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Perspectives in Cancer Research

Multiple Daily Fractionation in Radiotherapy: Biological Rationale and Preliminary Clinical Experiences*

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Abstract—*The biological bases of radiation dose fractionation are reviewed and discussed with special emphasis on reassortment. Experimental data on animal model systems are presented to clarify that reassortment has to be added to sublethal damage repair and reoxygenation in establishing the rationale for an optimized radiotherapy course according to tumor cell kinetics. Clinical results on several human tumors treated with twice or thrice daily fractions are described. These results show that some clinically "radioresistant" tumors (especially if not characterized by a relatively long clinical doubling time) can be satisfactorily dealt with multiple daily fractionation. Our clinical observations indicate that a relatively high cumulative daily dose* (200 + 150 + 150 rad) *can be safely administered.*

INTRODUCTION

It is not yet known if clinical tumor radioresistance is essentially due to a low radiosensitivity of cell clones. Survival curves of human normal and tumor cells assayed *in vitro* often exhibited survival parameters similar to those of established cultured cell lines [1, 2].

The clinical relevance of *in vitro* curves has been sometimes questioned being cell survival measured on isolated single cells, under conditions in which no cellular interactions and vascular factors are conditioning the radiation response, and the distribution of the cells in the phases of the generation cycle is different from that in the *in vivo* situation. On the other hand, quantitative data on survival and progression can be collected only in *in vitro* systems or in particular single-cell *in vivo* model systems. They indicate that the resulting radiosensitivity of a complex cell population is often due mainly to its kinetic situation (e.g., [3]).

Therefore, up to date, it is only on the basis of *in vitro* and *in vivo* experimental results that it is possible to obtain relevant information on how and to what extent are population cell kinetics involved in determining clinical radioresistance.

Tumor radiotherapy is historically and necessarily based on dose fractionation in order to obtain the best therapeutic ratio. It is therefore important to understand how the dose fractionation schema can be optimized according to the kinetics of both normal and tumor tissues. In fact, nowadays, nonconventional fractionation schemes are one of the most explored subjects by clinical radiation biology.

The lethal effects of a fractionated radiation dose depend upon a succession of partial overlapping radiobiological phenomena which are differentially relevant for either normal or tumor tissues.

To obtain the best therapeutic ratio, conventional fractionation schemes are essentially based on the different ability of normal [4–8] and neoplastic cells to repair from sublethal damage, and on the reoxygenation of tumor hypoxic cells. Further radiobiological reasons are in favor of using multiple daily versus conventional or other fractionation schemes (Table 1):

(1) reassortment in tumors may be greater

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Table 1. *The R's affecting fractionated radiation response*

1.	Repair from sublethal damage
2.	Repair from potentially lethal damage
3.	Reassortment:
	(a) age-response
	(b) age-dependent progression delay
	(c) cell progression throughout the cycle
4.	Repopulation and recruitment into cycle
5.	Reoxygenation
6.	"Intrinsic" cellular radiosensitivity [?] [21,22]

than in normal tissues if the latter are proliferating more slowly or not proliferating at all [5, 9, 10]

(2) the differential oxygen effect should be small at relatively low doses per fraction as survival curve in presence and absence of oxygen may aim to come closer in the low dose range [11–13];

(3) in the first part of the cell survival curve, at relatively low doses, a "single-hit" mode of killing is acting and it could be responsible for non-repairable sublethal damage [14, 15];

(4) recent evidence indicated a slower rate of repair in tumor cells than in normal tissues, such that little repair in tumors may occur over periods of few hours [16, 17];

(5) a compression in time of the cumulative dose should favour a reduction in the extent of repopulation.

SOME NEW *IN VITRO* EXPERIMENTAL MODEL DATA

To better explore the problem of optimal fractionation intervals, we performed some *ad hoc* experiments in collaboration with other authors (see Acknowledgements). Firstly, some classic experiments on murine hemopoietic stem cells tested for survival by the spleen-colony technique [18] were repeated. Such a population (CFU_s) has a fraction of cells in S-phase of about 5% [19], and thus is comparable, in proliferation terms, to some typical normal renewing populations in clinical situation.

New experiments on murine hemopoietic stem cells tested for survival by the agar culture technique [20] were then performed. The population monitored in this way (CFU_c) is characterized by a fraction of cells in S-phase higher than 50% [19], and can be assimilated, therefore, from a simulation point of view only, to a neoplastic population with this kind of kinetic features. In the CFU_c experiments (Figs. 1 and 2), the cells show a maximum of sublethal damage repair by about 2 hr. However, a survival minimum is reached by 5–6 hr and corresponds, after an appropriate conditioning dose, to a dramatic loss in survival (Fig. 2) even in respect to the survival initially recovered by fractionation.

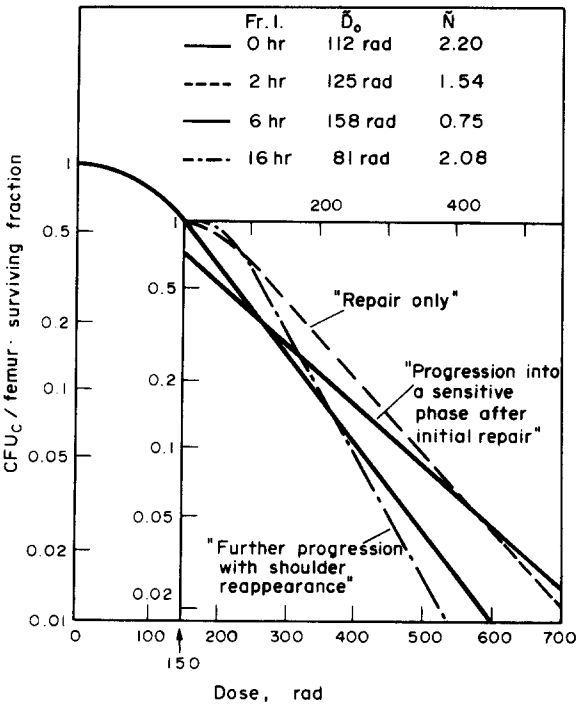

Fig. 1. *Survival curves of X-irradiated hemopoietic stem cells assayed as CFUc. Continuous, marked curve: single dose survival curve. Dashed line: second dose survival curve after a conditioning dose of* 150 rad *and an interval of* 2 hr. *Continuous, thin line: second dose survival curve after a conditioning dose of* 150 rad *and an interval of* 6 hr. *Note that the curve seems to begin at a value lower than* 100% *survival (on the scale of the second dose panel). Actually, the curve starts from* 100% *by definition, but its straight portion extrapolates to a lesser value (see text). The curve has been drawn as shown because of the scanty information on its early trend. Dotted(-----) line: second dose survival curve after a conditioning dose of* 150 rad *and an interval of* 16 hr.

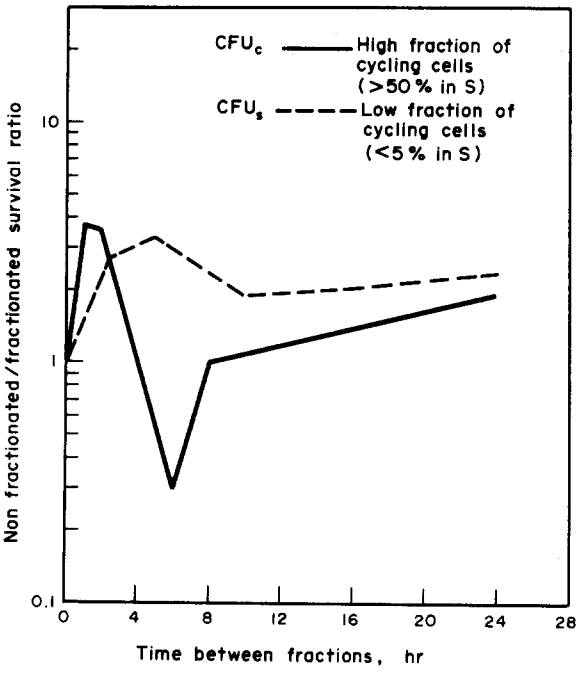

Fig. 2. Comparison of the repair curves for hemopoietic stem cells assayed either as CFU$_c$ (continuous line) or as CFU$_s$ (dashed line). The data refer to response to a second dose of 150 *or* 200 rad *as a function of time after a conditioning dose of* 150–200 rad, *(the lower doses referring to CFU$_c$). Experimental points are not shown.*

In other words, in the experimental conditions described, drastic cycle variations in radiation response can be observed after fractionation, due to repair and to cell accumulation in the (most) sensitive phase(s) of the generation cycle. These variations are illustrated in Fig. 1, where dose–survival curves are reported for a single dose experiment and for various fractionation intervals after an initial conditioning dose of 150 rad. The variations in D_o and D_Q (here expressed as n) are evident. For the 6 hr interval, the value of n appears even lower than 1 (that is D_Q is negative)—clearly a result, in the composition of the curve, of the overlapping of the response of various subpopulations.

The comparison of the repair curves, as a function of the fractionation interval, for CFU$_s$ and CFU$_c$ is shown in Fig. 2. The survival trends suggest that, assuming normal and malignant tissues of clinical interest as having the same kinetic features than those "simulated" here, by using a dose fractionation interval around 5 hr, the maximum of repair for both normal and neoplastic populations should have been accomplished. At the same time, cycling cells should have already progressed through the cycle, thus reaching a region of renewed sensitivity. In a fractionated course, this should lead to an increasing likelihood of "radiosensitization" through reassortment.

DOSE FRACTIONATION EXPERIMENTS IN A MOUSE TUMOR

In Fig. 3 data on a relatively radioresistant mouse tail sarcoma of spontaneous origin is shown. A 250 keV X-irradiation with a conventional fractionation (200 rad/day, 5 days/week, to a total of 2500 rad) resulted in a downward displacement of the tumor growth curve without any growth delay. Multiple daily fractionation (200 + 150 + 150 rad daily at 5 hr intervals, to a total of 2500 rad) induced a growth delay lasting the time of irradiation.

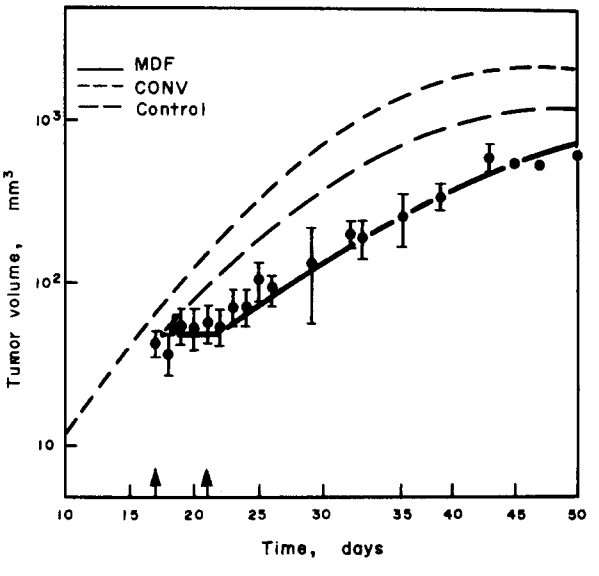

Fig. 3. Effects of irradiation on the volume of a mouse tail sarcoma. Continuous line (closed points): 200 + 150 + 150 rad *daily at* 5 hr *interval between fractions to a total of* 2500 rad *(arrows indicate the first and the last day of treatment). Dotted line (points not shown):* 200 rad/day, 5 days/week, *to a total of* 2500 rad. *Dashed line (points not shown): control growth curve.*

CLINICAL OBSERVATIONS

Our clinical experience is based on irradiation (6 MeV Photons) of selected patients with tumors in late stages and/or commonly defined as clinical "radioresistant" [21, 22]. The patients have been allotted to treatment groups as reported in Table 2.

In a selected group with multiple lung metastases, comparison on the same patient between thrice daily fractionation and conventional fractionation was carried out.

Table 2. Patients treated with twice or thrice daily fractions

1st group of patients (MDF, twice: 200+200 rad)			2nd group of patients (MDF, thrice: 200+150+150 rad)		
Number of patients		Total dose	Number of patients		Total dose
6	Head and neck	6000–7400	8	Head and neck	4650–7400
4	Astrocytoma (III and IV)	5870–6650	3	Lung	5000–5500
2	Ovary	4000	4	Breast (inflammatory)	6500–6800
4	Uterus { sarcoma (1) carcinoma (3)	6000–7000	5	Uterus { sarcoma (1) carcinoma (4)	6000–7000
4	Prostate	7000	3	Bone sarcoma	6000–9350
3	Bone sarcoma	6300–8000	6	Bone metastases	4000
10	Bone metastases	4000	3	Lung metastases	5000
8	Brain metastases	4000–4600			
3	Lung metastases	5000–5400			

Table 3. Results of treatments

Treatment	End XRT	4 months	10 months
Convent. XRT (historical)	38/166 (0.23)		53/160 (0.33)
MDF (twice)			
total	11/46 (0.24)	32/44 (0.73)	10/14 (0.71)
rapidly growing	8/24 (0.33)	18/24 (0.75)	4/6 (0.67)
MDF (thrice)			
total	8/26 (0.31)	19/23 (0.83)	8/10 (0.80)
rapidly growing	8/18 (0.44)	15/17 (0.88)	7/8 (0.88)

In Table 3 the results obtained with twice and thrice daily fractions are shown and compared, at the present stage, not with a randomized group, but with our historical experience in treating the same type of tumors with a conventional fractionation of 200 rad/day. The results indicate an appreciable enhancement in the rate of complete local response at the end of treatment. The enhancement is still higher and stabilized after 10 months follow-up where the success in respect to the conventional fractionation can be definitely appreciated.

In the same table, from the same data, the results for tumors with relatively fast clinical doubling time only (less than 30 days) are evidenced. A correlation between MDF and the proliferation features of the tumors and further enhancement of the local response rate can be observed.

In Fig. 4 the results for a group of 4 patients with multiple lung metastases are shown in terms of shrinkage curves. The data appear to follow two different trends according to the same criterion of clinical doubling time. The response of slowly growing nodules with thrice daily fractions is similar to that of rapidly growing nodules treated with conventional fractionation.

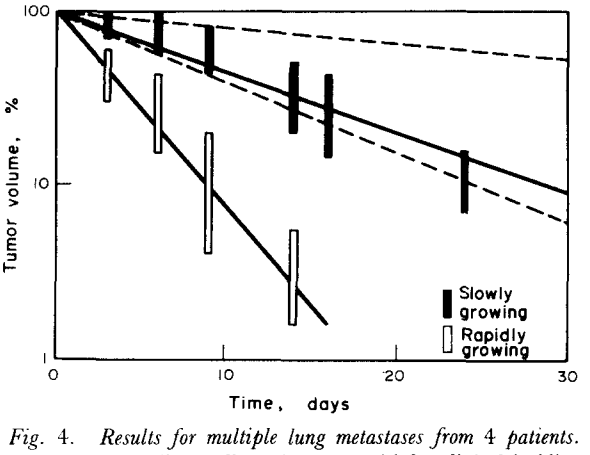

Fig. 4. Results for multiple lung metastases from 4 patients. Lower continuous line: well reacting group with fast clinical doubling times (less than 30 days). Higher continuous line: poorly reacting group with slow clinical doubling times. These results have been obtained with 200+150+150 rad daily at 5 hr intervals between fractions, 5 days/week, to a total of 5000 rad. The results are compared with similar groups treated with conventional fractionation (200 rad/day, 5 days/week).

The number of cases reported does not allow, for the moment, a statistical com-

parison of twice daily vs thrice daily fractionations. However, at present time, the latter modality seems to be the most effective and, in our clinical practice, we tend to prefer thrice daily fractions just on the grounds of immediate patient tolerance.

A PECULIAR CASE OF BIOLOGICAL AND CLINICAL INTEREST

This case is of interest as an example of both the advantages and the concomitant problems of multiple fractionation treatment, in spite of the relatively scarce success achieved in this particular instance.

A patients (S.A.M.) was affected by inflammatory mammary carcinoma with large axillary and subclavear adenopathies. During multiple (thrice daily) fractionation, after 4800 rad for 29 fractions in 15 days, the axillary adenopathy shrunk of an 80%. We were expecting an easy and persistent local control but, at that point, with the treatment still going on, we began to observe a rapid regrowth on the site with a clinical doubling time of about 7 days. Assuming that the correct hypothesis was that of the selection of a cycling (and/or recruited) radioresistant population, we suspended radiotherapy and began chemotherapy with specifically anti-cycling drugs (Table 4). The effect of chemotherapy was very good: the tumor shrunk with a rate similar to the regrowth rate, reaching a plateau corresponding to about 25% of the initial volume (Fig. 5). Nowadays, 10 months later, the plateau is still going on, in spite that chemotherapy had to be discontinued because of the denudation level of epidermitis suddenly observed in the irradiated area. In the same patient, conventional fractionation had no effect on the subclavear adenopathy, where, as a matter of fact, some further growth could be noted (Fig. 5).

TOLERANCE TO MULTIPLE DAILY FRACTIONATION COURSE

In most instances, multiple fractionation was well tolerated. As expected, in comparison to conventional fractionation, the treatment is not only more effective, but also better tolerated in terms of side effects, especially in particular sites. Patients with abdomino-pelvic tumors treated with large portals covering the pelvis and/or large portions of the abdomen, exhibited only mild intestinal symptoms.

In patients with head and neck cancers the usual acute 2nd degree mucositis tends to appear between the 10th and 14th day of treatment, as for conventional fractionation. The mucositis shows up with the same intensity and recovers a little later, in spite that the administered dose is already at least twice as much. In this way, patients suffer only one mucositis during thrice daily fractionation courses.

No abnormal effects on the hemopoietic system or on the skin were observed.

No late radiation effects on normal renewing tissues have been seen until now. We may not expect them as repair should be dependent upon the size of dose per fraction independently from the overall time [23], and reassortment should be (by definition) absent in nonrenewing tissues [10].

CONCLUSIONS

In conclusion, our initial clinical results suggest that, with this approach, a better therapeutic ratio could be achieved in human tumor radiotherapy, thus encouraging further clinical exploration.

Recently, several suggestions of improving therapeutic ratio by reduction of dose fraction intervals have been advanced and, sometimes,

*Table 4. Anti-cycling chemotherapy**

	†6 hr	8 hr	8 hr	12 hr	12 hr
	ADR MTX VLB	CTX VLB	CTX HOU	HOU HOU	HOU
Day	1st	2nd	3rd	4th	5th

*ADR: adriamycin.
CTX: cytoxan.
HOU: hydroxyurea.
MTX: methotrexate.
VLB: vinblastine.
†Hours between two drug administrations on the same day.

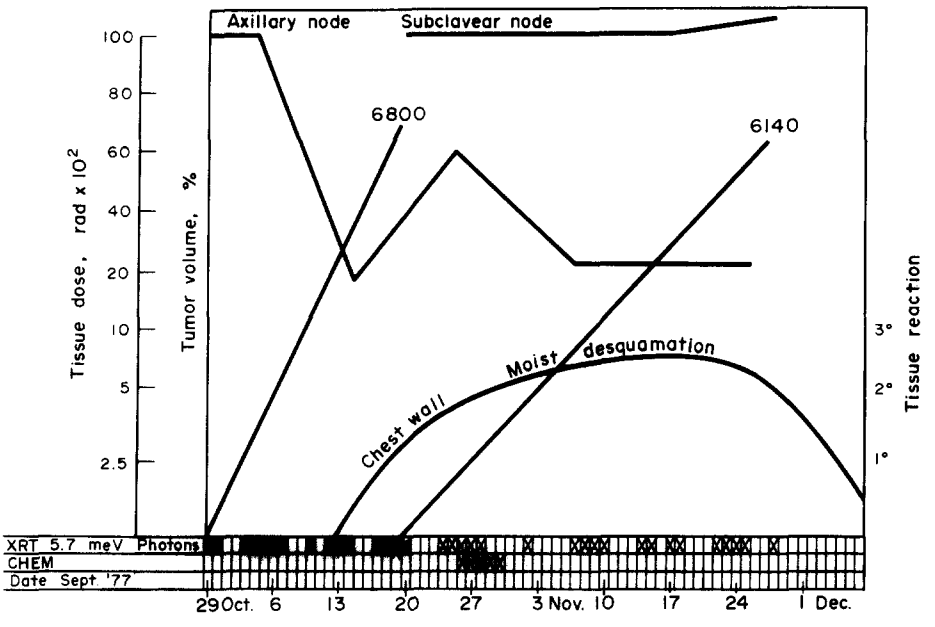

Fig. 5. Patient S.A.M. clinical chart. Regression curves and cumulative total doses for the axillary node (to the left) and the subclavear node (to the right), respectively. In the lower part of the chart, the days of thrice daily fractionation are induced with filled boxes, and of conventional fractionation (and of chemotherapy) with crossed box. Multiple fractionation refers to the axillary node, and conventional fractionation to the subclavear node. Note the regrowth of the axillary node during treatment, and its further regression after chemotherapy (see text).

clinically supported [11, 24–28]. We confirm the relevance of these suggestions, clarifying that, after repair [29, 30] and reoxygenation [11], reassortment too should be taken as a base for optimizing radiotherapy courses. Finally, our results stress the evidence that a relatively high cumulative dose can be safely administered and, as a matter of fact, is to be related to the satisfactory tumor response observed. The compression of the overall treatment time represents, anyhow, *per se*, a further advantage to counteract possible repopulation and to allow concomitant and/or sequential hyperthermia or cytotoxic drug administration.

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