

A Computational Model for Oxygen Depletion Hypothesis in FLASH Effect

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Experiments have reported low normal tissue toxicities during FLASH irradiation, but the mechanism has not been elaborated. Several hypotheses have been proposed to explain the mechanism. One hypothesis is oxygen depletion. We analyze the time-dependent change of oxygen concentration in the tissue to study the oxygen depletion hypothesis using a computational model. The effects of physical, chemical and physiological parameters on oxygen depletion were explored. The kinetic equation of the model is solved numerically using the finite difference method with rational boundary conditions. Results of oxygen distribution is supported by the experiments of oxygen-sensitivity electrodes and experiments on the expression and distribution of the hypoxia-inducible factors. The analysis of parameters shows that the steady-state oxygen distribution before irradiation is determined by the oxygen consumption rate of the tissue and the microvessel density. The change of oxygen concentration after irradiation has been found to follow a negative exponential function, and the time constant is mainly determined by the microvessel density. The change of oxygen during exposure increases with dose rate and tends to be saturated because of oxygen diffusion. When the dose rate is high enough, the same dose results in the same reduction of oxygen concentration regardless of dose rate. The analysis of the FLASH effect in the brain tissue based on this model does not support the explanation of the oxygen depletion hypothesis. The oxygen depletion hypothesis remains controversial because the oxygen in most normal tissues cannot be depleted to radiation resistance level by FLASH irradiation. © 2022 by Radiation Research Society

INTRODUCTION

Some animal experiments have reported unexpectedly low normal tissue toxicities during ultra-high dose rate irradiation, which is called FLASH effect (1–3). Because of

the great potential clinical benefit resulted from the low damage to normal tissue, FLASH radiotherapy is attracting great attention in the radiation oncology community (4). However, a mechanism explaining the FLASH effect has not yet been demonstrated (3, 4). Oxygen depletion hypothesis has been proposed to explain the FLASH effect (5–8). The hypothesis assumes that cells irradiated using ultra-high dose rate radiation become hypoxic, showing radiation resistance. P Wilson et al. (3) and J Wilson et al. (9) pointed out that the oxygen depletion may be the mechanism of FLASH effect. Some experiments were conducted to explore the relationship between oxygen depletion and FLASH effect. Montay-Gruel et al. indicated that the FLASH effect was related to oxygen, based on their results of animal experiments and H₂O₂ measurements after FLASH irradiation (5). Wardman commented that yields of H₂O₂ reported in the FLASH versus conventional comparison differ from what would have been expected (10). Adrian et al. concluded that the FLASH effect depended on oxygen, based on the experiment of prostate cancer cell line DU145 (11). However,

Acharya et al. found reduced DNA damage after ultra-high dose rate irradiation in normal cells grown in vitro in equilibrium with 200 μmol/L O₂, which indicated that FLASH radiation could lead to protective effect for normoxic cells (12). Besides the oxygen depletion hypothesis, different views have also been proposed (13, 14). Labarbe et al. proposed that the reduction of ROO[•] lifetime is the main root of FLASH effect instead of the hypothesis of transient oxygen depletion (15). Abolfath et al. proposed that FLASH may be explained by the reaction between reactive oxygen species (ROS) (16).

To study the oxygen depletion hypothesis, a computational model is required to describe the spatial and time dependent distribution of oxygen concentration during the FLASH irradiation, because oxygen diffusion, reaction and metabolism are dynamic processes. The qualitative analysis cannot provide enough results to understand the role of oxygen depletion in FLASH effect. Some researchers proposed models to study the oxygen depletion in FLASH irradiation. A computational model has been introduced by Prax and Kapp (6). They solved their equation with only

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one boundary condition which fixed the oxygen concentration at the boundary of the capillary. However, generally, the second order differential equation must be solved with two independent boundary conditions to get one unique solution. Moreover, they set 3 mmHg/s as the value of oxygen consumption rate of the tissue, which was not a typical value or a universal value. Petersson et al. proposed a model to analyze the oxygen depletion in FLASH effect, setting the parameter of oxygen recovery rate λ as 1 s^{-1} without any explanation (17). Spitz et al. added Fenton reaction, chain reaction of lipid peroxidation and other oxygen-related reactions to estimate the oxygen consumed by radiation qualitatively and estimated that a 10 Gy pulse could deplete 25 $\mu\text{mol/L}$ oxygen (18). To overcome the limitations of the previous studies, we performed a study on the oxygen concentration surrounding a capillary using the computational model and solved the model with more rational boundary conditions and important parameters based on the experimental measurements.

Our study directs to the kernel of the oxygen depletion hypothesis: the oxygen in the tissue can be depleted or not. The kernel contains two important aspects: oxygen distribution before the irradiation and the change of oxygen during the irradiation. The physiological parameters affect the oxygen distribution before the irradiation and the oxygen recovery. The physical and chemical parameters are related to the oxygen depletion during the irradiation. By exploring the effect of physiological, physical and chemical parameters on oxygen depletion, we evaluate the oxygen depletion hypothesis in detail.

Oxygen Distribution Before Irradiation

The oxygen distribution in the tissue before irradiation is the primary state of the oxygen depletion. It also reflects the oxygen distribution during the conventional irradiation because the dose rate is too low to affect the oxygen distribution. The oxygen distribution is mainly affected by two physiological parameters: metabolic oxygen consumption of tissue and the microvessel density.

Metabolic Oxygen Consumption of Tissue

The oxygen distribution in the tissue is strongly affected by the oxygen consumption rate (m , to keep the same expression as Prax and Kapp) of the tissue (19). Oxygen consumption rate (m) of the tissue tends to be zero when the oxygen tension tends to be zero and shows a tendency of saturation when the oxygen concentration increases (20). We choose a negative exponential function as the m function, which shows the same tendency of the oxygen consumption rate with oxygen concentration and is easy to calculate its derivative. The m of the tissue is given by

$$m(p) = OCR_{\max}(-\exp(-\lambda p)), \quad (1)$$

where p is the oxygen concentration, $OCR_{\max}$ is the maximum oxygen consumption rate and λ is a constant.

When the oxygen concentration is about 1.4 $\mu\text{mol/L}$ (1 mmHg), m is observed at the half level of $OCR_{\max}$ typically (21). The value of λ is calculated by the data. We compared our m function with the hyperbolic curve, finding that the differences are within 10% for oxygen concentration ranging from 10 to 200 $\mu\text{mol/L}$. Some models regard the m as a constant (6, 21), which may lead to a negative value of oxygen tension in the reaction-diffusion model.

Average cerebral metabolic rate of oxygen was measured by the Kety-Schmidt technique, which can be regarded as the reference method for the measurement. According to the measurements, the average cerebral metabolic rate of oxygen for the healthy young adult is 3.0 ml/100 g/min. For 1L tissue, the oxygen consumption is 30 ml/min, which is equal to 1.2 mmol/min so that we can calculate that the oxygen consumption rate of brain is about 20 $\mu\text{mol/L/s}$.

To use the chemical reaction rate equation to analyze the oxygen depletion induced by FLASH irradiation directly, we employed oxygen molar concentration ($\mu\text{mol/L}$) rather than oxygen tension (mmHg) to describe the oxygen in the tissue. The oxygen tension (mmHg) in the tissue could be transformed to the oxygen concentration ($\mu\text{mol/L}$) by multiplying a factor of 1.4.

Simplified Tissue-Capillary Model

The structure of the capillary system is quite complicated. There exist some problems to calculate the distribution of oxygen in the tissue accurately, such as establishing the detailed model of capillaries in the tissue and solving the kinetic equation of oxygen in such complex geometry. We use the Krogh geometrical model, a widely used model, to describe the capillary-tissue system (22–24). The model describing the oxygen diffusion given by Prax and Kapp is also the Krogh cylinder model. The model regards a capillary and the surrounding tissue as two coaxial cylinders. The inner cylinder represents the capillary and the outer cylinder represents the tissue around the capillary. The oxygen diffused from the capillary to the tissue.

We further assume that the tissue around the capillary is homogeneous. For the substance which cannot freely cross the cell membrane, an effective diffusion constant determined by the tortuosity in the extracellular space is introduced (25). The oxygen can cross the membrane freely, so we think the diffusion is slightly affected by the tortuosity of the extracellular space so that we use the diffusion rate constant in water directly. Then the oxygen diffusion and consumption in the tissue can be described by

$$\frac{\partial p}{\partial t} = D\nabla^2 p - m(p), \quad (2)$$

where p is the molar concentration of O_2 in the tissue, D is the diffusion coefficient of O_2 , and $m(p)$ is the function of oxygen consumption rate related to the p . The steady-state transport of O_2 is governed by

$$D\nabla^2 p - m(p) = 0. \quad (3)$$

Considering that the radius of the outer cylinder (<100 μm) is far less than the length of the capillary ($\sim 1\text{ mm}$), we assume that the axial diffusion of O_2 in the tissue is negligible compared to the radial diffusion. Oxygen distribution in the tissue can be described in the cross-section plane with polar coordinates. The steady-state equation is simplified to

$$D\left(\frac{\partial^2 p}{\partial r^2} + \frac{1}{r}\frac{\partial p}{\partial r}\right) - m(p) = 0. \quad (4)$$

Equation (4) is subject to the following boundary conditions

$$\begin{aligned} p &= p_w, r = r_c \\ \frac{\partial p}{\partial r} &= 0, r = r_t, \end{aligned} \quad (5)$$

where p_w is the molar concentration of O_2 at the capillary-tissue interface, r_c is the radius of the capillary, and r_t is obtained from the value of microvessel density. The value of r_t is approximately equal to the half distance between two adjacent capillaries. The fluxes of oxygen from these two adjacent capillaries are equal at the position $r = r_t$, so that the first derivative of p vs. r is equal to zero. The boundary condition r_t considers the density of capillaries in the tissue. One of the main differences between the model in this study and that of Pratz and Kapp is the boundary condition (6). A finite difference method was adopted to solve Eq. (3) with the boundary conditions. The steady-state oxygen distribution in the tissue can be obtained from the solution.

Change of Oxygen During the FLASH Irradiation

The change of oxygen during the irradiation depends on the radiolytic oxygen consumption and the oxygen diffusion. We extended the steady-state equation to dynamic model by adding the reaction term of radiolytic products. Noted that oxygen diffusion is strongly affected by the physiological parameters, we explore the effect of radiolytic oxygen consumption and physiological parameters on time-dependent change of oxygen.

Radiolytic Oxygen Consumption

The radiolytic oxygen consumption is a key parameter in oxygen depletion hypothesis. However, detailed analysis by considering every relative reaction is almost impossible because of complex composition in the cell especially for living tissue (10). We try to simplify the complex routes to several types of reactions and estimate the radiolytic oxygen consumption based on some experiments. Radiation generates radicals, which can react with oxygen leading to oxygen consumption. Radiation interacts with water and cellular constituents to generate radicals such as e_{aq}^- , $\text{H}^\bullet$, $^\bullet\text{OH}$ and organic radicals. Then most radicals (including most

part of e_{aq}^- , $\text{H}^\bullet$ and $^\bullet\text{OH}$) react with cellular constituents to generate organic radicals (26). Organic radicals react with oxygen to generate peroxy radicals, which is the main process of transient oxygen depletion by irradiation (10, 15caa). The rate constant of this process ranges from $k = 10^7$ to 10^{10} L/mol/s in water, depending on the electronic properties of radicals (27). We chose the value $k = 10^8$ L/mol/s in this study to represent the consumption rate of oxygen and the difference between the actual value and the chosen does not contribute significantly to oxygen depletion, because the time scale of oxygen diffusion in the tissue is at least the magnitude of ten milliseconds (described below). The reactions in the cell after irradiation are too complex for the model to specifically describe individually. A general and simplified process is used to describe the quantity and kinetic of oxygen depleted by radiation. To analyze the transient oxygen depletion by the pulse change affected by the parameter, we solved the model by setting the parameter as 0.2–0.6 $\mu\text{mol/L/Gy}$. Other routes for oxygen depletion such as lipid peroxidation and Fenton reaction are disregarded in this study because these reactions are too slow to affect the oxygen concentration in the tissue. The lipid peroxidation is started by peroxy radicals ($\text{ROO}^\bullet$), which is relatively long-lived with a second-time half-life (15). The reaction rate constant of Fenton reaction is ~ 10 L/mol/s. The time scale of these routes is longer than the oxygen diffusion and much longer than the critical time window (1–2 ms) of oxygen-sensitive effect so that the oxygen concentration is not affected by these routes because oxygen in the tissue is largely supplied from the capillaries (10).

Time-Dependent Change of Oxygen Distribution During FLASH

The steady-state equation gives the oxygen distribution in unirradiated tissue. To consider the change of oxygen distribution in FLASH irradiated tissue, we add a reaction term to the steady-state equation. The reaction term is expressed in the form of the chemical reaction rate equation, instead of $L_{\text{ROD}}Dp/T$ given by Pratz and Kapp, which would fail when the oxygen concentration is quite low. As described above, radicals generated by radiation consume the oxygen in the tissue. We assume that the radicals yielded by low-LET radiation are homogeneous in the tissue (7) on the scale of capillary. The time-dependent change of oxygen distribution is governed by

$$\frac{\partial p}{\partial t} = D\left(\frac{\partial^2 p}{\partial r^2} + \frac{1}{r}\frac{\partial p}{\partial r}\right) - m(p) - kpL(t). \quad (6)$$

where k is the reaction rate constant of oxygen-radical reaction and $L(t)$ is the molar concentration of the radicals. The molar concentration of the reducing radicals $L(t)$ is described by

$$\frac{\partial L(t)}{\partial t} = R(t) - kpl(t), \quad (7)$$

where $R(t)$ is the yielding rate of radicals, which is directly proportional to the dose rate.

We assume that the oxygen in capillaries can hardly be affected by radiation because the hemoglobin carries a large amount of oxygen compared with the dissolved oxygen in the tissue (23). We solved Eqs. (6) and (7) with the same boundary conditions used in the steady-state equation. Moreover, the steady-state oxygen distribution is used as the initial value, and the initial value of the reducing radicals is set to be zero.

The Crank-Nicolson method is used to solve the time-dependent equation of oxygen distribution. Solutions of the time-dependent equation reflect the effect of radiation on oxygen distribution. We can quantitatively analyze the oxygen depletion hypothesis using these solutions.

To explore the relationship between the time-dependent change of oxygen distribution and some biological parameters such as microvessel density and oxygen consumption rate, we set different values of r_t and $OCR_{\max}$.

Biological Effect Predicated by Oxygen Enhancement Model

The radiosensitivity is significantly affected by the oxygen concentration for low-LET radiation. The oxygen concentration affects the acute and chronic effects induced by radiation. However, there is limited data to predict the survival fraction and chronic effect of the tissue. Therefore, a classical oxygen enhancement model to predict the biological effect of FLASH radiation was chosen in the present work. The oxygen related effect is solely represented by oxygen enhancement ratio (OER). The values of OER are measured by the survival fraction of the irradiated cells. In the mentioned classical model (28), OER is given by

$$OER = \frac{mp + K}{p + K}, \quad (8)$$

where p is the molar concentration of oxygen, $\mu\text{mol/L}$; m and K are constant parameters, 2.9 and 7.2 $\mu\text{mol/L}$, respectively. The parameters in this model were calculated by the fitting of experiment data. Recent OER models (29) are given by introducing parameters to link the OER to some mechanisms. They reflect the similar phenomenon with the classical OER model used in this study.

We can calculate the value of OER of each position when receiving FLASH radiation and compare it with the OER under steady-state oxygen concentration. The oxygen concentration in conventional irradiation is considered to keep the value of the steady-state oxygen concentration. For the FLASH irradiation, the OER is calculated in accordance with (6)

$$OER = \frac{\int_0^T OER(p(t)) \cdot Rate(t) dt}{\int_0^T Rate(t) dt}$$

where T is the width of the pulse and $Rate(t)$ is the function of dose rate.

Parameters of the Model

Studies on brain tissue have measured the data of biological parameters of the brain such as the OCR and the microvessel density of brain tissue (30, 31). The model is solved with typical values of microvessel density, $OCR_{\max}$, and oxygen concentration at the venous end of the capillary in the brain tissue. If we assume that capillaries are arranged in parallel, the boundary condition r_t can be calculated by length density of capillary. The microvessel density is converted to the boundary condition r_t by

$$r_t = \frac{1}{f\sqrt{L_v}}, \quad (9)$$

where L_v is the length density of capillary (30), and f is a factor related to the form of arrangement. If the capillaries are arranged in the form of square lattice in the cross-section plane, f is equal to 2.0. If the capillaries are arranged in the form of a hexagonal lattice, f is equal to 2.278. We choose 2.0 as the value of f , which leads to more zones at the low oxygen level. If the result of the model using this parameter showed no hypoxic zone, the model using a larger value of f would not give the result showing a hypoxic zone. A typical value of L_v in the cerebral cortex, 500 mm^{-2} , is chosen to calculate the boundary condition (30).

The constant dose rate during the pulse is assumed for its simplicity. The yield of radicals reacting with oxygen, G , is 0.2–0.6 $\mu\text{mol/L/Gy}$ (6). Yielding rate of radicals, $R(t)$, is given by

$$R(t) = G \cdot Rate(t), \quad (10)$$

where $Rate(t)$ is the dose rate, Gy/s. Parameters of the model are listed in Table 1.

With these typical parameters, we analyze the effects of FLASH irradiation on the brain tissue.

RESULTS AND DISCUSSION

Oxygen Distribution in the Tissue

We solved the steady-state equation of oxygen distribution with different values of r_t and $OCR_{\max}$ to evaluate the relationship between oxygen distribution and biological parameters. The results are shown in Fig. 1.

Results show that oxygen distribution is significantly affected by oxygen consumption rate and the boundary condition. The oxygen concentration is lower when a larger r_t is set, because large distance between two capillaries leads to low-oxygen-concentration level in the tissue. The high level of oxygen consumption rate results in the low oxygen concentration because of fast oxygen consumption. The

TABLE 1
Parameters of the Model for the Brain Tissue (6)

Parameter	Value
OCR_{max}	$20 \times 10^{-3} \mu\text{mol/L/ms}$ for brain tissue (31) $(10-30) \times 10^{-3} \mu\text{mol/L/ms}$
λ	$0.485 \text{ L}/\mu\text{mol}$ (21)
D	$2 \mu\text{m}^2/\text{ms}$ (6)
p_w	$57 \mu\text{mol/L}$ (6)
r_c	$3 \mu\text{m}$ (6)
r_i	$26 \mu\text{m}$ for brain tissue (30) $26-66 \mu\text{m}$
f	2.0
k	$1 \times 10^8 \text{ L/mol/s}$ (7)
G	$0.2-0.6 \mu\text{mol/h/Gy}$ (6)
m	2.9 (28)
K	$7.2 \mu\text{mol/L}$ (28)

boundary condition r_i and oxygen consumption rate are two important parameters deciding the steady-state oxygen concentration in the tissue. For the brain tissue, the value of oxygen concentration is higher than the level ($<20 \mu\text{mol/L}$) at which the radiation-resistant phenomenon can be observed. Our result shows that the oxygen concentration of the cerebral cortex is about $50 \mu\text{mol/L}$, which is equivalent to 5 kPa oxygen tension measured by the oxygen-sensitivity electrodes (32). The oxygen concentration in the cerebral cortex is in accordance with the experimental results of the oxygen-sensitivity electrodes.

Characteristic of Time-Dependent Change of Oxygen Distribution

Many types of facilities have been used in the study of FLASH effect. Some facilities generated continuous beams such as proton cyclotrons and the superconducting linear accelerator on CTFEL (in Chengdu, China. The accelerator

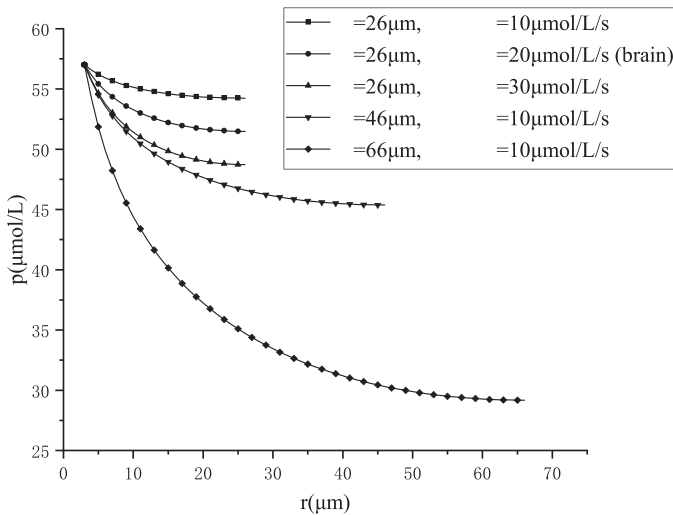


FIG. 1. Oxygen distribution for different boundary condition (r_i) and metabolic oxygen consumption rate (OCR_{max}), where r is the distance to the capillary and p is the oxygen concentration. The steady-state oxygen distribution is calculated by setting different values of r_i and OCR_{max} .

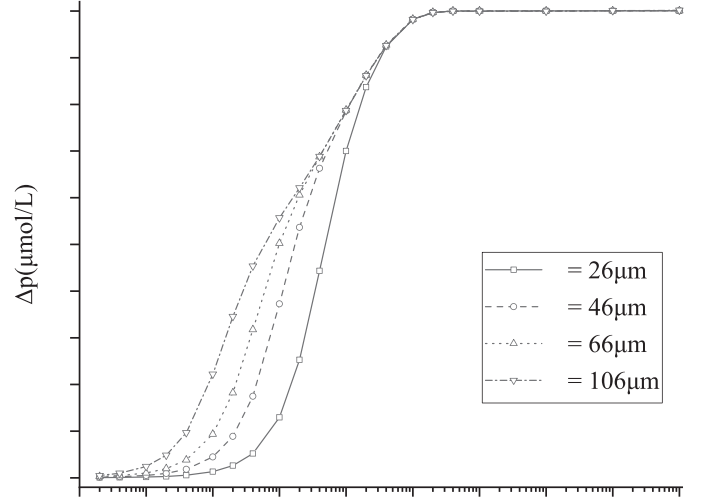


FIG. 2. Maximum change of oxygen (Δp) by different dose rate pulse for tissue with different r_i (related to the microvessel density). The oxygen consumption rate is setting to $0.5 \mu\text{mol/L/Gy}$ as an example.

can generate several milliseconds macro pulse and the macro pulse contains millions of micro pulse with 18.5 ns pulse width) (33). Other facilities work at pulse mode such as electron linear accelerators (they generate $\sim \mu\text{s}$ RF pulse with frequency of $\sim 100 \text{ Hz}$). We simulate pulses with 20 Gy dose and pulse width ranging from $1 \mu\text{s}$ to 2000 s to study the effect of pulse width on the oxygen consumption to consider different types of facilities. We summary the relationship between the maximum oxygen change and dose rate for different values of r_i in Fig. 2 when setting the oxygen consumption rate of $0.5 \mu\text{mol/L/Gy}$ as an example. It indicates that oxygen consumed by the radiation pulse is no longer related to the width of the pulse when the dose rate is high enough.

The time-dependent equation was solved with different parameters to evaluate the influence of biological parameters. The continuous pulse is chosen as an example. For each situation, the tissue receives a pulse with a total dose of 20 Gy and a pulse width of 50 ms, which is set to keep the computing time at a rational level. The dose of the pulse is higher than most FLASH experiments, and the dose rate is up to 400 Gy/s, which is higher than 40 Gy/s. The pulse represents a typical situation of FLASH irradiation even if it does not correspond to the pulse in the experiment. The time-dependent change of oxygen at the position $r = 18 \mu\text{m}$ is shown in Fig. 3.

We found that the change of oxygen concentration is approximately a negative exponential curve after the pulse. The negative curve reflects the oxygen recovery resulted by oxygen diffusion from the capillary. The curve can be described by

$$P(t) = p_s - \Delta p \cdot e^{-t/\tau}, \quad (11)$$

where p_s is the steady-state oxygen concentration, Δp is the oxygen consumed by radiation pulse, and τ is the time

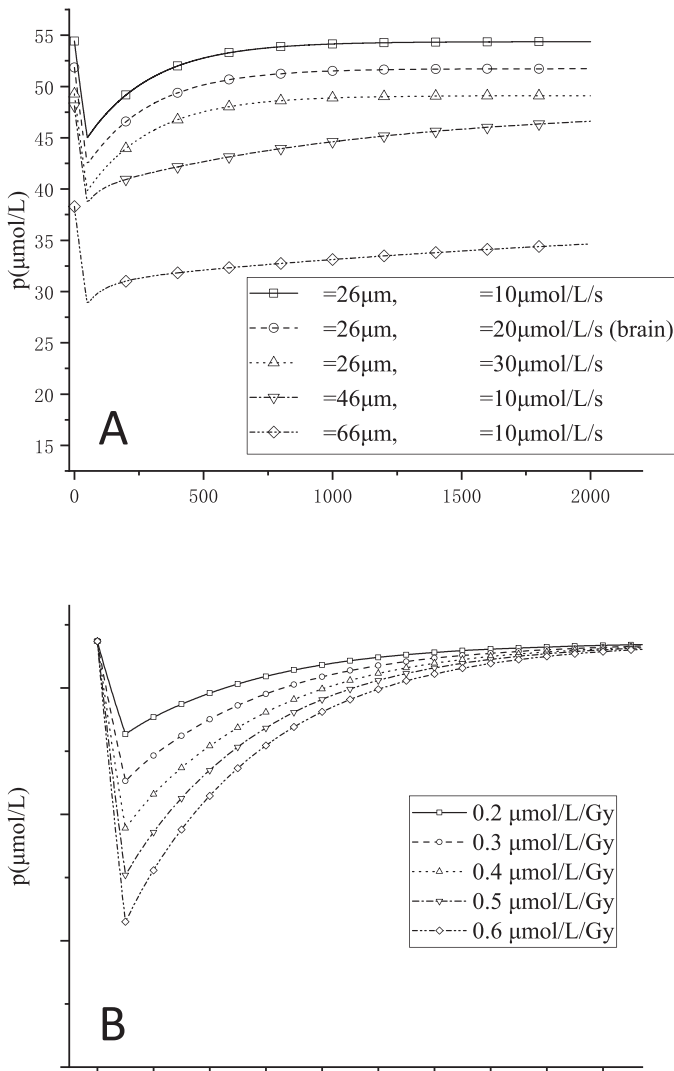


FIG. 3. Panel A: Time-dependent change of oxygen concentration for different biological parameters. Curves of change of oxygen concentration at $18\mu\text{m}$ under different biological parameters (r_t and OCR_{max}) and fixing the $G = 0.5\mu\text{mol/L/Gy}$ are shown in this figure. The initial values of the curves are the values of steady-state oxygen concentration determined by the related values of r_t and OCR_{max} . Panel B: Time-dependent change of oxygen concentration for different G values. The biological parameters are the same as the brain tissue $r_t = 26\mu\text{m}$, $OCR_{\text{max}} = 20\mu\text{mol/L}$. The maximum change of oxygen is determined by G value.

constant of oxygen concentration recovering. Besides, the values of time constant at the positions $r = 23, 43$ and $63\mu\text{m}$ for $r_t = 66\mu\text{m}$ are calculated. Table 2 shows the values of τ at different positions for different biological parameters.

The extent of oxygen consumption (Δp in formula 11) is proportional to the values of oxygen consumption rate constant. We further found that τ is independent of OCR_{max} but related to r_t and the position r . The distance to the capillary r affects τ slightly at the position far away from the capillary, compared to the influence of the r_t . It indicates that the time-dependent change of oxygen concentration is

TABLE 2
Values of Time Constant at Different Positions for Different Biological Parameters

r_t (μm)	OCR_{max} ($\mu\text{mol/L/s}$)	r (μm)	τ (ms)
26	10	18	255
26	20	18	255
26	30	18	255
46	10	18	1,072
66	10	18	2,479
66	10	23	2,502
66	10	43	2,607
66	10	63	2,660

closely related to r_t , which is determined by the value of microvessel density.

Moreover, formula (11) can be regarded as the impulse response function of a system so that the time-dependent curve can be obtained by convolution for radiation with longer pulse width and multi-pulses. The changes of oxygen concentration in these two situations are smaller than the pulse with short pulse width because of the recovery induced by the oxygen diffusion. The analysis on the change of oxygen concentration in this study well represents the typical change of the oxygen concentration in FLASH irradiation.

Biological Effect Predicted by Oxygen Enhancement Model

We calculated the OER of the brain tissue in the previously calculated FLASH pulse (20 Gy, 50 ms), and the OER of conventional irradiation was calculated by using the oxygen distribution before irradiation. The differences of OER values between FLASH and conventional irradiation at different positions of the brain tissue are shown in Fig. 4.

The maximum differences of OER values is about 1% (0.03/2.7) between FLASH irradiation and conventional irradiation under the steady-state oxygen concentration. There is no obvious change of OER value for the brain tissue receiving FLASH irradiation. In well-oxygenated tissue such as brain and lung, FLASH irradiation changed the survival fraction of the cell slightly (reflected by the change of OER). It must be noted that the oxygen is heterogeneous in tissues, some cells that may be at intermediate oxygen tensions. The survival fractions of these cells are influenced by the radiolytic oxygen consumption. This issue is further discussed in the section "Limitations of this Study".

Discussion on Boundary Conditions of the Model

As the aforementioned analysis of oxygen concentration in the tissue, the spatial distribution and the time-dependent change of oxygen concentration are both strongly affected by the boundary conditions. A comparison between our model and the model by Pratz and Kapp (shown below).

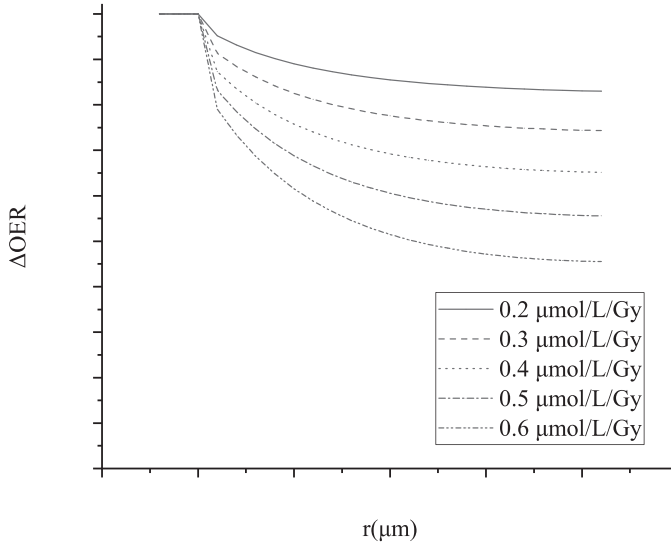


FIG. 4. Differences on OER between conventional irradiation and FLASH irradiation at different positions of the brain tissue under oxygen depletion hypothesis.

The oxygen consumption function $m(p)$ in Eq. (4) is set to be a constant OCR so that we can compare the difference in boundary conditions between two mentioned models. There is an analytic solution of equation with the constant oxygen consumption rate, given as formula (12).

$$p_{ss}(r) = C_1 \ln r + \frac{OCR}{4D} r^2 + C_2 \quad (12)$$

C_1 and C_2 are constant determined by the boundary conditions. The function in formula (12) will become infinity when r tends to be infinity. Two independent boundary conditions are necessary to calculate C_1 and C_2 while solving the equation. However, Pratz and Kapp solved the equation with the boundary condition $p(r \leq r_c) = p_0$. The oxygen concentration in the capillary cannot be described by Eq. (4) because hemoglobin releases oxygen to the blood is not considered by Eq. (4). For the above reason, the first derivative of $p(r)$ is not continuous at r_c . The boundary condition $p(r \leq r_c) = p_0$ is equivalent to $p(r = r_c) = p_0$. It means that there is only one boundary condition to solve the equation in that study. As two boundary conditions are indispensable to solve the equation, the solution may be determined by an implicit boundary condition which existed but not illustrated in the article. Our result on time-dependent change of oxygen concentration further indicates that the recovery time of oxygen concentration is strongly affected by the boundary condition. The boundary condition is also an important factor for the analysis of oxygen changing during radiation exposure.

Discussion on Oxygen Depletion Hypothesis

The original oxygen concentration and oxygen consumption postirradiation are two important factors of the oxygen depletion hypothesis. Computational model research and

experiments have been conducted to study the oxygen change and related biological effect in the FLASH effect.

Our results show that the values of original oxygen concentration at all positions in the brain are enough and a 20 Gy pulse affect the oxygen related effect slightly, but FLASH effect has been observed in the brain tissue. Moreover, our results of the oxygen concentration in the tissue are calculated for the tissue around the venous end of the capillary. The oxygen concentration at the arterial end of the capillary is 140 $\mu\text{mol/L}$ ($\sim 100\text{mmHg}$), which is much higher than that at the venous end of the capillary (57 $\mu\text{mol/L}$, 40 mmHg). The oxygen concentration levels in other areas are higher than that of our results because the oxygen concentration at the capillary between the arterial end and the venous end is higher than that at the venous end. The oxygen in the tissue between the arterial end and the venous end cannot be depleted to radiation resistance level by FLASH irradiation, either.

Recent experiments have reported FLASH effect in lung and brain. There is no significant evidence showing that a large zone of hypoxic tissue can be observed in these organs. Results of experiments on expression and distribution of the hypoxia-inducible factors (HIF) showed that most zones in the normal tissue are not hypoxic (34). Measurements of oxygen tension in the tissue via oxygen-sensitive electrodes also showed that the oxygen levels in the normal tissue ($\sim 5\text{ kPa}$ at the cerebral cortex) are much higher than the interval in which the oxygen tension can be consumed to make the tissue radiation-resistant using 20 Gy FLASH irradiation. Cao et al. (35) performed *in vivo* experiments to measure oxygen consumption by FLASH irradiation, their results also show that the oxygen in the tissue cannot be depleted by FLASH irradiation. The oxygen depletion hypothesis is not supported by the computational model and results of measurements.

Limitations of this Study

It should be noted that there are still limitations in our study. The main limitation is brought by the Krogh cylinder model. The simple Krogh cylinder model describing the capillary-tissue system cannot consider the complicated geometry of the real capillary system. Some experiments have reported that there are hypoxic microenvironments in neural stem cell niches (oxygen concentration in the stem cell niches between 10–80 $\mu\text{mol/L}$) (36), which cannot be explained by the Krogh cylinder model. However, the oxygen concentration in the brain tissue calculated by our model is larger than 50 $\mu\text{mol/L}$ at every position. The value is far beyond the range in which OER changes obviously ($< 20\text{ } \mu\text{mol/L}$). Therefore, these limitations can hardly affect the conclusion.

The oxygen concentration in the tissue is one of the key problems of the oxygen depletion hypothesis. Electrode measurements show that the oxygen is heterogeneous. According to our model, the heterogeneity of oxygen could

be explained by the distribution of capillaries. Our model focuses on the relatively microscopic area around a capillary. The relatively high oxygen concentration in this area does not conflict with the heterogeneity of oxygen for a macroscopic scale. For the parts of tissue with low microvessel density, there are some cells at intermediate oxygen tensions that most likely to be influenced by a transient decrease in oxygen. It should bear in mind that the present result of oxygen depletion in the tissue is applicable to the tissue with a high microvessel density, in which the FLASH effect is observed however cannot be explained by the oxygen depletion hypothesis.

Analysis of other tissues is not performed due to the lack of data on biological parameters. Moreover, the model of biological effect affected by oxygen is limited to cell survival. There is no widely accepted model to describe the relationship between chronic effects and oxygen in radiation because of the complexity of chronic effects. Some experiments showed that FLASH irradiation led to the same result of cell survival as conventional irradiation (13), it indicated that cell survival may not be the sole determinant of any FLASH effect. Other explanations still need further research.

CONCLUSION

A detailed analysis was performed to study the oxygen distribution and the kinetics of oxygen in the tissue on the scale of microvessel using a computational model. The model with the new boundary conditions and more realistic term in the equation in our study overcomes the limitations of ignoring the influence of surrounding capillaries and regarding the oxygen consumption rate of tissue as a constant in previous models. We explore the relationship between the FLASH effect and oxygen concentration with the computational model and take the FLASH effect in brain tissue as an example.

The study on the oxygen concentration in the tissue using this model gives some conclusions on the time-dependent change of distribution: 1. The steady-state oxygen distribution is determined by the values of oxygen consumption rate and distance between capillaries. 2. The oxygen concentration recovers to the steady state with a negative exponential format postirradiation. 3. The extent of radiolytic oxygen consumption is proportional to oxygen consumption rate constant. It is a key parameter in oxygen depletion hypothesis, but the value remains uncertain for real cellular condition. 4. The time constant of the exponential format is determined by the distance between capillaries, which means that the oxygen recovery is mainly determined by the microvessel density. 5. The same dose results in the same reduction range of oxygen concentration regardless of dose rate, if the pulse width is much less than the time constant. From the perspective of the oxygen consumption, a pulse with several milliseconds' width is equal to the pulse with several microseconds even nanoseconds width.

The computational analysis for brain tissue in this work does not support the oxygen depletion hypothesis to explain the observed FLASH effect. The results of oxygen concentration in brain tissue calculated by the model are supported by the measurements of HIF and oxygen tension in the brain tissue. Therefore, we suppose the oxygen depletion hypothesis remains controversial.

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