Molecular MRI of neurovascular inflammation in a mouse stroke model using bimodal ICAM-1 targeted nanoparticles

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Introduction:

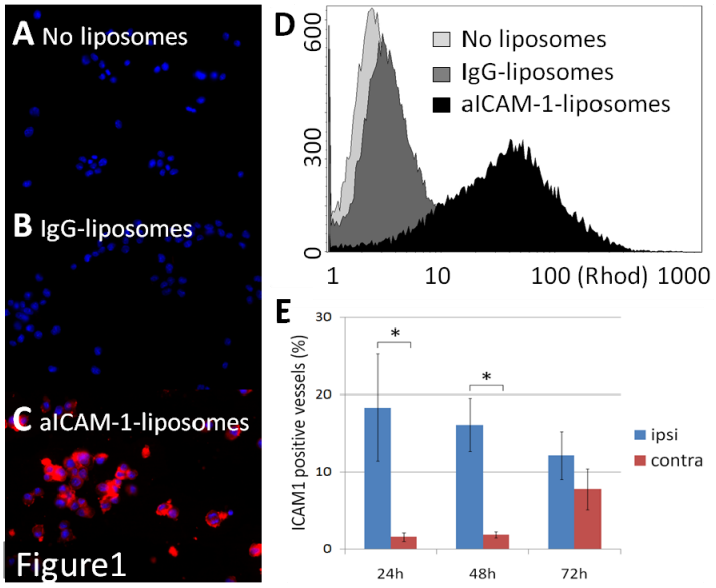
Neuroinflammation is significantly involved in stroke pathophysiology, but underlying processes are still largely unraveled. Target-specific MR imaging of inflammatory markers may improve our insights into the explicit involvement of distinct neuroinflammatory events, which could make way for new anti-inflammatory treatment strategies [1,2]. In this study we focused on intercellular adhesion molecule 1 (ICAM-1), which is upregulated on inflamed cerebrovascular endothelium and therefore may serve as a potential marker for the (sub)acute stage of neuroinflammation. To that end we applied an ICAM-1 specific bimodal liposomal nanoparticle to allow *in vivo* molecular MRI of neurovascular inflammation in combination with post mortem fluorescence microscopy.

Methods:

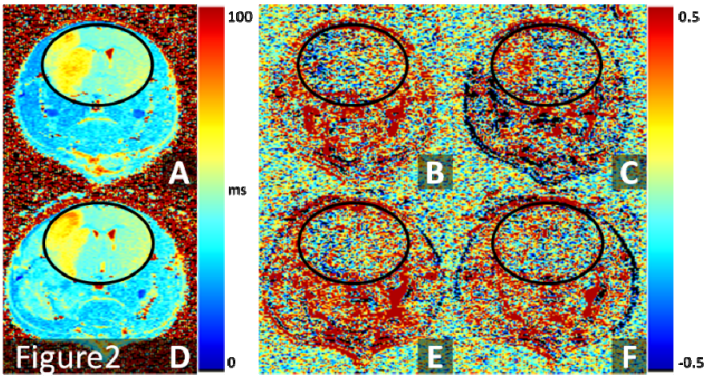
Liposomes of 200 nm containing 25 mole% Gd-DTPA-DSA and 0.2 mole% rhodamine-PE were synthesized, and anti ICAM-1 antibodies (aICAM-1) or irrelevant immunoglobulin G antibodies (IgG) were coupled as described previously [3].

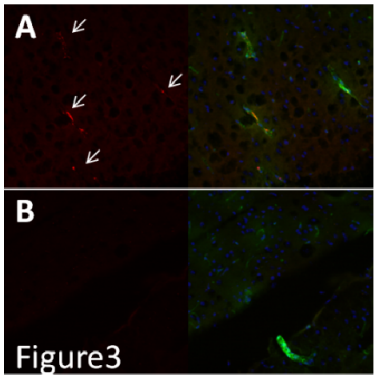
Mouse endothelial cells (bEnd5) were incubated without or with 1 µmol/ml IgG- or aICAM-1-liposomes for 4h at 37 °C, after which cells were thoroughly washed, trypsinized and fixed in a 4% paraformaldehyde solution. Cells were analyzed with fluorescence microscopy and flow cytometry.

Adult mice (C57Bl6) underwent a 30 min transient intraluminal occlusion of the right middle cerebral artery. First, the temporal upregulation of ICAM-1 was evaluated histologically. To that end, tissue sections of *ex vivo* mouse brains, sacrificed at 24, 48 and 72h after stroke onset, were stained for ICAM-1 and the endothelium marker CD31. ICAM-1-positive vessels were counted in the ipsi- and contralateral hemispheres.



In vivo, mice were MR scanned 24h after the onset of stroke. Spin-echo MRI was applied for T_2-mapping (TR 2300ms, TE 12ms, NE 8, NA 4, matrix 192x96) and for T_1-weighted imaging (TR 800ms, TE 8.6ms, NA 16, matrix 192x96), while an inversion recovery Look-Locker sequence was used for T_1-mapping (TR 9000 ms, τ 10 ms, number of images 40, TE 4.5 ms, α 10°, NA 2, matrix 96x64). All scans were conducted on a 9.4 T horizontal 20-cm bore MR system (Varian Inc., Palo Alto, CA) with a field-of-view of 2x2 cm^2 and 21 slices of 400 µm thickness. During the 24h scan session, mice received an intravenous injection of IgG- (n=3) or aICAM-1-liposomes (n=3) (lipid concentration 5µmol) after baseline MRI measurements, and T_1-weighted sequences were repeated for an additional 3h. Mice were sacrificed after 48h. Brains were snap-frozen in liquid N_2 and tissue sections were stained with a CD31 antibody and Hoechst to visualize the vasculature and cell nuclei, respectively. Sections were analyzed with fluorescence microscopy.





Results:

Fluorescence microscopy of *in vitro* incubated endothelial mouse cells showed a strong increase in fluorescent signal in cells incubated with aICAM-1-liposomes compared to cells incubated without or with IgG-liposomes (Fig1A-C). These results were confirmed by flow cytometry (Fig1D).

ICAM-1 staining demonstrated significant upregulation in the ipsilateral hemisphere at 24 and 48h after stroke (Fig1E). We measured significant blood-brain barrier (BBB) leakage (with post-Gd-DTPA T_1-weighted MRI) at 48h but not at 24h in this mouse model (data not shown). Therefore, liposomes were injected at 24h after stroke to minimize non-specific accumulation. Typical examples of a coronal mouse head slice after injection with aICAM-1- or IgG-liposomes are shown in Fig2A-C and Fig2D-F, respectively. Lesion location and progression were similar, as shown by T_2-maps at 24h (Fig2A, D). T_1-weighted images acquired directly after liposome injection and subtracted with pre-contrast T_1-weighted images (i.e. T_1-weighted difference images) (Fig2B, E) showed increased signal in the entire brain, indicating global presence of liposomes in circulation. T_1-weighted difference images after 3h showed a strong increase in signal in the lesion after injection of aICAM-1-liposomes (Fig2C), which was absent when IgG-liposomes were injected (Fig2F). Analysis of brain tissue sections showed liposomes associated with the neurovasculature inside the lesion after aICAM-1-liposomes injection (Fig3A), but not after IgG-liposomes injection (Fig3B).

Conclusion:

Our data show that aICAM-1-liposomes bind specifically to the inflamed vasculature in mouse brain at 24h after the onset of stroke, which was visualized by *in vivo* MRI and *ex vivo* fluorescent microscopy.

Literature:

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