Early detection of brain metastasis using novel MRI contrast agent targeting VCAM-1

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Background: Contrast-enhanced magnetic resonance imaging (MRI) is currently the most sensitive method for brain metastasis detection, but relies on blood-brain barrier (BBB) compromise and, consequently, is sensitive to late-stage metastases only. We have developed an MRI-detectable contrast agent targeted specifically at the endothelial adhesion molecule VCAM-1 (VCAM-MPIO) and have shown that this agent enables detection of endothelial activation early in brain pathology [1]. Based on our recent findings that brain metastases develop in close association with existing brain vessels [2], we hypothesised that VCAM-1 is upregulated during metastasis development and that our VCAM-MPIO may enable early detection of brain metastases.

Methods: Female balb/c mice (8-10 weeks) were injected intracardially with $1x10^4$ 4T1 cells, a metastasising murine mammary carcinoma line. Purified monoclonal rat antibodies specific to mouse VCAM-1 (clone M/K2, Cambridge Bioscience) or control IgG-1 (clone Lo-DNP-1, Serotec) were conjugated to myOne tosylactivated MPIO (1-µm diameter; iron content 26%; Invitrogen) as described previously [1]. Either 5 or 10 days after 4T1 cell injection animals were anaesthetised and injected intravenously with either VCAM-MPIO or control IgG-MPIO (4×10^8; 4.5mg iron / kg body weight; n = 3-4 per group). After 1h animals underwent MRI at 7T and a T2*-weighted 3D gradient-echo dataset was acquired (acquisition ~1h; isotropic resolution 88mm). Post-gadolinium T1-weighted images were acquired to assess BBB integrity. T2*-weighted images were processed into a 3D isotropic dataset and converted into tiff images. For each image, the brain was manually masked to exclude extracerebral structures. Quantification of VCAM-MPIO binding (defined as focal hypointensities) was performed by observers blind to the identity of the dataset. Volumes are expressed as raw volumes in µL.

Results: Immunohistochemically, upregulation of VCAM-1 was co-localised to brain metastases. *In vivo* MRI revealed focal areas of signal hypointensity throughout the brain, indicating VCAM-MPIO accumulation. Immunohistochemical analysis demonstrated co-localisation of the MRI hypointensities with metastases. Quantitatively, the volume of hypointensities in the VCAM-MPIO injected animals was greater than in IgG-MPIO-injected animals (i.e. background). None of the animals showed BBB breakdown.

Conclusion: Upregulation of VCAM-1 during metastasis development enables earlier detection of metastases in the brain, using our novel VCAM-1-targeted contrast agent, than is currently possible clinically. Early detection of brain metastases may significantly alter patient prognosis.

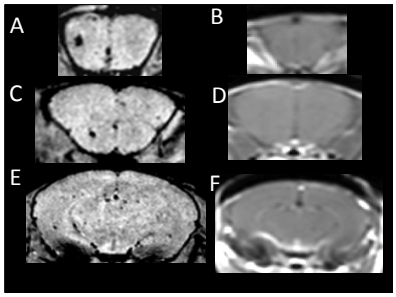

Figure 1. Accumulation of VCAM-MPIO.
In vivo MRI reveals focal areas of signal hypointensity throughout the brain and with large hypointense regions in the olfactory bulb, indicating VCAM-MPIO accumulation (A, C, and E). Following intravenous Gd-DTPA injection, T1-weighted images indicated no blood-barrier breakdown where VCAM-MPIO is accumulated (B, D and F).

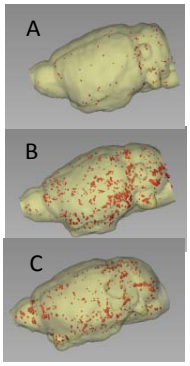

Figure 2. 3D construction of *in vivo* MRI data.
3D construction of the MRI images reveal the presence of VCAM-MPIO accumulation throughout the brain with much more signal (in red) in animals with brain metastases at 5 (B) and 10 days (C) after intracardiac injection than in naive animal (A) (no intracardiac injection).

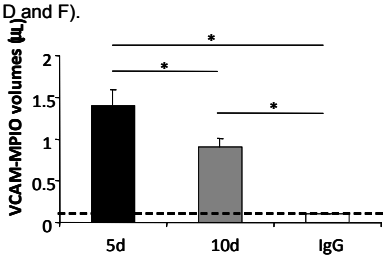

Figure 3. Significant accumulation of VCAM-MPIO in animal with brain metastases.
Quantitatively, the volume of hypointensities was significantly greater at both days 5 (black bar) and 10 (greay bar) than in IgG-MPIO injected animals (white bar). * denotes a significant difference between groups with $p<0.05$. ANOVA followed by Bonferroni's t tests. Dotted line indicates the average volume obtained in naïve animals injected with VCAM-MPIO.

References

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