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Proton irradiations at ultra-high dose rate vs. conventional dose rate: Strong impact on hydrogen peroxide yield

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ABSTRACT:

Background: During ultra-high dose rate (UHDR) external radiation therapy, healthy tissues appear to be spared while tumor control remains the same compared to conventional dose rate. However, the understanding of radiochemical and biological mechanisms involved is still to be discussed.

Methods: This study shows how the hydrogen peroxide (H₂O₂) production, one of the Reactive Oxygen Species (ROS), could be controlled by early heterogeneous radiolysis processes in water during UHDR proton beam irradiations. Pure water was irradiated in the plateau region (T.S. : track-segment) with 68 MeV protons under conventional (0.2 Gy/s) and several UHDR conditions (40 Gy/s to 60 kGy/s) at the Arronax cyclotron. Production of H₂O₂ was then monitored using the Ghormley triiodide method.

Results: New values of $G_{TS}(H_2O_2)$ were added in conventional dose rate. A substantial decrease in H_2O_2 production was observed from 0.2 to 1.5 kGy/s with a more dramatic decrease below 100 Gy/s. At higher dose rate, up to 60 kGy/s, the H_2O_2 production stayed stable with a mean decrease of $38\% \pm 4\%$.

Conclusions: This finding, associated to the decrease in the production of hydroxyl radical ($^{\circ}OH$) already observed in other studies in similar conditions can be explained by the well known spur theory in radiation chemistry. Thus, a two step FLASH-RT mechanism can be envisioned: an early step at the microsecond scale mainly controlled by heterogenous radiolysis, and a second, slower, dominated by O_2 depletion and biochemical processes. To validate this hypothesis, more measurements of radiolytic species will soon be performed, including radicals and associated lifetimes.

Background

Radiation therapy at ultra-high dose rate (UHDR, > 40 Gy/s), has recently shown promising benefits with a reduction in normal tissue toxicity while maintaining the tumor control. This observation, also called FLASH effect, was first shown with electron beams on mammalian cells [1] and more recently on various animal models: mice [2-5], zebrafish embryos [6, 7], cats and mini-pigs [8]. The first patient was treated in 2019 [9]. The FLASH effect was also demonstrated with X-Rays [10, 11], protons [12-15] and helium ions [16].

The mechanisms underlying the FLASH effect are still unclear and appear to be multiple and interrelated [17, 18]. A direct consequence of UHDR irradiations, compared to irradiations at conventional dose rate is a local higher concentration of free radicals within a short time interval, that may modify the chemical pathways. Numerous studies have shown the role of oxygen

concentration in the FLASH effect [6, 7, 19] and this assumption was integrated in various models [20-22]. However, recent studies came to the conclusion that oxygen depletion and transient hypoxia might not be the main mechanism of the FLASH effect [23-25]. Among the other explored mechanisms, the impact on radical production and recombination could have an important role in the FLASH effect [7, 10, 17, 25]. To corroborate the hypothesis that ultra-high and conventional dose rates do not produce the same levels of specific radicals, Montay-Gruel et al. [6] compared Hydrogen Peroxide (H_2O_2) produced after UHDR and conventional dose rate irradiations for electron beams (6 MeV) in water. They measured a significant decrease of H_2O_2 after UHDR (~ 500 Gy/s) compared to conventional dose rate (~ 0.3 Gy/s). Similarly, Kusumoto et al. [26] evaluated radiolytic yield (G values) of 7-hydroxy-coumarin-3-carboxylic acid (7OH-C3C4) as a radical scavenger of hydroxyl radicals ($^{\circ}OH$) while varying the mean dose rate of proton beam (27.5 MeV) from 0.05 to 160 Gy/s. The G value of produced 7OH-C3C4, which is strongly associated to $^{\circ}OH$, decreases with an increasing dose rate.

This study aims to investigate the decrease in the production of H_2O_2 in UHDR irradiations conditions with proton beams. Moreover, we studied the variation of track-segment H_2O_2 production yield with the mean dose rate for 10 dose rates between 0.2 Gy/s to 60 kGy/s.

Materials and Methods

ARRONAX facilities and experimental setup

ARRONAX is an isochronous cyclotron (IBA Cyclone 70XP) that produces protons from 30 MeV up to 70 MeV, deuterons from 15 MeV up to 35 MeV and alpha particles at a fixed energy

of 68 MeV. It offers the possibility of delivering protons beams at dose rates ranging from conventional dose rate to UHDR thanks to available beam intensities (proton intensity can vary from 1 pA to 350 μ A) and a homemade pulsing device developed and validated in house [27]. This system allows from bunches of protons interspaced by 32.84 ns (micro-pulse, RF = 30.45 MHz) to adjust the duration of the irradiation (>10 μ s) and the frequency rate of the macro-pulse repetition, allowing an easy shift between conventional dose rate and UHDR irradiations and a flexible beam structure.

Experimental setups

Our experimental setups for H₂O₂ measurements with 68 MeV proton beams were directly adapted from our irradiation setups designed for the irradiation of zebrafish embryos (Figure 1). A first setup (#1), with a large source-target distance (Figure 1A) enabled a maximal pulse dose rate of 7.5 kGy/s. A second setup (#2) with a shorter source-target distance (Figure 1B) allowed to increase the pulse dose rate up to 60 kGy/s. On Figure 1, K corresponds to a kapton beam exit window, TF to a 50 μ m tungsten foil and C1 (\varnothing 15 mm) and C2 (\varnothing 10 mm) to aluminum collimators. This set of elements were used to spread and homogenize the beam. An online dosimetry was performed with a R928 Hamamatsu Photonics photomultiplier tube (PMT) measuring the UV photons emitted from excited nitrogen of the air present on the path of the incident beam [28], together with an in-transmission parallel-plate ionization chamber (IC) (model 34058, PTW, Freiburg, Germany). Both detectors were calibrated at the beginning of each experiment using a Faraday cup (FC) with an electron repeller positioned after the target, and connected to a MULTIDOS high-precision electrometer (PTW Freiburg GmbH). A dose

uncertainty of 1.5% was assessed in conventional dose rate with the IC, whereas a 3% dose uncertainty was estimated in UHDR conditions using the PMT.

A rack was designed and 3D-printed to support twelve 1.5 mL Eppendorf tubes. Each Eppendorf tube was filled with 1.4 mL of ultrapure purewater ($\rho=18.2\text{M}\Omega\cdot\text{cm}$), closed under air condition and placed far enough from the other tubes (6 cm center to center) to avoid the impact of scattered radiations from one tube to the other (Figure 2A). The beam was directed to the cylindrical part of each tube and laterally centered (Figure 2B). Sample tubes received a track-segment irradiation, while Bragg peak was deposited inside the faraday cup (FC). The average L.E.T. is calculated at $1.17\text{keV}\cdot\mu\text{m}^{-1} \pm 6.5\%$ using a monte-carlo calculation [29]. The rack was placed on an in-house automatic XY translator built using two high-precision linear stages with step motor.

Beam structures

With these experimental setups and a single pulse, the maximal mean dose rate achieved in Eppendorf tubes was around 7.5 kGy/s for setup #1 and 60 kGy/s for setup #2. Using the minimal intensity, the corresponding minimal pulse dose rates were 2.5 Gy/s and 40 Gy/s for setup #1 and #2 respectively. To set a conventional dose rate around 0.2 Gy/s (12 Gy/min), multiple pulses were used and both intensity and frequency were adjusted (Table 1). With setup #1, five UHDR proton irradiations were performed considering a single pulse and mean dose rates from 50 Gy/s to 7.5 kGy/s. While with the setup #2, four UHDR proton irradiations were performed using a single pulse and mean dose rates from 40 Gy/s to 60 kGy/s.

H₂O₂ measurements

Concentrations of H₂O₂ have been determined post-irradiation (about 15 min after irradiation), using the Ghormley triiodide method [30] and two reagents. One is a mixture of ammonium molybdate (Mo₇O₂₄(NH₄)₂H₂O), potassium iodide (KI) and sodium hydroxide (NaOH) and the second one is a buffer solution (pH 4–5) of acid potassium phthalate (C₈H₅KO₄). For a total volume of 2.8 mL, 0.7 mL of both reagents were mixed with 1.4 mL of the sample solution. The concentration of H₂O₂ was obtained indirectly by measurement of I₃⁻ absorbance using a CARY4000 (VARIAN) spectrophotometer. The molar extinction coefficient of I₃⁻ at 351 nm wavelength was previously determined at 21260 L.mol⁻¹.cm⁻¹ in the studied solution at 298 K.

Radiolytic yield calculation

The radiolytic yield (G) is defined as the number of species formed or consumed per unit of deposited energy. It is expressed in the international system in mol.J⁻¹ and is calculated at a time *t* after transition of the ionizing irradiation according to:

$$G(X) = \frac{X_t}{\rho D} \quad (1)$$

where X_{*t*} is the concentration of the species X at time *t* (mol.L⁻¹), ρ is the volumic mass of the irradiated solution (kg.L⁻¹) and D the absorbed dose (in Gy; 1 Gy = 1 J.kg⁻¹ of water).

Results

We observed a significantly lower concentration of H₂O₂ for all doses above 10 Gy in UHDR proton irradiations compared to conventional ones with setup #1 (Figure 3) and setup #2 (Figure 4). These measurements were repeated on independent experiments (2 or 3 times). Track

segment $G_{TS}(H_2O_2)$ values were extracted from the linear fit of these measurements and mean values from repeated measurements are shown in Figure 5 and Table 2.

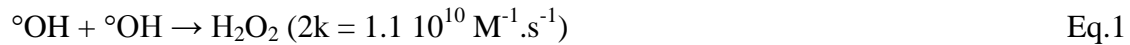
At a conventional dose rate, we obtained a new $G_{TS}(H_2O_2)$ equal to $0.98 \pm 0.05 \cdot 10^{-7} \text{ mol J}^{-1}$ (Table 2). As proton dose rate increases, $G_{TS}(H_2O_2)$ decreases as function of dose rate up to 1.5kGy/s and then remains constant above 7.5 kGy/s with a mean decrease of $38\% \pm 4\%$ (Figure 5 and Table 2). Decrease is already seen at 40Gy/s (-23%).

Discussion

Our experiments with proton beams confirmed the results of lower H_2O_2 production yields in UHDR irradiation compared to conventional dose rate irradiation obtained with electron beams by Montay-Gruel et al [6]. We found a slightly higher decrease in H_2O_2 production yields in the present study. This finding supports the hypothesis that UHDR irradiations have an impact on the ROS production.

This is particularly interesting when considering the major differences between the beams of these two studies, besides the particle type. The conventional mean dose rate used by Montay-Gruel *et al.* [6] (0.29 Gy/s) was quite similar to our conventional dose rate of 0.20 Gy/s. The mean dose rate in UHDR conditions applied by Montay-Gruel *et al.* [6] was around 500 Gy/s, which was reproduced in our experiment. However, beam structures were very different in the two studies. For UHDR irradiations, Montay-Gruel *et al.* [6] used multiple very short pulses (pulse width $< 2 \mu\text{s}$) while we applied longer single pulses, in the millisecond range. Electrons intra-pulse dose rates from Montay-Gruel *et al.* [6] were much higher than ours in UHDR conditions ($2.8 \cdot 10^6 \text{ Gy/s}$ vs. 40 Gy/s to 60 kGy/s) as well as for conventional dose rates ($2.8 \cdot 10^4 \text{ Gy/s}$ vs. 2.5 and 40 Gy/s, depending on the setup used in our experiments).

In this study, we investigated the variation of $G_{TS}(H_2O_2)$ for a large range of mean dose rates in UHDR conditions, from the minimal dose rate of 40 Gy/s ever considered for FLASH radiotherapy in the literature [31]. We find a decrease of $G_{TS}(H_2O_2)$ with an increasing dose rate up to 1.5 kGy/s, with a more dramatic decrease below 100 Gy/s, and followed by a plateau (38% \pm 4%) up to 60 kGy/s. In the study of Kusumoto *et al.* [26], on 7OH-C3C4 giving information on $^{\circ}OH$ production, they used three low mean dose rates (0.05, 0.8 and 7.7 Gy/s) and two UHDR (80 and 160 Gy/s). The G value of produced 7OH-C3C4 decreased steadily with an increasing dose rate and tended to saturate above 80 Gy/s. These findings are in line with our results concerning H_2O_2 and give some hints on the ROS production landscape with a reduced production of $^{\circ}OH$ and H_2O_2 in proton conditions that reached a plateau above 160 Gy/s in both cases. These behaviours could be explained considering the long-established water radiolysis reactions [32]. A lack of $^{\circ}OH$, and therefore a lack of H_2O_2 which is produced through the $^{\circ}OH - ^{\circ}OH$ reaction (Eq.1), could be explained by a faster reaction of $^{\circ}OH$ with aqueous electron radicals (e^-_{aq}) (Eq.2) inside the spurs and overlapping spurs, created by the interaction of the incident particle with water. With this spur theory, we suggest that UHDR conditions would favor the $^{\circ}OH - e^-_{aq}$ reaction (Eq.2) vs.the $^{\circ}OH - ^{\circ}OH$ one (Eq.1), due to a higher reaction kinetic rate and the high density of radiolytic species created in these conditions:



Indeed, in UHDR conditions, there is an increased local dose deposition in time and space coming from the short duration of the irradiation that leads to increased local radical concentrations and that would emphasize this phenomenon. Remaining radicals could then diffuse in medium leading to conditions that are close to that obtained in conventional dose rate

irradiations outside spurs (homogeneous chemistry step) and process as usual to recombinations with the medium. However, even with higher constant rates in Eq.2, all $^{\circ}\text{OH}$ radicals will not be scavenged, which could explain why H_2O_2 production is never completely avoided, even at 60 kGy/s in the present study. Further experiments will be performed to scavenge aqueous electrons and measure the impact on H_2O_2 production in order to test this hypothesis. In addition, H_2O_2 measurements for intermediate mean dose rates between 0.2 and 40 Gy/s will be studied to allow more comparison with Kusumoto et al.[26].

Conclusions

In this work, impact of protons at ultra-high dose rates on the hydrogen peroxide concentration was studied for a wide range of dose rates (from 40 Gy/s up to 60 kGy/s). A decrease in H_2O_2 production yields ($38\% \pm 4\%$) was observed in UHDR conditions compared with conventional ones, which seems to be induced by chemical reactions between several radiolytic species such as e^-_{aq} , $^{\circ}\text{OH}$ and H° . In particular, two steps mechanisms might be involved in the mechanism: (1) a faster one (under the μs scale) with reactions between highly locally concentrated radicals (e^-_{aq} , $^{\circ}\text{OH}$ and H°) in spurs that lead to produce less ROS, (2) a slower one (at the ms scale) which involves radiolytic molecules (H_2O_2 , H_2), remaining radicals and oxygen present in biological tissues. Therefore, in order to understand the mechanisms underlying the FLASH effect, the radiolytic mechanisms should first be investigated extensively. Experimental data should be used as input for models that intend to simulate the production and diffusion of ROS after irradiation under UHDR conditions. Our next efforts will focus on the determination of $G(e^-_{\text{aq}})$ in conventional and UHDR conditions and its comparison with the G of other radicals such as $^{\circ}\text{OH}$ and H° .

Acknowledgment

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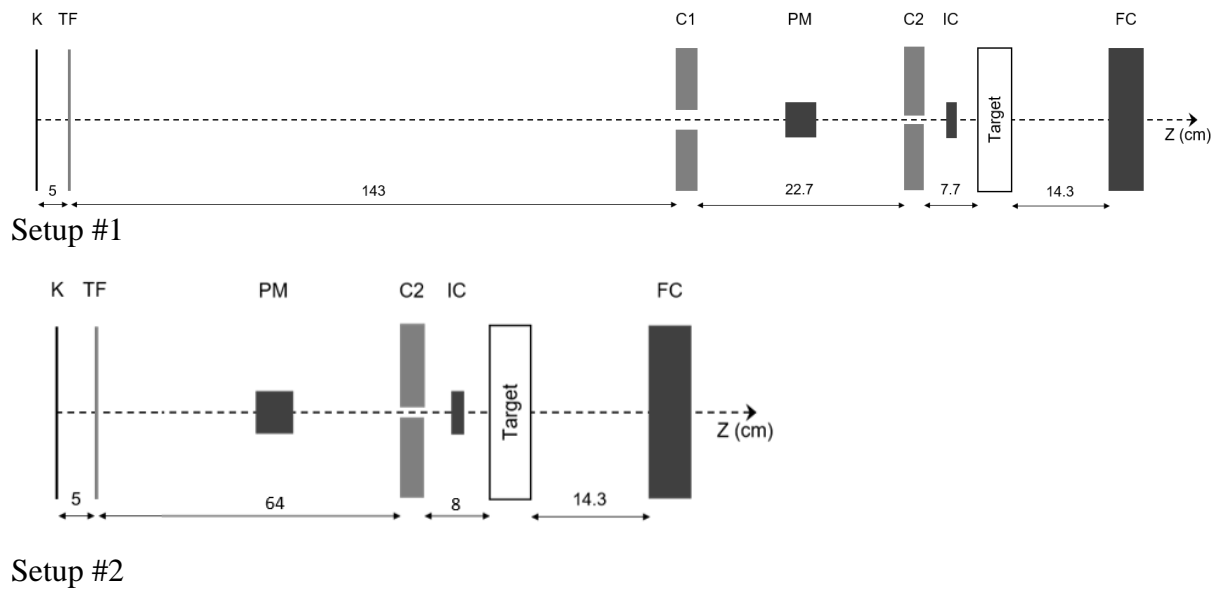
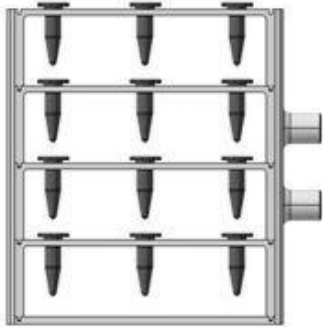


Figure 1. Proton beam experimental setup #1 with a large distance allowing a maximal pulse dose rate about 7.5 kGy/s and experimental setup #2 with a shorter distance allowing a maximal pulse dose rate about 60 kGy/s. K = Kapton (beam exit window); TF = Tungsten foil; C1 = 1st collimator Ø 15 mm; PM = Photomultiplier tube; C2 = 2nd collimator Ø 10 mm; IC = ionization chamber; FC = Faraday cup Ø 30 mm.



A.



B.

Figure 2. (A) 3D-printed Eppendorf tubes rack to be fixed on the in-house automatic XY translator. (B) 1cm diameter beam presented on a 1.5 mL Eppendorf tube, filled with 1.4 mL of water.

Table 1: Beam structures to deliver 6 doses values in conventional and UHDR irradiation modes for setup #1 and #2.

Setup	Mean dose rate (Gy/s)	Pulse Dose rate (Gy/s)	Number of pulses	Pulse width (ms)	Frequency (Hz)	Doses (Gy)
Setup #1	0.2	2.5	[2400 – 40500]	0.80	100	[5, 10, 20, 30, 40, 80]
	$7.5 \cdot 10^3$	$7.5 \cdot 10^3$	1	[0.67 – 10.67]	-	
	$1.5 \cdot 10^3$	$1.5 \cdot 10^3$		[3.30 – 53.30]		
	500	500		[10.0 – 160.0]		
	100	100		[50.0 – 800.0]		
	50	50		[100.0 – 1600.0]		
Setup #2	0.20	40	[1500 – 20550]	0.10	50	[10, 20, 30, 40, 60, 80]
	$60 \cdot 10^3$	$60 \cdot 10^3$	1	[0.167 – 1.33]	-	
	$40 \cdot 10^3$	$40 \cdot 10^3$		[0.25 – 2.0]		
	$20 \cdot 10^3$	$20 \cdot 10^3$		[0.50 – 4.0]		
	40	40		[250.0 – 2000.0]		

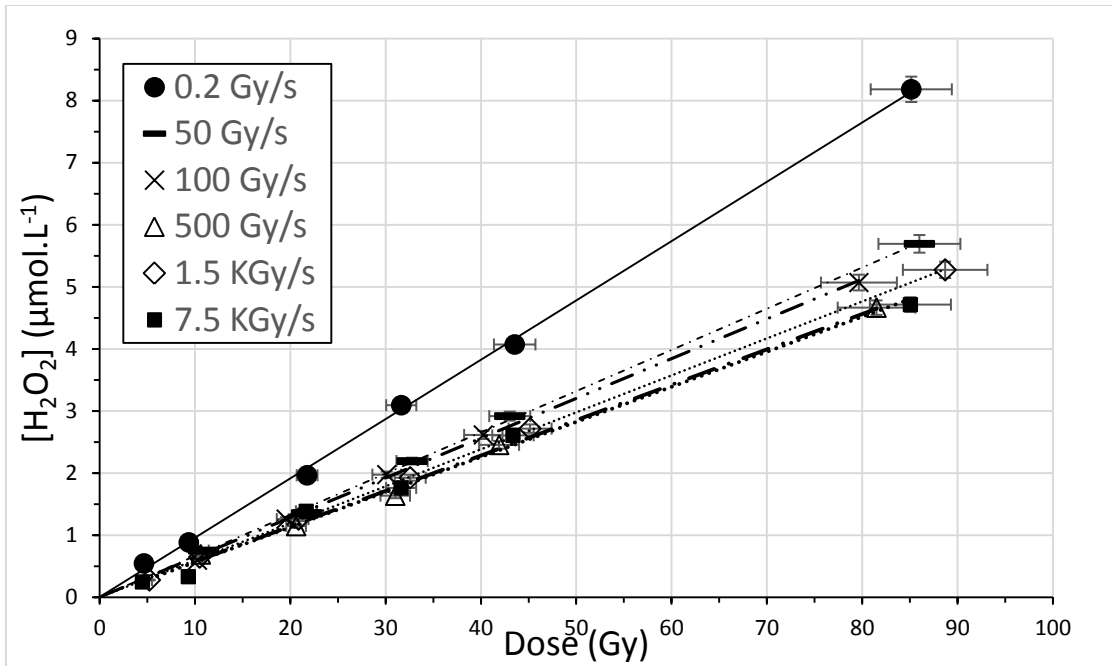


Figure 3: H_2O_2 measurement with the setup #1: five ultra high dose rates from 50Gy/s to 7.5 kGy/s with the same beam structure (single pulse), and one conventional dose rate of 0.2 Gy/s (multiple pulses). For the sake of readability, only one dataset per dose rate is shown among all measured data.

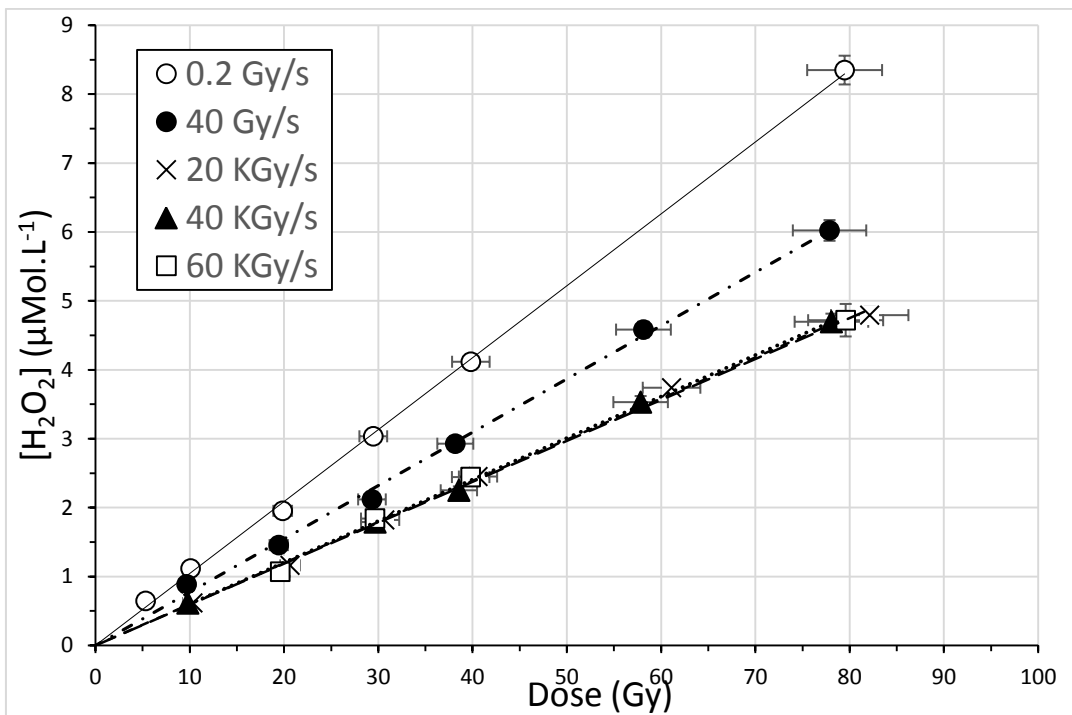


Figure 4: H₂O₂ measurement with the setup #2: four UHDR from 40Gy/s to 60 kGy/s with the same beam structure (single pulse) and one conventional dose rate of 0.2 Gy/s (multiple pulses). For the sake of readability, only one dataset per dose rate is shown among all measured data.

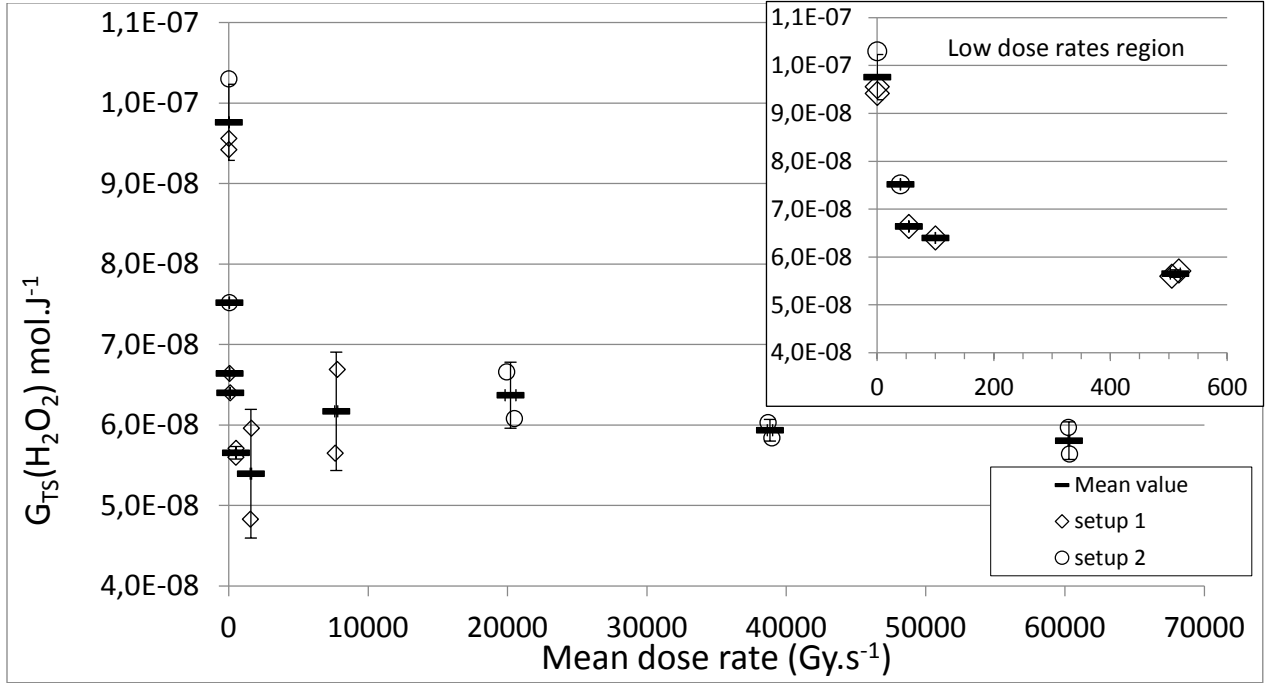


Figure 5: $G_{TS}(H_2O_2)$ in $mol.J^{-1}$ for all experiments (setup #1 and #2).

Table 2: $G_{TS}(H_2O_2)$ obtained for conventional and UHDR irradiations. G values are obtained from experiments presented on Figures 3 and 4 for setup #1 and #2 for six different doses and a linear trend line.

Mean dose rate (Gy/s)	$G_{TS}(H_2O_2)$ 10^{-7} mol/J	Mean $G_{TS}(H_2O_2)$ 10^{-7} mol/J (SD)	R ²	Decrease (%) in UHDR $G(H_2O_2)$ from mean conventional $G_{TS}(H_2O_2)$
0.20	0.96	0.98 (4.7e-3)	0.999	-
	1.03		0.999	
	0.94		0.997	
40	0.75	-	0.997	23
50	0.66	-	0.999	32
100	0.64	-	0.999	34
500	0.56	0.57 (7.8e-4)	0.997	43
	0.57		0.997	41
1.5 10 ³	0.48	0.54 (8.0e-3)	0.996	51
	0.60		1.000	39

7.5 10³	0.57	0.62 (7.4e-3)	0.993	42
	0.67		0.994	31
20 10³	0.67	0.64 (4.1e-3)	0.990	32
	0.61		0.999	38
40 10³	0.58	0.59 (1.3e-3)	0.999	40
	0.60		0.999	38
60 10³	0.60	0.58 (2.3e-3)	0.997	39
	0.56		0.986	42

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