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FROM THE UNIVERSITY OF IOWA, RADIATION RESEARCH LABORATORY, IOWA CITY, IOWA, USA.

INTESTINAL COMPLICATIONS FOLLOWING ACCELERATED FRACTIONATED X-IRRADIATION

An experimental study in the rat

M. HAUER-JENSEN, L. POULAKOS and J. W. OSBORNE

Abstract

Due to paucity of suitable animal models, it has been difficult to study the development of long-term intestinal complications following fractionated irradiation. We recently developed a model which allows multiple radiation exposures of a short segment of rat ileum without the need for repeated surgery. In the present series, this model was used to study the influence of shortening the total treatment time (accelerated fractionation) on development of radiation enteropathy. Male rats were orchiectomized and a short segment of distal ileum was transposed to the scrotum. Starting 3 weeks after surgery, the scrotum containing the intestinal segment was x-irradiated with 20 fractions of 2.8 Gy (total dose 56 Gy). Two fractionation schedules were compared: One fraction per day (total treatment time 26 days) and 3 fractions per day (total treatment time 7 days). Actuarial survival curves were obtained, and the degree of radiation injury was assessed 2, 8, and 26 weeks after the last radiation exposure using a semiquantitative histopathologic scoring system. There was no mortality from acute radiation injury in either treatment group. All animals of the 1-fraction/day group survived the observation period (26 weeks). In the 3-fraction/day group, there was significant mortality due to intestinal obstruction, and cumulative mortality at 26 weeks was 100%. Radiation injury, as assessed by the histopathologic scoring system, was also more pronounced in this group than in the 1-fraction/day group. We conclude that shortening the total treatment time significantly increases the severity of late intestinal complications. Our data are suggestive of an association between acute mucosal damage and chronic radiation injury of the small intestine.

Key words: Radiation injury, experimental, small intestine, fraction.

Normal tissue tolerance is the main dose-limiting factor of radiation therapy. In the search for methods to improve tumor control while maintaining an acceptable level of normal tissue damage, various modifications of the standard fractionation regimens are currently being investigated (1, 2). In accelerated regimens, the overall treatment time is shortened by giving 2 or 3 fractions per day. This allows less time for proliferation between fractions and may thus be beneficial for patients with tumors in which the clonogenic cells have a short doubling time (3–6). Although acute reactions in normal tissues with rapidly proliferating target cells may also be aggravated, it is assumed that the degree of late radiation injury is not affected. However, whether accelerated fractionation actually improves the long-term therapeutic ratio, i.e. tumor control relative to the incidence of late complications, is still a controversial issue (4, 7, 8).

Acute radiation injury of the small intestine is primarily due to killing of rapidly proliferating crypt cells, whereas late radiation enteropathy is believed to be secondary to vascular damage and/or killing of slowly proliferating cells (9–11). 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 (12, 13). Thus, when the small bowel is the organ at risk, it is conceivable that shortening the treatment time may not only aggravate the acute reactions, but may also result in more severe late complications. The present investigation addresses this issue.

Material and Methods

Surgical procedure and irradiation. A detailed description of the surgical and irradiation procedures has recently been published (14).

In brief, male Sprague-Dawley rats were anesthetized

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with ketamine-acetylpromazine and orchiectomized. A 3–4 cm segment of ileum, located 10–15 cm proximal to the ileocecal junction, was transposed to the left side of the scrotum and fixed with a short, running polypropylene suture. Thus, a 'scrotal hernia' containing a loop of small intestine was prepared. Three weeks were allowed for postoperative recovery before irradiation was commenced.

Prior to irradiation, the rats were anesthetized with methoxyflurane and strapped to a lucite board in the supine position. The side of the scrotum containing the segment of small bowel was irradiated with orthovoltage x-rays (250 kVp, FSD 46 cm, 30 mA, filtration 0.5 mm Cu +1.0 mm Al, HVL 1.5 mm Cu, dose rate 1.51 Gy/min). A cylindrical collimator with an internal diameter of 25 mm was used to limit the radiation beam.

In the present series, 50 rats were surgically prepared as described above. In 38 animals, a total dose of 56 Gy was delivered as 20 fractions of 2.8 Gy. Two different treatment schedules were used: standard fractionation with 1 fraction per day (1 F/d, interval 24 h) and accelerated fractionation with 3 fractions per day (3 F/d, interval 4 h). The total treatment times were 26 days for the 1 F/d group and 7 days for the 3 F/d group. Two groups of 6 rats were anesthetized according to the same schedules and sham-irradiated to serve as controls.

Assessment of radiation injury. For each animal, the time between the last radiation exposure to sacrifice or death was recorded. Cumulative survival was calculated within each group, and actuarial survival curves were constructed and compared.

Groups of animals were anesthetized and killed by induction of bilateral pneumothorax at 2, 8, and 26 weeks after the last irradiation. Specimens of irradiated, sham-irradiated, and unirradiated intestine were removed, and histological sections were prepared, stained with hematoxylin and eosin, and independently evaluated by two of the investigators.

The degree of radiation injury was assessed using a semiquantitative histopathologic scoring system (13): Seven different parameters of radiation injury were graded according to severity (Table 1). For each animal, a radiation injury score (RIS) was calculated by adding the scores for the individual parameters (maximum score 18). A detailed description and evaluation of this scoring system has been published elsewhere (15). In addition to RIS, cumulative prevalence (CP) was used as a measure of the totality of injury within a group of animals following a particular radiation dose. This parameter is expressed (in percentage) as the observed number of pathologic alterations relative to the maximum number of pathologic alterations at a particular radiation dose level (Poulakos et al., submitted).

Statistical methods. The data were analyzed using non-parametric statistical tests. Survival functions were estimated by the Kaplan-Meier product limit method. The difference between the resulting survival curves was assessed by the log-rank test. The Mann-Whitney U-test was used to compare RIS-values among treatment groups. Comparisons of cumulative prevalence values were performed with the Fisher exact test. p-vales of less than 0.05 were considered statistically significant.

Table 1

Histophatologic radiation injury parameters used to calculate the radiation injury score

Mucosal ulcerations
1 = Small, superficial ulcerations
2 = Ulcerations involving more than half of the intestinal circumference
Epithelial atypia
1 = Abnormally oriented crypts
2 = Irregular crypt regeneration with atypical epithelial cells
3 = Adenocarcinoma
Thickening of subserosa
1 = Slight thickening. Hyperplasia of peritoneal mesothelium
2 = Marked thickening
3 = Extreme thickening and fibrosis
Vascular sclerosis
1 = Slight thickening and hyalinization of vessel wall
2 = Vessel wall double normal thickness. Hyalinization and stenosis
3 = Extreme sclerosis with marked stenosis or complete occlusion. Fibrinoid necrosis
Intestinal wall fibrosis
1 = Submucosa double normal thickness. Broadened and hyalinized collagen fibres
2 = Submucosa 3–4 times normal thickness. Abnormal collagen fibres
3 = Massive fibrosis including muscularis
Ileitis cystica profunda
1 = Glandular inclusions in submucosa
2 = Submucosal cysts with polypoid elevation of mucosa
3 = Large cysts extending into muscularis
Lymph congestion
1 = Dilated lymph vessels or cystic collections of lymph

Results

The overall anesthetic mortality was approximately 0.1% (1 of 990 anesthesias). Actuarial survival curves for the 1 F/d group and the 3 F/d group are shown in Fig. 1. There was no mortality from acute radiation injury in either group of animals. However, the two treatment groups differed significantly with regard to long-term survival ($\chi^2=14.0$, $p<0.0002$). All animals of the 1 F/d group and all sham-irradiated control animals survived the entire observation period of 26 weeks. In contrast, in the 3 F/d group, there was a gradual decline in the number of surviving animals, resulting in a cumulative mortality of 100% at 26 weeks. Two animals died from malnutrition secondary to enterocutaneous fistulae; the remainder died from intestinal obstruction caused by fibrotic stricture formation.

The occurrence of radiation-induced changes in the 1

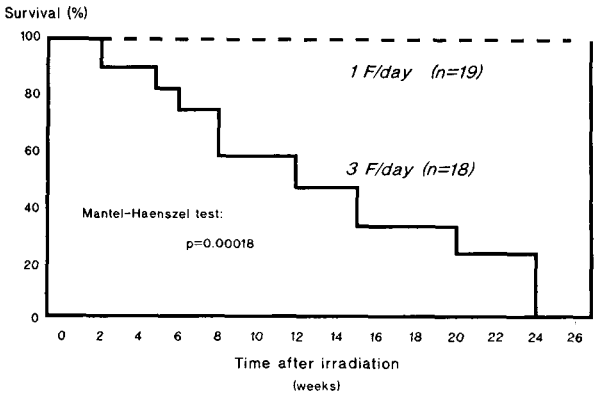

Fig. 1. Actuarial survival curves for animals irradiated with 20 fractions of 2.8 Gy. 1 F/day: 1 fraction per day, 3 F/day: 3 fractions per day.

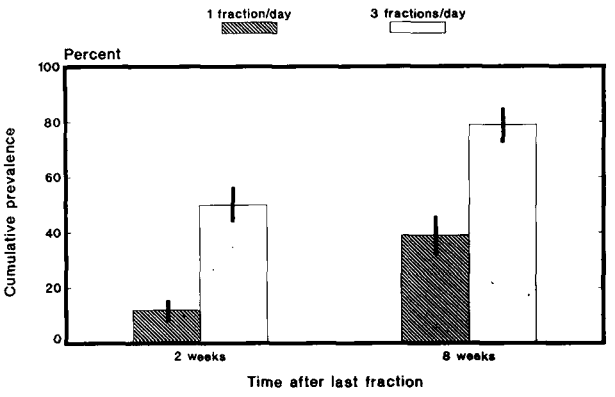

Fig. 2. Cumulative prevalence of radiation injury parameters 2 and 8 weeks after the last dose fraction. (Error bars: ±1 SD.)

F/d group and 3 F/d group is shown in Table 2, and median radiation injury scores are shown in Table 3. There was a statistically significant difference in RIS between the 1 F/d and 3 F/d groups. The median RIS of specimens from 1 F/d animals was not significantly different from that of sham-irradiated controls at either observation time. However, the 3 F/d animals had higher values than controls and 1 F/d animals at both 2 and 8 weeks. The most prominent features of radiation injury in the 3 F/d group were subserosal thickening, mucosal ulcerations, intestinal wall fibrosis, and vascular sclerosis. At 26 weeks, all animals belonging to the 3 F/d group had died from radiation-induced intestinal complications, thus precluding calculation of the RIS at this time.

Cumulative prevalence (CP) of the radiation injury parameters are shown in Fig. 2. At 2 weeks, CP was 12% for the 1 F/d group and 50% for the 3 F/d group ($p<0.001$). At 8 weeks the CP values were 39% and 79% respectively ($p<0.01$).

Discussion

Experiments involving a single radiation exposure or a relatively small number of fractions may be performed by temporarily exteriorizing a segment of intestine (13, 16–18). In contrast, the model used in the present study allows multiple radiation exposures of a localized segment of rat small bowel without a need for repeated surgery. The surgical procedure does not alter intestinal morphology and does not interfere with intestinal transit (14). Only a short row of sutures fixes the intestine to the inside of the scrotum, and the bowel thus remains mobile and within the peritoneal cavity.

It is generally assumed that, in most organs, there is no causal relationship between acute and late radiation effects. This is due to the difference in pathogenesis of the two types of injury: killing of rapidly proliferating target cells causing acute injury, as opposed to depletion of slowly proliferating target cells and/or vascular damage being responsible for late effects (10, 11, 19). In the small intestine, killing of the rapidly proliferating crypt cells results in denudation of the villi during the acute phase following irradiation. Late radiation enteropathy is caused by changes in various other compartments, the main histopathologic features being chronic ulcerations, intestinal wall fibrosis, vascular sclerosis, and lymph congestion (9, 13).

In the small intestine, there is evidence from clinical and experimental studies suggesting a relationship between acute and late changes which can not be explained solely from dose dependence (12, 13, 20). It is possible that extensive mucosal damage predisposes to development of chronic ulcerations which may ultimately cause stenosis of the intestinal lumen due to reactive fibrosis (20, 21). Thus, in some cases, intestinal obstruction and fistula formation may represent sequelae to severe acute damage, rather than radiation injury of late reacting tissue compartments. This theory is in agreement with a recent report by Peck & Gibbs (22) suggesting two separate pathogenetic mechanisms of intestinal wall fibrosis: one depending on mucosal damage (alpha/beta ratio 13–14), the other occurring in cases with preservation of epithelial integrity (alpha/beta ratio 2–3). In a study of chronic rectal radiation injury in the rat, van der Kogel et al. (23) observed an early type consisting of deep ulcers and fistula formation and a late type characterized by vascular injury, fibrosis, and mucosal cysts. Furthermore, increasing total treatment time resulted in a significant rise in isoeffective doses, suggesting a relationship between delayed injury and acute mucosal changes. Both reports are in agreement with our concept of 'early appearing' and 'late appearing' alterations of chronic radiation enteropathy introduced previously (13). In the present study we tested the hypothesis that severe acute mucosal damage increases the incidence of late intestinal complications by comparing two fractionation schedules resulting in a dif-

ferent degree of depletion of rapidly proliferating cells but not of slowly proliferating cells.

In contrast to earlier models for calculation of radiation isoeffectiveness, the currently popular alpha-beta isoeffect curve model does not include a time factor (24–26). According to this model, cell killing following fractionated irradiation does not depend on the total treatment time, provided that 1) there is sufficient time between fractions for 'complete' repair of repairable sublethal and potentially lethal damage, and 2) no cell proliferation occurs during the course of treatment (26).

In the present study, total treatment time significantly influenced the development of long-term complications, indicating incomplete repair of repairable damage between fractions in the 3 F/d group and/or significant proliferation of cells involved in the pathogenesis of radiation enteropathy during the course of treatment.

The time required for 'complete' repair in a given situation depends on the dose per fraction, the alpha/beta ratio of the target cells, and the sensitivity of the endpoints used (27). In a study of intestinal radiation death in mice following fractionated abdominal irradiation Wambersie et al. (28) found no significant difference in repair for intervals of 3.5 h and 24 h between fractions. In an investigation by Withers et al. (29), recovery in mouse jejunum did not differ between groups exposed with 3 h and 6 h fractionation intervals. However, the halftime for repair may be longer in late reacting tissues than in acutely reacting tissues (30, 31). Also, in spite of similar halftimes, complete repair may take longer in late reacting tissues than in acutely reacting tissues, due to a greater relative proportion of repairable damage (32). In a study of late radiation enteropathy following split-dose irradiation of exteriorized rat small intestine, however, only slight sparing was found when the interval between fractions was increased beyond 4 h (33). It is thus not likely that a relative difference in repair was the main cause of the difference in the incidence of late complications in the present study: with a dose per fraction of 2.8 Gy, an interval of 4 h is probably adequate, considering the sensitivity of the endpoints used (lethality and RIS). This is in agreement with the 'fading time' concept as discussed by Fowler (27).

Intestinal crypt cells have a high turnover-rate, and the second requirement for validity of the alpha/beta model, i.e. no cell proliferation during treatment, is clearly not met. Shortening of the mitotic cycle of crypt cells, mainly by a decrease in the duration of the G_1 phase, and an increase in the absolute size of the stem cell compartment contribute to increased cell production during fractionated irradiation (29, 34). Wambersie et al. (35) demonstrated that increasing total treatment time results in an increase in LD_{50} of about 0.75 Gy per day, independent of the number of fractions. We have previously compared acute intestinal radiation injury following regimens of 20 fractions of 2.8 Gy each, given as 1, 2, or 3 fractions per day.

Table 2

Distribution of rats according to severity (grade) of the individual radiation injury parameter

	2 weeks after irradiation							
	1 fraction/day				3 fractions/day			
	Grade				Grade			
	0	1	2	3	0	1	2	3
Mucosal ulceration	6	1	0	–	2	2	2	–
Epithelial atypia	6	1	0	0	3	2	1	0
Serosal thickening	5	2	0	0	2	1	2	1
Vascular sclerosis	6	0	1	0	1	1	4	0
Fibrosis	6	1	0	0	3	3	0	0
Ileitis cystica	7	0	0	0	4	2	0	0
Lymph congestion	7	0	–	–	5	1	–	–
	8 weeks after irradiation							
	1 fraction/day				3 fractions/day			
	Grade				Grade			
	0	1	2	3	0	1	2	3
Mucosal ulceration	5	0	1	–	1	1	2	–
Epithelial atypia	3	3	0	0	2	1	1	0
Serosal thickening	2	4	0	0	0	0	1	3
Vascular sclerosis	4	2	0	0	0	1	1	2
Fibrosis	2	4	0	0	0	0	2	2
Ileitis cystica	6	0	0	0	1	2	1	0
Lymph congestion	5	1	–	–	2	2	–	–
	26 weeks after irradiation 1 fraction/day							
	Grade							
	0	1	2	3				
Mucosal ulceration	6	0	0	–				
Epithelial atypia	4	2	0	0				
Serosal thickening	2	3	1	0				
Vascular sclerosis	6	0	0	0				
Fibrosis	2	4	0	0				
Ileitis cystica	6	0	0	0				
Lymph congestion	4	2	–	–				

Table 3

Radiation injury score of the experimental groups (median and range)

Group	Time after last exposure		
	2 weeks	8 weeks	26 weeks
1-fraction/day — CTR	0 (0–2)	–	2 (0–3)
1-fraction/day — XR	0 (0–4)	2 (0–6)	2.5 (1–3)
3-fraction/day — CTR	0 (0–1)	–	0.5 (0–1)
3-fraction/day — XR	5.5 (2–9)	12 (6–14)	–

CTR:Controls XR:x-irradiated

There was a pronounced difference in the degree of acute damage between the 3 treatment groups as assessed by histopathologic changes (14) and crypt cytokinetic parameters (Poulakos et al., submitted). Compensatory proliferation was sufficient to maintain epithelial integrity in the groups receiving 1 and 2 fractions per day, but not in the 3-fraction per day group.

In contrast to target cells responsible for acute effects, cells involved in the development of late effects have slow proliferation rates (11). Thus, proliferation of such cells during the course of irradiation is negligible, and the degree of late radiation injury should be relatively independent of total treatment time. This is the biological rationale for assuming that regimens of accelerated fractionation increase the long-term therapeutic ratio when applied to tumors with rapidly proliferating cologenic cells (3, 5).

The present study, however, shows that shortening the treatment time significantly increases late intestinal complications, suggesting that damage to cells having a relatively rapid proliferative rate may influence the development of radiation enteropathy. The two cell types most likely involved are vascular endothelial cells and crypt stem cells.

Vascular damage has been suggested as an important factor in the pathogenesis of late radiation injury in many organs, including the small intestine (10). Although the turnover of vascular endothelium is generally considered to be relatively slow, experimental evidence suggests that a subpopulation of endothelial cells may have a rapid proliferative rate (36). However, rather than differential killing of endothelial cells, it is more likely that the difference between the two treatment groups is caused by a difference in the degree of mucosal damge secondary to depletion of rapidly proliferating crypt cells: The time between fractions in the 3 F/d group was not sufficient for cellular proliferation to maintain epithelial integrity, predisposing to late complications from chronic ulcerations with reactive fibrosis. These findings are in agreement with data from our previous study, in which the 1 F/d group exhibited a virtually normal histopathologic picture and normal crypt cytokinetics 6 h after the last radiation exposure (14). However, our results are somewhat different from those obtained by Dewit & Oussoren (37) following partial abdominal irradiation of mice with 14 fractions of 3 Gy. In their study, in spite of very little acute damage, significant late changes occurred at 6 months. It is possible that this may be explained by the larger volume of irradiated intestine and/or to an indirect effect of damage to other intraabdominal organs/tissues.

We conclude that shortening of the total treatment time significantly increases the incidence of intestinal complications. This indicates that cells having a high proliferative rate may be indirectly involved in the pathogenesis of radiation enteropathy. Our data thus suggest the presence of an association between early and late radiation injury of the small bowel. Consequently, accelerated fractionation schedules for intraabdominal tumours should be used with caution to avoid serious late intestinal complications.

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Request for reprints: Dr Martin Hauer-Jensen, the Norwegian Radium Hospital, N-0310 Oslo 3, Norway.

REFERENCES

1. Fowler JF. Fractionated radiation therapy after Strandqvist. Acta Radiol Oncol 1984; 23: 209–16.
2. Withers HR. Biologic basis for altered fractionation schemes. Cancer 1985; 55: 2086–95.
3. Arcangeli G, Mauro F, Morelli D, Nervi C. Multiple daily fractionation in radiotherapy: Biological rationale and preliminary clinical experience. Eur J Cancer 1979; 15: 1077–83.
4. Saunders MI, Dische S, Fowler JF, et al. Radiotherapy employing three fractions on each of twelve consecutive days. Acta Oncol 1988; 27: 163–7.
5. Thames HD, Peters LJ, Withers HR, Fletcher GH. Accelerated fractionation vs. hyperfractionation: Rationales for several treatments per day. Int J Radiat Oncol Biol Phys 1983; 9: 127–38.
6. Wang CC, Blitzer PH, Suit H. Twice-a-day radiation therapy for cancer of the head and neck. Cancer 1985; 55: 2100–4.
7. Peters LJ, Ang KK, Thames Jr HD. Accelerated fractionation in the radiation treatment of head and neck cancer. A critical comparison of different strategies. Acta Oncol 1988; 27: 185–94.
8. Turesson I, Notter G. Accelerated versus conventional fractionation. The degree of incomplete repair in human skin with a four-hour-fraction interval studied after post mastectomy irradiation. Acta Oncol 1988; 27: 169–79.
9. Berthrong M, Fajardo LF. Radiation injury in surgical pathology. Part II. Alimentary tract. Am J Surg Pathol 1981; 5: 153–78.
10. Law MP. Radiation-induced vascular injury and its relation to late effects in normal tissue. Adv Radiat Biol 1981; 9: 37–73.
11. Withers HR, Peters LJ, Kogelnik HD. The pathobiology of late effects of irradiation. In: Meyn RE, Withers HR, eds. Radiation biology in cancer research, New York: Raven Press 1980: 439–48.
12. Bourne RB, Kearsley JH, Grove WD, Roberts SJ. The relationship between early and late gastrointestinal complication of radiation therapy for carcinoma of the cervix. Int J Radiat Oncol Biol Phys 1983; 9: 1445–50.
13. Hauer-Jensen M, Sauer T, Devik F, Nygaard K. Late changes following single dose roentgen irradiation of rat small intestine. Acta Radial Oncol 1983; 22: 299–303.
14. Hauer-Jensen M, Poulakos L, Osborne JW. Effects of accelerated fractionation on radiation injury of the small intestine: A new rat model. Int J Radiat Oncol Biol Phys 1988; 14: 1205–12.
15. Hauer-Jensen M. Late radiation injury of the small intestine (Dissertation). Oslo: University of Oslo, 1988.
16. Geraci JP, Jackson KL, Christensen GM, Thrower PD, Weyer BJ. Acute and late damage in the mouse small intestine following multiple fractionation of neutrons or X-rays. Int J Radiat Oncol Biol Phys 1977; 2: 693–9.

17. Hauer-Jensen M, Sauer T, Devik F, Nygaard K. Effects of dose fractionation on late roentgen radiation damage of rat small intestine. Acta Radiol Oncol 1983; 22: 381–4.
18. Osborne JW, Prasad KN, Zimmerman GR. Changes in the rat intestine after X-irradiation of exteriorized short segments of ileum. Radiat Res 1970; 43: 131–42.
19. Withers HR, Thames HD, Flow PL, Mason KA, Hussey DH. The relationship of acute to late skin injury in 2 and 5 fraction/week gamma-ray therapy. Int J Radiat Oncol Biol Phys 1978; 4: 595–601.
20. Hauer-Jensen M, Sauer T, Berstad T, Nygaard K. Influence of pancreatic secretion on late radiation enteropathy in the rat. Acta Radiol Oncol 1985; 24: 555–60.
21. Trott KR. Chronic damage after radiation therapy: Challenge to radiation biology. Int J Radiat Oncol Biol Phys 1984; 10: 907–13.
22. Peck JW, Gibbs FA. Therapeutic implications of thermal radiosensitization and alpha-beta analysis of intestinal fibrosis. Abstracts of papers for the 36th annual meeting of the Radiation Research Society 1988; 155.
23. van der Kogel AJ, Jarrett KA, Paciotti MA, Raju MR. Radiation tolerance of the rat rectum to fractionated X-rays and pi-mesons. Radiother Oncol 1988; 12: 225–32.
24. Barendsen GW. Dose fractionation, dose rate, and iso-effect relationships for normal tissue response. Int J Radiat Oncol Biol Phys 1981; 8: 1981–97.
25. Douglas BG, Fowler JF. Fractionation schedules and a quadratic dose—effect relationship. Br J Radiol 1975; 48: 502–4.
26. Thames HD, Withers HR, Peters LJ, Fletcher GH. Changes in early and late radiation responses with altered dose fractionation: Implications for dose—survival relationships. Int J Radiat Oncol Biol Phys 1982; 8: 219–26.
27. Fowler JF. 'Fading times' required for apparently complete repair in irradiated tissues assuming the linear quadratic model of dose response. Int J Radiat Biol 1986; 50: 601–7.
28. Wambersie A, Dutreix J, Gueulette J, Lellouch J. Early recovery for intestinal stem cells, as a function of dose per fraction, evaluated by survival rate after fractionated irradiation of the abdomen of mice. Radiat Res 1974; 58: 498–515.
29. Withers HR, Chu AM, Reid BO, Hussey DH. Response of mouse jejunum to multifraction radiation. Int J Radiat Oncol Biol Phys 1975; 1: 41–52.
30. Elkind MM. DNA repair and cell repair: Are they related? Int J Radiat Oncol Biol Phys 1979; 5: 1089–94.
31. Thames HD. Effect-independent measures of tissue responses to fractionated irradiation. Int J Radiat Biol 1984; 45: 1–10.
32. Fowler JF. Intervals between multiple fractions per day. Differences between early and late radiation reactions. Acta Oncol 1988; 27: 181–3.
33. Hauer-Jensen M, Sauer T, Reitan JB, Nygaard K. Late radiation enteropathy following split-dose irradiation of rat small intestine. Acta Radiol Oncol 1986; 25: 203–06.
34. Maisin J, Wambersie A, Lambiet-Collier M, Gueulette J. Intestinal proliferation after multiple fractions of gamma irradiation. Radiat Res 1974; 71: 338–54.
35. Wambersie A, Dutreix J, Maisin J, Gueulette J. Reponse de la muqueuse digestive à une irradiation unique et fractionnee. Bull Cancer (Paris) 1976; 63: 175–90.
36. Hirst DG, Denekamp J, Travis EL. The response of mesenteric blood vessels to irradiation. Radiat Res 1979; 77: 259–75.
37. Dewit L, Oussoren Y. Late effects in the mouse small intestine after clinically relevant multifractionated radiation treatment. Radiat Res 1987; 110: 372–84.