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Study on the Health Recovery Effects of Injection Omega-3 Fatty Acid to Mouse by Exposing Radiation

Kyung-Rae Dong[1], Jong-Gil Kwak[1] and Chang-Gi Kong[2,*]

[1]*Department of Radiological Technology, Gwangju Health University, 73, Bungmun-daero 419 beon-gil, Gwangsan-gu, Gwangju 62271, Republic of Korea*
[2]*Department of Radiology, Cheomdan Hospital, 59, Cheomdanjungang-ro 170 beon-gil, Gwangsan-gu, Gwangju 62274, Republic of Korea*

Abstract - This study is to determine the radioprotective effects of Squalene (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. 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. 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^{-1}. It has been confirmed that squalene with good antioxidant effects gives radioprotection by promoting NO generation and by inhibiting Caspase that a natural product with a lower level of chemical toxicity can be used as a radioprotector.

Key words : SQ, Caspase-3, Caspase-9, NO, Radioprotective

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. 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. 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.) (Asa *et al*. 2000; Asa *et al*. 2002; Kim *et al*. 2003; Kuroda *et al*. 2004; Lim 2006). 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 protec-

* Corresponding author: Chang-Gi Kong, Tel. +82-62-601-8114, Fax. +82-62-601-8199, E-mail. changgi26@naver.com

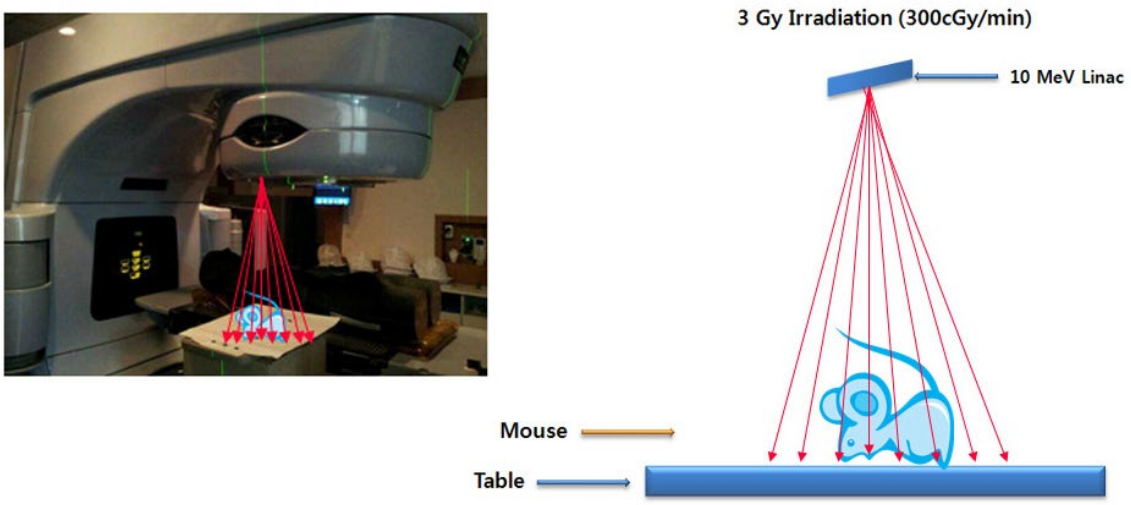

Fig. 1. Irradiation in mice using the 10 MeV Linac X-ray.

tive effects in case of administration one to three days before irradiation (Vuorio *et al*. 2002). 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 (Clarke *et al*. 2004; Veen *et al*. 2004). 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 (Nishimoto *et al*. 2003). 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 (Nakamura *et al*. 1997; Relas *et al*. 2000; Matyas *et al*. 2004). Research on radioprotection is being very positively conducted abroad as well. An acidic fibroblast growth factor (FGF1) 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 (Senthilkumar *et al*. 2006). 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 (Sabeena *et al*. 2004). 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 (Ahn *et al*. 1991).

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.

MATERIALS AND METHODS

1. Experimental Animals and Irradiator

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°C and humidity at 45 ± 5% and were allowed to have feed (manufac-

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

2. 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 $cGy \cdot min^{-1}$ (Fig. 1).

3. Specimen Administration

8 $mL \cdot kg^{-1}$ 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).

4. Measurement

4.1. 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 4 mM LEHD-pNA substrate to each tube and incubate it at 37°C for 1~2 hours. Then, perform measurement in a microplate reader (microreader: 405 nm, U.S.A.).

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

4.2. 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 NO_2^- 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 0.5 N 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.

5. 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

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. *$p<0.05$.

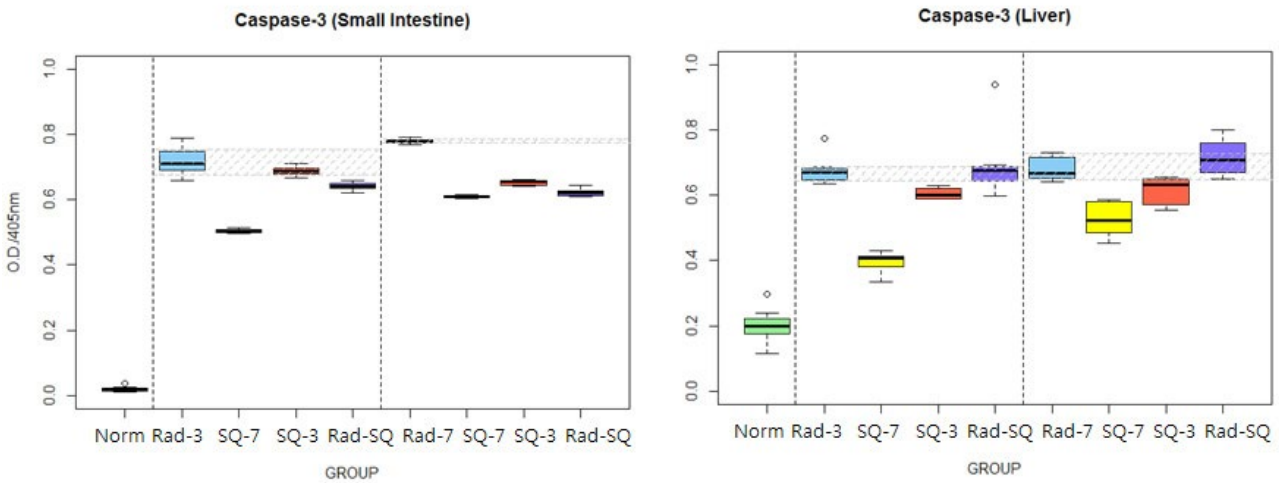

Fig. 2. Caspase-3 in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

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.

RESULTS

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

1.1. 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 nm, 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 be-

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. *$p < 0.05$.

fore 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. 2).

1.2. 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. 2).

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

2.1. Small Intestines

SQ was administered to irradiated mice to observe

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. *$p < 0.05$.

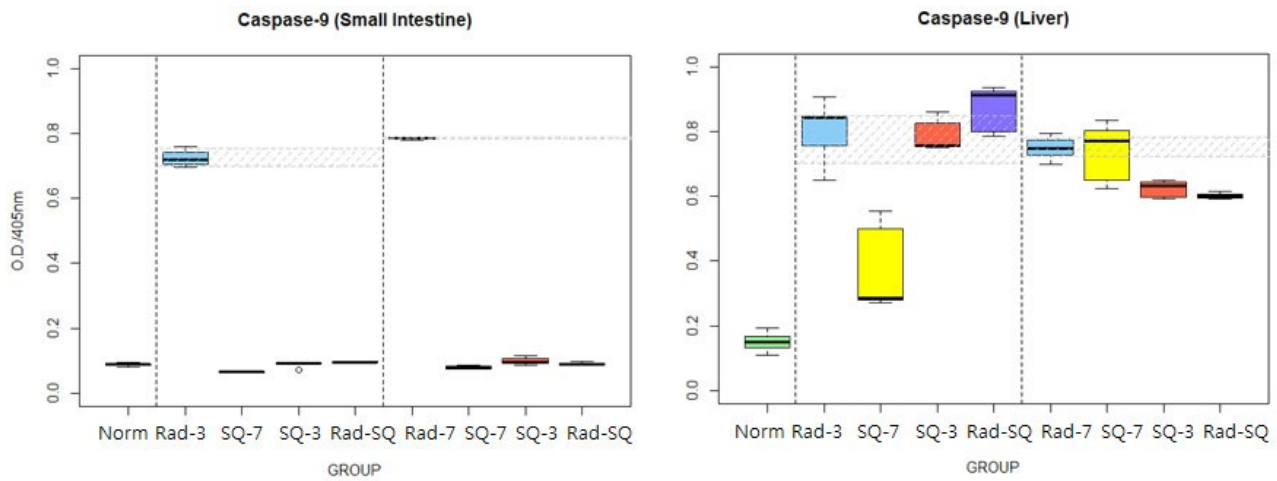

Fig. 3. Caspase-9 in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

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

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. *$p < 0.05$.

receiving SQ administration for seven days before irradiation (Table 4, Fig. 3).

2.2. 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. 3).

3. Nitric Oxide (NO)

3.1. 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 admin-

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^{-1}. *$p < 0.05$.

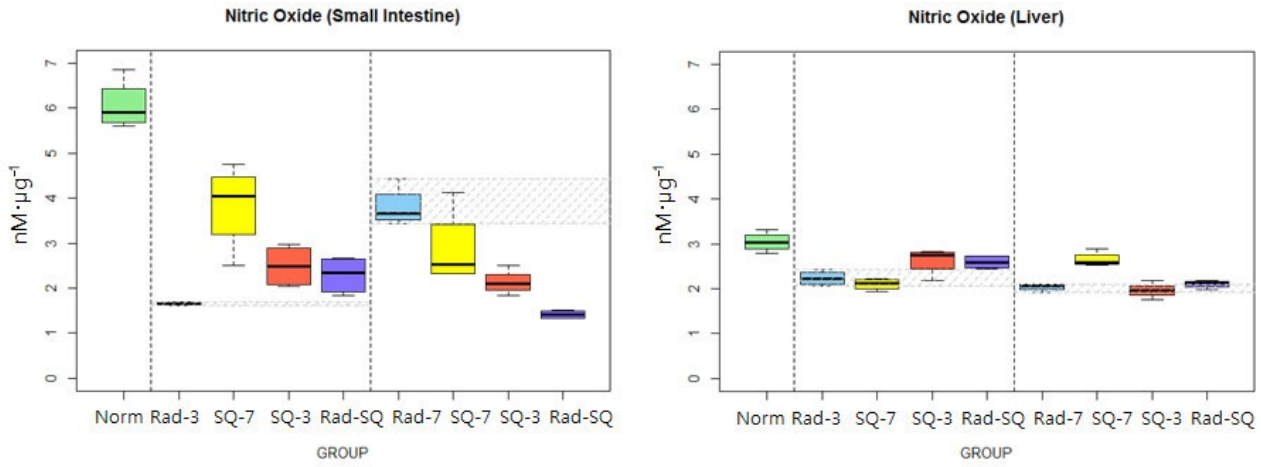

Fig. 4. NO (Nitric Oxide) in small intestine and liver of 3 Gy irradiated mice with SQ treatment.

istration 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. 4).

3.2. 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

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 $nM \cdot \mu g^{-1}$. *$p < 0.05$.

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. 4).

DISCUSSION

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 (Cohen 1962; Byyny *et al*. 1972; Taylor *et al*. 1972; Cohen and Carpenter 1975; Barrando and Green 1987; Fisher and Lakshmanan 1990; Nanney *et al*. 1990; Bennett *et al*. 1993; Storm *et al*. 1993; Kohno *et al*. 1995). 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).

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 (Kesaniemi and Miettinen 1987). Value decreased by SQ, which provided radioprotection through antioxidation. 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 $mJ \cdot cm^{-2}$) 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 (Nakagawa *et al*. 2001; Bae *et al*. 2002; Wang *et al*. 2002; Sethi and Sodhi 2004). 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 ($M = 1.652$). 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 (Zaidi *et al*. 2004).

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. 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, 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) ($p < 0.05$) in the Caspase-3 test in the small intestines and 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 in the Caspase-9 test in the small intestines ($p < 0.05$). 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 ($p < 0.05$). It was confirmed, therefore, that SQ promoted NO generation. In conclusion, it has been confirmed that squalene with good antioxidant effects gives radioprotection by promoting NO generation and by inhibiting Caspase that a natural product with a lower level of chemical toxicity can be used as a radioprotector.

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