MR Imaging based detection of glial brain tumors in mice after anti-angiogenic treatment.

B. C. Hamans[1], A. Claes[2], G. Gambarota[1], O. van Tellingen[3], P. Wesseling[2], C. N. Maass[2], A. Heerschap[1], and W. P. Leenders[2]

[1]Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [2]Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [3]Clinical Chemistry, Dutch Cancer Institute, Amsterdam, Netherlands

Introduction

Glioblastoma multiforme (GBM) is the most frequent and most malignant glial brain tumor with poor prognosis and survival, usually less than two years, despite development of novel chemotherapeutic compounds (1). Proper delineation of gliomas using conventional contrast-enhanced magnetic resonance imaging (CE-MRI) poses a problem in neuro-oncology. The blood brain barrier (BBB) in areas of diffuse infiltrative growth may be intact, precluding extravasation and subsequent MR-based detection of the contrast agent Gd-DTPA (2-4). Treatment with anti-angiogenic compounds may further complicate tumor detection as such compounds can restore the BBB in angiogenic regions of tumors, which thus converts to co-opting growth along existing vessels (5). The increasing number of clinical trials with anti-angiogenic compounds for treatment of gliomas calls for the development of alternative imaging methods. Imaging with blood pool contrast agents may therefore be an attractive complementary tool to detect glioma invasion and to evaluate response to anti-angiogenic therapy.

Aim

To investigate whether CE-MRI using ultrasmall particles of iron oxide (USPIO) as blood pool contrast agent has additional value for detection of glioma in the brain of mice after treatment with vandetanib.

Material and methods

We compared conventional T1-weighted Gd-DTPA-enhanced MRI with T2*-weighted USPIO-enhanced MRI in nude mice carrying orthotopic U87 glioma which were either or not treated with the anti-angiogenic compound vandetanib (ZD6474, ZACTIMA™). Balb/c nude mice (6–8w, 18–25g) were used in all experiments. 100,000 U87 glioma cells in 2 µl phosphate buffered saline were injected through the skull of anaesthesized mice (1.3% Isoflurane in N2O/O2) at a depth of 3 mm. Mice carrying intracerebral U87 xenografts received vandetanib [0mg/kg (n=8), 50mg/kg (n=8), 100mg/kg (n=9) as a suspension in 1% polysorbate-80] once daily by oral gavage in a volume of 100µl. Mice were treated from day 7 till day 21 after tumor injection. After 16 to 20 days, when tumor-related symptoms became apparent, MRI was performed. Gd-DTPA CE-MRI was performed as described in (6). Animals were anesthetized and the tail vein was catheterized for injection of the two contrast agents. MR imaging was performed on a 7T 200mm MR system (MR Research Systems Ltd, Guildford, UK). Sixteen T1-weighted coronal images (TE=8ms; TR=100ms; α=90º; fov=25x25mm; matrix=256x256; thk=1mm) were acquired. A bolus of 0.2ml of Gd-DTPA (20mMol/l, Magnevist®, Schering, Germany) was injected intravenously and additional sets of T1-weighted images were acquired immediately after and at 2 and 10 minutes after administration. Subsequently the animal was allowed to recover for a period of two hours. Then, a reference T2*-weighted multi slice gradient echo (TE=7ms; TR=1500ms; fov=35x35mm; matrix=256x256; thk=1mm) was acquired, followed by an additional acquisition 2 min after injection of USPIO (Sinerem, Guerbet, France) at a dose of 12.5mg/kg. After MRI the mice were sacrificed, brains removed and formalin-fixed and cut for (immunohisto-) chemical stainings. Quantitative measurements for hypoxia and micro vessel density were performed on these slices. For analysis in two mice of each group, regions of interest, retrospectively defined from histology and the appearance on the MR images, were selected that encompassed a complete lesion and intensity within such regions was measured pre-contrast and at different time points post contrast agent injection.

Results

In untreated animals, vessel leakage within the tumor and a relatively high tumor blood volume resulted in good MRI visibility with Gd-DTPA- and USPIO-enhanced MRI, respectively (Fig. 1). Consistent with previous findings, vandetanib treatment restored the BBB in the tumor vasculature, resulting in loss of tumor detectability in Gd-DTPA MRI. However, vessel density also decreased due to this treatment causing a lower blood volume in the lesion compared to normal brain. Therefore, treated tumors could be readily detected in USPIO-enhanced MRI scans (Fig. 2).

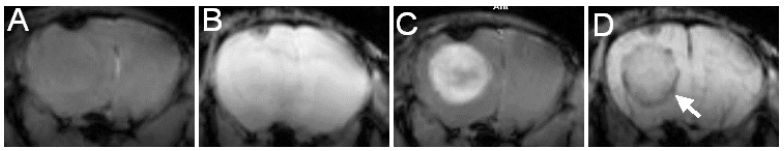

Figure 1: CE-MRI of intracerebral U87 glioma lesions of vehicle-treated mouse. Representative images of intracerebral lesions before (A,B) and after (C,D) injection of contrast agent. Tumor lesions are not visible pre-contrast, but become visible as hyper-intense lesions in Gd-DTPA-enhanced MR images (C) due to leakage of contrast agent in and immediately around the tumor, and as hypo-intense lesions in USPIO-enhanced images (D) due to a higher vessel density in the tumor. These latter vessels cause a a dark rim around the tumor lesion in the Sinerem-enhanced MR image (D, arrow).

Conclusion

Our findings show that Gd-DTPA enhanced MRI may result in an overestimation of the effect of anti-angiogenic therapy of glioma. Because blood vessel density and blood volume are low in treated tumours relative to normal brain parenchym USPIO enhanced MRI can be used as an important complementary diagnostic tool to evaluate response to anti-angiogenic therapy of these tumours. Therefore, complementary imaging of brain tumours using USPIOs will reduce the chance on false conclusions about the efficacy of anti-angiogenic therapy that is based on Gd-DTPA MR scans.

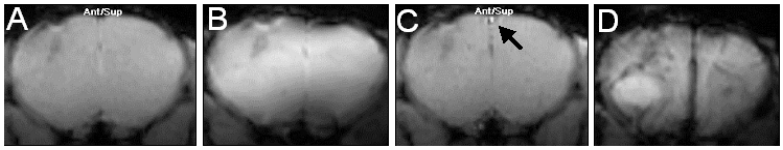

Figure 2: CE-MRI of cerebral U87 lesions in mice treated with 100 mg/kg vandetanib. Representative images of intracerebral lesion before (A,B) and after (C,D) injection of contrast agent. Tumor lesions are not visible pre-contrast and stay invisible after injection of Gd-DTPA (C). Note that contrast agent did reach the brain, as indicated by contrast enhancement of large meningeal vessels (arrow). After USPIO injection, tumors become visible as hyper-intense lesions due to a relatively lowvascular volume as compared to the surrounding normal brain parenchyma (D).

References

1. Stupp R,et al. N Engl J Med 2005;352(10):987-996. **2.** Gerlowski LE, Jain RK. Microvasc Res 1986;31(3):288-305. **3.** Ludemann L, et al. MRI 2005;23(8):833-841. **4.** Wesseling P, et al. J Neurooncol 1997;32(3):253-265. **5.** Ponce P, et al. Eur J Radiol 1998;28(3):226-229. **6.** Leenders W, et al. Int J Cancer 2003;105(4):437-443.