Published in final edited form as:

Radiat Res. 2020 January ; 193(1): 1–4. doi:10.1667/RR15537.1.

The Importance and Clinical Implications of FLASH Ultra-High Dose-Rate Studies for Proton and Heavy Ion Radiotherapy

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Abstract

The goal of radiation therapy is to provide the highest probability of tumor control while minimizing normal tissue toxicity. Recently, it has been discovered that ultra-high dose rates of ionizing radiation may preferentially spare normal tissue over tumor tissue. This effect, referred to as FLASH radiotherapy, has been observed in various animal models as well as, more recently, in a human patient. This effect may be related to the cell sparing found *in vitro* at ultra-high dose rates of photons and electrons dating back to the 1960s. Conditions representative of physiologic oxygen were found to be essential for this process to occur. However, there is no conclusive data on whether this effect occurs with protons, as all results to date have been in cells irradiated at ambient oxygen conditions. There have been no ultra-high dose-rate experiments with heavy ions, which would be relevant to the implementation of FLASH to carbon-ion therapy. These basic science results are critical in guiding this rapidly advancing field, since clinical particle therapy machines capable of FLASH dose rates have already been promoted for protons. To help ensure FLASH radiotherapy is reliable and maximally effective, the radiobiology must keep ahead of the clinical implementation to help guide it. In this context, *in vitro* and *in vivo* proton and heavy ion experiments involving FLASH dose rates need to be performed to evaluate not only short-term consequences, but also sequelae related to long-term health risks. Critical to these future studies is consideration of relevant oxygen tensions at the time of irradiation, as well as appropriate *in silico* modeling to assist in understanding the initial physicochemical events.

The use of radiation therapy in the treatment of cancer relies on various techniques for sparing the normal tissue relative to the tumor (1). These methods capitalize on biological differences in the responses to radiation between normal and tumor tissues as well as technical approaches allowing relative sparing of normal tissue. Fractionation, the primary biological approach, involves the delivery of radiation in a series of small doses spaced out over time to reduce the incidence of radiation-induced side effects. The mechanistic underpinnings of fractionation have been explained by the four Rs of radiobiology: repair, reoxygenation, redistribution and repopulation. Advances in physics and technology, such as imaging, delivery of radiotherapy in an intensity-modulated pattern to conform to the shape of the tumor, computer treatment planning techniques and the generation of multi-leaf

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collimators, have contributed to reduced radiation-induced side effects by minimizing the dose to normal tissue. Proton and heavy-ion therapies capitalize on the physical and radiobiological properties of charged particles for an improved dose distribution and increased cell killing efficacy (2). Variations on these three strategies have been the standard approach to increasing the therapeutic ratio of tumor control over normal tissue toxicity.

A recent advance in the field, called FLASH radiotherapy, may be the next addition to the arsenal (3). It achieves increased sparing of the normal tissue by using ultra-high dose rates of ionizing radiation. These dose rates are on the order of 100 Gy/s, while conventional radiation therapy uses approximately 0.03 Gy/s. Although the phenomenon of cell sparing at ultra-high dose rates has been known since the late 1960s (4), its clinical utility has only recently started to receive recognition. There are now examples of FLASH radiotherapy allowing dose escalation in mouse (5–7) and porcine (8) models, felines (8) and even a human patient (9). However, full clinical implementation of this technique will require improvements in the physical aspects of radiation delivery (10). Beyond this, much more basic science work is required. Early published studies suggest that ultra-high dose rates can spare cells *in vitro* via oxygen depletion, as the effects were only observed at oxygen tensions close to physiologic (11). Moreover, although classical radiobiological principles cannot readily explain why this would spare the normal tissue but not the tumor, new models are being proposed to provide more testable hypotheses for this difference (12, 13).

The FLASH effect, or the relative sparing of cells and tissues at high dose rates, has been found after exposure to photon and electron radiations; however, it remains an open question as to whether this effect occurs with other therapeutic modalities, namely proton and heavy ion radiotherapies. These approaches have unique physical and radiobiological characteristics. Ion-based radiotherapies increase their energy deposition with depth, reaching a maximum toward the end of their path. In the context of cancer therapy, this means the normal tissue that is traversed by an ion in transit to the tumor receives a lower absorbed dose than the tumor. Particularly for heavy ions like carbon ions, there is also an improved relative biological effectiveness, resulting in an increased cell killing for a given dose of radiation. These normal tissue sparing effects associated with hadron therapy may be additive with FLASH radiotherapy, or perhaps even synergistic.

Currently, there are nine publications for ultra-high dose-rate irradiation with protons (Table 1) (14–24). Although the original *in vitro* ultra-high dose-rate studies with X rays and electrons found that only oxygen tensions closer to physiologic had a cell sparing effect (11, 25), as determined by clonogenic survival, all the proton studies were performed under ambient atmospheric conditions (21% O₂). This may provide the reason that all of these studies found no effect of ultra-high dose rates for acute toxicity end points. Notably, the one study in which proxy markers for late toxicities were examined showed that TGF β and cellular senescence were decreased at FLASH dose rates (20), suggesting that the toxic effects induced by inflammation and reduced cellular replication may be independent of the oxygen effects of FLASH radiotherapy. Overall, future *in vitro* studies will require examination of FLASH effects for protons at a range of oxygen tensions to determine the ideal conditions, as has been done for photons and electrons.

There are no published studies on the effect of ultra-high dose-rate irradiations using heavy ions, such as carbon ions, although this is possibly the most fascinating area for ultra-high dose-rate studies. Examination of the effects of oxygen will be even more important here than for protons. For heavy ions, oxygen enhancement of radiation-induced damage is most prominent at the end of their track (26), possibly due to the ability of heavy ions to generate molecular oxygen in their path (27). If the tissue sparing in FLASH radiotherapy is believed to be due to the depletion of oxygen in the cells (12), then an increased generation of oxygen at the heavy-ion flight terminus would result in an improved therapeutic ratio. For carbon-ion therapy, this could mean that theoretically, the normal tissue, being in the plateau region, would have the FLASH effect; however, the tumor cells would not, due to the molecular oxygen generated at the Bragg peak. Moreover, FLASH effects may be additive or even synergistic with the increased radiobiological effectiveness of carbon-ion therapy. Looking to the future, these added benefits of FLASH effects may help to encourage the clinical application of carbon-ion therapy in the U.S. and elsewhere, and may influence how the facility is constructed (28).

The studies exploring the underlying mechanism of FLASH and its role in ion therapies are timely. Current standard clinical linear accelerators cannot achieve FLASH dose rates in a way that would be meaningful for most radiotherapy treatments (10). With only small volumes close to the source able to be irradiated, they are still largely relegated to *in vitro* and *in vivo* experiments. To treat human cancers, larger fields and deeper penetration will be required. The intense heat produced from generating photons at ultra-high dose rates by Bremsstrahlung techniques presents a challenge with traditional clinical linear accelerators (29), although there are promising technical advances to overcome this (30). Similarly, proton and heavy-ion irradiations have their own questions and challenges to be overcome (31). For example, the delivery of these ions as a passively scattered beam versus a pencil beam may be significant in terms of achieving a FLASH effect (32). Despite this, FLASH proton therapy is already being promoted (33). The rapid pace of the field towards clinical implementation highlights the importance of understanding the underlying mechanisms for the FLASH effect. There must be a radiobiological basis to optimize this phenomenon to achieve the greatest likelihood of success, as well as to ensure that the FLASH effect can be reliably obtained. However, the focus cannot be exclusively on photons and electrons, as proton and heavy-ion facilities may be capable of generating clinically relevant FLASH dose rates. For instance, using a ridge filter on a passively scattered high-fluence beam is one elegant approach to achieving a spread-out Bragg peak at ultra-high dose-rates with protons (34). Thus, it will be important to determine if protons and heavy ions are equally capable of a FLASH effect. In this context, simulation methods employing Monte-Carlo techniques would greatly facilitate our physicochemical understanding of why FLASH occurs, and whether it should be expected in the context of protons or heavy ions. Computer modeling will help us estimate the yield of water radiolytic species as a function of time postirradiation and shed light on the spatial distribution of these species at times ranging from pico- to micro-seconds (35). The effects that microenvironmental parameters may exert in the biological responses (e.g., redox environment, pH, osmolality) need to be integrated in the models. The theoretical support provided by this approach, together with experimental results obtained *in vitro* and *in vivo*, will shed light on the relevance of different types of

ionizing radiation delivered at ultra-high dose rates in enhancing the therapeutic gain. They will also help us understand the extent of cellular damage induced by the direct and indirect effects of radiation under FLASH conditions.

Ultimately, whether using photons, electrons, protons or heavy ions, the integration of FLASH techniques is likely to have several clinical benefits. One benefit is that the reduced number of fractions resulting from the ability to apply larger acute doses with less normal tissue toxicity would spare patients repeated trips to the clinic. In addition, there may be advantages in outcomes from larger acute doses of radiation. Finally, if the FLASH effect truly gives a relative sparing of the normal tissue compared to the cancer cells, then larger fields could be used to treat microscopic disease with less toxicity. This last point would be significant for whole-brain irradiations to treat metastatic disease to the brain, whole-breast irradiations in breast cancer and whole-pelvis irradiations in prostate cancer. FLASH radiotherapy may allow the variables of fewer fractions, higher doses and larger field sizes to be incorporated into treatment protocols to attempt to improve patient outcomes and quality of life.

In conclusion, as the field moves toward clinical integration of ultra-high dose rates, the basic science needs to keep pace with the advances to ensure maximum benefit and reliability is obtained. In the context of proton and heavy-ion therapies, there is a need for *in vitro* and *in vivo* experiments performed at relevant oxygen tensions, as well as *in silico* experiments to help determine the early physicochemical events at ultra-high dose rates. Sensitive imaging that records in real time the rise and decrease of tissue oxygen concentration during and after irradiation will further enhance our understanding. The effects on later- and early-responding tissues, as well as the long-term risks of second cancers and the development of degenerative conditions in survivors would also need to be evaluated. Radiobiology, together with radiation physics, radiation chemistry and technological innovations, promises to make further additional contributions to cancer radiotherapy.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (NIH grant no. F30CA206389 to NWC) and the National Aeronautics and Space Administration (NASA grant no. NNX15AD62G to EIA and NWC).

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TABLE 1

Literature Review of Ultra-High Dose-Rate Experiments Using Protons or Heavy Ions

Ref.	Date	Model	Radiation type	Dose rate (Gy/s)	D _{max} (Gy)	O ₂	FLASH effect
(14)	2011	HeLa human cervical cancer cells	Proton	>10 ⁹ vs. 30 (pulse: <1 ns or 100 ms)	3	21%	No dose effect on G ₂ arrest, apoptosis, clonogenic survival
(15)	2012	V79 Chinese hamster lung fibroblasts	Proton (compared to 225 kVp X rays)	>10 ⁹ (for proton)	5	21%	Not determined
(16)	2012	HeLa human cervical cancer cells	Proton	>10 ⁹ vs. 50 (pulse: <1 ns or 100 ms)	5	21%	No for γ-H2AX foci
(17)	2014	FaDu human hypopharyngeal SCC cells implanted subcutaneously NMRI mice	Proton	>10 ⁹ vs. 50 (Pulse: <1 ns or 100 ms)	17.4	Physiologic	No for tumor growth delay
(18)	2017	HUVEC human umbilical vein endothelial cells	Proton	>10 ⁹ vs. "conventional"	4.5	21%	No for clonogenic survival
(19)	2017	HCT116 human colorectal cancer cells	Proton	>10 ⁸ vs. 0.05	~10	21%	No for clonogenic survival
(20)	2019	IMR90 human lung fibroblasts	Proton	0.05, 100, 1,000	20	21%	No for clonogenic survival and γ-H2AX foci formation Yes for senescence and TGFβ expression (20 Gy at 1,000 Gy/s)
(21)	2019	AG01522B human skin fibroblasts	Proton (compared to 225 kVp X rays)	>10 ⁹	1–2	21%	Yes for senescence and TGFβ expression (20 Gy at 1,000 Gy/s)
(22)	2019	Zebrafish embryo	Proton	0.08 vs. 100	43	21%	Not determined
(23)	2019	SF763 and U87 human glioblastoma cells, HCT116 human colorectal cancer cells	Proton	>10 ⁹ vs. "conventional"	10	21%	No for embryo survival, spinal curvature, and pericardial edema No for clonogenic survival or γ-H2AX foci formation

Notes. Results from a literature search of proton and heavy-ion experiments using ultra-high dose-rates. Nine unique studies were identified using protons at dose rates consistent with the FLASH effect. Notably, all the studies, except the one *in vivo* study by Zlobinskaya *et al.* (17), used ambient atmospheric oxygen conditions (21%). Only the study by Buonomo *et al.* (20) had positive findings under these conditions. There were no heavy-ion studies found that had dose rates consistent with a FLASH effect. Of note, one study, by Matsuura *et al.* (24), referenced ultra-high dose rate, but at 5.4 Gy/s this may not be enough to find a FLASH effect so was not included in the table. D_{max} = highest dose used at FLASH dose rates; SCC = squamous cell carcinoma.