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Overview

A Brief Overview of the Preclinical and Clinical Radiobiology of Microbeam Radiotherapy

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Abstract

Microbeam radiotherapy (MRT) is the delivery of spatially fractionated beams that have the potential to offer significant improvements in the therapeutic ratio due to the delivery of micron-sized high dose and dose rate beams. They build on longstanding clinical experience of GRID radiotherapy and more recently lattice-based approaches. Here we briefly overview the preclinical evidence for MRT efficacy and highlight the challenges for bringing this to clinical utility. The biological mechanisms underpinning MRT efficacy are still unclear, but involve vascular, bystander, stem cell and potentially immune responses. There is probably significant overlap in the mechanisms underpinning MRT responses and FLASH radiotherapy that needs to be further defined.

Key words: Bystander effects; grid therapy; microbeam radiotherapy; vascular damage

Statement of Research Strategies

This overview was based on a search of the literature for the terms "GRID and radiotherapy", FLASH and radiotherapy" and "Microbeam and radiotherapy".

Introduction

Over many years, improvements in radiotherapy technologies have focussed on increasing the precision and accuracy with which uniform radiation doses are delivered to target tumour volumes. Recent advances have included intensity-modulated techniques such as intensity-modulated radiotherapy and volumetric-modulated arc therapy and, more recently, particle therapies using protons and carbon ions. Significant improvements in the accuracy and precision of radiotherapy delivery now means that smaller volumes can be more precisely targeted with higher doses, for example using stereotactic ablative radiotherapy. However, despite these advances, normal tissue toxicity in organs at risk still remains a limiting factor for the efficacy of most external beam radiotherapy [1].

For several decades it has been recognised that the delivery of non-uniform radiation beams has the potential to reduce normal tissue damage. This concept is now a major research area across preclinical and clinical studies, yet significant challenges remain in optimising the use of techniques involving the delivery of non-uniform radiation fields in the clinic. Radiotherapy techniques in which doses are spatially fractionated have been evaluated clinically using GRID therapy. Dose delivery can also be modulated at the microscale, which has led to the development of microbeam radiotherapy (MRT, not to be confused with molecular radiotherapy, which is sometimes also known as MRT), although this has yet to translate to clinical trials. Here we provide a primer on the principles and development of spatially fractionated radiotherapy techniques focussing on GRID and MRT. We discuss the current evidence base supporting the use of these techniques and the challenges of translating these approaches into the clinic.

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GRID Therapy

The first historical use of spatially fractionated or GRID therapy was reported in 1909 by Alban Kohler [2], who used an attenuating grid directly placed on the skin to reduce tissue damage during the treatment of tumours at depth. In the kilovoltage era, this was an innovative approach to reduce normal tissue toxicity [3], but with the advent of megavoltage linear accelerator-generated beams, its utility was further explored in a series of elegant studies by Mohiuddin and colleagues [4–8]. In these studies, a 7 cm thick metal-alloy block of Cerrobend with 256 holes configured in a 16 × 16 cm matrix was used to deliver 50% open and closed beam areas (Figure 1a). The authors delivered single treatments using this beam configuration to doses of 50–70 Gy, some in combination with conventionally fractionated radiotherapy, to patients with different tumours in the abdomen and pelvis, head and neck region, thorax and extremities. This study showed the efficacy of GRID in patients with large bulky tumours (>8 cm). An important observation with GRID approaches, for skin response, is that there is a significant dependency on field size related to the potential for necrosis [9].

GRID studies were then shown using a multileaf collimator to shape the beams and deliver spatially fractionated dose distributions [8]. This approach was also shown by other groups in patients receiving combined GIRD and chemoradiotherapy in head and neck tumours [10]. Subsequent studies have highlighted the potential use of electron beams with this approach [6]. Radiobiological modelling studies have predicted that significant increases in the therapeutic index could be delivered independently of the GRID design relative to uniform exposures [7].

Although they have shown utility, GRID therapies have been limited due to their inherent two-dimensional delivery, which brings significant constraints if critical normal tissues are in the beam path. To overcome this, recent developments in the delivery of spatially fractionated radiotherapy have included three-dimensional modulation of dose in a series of high dose regions created within tumours by converging photon beams, known as lattice radiotherapy (Figure 1b) [11]. These geometries give rise to significant peak to valley dose ratios (PVDR) equivalent to typical dose distributions delivered by GRID radiotherapy [12]. It has also been proposed that this approach can be delivered with charged particle beams using spot scanning approaches [11,12]. The lattice approach has the advantage of allowing dose sparing in surrounding normal tissue but maximising dose to the tumour.

Microbeam Radiotherapy

In 1992, Slatkin and colleagues [13] first defined the term microbeam radiotherapy (MRT) to refer to the use of parallel 50–150 keV X-ray beams of micron dimensions width for potential therapeutic use, specifically in the treatment of brain lesions in children. The concept was built on pioneering approaches introduced by Curtis [14,15] in the late 1960s using deuteron beams that were restricted into 25 μm circular or rectangular incident beams. In contrast to macroscopic beam exposures, much higher doses were required to produce tissue necrosis in the mouse brain with these deuteron beams. Using Monte Carlo simulations, Slatkin and colleagues [13] predicted that synchrotron-generated X-rays of 50–150 keV at high dose rates of 300–600 Gy/s could be used for MRT and that this could be achieved by crossfiring an array of parallel microbeams, each with a 25 μm wide cross-section. The key physical parameters defining these beams are related to the beam cross-sections. This includes the peak dose of each of the microbeams, its diameter and the interbeam distance (i.e. peak to peak spacing) between each of the microbeams. From these parameters, other parameters of the beam profile can be measured to allow a comparison between different beam configurations. This includes the valley dose

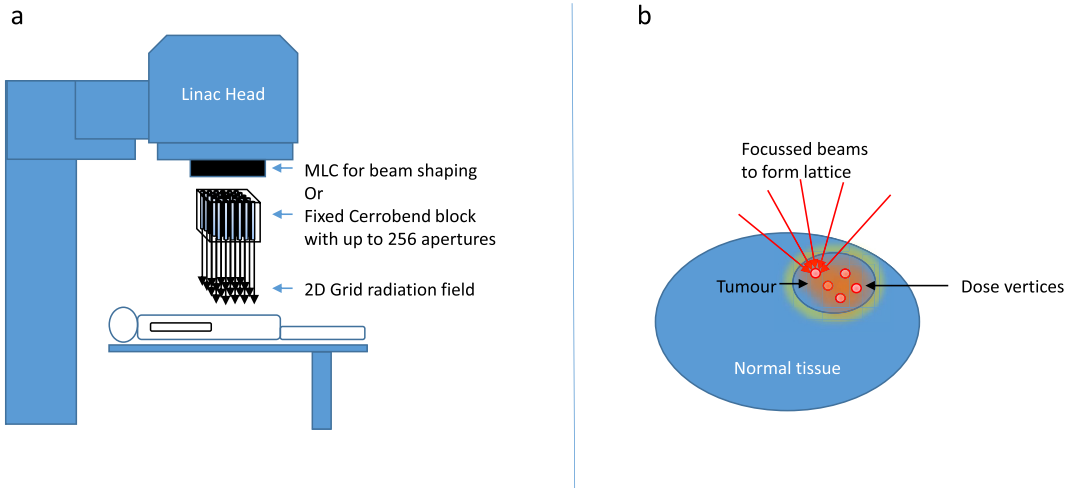

Fig 1. Schematic illustration of GRID radiotherapy: (a) where two-dimensional delivery of photons via a Cerrobend block with apertures or the multileaf collimator of a linear accelerator is used or (b) where focussed beams are used to produce individual high dose vertices within a tumour via a three-dimensional lattice delivery.

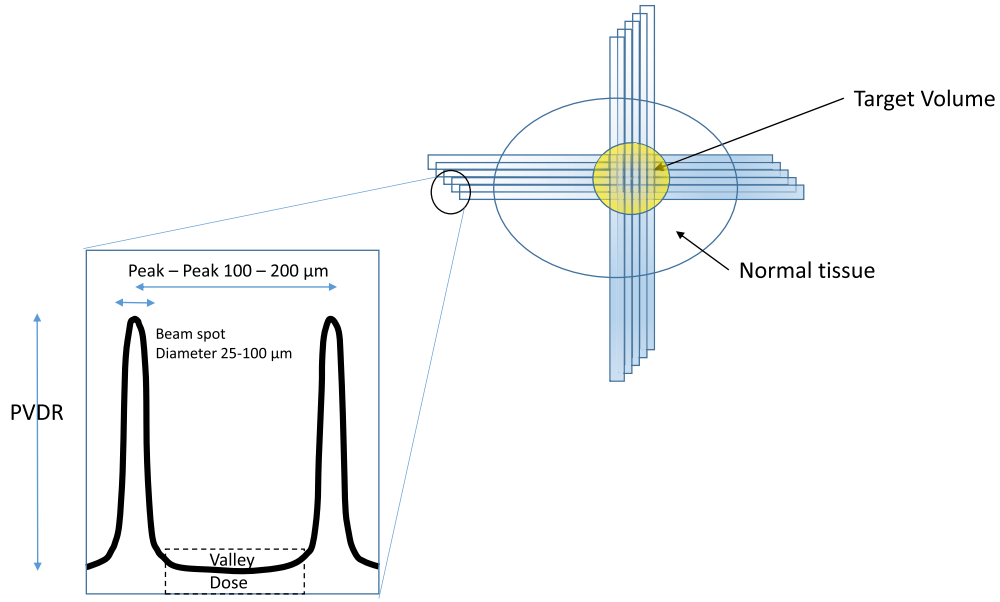

Fig 2. Schematic illustration of the dose delivery patterns used for microbeam radiotherapy, showing two interlaced beams, and a typical beam profile showing the peak to peak spacing, peak to valley dose ratio and the valley dose (not to scale).

delivered between the microbeams from which the PVDR can be calculated. The PVDR and the valley doses delivered relate to beam scattering (Figure 2).

The potential of MRT was confirmed experimentally by Slatkin *et al.* in 1995 [16], when normal rat brain was exposed to an array of parallel X-ray beams produced using a multi-slit collimator. The X-ray beam energy ranged from 32 to 126 keV and 3, 20 or 21 of these beams slices were delivered to each animal. The individual beams consisted of MRT patterns of either 20 or 37 μm beam thickness with an interval between the beams of either 75 or 200 μm. These exposures were delivered at a high dose rate (310–650 Gy/s) and total doses delivered, quantified as skin entrance doses, ranged from 312 to 10 000 Gy. Despite this, only two of the animals that received the 10 000 Gy exposures showed brain necrosis, and although some loss of cell nuclei was observed at lower exposures, a significant resistance overall to brain necrosis was seen for all the other exposures. A range of preclinical studies since then have outlined some of the key parameters for these beams to show reduced normal tissue toxicity in a range of models.

Extensive validation in preclinical models has included a range of normal tissue and tumour models [17], such as the cerebellum [18], skin [19,20], muscle [21] and lung [22]. Most tumour studies to date have been carried out in rat glioma models using 9LGS cells in the Fisher 344 rat model. Using this model, Dilmanian *et al.* [23] showed a significant increase in therapeutic index of unidirectional microbeams, with 50, 75 or 100 μm spacing and doses of up to 500 Gy in comparison with uniform exposure. The optimal dose tolerability was a 75 μm beam spacing with a 250 Gy entrance dose and 100 μm spacing with a 500 Gy entrance dose. They proposed that a biological sparing effect was playing a role beyond the volume effect predicted from reducing the amount of normal tissue being irradiated. Likewise, for the tumour response, they predicted that the response of the vasculature was a key factor. Further studies have tested the impact of applying multiple beam arrays as bidirectional beams ranging from 25 to 75 μm and peak doses of 320–860 Gy while maintaining the valley dose to <20 Gy, all of which were effective at reducing tumour growth [24,25]. In general, maintaining a low valley dose seems to be critical for delivering good tumour response and minimal normal tissue toxicity. In its most recent advanced form, multidirectional exposures from up to five different directions and maintaining a valley dose of 10 Gy led to a 2.5-fold increase in biological equivalent tumour doses with a direct relationship between the number of beam directions and animal survival [26].

Dalmanian and colleagues [27] proposed an extension of the principles of MRT to larger beam sized approaches that could offer the potential of moving away from the use of synchrotron energies to higher energies. This utilised 0.68 mm microbeams spaced 4 or 1.36 mm apart and, crucially, these beams were overlapped with each other using an interlaced technique, in an approach defined as minibeam radiation therapy. In a normal rat brain model, in-beam depth doses of 90 and 120 Gy in a 34 mm^3 volume produced minimal damage outside the target volume.

Prezado and Fois [28] extended this approach to protons and have shown the potential advantages of these beams in preclinical studies. With charged particles, as the minibeams slow down and lose energy as they enter the target volume, lateral scattering and increasing linear energy transfer leads to potentially highly effective uniform dose distributions at depths that may have significant advantages [29,30]. A summary of the key differences between GRID and MRT is given in Table 1.

Table 1
Comparison of key differences between GRID and microbeam radiotherapy

Parameter	GRID	MRT/MBRT
Sources used	X-rays (MV), gamma-rays	X-rays (kV), protons (MeV)
2D/3D	2D grid/3D lattice	3D
Beam spot sizes	0.5–2 cm	25–100 μm MRT, 0.5–1 mm MBRT
Beam spot spacings	0.5–2 cm	50–200 μm MRT or 1–4 mm MBRT
Dose rate	1–6 Gy/min	>100 Gy/s (FLASH and above)
Total dose per fraction	15–20 Gy	50–1000 Gy
Clinical application	Yes, GRID and lattice	Preclinical at present

2D, two-dimensional; 3D, three-dimensional; MBRT, minibeam radiotherapy; MRT, microbeam radiotherapy.

Biological Mechanisms Underpinning Microbeam Radiotherapy

One defining characteristic of non-uniform exposures typically delivered during spatially fractionated radiotherapy is that the observed dose–response relationships cannot be predicted from uniform exposures and based on the standard radiobiological DNA damage and repair model. It is clear that additional biological mechanisms play a role in mediating responses to spatially fractionated radiotherapy to potentially increase tumour cell kill or reduce normal tissue damage. Several potential mechanisms of tissue sparing have been proposed based on our understanding of normal tissue damage pathogenesis after radiation exposure [31].

Vascular Effects

One of the major modes of action defined for the effectiveness of MRT is the impact on tissue vasculature. Interestingly, this was first observed in the 1960s, with the early deuteron studies [32]. There are significant differences in the vasculature of normal tissue relative to tumours related to the strong pro-angiogenic signalling, which leads to abnormal vasculature in tumours. Many studies have now shown that this is more radiosensitive than that of normal tissue vasculature, and there is now evidence suggesting that this plays an important role in the response to radiation exposure, including more recent studies with MRT [25,33–36]. This has been clearly articulated in model studies using, for example, the chicken chorioallantoic membrane model [37] and zebra fish [38]. These studies have shown that the immature vasculature found within tumours is particularly sensitive to radiation, leading to vascular collapse and reduced blood flow [37]. At the molecular level, this is probably mediated via endothelial cell apoptosis [39]. A secondary consequence of this is the induction of hypoxia and subsequent necrosis [40].

Stem Cell Migration

For normal tissues, loss of stem cells has the potential to cause major damage and limit wound healing responses [31]. A pioneering study by Dilmanian and colleagues [41] followed the response of rat spinal cord to microbeam exposures. The spinal cord study revealed a loss of oligodendrocytes, astrocytes and myelin 2 weeks after exposure, but by 3 months, repopulation and remyelination were nearly complete. They suggested that non-exposed progenitor/stem cells migrated into the irradiated region to recover function as part of a bystander response.

In recent studies, the impact of MRT exposure on spermatogenesis has been tested in *ex vivo* mouse testes models, where spermatogonial function is maintained. The effect of conventional broad-beam versus MRT exposures has been compared using a GFP-tagged mouse model, where functional meiosis can be assessed. A significant tissue-sparing effect (TSE) was observed with MRT exposures and evidence points to stem cell migration from non-irradiated to irradiated areas playing a role [42]. Also, the TSE in irradiated testicular tissue was more effective when more spermatogonial stem cells survived after exposure to spatially fractionated radiation [43]. In addition, the TSE of specially fractionated radiation has promise for clinical application, as it responds to a wide range of energy of X-rays [44]. This highlights the potential for spatial irradiation approaches to potentially preserve male fertility but further mechanistic studies *in vivo* are required.

According to the report recently published by the International Commission of Radiological Protection [45], the stem cell niche usually provides shelter from various genotoxic stresses. In addition, there is a competition among tissue stem cells to occupy the niche, with the retention of favourable cells and the elimination of unwanted cells. The TSE efficiency for maintaining male fertility is dependent on the size of the non-irradiated area, indicating that stem cell migration/competition is possibly involved in the underlying mechanisms of TSE. In addition, the ‘stem cell pool’ would define the tissue-sparing ability following exposure to non-uniform radiation. This tissue microenvironment-based selection will probably function as a tissue-based quality control. Further investigations on the underlying mechanism of stem cell migration following irradiation are needed to better understand not only stem cell biology but radiation biology.

Bystander, Abscopal and Immune Effects

At the cellular level, it is known that both targeted and non-targeted effects play central roles in the radiobiological response. Non-targeted bystander effects occur in cells that

are not directly traversed by radiation tracks and are mediated by intercellular signalling through gap junction intercellular signalling and by systemic signalling [46]. Bystander effects have been postulated to play a role in GRID therapy [47]. For MRT exposures, *in vitro* studies have shown increased levels of residual damage (using the γH2AX assay) in the low dose valley regions between peak valley doses of MRT beams [48]. Extensive work at the European Synchrotron Radiation Facility (ESRF) has tested for the role of bystander responses *in vivo* after MRT exposure [49]. Using a cell-based reporter assay, they showed that after direct MRT irradiation of one brain hemisphere, bystander signals could be detected in the non-irradiated brain hemisphere and bladder, indicative of a systemic response [50]. In follow-up studies, the presence of a tumour in immunocompromised mice reduced the production of these signals [51]. Experiments were carried out in mouse tumours following either localised irradiation with 50 Gy 160 kVp MRT (a single 300 μm microplanar beam) or broad field exposures, using a window chamber approach to monitor the effects on the environment non-invasively. The authors reported significant differences in the microvasculature between the treatments related to differences in the spatial and temporal patterns of HIF-1 expression induced through radiation bystander effects leading to increased vascular recovery in the MRT exposed cells. In this study, no evidence of tumour vascular collapse was reported in the MRT arm in contrast to the broad field exposures [52].

Although bystander responses have been defined as short range signalling effects after localised radiation exposure, it has been known for many years that longer range, out-of-field or abscopal effects can occur after irradiation [53], including in patients receiving radiotherapy, although much of this evidence has been anecdotal [54]. More recently, however, it has been found that there is a significant immune signalling component playing a role in radiotherapy response that can be harnessed to amplify these effects [55]. MRT studies have shown clear evidence of an immune-mediated response on the transcriptomic level, alongside inflammatory signalling markers showing involvement of macrophage, dendritic, natural killer, T and B lymphocyte populations [56,57]. Specifically, in MRT-treated rats with intracranial glioma, a higher induction of cell death and immune activation in tumours was observed relative to broad-field exposure [58]. In a radioresistant murine melanoma model, MRT exposure led to blood vessel disruption and immune infiltration [59]. Studies in mammary tumour models also reported differential immunomodulatory responses between MRT and conventional exposures [60].

Implications of Dose Rate

MRT approaches all typically involve a high dose rate (~100–1000 Gy/s) and in most cases high peak dose exposures (~100–1000 Gy). Our understanding of the importance of dose rate in radiation response is going through a period of revision as our previous knowledge based on a reduced biological response with decreasing dose rate [61,62] is being challenged by new data at higher dose rates (typically >40 Gy/s) showing specific protection of normal tissues via so-called 'FLASH' effects [63,64]. Significantly, these FLASH effects do not impact on tumour responses. Although previous studies in the 1970s and 1980s had suggested that increased dose rates may have potentially protective effects in normal tissues [64], it is only recently that these effects have been better defined. There is probably a considerable overlap in the underlying mechanisms of action of MRT and FLASH exposures [65]. To date, however, the mechanisms of FLASH protection of normal tissues are unclear. Much has been proposed around the potential for high dose and dose rate exposure to deplete oxygen [66,67], which normally sensitises tissue to radiation exposure, but recent evidence suggests that this is not the only factor [68].

Future Developments and Clinical Utility

From a clinical perspective much of the emphasis on the potential application of MRT has been for the treatment of brain tumours and there is now extensive preclinical evidence to support the utility of this. For other tumour sites, preclinical evidence is lacking and further work needs to be carried out to ascertain if other sites, such as the lung, where normal tissue damage after irradiation is a limiting factor, could also benefit from MRT approaches.

The clinical utility and application of MRT is currently limited due to the reliance on large infrastructure synchrotron sources for the delivery of these beams. These are large facilities not optimised for patient treatment. All of the preclinical work in this area has taken place at Brookhaven National Laboratory in the US, ESRF in Geneva, Switzerland and the Australian National Synchrotron in Melbourne, Australia [69]. At the ESRF, beamline ID17 is the medical beamline and has already been used for patient treatments, with non-MRT exposures termed stereotactic synchrotron radiation therapy [70] but, to date, no clinical trials of MRT have been undertaken. Another challenge is the extreme high dose and dose rate that is required, although recent preclinical studies have shown that temporal fractionation of the dose delivery, into three sessions delivered over 3 days, may provide the same benefits [71]. Development of suitable treatment planning systems prior to studies in veterinary trials is also underway [72]. The recent interest in FLASH radiotherapy has led to renewed interest in the development of new compact accelerators that can deliver radiation at the high dose rates required for FLASH and potentially these could also be used to deliver MRT in the future in a more cost-effective manner [73,74]. Overall, there is probably considerable overlap between the utilities of FLASH, GRID and MRT if the underpinning principles governing their impact on the therapeutic ration can be defined.

Conclusions

MRT is the application of parallel micron-sized beams for delivering advanced spatially patterned irradiation. These spatial patterns have unique features that affect the protection of normal tissues, even after high dose exposure. When delivered as a series of overlapping arrays they can also increase the dose to the targeted tumours, leading to vascular collapse and the potential for abscopal/immune-mediated responses. Technological challenges currently limit the clinical application of these approaches, but alongside GRID and FLASH they have the potential to give significant improvements to the therapeutic ratio of spatially constrained radiotherapies in the future.

Conflicts of interest

The authors declare no conflict of interest.

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