Short Communication

Effects of Orlistat on white adipose tissue (WAT) in the γ-irradiated mouse model

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In this study, we showed that γ-irradiation could trigger biological response like the fat accumulation of gonadal white adipose tissue (gWAT) in mice. The irradiated animal can be used as a useful model for evaluation of anti-obesity drugs. To induce the fat accumulation by γ-irradiation, 2-months-old female C57BL/6 mice were irradiated at 5 Gy and further raised for 6 months. Subsequently, the mice were i.p. injected daily with Orlistat (25 mg/kg) or vehicle for 3 wk and analyzed for the adipose tissue weight and serum triglyceride and T-cholesterol levels. The abdominal WAT of the γ-irradiated mice weighed an average of 3.9/100 g body weight, 1.7-fold higher than what was seen in normal mice (2.3/100 g body weight), indicating that γ-irradiation induced the fat accumulation in the adipose tissue. However, the administration of Orlistat, a well-known anti-obesity agent, significantly reduced the adipose tissue weight to 1.7/100 g body weight in irradiated mice. In addition, in these Orlistat-treated mice, a significant reduction of serum triglyceride and T-cholesterol was observed up to 14% and 27%, respectively.

Keywords: Animal model / Fat accumulation / Gonadal white adipose tissue (gWAT) / Ionizing radiation / γ-irradiation

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1 Introduction

Ionizing radiation has become an inevitable health concern, emanating from natural sources during space travel and from artificial sources like medical therapies. Although the risk of degenerative diseases by ionizing radiation has been reported [1–4], the degeneracy of adipocytes during exposure to radiations is poorly understood. The regulation of adipocytes can be considered as a way to balance both glucose uptake and energy expenditure in order to derive the amount of fat stored. Thus, increases in glucose uptake or decreases in energy expenditure result in elevated fat deposition. Exposure to ionizing radiation such as γ-rays is one of the methods currently used to stress specific model system. In 2003, the metabolic syndromes caused by radiation exposure have been reported in the Japanese atomic bomb survivors [5–7]. Akahoshi group reported that radiation dose was positively associated with fatty liver from metabolic coronary heart disease (CHD) with insulin resistance syndrome, cholesterol and hypertriglyceridemia [5].

Orlistat is a member of a class of drugs known for inhibition of obesity and commercially available as Xenical™. Chemically, Orlistat is [2S-[2alph, 3 beta]-*N*-formyl-L-leucine 1-[(3-hexyl-4-oxo-2-oxetanyl)methyl]dodecyl ester. It is also known as *N*-formyl-L-leucine ester with (3S,4S)-3-hexyl-4-[(2S)-2-hydroxy-tridecyl]-2-oxetanone, or (−)-tetrahydrolipstatin [8–10].

In this study, we present our observation of the biological response resulting in fat accumulation in adipose tissue by ionizing radiation [11]. Until now, there was no report about changes in lipid metabolism disorder to growth and development of γ-irradiated animal. We suggest that γ-irradiation in animal model might result in fat accumulation and Orlistat treatment as an anti-obesity agent that inhibits the accumulated fat in C57BL/6 mice. In this regard, γ-irradiated mice could be evaluated as a useful model for the development of novel therapeutic approach for obesity.

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Abbreviations: gWAT, gonadal white adipose tissue; **STD,** standard diet

2 Materials and methods

2.1 Reagents

Orlistat was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest grade available.

2.2 Animal experiments

C57BL/6 female mice were used in the present study. Young mice (6 wk of age) were purchased from Orient Bio Co., Ltd. (Charles River Technology, South Korea). They were housed in 5–10 numbers per cages under specific pathogen-free conditions at 23 ± 3°C and 50 ± 10% relative humidity, and were provided with nutritional chow (5L79, PMI Nutrition LLC, USA). The mice were given free access to water and were fed on a standard diet (STD). All animal experiments were approved by the Ethics Committee for Animal Experimentation of Korea Food Research Institute. All procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals (Institute of Laboratory Animal Research, National Research Council, 1996. Guide for the care and use of laboratory animals, seventh ed. Washington, D.C.). Food intake and body weight were monitored on a weekly basis.

2.3 Ionizing radiation

The mice were exposed to whole-body γ-irradiation in a plastic chamber to ^{137}Cs γ-rays with Gamma Cell 40 Exactor (Nordion International Inc., Canada) without physical restraint or anesthesia. This device has been used in previous biological studies and radiation procedure has been described in more details [12, 13]. The 2-month-old C57BL/6 mice were exposed to ^{137}Cs γ-rays at a single (5 Gy) dose. After exposure to whole-body γ-irradiation, the mice were sorted into individual cages and monitored daily for the development of symptoms of radiation sickness and mortality. The mice were divided into two groups with three mice in each group. The experimental design and treatment protocol were as follows: Normal group: 8-month-old C57BL/6 mice without γ-irradiation served as reference; Radiation group: 8-month-old C57BL/6 mice, the age of 2-month-old was exposed to ^{137}Cs γ-rays at a single 5 Gy (1.1 Gy/min) dose and sustained for 6 months.

2.4 Administration of Orlistat

To examine the effect of Orlistat, two groups of irradiated mice were allocated into group 1: mice fed with STD; group 2: STD mice were treated with Orlistat (25 mg/kg/day). The mice were treated with Orlistat (25 mg/kg body weight, dissolved in 30% ethanol, i.p. injection volume of 200 μL/mouse) for 3 wk. Mice in the control group 1 were administrated 200 μL of 30% ethanol.

2.5 Quantification of serum triglyceride and T-cholesterol

To measure biochemical analysis in serum, the animals were deprived of food for 15 h, and then blood samples were collected from the veins of mice under ether anesthesia. Triglyceride concentration was measured enzymatically using commercial kits and following manufacturer's procedures (ASAN Pharm. Co., Seoul, South Korea). T-cholesterol in serum was measured by an enzymatic colorimetric method using a commercial kit (ASAN Pharm. Co., Seoul, South Korea).

3 Results and discussion

Figure 1 represents that the scheme of fat accumulation by ionizing radiation and the effects of Orlistat as an anti-obesity agent in the γ-irradiated animal model. To observe the fat accumulation by γ-irradiation, 2-month-old female C57BL/6 mice were irradiated at a single 5 Gy (1.1 Gy/min) dose and further sustained for 6 months. In Fig. 2A,B, the abdominal white adipose tissue (WAT) of the γ-irradiated mice weighed an average of 3.9/100 g body weight, 1.7-fold higher than what was seen in the normal mice (2.3/100 g body weight), indicating that γ-irradiation triggered the fat accumulation in the adipose tissue. We monitored the food intake on a weekly basis. During the monitoring period, the average food intake of mouse was 3.53 ± 0.5 g/day. For food intake, there was no

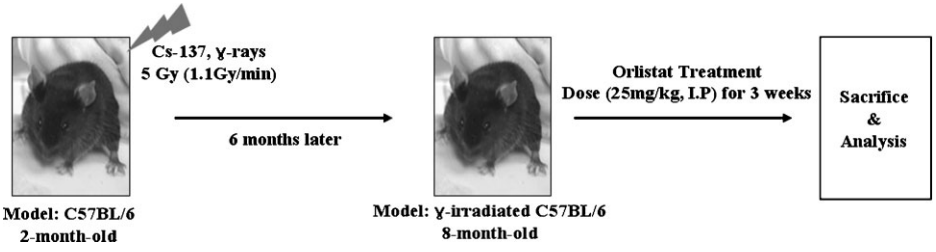

Figure 1. Scheme of fat accumulation by ionizing radiation and the effects of Orlistat as an anti-obesity agent in the γ-irradiated animal model.

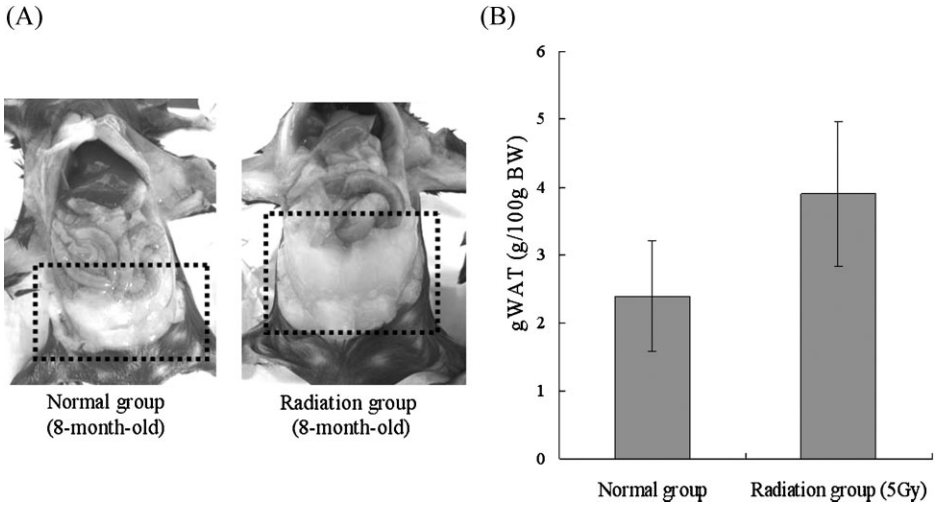

Figure 2. Effects of γ-irradiation on the gonadal white adipose tissue (gWAT) in mice. Normal group (8-month-old) C57BL/6 mice and Radiation group (5 Gy, 8-month-old) irradiated mice were observed for their white adipose tissue development. Radiation induced the size increase of the gonadal white adipose tissue (gWAT) in gross findings (A) and the adipose tissue weight (B).

noticeable difference in normal and γ-irradiation group (data not shown).

The purpose of the present study was to determine the effect of the Orlistat on fat accumulation induced by ionizing radiation and to elucidate the underlying lipid metabolism. To determine the effect of the Orlistat on C57BL/6 mice, the mice were separated into two groups: irradiated untreated group, and irradiated Orlistat-treated group. We speculate that the Orlistat-treated group exhibits a lipid-lowering effect. The administration of Orlistat (25 mg/kg/day), a well-known anti-obesity agent, significantly reduced the adipose tissue weight to 1.7 g (approximately 56%) per 100 g body weight in irradiated mice in Fig. 3A,B. In Fig. 4A,B, a significant reduction of serum triglyceride and T-cholesterol in these Orlistat-treated mice was observed up to 14%, 27% level, respectively. For monitoring of the

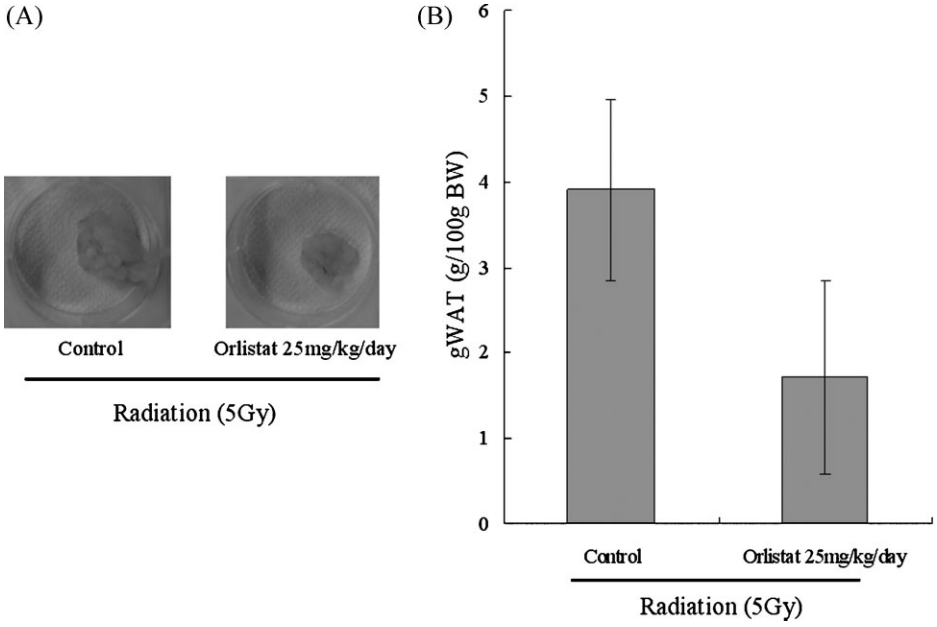

Figure 3. Effect of Orlistat on the white adipose tissue (WAT) in irradiated mouse model. (A) Isolated gonadal white adipose tissue (gWAT) and (B) weight of gonadal white adipose tissue (gWAT) treated and untreated mice with Orlistat (25 mg/kg/day).

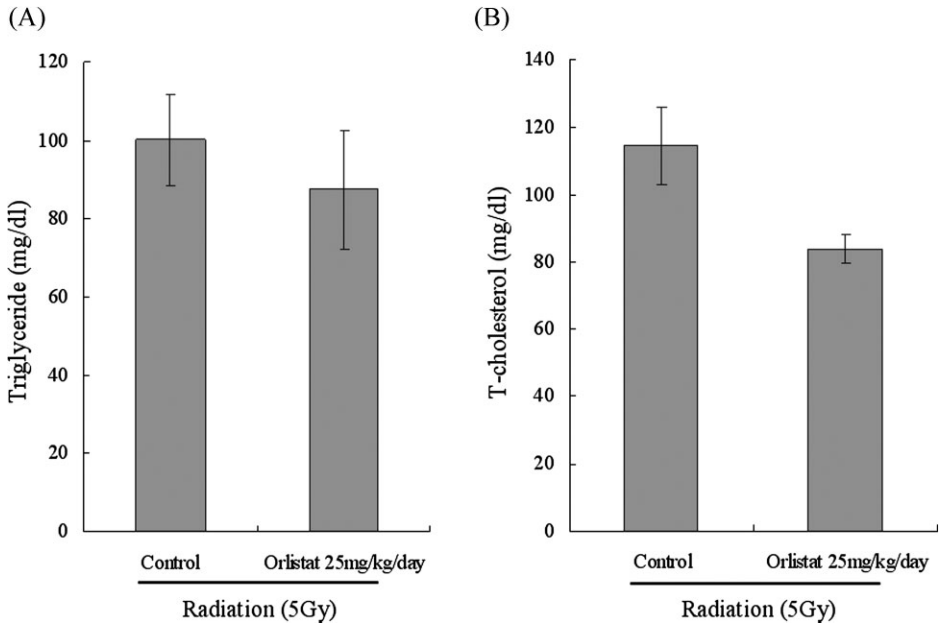

Figure 4. The amount of triglyceride, total-cholesterol present in serum for 21 days treated and untreated mice with Orlistat (25 mg/kg/day).

food intake on a weekly basis, there was no noticeable difference in Orlistat-untreated and Orlistat-treated group (data not shown).

Many scientific communities have become increasingly interested in the molecular regulation of triglyceride synthesis and in pharmaceutical approaches to reduce fat storage. Accordingly, many efforts are currently directed towards the identification of molecular targets for fat storage. Driven by this need, the study of model induced by ionizing radiation might lead to the development of drugs that specifically reduce WAT mass.

In this study, the changes of WAT weight and serum triglyceride by Orlistat administration in animal model induced by radiation exposure were investigated. The findings in this study suggest that the senescence of fat accumulation induced by radiation exposure might contribute to the development of novel therapeutic approach for the treatment of lipid metabolism disorders such as obesity.

In summary, we showed that γ-irradiation could trigger the biological response resulting in the fat accumulation of WAT in mice, and that this γ-irradiated mice could be used as a useful model for evaluation of anti-obesity agent.

4 Conclusion

The ionizing radiation triggers fat accumulation and Orlistat as an anti-obesity agent could ameliorate these effects in γ-irradiated animal model. Thus γ-irradiated mice could be applied as a useful model for the development of novel therapeutic approaches for obesity.

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The authors have declared no conflict of interest.

References

[1] White, R. J., Averner, M., Humans in space. *Nature* 2001, *409*, 1115–1118.

[2] Henshaw, P. S., Riley, E. R., Stapleton, G. E., The biological effects of pile. *Radiologie* 1947, *49*, 349–364.

[3] Mewissen, D. J., Comar, C. L., Trum, B. F., Rust, J. H., A formula for chronic radiation dosage versus shortening of life span:application to a large mammal. *Radiat. Res.* 1957, *6*, 450–459.

[4] Upton, A. C., Kimball, A. W., Furth, J., Christenberry, K. W., Benedict, W. H., Some delayed effects of atom-bomb radiations in mice. *Cancer Res.* 1960, *20*, 1–60.

[5] Akahoshi, M., Amasaki, Y., Soda, M., Hida, A., *et al*. Effects of radiation on fatty liver and metabolic coronary risk factors among atomic bomb survivors in Nagasaki. *Hypertens Res.* 2003, *26*, 965–970.

[6] Wong, F. L., Yamada, M., Sasaki, H., Kodama, K., Hosoda, Y., Effects of radiation on the longitudinal trends of total serum cholesterol levels in the atomic bomb survivors. *Radiat. Res.* 1999, *151*, 736–746.

[7] Finch, S. C., Beebe, G. W., Review of thirty years study of Hiroshima and Nagasaki atomic bomb survivors. II. Biological effects. F. Aging. *J. Radiat. Res. (Tokyo)* 1975, *16*, 108–121.

[8] Harp, J. B., Orlistat for the long-term treatment of obesity. *Drugs Today* 1999, *35*, 139–145.

[9] Ballinger, A., Peikin, S. R., Orlistat: Its current status as an antiobesity drug. *Eur. J. Pharmacol.* 2002, *440*, 109–117.

[10] Heck, A. M., Yanovski, J. A., Calis, K. A., Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacotherapy* 2000, *20*, 270–279.

[11] Jo, S. K., Roh, C. H., Park, H. R., Jung, U., Method of fat accumulation by ionizing radiation in animal and its application for obesity-related animal model, Patent, Korea/2010-0037 873.

[12] Park, H. R., Jo, S. K., Paik, S. G., Factors effecting the Th2-like immune response after gamma-irradiation: Low production of IL-12 heterodimer in antigen-presenting cells and small expression of the IL-12 receptor in T cells. *Int. J. Radiat. Biol.* 2005, *81*, 221–231.

[13] Park, H. R., Jung, U., Jo, S. K., Impairment of natural killer (NK) cells is an important factor in a weak Th1-like response in irradiated mice. *Radiat. Res.* 2007, *168*, 446–452.

