Radiotherapy and Oncology 139 (2019) 62–63

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Letter to the Editor

Re: Differential impact of FLASH *versus* conventional dose rate irradiation: Spitz et al.,

The article by Spitz and colleagues describes an integrated physico-chemical approach to understanding the relative protection of normal tissue *versus* tumor tissue after ionizing radiation (IR) by the use of FLASH (>40 Gy/s) *versus* conventional (~0.05 Gy/s) dose-rates [1]. Due to the potential importance of FLASH radiotherapy [2,3], and inspired by the Spitz analysis, this letter suggests specific criteria and investigations that may improve future assessment. https://doi.org/10.1016/j.radonc.2019.03.028

1) Specify the time scales of interest [4]

A substantial portion of the Spitz paper compares 10 Gy in a single 1.8 μs pulse of electrons (FLASH) with the individual 3.5 μs pulses of a clinical LINAC. This comparison is somewhat misleading: the FLASH dose rate is ~5 x 10^6 Gy/s (>>>40 Gy/s), and the pulsatile nature of LINACS is likely irrelevant, since there are no reported differences between irradiation by LINAC *versus* constant dose-rate sources (such as X-rays and Cs-137) except for depth-dose changes and minor RBE decreases at high energy. If 10 Gy were delivered at ~40 Gy/s (FLASH) *versus* 0.05 Gy/s (Conventional), the total radiation times would be 0.25 s versus 200 s. Thus, emphasizing (radio)chemical or physiological events in these time-frames might be the most useful.

2) Specify the type(s) of cell killing being compared [5]

The references to the classical view of cell killing (loss of clonogens) include three books that do not properly encapsulate the detail of current knowledge derived from intense collaborations between radiation chemists and biologists in the 70's and 80's. While the Spitz paper correctly describes the generation of neutral target radicals after hydrogen abstraction by hydroxyl radicals, some important details are missing. As described, 10 Gy would generate about 3 μM of OH**.** implying a roughly 100 nm separation for individual, random OH**.** generation. This separation was used to rule out radical–radical interactions based on their diffusion-controlled rate constants within the local cellular milieu. However, a 100 nm separation was previously shown to directly conflict with the strong relationship between chromosome aberrations, DNA double-strand breaks (DSB's) and cell killing [6,7] (e.g. there was essentially zero probability to generate two or more OH· within the few nm required to make a DNA–DSB). This microdosimetric paradox was resolved by the realization that electron track ends produced spurs and blobs of ionizations at appropriate local densities, orders of magnitude larger than the average specified above [8]. Thus, one cannot necessarily rule out additional radical–radical interactions at FLASH compared with conventional dose-rates. These would have the effect of reducing the biological dose, compared with the physical dose, and might be identified by, for example, an increased production rate of hydrogen peroxide under FLASH *versus* conventional dose-rate conditions. Since such a dose reduction might be small, one would need substantial information on the dose–response for the normal tissue protection of interest (e.g. for an observable effect of a relatively small dose change, the dose–response would need to be quite steep).

3) Tissue pO_2 requirements for FLASH protection of normal tissue by radiochemical oxygen consumption (ROC) [9]

In contrast to (2) above, one might expect a substantially larger protection factor if the mechanism of normal tissue protection were ROC, as suggested in the Spitz paper. For this to apply, the FLASH dose would have to move the survival response from low to moderate resistance. Since the oxygen effect also takes place over a very short time frame (<3 ms – [10,11]) this response change would be continuous over the period of the dose administration. As long as the replacement of oxygen consumed by the radiation dose is limiting, this protection effect can been observed at both conventional as well as high dose-rates [11,12]. For cells with low thiol-content, such as the V79 or CHO cells used in most of the original determinations, the K_m for radiation resistance is about 3 mm Hg (this corresponds to about 4 μM oxygen in air-equilibrated physiological saline at room temperature). There are very few reports that specifically document ROC, but we showed that ROC occurred almost independently of initial oxygen content and with a rate of roughly −4 μM/10 Gy [9]. Thus, to get the maximum protective effect of ROC for 10 Gy (the dose considered in the Spitz paper) one would require that the initial pO_2 be somewhat above the K_m. The authors considered brain as a normal target tissue and a much higher initial pO_2 (20 mm Hg), but also postulated a roughly six-fold higher rate of ROC due to chain-oxidations in membrane lipids. While this would need to be confirmed experimentally, the authors have not considered that oxygen solubility is also much higher in lipids (~10-fold). This has the effect of causing roughly similar (and much lower) decreases in oxygen partial pressure for the local microenvironment of membranes *versus* the spatially distinct DNA. Additionally, it should be remembered that lipid chain oxidations are enhanced at high pH and would be limited by chain terminators such as vitamin E; they are known to be enhanced by low, not high, dose rates [13]. Hydroxyl radical scavengers such as DMSO, while very effective at protecting against radiation-induced lethality, do not protect against lipid peroxidation [14], suggesting a lack of relationship between DNA damage and lipid damage. Finally, there are much less invasive and more accurate measurements of brain pO_2 than the microelectrode measurements described ([1] – Ref. [35]). Using either phosphorescence decay or EF5 binding, pO_2

https://doi.org/10.1016/j.radonc.2019.07.004

levels in normal brain have been estimated to be more than 35 mm Hg [15,16]. This seems a more reasonable starting point considering that the mixed venous pO_2 is roughly 40 mm Hg.

Other potential normal tissue endpoints (gut and bone-marrow death) are even more likely attributable to clonogenic death, and their dose limitations (roughly 15 Gy and 10 Gy in mice, respectively) are not suggestive of a hypoxia-based underlying radiation response. Most importantly, tumors grow in the normal tissue at risk, and one would predict that their average pO_2 would always be lower than that in the encompassing normal tissue. Thus, it would seem as if tumors, not normal tissue, have the key advantage in terms of possible protection by ROC. Finally, if ROC is ~ 4 μM/10 Gy, or even higher, it is essential to compare this with endogenous cellular oxygen consumption. For human and mouse brain tissue this is about 22 and 42 μM/sec, respectively [17,18]. Thus, ROC by 10 Gy over a time frame of 0.25 second is less than normal-tissue oxygen consumption.

4) Peroxides and peroxyl radicals are the damaged species, not the damaging species [19]

One often finds oxygen-derived ROS and their reactions described in the modern literature as being comparable in toxicity to the water-derived free-radicals produced by IR. Whenever this possibility has been tested however, the latter have been found to have greatly differing characteristics and much greater toxicity. For example, the average toxicity of 10 Gy, causing about 10^6 OH radicals per cell, is equaled by about 50,000-fold higher numbers of H_2O_2 molecules, the main peroxide produced by radiation [20,21]. The principle reasons for this vast discrepancy are the very high degree of protection afforded by catalase and glutathione peroxidase against hydrogen peroxide and the inability of peroxides to cause DNA-DSB's [19]. Thus it is critically important, when describing the sequellae that occur following IR, to keep in mind (1) that ROC occurs through multiple reactions of primary and secondary radicals (e.g. e^-aq., H., R–CH.–R′) with oxygen, producing new radicals. Oxidation of the last of these is generally in competition with reduction by a thiol, and in the particular case that the carbon radical is part of the DNA backbone is the precise chemical mechanism for the oxygen effect [9].

Summary

FLASH protection by ROC is an interesting hypothesis that is not easily testable *in vivo*. Considerations of ROC as the mechanism of normal-tissue protection by FLASH, described above, seem at odds with several well-documented concepts of radiation chemistry and physics, as well as normal-tissue physiology.

Acknowledgement

Work supported by Department of Radiation Oncology, University of Pennsylvania.

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Available online 17 August 2019