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Single high-dose vs. fractionated radiotherapy: Effects on plant growth rates

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ABSTRACT

Aim: To evaluate the differential effects of fractionated vs. high-dose radiotherapy on plant growth.

Background: Interest in hypofractionated radiotherapy has increased substantially in recent years as tumours (especially of the lung, prostate, and liver) can be irradiated with ever greater accuracy due to technological improvements. The effects of low-dose ionizing radiation on plant growth have been studied extensively, yet few studies have investigated the effect of high-dose, hypofractionated radiotherapy on plant growth development.

Materials and methods: A total of 150 plants from the genus *Capsicum annuum* were randomized to receive fractionated radiotherapy (5 doses of 10 Gy each), single high-dose (SHD) radiotherapy (single 50 Gy dose), or no radiotherapy (control group). Irradiation was delivered via linear accelerator and all samples were followed daily for 26 days to assess and compare daily growth.

Results: On day 26, plants in the control, fractionated, and SHD groups had grown to a mean height of 7.55 cm, 4.32 cm, and 2.94 cm, respectively. These differences in overall growth were highly significant ($P = 0.005$). The SHD group showed the least amount of growth.

Conclusions: SHD effectively stunts plant growth and development. Despite the evident differences between plant and animal cells, ionizing radiation is believed to work in a similar manner in all biological cells. These findings highlight the need to continue investigating the use of hypofractionated schemes in humans to improve cancer treatment outcomes.

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1. Background

Interest in hypofractionated radiotherapy has increased substantially in recent years, particularly for certain tumour localizations (lung, prostate, liver, among others).[1] Highly sophisticated technological developments, such as intensity modulated radiotherapy (IMRT) and stereotactic body radiotherapy (SBRT) have given clinicians the ability to deliver increasingly precise doses that conform closely to the target while minimizing the dose to surrounding healthy tissue.[2,3]

The precision of these new technologies permits the use of higher doses delivered in fewer fractions, an approach known as hypofractionation, because doses are higher and fewer compared to normofractionated treatment regimens. These hypofractionated schemes offer the potential for better local control and improved outcomes, with less cost and a lower burden on the patient and a reduced demand on radiotherapy services. However, there is a trade-off: hypofractionation has a higher risk of adverse effects due to the potential for irreversible damage to healthy tissue.

Meristematic plant cells, like human cancer cells, undergo a rapid cell division.[4] Meristematic cells are the drivers of plant growth and when these cells are stressed—by bacterial or viral invasion, or by lack of water or nutrients, growth will be slowed or even halted. Human cancer cells and meristematic plant cells (particularly in immature plants that have not yet reached full size) both share a common characteristic: rapid growth. This shared trait makes plant cells an interesting material with which to study the effects of hypofractionated radiotherapy, as studying the effects of ionizing irradiation on the growth of plant cells could shed more light on the effects of hypofractionation on rapidly growing cells.

Much research has been carried out on the effects of low-dose ionizing radiation on plant growth.[5,6] In most cases, however, the focus of this research is on stimulating growth and increasing yields. In contrast, very little research has been done on the use of high dose radiation to slow or halt plant growth.

Aim: In this study, we used a plant model to evaluate the differential effects of fractionated and single high-dose (SHD) radiotherapy on plant growth.

2. Materials and methods

The study sample consisted of 150 plants from the genus *Capsicum annuum* (pepper plant) grown in a local greenhouse. All the plants were grown in exactly the same conditions: same container type, climate (temperature, light, humidity), and feeding (soil type, amount of water).

The 150 plants were randomly allocated to one of three groups (50 plants per group). Plants assigned to group 1 were considered controls and were not irradiated. Plants in group 2 received 50 Gy of radiation delivered in a fractionated schedule of 10 Gy per dose over 5 consecutive days. Plants allocated to group 3 received a single, high-dose fraction of 50 Gy. Irradiation was delivered via linear accelerator.

Fig. 1 – Image of young plants and metric ruler.

Dose simulation was performed with a 30 cm × 30 cm × 20 cm slab phantom using the ELEKTA PrecisePlan planning system using a 40 cm × 40 cm field to encompass both treatment groups. We used 6 MV photon beams and the dose was prescribed to the depth of maximum dose (1.5 cm).

All plants were followed daily for 26 days to assess day-by-day growth. Growth was measured daily (Figs. 1 and 2) using a metric ruler.

2.1. Statistical analysis

This was a descriptive, randomized study. Plant growth was measured in cm/day and a simple mean daily growth was calculated with a standard deviation. A level of $P \leq 0.05$ was used to assess significance.

3. Results

The tables show results obtained in each group each day. Results for the control group (group 1) are shown in Table 1 and Fig. 3, while results for fractionated radiotherapy (group 2) are shown in Table 2 and Fig. 4. Table 3 and Fig. 5 show the results for the SHD group (group 3). Finally, Fig. 6 shows a

Fig. 2 – Close-up of plant.

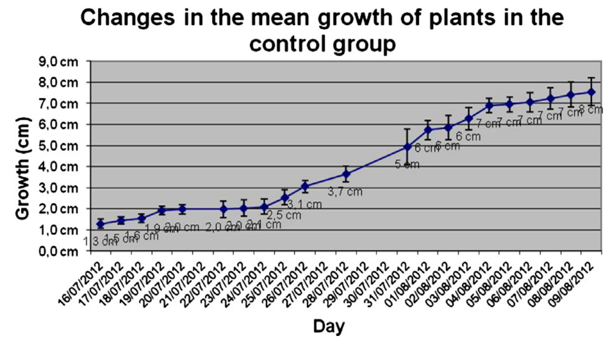

Fig. 3 – Daily changes in mean growth of control plants.

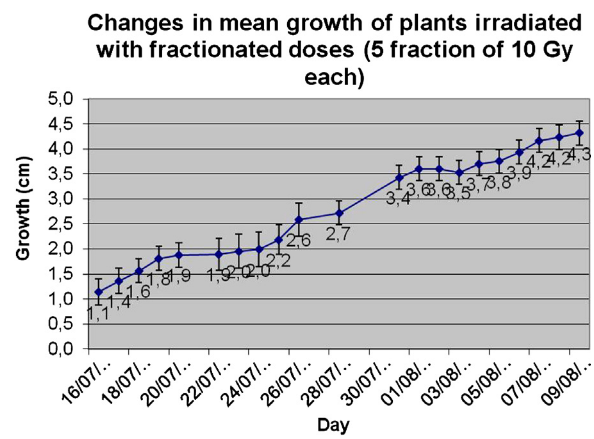

Fig. 4 – Daily changes in mean growth of plants irradiated on a fractionated schedule.

Table 1 – Daily growth of group 1 (control group).

Day	Group 1 (control group)				
	Mean height (cm)	Standard deviation (cm)	Number (*n*)	Number of plants with zero growth	No. of plants dead on day of measurement
16-07-12	1.29	0.23	47	3	0
17-07-12	1.45	0.18	47	3	0
18-07-12	1.56	0.20	47	3	0
19-07-12	1.92	0.21	47	3	0
20-07-12	1.98	0.22	47	3	0
22-07-12	1.98	0.40	47	3	0
23-07-12	2.03	0.39	47	3	0
24-07-12	2.10	0.36	47	3	0
25-07-12	2.54	0.35	46	3	1
26-07-12	3.06	0.29	46	4	0
28-07-12	3.65	0.37	46	4	0
31-07-12	4.94	0.85	46	4	0
01-08-12	5.73	0.47	46	4	0
02-08-12	5.85	0.57	46	4	0
03-08-12	6.27	0.51	46	4	0
04-08-12	6.89	0.34	46	4	0
05-08-12	6.95	0.36	46	4	0
06-08-12	7.05	0.45	46	4	0
07-08-12	7.24	0.51	46	4	0
08-08-12	7.42	0.59	46	4	0
09-08-12	7.55	0.67	46	4	0

Table 2 – Growth of plants in group 2 (fractionated radiotherapy).

Day	Mean height (cm)	Standard deviation (cm)	Number (*n*) of plants receiving fractionated dose	No. of plants showing zero growth	No. of plants dead on day of measurement
16-07-12	1.14	0.26	49	1	0
17-07-12	1.36	0.26	49	1	0
18-07-12	1.57	0.23	49	1	0
19-07-12	1.81	0.24	49	1	0
20-07-12	1.88	0.25	49	1	0
22-07-12	1.90	0.32	48	1	0
23-07-12	1.96	0.34	49	1	0
24-07-12	1.99	0.35	49	1	0
25-07-12	2.19	0.29	49	1	0
26-07-12	2.59	0.34	49	1	0
28-07-12	2.72	0.24	48	1	1
31-07-12	3.43	0.24	48	2	0
01-08-12	3.60	0.24	48	2	0
02-08-12	3.60	0.24	48	2	0
03-08-12	3.53	0.24	48	2	0
04-08-12	3.70	0.24	48	2	0
05-08-12	3.76	0.24	47	2	0
06-08-12	3.93	0.24	47	3	0
07-08-12	4.17	0.24	47	3	0
08-08-12	4.23	0.24	46	3	0
09-08-12	4.32	0.24	46	4	0

comparison of the 3 groups in terms of changes in mean growth. Table 4 shows the mean daily differences in total growth (in cm) between groups (Fig. 7).

As Table 4 shows, on day 30, while the control group had grown to a mean height of 7.55 cm, the fractionated group (group 2) had grown to only 4.32 cm and the hypofractionated group to only 2.94 cm. The unirradiated plants used as a control showed a significantly greater growth than both irradiated groups ($P = 0.005$). The group that showed the least amount of growth was the SHD group.

Table 3 – Growth of plants in group 3 (single high dose of 50 Gy).

Day	Group 3: single dose of 50 Gy				
	Mean height (cm)	Standard deviation (cm)	Number (*n*)	No. of plants showing zero growth	No. of plants dead on the day of measure
16-07-12	1.12	0.17	48	2	0
17-07-12	1.33	0.20	48	2	0
18-07-12	1.56	0.18	48	2	0
19-07-12	1.72	0.21	48	2	0
20-07-12	1.82	0.23	48	2	0
22-07-12	1.68	0.24	48	2	0
23-07-12	1.73	0.25	48	2	0
24-07-12	1.78	0.26	48	2	0
25-07-12	1.89	0.22	48	2	0
26-07-12	1.91	0.37	48	2	0
28-07-12	2.07	0.30	48	2	0
31-07-12	2.64	0.43	47	2	1
01-08-12	2.69	0.41	47	3	0
02-08-12	2.68	0.42	47	3	0
03-08-12	2.67	0.41	47	3	0
04-08-12	2.94	0.38	47	3	0
05-08-12	2.95	0.35	47	3	0
06-08-12	2.89	0.37	47	3	0
07-08-12	2.93	0.47	37	3	10
08-08-12	2.96	0.46	35	13	2
09-08-12	2.94	0.45	35	15	0

Table 4 – Mean daily differences in total growth (cm) between groups.

Day	Differences in mean plant height between groups		
	Single high dose of 50 Gy vs. control group (cm)	Fractionated dose vs. control group (cm)	Fractionated dose (group 2) vs. single high dose (group 3) (cm)
16-07-12	0.17	0.15	0.02
17-07-12	0.12	0.09	0.03
18-07-12	0.00	0.01	0.01
19-07-12	0.20	0.11	0.09
20-07-12	0.17	0.10	0.06
22-07-12	0.30	0.08	0.21
23-07-12	0.31	0.08	0.23
24-07-12	0.32	0.11	0.22
25-07-12	0.64	0.35	0.29
26-07-12	1.15	0.47	0.68
28-07-12	1.59	0.93	0.66
31-07-12	2.30	1.51	0.79
01-08-12	3.04	2.13	0.91
02-08-12	3.17	2.25	0.92
03-08-12	3.60	2.74	0.86
04-08-12	3.96	3.19	0.77
05-08-12	4.01	3.20	0.81
06-08-12	4.16	3.12	1.04
07-08-12	4.31	3.08	1.23
08-08-12	4.46	3.18	1.28
09-08-12	4.61	3.24	1.37

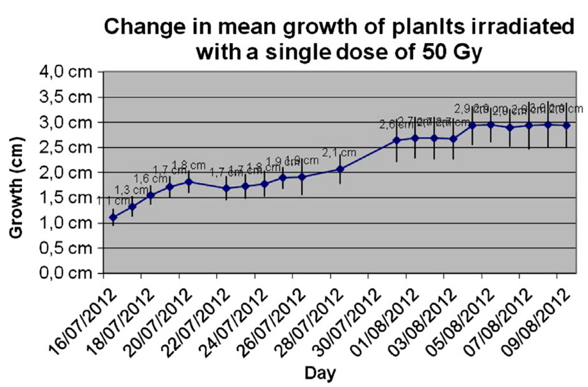

Fig. 5 – Daily changes in mean growth of plants irradiated with a single 50 Gy dose fraction.

Fig. 7 – Image of all three groups on day 26: control, high-dose, and fractionated, respectively.

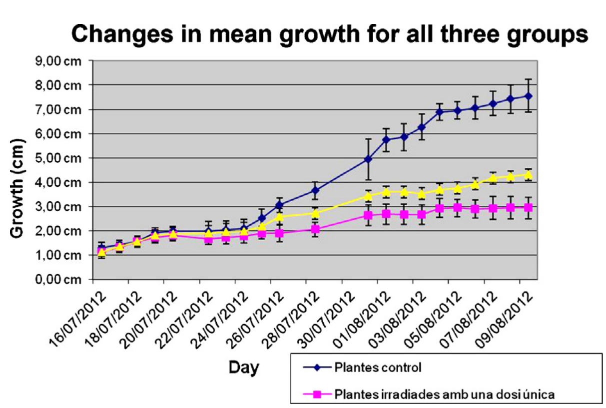

Fig. 6 – Comparative chart depicting mean growth in both treatment groups and the control group.

4. Discussion

Our results clearly illustrate that single high-dose radiotherapy is much more effective in slowing plant growth than the fractionated schedule. Such a result, while not unexpected, supports efforts to further investigate the potential value of hypofractionated radiotherapy.

Although there are many differences between plant and animal cells—particularly the fact that animal cells do not contain cell walls—the effect of ionizing radiation is similar in both. Esnault et al., in a review of the effects of ionizing radiation on genetic material in higher plants, described the mechanism of action of ionizing radiation on plant DNA.[7] According to the authors, ionizing radiation causes direct and indirect damage to DNA through water radiolysis and the resulting creation of reactive hydroxyl radicals. This process occurs in a similar manner in all biological systems (animal and plant), as the initial absorption of ionizing radiation leads to a cascade of effects that ultimately end in the final biological injury. Because all biological organisms contain water molecules, water radiolysis is the most important factor in causing damage to biological organism. As in human tissue, chromosomal damage is dose-dependent.

An interesting similarity between plant and human cells is that repeated use of ionizing radiation (either acute or chronic) triggers radioresistance,[8] which may reflect an adaptive response to radiation.[9] This phenomenon suggests that fractionated schedules may generate radioresistance, and may explain why plants in our experiment that underwent a fractionated treatment schedule managed to achieve a greater mean growth than those in the SHD group. This observation supports the use of hypofractionation to avoid generating radioresistance, although the adverse effects of high dose radiotherapy on healthy cells is an important limiting factor that must be considered. In addition, it is evident that the results that we report here cannot be directly extrapolated to human tissue cells. However, they do provide further confirmation of the differences between conventional and extreme hypofractionated radiotherapy in cell division.[10] As our study shows, these differences hold true for both plant and human cells.

Ours is not the only study to evaluate the effects of ionizing radiation on plant growth. For example, Kahan et al.[11] evaluated the differential effects of small doses of ionizing radiation on the growth of onion seeds and bulbs. They found that doses of 6–10 Gy to onion bulblets applied 8–25 days before planting reduced crop yields by 25% and the number of large onions by 75%. Similarly, Kovalchuk et al. analysed the influence of acute and chronic ionizing radiation on plant genome stability and global genome expression. They found that plants exposed to chronic but not acute radiation showed early flowering. Transcriptome analysis showed fundamental differences in plant response to acute and chronic exposure to ionizing radiation.[12]

Hypofractionation began in the 1960s as physicians sought to reduce patient discomfort arising from the extended treatment schedules (requiring daily hospital visits) and to save machine time.[13] However, the resulting increase in complications and late sequelae quickly convinced clinicians to abandon such extreme regimens. It has only been in the last 10–20 years that hypofractionation has once again become popular with the advent of more sophisticated stereotactic, intensity-modulated and image-guided techniques. Similarly, advances in our understanding of radiobiological characteristics of different cell types has helped to identify those tumours that are most amenable (i.e., responsive) to accelerated fractionation schemes. Emerging clinical evidence is showing that, for several of the most common cancers needing radiotherapy, the total length of treatment can be significantly shortened without sacrificing efficacy and tolerability.

In humans, the improved target localization of IMRT, combined with advances in our understanding of radiobiology, have led to an increased interest in hypofractionated treatment schedules in recent years,[14] particularly in prostate and lung cancer, but also in breast, liver, and kidney cancers. In a recent study, Arcangeli et al. found that, at a median follow-up of 32 months, prostate cancer patients who received hypofractionated treatment had significantly less biochemical failure rates than patients who received conventional fractionation. Moreover, no significant differences in Grade 2 gastrointestinal (GI) or genitourinary (GU) toxicity were observed. The authors conclude that these findings support the role of hypofractionation in increasing tumour control without increasing toxicity.[15] Zelefsky et al. recently reported outcomes of treatment with a high single-dose image-guided IMRT for metastatic renal cell cancer, with very good results.[16] Similarly, SBRT is now the standard of care for inoperable lung tumours.[17] However, despite the good results achieved with IMRT, SBRT, and other new technologies, toxicity continues to be an important concern due to the high doses used.[18]

Another issue surrounding hypofractionation is related to difficulties identifying the optimal fractionation schedule. As in the study by Arcangeli and colleagues, most phase 1 and phase 2 studies of prostate cancer treated with hypofractionated IMRT have reported acceptable levels of late GI and GU toxicity. However, in many of these studies follow up is too short.[19] The same applies to studies of hypofractionated radiotherapy in breast cancer, in which published randomized controlled studies have shown the effectiveness and safety of modest hypofractionation. However, once again the relatively short follow-up (5–10 years) precludes any definitive conclusions.[20] That said, the use of hypofractionated schemes in breast cancer, particularly accelerated partial breast irradiation (APBI) continues to increase. Several large randomized studies are currently in progress, with encouraging early results.[21]

5. Conclusion

The potential clinical benefits of a short course of radiotherapy are myriad, not only in terms of local and distant control and survival, but in other ways as well. For example, fewer fractions would ease the burden on the patient by reducing the number of treatment sessions vs. conventional fractionation. In addition to these time savings, hypofractionation can also reduce costs.

The study presented here adds incremental knowledge to our understanding of the powerful effects of hypofractionation. As our findings confirm, extreme hypofractionation is much more effective than conventional fractionation in slowing and halting cell growth. This suggests a need to continue investigating the use of hypofractionation schemes to achieve better results. However, the use of such schemes will require furtherer improvements in the accuracy of radiotherapy delivery so that surrounding healthy tissues are protected while greater amounts of radiation are delivered.

Conflict of interest

All authors hereby declare that they have no conflict of interest related to this paper.

Financial disclosure

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