (Molecular Devices, Sunnyvale, CA, U.S.A) to measure the concentration of nitrite at 540 nm wavelength. Use sodium nitrate (0.5-100 M) as standard nitrite.

c. Cytokines: (TNF-α: tumor necrosis factor; IL-6 and IL-10: immune factors)

Cytokines, such as TNF-α, IL-6, and IL-10, were measured according to the manufacturer's instruction. First, put a 50 μL assay diluent in each well, add 50 μL of a standard solution and 50 μL of an experimental solution to the center of the well for each cytokine, hit the plate gently on the floor to mix well, cover them with airtight tapes, and leave them at room temperature for two hours to make a reaction. Then, remove the tapes and give five sessions of washing with the washing buffer. Put a 100 μL conjugate solution of cytokine to measure in each well and cover it with airtight tapes to make a reaction for two hours; then, give five sessions of washing with the washing buffer. Put a 100 μL substrate solution in each well and keep it at room temperature in a light-shielded environment for thirty minutes to make a reaction. Then, put a 100 μL stop solution in each well and perform measure-

ment within thirty minutes (microreader: 450 nm, wavelength correction: 570 nm, U.S.A.).

d. Protein Content (Serum Measurement)

The protein concentration was measured using a BCA protein assay kit. Use BCA as a standard to elute 25 μL of each protein specimen to 96 well plates, add a 200 μL BCA agent composed of reagents A and B (50 : 1) to each of the plates, and incubate it at 37℃ for one hour. After the incubation, use a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to measure absorbance at 540 mm.

D. Statistical Analysis

The results of each experiment were presented in the mean and standard deviation. Since there were only a few samples that constituted the experimental groups, significance was tested among experimental groups by using a bootstrap method to estimate an asymptotic 95% confidence interval. The bootstrap method developed by B. Efron, which is to estimate a confidence interval through sampling without specific supposition of a probability model, is useful in estimating a more robust and accurate confidence interval in case of asymmetrical data, as compared with traditional methods reliant on distribution. Typical methods to estimate a confidence interval by bootstrap include a percentile method and a Bias-corrected and accelerated (BCa) method. Since the latter, which gives correction of skewness, has a problem with estimation of a confidence interval with an asymmetrical type of large distribution, the former was used in this study. Kruskal-Wallis test was carried out to get the mean between groups on the basis of ordinal variables in testing hypotheses. The statistical test was processed at the $p < 0.05$ significance level.

III. Results

A. Cell Signaling Pathways Leading to Apoptosis

1. Caspase-3 Observation in Cell Signaling Pathways Leading to Apoptosis

a. Small intestines

SQ was administered to irradiated mice to observe Caspase-3 in the tissues from the small intestines. As for the O.D. value at 405 ㎚, Caspase-3 was estimated to be 0.020 in the normal small intestines. Caspase-3 was estimated to be 0.720 and 0.779—higher than the normal level of 0.020—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.505 and 0.688 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.642 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.720). It was estimated to be 0.610 and 0.652 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.622 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.779). At the 95% confidence interval, there were statistically significant differences between both the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the group receiving irradiation alone (Rad control group) ($p < 0.05$) but not between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) and the Rad control group. Statistically significant differences were found between both the groups with measurement three days after

irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of Caspase-3 in the small intestines were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 2, Fig. 3).

Table 2. Caspase-3 in Small intestine of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.020	0.009	(0.012 0.024)
3 day	Irradiation control	7	0.720	0.046	(0.675 0.755)
	SQ(pre 7day)+Rad	7	0.505	0.005	(0.501 0.508)*
	SQ(pre 3day)+Rad	7	0.688	0.016	(0.672 0.706)
	Rad+SQ	7	0.642	0.013	(0.633 0.653)*
7 day	Irradiation control	7	0.779	0.008	(0.775 0.786)
	SQ(pre 7day)+Rad	7	0.610	0.004	(0.606 0.614)*
	SQ(pre 3day)+Rad	7	0.652	0.009	(0.642 0.659)*
	Rad+SQ	7	0.622	0.011	(0.612 0.627)*

Note: The interaction effect was determined using boostrap method. The unit is the number of O.D. 405 nm. *$p < 0.05$.

b. Liver

SQ was administered to irradiated mice to observe Caspase-3 in the tissues from the liver. As for the O.D. value at 405 nm, Caspase-3 was estimated to be 0.201 in the normal liver. Caspase-3 was estimated to be 0.676 and 0.683—higher than the normal level of 0.201—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. Three days after irradiation following SQ administration seven days and three days before irradiation, it was estimated to be 0.395 and 0.606, respectively, both of which were lower than that of the group receiving irradiation alone (M=0.676). In contrast, it was estimated to be 0.697 in case of SQ administration after irradiation, which was higher than that of the group receiving irradiation alone (M=0.676). Seven days after irradiation following SQ administration seven days and three days before irradiation, it was estimated to be 0.527 and 0.613, respectively, both of which were lower than that of the group receiving irradiation alone (M=0.676). In contrast, it was estimated to be 0.718 in case of SQ administration after irradiation, which was higher than that of the group receiving irradiation alone (M=0.779). At the 95% confidence interval, there were statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$) but not between the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. Statistically significant differences were found between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$) but not between the group with measurement seven days after irradiation following SQ administration three days before irradiation

(SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of Caspase-3 in the liver were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 3, Fig. 3).

Table 3. Caspase-3 in Liver of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.201	0.057	(0.164 0.239)
3 day	Irradiation control	7	0.676	0.047	(0.644 0.686)
	SQ(pre 7day)+Rad	7	0.395	0.035	(0.355 0.418)*
	SQ(pre 3day)+Rad	7	0.606	0.018	(0.588 0.627)*
	Rad+SQ	7	0.697	0.113	(0.614 0.694)
7 day	Irradiation control	7	0.683	0.038	(0.648 0.727)
	SQ(pre 7day)+Rad	7	0.527	0.058	(0.456 0.586)*
	SQ(pre 3day)+Rad	7	0.613	0.044	(0.567 0.656)
	Rad+SQ	7	0.718	0.062	(0.664 0.800)

Note: The interaction effect was determined using boostrap method. The unit is the number of O.D. 405 nm. $^{*}p < 0.05$.

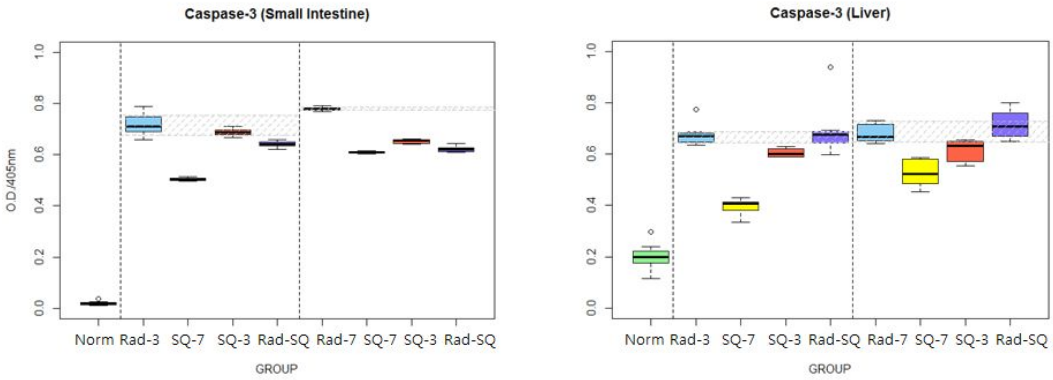

Fig. 3. Caspase-3 in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

2. Caspase-9 Observation in Cell Signaling Pathways Leading to Apoptosis

a. Small intestines

SQ was administered to irradiated mice to observe Caspase-9 in the tissues from the small intestines. As for the O.D. value at 405 nm, Caspase-9 was estimated to be 0.088 in the normal small intestines. Caspase-9 was estimated to be 0.725 and 0.786—higher than the normal level of 0.088—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.065 and 0.090 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.096 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.725). It was estimated to be 0.079 and 0.099 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.090 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.779). At the 95% confidence interval, there were statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). Statistically significant differences were found between both the groups with measurement seven days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of Caspase-9 in the small intestines were found to be greatest in the group

receiving SQ administration for seven days before irradiation (Table 4, Fig. 4).

Table 4. Caspase-9 in Small intestine of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.088	0.004	(0.084 0.090)
3 day	Irradiation control	7	0.725	0.025	(0.700 0.753)
	SQ(pre 7day)+Rad	7	0.065	0.002	(0.063 0.067)*
	SQ(pre 3day)+Rad	7	0.090	0.007	(0.090 0.093)*
	Rad+SQ	7	0.096	0.002	(0.094 0.098)*
7 day	Irradiation control	7	0.786	0.003	(0.784 0.789)
	SQ(pre 7day)+Rad	7	0.079	0.006	(0.073 0.084)*
	SQ(pre 3day)+Rad	7	0.099	0.012	(0.088 0.115)*
	Rad+SQ	7	0.090	0.004	(0.087 0.092)*

Note: The interaction effect was determined using boostrap method. The unit is the number of O.D. 405 nm. *$p < 0.05$.

b. Liver

SQ was administered to irradiated mice to observe Caspase-9 in the tissues from the liver. As for the O.D. value at 405 nm, Caspase-9 was estimated to be 0.150 in the normal liver. Caspase-9 was estimated to be 0.801 and 0.750—higher than the normal level of 0.150—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. Three days after irradiation following SQ administration seven days and three days before irradiation, it was estimated to be lower than that of the group receiving irradiation alone (M=0.801): 0.382 and 0.791, respectively. In contrast, it was estimated to be 0.870, which was higher than that of the group receiving irradiation alone (M=0.801), in case of SQ administration after irradiation. It was estimated to be 0.734 and 0.622 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.602 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.750). At the 95% confidence interval, there were statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$) but not between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. Statistically significant differences were found between both the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$) but not between the group with measurement seven days after irradiation following SQ administration

seven days before irradiation (SQ(pre-seven days)) and the Rad control group. In particular, radioprotective effects of Caspase-9 in the liver were found to be greatest in the group with measurement three days after irradiation following SQ administration seven days before irradiation and in the group receiving SQ administration seven days after irradiation (Table 5, Fig. 4).

Table 5. Caspase-9 in Liver of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.150	0.030	(0.120 0.182)
3 day	Irradiation control	7	0.801	0.091	(0.702 0.850)
	SQ(pre 7day)+Rad	7	0.382	0.129	(0.274 0.501)*
	SQ(pre 3day)+Rad	7	0.791	0.050	(0.753 0.857)
	Rad+SQ	7	0.870	0.070	(0.798 0.936)
7 day	Irradiation control	7	0.750	0.034	(0.721 0.783)
	SQ(pre 7day)+Rad	7	0.734	0.090	(0.650 0.832)
	SQ(pre 3day)+Rad	7	0.622	0.026	(0.596 0.650)*
	Rad+SQ	7	0.602	0.008	(0.596 0.609)*

Note: The interaction effect was determined using boostrap method. The unit is the number of O.D. 405 nm. *$p < 0.05$.

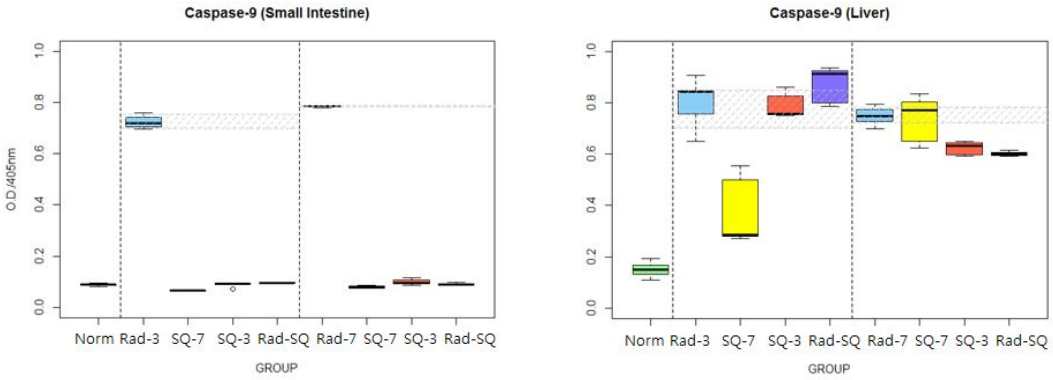

Fig. 4. Caspase-9 in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

B. Inflammatory Response Measurement

1. Nitric Oxide (NO)

a. Small intestines

SQ was administered to irradiated mice to observe NO in the tissues from the small intestines. NO was estimated to be 6.064 in the normal small intestines. NO was estimated to be 1.652 and 3.802—lower than the normal level of 6.064—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 3.835 and 2.492 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 2.291 in case of SQ administration after irradiation, all of which were higher than that of the group receiving irradiation alone (M=1.652). It was estimated to be 2.873 and 2.136 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 1.414 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=3.802). At the 95% confidence interval, there were statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, there was no statistically significant difference between the groups with measurement seven days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of NO in the small intestines were found to be greatest three days after irradiation following SQ administration seven days before irradiation (Table 6, Fig. 5).

Table 6. NO(Nitric Oxide) in Small intestine of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	6.064	0.559	(5.596 6.863)
3 day	Irradiation control	7	1.652	0.035	(1.606 1.693)
	SQ(pre 7day)+Rad	7	3.835	0.949	(2.514 4.744)*
	SQ(pre 3day)+Rad	7	2.492	0.473	(2.034 2.970)*
	Rad+SQ	7	2.291	0.426	(1.835 2.660)*
7 day	Irradiation control	7	3.802	0.440	(3.439 4.439)
	SQ(pre 7day)+Rad	7	2.873	0.853	(2.315 4.125)
	SQ(pre 3day)+Rad	7	2.136	0.276	(1.835 2.505)
	Rad+SQ	7	1.414	0.096	(1.328 1.520)

Note: The interaction effect was determined using boostrap method. The unit is the number of nM/μg. $^{*}p < 0.05$.

b. Liver

SQ was administered to irradiated mice to observe NO in the tissues from the liver. NO was estimated to be 3.044 in the normal liver. NO was estimated to be 2.232 and 2.034—lower than the normal level of 3.044—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 2.626 and 2.593 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=2.232). In contrast, it was estimated to be 2.100 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=2.232). It was estimated to be 2.649 and 2.107 seven days after irradiation following SQ administration seven days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=2.034). In contrast, it was estimated to be 1.965 seven days after irradiation following SQ administration three days before irradiation, which was lower than that of the group receiving irradiation alone (M=2.034). At the 95% confidence interval, there were statistically significant differences between the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement three days after irradiation following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) and the Rad control group. There were statistically significant differences between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was

found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of NO in the liver were found to be greatest in the group receiving SQ administration after irradiation in case of measurement three days after irradiation and in the group with measurement seven days after irradiation following SQ administration seven days before irradiation (Table 7, Fig. 5).

Table 7. NO(Nitric Oxide) in Liver of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	3.044	0.222	(2.788 3.324)
3 day	Irradiation control	7	2.232	0.167	(2.067 2.416)
	SQ(pre 7day)+Rad	7	2.100	0.127	(1.934 2.222)
	SQ(pre 3day)+Rad	7	2.626	0.305	(2.173 2.822)
	Rad+SQ	7	2.593	0.154	(2.455 2.737)*
7 day	Irradiation control	7	2.034	0.083	(1.917 2.101)
	SQ(pre 7day)+Rad	7	2.649	0.163	(2.524 2.890)*
	SQ(pre 3day)+Rad	7	1.965	0.169	(1.766 2.179)
	Rad+SQ	7	2.107	0.088	(1.989 2.190)

Note: The interaction effect was determined using boostrap method. The unit is the number of nM/μg. *$p < 0.05$.

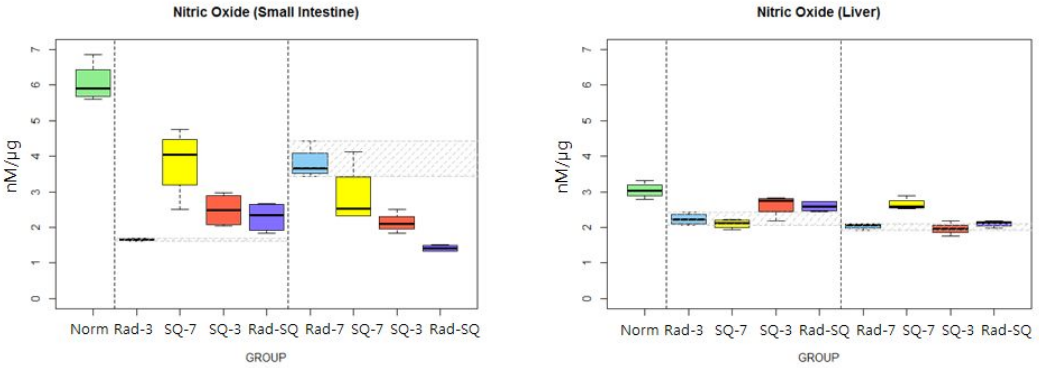

Fig. 5. NO(Nitric Oxide) in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

C. Cytokine Measurement

1. TNF-α (Tumor necrosis factor - α)

a. Small intestines

SQ was administered to irradiated mice to observe TNF-α in the tissues from the small intestines. TNF-α was estimated to be 0.046 in the normal small intestines. TNF-α was estimated to be 0.454 and 0.550 —higher than the normal level of 0.046—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.295 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=0.454). In contrast, it was estimated to be 0.504 and 0.475 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.454). It was estimated to be 0.305 and 0.472 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.412 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.550). At the 95% confidence interval, there were statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. There were statistically significant differences between the groups with measurement seven days after irradiation following SQ administration seven days and three days

before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of TNF-α in the small intestines were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 8, Fig. 6).

Table 8. TNF-alpha in Small intestine of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.046	0.016	(0.036 0.064)
3 day	Irradiation control	7	0.454	0.108	(0.385 0.542)
	SQ(pre 7day)+Rad	7	0.295	0.037	(0.264 0.317)*
	SQ(pre 3day)+Rad	7	0.504	0.039	(0.502 0.528)
	Rad+SQ	7	0.475	0.017	(0.461 0.493)
7 day	Irradiation control	7	0.550	0.080	(0.526 0.601)
	SQ(pre 7day)+Rad	7	0.305	0.040	(0.265 0.352)*
	SQ(pre 3day)+Rad	7	0.472	0.035	(0.433 0.501)*
	Rad+SQ	7	0.412	0.018	(0.397 0.429)*

Note: The interaction effect was determined using boostrap method. The unit is the number of pg/mG. *$p < 0.05$.

b. Liver

SQ was administered to irradiated mice to observe TNF-α in the tissues from the liver. TNF-α was estimated to be 0.014 in the normal liver. TNF-α was estimated to be 0.329 and 0.280—higher than the normal level of 0.014—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.169 and 0.239 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.319 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.014). It was estimated to be 0.205 and 0.248 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.258 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.280). At the 95% confidence interval, there were statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$). In contrast, there was no statistically significant difference between the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. Statistically significant differences were found between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of TNF-α in the liver were found to be greatest

in the group receiving SQ administration for seven days before irradiation (Table 9, Fig. 6).

Table 9. TNF-alpha in Liver of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.014	0.010	(0.006 0.022)
3 day	Irradiation control	7	0.329	0.045	(0.281 0.371)
	SQ(pre 7day)+Rad	7	0.169	0.016	(0.154 0.180)*
	SQ(pre 3day)+Rad	7	0.239	0.042	(0.197 0.265)*
	Rad+SQ	7	0.319	0.049	(0.264 0.361)
7 day	Irradiation control	7	0.280	0.031	(0.249 0.315)
	SQ(pre 7day)+Rad	7	0.205	0.050	(0.155 0.234)*
	SQ(pre 3day)+Rad	7	0.248	0.024	(0.236 0.260)
	Rad+SQ	7	0.258	0.114	(0.281 0.299)

Note: The interaction effect was determined using boostrap method. The unit is the number of pg/mG. *$p < 0.05$.

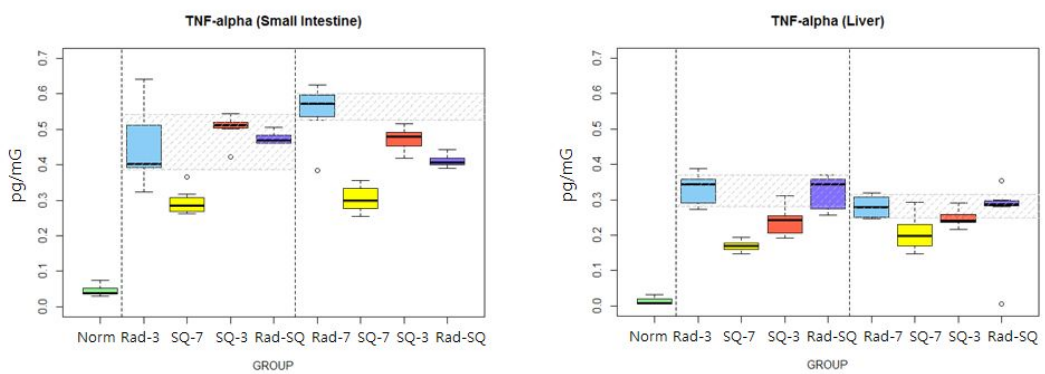

Fig. 6. TNF-alpha in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

2. IL-6 (Interleukin-6)

a. Small intestines

SQ was administered to irradiated mice to observe IL-6 in the tissues from the small intestines. IL-6 was estimated to be 0.0051 in the normal small intestines. IL-6 was estimated to be 0.0328 and 0.0401—higher than the normal level of 0.0051—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.0205 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=0.0328). In contrast, it was estimated to be 0.0369 and 0.0367 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.0328). It was estimated to be 0.0228 and 0.0360 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0350 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0401). At the 95% confidence interval, there were statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. There were statistically significant differences between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast,

no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of IL-6 in the small intestines were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 10, Fig. 7).

Table 10. IL-6 in Small intestine of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.0051	0.0030	(0.0020 0.0074)
3 day	Irradiation control	7	0.0328	0.0067	(0.0264 0.0389)
	SQ(pre 7day)+Rad	7	0.0205	0.0050	(0.0158 0.0256)*
	SQ(pre 3day)+Rad	7	0.0369	0.0034	(0.0333 0.0396)
	Rad+SQ	7	0.0367	0.0053	(0.0305 0.0404)
7 day	Irradiation control	7	0.0401	0.0081	(0.0326 0.0443)
	SQ(pre 7day)+Rad	7	0.0228	0.0043	(0.0217 0.0249)*
	SQ(pre 3day)+Rad	7	0.0360	0.0036	(0.0332 0.0390)
	Rad+SQ	7	0.0350	0.0046	(0.0306 0.0388)

Note: The interaction effect was determined using boostrap method. The unit is the number of pg/mG. *$p < 0.05$.

b. Liver

SQ was administered to irradiated mice to observe IL-6 in the tissues from the liver. IL-6 was estimated to be 0.0088 in the normal liver. IL-6 was estimated to be 0.0160 and 0.0303—higher than the normal level of 0.0088—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.0096 three days after irradiation following SQ administration seven days after irradiation, which was lower than that of the group receiving irradiation alone (M=0.0160). In contrast, it was estimated to be 0.0237 and 0.0272 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.0160). It was estimated to be 0.0147 and 0.0251 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0232 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0303). At the 95% confidence interval, there were statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). There were statistically significant differences between both the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) and the Rad control

group. In particular, radioprotective effects of IL-6 in the liver were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 11, Fig. 7).

Table 11. IL-6 in Liver of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method	
	Normal(Intestine)	7	0.0088	0.0017	(0.0079	0.0102)
3 day	Irradiation control	7	0.0160	0.0036	(0.0122	0.0187
	SQ(pre 7day)+Rad	7	0.0096	0.0016	(0.0075	0.0113)*
	SQ(pre 3day)+Rad	7	0.0237	0.0028	(0.0208	0.0262)*
	Rad+SQ	7	0.0272	0.0047	(0.0236	0.0294)*
7 day	Irradiation control	7	0.0303	0.0036	(0.0268	0.0319
	SQ(pre 7day)+Rad	7	0.0147	0.0021	(0.0124	0.0164)*
	SQ(pre 3day)+Rad	7	0.0251	0.0022	(0.0228	0.0277)
	Rad+SQ	7	0.0232	0.0008	(0.0224	0.0238)*

Note: The interaction effect was determined using boostrap method. The unit is the number of pg/mG. *$p < 0.05$.

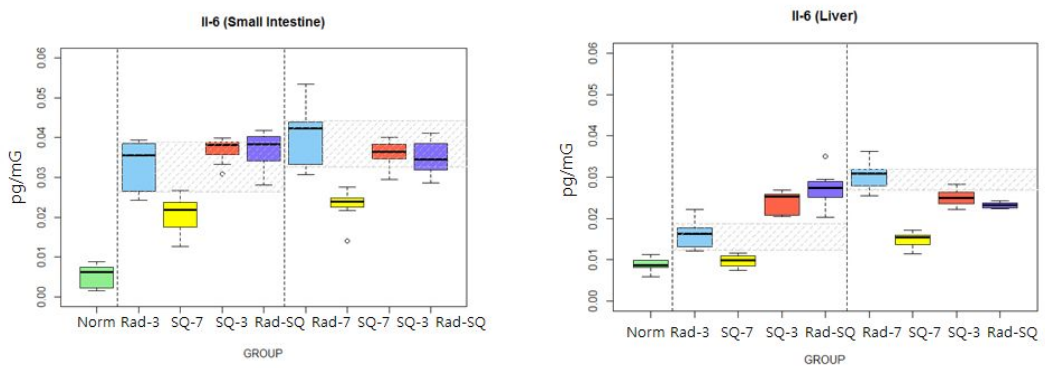

Fig. 7. IL-6 in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

3. IL-10 (Interleukin-10)

a. Small intestines

SQ was administered to irradiated mice to observe IL-10 in the tissues from the small intestines. IL-10 was estimated to be 0.0124 in the normal small intestines. IL-10 was estimated to be 0.0460 and 0.0242—higher than the normal level of 0.0124—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.0166 and 0.0308 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0412 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0460). It was estimated to be 0.0207 and 0.0211 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0240 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0242). At the 95% confidence interval, there were statistically significant difference between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). No statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of IL-10 in the small intestines were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 12, Fig. 8).

Table 12. IL-10 in Small intestine of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.0124	0.0024	(0.0092 0.0149)
3 day	Irradiation control	7	0.0460	0.0005	(0.0454 0.0466)
	SQ(pre 7day)+Rad	7	0.0166	0.0033	(0.0126 0.0198)*
	SQ(pre 3day)+Rad	7	0.0308	0.0028	(0.0267 0.0327)*
	Rad+SQ	7	0.0412	0.0019	(0.0391 0.0432)*
7 day	Irradiation control	7	0.0242	0.0033	(0.0194 0.0265)
	SQ(pre 7day)+Rad	7	0.0207	0.0032	(0.0183 0.0255)
	SQ(pre 3day)+Rad	7	0.0211	0.0018	(0.0188 0.0229)
	Rad+SQ	7	0.0240	0.0034	(0.0199 0.0281)

Note: The interaction effect was determined using boostrap method. The unit is the number of pg/mG. *$p < 0.05$.

b. Liver

SQ was administered to irradiated mice to observe IL-10 in the tissues from the liver. IL-10 was estimated to be 0.0070 in the normal liver. IL-10 was estimated to be 0.0131 and 0.0132—higher than the normal level of 0.0070—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.0127 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=0.0070). In contrast, it was estimated to be 0.0149 and 0.0160 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.0131). It was estimated to be 0.0134 and 0.0192 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0163 in case of SQ administration after irradiation, all of which were higher than that of the group receiving irradiation alone (M=0.0132). At the 95% confidence interval, there were statistically significant differences between the group with measurement three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) and the Rad control group. There was no statistically significant difference between the group with measurement seven days after irradiation following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. In particular, radioprotective effects of IL-10 in the liver were found to be greatest in the group receiving SQ

administration for seven days before irradiation (Table 13, Fig. 8).

Table 13. IL-10 in Liver of 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.0070	0.0013	(0.0054 0.0084)
3 day	Irradiation control	7	0.0131	0.0007	(0.0122 0.0139)
	SQ(pre 7day)+Rad	7	0.0127	0.0014	(0.0118 0.0147)
	SQ(pre 3day)+Rad	7	0.0149	0.0017	(0.0127 0.0167)
	Rad+SQ	7	0.0160	0.0017	(0.0139 0.0180)*
7 day	Irradiation control	7	0.0132	0.0019	(0.0114 0.0158)
	SQ(pre 7day)+Rad	7	0.0134	0.0017	(0.0118 0.0151)
	SQ(pre 3day)+Rad	7	0.0192	0.0010	(0.0183 0.0203)*
	Rad+SQ	7	0.0163	0.0011	(0.0150 0.0175)

Note: The interaction effect was determined using boostrap method. The unit is the number of pg/mG. $^{*}p < 0.05$.

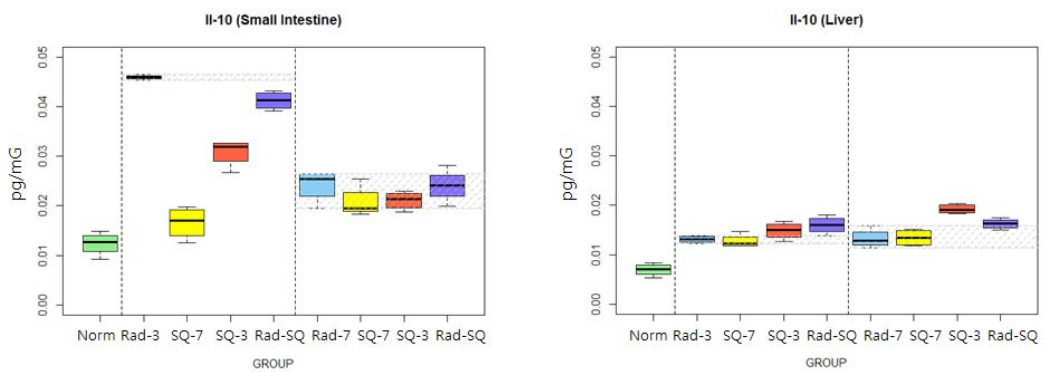

Fig. 8. IL-10 in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

D. Serum Analysis

To obtain supplementary data about the radioprotective effects of SQ, the following fourteen bio-chemical tests with the serum from mice exposed to one session of 3 Gy whole-body irradiation:

1. Total Protein (TP)

SQ was administered to irradiated mice to observe TP in the serum. TP was estimated to be 4.14 in the normal state and 4.04 and 4.16—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 4.45 and 4.12 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 4.08 in case of SQ administration for three days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=4.04). It was estimated to be 4.42 and 4.06 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 4.15 in case of SQ administration for seven days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=4.16). At the 95% confidence interval, there were statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of TP in the serum were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 14, Fig. 9).

Table 14. TP in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	4.14	0.23	(3.96 4.32)
3 day	Irradiation control	7	4.04	0.15	(3.92 4.16)
	SQ(pre 7day)+Rad	7	4.45	0.25	(4.20 4.65)*
	SQ(pre 3day)+Rad	7	4.12	0.22	(4.00 4.32)
	Rad+SQ	7	4.08	0.26	(3.88 4.28)
7 day	Irradiation control	7	4.16	0.19	(4.04 4.34)
	SQ(pre 7day)+Rad	7	4.42	0.28	(4.18 4.62)
	SQ(pre 3day)+Rad	7	4.06	0.34	(3.80 4.32)
	Rad+SQ	7	4.15	0.31	(3.85 4.35)

Note: The interaction effect was determined using boostrap method. The unit is the number of g/dL. $^{*}p < 0.05$.

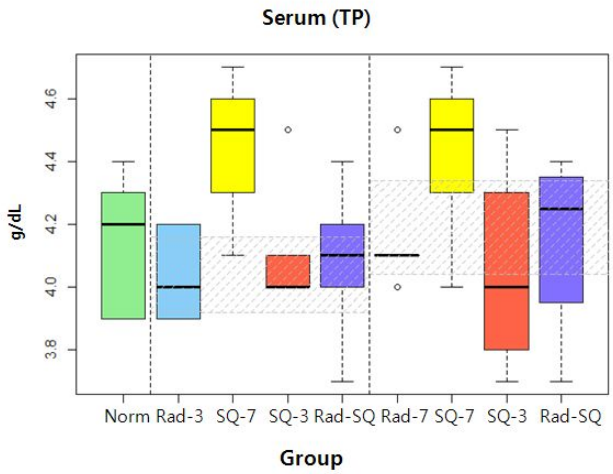

Fig. 9. TP in 3 Gy irradiated mice with SQ treatment.

2. Albumin

SQ was administered to irradiated mice to observe albumin in the serum. Albumin was estimated to be 3.18 in the normal state and 2.88 and 3.08—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 3.70 and 2.92 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 3.32 in case of SQ administration for three days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=2.88). It was estimated to be 3.08 and 3.46 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 3.30 in case of SQ administration for seven days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=2.70). At the 95% confidence interval, there were statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$). There were statistically significant differences between both the groups with measurement seven days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of albumin in the serum were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 15, Fig. 10).

Table 15. ALB in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	3.18	0.41	(2.88 3.54)
3 day	Irradiation control	7	2.88	0.31	(2.64 3.12)
	SQ(pre 7day)+Rad	7	3.70	0.70	(3.15 4.25)*
	SQ(pre 3day)+Rad	7	2.92	0.22	(2.76 3.10)
	Rad+SQ	7	3.32	0.47	(2.96 3.66)
7 day	Irradiation control	7	2.70	0.12	(2.62 2.80)
	SQ(pre 7day)+Rad	7	3.08	0.13	(2.98 3.18)*
	SQ(pre 3day)+Rad	7	3.46	0.68	(2.94 3.96)*
	Rad+SQ	7	3.30	0.62	(2.90 3.88)*

Note: The interaction effect was determined using boostrap method. The unit is the number of g/dL. *$p < 0.05$.

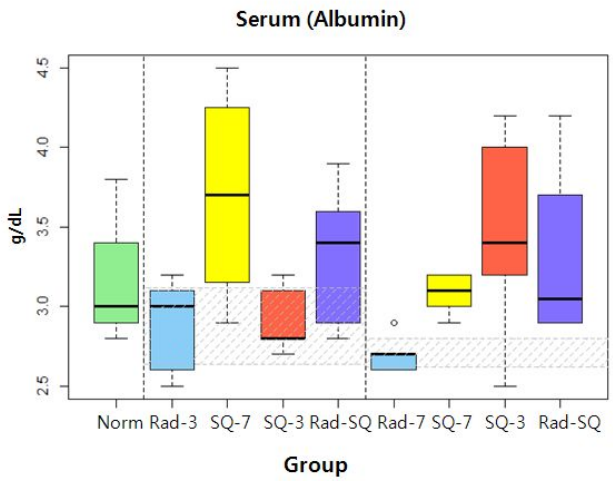

Fig. 10. ALB in 3 Gy irradiated mice with SQ treatment.

3. Glutamine Oxaloacetic Acid Transaminase (GOT)

SQ was administered to irradiated mice to observe GOT in the serum. GOT was estimated to be 170.60 in the normal state and 250.00 and 170.20 —similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 135.25 and 215.60 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 243.00 in case of SQ administration for three days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=250.00). It was estimated to be 192.40 and 242.40 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, both of which were lower than that of the group receiving irradiation alone (M=170). It was estimated to be 116.75 in case of SQ administration for seven days after irradiation, which was higher than that of the group receiving irradiation alone (M=170.20). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of GOT in the serum with SQ administration (Table 16, Fig. 11).

Table 16. GOT in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	170.60	66.33	(124.40 230.20)
3 day	Irradiation control	7	250.00	229.17	(80.60 427.12)
	SQ(pre 7day)+Rad	7	135.25	51.24	(91.50 179.00)
	SQ(pre 3day)+Rad	7	215.60	129.19	(129.40 323.40)
	Rad+SQ	7	243.00	83.53	(168.80 302.00)
7 day	Irradiation control	7	170.20	132.79	(86.40 274.40)
	SQ(pre 7day)+Rad	7	192.40	138.53	(90.80 311.40)
	SQ(pre 3day)+Rad	7	242.40	198.90	(133.00 419.80)
	Rad+SQ	7	116.75	21.70	(104.00 138.00)

Note: The interaction effect was determined using boostrap method. The unit is the number of IU/L $^{*}p < 0.05$.

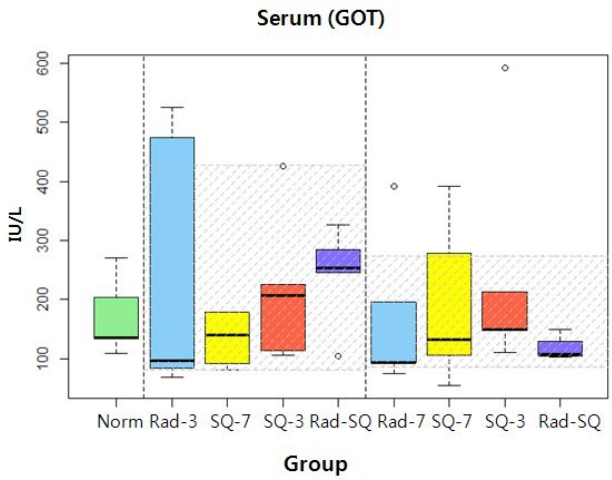

Fig. 11. GOT in 3 Gy irradiated mice with SQ treatment.

4. Glutamine Pyruvic Acid Transaminase (GPT)

SQ was administered to irradiated mice to observe GPT in the serum. GPT was estimated to be 25.71 in the normal state and 23.00 and 26.86—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 24.17 and 28.29 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=23.00). It was estimated to be 20.86 in case of SQ administration for three days after irradiation, which was lower than that of the group receiving irradiation alone (M=23.00). It was estimated to be 25.71 and 19.00 seven days after irradiation following SQ administration seven days before irradiation and in case of SQ administration for seven days after irradiation, respectively, both of which were lower than that of the group receiving irradiation alone (M=26.86). It was estimated to be 29.86 seven days after irradiation following SQ administration three days before irradiation, which was higher than that of the group receiving irradiation alone (M=26.86). At the 95% confidence interval, there were statistically significant differences in the radioprotective effects of GPT in the serum with SQ administration for seven days after irradiation ($P < 0.05$)(Table 17, Fig. 12).

Table 17. GPT in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	25.71	5.96	(21.71 30.00)
3 day	Irradiation control	7	23.00	7.75	(18.57 29.14)
	SQ(pre 7day)+Rad	7	24.17	4.36	(21.17 27.33)
	SQ(pre 3day)+Rad	7	28.29	15.64	(20.00 40.86)
	Rad+SQ	7	20.86	6.49	(16.43 25.43)
7 day	Irradiation control	7	26.86	6.23	(22.57 31.00)
	SQ(pre 7day)+Rad	7	25.71	5.77	(22.14 30.00)
	SQ(pre 3day)+Rad	7	29.86	10.61	(23.29 37.57)
	Rad+SQ	7	19.00	1.83	(17.86 20.28)*

Note: The interaction effect was determined using boostrap method. The unit is the number of IU/L $^{*}p < 0.05$.

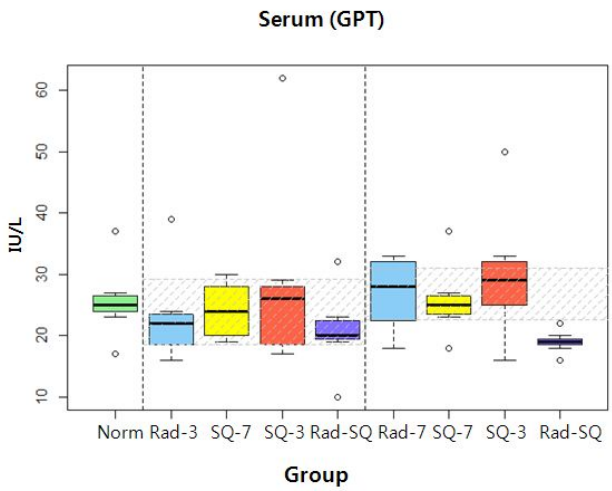

Fig. 12. GPT in 3 Gy irradiated mice with SQ treatment.

5. Alkaline Phospatase (ALP)

SQ was administered to irradiated mice to observe ALP in the serum. ALP was estimated to be 466.29 in the normal state and 476.86 and 406.71 —similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 441.83 and 463.71 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 381.14 in case of SQ administration for three days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=476.86). It was estimated to be 376.29 and 376.29 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 388.71 in case of SQ administration for seven days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=406.71). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of ALP in the serum with SQ administration (Table 18, Fig. 13).

Table 18. ALP in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	466.29	68.73	(422.87 516.85)
3 day	Irradiation control	7	476.86	43.49	(446.44 506.57)
	SQ(pre 7day)+Rad	7	441.83	29.06	(421.00 464.17)
	SQ(pre 3day)+Rad	7	463.71	45.32	(433.43 496.00)
	Rad+SQ	7	381.14	58.16	(340.57 419.14)
7 day	Irradiation control	7	406.71	74.24	(353.57 455.69)
	SQ(pre 7day)+Rad	7	376.29	97.00	(307.01 436.69)
	SQ(pre 3day)+Rad	7	280.00	102.81	(205.00 347.84)
	Rad+SQ	7	388.71	31.71	(368.00 411.14)

Note: The interaction effect was determined using boostrap method. The unit is the number of IU/L $^{*}p < 0.05$.

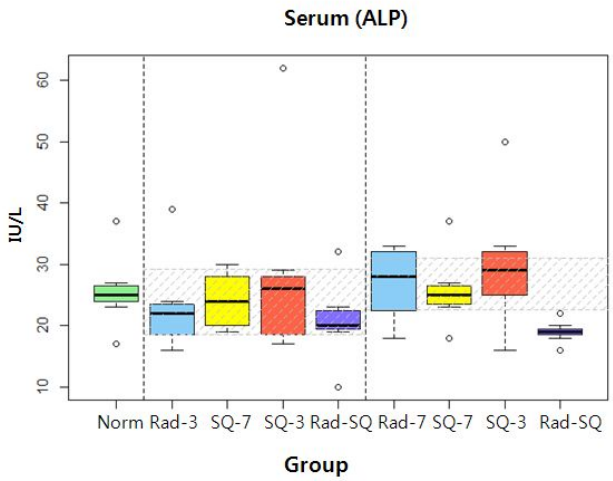

Fig. 13. ALP in 3 Gy irradiated mice with SQ treatment.

6. Lactate Dehydronase (LDH)

SQ was administered to irradiated mice to observe LDH in the serum. LDH was estimated to be 1436.86 in the normal state and 1446.29 and 820.57—lower than the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 2363.00 and 1657.86 three days after irradiation following SQ administration seven days before irradiation and in case of SQ administration for three days after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=1446.29). It was estimated to be 1375.00 three days after irradiation following SQ administration three days before irradiation, which was lower than that of the group receiving irradiation alone (M=1446.29). It was estimated to be 1093.00 and 1193.14 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, both of which were lower than that of the group receiving irradiation alone (M=1446.29). It was estimated to be 583.86 in case of SQ administration for seven days after irradiation, which was lower than that of the group receiving irradiation alone (M=820.57). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of LDH in the serum with SQ administration. However, radioprotective effects of LDA in the serum were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 19, Fig. 14).

Table 19. LDH in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method	
	Normal(Intestine)	7	1436.86	398.13	(1138.59	1677.13)
3 day	Irradiation control	7	1446.29	647.82	(1042.91	1906.00)
	SQ(pre 7day)+Rad	7	2363.00	525.87	(2002.37	2774.00)
	SQ(pre 3day)+Rad	7	1375.00	307.22	(1171.31	1576.28)
	Rad+SQ	7	1657.86	333.04	(1436.15	1890.14)
7 day	Irradiation control	7	820.57	381.30	(586.43	1086.84)
	SQ(pre 7day)+Rad	7	1093.00	593.16	(707.72	1520.10)
	SQ(pre 3day)+Rad	7	1193.14	683.95	(775.87	1689.11)
	Rad+SQ	7	583.86	131.47	(506.32	684.57)

Note: The interaction effect was determined using boostrap method. The unit is the number of IU/L $^{*}p < 0.05$.

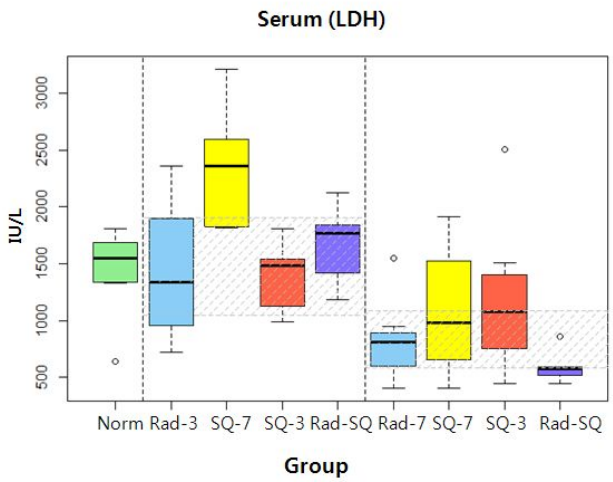

Fig. 14. LDH in 3 Gy irradiated mice with SQ treatment.

7. Total Cholesterol (TC)

SQ was administered to irradiated mice to observe TC in the serum. TC was estimated to be 86.00 in the normal state and 88.00 and 89.14—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 89.83 and 118.29 three days after irradiation following SQ administration seven days before irradiation and in case of SQ administration for three days after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=88.00). It was estimated to be 74.00 seven days after irradiation following SQ administration three days before irradiation, which was lower than that of the group receiving irradiation alone (M=88.00). It was estimated to be 77.86 and 73.71 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 81.14 in case of SQ administration for seven days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=89.14). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of TC in the serum with SQ administration (Table 20, Fig. 15).

Table 20. T.CHO in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	86.00	7.35	(80.86 90.71)
3 day	Irradiation control	7	88.00	3.65	(85.58 90.57)
	SQ(pre 7day)+Rad	7	89.83	5.81	(85.67 94.33)
	SQ(pre 3day)+Rad	7	74.00	11.80	(65.57 81.29)
	Rad+SQ	7	118.29	67.31	(83.29 171.43)
7 day	Irradiation control	7	89.14	5.43	(84.86 92.29)
	SQ(pre 7day)+Rad	7	77.86	3.58	(75.71 80.57)
	SQ(pre 3day)+Rad	7	73.71	6.10	(69.57 78.00)
	Rad+SQ	7	81.14	4.95	(77.71 84.85)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

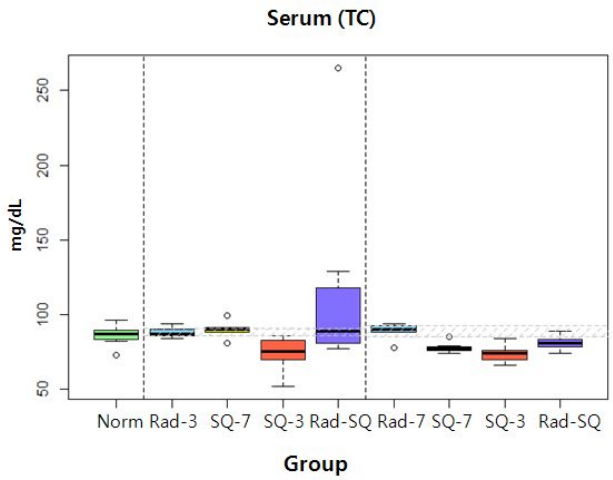

Fig. 15. T.CHO in 3 Gy irradiated mice with SQ treatment.

8. Triglycerides (TG)

SQ was administered to irradiated mice to observe TG in the serum. TP was estimated to be 31.29 in the normal state and 39.71 and 50.71—higher than the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 42.67 three days after irradiation following SQ administration seven days before irradiation, which was higher than that of the group receiving irradiation alone (M=39.71). It was estimated to be 18.29 and 37.29 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration for three days after irradiation, respectively, all of which were lower than that of the group receiving irradiation alone (M=39.71). It was estimated to be 76.29 and 70.29 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 55.14 in case of SQ administration for seven days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=50.71). At the 95% confidence interval, there were statistically significant differences in the radioprotective effects of TG in the serum in case of SQ administration for three days after irradiation following the administration three days before irradiation ($p < 0.05$)(Table 21, Fig. 16).

Table 21. TG in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	31.29	11.51	(23.43 39.00)
3 day	Irradiation control	7	39.71	20.59	(27.15 54.29)
	SQ(pre 7day)+Rad	7	42.67	11.98	(34.17 52.16)
	SQ(pre 3day)+Rad	7	18.29	8.22	(13.29 24.43)*
	Rad+SQ	7	37.29	26.81	(20.86 57.00)
7 day	Irradiation control	7	50.71	15.40	(40.43 61.14)
	SQ(pre 7day)+Rad	7	76.29	27.75	(55.43 93.86)
	SQ(pre 3day)+Rad	7	70.29	21.89	(54.72 83.71)
	Rad+SQ	7	55.14	16.73	(44.00 66.71)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

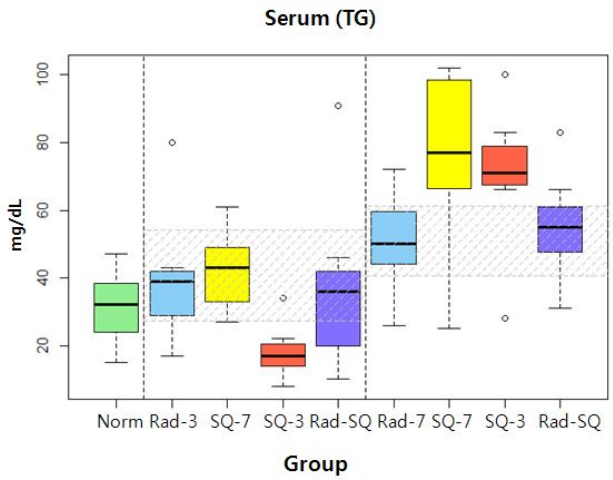

Fig. 16. TG in 3 Gy irradiated mice with SQ treatment.

9. High Density Lipoprotein Cholesterol (HDLC)

SQ was administered to irradiated mice to observe HDLC in the serum. HDLC was estimated to be 57.57 in the normal state and 55.14 and 55.29 —lower than the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 52.00 and 43.00 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 48.29 in case of SQ administration for three days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=55.14). It was estimated to be 46.29 and 46.00 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, both of which were lower than that of the group receiving irradiation alone (M=55.29). It was estimated to be 54.86 in case of SQ administration for seven days after irradiation, which was higher than that of the group receiving irradiation alone (M=55.29). At the 95% confidence interval, there were statistically significant differences between both the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). There were statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of HDLC in the serum were found to be greatest in the group receiving SQ administration seven days and three days before irradiation (Table 22, Fig. 17).

Table 22. HDL.CHO in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	57.57	4.12	(54.71 60.00)
3 day	Irradiation control	7	55.14	3.72	(52.57 57.71)
	SQ(pre 7day)+Rad	6	52.00	1.41	(51.00 53.00)
	SQ(pre 3day)+Rad	7	43.00	5.42	(39.29 46.57)*
	Rad+SQ	7	48.29	3.20	(45.86 50.29)*
7 day	Irradiation control	7	55.29	3.68	(52.71 57.86)
	SQ(pre 7day)+Rad	7	46.29	3.59	(44.00 48.86)*
	SQ(pre 3day)+Rad	7	46.00	7.81	(40.43 51.29)*
	Rad+SQ	7	54.86	5.27	(51.43 58.43)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

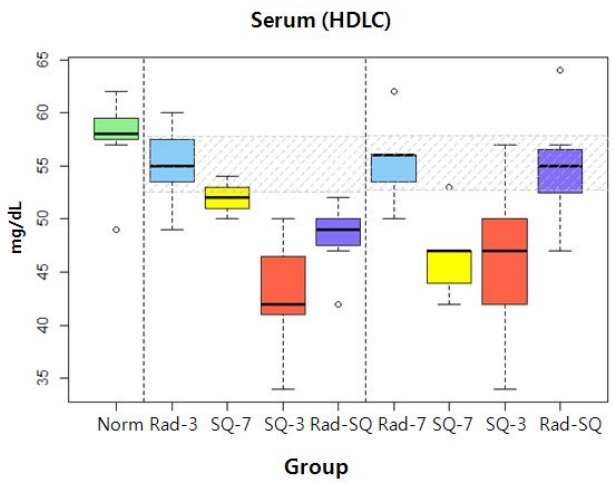

Fig. 17. HDL.CHO in 3 Gy irradiated mice with SQ treatment.

10. Low Density Lipoprotein Cholesterol (LDLC)

SQ was administered to irradiated mice to observe LDLC in the serum. LDLC was estimated to be 5.86 in the normal state and 6.00 and 5.29—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 9.83 and 6.14 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 8.29 in case of SQ administration for three days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=6.00). It was estimated to be 7.00 and 5.86 seven days after irradiation following SQ administration seven days before irradiation and in case of SQ administration for seven days after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=5.29). It was estimated to be lower seven days after irradiation following SQ administration three days before irradiation than that of the group receiving irradiation alone (M=5.29). At the 95% confidence interval, there were statistically significant differences between both the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of LDLC in the serum were found to be greatest in the group receiving SQ administration seven days before irradiation (Table 23, Fig. 18).

Table 23. LDL.CHO in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	5.86	1.68	(4.86 7.14)
3 day	Irradiation control	7	6.00	0.82	(5.43 6.57)
	SQ(pre 7day)+Rad	7	9.83	1.17	(9.00 10.67)*
	SQ(pre 3day)+Rad	7	6.14	1.68	(4.86 7.14)
	Rad+SQ	7	8.29	1.80	(7.29 9.57)*
7 day	Irradiation control	7	5.29	1.11	(4.57 6.00)
	SQ(pre 7day)+Rad	7	7.00	3.74	(5.00 9.86)
	SQ(pre 3day)+Rad	7	4.86	1.07	(4.29 5.71)
	Rad+SQ	7	5.86	1.35	(5.00 6.86)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

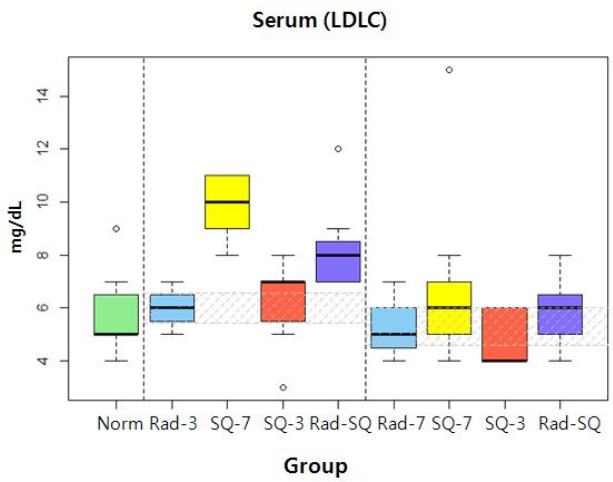

Fig. 18. LDL.CHO in 3 Gy irradiated mice with SQ treatment.

11. Glucose

SQ was administered to irradiated mice to observe glucose in the serum. Glucose was estimated to be 185.29 in the normal state and 208.57 and 215.00—higher than the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 208.83 three days after irradiation following SQ administration seven days before irradiation, which was slightly higher than that of the group receiving irradiation alone (M=208.57). It was estimated to be 193.29 and 184.14 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration for three days after irradiation, respectively, both of which were lower than that of the group receiving irradiation alone (M=208.57). It was estimated to be 205.71 and 179.14 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 210.14 in case of SQ administration for seven days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=215.00). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of glucose in the serum with SQ administration (Table 24, Fig. 19).

Table 24. GLU in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	185.29	16.22	(174.71 196.57)
3 day	Irradiation control	7	208.57	27.02	(190.14 227.43)
	SQ(pre 7day)+Rad	7	208.83	24.56	(192.84 227.66)
	SQ(pre 3day)+Rad	7	193.29	65.83	(142.71 228.57)
	Rad+SQ	7	184.14	20.70	(169.00 197.71)
7 day	Irradiation control	7	215.00	32.06	(194.43 239.42)
	SQ(pre 7day)+Rad	7	205.71	32.35	(185.14 228.00)
	SQ(pre 3day)+Rad	7	179.14	32.63	(157.72 201.71)
	Rad+SQ	7	210.14	23.15	(194.01 225.86)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

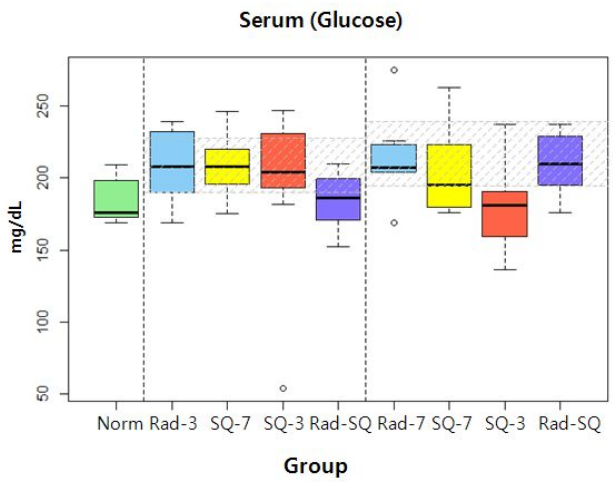

Fig. 19. GLU in 3 Gy irradiated mice with SQ treatment.

12. Blood Urea Nitrogen (BUN)

SQ was administered to irradiated mice to observe BUN in the serum. BUN was estimated to be 19.73 in the normal state and 15.79 and 20.34—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 16.17 and 16.76 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 16.94 in case of SQ administration for three days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=15.79). It was estimated to be 17.06 and 16.79 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 17.77 in case of SQ administration for seven days after irradiation, all of which were lower than that of the group receiving irradiation alone (M=20.34). At the 95% confidence interval, there were statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$). In particular, radioprotective effects of BUN in the serum were found to be greatest in the group receiving SQ administration seven days and three days before irradiation (Table 25, Fig. 20).

Table 25. BUN in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	19.73	1.66	(18.66 20.80)
3 day	Irradiation control	7	15.79	1.47	(14.60 16.67)
	SQ(pre 7day)+Rad	7	16.17	0.81	(15.53 16.73)
	SQ(pre 3day)+Rad	7	16.76	0.70	(16.31 17.29)
	Rad+SQ	7	16.94	1.45	(16.03 17.99)
7 day	Irradiation control	7	20.34	2.15	(18.87 21.83)
	SQ(pre 7day)+Rad	7	17.06	0.99	(16.43 17.77)*
	SQ(pre 3day)+Rad	7	16.79	1.90	(15.51 18.06)*
	Rad+SQ	7	17.77	2.24	(16.19 19.24)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL *$p < 0.05$.

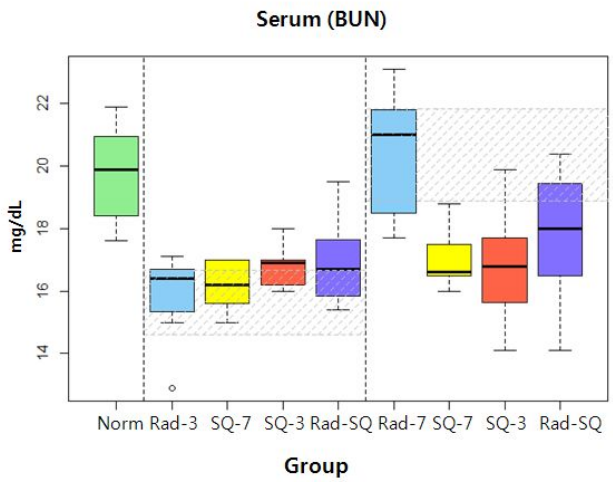

Fig. 20. BUN in 3 Gy irradiated mice with SQ treatment.

13. Creatinine

SQ was administered to irradiated mice to observe creatinine in the serum. Creatinine was estimated to be 0.29 in the normal state and 0.30 and 0.31—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.32 and 0.29 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.30 in case of SQ administration for three days after irradiation, all of which were similar to that of the group receiving irradiation alone (M=0.30). It was estimated to be 0.33 and 0.30 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.31 in case of SQ administration for seven days after irradiation, all of which were similar to that of the group receiving irradiation alone (M=0.31). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of creatinine in the serum with SQ administration (Table 26, Fig. 21).

Table 26. CREA in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	0.29	0.07	(0.24 0.33)
3 day	Irradiation control	7	0.30	0.08	(0.24 0.36)
	SQ(pre 7day)+Rad	7	0.32	0.08	(0.27 0.37)
	SQ(pre 3day)+Rad	7	0.29	0.07	(0.24 0.33)
	Rad+SQ	7	0.30	0.08	(0.24 0.36)
7 day	Irradiation control	7	0.31	0.07	(0.27 0.36)
	SQ(pre 7day)+Rad	7	0.33	0.08	(0.29 0.39)
	SQ(pre 3day)+Rad	7	0.30	0.06	(0.26 0.34)
	Rad+SQ	7	0.31	0.07	(0.27 0.36)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

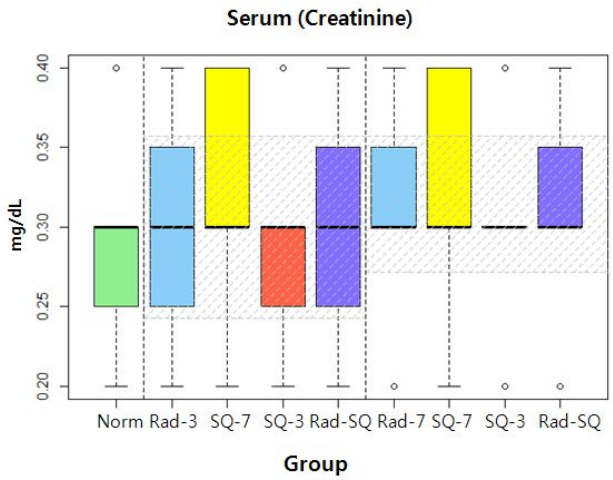

Fig. 21. CREA in 3 Gy irradiated mice with SQ treatment.

14. Uric Acid (UA)

SQ was administered to irradiated mice to observe UA in the serum. UA was estimated to be 1.89 in the normal state and 1.97 and 1.57—similar to the normal level—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 3.67 and 2.37 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 2.57 in case of SQ administration for three days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=1.97). It was estimated to be 3.29 and 2.09 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 2.00 in case of SQ administration for seven days after irradiation, all of which were higher than that of the group receiving irradiation alone (M=1.57). At the 95% confidence interval, no statistically significant difference was found in the radioprotective effects of UA in the serum with SQ administration. However, radioprotective effects of UA in the serum were found to be greatest in the group receiving SQ administration for seven days before irradiation (Table 27, Fig. 22).

Table 27. UA in 3 Gy irradiated mice with SQ treatment

Measured after irradiation	Group	N	Mean	S.D.	95% confidence interval using boostrap method
	Normal(Intestine)	7	1.89	0.56	(1.46 2.26)
3 day	Irradiation control	7	1.97	0.31	(1.73 2.17)
	SQ(pre 7day)+Rad	7	3.67	1.09	(2.98 4.58)
	SQ(pre 3day)+Rad	7	2.37	0.63	(1.93 2.79)
	Rad+SQ	7	2.57	0.43	(2.26 2.84)
7 day	Irradiation control	7	1.57	0.77	(1.04 2.07)
	SQ(pre 7day)+Rad	7	3.29	1.07	(2.53 4.03)
	SQ(pre 3day)+Rad	7	2.09	0.62	(1.71 2.56)
	Rad+SQ	7	2.00	0.98	(1.33 2.71)

Note: The interaction effect was determined using boostrap method. The unit is the number of mg/dL $^{*}p < 0.05$.

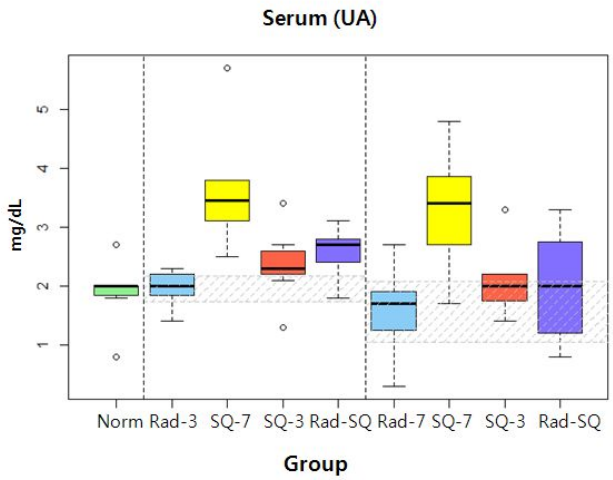

Fig. 22. UA in 3 Gy irradiated mice with SQ treatment.

IV. Discussion

George et al. noted that SQ is an isoprenoid compound with a similar structure to β-carotene with six double bonds and thirty carbons, passes through farnselyl pyrophophatase following phosphorylation of mevalonate during cholesterol synthesis, is an intermediate generated during the process of cholesterol synthesis through squalene epoxidase and lanosterol, is distributed over adipose tissues, the skin, lymphatic tissues, kidneys, skeletal muscles, cardiac muscles, and arteries, with the dry weight of 475 μg/g per 1 μg/g in the skin, about 275 μg/g in adipose tissues, 75 μg/g in the liver with active cholesterol synthesis, and about 42 μg/g in the small intestines, is unsaturated fatty acid widely distributed among animals and plants, and is most abundant in olive oil—7 mg/g per 1 μg/g—among plants and in the liver of centrophorusatromarginatus among animals and is also contained in foods[21]. It has been found that SQ synthesis and synthetase are located at the endoplasmic reticulum and microsome in liver cells of a white mouse and vary by a few low-fat medicines or diets[23]. When a person had a 900 mg daily intake of SQ in three doses of 300 mg for four weeks, it was found, on the basis of the sterol balance as well as the serum SQ level, the cholesterol level, and cholesterol synthesis, that 60% of it was absorbed from intake and the serum SQ level increased by 17 times but there was no change in serum triglyceride or cholesterol[25]; SQ intake increased cholesterol synthesis and LDL apoB metabolic activity[37]; and it reduced HMG CoA reductase activity in the liver of a white mouse[25]. SQ is known to act antioxidatively and have a mechanism to suppress free radicals and reactive oxygen generated from various stimuli and can be an effective protector of a living body by rapidly eliminating malignant neoplasms generated by ionization and excitation right after exposure to irradiation in case of good treatment before or after irradiation[38]. In terms of cell signaling pathways through which irradiation leads to apoptosis, Wang et al. reported that NF-kB, an important signal transmitter in the process of inflammatory response generation, is an essential element that leads to apoptosis or cell survival in a transduction pathway signals caused by ionizing radiation[39]; Zaidi et al. found that treatment

with gentle whole-body thermotherapy (39°C, 1h) twenty hours before whole-body irradiation of 8 Gy could prevent much of apoptosis from being caused by whole-body irradiation[40], and Kim noted that treatment with chitosan could inhibit lots of chromosomal abnormalities from being induced by contamination with radioactive mercury[41]. Hong et al. reported that c-fos and junB, acutely expressed protein genes in brain neurons, made a reaction through brain tissues which reacted to irradiation[42] and Claudia et al. noted that such enzymes as protein kinase C known to cause radiation injury and stress response protein kinase delayed cell division in the G1 phase by inducing acute response genes[43]. It has been reported that an active process requiring energy, apoptosis is caused by several stimuli to cells, such as ultraviolet rays, lack of cell growth factors, connection of ligans with a receptor, reactive oxygen metabolites, and DNA damage by ionized radiation, that degradation of actin and actin-binding protein, fordrin, forms air bubbles in plasma membranes, which leads to apoptosis, that it is possible to cause or inhibit artificial apoptosis by getting a good understanding of the process of signal transmission for apoptosis, and that it is possible to find pharmacological and physical means to inhibit apoptosis[44-46]. In this study, we examined Caspase-3 and Caspase-9 among cell signaling pathways to apoptosis after irradiation: while both of them had the O.D. value increased significantly in the irradiation control group, the former was estimated to be 0.505 and 0.688 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.642 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.720). It was estimated to be 0.610 and 0.652 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.622 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.779). Caspase-3 of Liver was estimated to be 0.676 and 0.683—higher than the normal level of 0.201—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. Three days after irradiation following SQ administration seven days and three days before irradiation, it was estimated to be 0.395 and 0.606,

respectively, both of which were lower than that of the group receiving irradiation alone (M=0.676). In contrast, it was estimated to be 0.697 in case of SQ administration after irradiation, which was higher than that of the group receiving irradiation alone (M=0.676). Seven days after irradiation following SQ administration seven days and three days before irradiation, it was estimated to be 0.527 and 0.613, respectively, both of which were lower than that of the group receiving irradiation alone (M=0.676). In contrast, it was estimated to be 0.718 in case of SQ administration after irradiation, which was higher than that of the group receiving irradiation alone (M=0.779). Caspase-9 of Small intestine was estimated to be 0.725 and 0.786—higher than the normal level of 0.088—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. It was estimated to be 0.065 and 0.090 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.096 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.725). It was estimated to be 0.079 and 0.099 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.090 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.779). Caspase-9 of Liver was estimated to be 0.801 and 0.750—higher than the normal level of 0.150—in the irradiation control group three days and seven days after 3 Gy irradiation to normal mice, respectively. Three days after irradiation following SQ administration seven days and three days before irradiation, it was estimated to be lower than that of the group receiving irradiation alone (M=0.801): 0.382 and 0.791, respectively. In contrast, it was estimated to be 0.870, which was higher than that of the group receiving irradiation alone (M=0.801), in case of SQ administration after irradiation. It was estimated to be 0.734 and 0.622 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.602 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.750). In particular, radioprotective effects of Caspase-9 in the small intestine and liver were found to be greatest in the group

with measurement three days after irradiation following SQ administration seven days before irradiation and in the group receiving SQ administration seven days after irradiation. In those cells with apoptosis being caused by irradiation, Caspase serves to induce apoptosis of severely damaged cells by degrading and eliminating intranuclear anti-apoptosis protein and it is assumed that the SQ treatment groups had the Caspase O.D. value decreased by SQ, which provided radioprotection through antioxidation[47-49]. In terms of an inflammatory response factor, nitric oxide (NO), Sethi et al. reported that abdominal macrophage activation facilitated generation of NO, which was greatest 24 hours after a mouse was exposed to ultraviolet rays (50 mJ/cm^2)[50] and Nakagawa et al. noted that NO was induced in lots of organs by ionizing radiation in case of whole-body irradiation to a mouse[51]. In this study, we administered SQ to 3 Gy-irradiated mice and examined NO: while the irradiation control group had NO generation decreased in the tissues both from the small intestines and from the liver, NO in the small intestines was estimated to be 3.835 and 2.492 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 2.291 in case of SQ administration after irradiation, all of which were higher than that of the group receiving irradiation alone (M=1.652). NO of small intestine was estimated to be 2.873 and 2.136 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 1.414 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=3.802). No of Liver was estimated to be 2.626 and 2.593 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=2.232). In contrast, it was estimated to be 2.100 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=2.232). It was estimated to be 2.649 and 2.107 seven days after irradiation following SQ administration seven days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone

(M=2.034). In contrast, it was estimated to be 1.965 seven days after irradiation following SQ administration three days before irradiation, which was lower than that of the group receiving irradiation alone (M=2.034). n particular, radioprotective effects of NO in the small intestines were found to be greatest three days after irradiation following SQ administration seven days before irradiation. After being generated in cells, NO passes through other cell membranes to inhibit inflammatory responses there and give a function of immune regulation to confront infection and it is assumed that SQ increases NO generation, thereby giving radioprotection through inflammatory response inhibition, in the SQ treatment groups[52-54]. In terms of TNF-α among radiation-induced cytokines, Kyrkanides et al. reported that ionizing radiation generated serious levels of TNF-α and ICAM-1 in an experiment with the brain of irradiated mice[55-60] and Fagiolo et al., who gave irradiation to a pouch of blood with active mitosis and examined the time-response relations in monocytes, reported that cell proliferation began to be inhibited 24 hours after irradiation and TNF-α generation increased significantly[61-65]. Rube et al., who analyzed cytokines expressed after treatment with an anticancer agent and thoracic radiotherapy, reported that the experimental group receiving both thoracic radiotherapy[66,67] and treatment using gemcitabine had a higher level of TNF-α expression in all time slots than the group treated with an anticancer agent alone or the group receiving radiotherapy alone and Akmansu et al., who measured TNF-α before and after five-week radiotherapy in the serum from 34 persons receiving local radiotherapy against head and neck cancer, found that the level of TNF-α was drastically raised in all the patients receiving radiotherapy alone without surgical treatment[68-70]. Rube et al., who examined TNF-α generation when an immunomodulator, pentoxifylline, was administered to lung tissues after 12 Gy thoracic irradiation, reported that while the group receiving irradiation alone saw TNF-α generation facilitated, the experimental group receiving both pentoxifylline administration[70-73] and irradiation had a low level of TNF-α generation since pentoxifylline lowered the level of TNF-α generation and Grandjean-Laquerriere et al. noted that if cells exposed to ultraviolet rays were treated with kinase A, TNF-α was significantly inhibited[74-77]. In this study, we examined TNF-α in the tissues

from mice treated with SQ after a session of 3 Gy whole-body irradiation, TNF-α in the small intestines was estimated to be 0.295 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=0.454). In contrast, it was estimated to be 0.504 and 0.475 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.454). It was estimated to be 0.305 and 0.472 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.412 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.550). TNF-α of Liver was estimated to be 0.169 and 0.239 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.319 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.014). It was estimated to be 0.205 and 0.248 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.258 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.280). In particular, radioprotective effects of TNF-α in the liver were found to be greatest in the group receiving SQ administration for seven days before irradiation.

Biological control protein with antitumor effects, TNF-α is induced by stimuli, such as radiation, and protects cells from being injured and it is assumed that the SQ treatment group had the level of TNF-α lowered by SQ, which provided radioprotection through antioxidation[78-80]. In terms of interleukin among radiation-induced cytokines, Seki et al. reported that the immune factors of IL-4 and IL-7 as well as IL-2 saved both CD+4 and CD+8 T cells from radiation-induced apoptosis[81-83]; Legue et al. noted that injection of cytokines, such as IL-6, before whole-body irradiation had radioprotective effects with increased cell survival and had radioprotective effects observed in sertoli cells[84]; and Frasca et al., who examined the effects of cytokines generated in DNA-binding process of

eukaryotic cell ku in monocytes of peripheral blood exposed to x-ray radiation in young and middle-aged groups, reported that DNA-binding activity of ku increased drastically, thereby generating IL-6 modified cytokine, K-7/D-6, and inducing unscheduled DNA restoration, in the young group[85]. Meeren et al. reported that irradiation to human endothelial cells increased IL-6 generation[86]; Ross et al. noted that generation of IL-6 mRNA in glioblastoma cell lines was more positively facilitated by low or very low doses than by high or moderate doses[87]; and Grandjean-Laquerriere et al. reported that when the human skin exposed to ultraviolet rays was treated with PMA, a pharmacologically active agent of protein kinase C, the level of the immune factor IL-8 was raised in NCTC 2544 cell lines of keratinocyte[88]. In this study, we administered SQ to mice exposed to a session of 3 Gy whole-body irradiation and examined IL-6, which was estimated in the small intestines to be 0.0205 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=0.0328). IL-6 of small intestine was estimated to be 0.0369 and 0.0367 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.0328). It was estimated to be 0.0228 and 0.0360 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0350 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0401). It was estimated to be 0.0096 three days after irradiation following SQ administration seven days after irradiation, which was lower than that of the group receiving irradiation alone (M=0.0160). IL-6 of Liver was estimated to be 0.0237 and 0.0272 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.0160). It was estimated to be 0.0147 and 0.0251 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0232 in case of SQ administration after irradiation, all of which

were lower than that of the group receiving irradiation alone (M=0.0303). In particular, radioprotective effects of IL-6 in the small intestines were found to be greatest in the group receiving SQ administration for seven days before irradiation. In this study, we administered SQ to mice exposed to a session of 3 Gy whole-body irradiation and examined IL-10, which was estimated in the small intestines to be 0.0166 and 0.0308 three days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0412 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0460). IL-10 of Small intestine was estimated to be 0.0207 and 0.0211 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0240 in case of SQ administration after irradiation, all of which were lower than that of the group receiving irradiation alone (M=0.0242). IL-10 of Liver was estimated to be 0.0127 three days after irradiation following SQ administration seven days before irradiation, which was lower than that of the group receiving irradiation alone (M=0.0070). In contrast, it was estimated to be 0.0149 and 0.0160 three days after irradiation following SQ administration three days before irradiation and in case of SQ administration after irradiation, respectively, both of which were higher than that of the group receiving irradiation alone (M=0.0131). It was estimated to be 0.0134 and 0.0192 seven days after irradiation following SQ administration seven days and three days before irradiation, respectively, and 0.0163 in case of SQ administration after irradiation, all of which were higher than that of the group receiving irradiation alone (M=0.0132). In particular, radioprotective effects of IL-10 in the liver were found to be greatest in the group receiving SQ administration for seven days before irradiation. An active substance with an immunomediatory function between cells, such as blood cells, immunocytes, endocrine cells, and neurons, IL-6 is induced by stimuli, such as radiation, and give radioprotection to cells and it is assumed that the SQ treatment groups had the values of IL-6 and IL-10 decreased by SQ, which provided radioprotection through antioxidation. Kim, who administered 0.1% chitosan oligosaccharide to see how chitosan oligosaccharide intake affected changes in the chemical composition of blood in irradiated mice,

found that the alkalinephosphatase (ALP) and aspartate aminotransferase (AST) groups recovered more quickly in case of treatment with chitosan oligosaccharide for 14 days before irradiation than the irradiation control group[89]. In this study, we centrifuged serum from mice exposed to one session of 3 Gy whole-body irradiation and performed 14 biochemical tests to obtain supplementary data about the radioprotective effects of SQ, the administration of which led to recovery in some cases: TP increased three days after irradiation following SQ administration seven days before irradiation; albumin increased three days after irradiation following SQ administration seven days before irradiation; LDH increased three days after irradiation following SQ administration seven days before irradiation; HDLC decreased three days after irradiation following SQ administration seven days and three days before irradiation; LDHC increased three days after irradiation following SQ administration seven days before irradiation; and UA increased three days after irradiation following SQ administration seven days before irradiation.

V. Conclusion

To determine the radioprotective effects of squalene, the experimental groups of mice exposed to one session of 3 Gy irradiation were divided into normal, irradiation control, SQ treatment before irradiation, and SQ treatment after irradiation groups; then, the irradiation control group was subdivided into irradiation + measurement after 3 days and irradiation + measurement after 7 days groups, the SQ treatment before irradiation group into SQ treatment for 7 and 3 days + irradiation + measurement after 3 days and SQ treatment for 7 and 3 days + irradiation + measurement after 7 days groups, and the SQ treatment after irradiation group into irradiation + SQ treatment for 3 days + measurement 3 days after irradiation and irradiation + SQ treatment for 7 days + measurement 7 days after irradiation groups. There were a total of seven experimental groups and a total of 49 mice, 7 per experimental group, were used in this study. First, Caspase-3 and Caspase-9 in the cell signaling pathways, which led to apoptosis, were measured to examine cell activity; second, nitric oxide (NO) was measured to observe inflammatory responses in cells; and third, TNF-α, IL-6, and IL-10 were measured to observe radiation-induced cytokine. In addition, biochemical analyses of the serum from the same mice were made as part of an attempt to obtain supplementary data about the radioprotective effects of SQ.

In Caspase-3 and Caspase-9 tests to examine cell activity, while both of them had the O.D. value increased significantly in the irradiation control group, Caspase-3 of Small intestine was statistically significant differences between both the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the group receiving irradiation alone (Rad control group) ($p < 0.05$) but not between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) and the Rad control group. Statistically significant differences were found between both the groups with measurement three days after irradiation following SQ administration seven days

and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). Caspase-3 of Liver was statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$) but not between the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. Statistically significant differences were found between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$) but not between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. Caspase-9 of small intestine was statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). Statistically significant differences were found between both the groups with measurement seven days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). Caspase-9 of Liver was statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$) but not between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. Statistically significant differences were found between both the group with measurement seven days after irradiation following SQ administration

three days before irradiation (SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$) but not between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group. On the basis of Caspase-3 and Caspase-9, therefore, Caspase generation was inhibited in the SQ administration group.

In the NO test to observe inflammatory responses in cells, statistically significant differences were found between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). NO of small Intestine was statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, there was no statistically significant difference between the groups with measurement seven days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. NO of Liver was statistically significant differences between the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement three days after irradiation following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) and the Rad control group. There were statistically significant differences between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three

days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. It was confirmed, therefore, that SQ promoted NO generation. In the test of TNF-α among radiation-induced cytokines, there were statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$).

TNF-α of small intestine was statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. There were statistically significant differences between the groups with measurement seven days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). TNF-α of Liver was statistically significant differences between the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the Rad control group ($p < 0.05$). In contrast, there was no statistically significant difference between the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. Statistically significant differences were found between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. It was confirmed, therefore, that TNF-α generation was inhibited in the SQ administration group. In

the test of IL-6 among radiation-induced cytokines, there were statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). IL-6 of small intestine was statistically significant differences between the group with measurement three days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement three days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group. There were statistically significant differences between the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. IL-6 of Liver was statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). There were statistically significant differences between both the group with measurement seven days after irradiation following SQ administration seven days before irradiation (SQ(pre-seven days)) and the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration three days before irradiation (SQ(pre-three days)) and the Rad control group. IL-10 of small intestine was statistically significant difference between both the groups with measurement three days after irradiation following SQ administration seven days and three days

before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). No statistically significant difference was found between the group with measurement seven days after irradiation following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. IL-10 of Liver was statistically significant differences between the group with measurement three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). In contrast, no statistically significant difference was found between the group with measurement following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) and the Rad control group. There was no statistically significant difference between the group with measurement seven days after irradiation following SQ administration seven days or three days before irradiation (SQ(pre-seven days) or SQ(pre-three days)) or the group receiving SQ administration for seven days after irradiation (Rad+SQ) and the Rad control group. It was confirmed, therefore, that IL-6 generation was inhibited in the SQ administration group. In the test of IL-10 among radiation-induced cytokines in the small intestines, there were statistically significant differences between both the groups with measurement three days after irradiation following SQ administration seven days and three days before irradiation (SQ(pre-seven days) and SQ(pre-three days)) and the group receiving SQ administration for three days after irradiation (Rad+SQ) and the Rad control group ($p < 0.05$). It was confirmed, therefore, that IL-10 generation was inhibited in the SQ administration group. In the serum test, SQ administration led to recovery in some cases: total protein (TP) increased three days after irradiation following SQ administration seven days before irradiation; albumin increased three days after irradiation following SQ administration seven days before irradiation; LDH increased three days after irradiation following SQ administration seven days before irradiation; HDLC decreased three days after irradiation following SQ administration seven days and three days before irradiation; LDHC increased three days after irradiation following

SQ administration seven days before irradiation; and UA increased three days after irradiation following SQ administration seven days before irradiation.

In conclusion, it has been confirmed that squalene with good antioxidant effects gives radioprotection by promoting NO generation and by inhibiting Caspase, TNF-α, IL-6, and IL-10 generation and that a natural product with a lower level of chemical toxicity can be used as a radioprotector.

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