Avastin is Effective in Reducing rCBV in Fast Growing Human Tumor Xenografts

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Introduction: Two vascular xenograft rat glioma models were studied: D54MG, derived from an adult grade III astrocytoma, is a fast growing tumor; and D456MG derived from a pediatric glioblastoma multiforme is a slower growing tumor. Avastin is an anti-VEGF agent that should prevent the development and recruitment of new vessels to the growing tumor. Dynamic susceptibility contrast (DSC) magnetic resonance imaging methods were used to track changes in angiogenesis in response to treatment.

Methods: Cell suspensions for both D54MG (n=4), and D456MG (n=7) were obtained by growing tumors subcutaneously in the flanks of athymic mice. When the tumors reached a diameter of approximately 2cm they were removed, ground with a tissue press, washed in MEM Zinc Option (Gibco), resuspended in Stemline Methocel (Sigma S-0189), and filtered with 70um tissue filter. Tumors were allowed to grow for 10 days (D54MG) or 14 days (D456MG) after which the rats were prepared for a series of scans by implanting a permanent catheter into the common femoral vein. This catheter will allow the gadolinium contrast agent (Omniscan) to be injected IV without the need of further invasive surgery. Treatment with Avastin (antiVEGF antibody, Genentech, South San Francisco, CA), began on day 10 (D54MG) or day 14 (D456MG) with 1mg/kg injected IP once a day, every day for 5 days or until rats show signs of distress. Control rats receive sterile water IP. Rats were scanned at days 10 and 15 (D54MG) and days 16 and 22 (D456MG) post inoculation. T1-weighted SE images were acquired before and after injecting gadolinium contrast agent (0.2mmolGdDTPA/kg rat), and post contrast. For the DSC study a 0.1 mmole/kg loading dose of Omniscan was administered just prior to the perfusion scan to diminish T1 leakage effects that may occur during the subsequent perfusion scan. Next, a 2 min simultaneous GE- EPI pulse sequence (64x64, TR = 1s, TE=30ms, 4 slices, slice = 2mm, 4cm FOV, matrix=64x64) was used for the DSC perfusion scan. At 1 min, a 0.2-mmol/kg bolus of Omniscan was administered via the femoral catheter. Using software developed at our Institution rCBV maps were created and corrected for any remaining T1 leakage effects as previously described (9).

Results: Representative untreated and treated image results are shown in figure 1 for the faster growing D54MG rat model. Though an obvious decrease in blood volume resulted in the treated rat, the total contrast enhancing area was not obviously lower. Overall, while the untreated tumors (n=2) demonstrated an approximate 100% increase in normalized rCBV, the treated tumor rCBV increased by only 25% from day 10 to 15 (Fig 2a). For the slower growing D456MG glioma, the results were less dramatic. In fact the Avastin appeared to have negligible effects in this model under the dosing and timing conditions studied (Fig 2b).

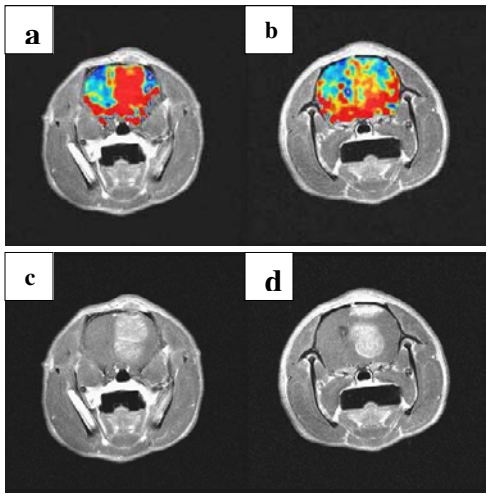

Figure 1. rCBV maps (a,b) and post-contrast images (c,d) from untreated (a,c) and treated (b,d) D54-inoculated rat brains.

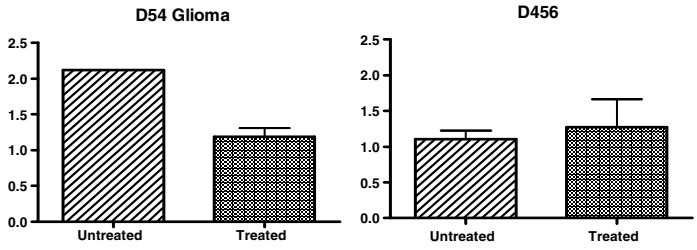

Figure 2. Normalized rCBV results in xenograft rat glioma models (a) D54 and (b) D456 untreated and treated with Avastin.

Conclusions: In this preliminary study of two different rat xenograft models, the results suggest that Avastin is more effective in the faster growing D54MG glioma model. Whether or not this is due to a greater expression of VEGF needs further determination with additional studies that also measure VEGF expression. Other possibilities for the differing effects of Avastin include timing and dosing effects. For example, a greater response to Avastin may result at later stages of tumor growth when the VEGF expression is higher. Each of these issues will be addressed with additional studies in these rat models.

Acknowledgements and References American Brain Tumor Association. (1) H. F. Dvorak: J of Thrombosis and Haemostasis, 3: 1835-1842. (2)H. F. Dvorak: J Clin Oncol 20:4368-4380, 2002. (3)R. Giavazzi: Am J Path 162, 6, 1913-1926. (4)K. H. Plate: J of Neuro-Oncology 35: 365-372, 1997. (5)H.J.J.A. Bernsen: J of Neuro-Oncology 45: 247-255, 1999. (6)A. B. Heimberger: Clinical Cancer Research 6: 4148-4153, Oct 2000. (7)S. N. Kurpad: Cancer Chemother Pharmacol (1997) 39: 307-316. (8) F. Yuan: Proc. Natl. Acad. Sci 93: 14765-14770, Dec 1996 (9) Schmainda KM et al., AJNR 25:1524-1532 (2004).