Vascular tumor response to synchrotron microbeam radiation therapy. A short term in vivo study.

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Introduction:

Microbeam radiation therapy (MRT), an experimental radiosurgery for tumors using micrometer-wide synchrotron-generated parallel X-ray beams, is based on the principle of spatial instead clinically used of temporal fractionation. MRT-damaged microsegments in the poorly differentiated neovasculature of brain tumors may not be efficiently repaired, in marked contrast to the rapid repair of radiation-damaged normal microvasculature by minimally irradiated cells surviving in the tissues slices situated between the microbeams [1, 2, 3]. The aim of this work was to study the tumor vessel response after microbeam exposure using magnetic resonance imaging.

Material and Methods:

Thirteen days after inoculation, rat 9L gliosarcomas implanted in nude mice brains (n=70) were irradiated (n=45) using two orthogonal arrays of 28 planar microbeams (width 25µm, interbeam spacing 211µm, in-microbeam entrance dose 500 Gy) Twenty-five unirradiated animals were kept as control. At different delays after MRT (1, 7, and 14 days after MRT *i.e.,* 14, 21 and 28 days after implantation) apparent diffusion coefficient (ADC), blood volume (BV) and vessel size index (VSI) were mapped at 2,35T in 38 anesthetized animals (30 MRT-treated and 8 non-irradiated) using a diffusion MR sequence (b=0 and b=700 s.mm^{-2}) and a multiple gradient echo-spin echo MR sequence (TR=6s, Gradient echoes=[6-42]ms, Spin-echo=102ms), the latter images being acquired before and after injection of Sinerem® (200-300 µmol Fe/Kg, tail vein injection). The voxel size was 234x234x800µm^3. Two regions of interest were defined: whole tumor and contralateral hemisphere. An ANOVA test was used to compare different data groups (*: p<0.05, **: p<0.01, ***: p<0.001). Tumor surfaces were calculated on the T_2 weighted image on which the tumor appeared the largest. A Mann Whitney test was used to compare tumor areas (***: p<0.001). Survival curves were plotted from 25 unirradiated animals and 45 MRT-treated animals.

Results and discussion:

At any time after implantation, ADC, BV and VSI were higher in tumors than in contralateral hemispheres (fig1a). A significant increase of ADC has been observed 24 hours (D14 and D14 MRT, fig1a) after MRT in irradiated tumors (1081 ± 73 × 10^{-6} mm².s^{-1}) versus unirradiated ones (978 ± 81 × 10^{-6} mm².s^{-1}) (p<0.001). This could reflect an increase in tumoral blood vessel permeability. During the 3rd week of tumor development, ADC and VSI measured in unirradiated tumors increased significantly (+14% (p<0.001) and 23% (p<0.05) respectively). In the irradiated group, no change in VSI was observed between the 14th and the 21st day after tumor inoculation but, thereafter, the VSI augmented significantly between 14 and 28 days of tumor growth (+26% (p<0.01)). There was no significant change in BV between irradiated and non irradiated groups which may suggest that MRT did not have important effects on tumor blood vessels. MRT inhibited tumor growth within the first week after treatment (fig1b). Indeed, there was no difference in tumor size between the 14th and the 21st day after tumor inoculation in MRT-treated group while, simultaneously, tumor size increased (×3) in the non-irradiated group (p<0.001). Fourteen days after MRT, *i.e.* 28 days after inoculation, tumor size started to increase again and all mice died in the following days. Survival time increased significantly in irradiated group versus non-irradiated mice (p<0.0001, median survival: 21 versus 28 days).

Conclusion:

9L gliosarcomas, in an heterologous system, present increased ADC, BV, and VSI like other glioma models which can be measured by MRI [4]. MRT slows 9L tumor growth in mouse brain but MRI results suggest that the increase in survival time after our MRT approach may be due to a cytoreduction rather than direct effect of ionizing radiation on tumor blood vessels. These results were confirmed by preliminary studies which have shown that at any time after radiation treatment, tumor vessels immunoreactive for type-IV collagen and PECAM-I proteins were detectable (data not shown) and perfused (decrease in T_2* after Sinerem® injection). These results suggest that MRT parameters could be honed to cause major damage to tumor vessels. The increase of ADC values 24h after MRT suggests an increase in blood tumor vessel permeability. The latter could be exploited to deliver cytotoxic or antiangiogenic agents specifically to tumor tissue *via* the circulatory system.

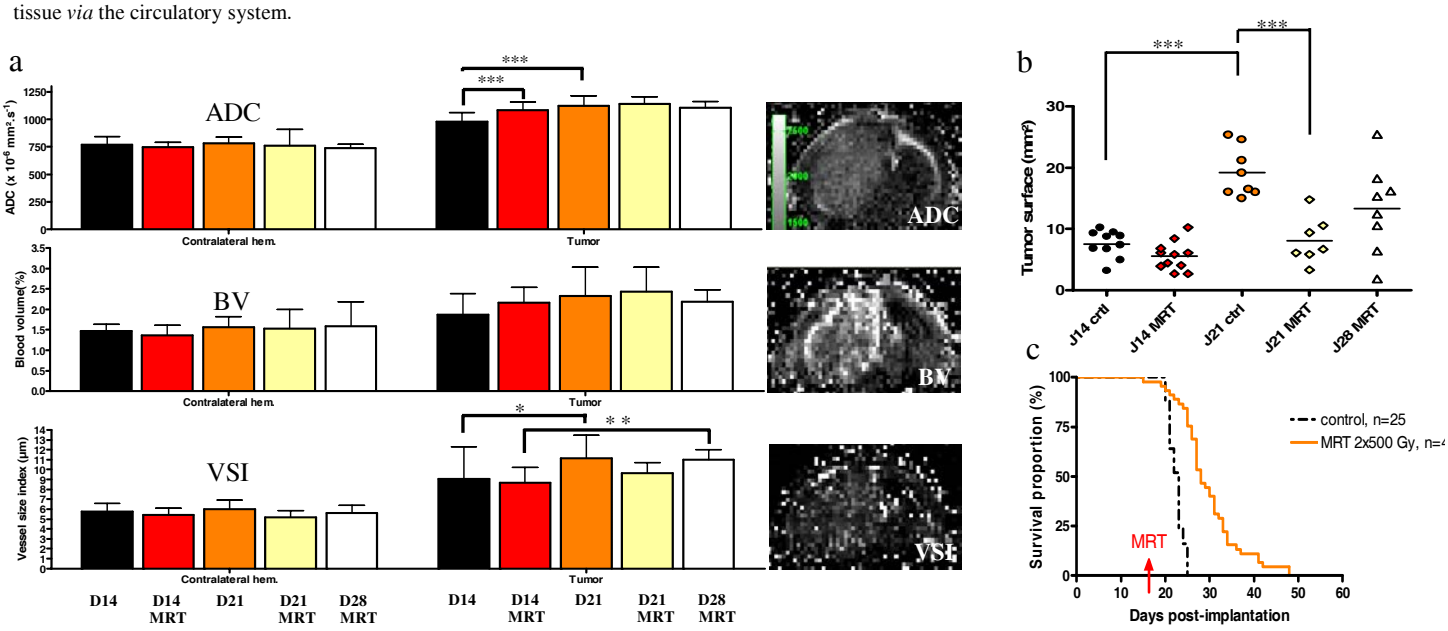

Fig 1: a-Means (±SD) of ADC, BV and VSI (and associated MR images (D21)) measured in contralateral hemispheres and in tumors at different times after tumor inoculation (14, 21 and 28 days). Black and orange bars represent the values for non irradiated control groups. Red, yellow and white bars represent the values for animal groups after MRT. b-Individual values (plots) and means (lines) of tumor areas measured on T_2 weighted images at different times after tumor inoculation ± MRT. c-Survival curves of unirradiated (broken line) and irradiated (orange line) mice bearing intracerebral 9L rat gliosarcomas.

[1] D.N. Slatkin, P. Spanne, F.A. Dilmanian*, et al.*, *Proc Natl Acad Sci U S A*, 92, 8783-7, (1995). [2] J.A. Laissue, G. Geiser, P.O. Spanne*, et al.*, *Int J Cancer*, 78, 654-60, (1998). R. Serduc, P. Verant, JC. Vial *et al.*, *Int J Rad Oncol Biol Phys,* 64, 1519-27 (2006). [4] I. Tropres, L. Lamalle, M. Peoc'h*, et al.*, *Magn Reson Med*, 51, 533-41, (2004).