Multiparametric characterization of the sex differences in a high-grade glioma rat model by in vivo magnetic resonance and post-mortem analysis.

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Target Audience

The present work may be addressed to people interested in testing animal models of brain tumor by using magnetic resonance (MR) approaches and people with interest in analyze physiological or physiopathological gender dependent differences detected by MR. We used for that a glioblastoma multiforme (GBM) animal model. GBM is the most frequent, aggressive and lethal intracranial tumor. Sex differences in the development of GBM have been little studied to date despite that men are 1,5-2 times more likely than women to develop it[2], regardless age or sexual maturity[3]. Genetics sex differences may play a role in the pathogenesis of gliomas, and surveying their molecular mechanism may improve our understanding of brain tumor and the development of therapies. Magnetic resonance (MR) is a widely used and versatile technique in neurooncology[4] that provides information about blood flow/volume, edema and cellularity[5].

Purpose

Our main objective was to analyze the sex dependence in the assessment of MR parameters, linked to brain tumorigenesis, by using a C6 high-grade glioma rat model.

Methods

High-grade gliomas were induced in Wistar rats by stereotaxic injection of 10^5 C6 cells in the right caudate nucleus. MR images were obtained on a 7T system with a ^{1}H selective birdcage resonator. Experiments were carried out 16-18 days after C6 cells (tumor bearing rats) or medium (sham rats) intracranial injection. MR protocol: a) T2W images with a RARE sequence (TR/TE=3000/60ms, Av=3, RARE factor=8) and b) CE-T1W images (TR/TE=600/10,467ms), both with an in plane resolution of 148µm/pixel, slice thickness=1.5 mm and number of slice =5; c) MT contrast imaging with two sets of acquisitions –the first one with the application of an MT train pulse and the second without it- to yield magnetization transfer ratio (MTR) maps (TR/TE = 2500/10ms, Av = 1, MT pulse train of N =50, bandwidth = 550 Hz, length=5ms, power=5,5 µT and offset=1500 Hz); d) DTI-EPI acquisitions (TR/TE=3000/40ms, 7 directions, b 300 and 1400 s/mm2, Δ/δ=20/4ms); FOV= 35x35 mm, mtx=128x128; plane resolution, 273µm/pixel); e) PWI by bolus tracking acquisition (TR/TE=250/7ms, single shot and 150 repetitions); f) MR spectroscopy of a voxel placed in the tumor -or analogous region in sham rats- using a PRESS sequence (TR/TE=3000/35ms, Av=128, volume=27µl). Images were computed in a pixel by pixel bases with a home-made software application written in MatLab (R2007a) to obtain parametric maps: MT ratio (MTR), fractional anisotropy (FA), mean diffusivity (MD), cerebral blood flow (CBF), volume (CBV) and mean transit time (MTT). Data were analyzed with Image J by manually selecting two tumoral regions: periphery and core. Spectra were processed with LcModel. Post-mortem analysis were performed to evaluated BBB integrity and glutamate and glutamine.

Results

Figure 1 presents the main obtained results from MR studies. Magnetization transfer maps allows to assess distribution of water molecules in the tissues depending on their environment. MTR values (panel A) were always lower in females than in males, and this phenomenon was more remarkable in rats with tumor in the regions assessed. Diffusion studies yielded parameters directly related to the microstructure of the brain by analyzing the higher or lower restriction to the transversal movement of the water molecules in the tissue. MD values were higher in males than in females in the tumor regions. FA was always decreased in females by comparing with male rats in all ROIs and experimental group (panel B). Perfusion imaging assesses the microvasculature of the brain and offers information about the angiogenesis in the glioma. Animals with GBM presented increased values of CBV (panel C) and CBF in both sexes, while MTT was superior for male rats in the core of glioma. Interestingly, MR spectra yielded a highlighted increase in Glx signal just in male animals with glioma compared with healthy groups. Ex-vivo analysis shown a clear increase in BBB permeability in glioma bearing males compared with female ones, and also a higher Gln concentration in this experimental group compared with sham (data not shown). Some parametric MRI maps as illustrative examples are depicted in figure 2.

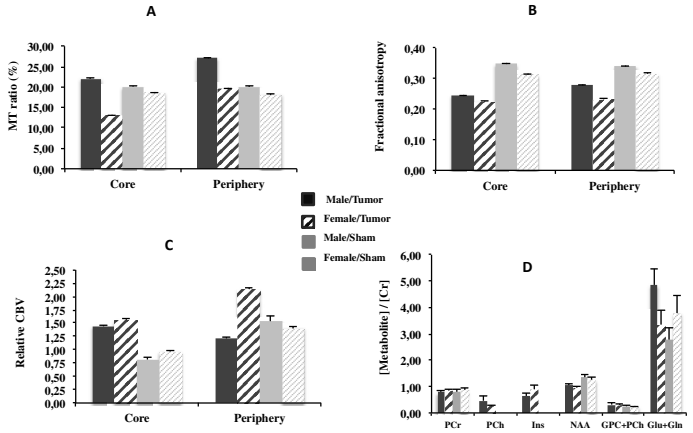

Figure 1. MRI parameters obtained in the analysis of the four experimental groups assessed in two tumoral regions, A: MTR values; B. FA data from DTI; C: Relative CBV from PWI; D: Metabolite concentrations from *in vivo* MR spectra.

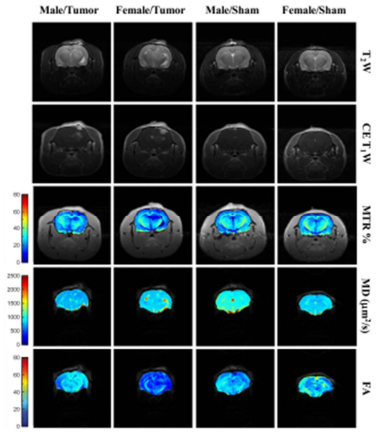

Figure 2. T1W and T2W (1st and 2nd raw) images, MTR (3rd raw), MD (4th raw) and FA (5th raw) maps corresponding to one rat of each experimental condition.

Discussion

MT and MD obtained results indicate higher cellular density in tumor males than females reflecting a higher proliferative behavior in male. Increased FA values in males may be due to a higher organization in the tumoral region on this gender. PWI results suggest an extensive disruption of the BBB in the glioma rim of tumor male rats that drove to an underestimation of CBV and CBF due to the notably extravasation of the CA. This was confirmed with post-mortem analysis. Additionally, MTT determinations showed a higher tortuosity and/or density of vessels on tumor male rats, which together with previous parameters indicates a higher malignancy in the tumor development in males.

Conclusion

We observed gender dependence in MR parameters, especially in those related to angiogenic, inflammatory and/or edematous processes. Sex differences should be taken into account in the study and characterization of tumoral behavior, and in the development of new therapeutic approaches.

References

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