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To cite this article: Guillem Pratx and Daniel S Kapp 2019 *Phys. Med. Biol.* **64** 185005

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PAPER

A computational model of radiolytic oxygen depletion during FLASH irradiation and its effect on the oxygen enhancement ratio

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Keywords: FLASH irradiation, computational model, oxygen enhancement ratio, hypoxia

RECEIVED
16 May 2019REVISED
10 July 2019ACCEPTED FOR PUBLICATION
31 July 2019PUBLISHED
11 September 2019**Abstract**

Recent results from animal irradiation studies have demonstrated the potential of ultra-high dose rate irradiation (also known as FLASH) for reducing radiation toxicity in normal tissues. However, despite mounting evidence of a ‘FLASH effect’, a mechanism has yet to be elucidated. This article hypothesizes that the radioprotecting effect of FLASH irradiation could be due to the specific sparing of hypoxic stem cell niches, which have been identified in several organs including the bone marrow and the brain. To explore this hypothesis, a new computational model is presented that frames transient radiolytic oxygen depletion (ROD) during FLASH irradiation in terms of its effect on the oxygen enhancement ratio (OER). The model takes into consideration oxygen diffusion through the tissue, its consumption by metabolic cells, and its radiolytic depletion to estimate the relative decrease in radiosensitivity of cells receiving FLASH irradiation. Based on this model and the following parameters (oxygen diffusion constant $D_{O_2} = 2 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, oxygen metabolic rate $m = 3 \text{ mmHg s}^{-1}$, ROD rate $L_{\text{ROD}} = 0.42 \text{ mmHg Gy}^{-1}$, prescribed dose $D_p = 10 \text{ Gy}$, and capillary oxygen tension $p_0 = 40 \text{ mmHg}$), several predictions are made that could be tested in future experiments: (1) the FLASH effect should gradually disappear as the radiation pulse duration is increased from $<1 \text{ s}$ to 10 s ; (2) dose should be deposited using the smallest number of radiation pulses to achieve the greatest FLASH effect; (3) a FLASH effect should only be observed in cells that are already hypoxic at the time of irradiation; and (4) changes in capillary oxygen tension (increase or decrease) should diminish the FLASH effect.

1. Introduction

For decades, radiobiological studies have sought to understand the acute and late effects ionizing radiation delivered at different dose rates. Clonogenic survival studies of bacterial and mammalian cells irradiated at ultra-fast dose rates ($>100 \text{ Gy s}^{-1}$) have shown that the slope of survival curves transition from aerobic to anaerobic response (Nias *et al* 1969, Berry and Stedeford 1972, Epp *et al* 1972)—a phenomenon attributed to radiolytic oxygen depletion (ROD), a transient decrease in oxygen tension caused by radiochemical reactions (Weiss *et al* 1974). However, since the effect was seen only in cells already at reduced levels of oxygen ($<4 \text{ mmHg}$), it was concluded by some that ‘no special advantages are likely to accrue from the use of radiations at ultra-high dose rates for the radiotherapy of human cancers’ (Berry and Stedeford 1972).

More recently, investigators at the Institut Curie in France and the Centre Hospitalier Universitaire Vaudois in Switzerland have reported unexpectedly low normal tissue toxicities in animals receiving ultra-high dose rate irradiation. Using short pulses ($<1 \text{ s}$) of radiation delivered at dose rates of 40 Gy s^{-1} or greater (known as FLASH), they and others have found less pulmonary fibrosis and less epithelial cell acute radiation apoptosis (Favaudon *et al* 2014), sparing of brain function (Montay-Gruel *et al* 2017, 2019, Simmons *et al* 2019), reduced depilation and fibronecrosis (Vozenin *et al* 2019a), and higher survival after abdominal radiation (Loo *et al* 2017). In addition, a FLASH effect has also been reported on the induction of alterations in the morphology of zebrafish following irradiation of fertilized zebrafish eggs (Vozenin *et al* 2019b). The potential of FLASH

irradiation for improving the therapeutic ratio has sparked significant interest in the radiation oncology community and the topic has been covered by several recent review articles (Durante *et al* 2018, Al-Hallaq *et al* 2019, Bourhis *et al* 2019, Vozenin *et al* 2019b).

However, despite mounting evidence of normal tissue sparing by FLASH radiation therapy, a mechanism of action has not yet been demonstrated (Durante *et al* 2018). Normal tissues are known to be well oxygenated and, based on existing *in vitro* evidence, no biologically significant ROD would be expected in these tissues at clinical dose levels (i.e. 10 Gy). However, normal tissue oxygenation is also highly heterogeneous, and the effect of FLASH irradiation may vary spatially within tissues. Recently, on the basis of a qualitative model of ROD (Pratz and Kapp 2019), we hypothesized that FLASH irradiation may protect normal tissues by specifically sparing hypoxic stem cell niches, which have been highlighted in several organs including the bone marrow and the brain. Stem cells are known for their powerful tissue regeneration capabilities. For instance, a previous study showed that the transfer of 4×10^5 unirradiated stem cells to the hippocampus of brain-irradiated mice was sufficient to rescue mice from significant cognitive impairments (Acharya *et al* 2009).

To better understand the response of hypoxic stem cell niches to FLASH irradiation, we developed a novel quantitative model of ROD in tissues. This model includes a number of parameters such as oxygen depletion rate, concentration, metabolism and diffusion. We assess the effect of FLASH irradiation by computing the relative decrease in the oxygen enhancement ratio (OER) due to ROD, a factor we call δ_{ROD} . Following this analysis, we make several predictions regarding the effect of FLASH radiotherapy for different radiation doses, dose rates, oxygen levels, and pulse structures. Testing of these predictions experimentally could be useful to help elucidate the mechanism of FLASH irradiation.

2. ROD at ultra-high dose rate ($> 100 \text{ Gy s}^{-1}$)

2.1. Theory

We first consider the case where radiation is delivered fast enough that the rate of ROD greatly exceeds changes in oxygen tension due to oxygen diffusion and metabolism in the tissue. In this first model, oxygen diffusion and metabolism are assumed to be negligible. A second model is presented in the next section where these effects are taken into consideration.

The effect of oxygen (or lack thereof) on the response to ionizing radiations has been extensively studied *in vitro*, in preclinical models, and in patients (Vordermark and Horsman 2016). This effect is typically quantified according to the OER, the ratio of the dose in anoxia to the dose in air required to achieve a defined rate of cell survival, and has been measured under a wide range of radiation dose rates (Ling *et al* 1985, 2010). Oxygen enhancement of radiation damage is achieved when oxygen is present at the time of the irradiation or up to a few milliseconds after irradiation (Michael *et al* 1973). The effect is due to two factors, (i) oxygen-enhanced formation of deleterious free radicals and (ii) rapid fixation of DNA damage—the so-called oxygen fixation hypothesis (Schwartz 1952, Alper and Howard-Flanders 1956). Several parameterizations have been proposed to fit experimental cell survival data according to oxygen level. For instance, the classical Alper-Howard-Flanders equation models the OER as a ratio of two parameters (Alper and Howard-Flanders 1956). A more recent parameterization is that of Grimes and Partridge (2015), used in this study,

$$\text{OER}(p) = 1 + \frac{\Phi_0}{\Phi_D} (1 - e^{-\varphi p}) \quad (1)$$

where $\Phi_0/\Phi_D = 1.63$ and $\varphi = 0.26$.

Radiochemistry experiments have shown that oxygen can be depleted during irradiation due to its reaction with two byproducts of water radiolysis, the solvated electron and the hydrogen radical (figure 1(a)). Irradiation of water with low linear-energy-transfer (LET) radiation produces solvated electrons and hydrogen radicals at a combined rate of 5.1 molecules per 100 eV, equivalent to $0.5 \mu\text{M Gy}^{-1}$ (Colliaux *et al* 2015). A water solution in equilibrium with 3.8 mmHg (0.5%) oxygen contains approximately $5.2 \mu\text{M}$ of dissolved oxygen, according to Henry's law ($K = 960 \text{ atm L mol}^{-1}$ at 37°C), thus a radiation dose of 10 Gy would generate a sufficient number of oxygen-depleting radicals to achieve stoichiometry with oxygen in a reaction that would yield the superoxide radical and its protonated form, HO_2 , as products (figure 1(a)). While cytotoxic, these radicals can also be neutralized by enzymes within cells. Importantly, oxygen depleted through this radiolytic process is no longer available to fix DNA damage, leading to a potential increase in clonogenic cell survival.

The depletion of oxygen through water radiolysis has also been reported experimentally in hermetically sealed solutions. Oxygen is depleted from these solutions in proportion to the radiation dose applied, at rate $L_{\text{ROD}} = 0.21\text{--}0.42 \text{ mmHg Gy}^{-1}$ (Weiss *et al* 1974, Whillans and Rauth 1980, Michaels 1986). This depletion rate is independent of initial oxygen concentration. Ignoring oxygen diffusion and metabolism, the oxygen tension following a FLASH dose of radiation D is

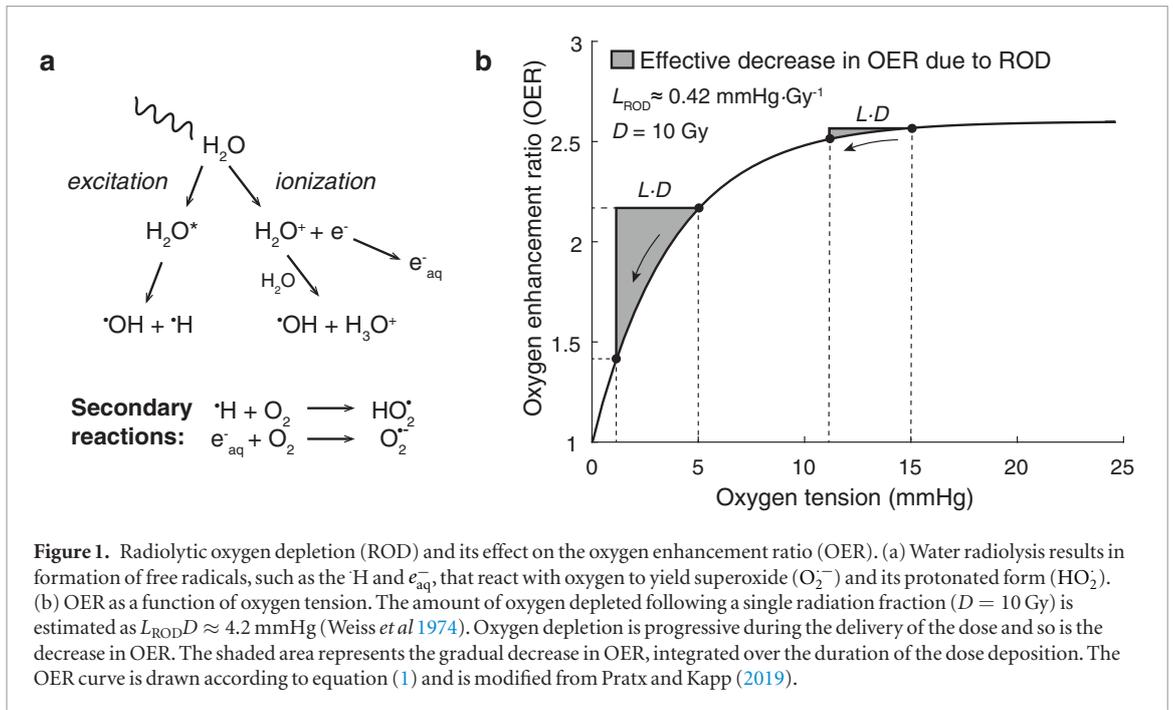


Figure 1. Radiolytic oxygen depletion (ROD) and its effect on the oxygen enhancement ratio (OER). (a) Water radiolysis results in formation of free radicals, such as the $\cdot\text{H}$ and e^-_{aq} , that react with oxygen to yield superoxide ($\text{O}_2^{\cdot-}$) and its protonated form (HO_2^\cdot). (b) OER as a function of oxygen tension. The amount of oxygen depleted following a single radiation fraction ($D = 10 \text{ Gy}$) is estimated as $L_{\text{ROD}}D \approx 4.2 \text{ mmHg}$ (Weiss *et al* 1974). Oxygen depletion is progressive during the delivery of the dose and so is the decrease in OER. The shaded area represents the gradual decrease in OER, integrated over the duration of the dose deposition. The OER curve is drawn according to equation (1) and is modified from Pratz and Kapp (2019).

$$p = p_0 - L_{\text{ROD}}D, \quad (2)$$

where p_0 is the oxygen tension before irradiation.

Accordingly, the effect of ROD can be qualitatively understood as a shift of the OER curve by an amount equal to $L_{\text{ROD}}D$ (Pratz and Kapp 2019). However, it is also important to consider that ROD occurs over microseconds, similar to the time scale of DNA damage fixation (Colliaux *et al* 2015). Starting from a baseline level p_0 , oxygen is gradually depleted according to equation (2), where D varies from 0 to a prescribed dose D_p . As this process takes place, the tissue is made progressively more resistant to radiation due to the continuous decrease in OER.

Considering an infinitesimal dose increment dD , the fractional cell kill can be expressed as:

$$\frac{dN}{N(D)} = -\alpha(p_0 - L_{\text{ROD}}D) dD, \quad (3)$$

where $N(D)$ is the number of surviving cells after a dose D and $\alpha(p)$ is the radiosensitivity under oxygen tension p . Since $L_{\text{ROD}}D$ may exceed p_0 , the radiosensitivity function is extended such that $\alpha(p) = \alpha(0)$ for $p < 0$ (total oxygen depletion).

It is important to note that radiochemical reactions that result in ROD are not instantaneous (Colliaux *et al* 2015). Equation (3) assumes that oxygen depletion resulting from the deposition of dose D is complete when estimating the fractional cell killing mediated by dose increment dD . For ultra-short dose delivery times (i.e. on the order of 10^{-6} – 10^{-4} s), the model may overestimate the effect of ROD on the OER, thus, it should only be applied to radiation treatments delivered over > 1 ms.

Next, the fractional cells killed is integrated up to prescribed dose D_p to yield the log-surviving fraction

$$\log\left(\frac{N_p}{N_0}\right) = -\int_0^{D_p} \alpha(p_0 - L_{\text{ROD}}D) dD, \quad (4)$$

where $N_p \equiv N(D_p)$ is the number of surviving cells after prescribed dose D_p . This formula can be applied to the conventional irradiation by setting $L_{\text{ROD}} = 0$ (no ROD), resulting in the classical exponential cell kill model

$$\log\left(\frac{N_p}{N_0}\right)\Big|_{L_{\text{ROD}}=0} = -\alpha(p_0) D_p. \quad (5)$$

To better understand the effect of ROD during FLASH irradiation relative to conventional irradiation, we reformulate equation (4) as

$$\log\left(\frac{N_p}{N_0}\right) = (1 - \delta_{\text{ROD}}) \log\left(\frac{N_p}{N_0}\right)\Big|_{L_{\text{ROD}}=0} \quad (6)$$

where δ_{ROD} is defined as

$$\delta_{\text{ROD}} = \frac{\alpha(p_0) D_p - \int_0^{D_p} \alpha(p_0 - L_{\text{ROD}} D) dD}{\alpha(p_0) D_p}. \quad (7)$$

This term is a function of two factors, the oxygen tension p_0 and the prescribed dose D_p . Because a linear radiosensitivity model is assumed, δ_{ROD} can also be expressed as a function of the OER:

$$\delta_{\text{ROD}} = \frac{\text{OER}(p_0) D_p - \int_0^{D_p} \text{OER}(p_0 - L_{\text{ROD}} D) dD}{\text{OER}(p_0) D_p}, \quad (8)$$

where OER is defined as a ratio of radiosensitivities

$$\text{OER}(p) = \frac{\alpha(p)}{\alpha(0)}. \quad (9)$$

In the following, we use δ_{ROD} to quantify the relative effect of ROD during FLASH irradiation on fractional cell survival. For conventional irradiation, ROD is negligible and, therefore, $\delta_{\text{ROD}} = 0$. More generally, the term δ_{ROD} can be interpreted as the relative decrease in radiosensitivity attributable to ROD.

2.2. Results

Using the formalism developed in section 2.1, we investigate how δ_{ROD} varies as a function of the prescribed dose (single fraction) and the partial pressure of oxygen in the tissue. In these experiments, we assume $L_{\text{ROD}} = 0.42 \text{ mmHg Gy}^{-1}$, based on previous measurements of ROD in cells irradiated at ultra-high dose rates (Weiss *et al* 1974). We also assume a dose delivery time of $\sim 1\text{--}10$ ms, longer than most radiochemical processes but shorter than the timescale of O_2 diffusion and metabolism.

We first plot δ_{ROD} according to various isoeffect lines, such as 1%, 10%, 20%, and so forth (figure 2(a)). As the amount of dose delivered to the tissue increases, oxygen is increasingly depleted and cells become more radioresistant. This is better seen in figure 2(b), where we have plotted the same data for five different radiation doses. At the low-dose level (1 Gy), the effect of ROD on effective dose is marginal and limited to very low oxygen levels. For a dose of 10 Gy, we observe a significant decrease in radiosensitivity of up to 30% due to ROD. However, radio-protection is only imparted to cells residing within a narrow range of oxygen levels of 3–5 mmHg. For higher radiation doses (≥ 30 Gy), this range broadens considerably but such high single-fraction doses are rarely used in the clinic.

We finally consider the effect of ROD on clonogenic cell survival at three different oxygen level, 0, 4 and 40 mmHg. Similar to equation (3), radiosensitivity is assumed to be linear with dose. Under this assumption, we make the following observations: for anoxic or normoxic cells (40 mmHg), there is no difference between FLASH and conventional dose-rate radiation. It is only for hypoxic cells ($p\text{O}_2 = 4 \text{ mmHg}$) that the two curves diverge (figure 2(c)). The survival curve obtained during FLASH irradiation is initially parallel to the curve obtained for the conventional treatment. Once oxygen is fully depleted (which occurs at dose $D_p = \frac{p_0}{L_{\text{ROD}}} = 9.5 \text{ Gy}$), a breakpoint is observed as the survival curve becomes parallel to the survival curve of anoxic cells.

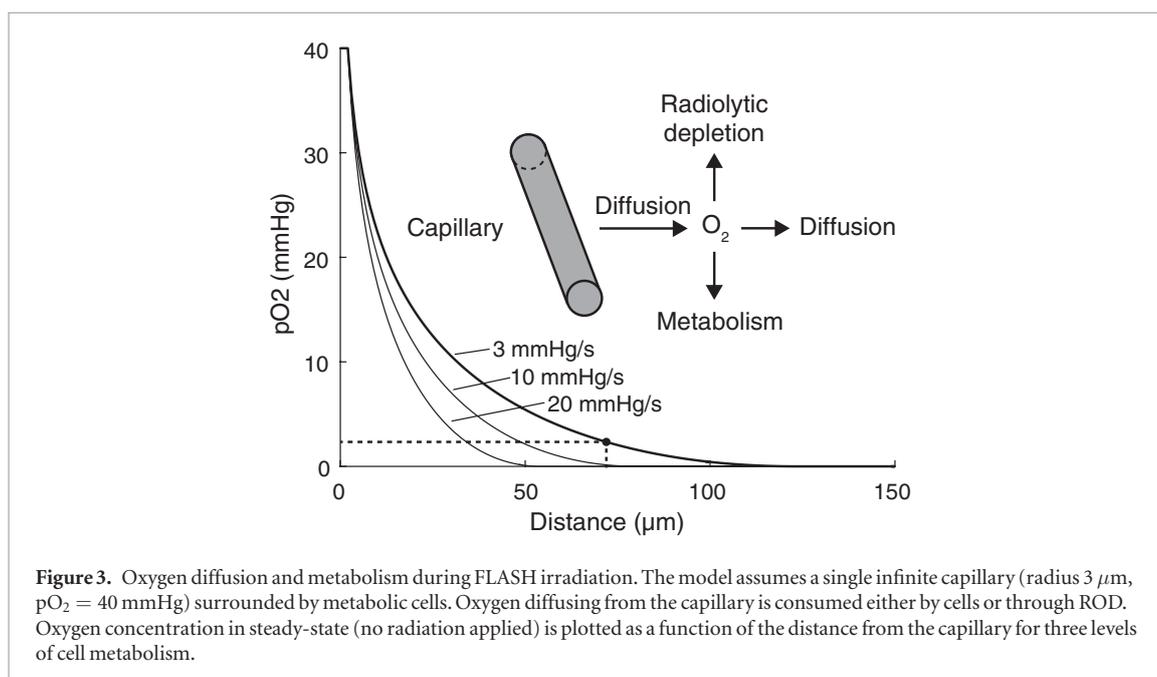
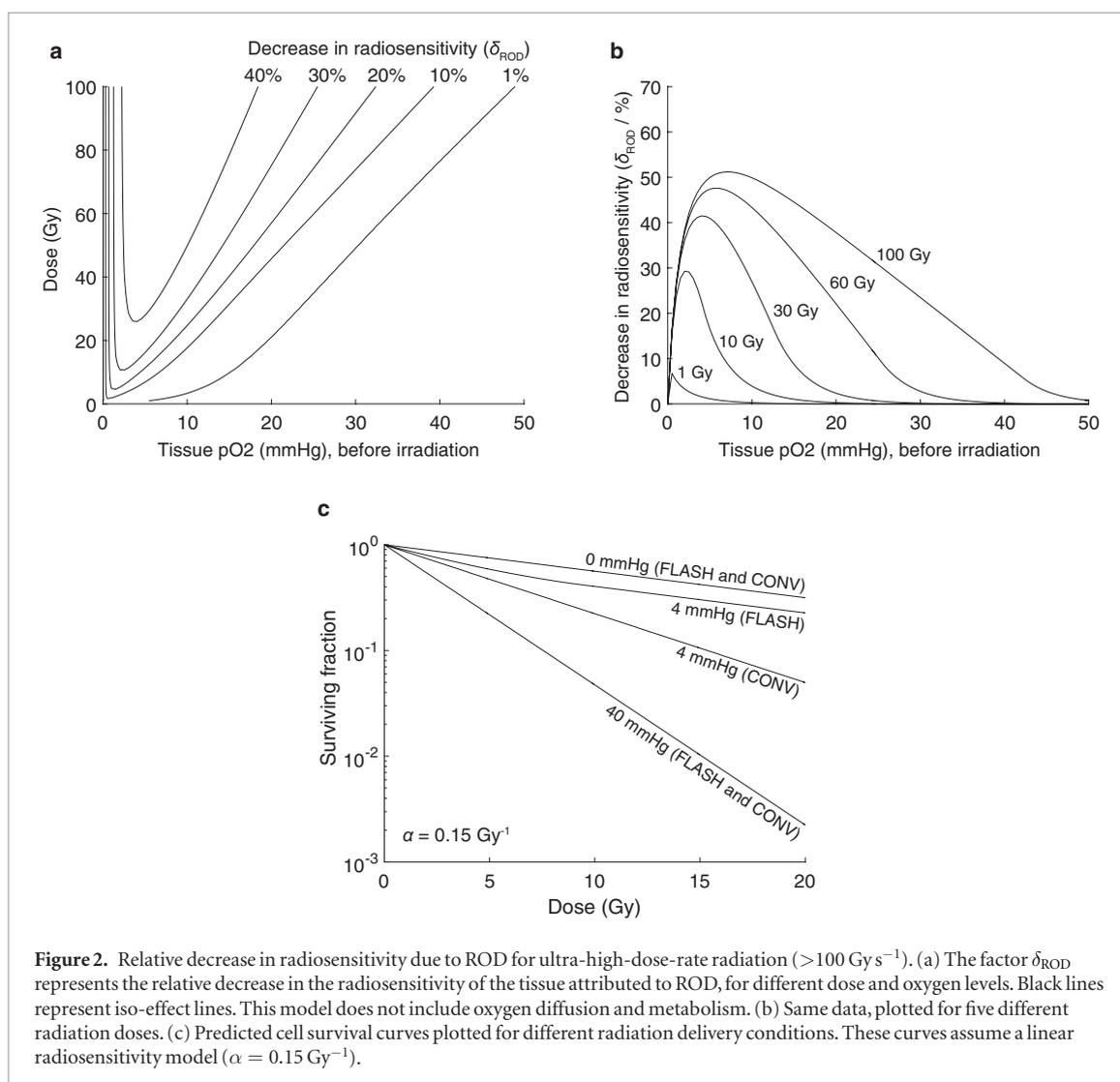
3. ROD at very-high dose rate ($> 10 \text{ Gys}^{-1}$)

3.1. Theory

In the previous section, we assumed that changes in oxygenation due to cell respiration and tissue diffusion were negligible in comparison to ROD due to the high dose rate. Here, we develop a spatiotemporal model that includes the effect of these two important factors. The diffusion of oxygen from a capillary into the surrounding tissue is modeled according to the diffusion equation in polar coordinates. We assume an infinitely long capillary (radius $r_0 = 3 \mu\text{m}$) and set oxygen tension within it to a fixed value, p_0 . Due to the equilibrium between dissolved oxygen and hemoglobin-bound oxygen, we can assume that ROD does not affect oxygen tension inside the capillary. We also assume a diffusion constant $D_{\text{O}_2} = 2 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Ferrell and Himmelblau 1967) and a rate m of oxygen consumption through cellular metabolism. As explained in the previous section, the rate of ROD is equal to $L_{\text{ROD}} D_p / T$, where L_{ROD} is the ROD rate constant, D_p is the prescribed dose, and T is the radiation pulse duration. The diffusion and metabolism of oxygen in the tissue is described by the following equation:

$$\frac{\partial p}{\partial t} = D_{\text{O}_2} \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial p}{\partial r} \right) - m - \frac{L_{\text{ROD}} D_p}{T}. \quad (10)$$

We first calculate the steady-state oxygen tension around the capillary before irradiation ($D_p = 0$), which we call $p_{\text{SS}}(r)$. We set the boundary condition as $p_{\text{SS}}(r \leq r_0) = p_0$. The rate of oxygen metabolism m is a parameter of the model. Based on these curves, we select a metabolic rate of oxygen of 3 mmHg s^{-1} (equivalent to $4.1 \mu\text{M s}^{-1}$) to achieve diffusion of oxygen up to $100 \mu\text{m}$ from the capillary (figure 3).



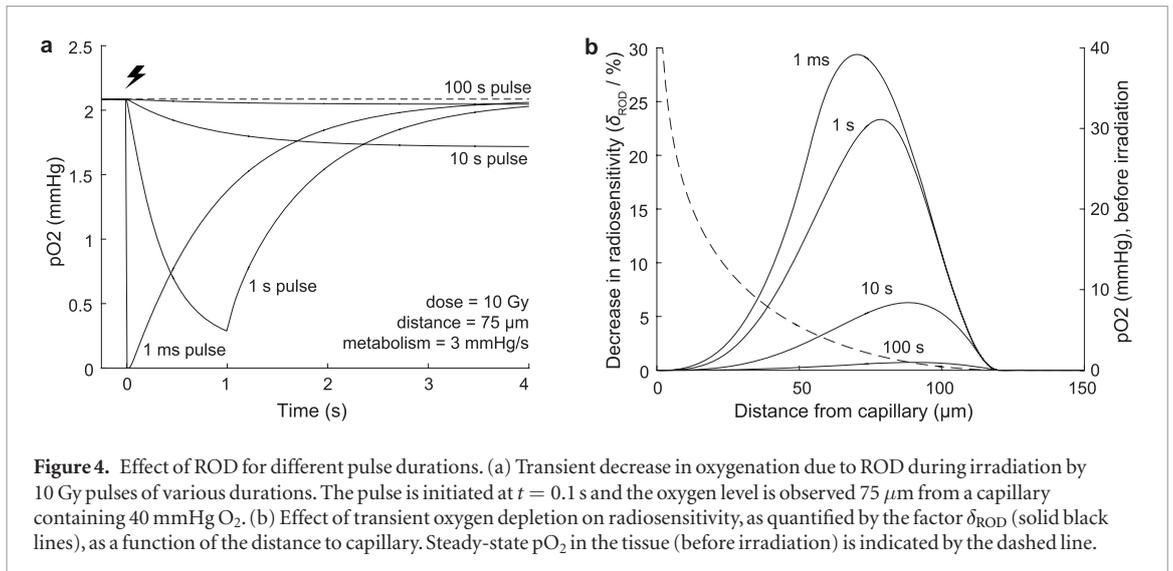


Figure 4. Effect of ROD for different pulse durations. (a) Transient decrease in oxygenation due to ROD during irradiation by 10 Gy pulses of various durations. The pulse is initiated at $t = 0.1$ s and the oxygen level is observed $75 \mu\text{m}$ from a capillary containing 40 mmHg O_2 . (b) Effect of transient oxygen depletion on radiosensitivity, as quantified by the factor δ_{ROD} (solid black lines), as a function of the distance to capillary. Steady-state pO_2 in the tissue (before irradiation) is indicated by the dashed line.

The diffusion equation is then solved again to model transient oxygen depletion during FLASH irradiation. The boundary conditions for this equation are $p(t=0, r) = p_{\text{SS}}(r)$ and $p(t, r \leq r_0) = p_0$. Oxygen tension in the tissue $p(t, r)$ is then computed as a function of time and distance from capillary using the finite difference method ($\Delta r = 2 \mu\text{m}$ and $\Delta t = 0.1 \mu\text{s}$) implemented in MATLAB (version R2015b).

Once the oxygen tension $p(r, t)$ is known, the survival cell fraction using the approach described in the previous section:

$$\log\left(\frac{N_p}{N_0}\right) = -\frac{D_p}{T} \int_0^T \alpha(p(r, t)) dt. \quad (11)$$

Here, the integration is performed over time, which is equivalent to equation (4) given that $D = \frac{L_{\text{ROD}} D_p}{T} t$. Similar in equation (6), we introduce δ_{ROD} to quantify the decrease in radiosensitivity relative to conventional irradiation (no ROD, $L_{\text{ROD}} = 0$):

$$\delta_{\text{ROD}} = \frac{\text{OER}(p_{\text{SS}}) - \frac{1}{T} \int_0^T \text{OER}(p(r, t)) dt}{\text{OER}(p_{\text{SS}})}. \quad (12)$$

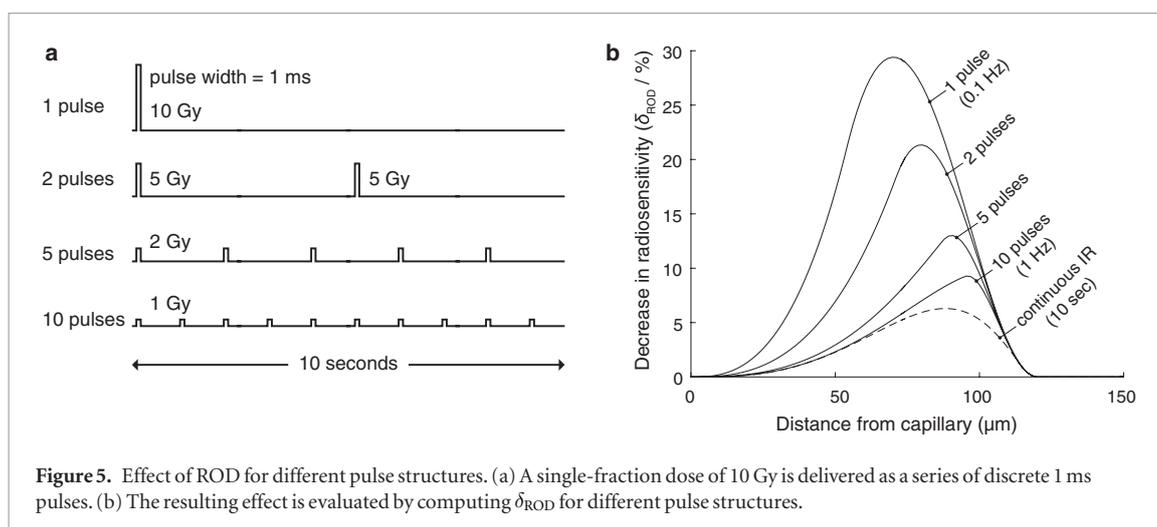
It should be noted that, according this definition, δ_{ROD} is a complicated factor that depends on several parameters, including oxygen pressure in the capillary (p_0), duration of the radiation pulse (T), total dose delivered (D_p), and distance to the capillary (r).

3.2. Results

At very high dose rates ($> 10 \text{ Gy s}^{-1}$), ROD remains a significant effect, yet oxygen diffusion and metabolism also affect the balance of oxygen within the tissue. Using the diffusion equation, we first simulate transient oxygen depletion during irradiation with 10 Gy pulses of different durations (figure 4(a)). To simplify our analysis of the results, we focus on a small volume of tissue located $75 \mu\text{m}$ from a capillary, thus hypoxic ($\text{pO}_2 = 2.1 \text{ mmHg}$, see figure 3). First, we consider a single 1 ms pulse of 10 Gy (dose rate = 10^4 Gy s^{-1}). At this high dose rate, the rate of ROD greatly exceeds oxygen diffusion and no significant replenishment occurs during the delivery of the pulse, and oxygen is entirely depleted. Full replenishment of the irradiated tissue occurs 2–3 s after irradiation. We then simulate a longer pulse of 1 s (dose rate = 10 Gy s^{-1}). Due to the lower dose rate, oxygen lost through ROD is partially replenished, preventing its complete depletion. Finally, longer pulses of radiation (10 and 100 s) are unable to deplete oxygen from the tissues as required for significant radioprotection.

We then consider the anticipated effect of pulse duration on radiosensitivity (as measured by δ_{ROD}) as a function of the distance to the capillary. For the shortest pulse (1 ms), the strongest decrease in radiosensitivity is achieved in tissues 60 – $100 \mu\text{m}$ from the capillary (figure 4(b)). As in the previous section, we assume a dose delivery time longer than most radiochemical processes. Based on radiosensitivity, these tissues would experience an effective decrease in cell radiosensitivity of 20%–25% compared to conventional dose rate irradiation. For the 1 s pulse duration, a similar albeit weaker effect is observed due to partial reoxygenation during dose delivery. Finally, only a marginal decrease in cell radiosensitivity ($< 5\%$) is expected for longer pulses (10 and 100 s).

We then turn to the effect of pulse structure on ROD and radiosensitivity. In this simulation, a total dose of 10 Gy is delivered over 10 s as a series of discrete 1 ms pulses (figure 5(a)). Results from these simulation shows that the effect of ROD on radiosensitivity becomes negligible as the pulse repetition rate is increased (figure 5(b)).



This is because oxygen is replenished between each pulse, limiting its depletion. The strongest effect on δ_{ROD} was measured for single pulses. Fractionation of the radiation into 10 pulses (each separated by 1 s) caused δ_{ROD} to drop below 10%, approaching the value observed for continuous irradiation (dashed line). As radiation was fractionated into an even greater number of pulses, the effect on ROD became undistinguishable from continuous dose delivery and no substantial difference could be observed between irradiation at 10 Hz and continuous irradiation.

Finally, we determine how changes in capillary oxygen tension may increase or decrease the magnitude of ROD during FLASH irradiation. Tissue oxygenation could be manipulated experimentally by carbogen and nitrogen breathing to help better understand the potential role of ROD to normal tissue sparing by FLASH radiotherapy.

According to the model, changing oxygen tension in the capillary shifts the location in the tissue where ROD yields the greatest radioprotecting effect but does not affect the magnitude of the effect (figure 6(a)). For instance, if capillary oxygen tension is decreased from 40 to 20 mmHg, the region in which ROD protects cells will move closer to the capillary, from 75 μm to 45 μm , but the decrease in radiosensitivity δ_{ROD} will remain the same. Conversely, if oxygen levels are increased, this region will move further away from the blood supply. This effect is shown in figure 6(b), where the effect of ROD on radiosensitivity is shown for a region of tissue distant 75 μm from the capillary. Overall, these data suggest that radioprotection of cells by ROD would be strongly affected by changes in capillary oxygenation.

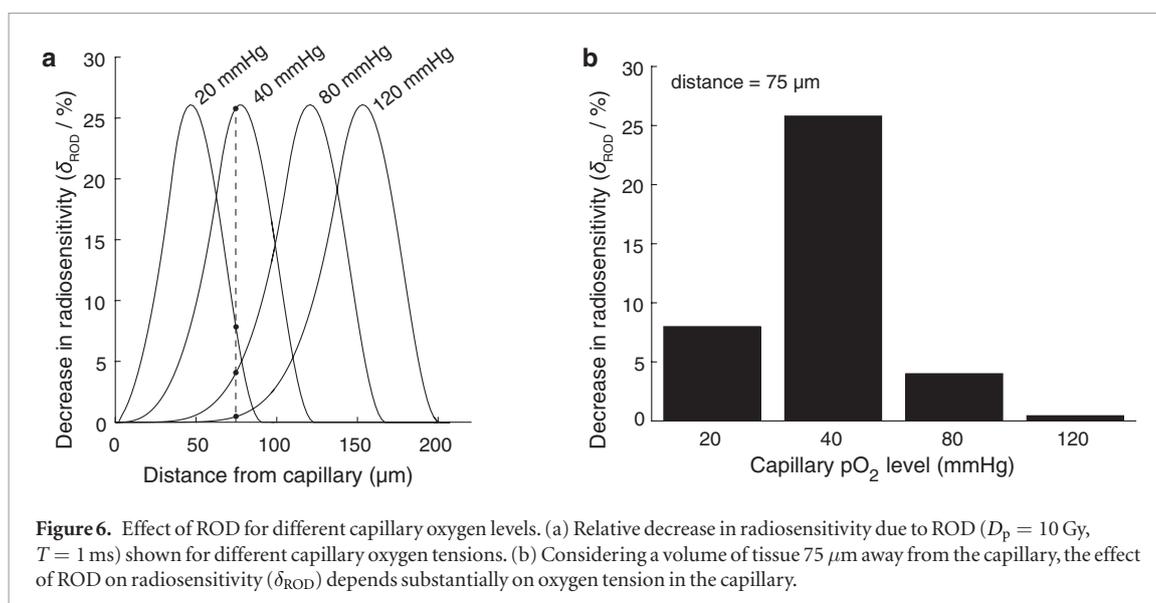
4. Discussion

We presented two different models of ROD during FLASH irradiation. In the first model, we consider radiation delivered at ‘ultra-high’ dose rate ($>100 \text{ Gy s}^{-1}$). For this type of irradiation, diffusion and metabolism of oxygen can be neglected and the effect of ROD on oxygen enhancement depends only on the prescribed single-fraction dose and initial oxygen concentration in the tissue. For a single-fraction dose of 10 Gy, the biological effect of ROD is expected only in hypoxic tissues (pO_2 of 2–5 mmHg) based on the expected ROD rate of $0.42 \text{ mmHg Gy}^{-1}$.

The second model considers the case where radiation is delivered at a ‘very high’ dose rate, defined as 10 Gy s^{-1} or higher. Using the diffusion equation to model the replenishment of interstitial oxygen, we find that the effect of ROD diminishes as radiation pulses increase in duration. While no clear threshold is observed, no substantial radioprotection is expected for pulses of radiation longer than 1–10 s.

Additionally, fractionation of the radiation into a series of discrete 1 ms pulses limited the benefits of ROD as oxygen was replenished in between pulses. Clinical linear accelerators typically deliver radiation as 5 μs pulses, with a repetition rate of 60–360 Hz. In a recent study, a clinical linear accelerator was modified to deliver electron radiotherapy at ultra-high dose rate to small animals and cells (Schüler *et al* 2017). In one experiment, a total dose of 30 Gy was delivered in $<0.16 \text{ s}$ as a train of 2 μs pulses and a repetition rate of 108–180 Hz to the brain of anesthetized mice (Simmons *et al* 2019). Our quantitative ROD model predicts that the radiobiological effect of such pulsed irradiation scheme is independent of the pulse structure and would be similar to the same dose delivered continuously.

Finally, assuming a single oxygenated capillary vessel, the results of our simulation suggest that only a narrow volume of tissue around the capillary would benefit from increased sparing by ROD during FLASH irradiation.



The exact location of these tissues being spared depends on the oxygen tension in the capillary and the oxygen metabolism of the tissue.

Initial findings of normal tissue sparing by FLASH irradiation were received with great interest given the potential for reducing normal tissue toxicities. However, the mechanism behind this effect remains subject to debate. Several biological explanations have been advanced, including sparing of circulating immune cells due to the rapid treatment time, changes in chromatin remodeling, DNA damage repair kinetics, and inflammatory/anti-inflammatory cell signaling (Durante *et al* 2018). Recently, the production of organic hydroperoxides and peroxy radicals derived through lipid peroxidation chain reactions and higher levels of redox-active iron have been advanced as possible explanations for the differential sparing of normal tissue by FLASH radiation (Spitz *et al* 2019).

This computational study focuses on the hypothetical role of ROD as an OER-modifying factor. A number of studies have shown increased cell survival *in vitro* when cells were irradiated at ultra-high dose rate under hypoxic conditions, corroborating our simulation results (Berry and Stedeford 1972, Epp *et al* 1972). In particular, simulations demonstrated a breakpoint in the slope of clonogenic survival curves around 9 Gy, consistent with *in vitro* data by Nias *et al* (1969) who experimentally observed a breakpoint in cell survival around 6–8 Gy for HeLa cells irradiated at ultra-high dose rates under 2.7 mmHg pO₂.

However, ROD may not explain the effect of FLASH on normal tissue cells, most of which are well oxygenated. Our simulations in perfused tissues show that cells must be sufficiently far from capillaries to experience a survival benefit from ROD. This finding led us to hypothesize that tissue sparing by FLASH irradiation may be driven by normal tissue stem cells, which may reside in hypoxic niches away from the vasculature.

Stem cells have been shown to help normal tissues recover from radiation-induced cognitive (Acharya *et al* 2009), gastrointestinal (Potten *et al* 1997) and bone marrow toxicities (Mauch *et al* 1995). Direct measurements have further shown that stem cell niches in the bone marrow maintain a hypoxic microenvironment with the lowest local pO₂ of approximately 9.9 mmHg reported (Spencer *et al* 2014). A hypoxic microenvironment may also exist in neural stem cell niches (8 mmHg), mesenchymal stem cell niches (15 mmHg), and hematopoietic stem cell niches (8 mmHg), as reviewed by Mohyeldin *et al* (2010) and Liu *et al* (2017). From our model, the oxygen tension in these hypoxic stem cell niches overlaps with the range where the model predicts significant radioprotection from ROD associated with FLASH.

The existence of normal tissue stem cells in a hypoxic niche and the impact of clinically applied modifiers of tumor hypoxia may offer at least partial explanations for previously observed clinical and translational research findings. These considerations are based on the following assumptions: (a) hypoxic normal tissue stem cells are relatively radioresistant; (b) when ‘activated’ (e.g. by tissue damage requiring repair), they become more oxygenated and therefore more radiosensitive; and (c) therapeutic gain by modification of oxygenation is dependent on the relative effect of the modification on tumor hypoxic cells compared with its effect on the hypoxic normal tissue stem cell fraction: more oxygenation of the tumor (or destruction of hypoxic tumor cells) than on normal tissue stem cells would result in better tumor control, while no improvement in tumor control would be expected for modifications that shift normal tissue hypoxic stem cells to a more oxygenated state—and perhaps an increase in normal tissue toxicity would be seen.

We consider three separate clinical studies for potential illustration of such effects:

- (i) *Carbogen breathing during radiation therapy*. A trial in patients with esophageal cancer involved administration of carbogen during treatment with high dose rate intraluminal brachytherapy (Hoskin *et al* 1996). Excessive acute mucosal normal tissue toxicity was observed. This is consistent with the carbogen increasing the oxygenation of the normal tissue and ‘driving’ hypoxic stem cells to a more oxygenated state and therefore increasing their radiosensitivity.
- (ii) *Tirapazamine (TPZ) chemoradiotherapy in head-and-neck (HN) cancers*. TPZ is a hypoxic cell toxin; it is a bioreductive alkylating agent that is selectively activated and toxic in hypoxic cells. An initial phase II trial in patients with advanced HN cancers (TROG 98.02) showed more febrile neutropenia and grade 3 or 4 late mucous membrane toxicity with TPZ/cisplatin/RT compared to patients treated with cisplatin/RT alone (Rischin *et al* 2005). This finding is consistent with killing of hypoxic normal tissue stem cells leading to worse radiation toxicity.
- (iii) *Hyperbaric oxygen*. Hyperbaric oxygen has long been used for the treatment of chronic tissue damage caused by radiation. It has been shown to increase stem cell proliferation (Peña-Villalobos *et al* 2018). This raises concern that hyperbaric oxygen might result in radiosensitization of normal tissue stem cells and contribute to increased normal tissue toxicity.

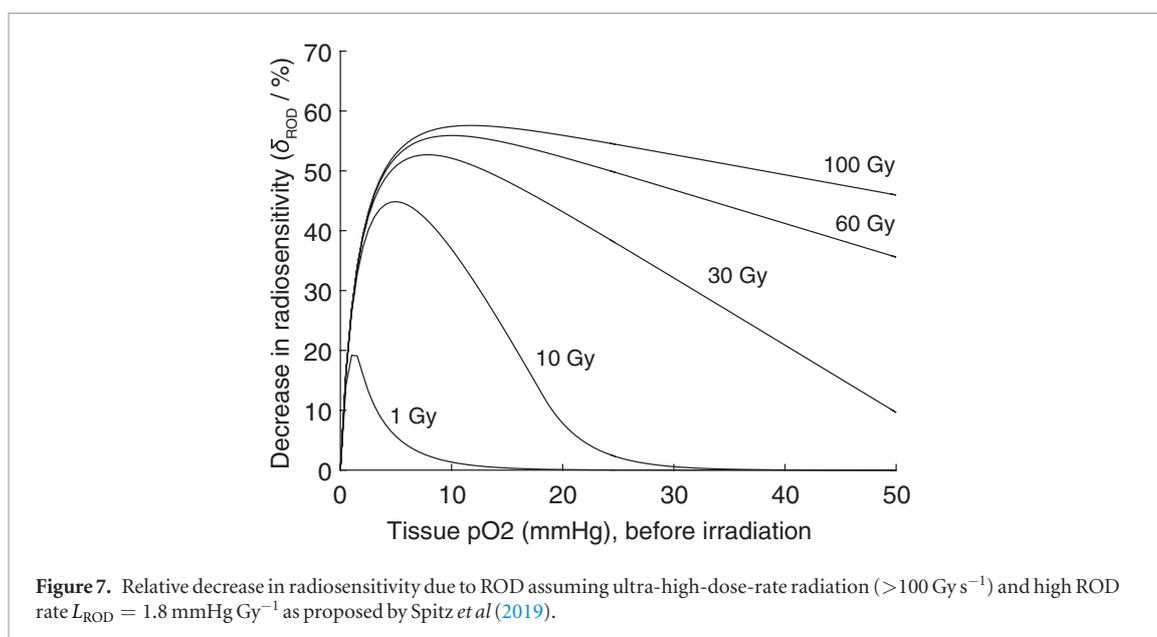
The model presented in this article relies on a few key assumptions. First, we assume a linear model for radiation cell survival, which is approximate since most clonogenic survival curves display a shoulder that is best modeled using a linear-quadratic (LQ) model parameterized by α and β . A linear model is accurate to describe cell survival beyond the initial shoulder. The use of a linear model is not a requirement of our approach and in fact any radiosensitivity model can be used in equation (3). A key methodological challenge, however, is the need for robust radiosensitivity data that covers a wide range of oxygen tensions. The seminal work of Alper and Howard-Flanders resulted in a simple analytical model describing the linear radiosensitivity of cells as a function of oxygen tension (Alper and Howard-Flanders 1956). Since then, various studies have attempted to estimate OER according to the LQ model (Skarsgard and Harrison 1991, Wouters and Brown 1997, Nahum *et al* 2003, Carlson *et al* 2006). However, several technical challenges have emerged from these studies. When OER is defined as a ratio of doses, the ratio becomes a function of the cell killing endpoint. Alternatively, OER can be defined in terms of radiosensitivity but then two independent ratios must be introduced, OER_{α} and OER_{β} . It is also unclear how these factors vary as a function of oxygen tension, as most studies have only considered the extreme cases of anoxia and normoxia. For these reasons, a linear radiosensitivity model was assumed in our simulations.

Second, our model assumes that ROD and DNA damage fixation occur instantaneously (relative to the treatment delivery time) once ionizing energy is deposited into the cell. In fact, these processes occur on the time scale of microseconds (Colliaux *et al* 2015) and therefore the proposed model may not be entirely accurate for radiation pulses substantially shorter than 1 ms. An improved model may include a physicochemical component that accounts for the kinetics of chemical reactions following the formation of water radiolysis products.

Third, we assumed that the rate of ROD in tissue was similar to the rate reported in water and cell culture medium. Recently, it has been hypothesized that the rate of ROD may be four times higher in brain tissue than in water due to lipid peroxidation chain reactions and reactions driven by redox-active iron (Spitz *et al* 2019). The computation model presented herein can be used to estimate oxygen depletion in brain tissues based on the higher rate of ROD proposed by Spitz *et al*. As shown in figure 7, a higher ROD rate of 1.8 mmHg Gy⁻¹ (equivalent to 2.5 μ M Gy⁻¹) would be sufficient to protect cells from a single-fraction dose of 10 Gy for oxygen tension up to 20 mmHg. Given the range of oxygen tension in normal peritumoral brain tissue (Collingridge *et al* 1999), radiation protection of normal tissues by FLASH could occur without invoking special hypoxic niches. The higher ROD calculated by Spitz *et al* is based in part on the high lipid and iron composition they have assumed for brain tissue; however, this might not hold uniformly throughout the brain (e.g. in regions of brain stem cells) and for other normal tissues. The rate of ROD in various tissues must be measured experimentally before a definite conclusion can be reached regarding the role of stem cell niches in FLASH irradiation.

Tumor hypoxia and its effect on the control of solid tumors by ionizing radiation have been widely studied (Vordermark and Horsman 2016). The confounding effect of acute hypoxia on tumor control has been less widely investigated. One study found that the removal of approximately 30% of blood immediately prior to irradiation of tumor-bearing mice caused a high degree of radioresistance equivalent to an increase in hypoxic fractions by factors of 10–30. Measurement of ¹⁴C-misonidazole binding to these tumors after acute hypoxia indicated changes in the number of hypoxic cells corresponding to the survival endpoint (Hirst and Wood 1987).

Studies in patients using Eppendorf electrodes to map tumors have shown significant heterogeneity in oxygen tension including significant regions of hypoxia (Wong *et al* 1997). The extent of intratumoral hypoxia has been associated with worse survival in cervical cancers treated with radiation. For example, disease-free survival rate was significantly worse for cancers where the percentage of pO₂ readings <5 mmHg (HP₅) was greater



than 50% compared to those tumors with $\text{HP}_5 < 50\%$ (Fyles *et al* 1998). Similarly, survival rates were lower in cervical cancer patients with median $\text{pO}_2 < 10 \text{ mmHg}$ compared with those with median $\text{pO}_2 > 10 \text{ mmHg}$ (Fyles *et al* 1998) and three-year local control rates were worse for patients with tumors with $\text{pO}_2 < 20 \text{ mmHg}$ compared with those with tumors with $\text{pO}_2 > 20 \text{ mmHg}$ (Suzuki *et al* 2006). For patients with head and neck carcinomas, worse disease-free survival was noted for patients with median tumor $\text{pO}_2 < 10 \text{ mmHg}$ compared to those with $\text{pO}_2 > 10 \text{ mmHg}$ (Brizel *et al* 1997). In addition, more hypoxic soft tissue sarcomas (median $\text{pO}_2 < 10 \text{ mmHg}$) had higher likelihood of distant metastases (Brizel *et al* 1996) and, in another study, hypoxic soft tissue sarcomas had a poorer disease-specific and overall survival at 5 years (Nordsmark *et al* 2001). Oxygen tension distributions were also sufficient to explain the local response of human breast tumors treated with radiation (Okunieff *et al* 1993).

In light of this significant association between tumor hypoxia and radioresistance, a transient increase in the extent of hypoxia due to ROD could render chronically hypoxic tumors more resistant to FLASH irradiation. This potentially detrimental effect of FLASH on the control of hypoxic tumors should be further investigated.

5. Conclusions

We presented two models of ROD during FLASH irradiation, a simplified one which is valid for ultra-high dose rate and a more detailed one that includes oxygen diffusion and replenishment during radiation. Neither of these models demonstrate a definitive mechanism for the FLASH effect, but they can be used to generate hypotheses that can be tested experimentally. For instance, the model predicts that the FLASH effect should gradually disappear as the radiation pulse duration is increased from $<1 \text{ s}$ to 10 s . Second, fractionation of the dose as a series of pulses should be more efficient than the delivery of the same dose as a single continuous pulse of equal duration. Finally, changes in capillary oxygen tension (increase or decrease) should result in a decrease of the FLASH effect. This last prediction could be tested using a hyperbaric chamber or through carbogen or nitrogen breathing.

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