Influence of Time Factor and Repopulation on Treatment Resistance

16

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CONTENTS

KEY POINTS

- Overall treatment time (OTT) is the time period between the first and the last day of treatment.
- Experimental and clinical evidence shows that OTT is an important parameter of curative radiotherapy and that many fractionated irradiated tumours exhibit a time factor.
- A meta-analysis of 12 randomized clinical trials including patients with squamous cell carcinomas of the head and neck revealed that accelerated radiotherapy resulted in significantly better local tumour control than conventional radiotherapy.
- Repopulation of tumour stem cells during fractionated irradiation is considered to be the major underlying mechanism of increased treatment resistance with longer OTT.
- The effective cell doubling time is determined by the rate of cell production and cell loss. Therefore, accelerated repopulation of tumour stem cells during radiotherapy could result from an increased production rate or reduced stem cell loss.
- While changes in microenvironment appear to passively affect repopulation, it has been postulated that an active regulatory element is involved in triggering accelerated repopulation in tumours. This view is supported by similar repopulation kinetics in squamous cell carcinomas and normal epithelium where reoxygenation is unlikely to contribute to accelerated repopulation. Several studies indicate a role of signalling via epidermal growth factor receptor (EGFR).
- In experimental studies, inhibition of EGFR by monoclonal antibodies has been shown to inhibit accelerated repopulation during fractionated irradiation.

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- Clinical studies suggest that the concept of dose-dense chemotherapy, e.g. 2-week cycles instead of 3-week cycles, is more successful in patients with lymphoma and breast cancer than in those with small cell lung cancer.

Abstract

Delivering radiation treatment with identical total dose over a shorter as compared to a longer time period influences the clinical effects on both normal tissue and tumour cells. The concept of dose-dense chemotherapy is also based on reduction of overall treatment time by shortening the interval between cycles. This chapter reviews preclinical and clinical data on the influence of treatment time and cell kinetics on outcome.

16.1 Introduction

Conventional curative radiotherapy is given in 30–35 daily fractions of 1.8–2 Gy in an overall treatment time (OTT) of 6–7 weeks. OTT is the time period between the first and the last day of treatment. This standard regimen has been developed to treat the tumour with a high radiation dose and with acceptable side effects to normal tissues. It has been recognized that normal tissue tolerance to radiotherapy increases with the use of small doses per fraction and with a time interval between fractions long enough for regeneration. On the other hand, it was generally accepted among radiation oncologists that prolonged OTT did not reduce antitumour efficacy of curative radiotherapy. This view was based on the observation that tumours usually grow at a slow rate with volume doubling times of several months (reviewed in Begg and Steel 2002). Therefore it was assumed that prolongation of OTT by several days, e.g. because of acute side effects or machine breakdown, would not result in inferior tumour control probability. However, this view has changed dramatically during the last 20 years by experimental and clinical evidence showing that OTT is an important parameter of curative radiotherapy and that many fractionated irradiated tumours exhibit a time factor.

16.2 Time Factor of Fractionated Radiotherapy

In their seminal article published in 1988, Withers and colleagues reported that in patients with head and neck squamous cell carcinomas local tumour control after fractionated radiotherapy decreases with prolonged OTT (Withers et al. 1988). The loss in radiation dose was estimated to be as high as 0.6 Gy per day. Several experimental studies on tumours in mice supported the early clinical observation that OTT matters (Baumann et al. 1994; Beck-Bornholdt et al. 1991; Speke and Hill 1995a, b; Suit et al. 1977). Consequently, the concept of shortening of the OTT (*accelerated radiotherapy*) as a therapeutic intervention counteracting the time factor of fractionated radiotherapy was tested in clinical trials. Today, data from numerous randomized clinical trials with several thousand patients, mainly with squamous cell carcinomas of the head and neck as well as with lung cancer, are available. A meta-analysis of 12 randomized clinical trials with 5,723 patients treated for squamous cell carcinomas of the head and neck revealed that accelerated radiotherapy resulted in significantly better local tumour control than conventional radiotherapy (Bourhis et al. 2006). Although cancer-specific and overall survival were only slightly improved, the local tumour control data strongly support the existence of a time factor. Similar findings are reported from randomized trials in lung cancer (Saunders et al. 1997, 1999; Turrisi et al. 1999). In other tumour types, such as bladder cancer, no benefit from accelerated radiotherapy was observed (Horwich et al. 2005). Today radiation oncologists are obliged to prescribe OTT as well as dose and number of fractions. It has become the standard of care in curative radiotherapy of tumour types with proven time factor to compensate for unplanned treatment breaks, e.g. because of machine breakdown or holidays.

16.3 Mechanisms Underlying the Time Factor

Repopulation of tumour stem cells during fractionated irradiation is considered to be the major underlying mechanism of increased treatment resistance with longer OTT. Mechanisms other than repopulation could theoretically contribute to or modulate the time factor (Table 16.1). However, systematic experiments did not reveal supportive evidence for alternative mechanisms

Table 16.1. Biological mechanisms other than repopulation which may result in an increase of radiation resistance of tumour stem cells during fractionated radiotherapy and thereby contribute to the time factor

Resistance factor	Possible underlying radiobiological mechanisms
Increased tumour hypoxia	Progressive destruction of the tumour vasculature by radiotherapy results in impaired oxygen supply and thereby in an increased radiobiological hypoxia
Selection of radioresistant clones	Subpopulations of radioresistant and rapidly proliferating clonogenic cells are selected during radiotherapy
Increased capacity to recover from sublethal damage	Clonogenic tumour cells adapt to the repeated radiation-induced damage/stress by an increased capacity to recover from sublethal damage
Accumulation in radioresistant phases of the cell cycle	During fractionated radiotherapy clonogenic cells stop to proliferate and are blocked at radioresistant phases of the cell cycle

of accelerated repopulation (Petersen et al. 2001, 2005; Zips et al. 2003). Each fraction of radiation inactivates a proportion of tumour stem cells, i.e. the population of tumour stem cells in a tumour is reduced (depopulation). A complete depopulation of tumour stem cells is the aim of curative radiotherapy. The desired therapeutic effect of depopulation is abrogated by the proliferation of surviving tumour stem cells during treatment. This process has been named repopulation. Assuming a linear relationship between tumour volume and the number of tumour stem cells in untreated tumours, the doubling time of tumour stem cells would be in the range of several weeks to months which could not explain the time factor observed in experimental and clinical studies. Findings from early experimental studies suggest that in some but not all tumour types the tumour stem cell doubling time after single-dose irradiation is shortened (Hermens and Barendsen 1969; Jung et al. 1990; Stephens et al. 1978), i.e. repopulation apparently accelerates in some tumour types after radiotherapy. The concept of accelerated repopulation stimulated a number of experimental and clinical studies to explore the kinetics and underlying mechanisms of accelerated repopulation during fractionated radiotherapy (Baumann et al. 2003).

16.4 Mechanisms of Accelerated Repopulation

The effective cell doubling time (*net doubling time*) is determined by the rate of cell production and cell loss (Begg and Steel 2002). Therefore, accelerated repopulation of tumour stem cells during radiotherapy could result from an increased production rate or reduced stem cell loss (Table 16.2). Methods to determine cell loss or cell production such as immunohistochemistry or flow cytometry are plagued by the fact that tumour stem cells are morphologically not distinguishable from non-tumour stem cells and represent only a very small fraction (about 1%) of all tumour cells (implications of the tumour stem cell concept for radiotherapy were recently reviewed in Baumann et al. 2008). Even major changes in the tumour stem cell compartment during acceleration of repopulation might be easily overlooked. Radiobiological methods (tumour control assay, excision assay) allow determination of tumour stem cell survival after irradiation in vivo (Baumann et al. 2008; Krause et al. 2006; Zips et al. 2005). Local tumour control data obtained from fractionated irradiated experimental tumours have been used to estimate repopulation rates of tumour stem cells (Hessel et al. 2003, 2004a, b; Petersen et al. 2001; Thames et al. 1996). However, using local tumour control assays, it still remains challenging to dissect mechanisms underlying accelerated repopulation of tumour stem cells. Therefore, data from local tumour control assays, studies into normal tissue response during fractionated irradiation and non-stem cell assays (histology, flow cytometry, etc.) were considered to hypothesize concepts of accelerated repopulation (Fowler 1991; Trott and Kummermehr 1991).

Table 16.2. Determinants of production and loss of tumour stem cells

Cell production	Cell loss
Growth fraction	Probability of self-maintenance
Cell cycle time	Necrotic/apoptotic cell death

16.4.1 Increased Cell Production Rate of Tumour Stem Cells During Fractionated Radiotherapy

Experimental and clinical data on normal epithelia suggest that acceleration of stem cell divisions might contribute to repopulation during fractionated irradiation (Dorr 1997). Modelling of cell kinetic data implies a shorting of the cell doubling time of surviving stem cells during fractionated irradiation from 3.5 to 1.4 days (Dorr and Kummermehr 1991). It has been speculated that in some tumours, e.g. well-differentiated squamous cell carcinomas, repopulation is reminiscent of the normal epithelium (Kummermehr et al. 1992; Trott and Kummermehr 1991). This seems to be supported by clinical data showing a more pronounced time factor of fractionated radiotherapy in well-differentiated primary tumours with high expression of epidermal growth factor receptor (EGFR, see below) than in less well differentiated primaries or lymph node metastases (Eriksen et al. 2004; Overgaard et al. 2003). Taking the experimental and clinical data together, it is conceptually possible that an increased production of tumour stem cells contributes to accelerated repopulation during fractionated radiotherapy.

16.4.2 Reduced Cell Loss of Tumour Stem Cells During Fractionated Radiotherapy

Applying the hierarchal structure of epithelial or haemopoietic normal tissues to malignant tumours, after each tumour stem cell division, the progeny either remain in the stem cell compartment or differentiate into a non-stem cell (Kummermehr and Trott 1997). As malignant tumours grow and the number of tumour stem cells increases with tumour volume (Baumann et al. 1990; Kummermehr and Trott 1997; Suit et al. 1965) the average probability for a tumour stem cell daughter to remain a tumour stem cell after cell division (average probability of self-maintenance) is higher than 50%. Assuming a tumour stem cell fraction of 1%, model calculations suggest that the average probability of tumour stem cell self-maintenance is 51–65%, i.e. the average probability of cell loss would equal 35–49% (Kummermehr and Trott 1997). Based on data obtained from normal epithelia (Dorr 1997) and from studies on tumour cell kinetics (Begg and Steel 2002) it has been proposed that during fractionated radiotherapy the loss of tumour stem cells decreases and more cells remain in the stem cell compartment, which would result in accelerated repopulation (Fowler 1991; Kummermehr et al. 1992; Trott and Kummermehr 1991). As an alternative mechanism of reduced cell loss, down-regulation of radiation-induced apoptotic cell death has been suggested as an underlying mechanism of accelerated repopulation (Thames et al. 1996).

16.4.3 Tumour Microenvironment and Accelerated Repopulation of Tumour Stem Cells

The microenvironment of malignant tumours is characterized by hypoxia, high interstitial fluid pressure, glucose and energy deprivation, high lactate levels and extracellular acidosis (Vaupel 2004). These hostile conditions contribute to the high cell loss occurring spontaneously in tumours. Cell loss factors between 89% and 97% have been estimated for carcinomas (Begg and Steel 2002). Experimental and clinical data indicate that tumours reoxygenate during fractionated irradiation (Horsman and Overgaard 2002). Based on these observations it has been hypothesized that reoxygenation during fractionated radiotherapy reduces cell loss and subsequently shortens the net doubling time of tumour stem cells (Fowler 1991). Experimental data support the hypothesis of a causative relationship between reoxygenation, cell loss and repopulation of tumour stem cells (Hessel et al. 2003, 2004a, b; Petersen et al. 2001, 2003; Speke and Hill 1995a, b). However, improved tumour microenvironment might also lead to a higher cell production rate.

16.4.4 Molecular Regulation of Accelerated Repopulation

While changes in microenvironment appear to passively affect repopulation, it has been postulated that an active regulatory element is involved in triggering accelerated repopulation in tumours (Trott and Kummermehr 1991). This view is supported by similar repopulation kinetics in squamous cell carcinomas and normal epithelium where reoxygenation is unlikely to contribute to accelerated repopulation. The molecular background of the hypothesized regulatory element has been explored in experimental and clinical studies. Several studies indicate that signalling via EGFR appears to be involved in accelerated repopulation of tumour stem cells during fractionated radiotherapy (Bentzen et al. 2005; Eriksen et al. 2004, 2005; Krause et al. 2005; Petersen et al. 2003; Schmidt-Ullrich et al. 1997; Zips et al. 2008). Activated EGFR signalling results in

multiple biological responses potentially relevant for accelerated repopulation, e.g. increased cell proliferation and reduced cell death by antiapoptotic signalling or by improved DNA repair (Baumann et al. 2007). However, EGFR expression and signalling might also be associated with the tumour microenvironment and reoxygenation during radiotherapy (Krause et al. 2005; Zips et al. 2008).

16.5 Time Factor of Fractionated Radiotherapy Combined with Other Treatment Modalities

Curative radiotherapy is often given combined with chemotherapy, surgery and biological modifiers. Radiotherapy after surgery is given to sterilize residual tumour stem cells. While it is clear that during the gap between surgery and start of radiotherapy the remaining tumour stem cells might repopulate, it remains controversial for example in patients with head and neck cancer whether accelerated postoperative radiotherapy improves locoregional control (Ang et al. 2001; Awwad et al. 1992, 2002; Sanguineti et al. 2005; Suwinski et al. 2008). Experimental data on repopulation rates of microscopic and macroscopic tumours suggest that in the postoperative situation the time factor of fractionated radiotherapy might be less pronounced (Beck-Bornholdt et al. 1991; Raabe et al. 2000).

In a large variety of advanced carcinomas curative radiotherapy is combined with chemotherapy. Experimental observations and some clinical studies indicate that chemotherapy as a single modality can induce accelerated repopulation in tumours (reviewed in Davis and Tannock 2000; Kim and Tannock 2005). Induced repopulation by induction chemotherapy may possibly explain the inferior results of induction chemotherapy before radiotherapy compared with concurrent chemoradiation in patients with non-small cell lung cancer (Fournel et al. 2005; Furuse et al. 1999; Zatloukal et al. 2004).

The evidence of a time factor of concurrent chemoradiation remains a controversial issue. A randomized clinical trial in patients with limited disease small cell lung cancer (SCLC) treated with chemoradiation demonstrated a significantly higher local tumour control when OTT was reduced from 33 to 19 days (Turrisi et al. 1999). A meta-analysis of four randomized clinical trials in patients with limited disease SCLC revealed that OTT is the most important predictive factor for outcome after chemoradiation (De Ruysscher et al. 2006). In contrast to the results of this meta-analysis, no impact of prolonged OTT on local tumour control rates after conventional fractionated chemoradiation for limited disease SCLC has been reported by others (Bogart et al. 2008). A time factor has been also hypothesized for postoperative chemoradiation in rectal cancer (Fietkau et al. 2007). Comparison of results from randomized clinical trials in head and neck cancer given with and without prolonged OTT supports the evidence of a significant time factor of chemoradiation (Budach et al. 2006). Experimental data on human squamous cell carcinoma indicates that concurrent chemotherapy inhibits tumour cell repopulation (Budach et al. 2002). Based on this observation it could be speculated that concurrent chemotherapy reduces the time factor of fractionated radiotherapy and thereby diminishes the benefit from accelerated radiotherapy. Taken together, most observations support the evidence of a time factor during chemoradiation. In contrast to radiotherapy alone, the underlying mechanisms of the time factor during chemoradiation are poorly understood. The clinical benefit of accelerated radiotherapy compared with conventional radiotherapy in the context of chemoradiation has been demonstrated in SCLC but requires further studies in other cancer types such as head and neck cancer.

Epidermal growth factor receptor inhibition in combination with fractionated radiotherapy in patients with head and neck cancer significantly improved locoregional tumour control and survival (Bonner et al. 2006). In experimental studies, inhibition of EGFR by monoclonal antibodies has been shown to inhibit accelerated repopulation during fractionated irradiation (Krause et al. 2005). Results from a subgroup analysis of a randomized clinical trial suggest that radiotherapy with and without EGFR inhibition is more effective when radiotherapy was given within shorter OTT, i.e. as accelerated and hyperfractionated-accelerated radiotherapy (Bonner et al. 2006). Although it is impossible to conclude on biological mechanisms of interaction from a subgroup analysis of a clinical trial, it appears that tumours treated with radiotherapy and EGFR inhibitor exhibit a time factor.

16.6 Dose-Dense Chemotherapy

Increased dose density is achieved by reducing the interval between each dose of chemotherapy. The cumulative drug dose remains constant, but the same amount of drug is administered over a shorter period. Mathematical models of tumour growth have provided the basis

for the clinical application of dose-dense chemotherapy (Norton 2005). The Norton-Simon model suggests that increasing the dose density of chemotherapy will increase efficacy by minimizing the opportunity for regrowth of tumour cells between cycles of chemotherapy. In patients with breast cancer, Intergroup trial 9741, coordinated by the Cancer and Leukemia Group B (CALGB), tested the two hypotheses that dose-dense and sequential administration of chemotherapy regimens incorporating doxorubicin, cyclophosphamide and paclitaxel would improve disease-free survival and overall survival. A statistically significant 4-year disease-free survival advantage was detected for the two dose-dense regimens compared with the regimens administered every 3 weeks (Citron et al. 2003; McArthur and Hudis 2007; Orzano and Swain 2005). In patients with non-Hodgkin's lymphoma, this concept has also been shown to improve the clinical outcome (reviewed in Broussais-Guillaumot and Coiffier 2007; Held et al. 2006), while disappointing results were reported from a trial that included 318 patients with better-prognosis SCLC treated with ifosfamide, carboplatin and etoposide (Lorigan et al. 2005).

16.7 Conclusion

Prolongation of overall treatment time has an adverse effect on outcome after fractionated radiotherapy. Accelerated repopulation of tumour stem cells during therapy, as the most likely explanation of this so-called time factor, is an established mechanism of treatment resistance. Understanding the underlying mechanisms and molecular regulation of accelerated repopulation resulted in successful therapeutic interventions. However, further investigations into accelerated repopulation in the context of combined treatments and into the clinical benefits of dose-dense chemotherapy without irradiation are necessary.

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