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Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100 Gy/s

Pierre Montay-Gruel [a,b,1], Kristoffer Petersson [c,1], Maud Jaccard [c], Gaël Boivin [a], Jean-François Germond [c], Benoit Petit [a], Raphaël Doenlen [d], Vincent Favaudon [b], François Bochud [c], Claude Bailat [c], Jean Bourhis [a,1], Marie-Catherine Vozenin [a,*,1]

[a] Department of Radiation Oncology/DO/CHUV, Lausanne University Hospital, Switzerland; [b] Institut Curie, INSERM U1021/CNRS UMR3347, Université Paris-Saclay, Orsay, France; [c] Institute of Radiation Physics (IRA), Lausanne University Hospital; and [d] Faculty of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Switzerland

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ABSTRACT

This study shows for the first time that normal brain tissue toxicities after WBI can be reduced with increased dose rate. Spatial memory is preserved after WBI with mean dose rates above 100 Gy/s, whereas 10 Gy WBI at a conventional radiotherapy dose rate (0.1 Gy/s) totally impairs spatial memory.
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Our recent publications have shown that irradiation at an ultra-high dose rate was able to protect normal tissue from radiation-induced toxicity. When compared to radiotherapy delivered at conventional dose rates (1–4 Gy/min), this so called "Flash" radiotherapy (>40 Gy/s; Flash-RT) was shown to enhance the differential effect between normal tissue and tumor in lung models [1,2] and consequently allowed for dose escalation. The biological interest of Flash-RT seems to rely essentially on a specific, yet undefined, response occurring in normal cells and tissues. We initially hypothesized that the protective effect of Flash was related to the high dose rate delivery, in other words related to the very short time of exposure. In order to further explore Flash-RT and to validate its protective effect on normal tissues, we decided to extend our observation from the lung to other organs. We decided to investigate brain response to Flash-RT as it is a well-defined and robust model in radiobiology [3–5].

When dealing with unexpected biological results, such as the ones previously described with Flash-RT, accurate dosimetry of the delivered irradiation is essential. However, dosimetry at (an ultra-)high dose rate in high dose-per-pulse beams is non-trivial as current radiotherapy dosimetry protocols are not designed for such conditions and because the detectors available for online measurements (i.e. ionization chambers, diodes, and diamond detectors) start to saturate when the dose rate/dose-per-pulse is increased beyond what is used in conventional radiotherapy [6–8]. Therefore, we needed to rely on dosimeters that had been previously validated to function accurately at more extreme irradiation conditions, i.e. mainly passive dosimeters. Among these options, we selected thermo-luminescent dosimeter (TLD) chips because of their small size ($3.2 \times 3.2 \times 0.9$ mm^3) so that they could be used for measuring dose in the brain of mice. By positioning the TLD inside the skull of a sacrificed mouse, we were able to validate the dose delivered to the brain during whole brain irradiation (WBI).

Brain injuries after WBI at sub-lethal doses delivered at conventional radiotherapy dose rates are well described [5,9,10]. They include functional alterations, neuronal [11], glial [12,13] and vasculature toxicities [14,15]. Cognitive impairments are the most described functional defects observed in mice and humans following WBI [4,16]. They are caused by an alteration of hippocampal neurogenesis, which can occur as early as one month post 10 Gy single fraction WBI [17]. These cognitive impairments can be evaluated using the "Novel Object Recognition test" [18] on WBI murine models [19]. Therefore, we used this assay to investigate the functional effect of Flash-RT on the normal brain of irradiated mice.

Using a combination of accurate dosimetry measurements and robust biological tests, we first aimed to investigate the potential neuroprotective effect of Flash-RT and indeed found memory preservation in mice after 10 Gy WBI with Flash-RT (delivered in

* Corresponding author at: Laboratoire de Radio-Oncologie, Centre Hospitalier Universitaire Vaudois, Bugnon 46, 1011 Lausanne, Switzerland.
E-mail address: marie-catherine.vozenin@chuv.ch (M.-C. Vozenin).
[1] Equal contribution.

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a single 1.8 μs electron pulse), whereas 10 Gy WBI delivered with a dose rate similar to what is conventionally used in radiotherapy (0.1 Gy/s) impaired mice memory. Then, we decided to further investigate the dose rate limits for Flash-induced neuropreservation. Using systematic dose rate escalation, 100 Gy/s was found to be the lower limit for full preservation of memory functions after 10 Gy WBI.

Materials and methods

Irradiation device

Irradiation was performed using prototype electron beam linear accelerators (LINACs) of type Oriatron 6e (6 MeV) and Kinetron (4.5 MeV) (PMB-Alcen, Peynier, France). This LINAC is able to produce electron beams at a mean dose rate ranging from 0.1 Gy/s (=6 Gy/min, i.e. similar to dose rates conventionally used for radiotherapy) to 1000 Gy/s, corresponding to a dose, in each electron pulse, ranging between 0.01 and 10 Gy. This wide range of dose rate is made possible by varying the linac gun grid tension, the pulse repetition frequency, pulse width, and the source-to-surface distance (SSD).

Dose prescription and measurement

The standard prescription dose for cognition assay is 10 Gy. Therefore, 10 Gy was used in this study as the prescription dose for the WBI. The irradiation settings, corresponding to the prescription dose, were defined according to surface dose measurement in a 30 x 30 cm^2 solid water phantom positioned behind a 1.7 cm in diameter aperture of a graphite applicator ($13.0 \times 13.0 \times 2.5$ cm^3). Beam profiles and percentage depth dose curves of the beam behind the applicator are presented in Fig. S1 in Sup. The measurements were performed for different linac set-ups (LINAC gun grid tension, pulse repetition frequency, number of pulses, and SSD) corresponding to different dose rates used in this study, i.e. 0.1, 1, 3, 10, 30, 100, and 500 Gy/s, as well as 10 Gy in a single 1.8 μs electron pulse (5.6 MGy/s). The pulse repetition frequency was kept at 100 Hz except for the lowest dose rate setting, for which a pulse repetition frequency of 10 Hz was used. The pulse width was kept at 1.8 μs except for the lowest dose rate setting, for which a pulse width of 1.0 μs was used. The absorbed dose at the surface of the solid water phantom was measured for the different dose rate settings with an ionization chamber (Advanced Markus, PTW-Freiburg GmbH, Freiburg, Germany) corrected for chamber saturation [20], with radiochromic film (Gafchromic™ EBT3, Ashland Inc., Covington, Kentucky, USA), with TLD (type: LiF-100), and with Alanine pellets. These different dosimeters, with appropriate correction factors, have all previously been reported to function correctly at the various dose rates used in this study [8,21–25].

Validation of the absorbed dose in the mouse brain

In order to validate that the dose measured at the surface of the solid water phantom actually corresponds to the absorbed dose in the mouse brain, TLD chips were positioned in the brain of one mouse, which had been sacrificed just prior to the experiment. The TLD chips (in vacuum sealed plastic bags) were positioned in the proximal part of the brain, between the two cerebral hemispheres, and in the lateral parts of the brain (left and right sides).

Mice irradiation

95 Female C57BL/6J mice (n = 5–13) were purchased from CRL at the age of eight weeks. Animal experiments were approved by the Ethics Committee for Animal Experimentation of France and Switzerland and performed within institutional guidelines.

Cognitive tests

Dose rate effect on neuroprotection was evaluated by "Novel Object Recognition test" [18], performed on the mice two months post-irradiation, as described by Acharya et al. [19]. All the experiments were video-recorded. Analysis was performed blindedly and the time spent on each object was measured in order to calculate the Recognition Ratio (RR) such as: $RR = \left(\frac{\text{time spent investigating the novel object}}{\text{time spent investigating the two objects}}\right)$.

Statistical Analysis

The statistical analyses of the Novel Object Recognition test and the BrdU data were performed using unpaired t-tests. Results were expressed as mean values ± standard deviations and the significance level chosen was 5%, with Bonferroni correction for multiple comparisons.

Results

Dose prescription and validation measurements

The absorbed dose measurements carried out at the surface of the solid water phantom for the various types of dosimeters showed that 10 Gy was accurately delivered, at the different dose rates used (Fig. S2 in Sup.). The TLD measurements in the brain of the mouse cadaver validated that 10 Gy was actually the dose delivered to the brain for the prescription of 10 Gy WBI, for the highest (10 Gy in a single 1.8 μs pulse) and the lowest dose rate (0.1 Gy/s) used in this study (Fig. 1a and b). The measured absorbed dose in the brain was 10 Gy (10.06 and 9.90 ± 8.2%, k = 2) in the center of the brain (proximal measurements) and slightly below 10 Gy (lateral left: 9.62 and 9.29 ± 8.2%, lateral right 9.56 and 9.72 ± 8.2%, k = 2) at the edge of the brain (lateral measurements in Fig. 1b).

Flash WBI preserves memory and neurogenesis in the hippocampus

A first set of *in vivo* assessments were conducted on mice following 10 Gy WBI with a conventional radiotherapy dose rate (0.1 Gy/s) or with Flash-RT (10 Gy in a single 1.8 μs pulse). Novel Object Recognition tests performed two months post-irradiation showed a significant drop in RR in mice irradiated with 10 Gy at a conventional radiotherapy dose rate, compared to the non-irradiated control group (53.0 ± 1.7% vs. 78.3 ± 2.6%). Interestingly, mice irradiated with 10 Gy in a single pulse did not show any change in RR compared to the control (75.9 ± 4.0% vs. 78.3 ± 2.6%) (Fig. 1c).

BrdU incorporation in the SGZ of the hippocampus was investigated in order to evaluate *de novo* neurogenesis. Multiple neurogenesis sites were found all over the non-irradiated SGZ with a mean (±standard deviation) of 771 ± 188 BrdU positive clusters (Fig. 2) Surprisingly, more than 37% (292 ± 80) of these clusters were preserved in brains irradiated with 10 Gy in a single pulse, whereas, as expected, mice irradiated with 10 Gy at 0.1 Gy/s only showed a low and significantly different preservation of 14% (108 ± 19) BrdU positive clusters. These results highlight a relative preservation of neurogenesis after Flash-RT WBI compared to conventional dose rate WBI. This *de novo* neurogenesis could partially explain the functional preservation described above.

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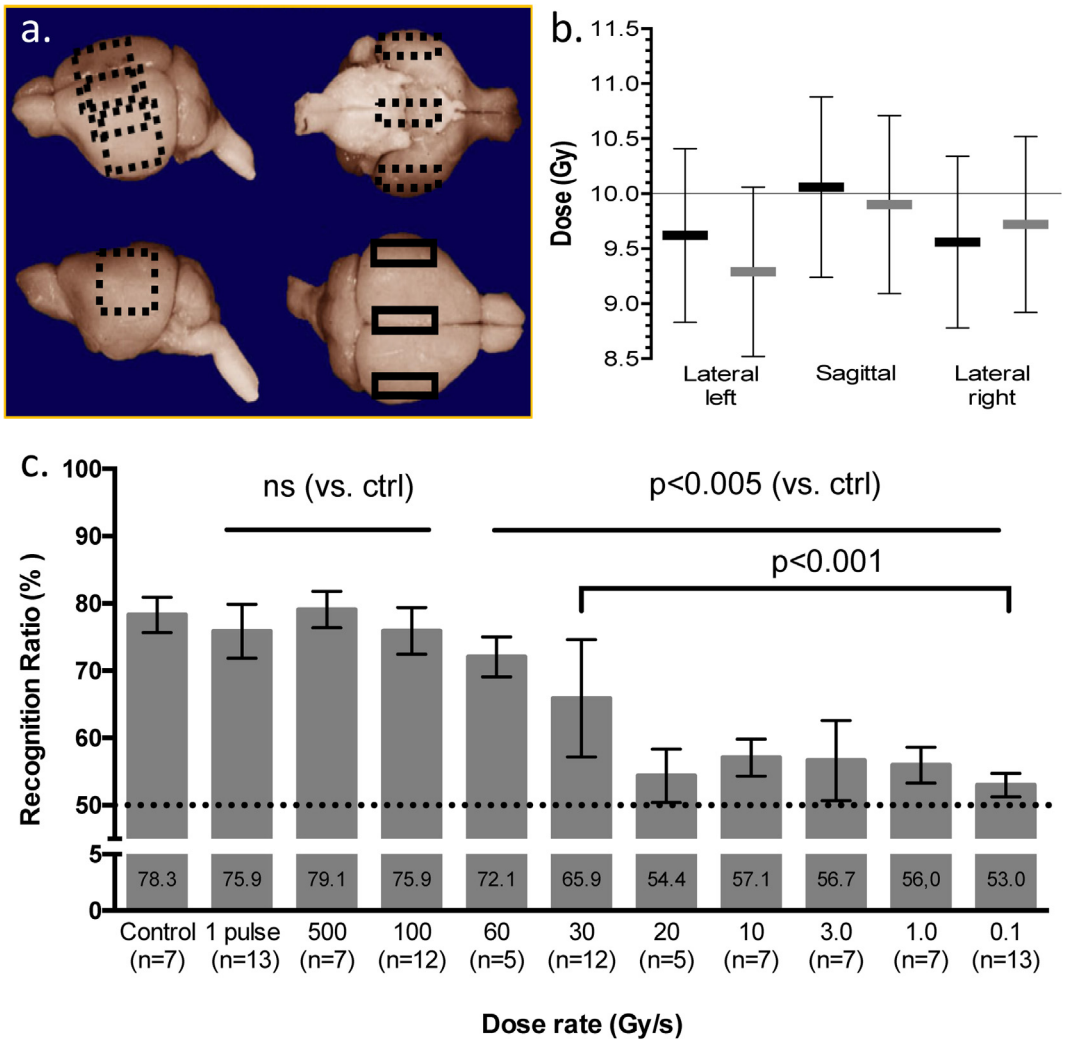

Fig. 1. TLD measurements in the brain of a mouse cadaver, a: TLD chips positions at the center of the brain (sagittal) and at either side of the brain (Lateral left and right); b: measurement results for a 10 Gy WBI delivery with a single 1.8 μs electron pulse (filled markers) and at a 0.1 Gy/s dose rate (open markers). Error bars represent the (expanded, $k = 2$) uncertainty in the absorbed dose measurements with the TLD; c: Evaluation of the Recognition Ratio (RR) two months post irradiation for groups of mice that received sham irradiation (Control) and 10 Gy WBI with a dose rate of 0.1, 1.0, 3, 10, 20, 30, 60, 100, or 500 Gy/s, or with a single 1.8 μs electron pulse (1 Pulse). Bars represent mean values and whiskers the standard deviations.

Flash-RT neuroprotective effect is lost below 30 Gy/s but fully preserved above 100 Gy/s

In order to further investigate the dose rate limits of the Flash-induced neuro-preservation, the experiment was repeated for intermediate dose rates. Interestingly, no memory alteration was observed in the groups irradiated with dose rates of 100 Gy/s or higher (RR were comparable to the ones of the control group), whereas a significant drop in the RR was observed for the group irradiated at 30 Gy/s (Fig. 1c). For the groups irradiated at dose rates below 30 Gy/s, the drop became even slightly larger as the dose rate was further lowered.

Discussion

We have for the first time been able to show that the damage to normal brain tissue, for a given absorbed dose of 10 Gy, can be reduced simply by increasing the dose rate to values 1000 times above what is used in conventional radiotherapy treatments. These unique results show a preservation of memory two months after a 10 Gy WBI with dose rates above 100 Gy/s, whereas 10 Gy WBI at a conventional radiotherapy dose rate (0.1 Gy/s) totally impaired memory.

TLD measurements of the absorbed dose in the mouse brain showed that 10 Gy (9.90–10.06 ± 8.2%, $k = 2$) was truly delivered to the brain center, and slightly below 10 Gy (9.29–9.72 ± 8.2%, $k = 2$) to its lateral parts. This slightly lower lateral dose was expected as the beam profiles (Fig. S1 in Sup.) clearly show a slight decrease in dose with distance from the beam center. As the dose prescription, which was based on surface measurements of a solid water phantom, was validated for the two extreme dose rate settings used in this study, we assumed its validity also for the intermediate dose rate settings.

Brain exposure to ionizing radiation is known to be responsible for long lasting and hardly reversible impairment of cognitive skills. Our results focus on hippocampal related memorization impairment two months post-WBI. Our results show relative hippocampal neurogenesis preservation after Flash-RT WBI assessed by BrdU incorporation, when irradiation at a conventional radiotherapy dose rate is known to directly impair neurogenesis. Neural Stem Cells (NSCs) have been identified in the adult brain as responsible for *de novo* neurogenesis [26]. Therefore, we suggest that the

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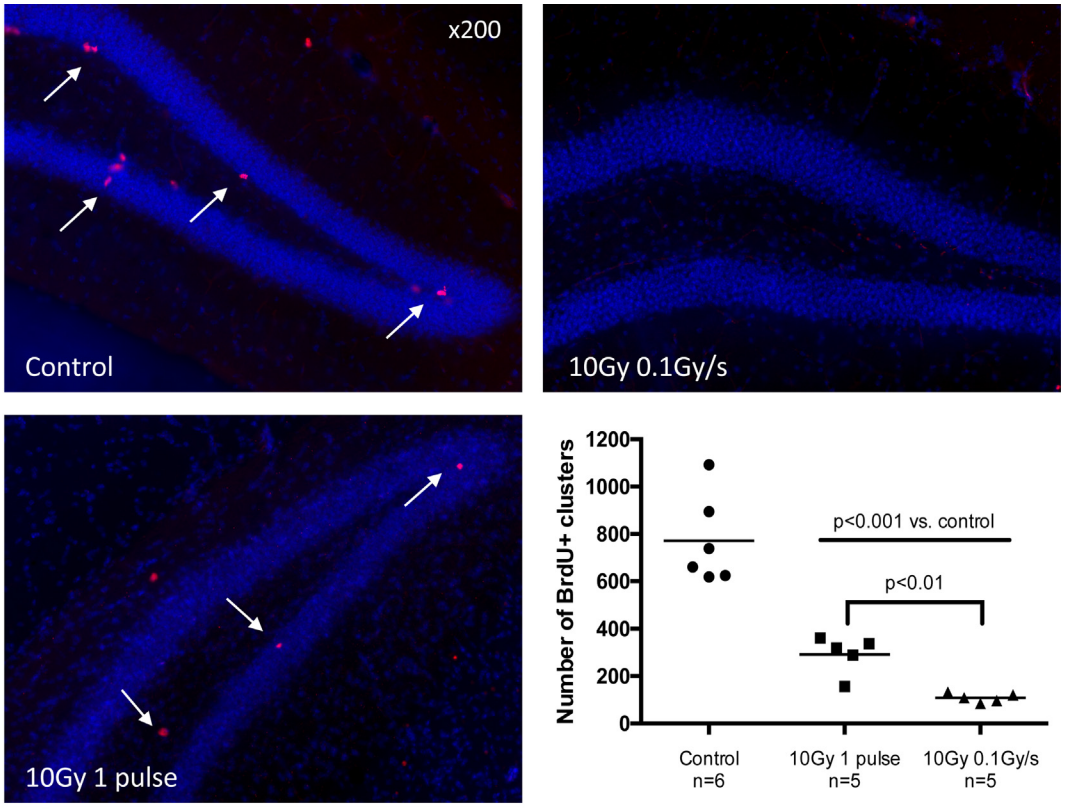

Fig. 2. BrdU immunostaining on brain hippocampal sections of non-irradiated mice (Control) and mice irradiated with 10 Gy at 0.1 Gy/s and mice irradiated with a single 1.8 μs pulse (1 Pulse). Arrows point at BrdU positive clusters in the SGZ. Blue: DAPI; Red: BrdU. Quantification was realized all over the hippocampal sections.

Flash-RT protective effect on neurogenesis relies partly on NSCs preservation. Nevertheless, the memory skills preservation certainly relies on other radiation-induced effects. Both neuroinflammation [27] and synaptic changes [28] are known to interfere with cognitive functions after WBI and could be differentially induced after Flash WBI.

The observed dose rate range of the Flash protective effect on normal tissue gives some physical and biological indications for further investigation regarding the antitumor effect. Despite recent technological developments in radiotherapy, radiation-induced neurotoxicity remains severe in both adult and pediatric patients treated for brain tumors. Our previous results show that Flash-RT demonstrates an antitumor effect similar to conventional radiotherapy [1] in various tumor types, including glioblastoma (preliminary *in vitro* and *in vivo* data). In this context, considering the use of a high dose rate in clinics could be an efficient way to increase the therapeutic ratio of radiation therapy. This radiobiological advantage, together with other practical considerations that benefit from rapid radiotherapy treatment delivery, such as minimizing intra-fractional motion, increased patient comfort, and improved treatment efficiency, makes Flash-RT a promising treatment modality.

Conflict of interest statement

None of the authors have any conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2017.05.003.

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