Effect of Radiotherapy Dose Rates on Early Intestinal Toxicity

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OBJECTIVE
The aim of the present study was to investigate early histopathological changes in small intestinal tissue caused by radiotherapy (RT) applications with varying dose rates in rats.

METHODS
Thirty rats were divided into 3 groups. The first and second groups were irradiated at 300 MU/min and 600 MU/min, respectively. The third was a control group. On day 7 following RT application, small intestine tissue samples were obtained.

RESULTS
Mucosal thickness was significantly lower in the 300 MU/min group, compared to the other groups, and thickness was significantly lower in the 600 MU/min group, compared to the control group. Villus length was significantly decreased in the 300 MU/min group, compared to the other groups, and villus width was significantly increased in the control group, compared to the other groups.

CONCLUSION
RT application with high dose may be less toxic for tissue with high risk for acute toxicity, such as that of the small intestine.

Keywords: Radiotherapy dose rate; rat; small intestine; toxicity.

Introduction

Radiotherapy (RT) is an important cancer treatment option, and is generally used in combination with other treatment alternatives, such as chemotherapy or tumor-removing surgery. Modern technology has allowed for the delivery of localized radiation to nearly every part of the body. However, normal tissue toxicity remains the most significant dose-limiting factor in clinical RT applications.[1] Radiation targets cellular DNA and can produce immediate cell death or loss of ability to sustain cell division.[2] In some cell types, cell death occurs rapidly, within several hours of irradiation.[3] This type of death, interphase death, is primarily limited to thymocytes, lymphocytes, spermatogonia, and other cells in rapidly proliferating tissue such

as that of the small intestine.[4]

Gastrointestinal toxicity can occur after irradiation of thoracic, abdominal, or pelvic malignancies if normal gastrointestinal structures are involved in the RT field. Early gastrointestinal side effects (i.e. acute toxicity) of RT include diarrhea, abdominal pain, nausea, and vomiting, which may develop during or shortly after the completion of a course of RT. In addition, late effects including ulceration, stricture formation, and bowel obstruction may be observed months to years after RT completion.[5] Gastrointestinal toxicity is the most significant dose-limiting side effect in patients receiving RT for pelvic and abdominal tumors.[6,7] These toxicities can limit maximum doses of RT and chemotherapy, and thus may reduce efficacy of treatment.[5]

At the acute (early) period, alterations of the intestinal mucosa, such as protein and fibrin precipitation, inflammation, and edema of the bowel wall, can be histologically detected.[8-10] The number and height of villi decreases, and the affected functioning of the bowel mucosa leads to the loss of proteins, electrolytes, and water.[8,11,12]

RT can be administered at different dose rates.[13] Acute and chronic side effects of different RT dose rates are still under investigation. The aim of the present experimental study was to investigate early histopathological changes in small intestinal tissue, which has high risk for acute toxicity of RT, caused by RT applications with different dose rates in rats.

Materials and Methods

Animals

Thirty adult female Sprague Dawley rats (133±8 gr) were used. Rats were housed in separate cages and given ad libitum access to food and water. The animals were acclimatized with a 12-hour light/dark cycle. The study was approved by the local Ethics Committee for Animal Studies (No: 15/72).

Study design and animal experimentation

Rats were divided into 3 groups of 10 each. Rats in the first group were irradiated at 300 MU/min, and those in the second group were irradiated at 600 MU/min by 6 MV external x-ray beams. Radiation of the total body was 800 cGy. The third group was designated as the control group and was not irradiated. Following RT application, the rats were clinically monitored for diarrhea. On day 7 following RT application, all rats were

sacrificed under general anesthesia, and small intestine tissue samples were obtained.

Small intestine tissues were fixed in 10% buffered formalin and embedded in paraffin blocks. Four-micron sections from each paraffin block were cut with a microtome, stained with hematoxylin and eosin, and histopathologically evaluated by light microscopy. Mucosal thickness, and villus length and width were measured, and villus length/crypt rates were calculated for the small intestine of each rat.

Statistical analysis

SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables were presented as mean±SD, and statistical analysis of variables was performed with one-way analysis of variance (ANOVA) and Tukey's post hoc test. Qualitative variables were noted in percentages, and the correlation between categorical variables was investigated using chi-square analysis (χ2); $p<0.05$ was considered significant.

Results

Histopathological comparison of intestinal findings and severity of diarrhea among the irradiated groups is shown in Table 1. Significant differences were found among the groups regarding mucosal thickness, villus length and width, and presence of diarrhea on day 3 following RT ($p<0.001$, $p=0.002$, $p=0.004$, $p=0.024$, respectively). Mucosal thickness was significantly decreased in the 300 MU/min group, compared to the other groups and was significantly decreased in the 600 MU/min group, compared to the control group (Figure 1). Villus length was significantly decreased in the 300 MU/min group, compared to the other groups, while there was no significant difference between the 600 MU/min group and the control group. Villus width was significantly increased in the control group, compared to the other groups, while there was no significant difference between the 300 MU/min group and the 600 MU/min group. No significant difference was found among the groups in terms of villus length/crypt rate ($p>0.05$).

On day 3 following RT, diarrhea developed in half of the 300 MU/min and 600 MU/min groups, while no diarrhea was observed in the control group. Though no significant difference regarding presence of diarrhea on day 7 was found among the groups, the p value bordered statistical significance ($p=0.082$). Diarrhea

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Table 1 Histopathological comparison of intestinal findings and severity of diarrhea among experiment groups

	300 MU/min, n: 10	600 MU/min, n: 10	Control, n:10	p value
Mucosal thickness (µm)[a]	19.7±3.2	24.4±4.4	28.6±3.3	<0.001
Villus length (µm)[b]	12.2±2.5	14.5±1.8	15.8±1.8	0.002
Villus width (µm)[c]	4.0±0.4	4.3±0.7	5.2±1.0	0.004
Villus length/crypt rate	3.2±1.1	3.0±0.7	2.9±0.6	0.791
Development of diarrhea on day 3 (%)	5 (50.0)	5 (50.0)	–	0.024
Presence of diarrhea on day 7 (%)	4 (40.0)	2 (20.0)	–	0.082

[a]Mucosal thickness was significantly lower in the 300 MU/min group, compared to the other groups, and was significantly lower in the 600 MU/min group, compared to the control group; [b]Villus length was significantly lower in the 300 MU/min group, compared to other groups, while no significant difference was found between the 600 MU/min group and the control group; [c]Villus width was significantly higher in the control group, compared to the other groups, while no significant difference was found between the 300 MU/min group and the 600 MU/min group.

continued to be observed in 40% of the 300 MU/min group and in 20% of the 600 MU/min group.

Discussion

In mammals exposed to radiation in dose ranges of 10-100 Gy, villi does not regenerate, due to mitotic activity loss in the crypt cells of the small intestine. Villi in the intestinal epithelium gradually disappear, and the inner surface of the intestine is eventually flattened. Time to intestinal damage varies, depending on species. In mice, for example, changes occur after a period of 3 to 4 days, whereas in humans they usually become evident in 10 post-irradiation days.[14,15] Therefore, in order to evaluate effects of various RT dose rates on small intestinal histopathology, rats were sacrificed and tissue samples were obtained on the seventh post-RT day. Results determined that early RT-induced intestinal toxicity was more prominent in animals irradiated with low dose rate (300 MU/min), compared to those irradiated with high dose rate (600 MU/min). Mucosal thickness, and villus length and width of the small intestine were decreased in animals irradiated with low dose rate, compared to those irradiated with high dose rate.

Gastrointestinal symptoms may begin within hours of radiation exposure but usually develop during the first or second week of RT. Acute injury to the small intestine following irridation is a frequent, dose-dependent event. Low doses produce mild, reversible disease, while higher doses may produce more serious or irreversible injury.[2] Diarrhea, abdominal cramping, nausea, vomiting, and lack of appetite are common early symptoms of radiation enteritis and may result from stimulation of the central nervous centers by substances such as free radicals or endotoxins produced by intestinal bacteria.[2,10] Early radiation-induced vomiting and diarrhea may also be related to increased motility of the small intestine.[2,16,17] Rats were monitored in the present study, and it was observed that diarrhea developed in half the animals in each irradiated group on day 3 following RT. Although no statistically significant difference between the 2 groups was determined, diarrhea was observed with higher frequency in the 300 MU/min group on day 7 following RT.

Dose rate term refers to the duration of administration of a particular dose. It is a primary factor in determining degree of biological effect generated by a certain dose of X and gamma radiation. Decrease in dose rate with prolongation of irridation time also generally reduces the degree of resultant biological effect.
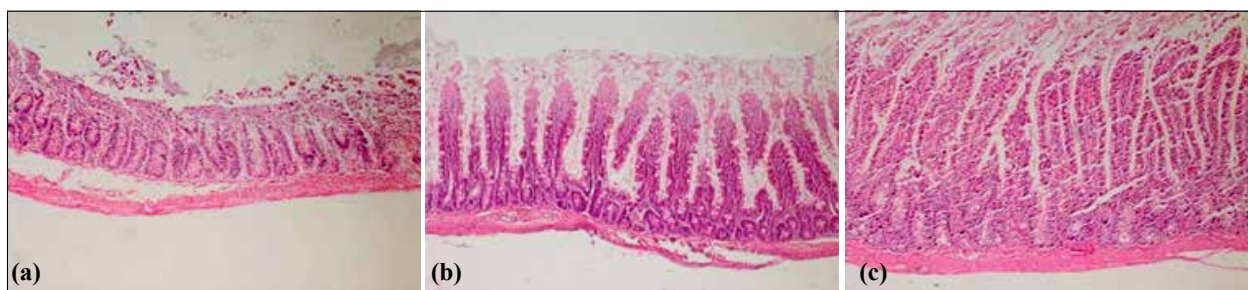

Fig. 1. Effect of radiotherapy dose rate on intestinal mucosal thickness. **(a)** 300 MU/min group, **(b)** 600 MU/min group, **(c)** control group [10 x hematoxylin and eosin].

Tumor and its microenvironment progress rapidly. Oxygenation of tumor is an important factor in treatment. Hypoxia of tumor affects response to RT, chemotherapy, and even surgery, not only for the treatment of large, bulky tumors, but also for the treatment of very small primary tumors.[18] Hypoxia is an important mechanism of resistance to RT in various tumour types.[19] Anti-cancer agents such as carbogen and nicotinamide, and RT approaches have been investigated for radioresistance of hipoxic tumor cells.[18] Hyperbaric oxygenation and accelerated radiotherapy with carbogen and nicotinamide (ARCON) therapy are examples of these approaches. Increase in effect of RT via simultaneous hyperoxygenation has been targeted in carbogen, nicotinamide, or their combinations, as in ARCON therapy. However, increase in early side effects of RT (i.e. toxicity of normal cells) has been observed in a majority of clinical reporting of carbogen plus nicotinamide or ARCON treatments.[20-25] For example, it has been reported that ARCON therapy for head and neck cancer increased early skin and mucosal reactions.[20-23] It has also been reported that carbogen plus nicotinamide or ARCON therapy of gliomas increased early toxicity of the central nervous system. [24,25] In other words, increased oxygenation during RT increases side effects, as hyperoxygenation of non-malignant cells may increase impression by RT. It is known that hypoxia enhances the radioresistance of non-maling cells such as mesenchymal stromal cells. [26] In applications of intensity-modulated radiotherapy (IMRT), decrease in response to treatment may occur due to overall prolongation of treatment time. However, it has been thought that this condition is compensated by rapid oxygenation.[27] The present results are supported by the afformentioned results. In the present study, the group with longer duration of treatment (i.e. 300 MU/min) had more severe intestinal side effects of RT, possibly because longer RT duration increased oxygenation of intestinal tissue. The authors believe that this finding indicates the significance of tissue oxygenation during RT. Vascularization and therefore oxygenation are normal in normal tissues (non-cancerous cells). Therefore, incerased oxygenization with prolongation of RT duration (even with limited minutes) increases RT toxicity in normal tissues such as those of the small intestine.

Conclusion

Results of the present study suggest that application of RT with high dose rate may be less toxic for tissues with high risk for acute toxicity, such as those of the small intestine.

Disclosure Statement

The authors declare no conflicts of interest.

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