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Tolerability of preoperative hyperfractionated pelvic radiotherapy of the small intestine: An experimental study in rat

BİLGE GÜRSEL[1], ŞABAN ÇAKIR[1], MAHMUT FİKRET GÜRSEL[2], LEVENT YILDIZ[3], DENİZ MEYDAN[1], NİLGÜN ÖZBEK[1], SEVGİ CANBAZ[4]

[1]Ondokuz Mayıs University Medical School, Department of Radiation Oncology, [2]Gazi Hospital, Department of General Surgery, Ondokuz Mayıs University Medical School, Departments of [3]Pathology and [4]Public Health, Samsun-Turkey

ABSTRACT

To investigate the tolerability of preoperative hyperfractionated pelvic irradiation on ninety male Wistar rats were used, 30 for sham irradiation (control), 30 underwent conventional pelvic irradiation, and the last 30 underwent hyperfractionated irradiation. During irradiation and follow up period of five weeks, weekly body weights were recorded. At the end of the follow up period animals were sacrificed and their ileal segments were examined histopathologically. Both irradiation groups displayed significant weight loss at 3rd-5th weeks of irradiation and at 1st-3rd weeks of the follow up. Four weeks after the end of irradiation; the weight loss in hyperfractionated group was significantly higher, but conventional groups was similar to the control group. The morphological assessment did not display statistically significant difference between the two irradiation groups. Hyperfractionated schedule, which was 20% higher dose than conventional group was applied with a protracted weight loss period but the morphological structure was similar to conventional group. [Turk J Cancer 2008;38(2):57-61]

KEY WORDS:

Hyperfractionation, radiotherapy, intestine

INTRODUCTION

There are clinical studies with hyperfractionated radiotherapy for rectum tumors. Especially the interest is on locally advanced tumors' down staging with hyperfractionated pelvic irradiation (1). Hyperfractionation can achieve higher doses with the same late toxicity for tissues (2). Following irradiation of rectal cancers, the small bowel is at particular risk due to its high radiosensitivity (3). Small intestine is the most dose restricting organ at pelvis and has a radiobiological structure almost similar to tumors. Experimentally, early and late changes occurring in the intestine following irradiation with a single or a small number of radiation doses have been well characterized. However, there is a comparative paucity of information about the influence of various fractionation schedules on the development of small intestine injury. Furthermore, small bowel is at risk for consequential radiation injury i.e., chronic injury secondary to mucosal barrier disruption (4). In spite of the difference in pathogenesis there is clinical and experimental evidence suggesting an association between acute mucosal damage and chronic radiation injury of the intestine (5). Therefore, the acute reactions of multicompartmental structured small intestine following hyperfractionated pelvic irradiation are important. In

this study we designed to compare small intestine's clinical and histopathological tolerance of hyperfractionated pelvic irradiation in which the fractionations schedules are applied like human beings.

MATERIAL AND METHODS

Animals

Ninety adult, male, Wistar rats with a median weight±standard deviation of 203.93±17.85 gram, were subjected to a 12 hours light - 12 hours darkness cycle in a temperature controlled room. The rats were housed in individual cages and were given free access to standard rat chow and water.

Randomization

After permission of Official Experimentation Committee of our Center for the supervision of animal trials the animals were randomized to three groups. In the first group there were 30 rats which were sham irradiated (control). The second group consisted of 30 rats which had conventional irradiation of 2 Gy per fraction and once a day, five times in a week with a total dose of 44 Gy. The hyperfractionated irradiation group also had 30 rats which were applied irradiation 1.2 Gy per fraction, twice a day with 8 hours interval, five days a week with a total irradiation dose of 52.8 Gy.

Irradiation

For all simulations and irradiations the rats were anesthetized with a subcutaneous injection of ketamine hydrochloride (Ketalar® Parke-Davis) 0.05 mg/g. A second injection was applied to control and conventional groups after 8 hours of their daily therapies. After anesthesia the animals' all four extremities were fixed to a special frame with adhesive bands. All animals' abdominal regions were shaved. The irradiation portals were designed by using simulator (Nucletron, System 300) after administration of rectal enema. One anterior field of 4x5 cm (width x length) was used in SSD 80 technique. The pelvic field was designed as in human pelvic tumors. The animals were irradiated with Cobalt-60 photons (Theratronics, Theratron 780 C).

Clinical assessment

Weights of the rats were measured weekly during the therapy and after completion till sacrificing at the 5th week of follow up (Shimadzu Libror EB-32KS). The mean values of the groups were calculated and compared.

Histopathological assessment

Rats were sacrificed by induction of bilateral pneumothorax at 5th week after completion of irradiation for assessment of intestinal injury morphologically. A 2-3 cm segment of ileum located 10-15 cm proximal to the ileoceacal junction was removed and fixed in 10% formalin. Following dehydration and embedding in paraffin, histological transverse sections (circumferences) were cut at 5 μm and stained with hematoxylin and eosin. The sections were coded to prevent bias during histopathological evaluation. The histopathological slides of all the animals were evaluated by the same pathologist.

Using a Reithezt-Jung microscope villous length, crypt depth was measured at x100 magnification. Fournine villi and crypts that had been found to be exactly horizontal were measured for each rat and the mean values were calculated. The mitotic figures were counted.

Other parameters used in histopathological assessment were qualitative alterations such as hemorrhage, ischemia, necrosis, inflammation, congestion, edema and lymphoid follicle.

Statistical analysis

Data were analyzed with a statistical software package (SPSS 9.0 for Windows). Statistical significance was assumed at $p \leq 0.05$. Quantitative data; villous depths and crypt lengths were expressed as mean±standard deviation of the mean, mitotic figures were expressed as median (min-max). Qualitative histopathological changes of the groups were presented in percentages and compared with z test for two proportions.

RESULTS

Groups

The overall anesthetic mortality was 0.18% (Seven in 3887 procedure). There was no statistical significant difference between the groups concerning death ratios ($p=0.28$).

Weight loss

There was no statistically significant difference between conventional and hyperfractionated groups for weight loss. At the 3rd week both irradiation groups had a significant weight loss when compared to control group. The difference continued to be significant at the 4th and 5th weeks of irradiation and also at the first 3 weeks after terminating irradiation. At the 4th week of follow up, the rats that were applied to conventional irradiation had a similar mean weight compared to the control group but the hyperfractionated group had still significant weight loss (p=0.01). At 5th week after irradiation all groups displayed similar values for mean weight (Figure 1).

Quantitative alterations

There was no statistically significant difference between the two irradiation groups for villous length, crypt depth and mitotic figures. However, the difference between hyperfractionated group and the control group was statistically significant. There was statistically significant difference between conventional and control groups for only villous length and mitotic figures (Table 1).

Qualitative alterations

There was no statistically significant difference between the two irradiation groups for qualitative parameters. However, the differences between irradiation groups and the control group were statistically significant for qualitative parameters such as edema, ischemia, necrosis and inflammatory reactions (Table 2). Hemorrhage was seen only in hyperfractionated group and there was a statistically significant difference between the hyperfractionated group and the control group (p=0.05).

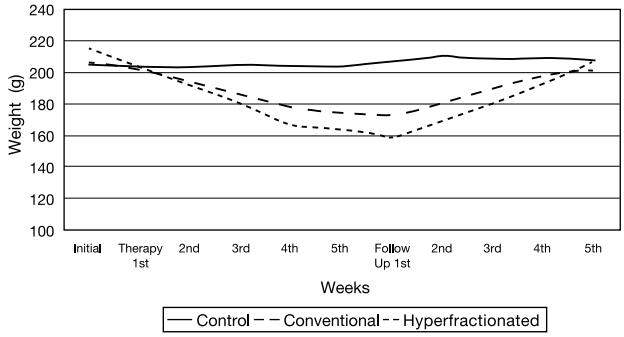

Fig 1. Weekly mean weight of the rat groups

DISCUSSION

The small intestine is unavoidably included in pelvic irradiation fields. The dose limiting normal tissue reactions in the intestine are therefore of clinical importance. Hyperfractionation has been advocated to improve local tumor control by increasing radiation dose (1,2,6). There

Table 1
Histopathological alterations*

		p values	
		Conventional	Hyperfractionated
	Length±SD		
Villous Lengths (μ)			
Control	426.20±93.56	0.02	0.001
Conventional	300.76±134.43		>0.05
Hyperfractionated	292.62±147.20		
	Depth±SD		
Crypt Depths (μ)			
Control	308.95±67.92	0.09	0.001
Conventional	230.80±104.71		>0.05
Hyperfractionated	212.92±104.12		
	Count (Min-Max)		
Mitotic Figure			
Control	1 (0 - 2)	0.01	0.01
Conventional	2 (0 - 3)		>0.05
Hyperfractionated	1 (0 -3)		

*Measurements and counts

are clinical studies of hyperfractionation on rectum cancer and most of them advocate the benefits of hyperfractionation (4,6,7).

There are also experimental studies that investigate effects of fractionation on the intestine. Brennan et al. (8) compared the responses of small intestinal morphological parameters after acute and protracted doses of radiation. Mice were examined 6, 24, and 72 hours after whole body gamma irradiation, given either as an acute 5 Gy dose, or as a protracted (continuous) dose of 20 cGy per day for 25 days to a total dose of 5 Gy. At 72 hours histological assessment revealed ultra structural changes more often in the single 5 Gy fractionation schedule.

Saclarides (9) reported radiation injuries of the gastrointestinal tract. During the acute phase of injury, characteristic changes are usually confined to the mucosa and include crypt cell damage, inflammatory cell infiltrate, mucosal slough, and loss of crypts. This study reported that these microscopic findings might still be present three months after therapy.

Hauer-Jensen and Langberg (3,5, 10-14) had a series of studies on the effects of time-dose-fractionation on rat small intestine. These studies showed that reduced overall irradiation time and increased fraction size greatly increased the frequency of intestinal complications, as well as histopathologic radiation injury. Epithelial damage was markedly increased in groups with shorter overall irradiation time. As evident from comparison of the regimens with 5.6 Gy fractions with the regimens with 2.8 Gy twice daily fractions, it was demonstrated that hyperfractionation reduced damage in all structures of the intestine (13). Langberg et al. (14) noted that increasing the interfraction interval from 0 to 6 hours was associated with a statistically significant reduction in intestinal complications (from 53% to 0%, $p < 0.001$).

Most of the experimental studies designed to show time-dose-fractionation effects on small intestine were with greater fractionation sizes and shorter overall treatment duration than normally used for treatment in men. So we planned to study the effects of exact pure hyperfractionated schedule over small intestine both clinically and morphologically.

In the weight evaluation the hyperfractionated group had a significantly longer weight loss period. Histopathologically, villous length and mitotic figure counts were statistically different compared to the control's but there were no differences in means between the two irradiation schedules for quantitative parameters. When compared with respect to the qualitative parameters, again there were no statistical differences between irradiation groups.

So as a conclusion, hyperfractionated pelvic irradiation was tolerated but weight loss lasted longer. There was no difference in terms of histopathological evaluation. This might be explained by the histopathological evaluations being made five weeks after the last day of irradiation. According to our results hyperfractionated irradiation was applied with tolerable significantly higher clinical toxicity but the difference was not significant histopathologically at the 5th week of follow up. It would be useful to study the subacute and chronic histopathological alterations and the tumor responses in the same irradiation schedules.

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