X-ray Micro-tomography Image Analysis for quantifying the Impact of Radiotherapy on Normal and Tumoral Brain Vessels

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Rationale and objectives

X-ray synchrotron micro-tomography is a high-resolution technique for providing accurate 3D images of brain vascular networks [1]. A previous experiment on non irradiated normal and tumorous rats brain samples brought interesting results concerning the quantitative structural properties of vessel networks [2]. Microbeam radiation therapy (MRT) is a new promising technique for brain tumour treatment. Its impact has been hypothesized to be different on normal or tumoral vessels [3]. We hereby present a new method for addressing the question of the MRT effect on functional 3D micro-vascular networks. A first quantitative estimate of MRT impact on both normal and tumoral vessels networks in rat brains is presented. Such study could lead to a better knowledge of MRT parameters influence on vessels damages and reparations.

Methods

14 days after inoculation of 9L gliosarcomas in rat caude nucleus, MRT was performed using 50 µm- or 25 µm-wide beams usingin two orthogonal arrays of 50 planar microbeams (50µm 480 Gy; 25µm: 860Gy). At different delays after MRT (2, 7, and 14 days after MRT i.e., 16, 21 and 28 days after implantation) the animals were euthanized and perfused with a Baryum sulfate solution, a x-ray contrast agent, providing three groups D+2, D+7 and D+14. Bi-irradiated tumors as well as mono-irradiated healthy tissues were then sampled from rat brains, providing cylindrical samples measuring 1 cm in height and 2,5 mm in diameter. A total of 44 samples were classified into 6 groups and imaged using X-ray micro-tomography on ID19 in absorption mode at 21 keV with a spatial resolution of 1.4 µm.

Results

Tumor vessels are recognizable from their much larger diameter and irregular shapes. In most cases, vessels direction follows a circular pattern around the tumor centroid. Damages caused by the microbeam path result in two kinds of leakages appearing in images of irradiated areas: minor leak (“milky” thread) and lakes. The former have a vaporous texture when the latter appears like a dense clouds. Qualitatively one can first observe that the 25 µm beam width damages seem most important in the D+2 group (rather than on D+7 and D+14) where lakes are most often observed, especially in tumoral tissue. Normal brain samples are almost free of any observable leak, except for minor leakages more or less lying along vertical planes through cortical regions.

Vascular network irradiated with 50 µm beam width appears quite different from those irradiated with 25 µm. Whatever the delay after irradiation there are systematically very sparse and sometimes, for D+2 (in 3 samples out of 6), massive leaky region with very large lakes are visible. This observation seems to indicate that the recovery to MRT-

damaged of tumoral micro-vascular networks is possible for 25 µm beam but very difficult for 50 µm beam width. One should nevertheless analyze these results relatively, using healthy tissue as references. We perform a first estimate of the contrast agent relative volume in tissues :

	Bi-irradiated tumoral tissue	mono-irradiated healthy tissue
50 µm	1.15%	0.75 %
25 µm	4.7%	1.69%

Conclusion

The drastic increase of observed leakages in tumor tissues comforts the hypothesis that MRT brings more damages on tumor vessels than on normal ones. Only minor leaks following straight vertical planes might be attributable to beam paths where vessels crossing them are clearly cut or damaged. When a 50 µm beam width is used, the sparsity of the vessel network could result from major damages on vessels providing upstream leaks and major dysfunction in flow distribution.

References

[1] Plouraboué F, Cloetens P, Fonta C, Steyer A, Lauwers F, Marc-Vergnes JP, X-ray high-resolution vascular network imaging, Journal of microscopy, 2004, vol. 215-2, pp 139-148.

[2] Risser L, Plouraboué F, Steyer A, Cloetens P, Le Duc G, Fonta C, From homogeneous to fractal normal and tumorous microvascular networks in the brain, Journal of cerebral blood flow and metabolism, 2007, vol. 27, pp 293-303.

[3] Laissue et al., Neuropathology of ablation of rat gliosarcomas and contiguous brain tissues using a microplanar beam of synchrotron-wiggler-generated X rays, Int. J. Cancer, 78, 5,1078-1083, 1998.