Plasmid DNA damages after FLASH vs conventional dose rate irradiations in various oxygen conditions

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Purpose

Ultra high dose rate (UHDR) irradiation, several orders of magnitude higher than in conventional dose rate (CONV) radiotherapy, causes less damage to healthy tissue without impacting tumor control (Montay-Gruel et al, 2020). The physico-chemical and biological mechanisms underlying this effect called FLASH are currently being investigated. In our work we thought to compare the effects of CONV and UHDR by quantifying DNA strand breaks (SB) using a plasmid (pBR322) under various oxygen concentrations.

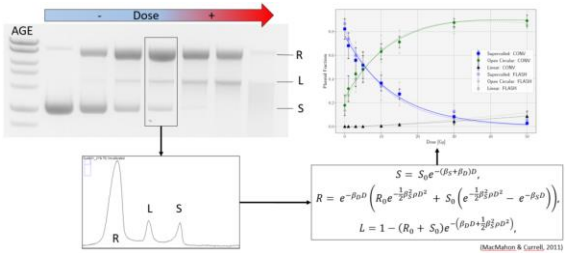

Figure 1. Plasmid forms can be separated and quantified by AGE. Through fitting, quantification allows to estimate SSB et DSB yields

Materials and Methods

pBR322 plasmid (4361 base pairs) was irradiated using a FLASH-validated 6 MeV electron beam (eRT6 Oriatron, PBM-Alcen) with increasing doses (1-100 Gy) and dose per pulse (0.01 Gy/s in CONV, $5.0*10^2$ to $5.6*10^6$ Gy/s in UHDR). pBR322 was irradiated dry or in water equilibrated at 21% (atmospheric level), 4% (physioxia) or 0.5% (severe hypoxia) using an hypoxia hood. The supercoiled (S), relaxed (R) and linear (L) plasmid forms were quantified by agarose gel electrophoresis (AGE). Yields of radio-induced single (SSB) or double SB (DSB) were then computed using a mathematical model (McMahon & Currell, 2011) (fig 1).

Results

High doses were required to produce measurable SB in dry pBR322 in absence of any free radicals. In this condition, UHDR and CONV irradiations produce similar yields of SB (fig 2).

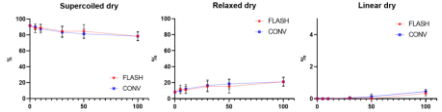

Figure 2. Plasmid forms after UHDR and CONV irradiations of dry plasmids.

In aqueous solution and under atmospheric condition, 50% of relaxed form was produced at 2 Gy and 50% of linear form at 18 Gy. The yield of SB was similar for UHDR and CONV. As expected, the plasmid was radio-protected in physioxia, still without difference between CONV and UHDR. Interestingly, hypoxia revealed a difference between UHDR and CONV: at 50 Gy, UHDR produced less than 5% of linear form whereas it produces more than 20% of them in CONV (fig 3). Subsequent computed SB yields are summarized in Table 1.

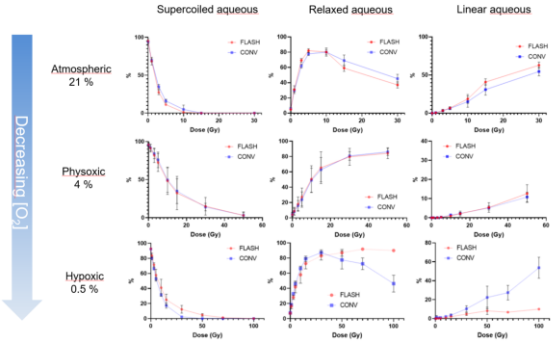

Figure 3. Plasmid forms after UHDR and CONV irradiations of plasmids in aqueous solution and various oxygen concentrations.

pBR322 irradiation	SSB yield (10^{-3} SSB / Gy / plasmid)		DSB yield (10^{-2} DSB / Gy / plasmid)	
Condition	FLASH	CONV	FLASH	CONV
21% oxygen	39.96 ± 9.12	34.22 ± 4.60	2.55 ± 0.62	2.11 ± 0.34
5% oxygen	6.32 ± 0.58	6.14 ± 0.68	0.21 ± 0.10	0.18 ± 0.12
1% oxygen	7.93 ± 0.78	10.99 ± 0.70	0.17 ± 0.10	0.38 ± 0.08
Dry	0.16 ± 0.02	0.18 ± 0.06	0.00 ± 0.02	0.01 ± 0.03

Table 1. SB yields in various conditions (mean +/- 2σ)

Conclusion

These experiments provide several informations about the chemical mechanisms involved after exposure to UHDR and might bring some insight to understand the FLASH effect. First, we showed that the direct effects are not enhanced at UHDR thus suggesting that they are not involved in the FLASH effect. Then, we showed that UHDR induce less SB than CONV under hypoxic conditions consistently with previous experiments published in bacteria as well as editorials and modelling papers published in the field (Weiss et al, 1974; Pratx & Kapp, 2019). However, in physioxia, no difference in DNA breaks was measured after UHDR and CONV. Then, assuming that 4% oxygen mimics healthy tissues while 0.5% oxygen mimics tumors, these results are opposite to the preclinical results showing the FLASH effect (protection of normal tissue and eradication of tumor). Thus, plasmid irradiation might be useful to understand DNA damage at UHDR but seems barely relevant to investigate the FLASH effect at the biology level.

Acknowledgments

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References

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