Investigation of genetically engineered orthotopic brain tumors using perfusion MRI provides new insights into the effect of VEGF on brain tumor physiology

B. A. Moffat[1], M. Kariaapper[1], J. Stojanovska[1], K. Lee[1], D. Hall[1], T. Chenevert[1], A. Rehemtulla[2], B. Ross[1]

[1]Radiology, University of Michigan, Ann Arbor, MI, United States, [2]Radiation Oncology, University of Michigan, Ann Arbor, MI, United States

Synopsis:

Genetically engineered rat glioma cells were produced to over or under express VEGF. Continuous arterial spin labeling (CASL) MRI was accomplished revealing higher blood perfusion in both tumor types as compared to wild type 9L tumors. These results give new insights into the effects of angiogenesis on tumor physiology that and have important implications for understanding the role of antiangiogenic cancer therapies.

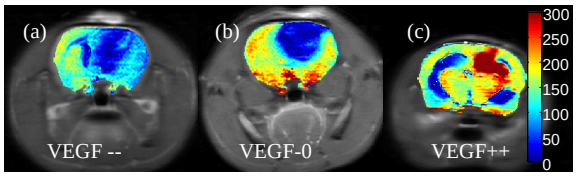
Introduction:

The last few years has seen an explosion in the development of antiangiogenic pharmaceuticals for tumor therapy. Currently the gold standard for measuring their efficacy is cessation of growth during a specific time period. However, the heterogeneity of tumor type, tumor location, and tumor morphology means that the results of such measurements are often inconclusive. Clearly then, there is a need to develop better surrogate markers of antiangiogenic efficacy. Significant effort over the last few years has been made to understand the effects antiangiogenic pharmaceuticals on tumor physiology in pre-clinical animal models through various types of imaging [1]. Already perfusion MRI has made significant contribution through the use of macromolecular gadolinium [2,3] and iron oxide [4,5] contrast agents. It was the hypothesis of this study that blood flow imaging of rat brain glioma models could provide complimentary information regarding the effect VEGF on tumor physiology. To test this hypothesis stably transfected 9L cell lines with wild type (VEGF-0), sense (VEGF++) and anti-sense (VEGF--) levels of VEGF were grown in cell culture and then transplanted intra-cerebrally into Fischer 344 rats (6 animals per cell line). Tumor diffusion, growth rate and tumor perfusion were then measured using an MRI protocol that included, T_2 weighted imaging, diffusion weighted imaging and CASL-MRI. The results of this imaging were then correlated to both histology and VEGF levels as measured by Northen blot analysis.

Methods:

Intracerebral 9L tumors were induced in Fischer 344 as previously described [6]. Briefly, 9L cells (10^5) that contained 3 different levels of VEGF expression were implanted in the right forebrain at a depth of 3 mm. Perfusion imaging was performed on a 7 tesla Varian Unity Inova imaging system. A single coil CASL imaging protocol [7] was used to obtain flow maps. Briefly perfusion weighted and control images were acquired using a fast spin echo imaging sequence (TR/TE=4000/15 ms, 128x128 matrix, 1.5 mm slice thickness, ETL = 8, and a 3 cm FOV) that were weighted using a train of hyperbolic secant inversion pulses to invert the inflowing blood spins. Blood flow images (below) were then calculated from these perfusion weighted images and a T_1 map.

Results:



Both the tumor perfusion and tumor growth rates were significantly different between the three tumor genotypes. The sense and antisense VEGF tumors both accelerated (doubling time of 32 hrs) and decelerated (doubling time of 85 hrs) tumor growth respectively as compared to the wild type 9L tumors (doubling time of 66 hrs). Both VEGF++ and VEGF—tumor types dramatically increased mean tumor blood flow values of 400 +/- 80 ml/100g/min and 70 +/- 25 ml/100g/min respectively over the VEGF-0 26 +/- 17 ml/100g/min. Tumor imunohistochemistry using von Willibrand factor staining for tumor vasculature revealed that tumor vascularity was highest in VEGF++ and lowest in VEGF--.

Discussion:

The use of molecular biology to genetically engineer glioma cells with 3 different vascular phenotypes provides an exciting opportunity to evaluate the effects of VEGF expression levels on perfusion MRI. Unexpected findings include tumor perfusion values which were higher in VEGF-- clones relative to wild type. These results indicate the probability that the tumor is recruiting neovasculature via a different mechanism. This will be further investigated using gene expression array technology. Finally, these results indicate that anti-angiogenic therapies targeting VEGF may not prove entirely beneficial due to alternative molecular pathways of tumor angiogenesis.

References

1. McDonald DM, Choyke PL.Nat Med., 9(6):713-25 (2003).
2. Bhujwalla ZM, et al., Neoplasia, 3(2):143 (2001).
3. Kobayashi, H. et al., 2001, Magn. Reson. Med. 45, 454–460 (2001).
4. Troprès I. et al., Magn. Reson. Med., 45, 397, (2001).
5. Kostourou V, et al., JR.Cancer Res. 63(16):4960-6 (2003).
6. Ross BD, et al., 1998, *Proc. Natl. Acad.Sci. USA*, **95**, 7012 (1998).
7. Moffat et al., Proc. Intl. Soc. Mag. Reson. Med. 10 (2002)

Acknowledgements: This work was supported in part by the following NIH/NCI grants:P01CA85878, R24CA83099, N01CO07013, and N01CO37123.