EARLY DETECTION OF BRAIN METASTASIS USING NOVEL MRI CONTRAST AGENT TARGETING VCAM-1

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Introduction: Metastatic spread of a primary tumour to the brain remains one of the greatest hurdles in cancer therapy, and prognosis is poor. 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 when therapeutic options are limited. 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 were injected intracardially with 1x105 4T1 cells, a metastasising murine mammary carcinoma line. Puri?ed monoclonal rat antibodies to mouse VCAM-1 (clone M/K2, Cambridge Bioscience) or control IgG-1 (clone Lo-DNP-1, Serotec) were conjugated to myOne tosylactivated MPIO (1µm diameter; iron content 26%; Invitrogen) [1]. At day 5, 10 or 13 after 4T1 cell injection animals were anaesthetised and injected intravenously with either VCAM-MPIO or IgG-MPIO. After 1h animals underwent MRI on a 7T Varian Inova system and a T2*-weighted 3D gradient-echo dataset was acquired. Subsequently, post-gadolinium T1-weighted images were acquired to assess BBB integrity. A second metastasis model was also studied; female SCID mice were injected intracardially with 1x105 MDA-231BR cells, a metastasising human breast adenocarcinoma line. These animals were imaged at 21 days post-cell injection, as described above. In all cases T2*-weighted images were processed into a 3D isotropic dataset and the brain was manually masked. Quantification of VCAM-MPIO binding (defined as focal hypointensities) was performed using Image-Pro software. Following MRI animals were transcardially perfusion-fixed and the brains sectioned and assessed immunohistochemically for VCAM-1 expression associated with tumour growth.

Results: Immunohistochemically, upregulation of VCAM-1 was co-localised to brain metastases in both models studied, and in many cases the presence of the VCAM-MPIO in VCAM-positive vessels was observed. Similarly, MRI revealed focal areas of signal hypointensity throughout the brain, indicating VCAM-MPIO binding, in both 4T1 and MDA-231BR injected animals. Assessment of the co-localisation of MRI hypointensities and metastases detected immunohistochemically indicated >90% positive identification by VCAM-MPIO MRI above a tumour diameter threshold of ca. 100?m. Quantitatively, the volume of VCAM-MPIO-induced hypointensities in 4T1 injected animals was greater than in IgG-MPIO-injected animals (i.e. background) and increased with disease progression. No 4T1 injected animals showed BBB breakdown.

Conclusions: 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. We believe that this new approach will open a therapeutic window in brain metastasis that currently does not exist.

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RESPONSIVE MAGNETIC RESONANCE IMAGING PROBES TO MONITOR SYNAPTIC GLUTAMATE FLUCTUATION IN THE BRAIN

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Introduction: The neurotransmitter glutamate is the major mediator of excitatory signals in the nervous system and is involved in nearly all aspects of normal brain functioning (cognition, memory, learning). Our idea was to develop glutamate 'responsive' magnetic resonance imaging (MRI) contrast agents (CAs) to image changes in specific brain regions upon neural activation. As CAs directly responsive to glutamate would not be feasible due to the very short half-life of glutamate in the extracellular space, we chose CAs that bind to glutamate receptors instead (to be specific metabotropic glutamate-receptor subtype 5 (mGluR5)), by this increasing image contrast. Ideally, upon glutamate-binding to the receptor (e.g. after glutamate-release at the synapse) the CA will be released, hence leading to a reduction in image-contrast, followed by a restoration of equilibrium and re-binding of the CA to the receptor. These events are believed to occur over a period of seconds allowing data acquisition using modern FLASH pulse techniques[1]. Here, we present a proof-of-concept study for such 'indirect' glutamate-responsive MRI CAs.

Methods: We have designed and synthesized different prospective CAs derived from various potent mGluR5-receptor antagonists (alkynes like MPEP, MTEP and dipyridyl/heterobiaryl amides) coupled to DOTA-derived macrocyclic lanthanide-chelates. The CAs were evaluated in cultured primary cortical rat astrocytes, expressing mGluR5 (verified by immunofluorescence). MRI-measurements to examine the ability of the CAs for cellular labeling were done with a 3T human whole body scanner. Antagonistic potency of the CAs was assessed with a calcium fluorescence assay, by which glutamate induced intracellular calcium-transients mediated by mGluR5 were measured. Antagonistic activity of the CAs was calculated as changes in EC50 of glutamate. Receptor binding was measured for the dipyridyl derivaties, as these compounds have an inherent fluorescence that changes upon binding. Commercially available receptor membrane preparations containing human mGluR5A were used for these experiments.

Results: Two of the gadolinium complexes retained significant antagonistic activity, one in each structural class. For the alkyne-derivative, about a threefold increase of the EC50(glutamate) (100µM CA, 15min, $P<0.001$) was found while under similar conditions the cellular relaxation rate R1,cell increased to 126% of control (100µM, 45 minutes incubation time, $P<0.001$). The CA derived from dipyridyl amides increased the EC50(glutamate) about fourfold ($p<0.001$) and the R1,cell to 115% ($p<0.05$). Fluorescence measurements of the latter CA showed enhanced emission upon binding to mGluR5-membrane preparations. This was reversed when increasing concentrations of glutamate were added, consistent with the a reversibility of CA-receptor binding

Conclusions: Using primary rat astrocytes as cellular model system to investigate newly developed glutamate-responsive MRI contrast agents, we were able to identify two promising candidates. These CAs are based on the structures of antagonists to mGluR5 and our studies establish the validity of the concept, by which it might be possible to use MRI to image transient changes in the neurotransmitter glutamate.

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