Vasculature characterization of angiogenic melanoma metastases in mouse brain by Gd-DTPA and USPIO contrast-enhanced MRI

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Introduction

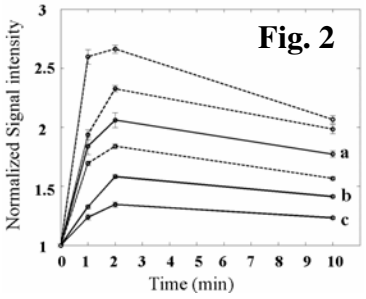
In order to enhance the success rate of anti-angiogenic therapies in the clinic, it is crucial to identify parameters for tumor angiogenesis which can predict response to these therapies [1]. In brain tumors, one such parameter is vascular leakage which is a response to tumor-derived Vascular Endothelial Growth Factor-A (VEGF-A) and can be measured by Gd-DTPA-enhanced MRI. However, as vascular permeability and angiogenesis are not strictly coupled, tumor blood volume may be another potentially important parameter. In this study, contrast enhanced MR imaging was performed in a brain tumor model of angiogenic melanoma (Mel57-VEGF-A_{165}) metastases using Gd-DTPA to detect vascular leakage and ultrasmall iron oxide particles (USPIO) to measure blood volume [2, 3]. Immunohistochemical analysis was performed on the same mice to validate the MRI findings.

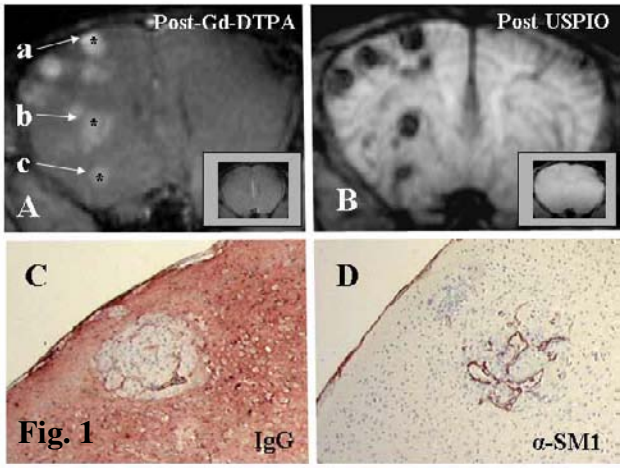
Materials and Methods

Tumor model. Stable VEGF-A_{165} transfectants of Mel57 cells were cultured in Dulbecco's Modified Essential Medium supplemented with 10% fetal calf serum [4]. Mel57-VEGF-A_{165} cells were injected in the internal carotid artery using a microsurgical procedure [5]. Using this melanoma metastasis model, multiple lesions develop in the brain parenchyma and display all hallmarks of angiogenesis (including leaky, highly dilated vessels).

MRI. Experiments were performed on a 7T MR-scanner. Multislice gradient-echo (TR/TE=1500/7ms, voxel-size=136x136x1000µm) and spin-echo (TR/TE=2000/9ms) imaging was performed prior to and following administration of a USPIO blood-pool agent (170 µg Fe/mouse, Sinerem, Guerbet, France) in four mice. Pixel-by-pixel ΔR2 and ΔR2* maps were generated and the average values in lesions were calculated. The protocol of the Gd-DTPA contrast-enhanced MRI consisted of T1-weighted multislice gradient echo images with TR/TE = 400/6 ms (other imaging parameters as in the USPIO protocol). The images were acquired before and 1, 2, 10 and 20 min after injection of Gd-DTPA (0.2 mmol/kg, Magnevist ®, Schering, Germany). For each animal, at least five lesions were analyzed.

Histological and immunohistochemical analysis. After the MRI experiments, animals were sacrificed and the brains were removed and fixed in formalin. Coronal slices were embedded in paraffin and processed for immunohistochemical analysis. Vessels in lesions were highlighted by staining for the endothelial marker CD34 (Hycult Biotechnology, Uden, the Netherlands) and the pericyte marker α-smooth muscle actin (α-SM1, Sigma Chemical Co, Zwijndrecht, The Netherlands) according to previously published protocols. To detect vascular permeability, sections were stained with HRPO-labeled anti-mouse IgG [4].



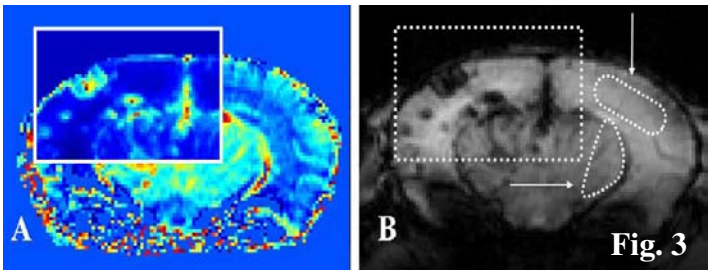


Results

The Mel57-VEGF-A_{165} metastases were not detectable in pre-contrast images (**Figure 1.A**, **1.B**, small insert in lower right corner). Following Gd-DTPA (**Figure 1.A**), metastases showed a high signal enhancement (> 2.5 times the signal intensity value pre-Gd-DTPA) which reached a maximum value at 2 minutes after Gd-DTPA administration, followed by a steady decrease thereafter (**Figure 2**, dotted lines refers to metastases in other slices). This correlated well with the high vascular permeability in the tumors, as assessed by anti-IgG staining (**Figure 1.C**). Following administration of USPIO (**Figure 1.B**), high values of ΔR2 (40 ± 13 Hz) and ΔR2* (284 ± 89 Hz) were measured in the metastases. Interestingly, this correlated well with the presence of highly dilated vessels (see α-SM1 staining, **Figure 1.D**). The values of ΔR2 and ΔR2* measured in healthy brain regions (Cortex: ΔR2 = 5 ± 2 Hz and ΔR2* = 21 ± 9 Hz; Striatum: ΔR2 = 9 ± 3 Hz and ΔR2* = 36 ± 10 Hz) were significantly lower than in metastases. The macrovascular blood volume map and the corresponding gradient echo image are shown in **Figure 3.A** and **3.B**, respectively. In the blood volume map, a high threshold for ΔR2* was used in the tumor areas (region inside the box). Outside the tumor region, a low cut off threshold for ΔR2* is used to maximize the contrast between striatum (ROI indicated by the horizontal arrow in the gradient echo image **Figure 3.B**) and cortex (ROI indicated by the vertical arrow, **Figure 3.B**).

Discussion

The ability to non-invasively detect aberrant vasculature is especially crucial for detection and delineation of brain tumors. In vivo Gd-DTPA and USPIO contrast-enhanced MR imaging provided a detailed characterization of the tumor vasculature, by imaging vascular leakage and vascular volume. The in vivo MRI results were in good agreement with immunohistochemical stainings. The vascular morphology and delineation of metastases was best assessed on post-USPIO gradient echo images. Quantitative assessment of blood volume, performed by calculating ΔR2*, has the potential to allow performance of longitudinal studies in order to assess, for example, treatment efficacy. The results of this study indicate that USPIO-induced ΔR2* values proved to be also highly sensitive to small changes in regional blood volume. Thus USPIO imaging may be a very attractive alternative to Gd-DTPA imaging and will at least have added value, especially for detection of brain tumors.



References

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