The effect of high dose rate laser driven radiation on the integrity of amino acids and implications in toxicity evaluation

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ABSTRACT

Lately, novel radiation therapy methods using advanced laser technologies with ultra-high dose rates ("FLASH"), such as laser-accelerated particle beams and laser-driven ionizing radiation in pulses, with time spans from fs to ns started to emerge, having encouraging results in numerous studies concerning toxicity characterization [1,2]. Significant parameters of radiation therapies like high radiation intensities and short irradiation times are intended to improve treatment's outcome and to have a better individualized treatment for each patients' needs. One of the most important issues in radiotherapy is to find the right compromise between radiation's efficacy to reduce the tumor and its toxicity. Also, it's necessary to explain the mechanisms underlying the effects induced by ionizing radiation in biological matrices, and for that reason, endogenous molecules that provide information on toxicity are required. Amino acids can be selected as viable candidates for biomarkers due to their involvement in many cellular metabolic processes determined by high dose-rate radiations.
As forerunner experiments of laser driven irradiation trials, in this study we have investigated experimental models of gamma irradiated lyophilised glioblastoma cells lines. The evaluation of amino acids was performed both from a qualitative and quantitative point of view, using a Reversed Phase Liquid Chromatography with diode-array detection (RPLC-DAD) employing a precolumn derivatization method. This inquiry consisting in characterizing and profiling, allows us to have an insight into amino acids as biochemical evidences and their pathways in metabolic processes stimulated by radiation, in order to anticipate whether low toxicity using FLASH radiation was achieved.

METHODS

Samples irradiation:
Source: ^{60}Co (GC 5000, IRASM, IFIN-HH)
Dose rates: 1.2; 5.07 Gy/s
Absorbed doses: 10; 20 Gy

Source: LINAC - X Rays (6MV)
(Amethyst Radiotherapy Centre)
Dose rates: 0.15; 0.58 Gy/s
Absorbed doses: 10;15;20;25;30;35;40 Gy

- **Equipment:** HPLC-DAD system, Waters Corp
- **Detection:** Diode array detector (wavelengths: 330, 260 & 241 nm)
- **Column:** AdvanceBio AAA, 4.6 x 100 mm (Agilent Scientific)
- Temperature: 40 °C
- Injection volume: 1 µL
- Flow rate: 1.5 mL/min
- DAD Gradient elution
- **Mobile phases:** Aq: 10 mM Na_2HPO_4, and 10 mM $Na_2B_4O_7$ w/ pH= 7.9; Organic: ACN:MeOH:H_2O (4:4:2)
- **Online derivatization**

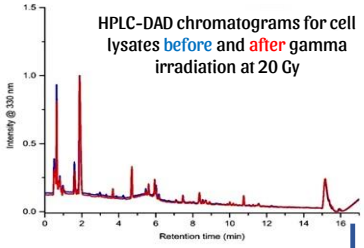
RESULTS

AA quantification - curve fit and LOD/LOQ results

Amino acid	alanine	arginine	citrulline	glutamic acid	glutamine	methionine	phenylalanine	sarcosine	threonine	tryptophan	tyrosine
Correlation coefficient	0.9999	0.9999	0.9998	0.9998	0.9996	0.9998	0.9999	0.9998	0.9999	0.9997	0.9999
LOD (nmol/ mL)	13.044	2.937	13.959	13.453	21.017	16.695	11.144	26.090	10.953	17.064	8.448
LOQ (nmol/ mL)	39.518	8.901	42.117	40.766	63.688	50.591	33.770	79.061	33.190	51.709	25.600

before & after irradiation @ 20 Gy
- threonine 57.58%
- arginine 18.60%
- alanine 22.78%
- tyrosine 76.01%

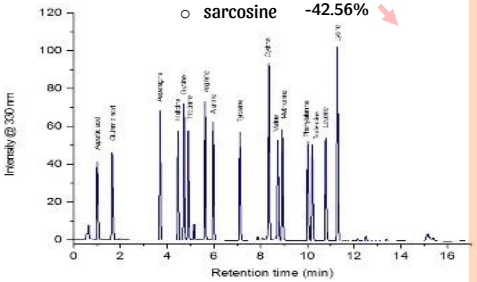
before & after irradiation @ 5.07 Gy/s
- isoleucine -53.53%
- sarcosine -42.56%



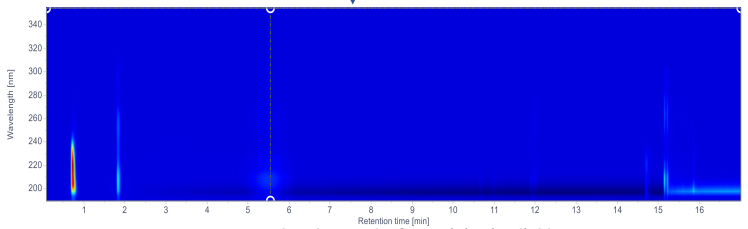
HPLC-DAD chromatograms for cell lysates before and after gamma irradiation at 20 Gy

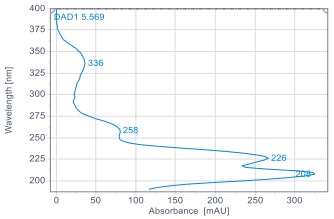
AA identified in cell medium and GB cells:
- asparagine
- tyrosine
- cystine
- phenylalanine
- isoleucine
- leucine
- lysine

AA identified only in GB cells:
- glutamic acid
- threonine
- arginine
- alanine
- methionine
- sarcosine



AA qualitative analysis





Isoabsorbance plot for arginine in glioblastoma

CONCLUSION

This study highlights the importance of chromatographic method development for accurate metabolomic profiling of GB cells after their gamma and Xray irradiation as preliminary experiments ahead high dose rate laser driven irradiations;

The HPLC-DAD method for amino acids achieved by pre-column derivatization was a simple, rapid and cost-effective one, while providing high sensitivity, repeatability and selectivity over the tested concentration range;

There were no significant differences before and after Xray irradiation, whereas gamma irradiation induced a decrease in the concentration of several AA;

Potential dose-dependent and dose rate-dependent irradiation biomarkers were identified and quantified.

References:
[1]Hofmann K.M., Schell S, Wilkens JJ., *J Biophotonics*, 2012, 5, 903–911;
[2]Tajima T., Habs D., Yan X., *Rev Accl Sci Tech*, 2009, 02, 201–228.

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