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Systematic analysis and modeling of the FLASH sparing effect as a function of dose and dose rate

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Abstract

Ultrahigh-dose-rate radiotherapy (FLASH-RT) is a revolutionary radiotherapy technology that can spare normal tissues without compromising tumor control. Although qualitative experimental results have been reported, quantitative and systematic analysis of data is necessary. Particularly, the FLASH effect response model to the dose or dose rate is still unclear. This study investigated the relationships between the FLASH effect and experimental parameters, such as dose, dose rate, and other factors by analyzing published in vivo experimental data from animal models. The data were modeled based on logistic regression analysis using the sigmoid function. The model was evaluated using prediction accuracy, receiver operating characteristic (ROC) curve, and area under the ROC curve. Results showed that the FLASH effect was closely related to the dose, mean dose rate, tissue type, and corresponding biological endpoints. The dose rate corresponding to a 50% probability of triggering cognitive protection in the brain was 45 Gy s^{-1}. The dose rate corresponding to a 50% probability of triggering intestinal crypt survival and regeneration was 140 Gy s^{-1}. For the skin toxicity effect, the dose corresponding to a 50% probability of triggering the FLASH effect was 24 Gy. This study helps to characterize the conditions underlying the FLASH effect and provides important information for optimizing experiments.

Keywords FLASH radiotherapy · Sparing effect · Systematic analysis · Dose rate · Dose · Biological endpoints

1 Introduction

Ultrahigh-dose-rate radiotherapy (FLASH-RT) causes less damage to normal tissues while maintaining antitumor efficacy [1] compared with the same dose of conventional dose-rate radiotherapy (CONV-RT)- the so-called FLASH effect. The FLASH effect has been confirmed in experiments with different beam modalities (X-rays [2, 3], electrons [4], very high-energy electrons [5, 6], protons [7–9], carbon ions [10]), tissues (skin [11], brain [12], small intestine [7], lung [4], bone [13], zebrafish embryos [14], etc.), and experimental endpoints [12] (fibrosis [4, 7], intestinal acute syndrome [15], etc.). Human patients have been treated with FLASH proton radiotherapy (FAST-01 trial [NCT04592887]) with prospective results, marking a milestone for the clinical application of FLASH-RT technology.

Understanding the conditions underlying the FLASH effect is necessary for its wide clinical application. Potential mechanisms, such as the oxygen depletion and immune response hypotheses [16], have been proposed, but none of them has been validated. Current experimental results show that the FLASH effect depends on various physical and biological conditions, such as dose, dose rate, tissue type, and observation endpoint. Although many experiments produced conclusive results, the data are sparse and need to be systematically and quantitatively analyzed. In particular, the response of the FLASH effect

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to key factors such as dose or dose rate is still unclear. For example, determining whether there is a threshold for the dose rate or dose response has always been the focus of academics. Owing to the limited availability of irradiation devices capable of producing ultrahigh dose rates, experiments on the FLASH effect have not been performed as extensively as experiments with conventional dose rates. Therefore, systematic and quantitative analysis of existing experimental data is highly important.

In this study, the relationships between the FLASH effect and experimental parameters, such as dose, dose rate, and other factors, were investigated by quantitatively analyzing published experimental data. Because it is difficult to control the oxygen environment and study the immune response in vitro, we focused mainly on in vivo experimental data from animal models. First, we conducted a systematic literature survey and summarized the results and key experimental parameters of each experiment. The data were subsequently grouped and analyzed using logistic regression analysis via the sigmoidal response model with quantitative evaluation results. Our study can promote the understanding of the conditions triggering the FLASH effect and provide important references to optimize subsequent experiments.

2 Materials and methods

2.1 Literature search and screening

Online searches for articles published after 1950 were conducted through Web of Science and PubMed on 15 September 2023 using the following terms: TS = (ultrahigh dose rate OR ultrahigh dose rate OR ultrahigh dose rate) AND TS = (in vivo OR animal model OR mice OR preclinical). The queries produced 980 results, with 564 results remaining after removing duplicate entries.

The titles and abstracts were reviewed manually by two authors, and the full texts of the suitable manuscripts were further screened considering factors such as topic, experimental conditions and methods, and research objects. The detailed record identification and screening flows based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines are summarized in Fig. 1. Finally, forty articles were used for our analysis.

2.2 Data extraction

The purpose of this study was to compare the effect of ultrahigh-dose-rate irradiation with that of conventional dose rate (CONV) irradiation on normal tissue. The FLASH effect was confirmed if there were significant differences in the experimental phenomena and data under the two radiation conditions. In the same article, the research items with

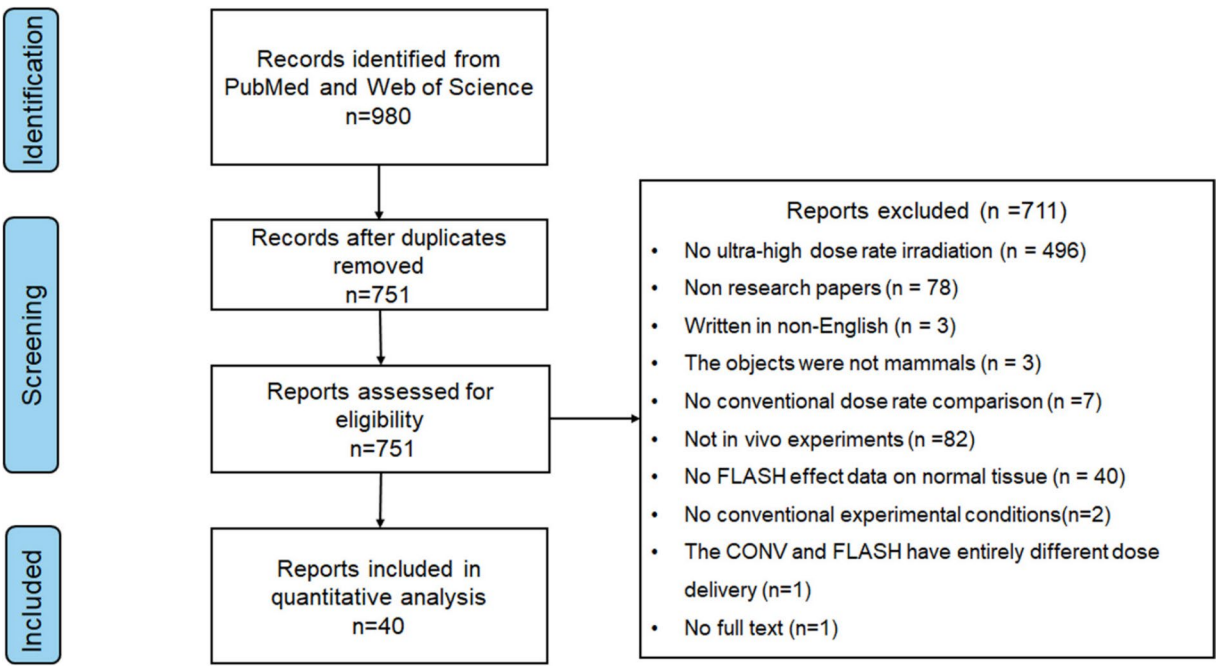

Fig. 1 Flow diagram of record identification and screening based on PRISMA

different endpoints but otherwise identical conditions were regarded as one item. As summarized in Table S1 of the supplementary material, a total of 131 items were extracted from 40 articles included in the analysis. For each item, the FLASH effect (1 represents a significant sparing effect, and 0 represents no sparing effect) and detailed parameters, including the type and energy of the radiation, dose, dose rate, experimental object, and pulse characteristics (if available), were recorded.

Since different tissues differ in terms of radiosensitivity, renewal, and repair capability, they exhibit different responses to ultrahigh-dose irradiation. Moreover, even with the same tissue, the observation of the FLASH effect can differ with early or late response endpoints. Thus, the research items were classified according to the tissue type and endpoints in the data analysis. The current most related studies focus on the brain, small intestine, and skin; the observation endpoints for these tissues are summarized in Table 1. For the brain, the cognitive effect was analyzed, which accounted for 77.8% of brain studies. Early responses, mainly crypt survival or regeneration, were considered for the small intestine. For the skin, late toxicity, including the skin late toxicity score, ulceration, fibrosis, and so on, was selected as the endpoint.

2.3 Data processing and analysis

According to the methods used to simulate the quantitative analyses of the normal tissue effect in the clinic (QUANTEC) [17], the probability of triggering the FLASH effect as a function of dose or mean dose rate was analyzed using a binary logistic regression model described as,

$$P = \frac{1}{1 + e^{-(\beta_0 + \beta_1(X))}}, \tag{1}$$

where P is the probability of triggering the FLASH effect, X is the mean dose rate or dose, and β_0 and β_1 are the parameters of the model. The analysis was performed using SPSS software.

For statistical data, there were large imbalances in the number of data entries with and without the FLASH effect (researchers are more inclined to report studies with positive results). Therefore, a more balanced dataset was obtained by oversampling using the K-Means SMOTE algorithm, which was implemented in Python based on the imblearn library.

For the dose rate factor, only the mean dose rate was analyzed because most of the published literature lack information on the pulse-dose rate. The data included both the pulsed beam and nonpulsed beam (e.g., kV X-ray irradiation). The data from experiments in which the irradiation was performed with only one pulse were excluded since the dose rate is the pulse dose rate, which is much higher than the mean dose rate in other studies.

When analyzing the dose factor, only the data from single-fraction experiments were considered. Although fractionated irradiation is commonly used clinics, studies on fractionated irradiation using FLASH are rare. Moreover, unlike single irradiation, the radiobiological effect of fractionated irradiation is related not only to the total dose but also to the biologically effective dose (BED).

2.4 Model evaluation

The prediction accuracy is the proportion of correct classifications and can be calculated as,

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}}, \tag{2}$$

where TP = true positives, TN = true negatives, FP = false positives, and FN = false negatives. A predicted result was considered positive if the probability was greater than 50%; otherwise, the result was considered negative.

Furthermore, a receiver operating characteristic (ROC) curve was plotted as the false-positive rate (FPR) against the true-positive rate (TPR) at different threshold values. The classification model was validated using the area under the receiver operating characteristic (ROC) curve (AUC) value, which is a threshold and scale invariant parameter.

Table 1 Endpoints of irradiation on normal tissues

Tissue	Response	Endpoint	Proportion
Brain	Early response	Inflammatory response (3)[1]	16.7%
	Late response	Cognitive function (14), percent survival (1)	83.3%
Intestine	Early response	Crypt survival or regeneration (21)	75.0%
	Late response	Probability of survival (4), fibrosis (2), muscularis thickness (1)	25.0%
Skin	Early response	Level of acute damage (7), epidermal necrosis (1)	25.8%
	Late response	Skin late toxicity score (14), Depth of normal skin (1), fibrosis (2), inflammation (2), ulceration (2), hyperplasia score (1), median survival (1)	74.2%

[1] The number in brackets refers to the quantity of items corresponding to that endpoint

3 Results and discussion

3.1 Dose rate factor

A single variable method was used to analyze the dose rate and dose factors. For dose-rate analysis, the dose distribution was limited to a narrow range. For the brain, most studies used a single dose of approximately 10 Gy, which is the standard prescription dose for cognitive experiments. As shown in Fig. 2, the relative changes in the FLASH and CONV groups were quantitatively evaluated and defined as *R*. As the mean dose rate increased, the *R* value increased, indicating a stronger ability to spare the brain. Furthermore, the prediction accuracy of our model reached 73%, with an AUC of 0.87. The probability of triggering the FLASH effect gradually increased with the increase in the mean dose and reached a plateau (Fig. 2). The dose rate corresponding to a 50% probability of triggering the FLASH effect was approximately 45 Gy s^{-1}, which is consistent with the experimental results reported in literature [12]. A dose-rate escalation study [12] showed significant cognitive sparing at an ultrahigh dose rate (30 Gy s^{-1}) compared to a conventional dose rate (0.1 Gy s^{-1}) for a single dose of 10 Gy. When the dose rate increased to 100 Gy s^{-1}, there was no significant difference in cognitive activity between the irradiated and nonirradiated groups, but the magnitude of the FLASH effect was unchanged.

For the small intestines, the single dose used in most studies was in the narrow range of 12 Gy to 14 Gy, with 12 Gy being the majority. Like in the brain, as the mean dose rate increased, the *R* value increased, suggesting that the intestinal crypts were more strongly protected (Fig. 3). Moreover, the prediction accuracy of our model was 75%, with an AUC of 0.91. The dose rate corresponding to a 50% probability of triggering the FLASH effect was approximately 140 Gy s^{-1} (Fig. 3).

Taken together, for the brain and small intestines, the probability of triggering the FLASH effect increased as a function of the mean dose rate. Similarly, the brain was likely to receive a lower dose rate than the small intestine, which may be related to differences in the characteristics of the tissues: the small intestine is an early-responding tissue, while the brain is a late-responding tissue. Additionally, these differences may be attributed to the observed endpoints. Brain cognition is usually assessed over a long period of time, whereas changes in the number of intestinal crypts are observed relatively earlier. For the skin, most experiments were performed with a narrow range of dose rate of approximately 200 Gy s^{-1}, providing insufficient data for analysis.

3.2 Dose factor

In addition to the dose rate, dose is another important factor that affects the FLASH effect. For a low dose, the difference between the ultrahigh dose rate and the conventional dose rate does not manifest owing to the low damage. The probability of triggering the FLASH effect as a function of dose was analyzed using a model similar to that used for the dose rate. For the skin, the mean dose rate used in most studies was 150–200 Gy s^{-1}, which is also a narrow distribution. As shown in Fig. 4, as the dose increased, the *R* value decreased, indicating decreased toxicity to the skin. Furthermore, the prediction accuracy of our model reached 89%, with an AUC of 0.93. The probability of triggering the FLASH effect gradually increased with the increase in dose and reached a plateau (Fig. 4). The dose corresponding

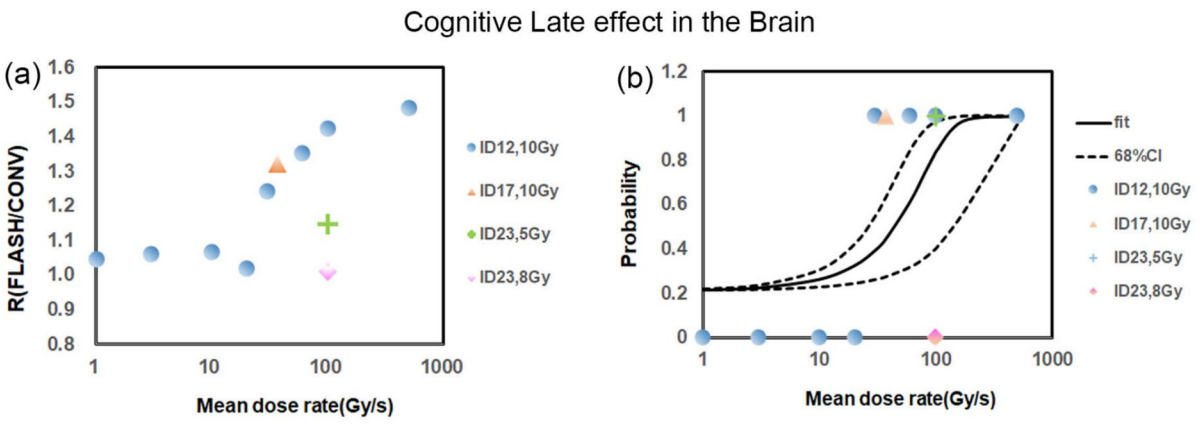

Fig. 2 (Color online) Relationship between the FLASH effect and mean dose rate for the brain. **a** Comparison of relative change in cognitive late effect between FLASH and CONV groups at different mean-dose rates. **b** Quantitative analysis of the probability of triggering a FLASH effect at different mean dose rates. Scatter points represent experimental results reported in different studies. The solid line shows the regression of the data using the sigmoid function with 68% confidence interval (dashed line)

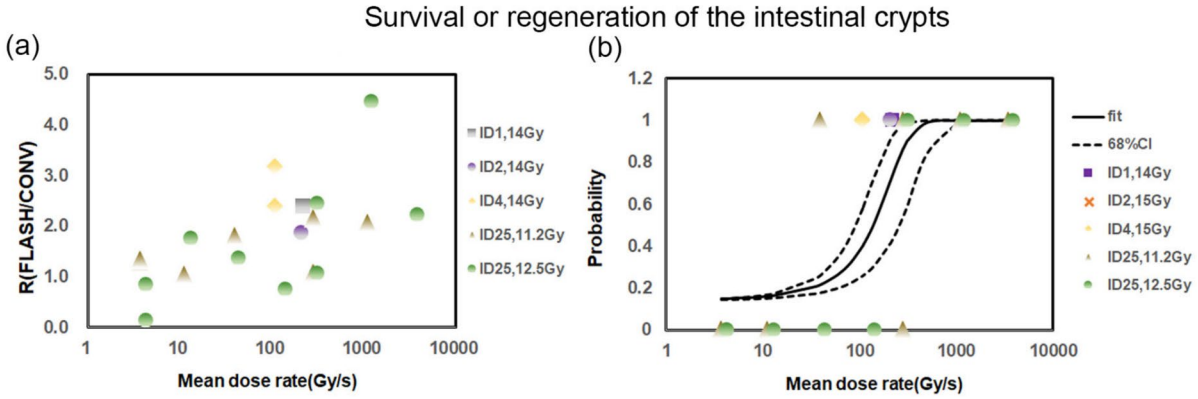

Fig. 3 (Color online) Relationship between the FLASH effect and mean dose rate for small intestines. **a** Comparison of the proportion of surviving or regenerating intestinal crypts between FLASH and CONV groups at different mean-dose rates. **b** Quantitative analysis of the probability of triggering a FLASH effect at different mean dose rates. Scatter points represent experimental results reported in different studies. The solid line shows the regression of the data using the sigmoid function with 68% confidence interval (dashed line)

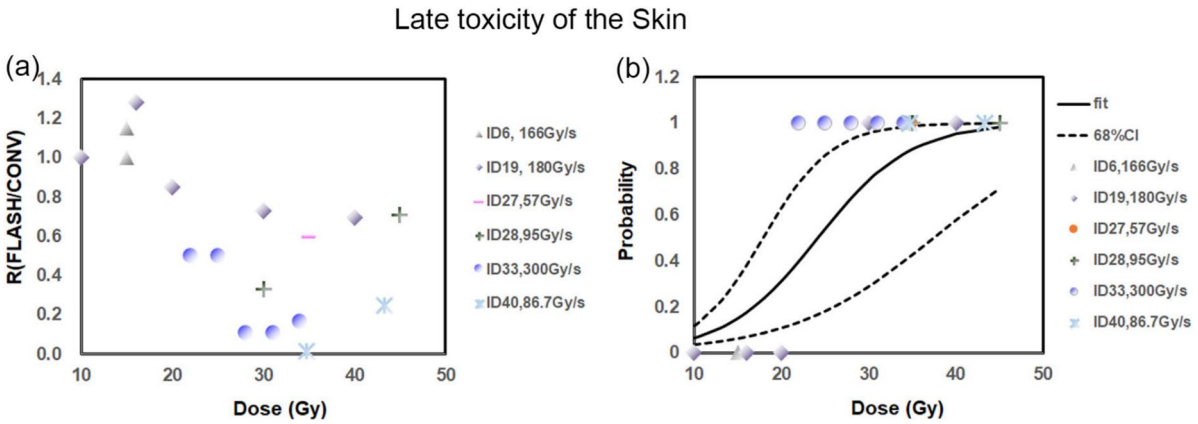

Fig. 4 (Color online) Relationship between the FLASH effect and dose for the skin. **a** Comparison of the relative change in skin late toxicity between FLASH and CONV groups at different doses. **b** Quantitative analysis of the probability of triggering a FLASH effect at different doses. Scatter points represent experimental results reported in different studies. The solid line shows the regression of the data using the sigmoid function with 68% confidence interval (dashed line)

to a 50% probability of triggering the FLASH effect was approximately 24 Gy (Fig. 4). In dose-escalation experiments reported in literature, a FLASH-sparing effect could be observed for a single dose greater than 23.5 Gy for toxicity scores ranging from 1.5 to 3.5 [18]. Taking the skin toxicity score of 1.5 as an example, the MDD50 (dose causing skin toxicity in 50% of mice) values for the conventional dose rate and ultrahigh dose rate were 24.7 Gy and 39.1 Gy, respectively.

Although the dose should reach a certain level to produce a FLASH effect, toxicity also increases when a single dose is too high, even at an ultrahigh dose rate. For example, cognitive impairment was observed at a dose of 10 Gy but disappeared when the dose was increased to 14 Gy [19]. Proton-based FLASH research has shown that the inhibitory effect is diminished when the dose is too high [7]. It has also been reported in large animal experiments that even at ultrahigh dose rates, a single irradiation dose of 30 Gy resulted in animal death [20]. This suggested that both the dose rate and dose need to be optimized to maximize the FLASH effect.

3.3 Other factors

In addition to the dose rate and dose, the FLASH effect is affected by other factors, such as the endpoint and time of observation. For this reason, we restricted our analyses to the same tissues and endpoints. Sorensen showed that the dose modifying factor (DMF) values were different when using skin fibrosis (DMF = 1.14) and acute toxicity (DMF = 1.4−1.58) as the endpoints, indicating a relatively weaker protective effect on the fibrosis endpoint [18]. Similarly,

some studies have shown that under the same dose and dose rate conditions, the magnitude of the FLASH effect decreases with increasing observation time [11, 21], which may be related to self-repair in the tissue.

Individual differences in the experimental subjects may also influence the results. A dose-rate escalation study reported that the dose rate of FLASH differs among mice of different ages [22]. The appropriate mouse strain should also be chosen. For example, studies have shown that C57BL/6J mice are not suitable for the study of pulmonary toxic injury [23].

Some studies have also compared the sparing effect on different irradiation areas. In contrast to hemibrain irradiation, the same dose and dose rate were given to the whole brain, and there was no FLASH effect [19]. The results indicated that the irradiation area is related to the FLASH effect.

Although dose-rate effects and inverse dose-rate effects have long been recognized as radiation responses of organisms, the effect of the dose rate in external beam radiotherapy was generally considered negligible until the advent of ultrahigh dose rate (>10 Gy min^{-1}) irradiation. In recent years, an increasing number of preclinical experiments have confirmed the protective effect of the ultrahigh dose rate on normal tissues. In this study, the inhibitory effect of the ultrahigh dose rate generally correlated positively with the dose and mean dose rate (Figs. 2–4). By analyzing the results of existing experiments with animal models, we found that the probability of triggering the FLASH effect increased and then reached a plateau as the mean dose rate increased. Similar results were found in different tissues, such as the brain and small intestine (Fig. 2 and Fig. 3).

In addition to the mean dose rate, the pulse dose rate had an important influence on the FLASH effect. Recent preclinical systematic studies have shown that with the same mean dose rate in the range of 0.01 Gy min^{-1} to 20 Gy min^{-1}, increasing the pulse dose rate results in more complex damage (unrepaired sublethal DNA damage), resulting in decreased survival of normal and tumor cells [24]. This indicates that the pulse dose rate affects the radiation response at the molecular, cellular, and tissue levels. Few published studies have provided information on the temporal properties of pulses. The relevant parameters, such as pulse dose rate, pulse width, time between pulses, dose per pulse, and number of pulses, should be provided in subsequent studies.

According to the analysis in our study, the dose should reach a certain level to produce the FLASH effect in addition to the dose rate requirement. The dose required for a 50% probability of triggering the FLASH effect in the skin was 24 Gy (Fig. 4). This finding is consistent with the results of dose escalation experiments [18]. Moreover, the results were similar to those of the statistical analysis in Ref. [25]. The authors found a dose threshold of approximately 10 Gy for the whole-mammalian data and approximately 20 Gy for the skin data alone [25]. Notably, some studies have shown that an excessive dose results in greater toxicity, diminishing the FLASH effect [7].

For fractionated treatments, a recent study reported no significant difference in memory skills between the ultrahigh-dose-rate group (mean dose rate between 1.9×10^6 Gy s^{-1} and 7.8×10^6 Gy s^{-1}) and the conventional dose rate group for a single dose of 14 Gy or 4×3.5 Gy, but memory skills were significantly protected with an ultrahigh dose rate of 2×7 Gy [19]. The results suggested that a larger fraction dose produced a greater probability of triggering the FLASH effect. However, studies on the effect of fractionated irradiation on FLASH cells are rare; more studies are needed.

The FLASH effect may also depend on the type of radiation. Given that most of the published studies were based on electron beams, data to analyze the role of radiation type in the FLASH effect are insufficient. It is worth investigating whether the Bragg effect of charged particles has a synergistic effect with FLASH. In addition, the clinical application prospects of X-rays, protons, and very high-energy electrons (VHEEs) have also attracted much attention.

Here, we used a sigmoid function with two parameters to analyze the relationship between the probability of triggering the FLASH effect and the dose rate or dose, whose advantages are simplicity and continuity. Our quantitative results based on this model are consistent with published experimental results. The sudden effect transition (SET) function was used to analyze the relationship between the FMF and dose in Ref. [25], and the results were consistent with the dose analysis results in this paper. The FLASH effect is the result of multiple factors. Therefore, univariate variables, such as limiting to a similar dose for the same tissue and endpoint, were used in the analysis of the relationship between the FLASH score and the mean dose rate. For other tissues, such as the lung, heart, spleen, and muscle tissue, there are too few studies and insufficient data for separate statistical analysis. More experimental data are needed for more comprehensive and accurate analysis.

In addition, novel FLASH accelerator technologies [26–31], online dose monitoring [32, 33], treatment planning [6, 34], and quality assurance [35] are urgently needed.

4 Summary

Elucidating the conditions of the FLASH effect is necessary for its wide application in clinical treatment. Published in vivo experimental data on the FLASH effect were systematically analyzed based on logistic regression

analysis, and we found that the FLASH effect was affected by multiple factors, such as dose, dose rate, tissue type, and the corresponding endpoints. The dose rate corresponding to 50% probability of triggering cognitive protection in the brain was 45 Gy s^{-1}. The dose rate corresponding to 50% probability of triggering intestinal crypt survival and regeneration was 140 Gy s^{-1}. The dose corresponding to 50% probability of triggering the FLASH effect in the skin was 24 Gy. This study helps characterize the conditions underlying the FLASH effect and provides important information for optimizing subsequent experiments. Limited by existing experimental data, other factors, such as the pulse dose, irradiation volume, and fraction dose, need to be further studied and analyzed. Although we addressed both acute and late toxicity effects, additional data on the long-term effects of irradiation at an ultrahigh dose rate are needed to assess its toxicity and side effects. In addition, how FLASH radiation combined with immunotherapy works merits further investigation.

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Data availability The data that support the findings of this study are openly available in Science Data Bank at https://cstr.cn/31253.11.sciencedb.j00186.00150 and https://doi.org/10.57760/sciencedb.j00186.00150.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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