Changes in the gastric ghrelin concentration after whole-abdominal irradiation in rats: Is this related to the radiation-induced anorexia and weight loss?

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ABSTRACT

Background: Ghrelin is a hormone related to food intake in rodents and humans, mainly produced in stomach. This study aimed to determine the effect of irradiation on ghrelin concentration in the gastric mucosa of rats. ***Materials and Methods***: Twenty-five rats were exposed to 15 Gy of whole-abdominal irradiation. Gastric tissue samples were obtained 1, 3, 7, 30, and 90 days after irradiation. Five non-irradiated rats were used as controls. The number of ghrelin cells that reacted with anti-ghrelin antibody was counted. Moreover, ghrelin mRNA expression was determined. Food intake and body weight changes were measured simultaneously. ***Results***: Compared to the controls, irradiated rats showed a significantly decreased gastric ghrelin cell count, i.e., 29%, 30%, 32%, and 32% at 1, 3, 30, and 90 days, respectively, after irradiation ($p < 0.05$). Irradiated rats also showed decreased ghrelin mRNA expression; the expression decreased by 54.1%, 58.8%, 52.0%, and 52.7% at 1, 3, 30, and 90 days, respectively ($p < 0.05$). Food intake of irradiated rats decreased continuously compared with the control rats, except at 90 days. Body weight of the irradiated rats was lower than that of the controls at 7 and 30 days. ***Conclusion***: This study demonstrated that abdominal irradiation can reduce gastric ghrelin concentration. Though decrease of food intake and body weight was observed simultaneously, further evaluation needs to find out the relationship between gastric ghrelin level and food intake after exposure to irradiation.

Keywords: *Ghrelin, weight loss, anorexia, whole-abdominal irradiation, rat.*

INTRODUCTION

Ghrelin, a 28-amino acid peptide secreted mainly from X/A-like cells (ghrelin cells) in the fundus of the stomach, was isolated in 1999 as the endogenous ligand for the growth hormone secretagogue receptor. Some researchers identified its orexigenic properties in rodents and humans [(1)]. In a recent study, administration of ghrelin to a group of cachectic and noncachectic cancer patients induced a 30% increase in appetite and food intake [(2)]. The discovery of ghrelin led to the establishment of the concept that signals for appetite induction could be transmitted through a pathway between the stomach and brain.

The GI tract is one of the most radiosensitive organs in the body because of constitutive rapid cell turnover. Radiation can induce damage of GI epithelium and affect GI tract functions, including movement (passage of the food), absorption, and especially secretion [3]. Previous studies demonstrated that the densities of GI endocrine cells, such as the G cell and somatostatin-releasing cell decrease after whole-body irradiation in the rat [4]. Therefore, abdominal irradiation may also reduce the concentration of ghrelin in the gastric mucosa, which may induce some biological reactions such as anorexia or weight loss consequently. To our knowledge, no experimental research has shown the effect of irradiation on ghrelin cells in the gastric tissue. The aim of this study was to elucidate how whole-abdominal irradiation affects ghrelin cells in the gastric tissue of rat. Ghrelin mRNA expression in the stomach was also evaluated to determine the degree of ghrelin synthesis at a molecular level. Additionally, the pattern of change in food intake and body weight was assessed during the experiment.

MATERIALS AND METHODS

Experimental animals

Male Sprague–Dawley rats, each weighing approximately 250–280 g, were used in this study. The rats were individually housed in a temperature-controlled (22°C ± 1°C) room illuminated from 08:00 to 20:00 (12-h light/dark cycle) with free access to standard laboratory chow diet (Purina lab diet 5001, PMI Nutrition International, Brentwood, MO) and water. Animal experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources). All experiments involving animals were approved by the review board for animal experimentation.

Study protocol

Twenty-five rats were divided into 5 groups (5 per group) and euthanized 1, 3, 7, 30, or 90 days after whole-abdominal irradiation. Five non-irradiated rats were assigned to a control group. Rats were weighed and evenly stratified according to body weight to ensure that the mean weight of each group was similar. Each rat was euthanized under ketamine anesthesia between 08:00 and 09:00 before a meal to collect the gastric tissues. The mean ghrelin cell count in each group was determined using a light microscope after immunohistochemical staining. Ghrelin mRNA expression was evaluated by real-time reverse-transcription polymerase chain reaction (RT-PCR). At the same time, body weight and the amount of 24-h food intake were measured.

Whole abdominal irradiation

In total, 25 rats were anesthetized with intra-peritoneal ketamine (50 mg/kg) and subjected to whole abdominal irradiation with a Linear Accelerator (Clinac® 21EX, Varian Medical Systems, Palo Alto, CA) at a dose rate of 600 cGy/min for 2.5 min, a total of 15 Gy per animal. During irradiation, the rats were mounted to fitted wallpaper with plastic wrap in the supine position. Anteroposterior and posteroanterior (AP/PA) fields were used with both anterior and posterior compensators (1-cm thick, 100% acrylic plate) for even radiation exposure. The radiation field extended from the xiphoid process to both iliac crests of the rat.

Determination of ghrelin cell count

Gastric tissues approximately 2 cm in length were resected and fixed in 10% neutral-buffered formalin for immunohistochemical analysis. Sections from each block were transferred to glass slides coated with poly-L-lysine and were air dried at 37°C overnight. They were then dewaxed in xylene (3 changes), hydrated in decreasing concentrations of ethanol, and rinsed in Tris buffered saline (TBS; pH 7.4). Endogenous peroxidase activity was inactivated with 5% hydrogen peroxide in methanol at room temperature for 20 min. Antigen retrieval was performed using a 5-min microwave treatment in TBS. Gastric tissue sections were incubated with anti-ghrelin antibody (Phoenix

Pharmaceuticals, Belmont, CA), which was diluted 1:500, at room temperature for 1 h. Immunohistochemical procedures were performed using the Envision detection kit (DAKO, Glostrup, Denmark). The sections were exposed to 3-amino-9-ethylcarbazole or diaminobenzidine to visualize the reaction products. Nuclei were lightly counterstained for approximately 20 s with Mayer's hematoxylin. Sections were mounted in diluted malinol after the application of Universal Mount (DAKO, Carpentaria, CA). Cells that were stained brown in the mucosal layer of the stomach, as observed by light microscopy, were counted in 10 different high-power fields (40´ objective), and the average number of ghrelin cells per 10 high-power field was calculated. To eliminate interobserver variability, a single pathologist counted the number of ghrelin cells on all slides.

Determination of ghrelin mRNA expression

Total RNA from the gastric tissue was obtained by the TriReagent^{R} method (Molecular Research Center Inc., Cincinnati, OH). Ghrelin mRNA content was detected using real-time RT-PCR kit (Retrotools cDNA/DNA polymerase; Biotools, Madrid, Spain). Reverse transcription was carried out using 1 µg of total RNA in a 20-µl reaction volume at 42 °C for 30 min. PCR was performed in a 50-µl reaction volume. β-Actin mRNA was used as an internal control. Amplification involved 38 cycles of denaturation (94 °C, 5 s), annealing (65 °C, 10 s), and extension (72 °C, 1 min). The PCR products were separated on a 2% agarose gel, the bands were visualized with ethidium bromide staining, and the band densities were normalized by the value of β-actin.

Statistical analysis

SPSS software for Windows (version 12.0, SPSS Inc., Chicago, IL) was used for statistical analysis. Quantitative parameters were expressed as mean ± standard deviation (SD). Statistical comparisons among the groups were performed using one-way analysis of variance (ANOVA) followed by the Mann-Whitney test for comparisons between 2 groups or Tukey post hoc multiple comparisons test. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

Ghrelin cell count in the gastric tissue after whole abdominal irradiation

As shown in figure 1 and 2, compared to ghrelin cell count of non-irradiated group, whole abdominal irradiation significantly decreased the number of gastric ghrelin cells by 29% (17.7 ± 3.7) and 30% (17.6 ± 7.7)

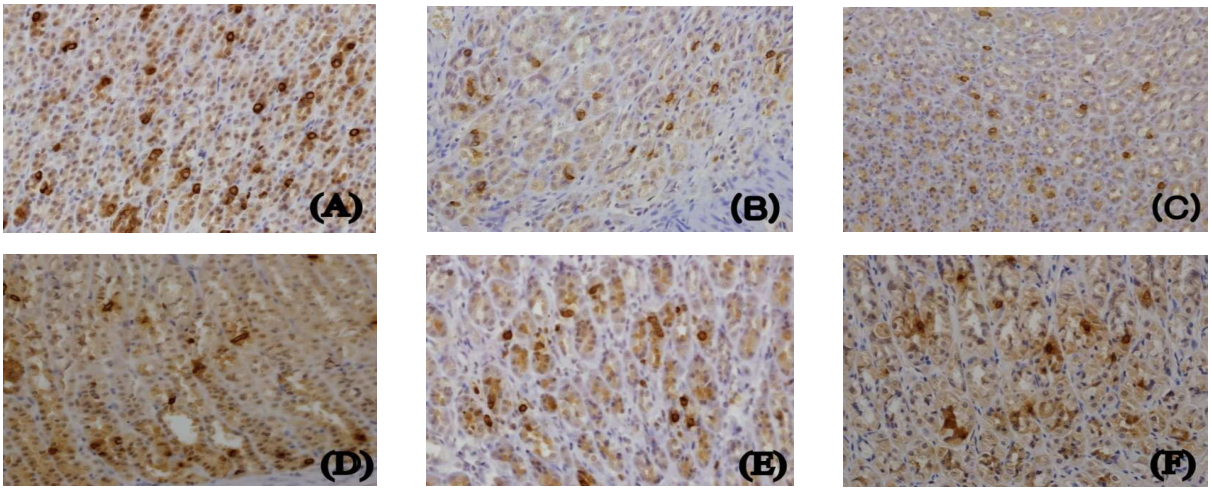

Figure 1. Light microscopy results of ghrelin cells (stained brown) in the oxyntic gland of the stomach (400´). (A) Non-irradiated group, (B) 1 day, (C) 3 days, (D) 7 days, (E) 30 days, and (F) 90 days after whole abdominal irradiation.

on days 1 and 3 after irradiation, respectively (25.0 ± 5.1; $p < 0.05$). On day 7 after exposure to 15 Gy, the cell count (22.7 ± 5.8) recovered temporarily; the difference in the cell count between the 2 groups was statistically insignificant ($p = 0.366$). However, the number of ghrelin cells in the gastric tissue then dropped again by 32% at 30 (16.9 ± 5.1) and 90 days (17.0 ± 4.4) after whole abdominal irradiation ($p < 0.05$).

Ghrelin mRNA expression in the gastric tissue after whole abdominal irradiation

As shown in figure 3, ghrelin mRNA expression in the gastric tissue of the irradiated rats decreased by 54.1% (0.68 ± 0.1) and 58.8% (0.61 ± 0.1) on days 1 and 3 after irradiation, respectively, compared to mRNA expression of the control group (1.48 ± 0.2; $p < 0.05$). However, the ghrelin mRNA level in the irradiated rats was 29.1% higher than that in the control group on day 7 (1.91 ± 0.3) after whole abdominal irradiation ($p < 0.05$). Ghrelin mRNA expression then decreased by 52.0% (0.71 ± 0.3) and 52.7% (0.70 ± 0.2) on days 30 and 90, respectively ($p < 0.05$).

Body weight and food intake after whole abdominal irradiation

The average daily food intake of irradiated rats was significantly lesser than that of control rats at nearly all experimental time points, except 90 days ($p = 0.236$), after whole abdominal irradiation (figure 4A). Compared to the average weight of the control group, that of irradiated group was significantly low at 7 and 30 days after irradiation ($p = 0.009$ for 7 days, ≤ 0.001 for 30 days; figure 4B).

DISCUSSION

This study showed for the first time that radiation affects the tissue concentration of ghrelin in the rat stomach. The number of ghrelin cells and the level of ghrelin mRNA expression in the gastric mucosa were reduced 1 and 3 days after whole abdominal irradiation. We assumed that this acute response was due to either the functional impairment of the ability of the irradiated mucosal cells to produce ghrelin by disturbing the signal transduction pathway, or the apoptotic cell death in some acute radiation-responsive gastric tissues. Both the cell count and mRNA expression of ghrelin temporarily increased 7 days after irradiation, but then decreased again at 30 and 90 days. This late reduction could be the result of classical mitotic cell death by high-dose radiation

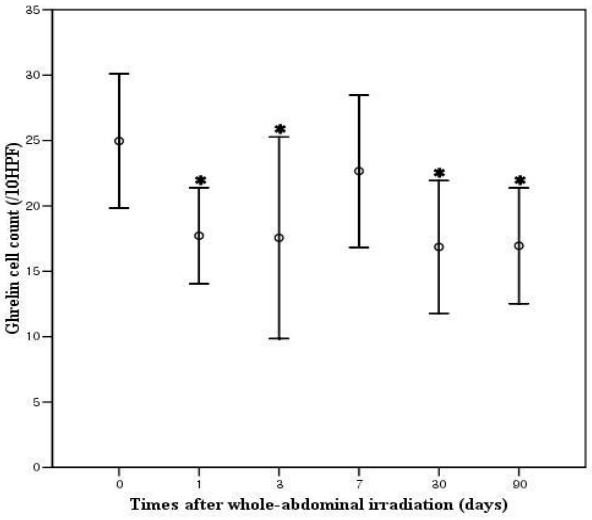

Figure 2. Changes in ghrelin cell count (per 10 high-power fields) according to days elapsed after whole abdominal irradiation. Data are presented as the mean ± SD. * P<0.05 versus control group.

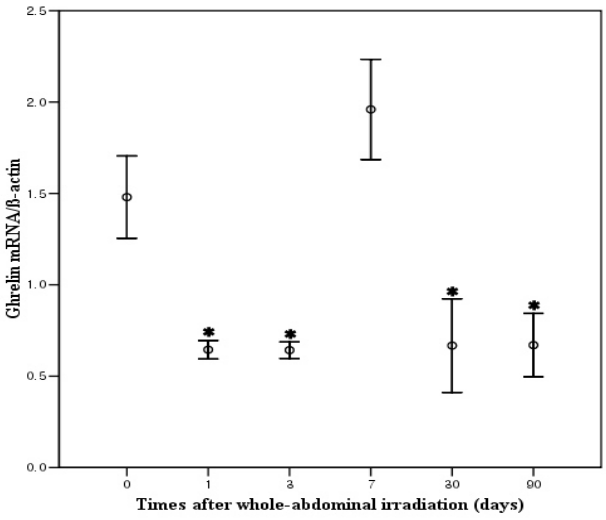

Figure 3. Alteration of ghrelin mRNA expression in the gastric mucosa according to days elapsed after whole abdominal irradiation. Data are presented as the mean ± SD. * P<0.05 versus control group.

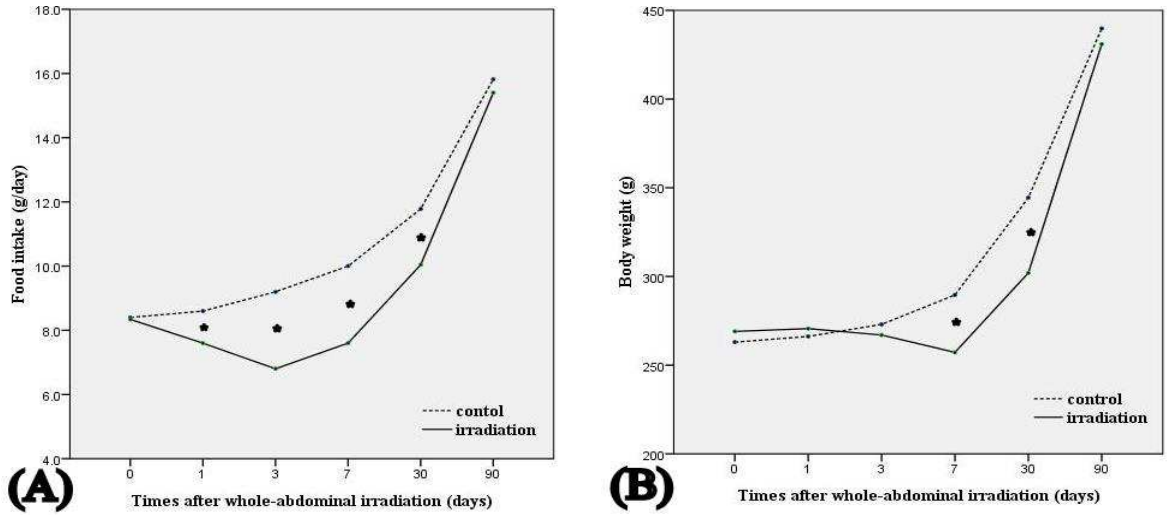

Figure 4. Average daily food intake (A) and body weight (B) in 5 irradiated and 5 non-irradiated rats according to days elapsed after whole abdominal irradiation. * P<0.05 versus control group.

exposure and irreversible long-term damage to the surrounding tissues.

In the gastric mucosa of the rat, a radiation dose of 6 Gy decreased the density of some functional cells such as enterochromaffin-like cells, gastrin cells, and somatostatin cells, which was accentuated 7 days after irradiation [4]. However, in this study, a recovery in the number of ghrelin cells and an increase in ghrelin mRNA expression were observed 7 days after whole abdominal irradiation. Although it is difficult to determine the exact mechanism from our experiment, we can present some speculations based on the function of ghrelin. Acute radiation-induced stressful conditions may increase the number of gastric ghrelin cells or the productive ability of these cells temporarily [5]. Low plasma ghrelin levels after gastric irradiation may stimulate the synthesis of ghrelin in the gastric tissue as a feedback mechanism.

We reviewed some recent studies in order to determine the optimal radiation dose for this study. In the literature, it has been reported that the plasma ghrelin level was not significantly changed in rats after 5-Gy whole-body irradiation. [6] However, one study demonstrated that a total dose of 15 Gy could modify the levels of enteric neuroendocrine products [4]. We know that there are differences in the radiation sensitivity of GI endocrine cells and that, compared to sensitivity of the intestinal mucosa, the gastric mucosa is relatively insensitive to irradiation. So a single dose of 15 Gy was selected to irradiate whole abdomen with expecting rapid and apparent tissue reaction in this study.

In animal experiments, a reduction of food intake after X-ray irradiation has been shown [7]; however, the mechanism is poorly understood. X-ray irradiation of the abdomen was reported to reduce food intake to a greater extent than irradiation of the head was [8]. Furthermore, clinical data showed that most patients who received abdominal irradiation for the treatment of gastric lymphoma suffered from anorexia [9]. These results suggest that peripheral afferent information from the GI tract play an important role in the loss of appetite due to X-ray irradiation.

The amount of 24-h food intake and body weight also decreased as the gastric ghrelin levels reduced in this study. There is an interesting report that rats which were vaccinated using anti-ghrelin vaccine showed slower body weight gain and reduced feed efficiency during the 7-day after immunization [10]. These results demonstrate that decrease of ghrelin activity may actually affect food intake and body weight. Is food intake or body weight affected directly by the amount of ghrelin secretion? Radiation can induce gene

expression and secretion of some cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α, which are involved in the acute inflammatory response to radiation injuries [11]. These cytokines also play a role in the suppression of appetite and cause some degree of weight loss through various pathways. Ghrelin can antagonize the effect of these cytokines on appetite and body weight. So we may assume that amount of ghrelin production after gastric irradiation is not enough to compensate these inflammatory conditions sufficiently.

However, it is still very difficult to declare that the reduction of ghrelin cell count is closely related to anorexia and sequential reduction of body weight. Because it remains uncertain whether a 30% decrease in the concentration of ghrelin in the gastric tissue is associated with a clinically significant reduction in the plasma level of ghrelin. Furthermore, there are many uncontrolled factors which can be open to a variety of interpretations of our results. Food intake is regulated via various neural and hormonal mechanisms, which involve hypothalamic neurons, gastrointestinal (GI) afferents, leptin from adipose tissue, blood glucose, and GI hormones [12]. Ghrelin level can be influenced by other materials including glucagon-like peptide, cholecystokinin, insulin and bombesin/GRP (gastrin-releasing peptide) [5]. Also, irritation of the GI tract after direct exposure to radiation may be manifested by nausea or diarrhea, which can also cause loss of food intake or body weight.

In this study, we observed that radiation can decrease the concentration of ghrelin and its mRNA expression in the gastric tissue and that this reduction manifests very quickly and lasts for at least 90 days. According to the changes in gastric ghrelin level, body weight and food intake were also reduced in the irradiated group compared to the non-irradiated control group. It is unsure that radiation-induced anorexia and weight loss is directly related to the reduction in the gastric ghrelin level. Our study suggests that the reduction of ghrelin production after irradiation can be a potential influencing factor for anorexia and weight loss related to abdominal radiation therapy in the clinical field of cancer patient treatment. Further studies are required to verify the relationship between ghrelin level (in both the gastric tissue and plasma) and anorexia in clinical situations.

REFERENCES

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature,* ***402:*** *656-660.*
2. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C *et al.* (2004) Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab,* ***89:*** *2832 -2836.*
3. Hauer-Jensen M, Wang J, Boerma M, Fu Q, Denham JW (2007) Radiation damage to the gastrointestinal tract: mechanisms, diagnosis, and management. *Curr Opin Support Palliat Care,* ***1:*** *23-29.*
4. Lehy T, Dessirlier V, Attoub S, Bado A, Griffiths NM, Linard C (1998) Exposure to ionizing radiation modifies circulating gastrin levels and gastrointestinal endocrine cell densities in the rat. *Int J Radiat Biol,* ***73:*** *331-340.*
5. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP *et al.* (2002) Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med,* ***346:*** *1623-1630.*
6. Katanyutanon S, Wu R, Wang P (2008) The effect of whole-body radiation on blood levels of gastrointestinal peptides in the rat. *Int J Clin Exp Med,* ***1:*** *332-337.*
7. Winsauer PJ, Verrees JF, O'Halloran KP, Bixler MA, Mele PC (1994) Effects of chlordiazepoxide, 8-OH-DPAT and ondansetron on radiation-induced decreases in food intake in rats. *J Pharmacol Exp Ther,* ***270:*** *142-149.*
8. Smith DE, Tyree EB (1954) Influence of X-irradiation upon body weight and food consumption of the rat. *Am J Physiol,* ***177:*** *251-260.*
9. Schechter NR, Portlock CS, Yahalom J (1998) Treatment of mucosa-associated lymphoid tissue lymphoma of the stomach with radiation alone. *J Clin Oncol,* ***16:*** *1916-1921.*
10. Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K *et al.* (2006) Vaccination against weight gain. *Physiology National Academy of Sciences,* ***103:*** *13226-13231.*
11. Hong JH, Chiang CS, Tsao CY, Lin PY, McBride WH, Wu CJ (1999) Rapid induction of cytokine gene expression in the lung after single and fractionated doses of radiation. *Int J Radiat Biol,* ***75:*** *1421-1427.*
12. Unno T, Hashimoto M, Arai S, Kurosawa M (2002) Reduction of food intake following X-ray irradiation of rats -involvement of visceral afferent nerves. *Auton Neurosci,* ***96:*** *119-125.*