PECAM-1-targeted MPIO as a molecular MRI contrast agent for detection of vascular remodeling after experimental stroke

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INTRODUCTION

An increasing amount of studies have provided evidence for formation of new blood vessels after cerebral ischemia, and a possible significant role of angiogenesis in post-stroke recovery [1,2]. However, the exact pattern of revascularization and its relation to restoration of function after stroke are still largely unresolved. Therefore, we are developing novel MRI approaches that may enable direct detection of vascular changes with use of contrast agents that are targeted to specific molecular vascular markers. As a possible target for molecular MRI of post-stroke vascular changes we here focus on platelet endothelial cell adhesion molecule 1 (PECAM-1) [3], which is constitutively expressed on endothelial cells.

METHODS & RESULTS

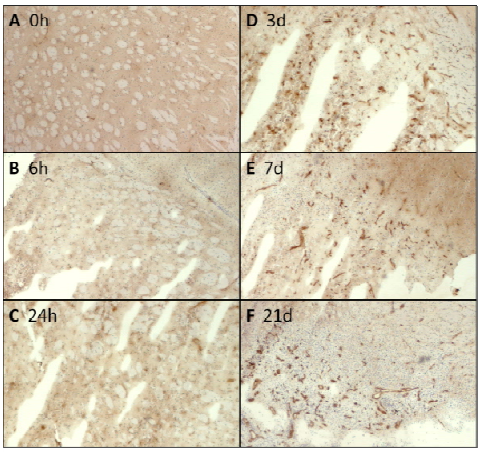
To validate our hypothesis, post mortem mouse brain tissue at different time points post-stroke was stained for PECAM-1. C57Bl6 mice underwent 30 min transient intraluminal right middle cerebral artery occlusion (MCAo) and were sacrificed at 6 h, 24 h, 3 days, 7 days or 21 days after reperfusion (n=4 per time point). Two healthy mice were included as control (0 h). 10 µm coronal cryo sections (6 per mouse, ranging from approx. -1 to +1.5 mm from bregma) were aceton fixated and incubated with αPECAM-1-biotin (1 h), followed by HRP-streptavidin (1 h), and subsequently with diaminobenzidin (DAB) reagent (3 min). Nuclei were stained with hematoxylin. Figure 1 shows typical examples of PECAM-1-stained tissue sections. PECAM-1 was expressed on vessels at all time points, but expression was markedly increased at 3, 7 and 21 days after stroke. To verify these immunohistochemical results we performed quantitative polymerase chain reaction (qPCR) on subsequent tissue sections. Tissue sections were divided in ipsi- and contralesional tissue after which mRNA was extracted (RNA capture kit). Subsequently cDNA was synthesized (Reverse Transcription Synthesis kit) and qPCR was performed with the SYBR Green method (Applied Biosystems, CA). Obtained expression levels of transcripts were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. No significant changes could be detected in the contralesional hemisphere over time, however, a significant increase ($p<0.05$) of ipsilesional PECAM-1 mRNA expression was detected at 6 and 24 h compared to healthy tissue (0 h) (Figure 2). Ipsilesional PECAM-1 mRNA levels at 6 h, 24 h and 3 days were also significantly increased ($p<0.001$) compared to contralesional expression.

Since both mRNA and protein levels of PECAM-1 were upregulated after stroke, we hypothesized that PECAM-1 could be a suitable target for molecular imaging of post-stroke vascular changes. To this extent, we synthesized antibody-functionalized micron-sized particles of iron oxide (MPIO) by coupling irrelevant immunoglobulin G antibody (IgG) or anti-PECAM-1 antibody (αPECAM-1) to 1.05 µm MPIO (ProMag™ 1 Series Bind-IT™, Bangs Laboratories, Inc., IN) as described by the manufacturer. T_1-mapping (inversion recovery Look-Locker: TR/TE 20000/3.18 ms, 40 images with 250 ms intervals, α 5°, NA 4, 117x117 µm^2) and T_2-mapping (multiple spin echo: TR/TE 5000/9-900 ms, NE 100, NA 4, 117x117 µm^2) were performed on dilution series of MPIO to determine r_1 and r_2. MPIO showed typical $T_2^{(*)}$ contrast agent properties, with a relatively small effect on T_1 (r_1=0.28 mM^{-1}s^{-1}), but a large effect on T_2 (r_2=91 mM^{-1}s^{-1}).

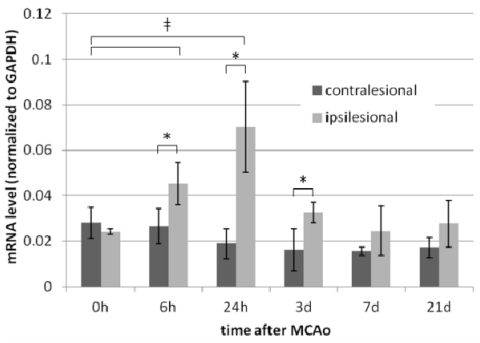
To explore the suitability of this contrast agent platform for molecular imaging of cerebrovasculature in vitro, murine cerebrovascular bEnd5 cells were incubated at 37 °C without, or with IgG-functionalized or PECAM-1-targeted MPIO (1 h), after which cells were thoroughly washed and embedded in agar for MRI analysis. A small amount of cells was used to prepare cytospins. Visual examination of cytospins with Perls' staining enhanced with diaminobenzidin (DAB) staining [4] showed an increased binding of αPECAM-1-MPIO (Figure 3C) when compared to IgG-MPIO (Figure 3B). Quantitative data of MRI on cells suspended in agar are shown in Figure 3A. No significant change in T_1 for αPECAM-1-MPIO- (T_1=2.06±0.32 s) or IgG-MPIO-incubated cells (T_1=2.27±0.05 s) was shown when compared to control cells (T_1=2.27±0.07 s) or agar without cells (T_1=2.31±0.09 s). However, cells incubated with αPECAM-1-MPIO showed a significant T_2-shortening effect ($p<0.05$, T_2=28±5 ms) when compared to IgG-MPIO-incubated cells (T_2=113±7 ms), control cells (T_2=109±3 ms) or agar without cells (T_2=127±5 ms). IgG-MPIO-incubated cells did not show a significant difference in T_2 compared to control cells or agar without cells, indicative of specific binding of αPECAM-1-MPIO to cells.

CONCLUSION

Based on these results we conclude that αPECAM-1-MPIO can be used as targeted $T_2^{(*)}$-based contrast agent for molecular MRI of vascular remodeling after experimental stroke. In vivo molecular MRI of PECAM-1 and other vascular markers may aid in improved characterization of the development of vascular inflammation and revascularization in relation to stroke pathophysiology and plasticity.

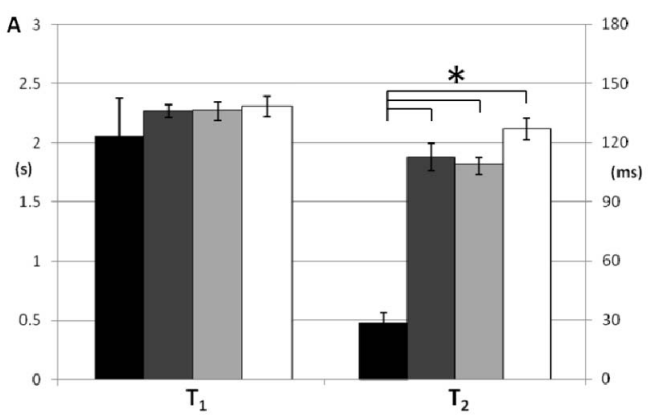


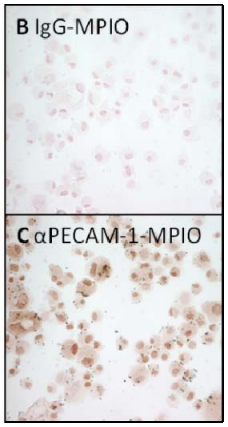
↑↑ Figure 1. Immunohistochemical PECAM-1 staining in mouse brain tissue at different time points after stroke.



↑ Figure 2. PECAM-1 mRNA levels in contra- and ipsilesional mouse brain hemispheres at different time points after stroke. □: $p<0.05$, *: $p<0.001$

↓ Figure 3. Quantitative MRI data (A) of agar (white bars) or agar with control cells (light grey bars), IgG-MPIO-incubated cells (dark grey bars) or αPECAM-1-MPIO-incubated cells (black bars) and complementary images of Perls' staining enhanced with DAB of IgG-MPIO-incubated cells (B) or αPECAM-1-MPIO-incubated cells (C)





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