Micron-sized particles of iron oxide and gadolinium-containing liposomes as targeted contrast agent for molecular MRI of neuroinflammation after stroke: A comparative study.

Lisette H. Deddens[1], Geralda A. F. Van Tilborg[1], Annette Van der Toorn[1], Leonie E.M. Paulis[2], Gustav J. Strijkers[2], Gert Storm[3], Willem J. M. Mulder[4], Helga E. De Vries[5], and Rick M. Dijkhuizen[1]

[1]Biomedical MR Imaging and Spectroscopy Group, Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands, [2]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [3]Biopharmacy and Pharmaceutical Technology, Utrecht University, Utrecht, Netherlands, [4]Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, New York, United States, [5]Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, Netherlands

INTRODUCTION

Neuroinflammation is significantly involved in stroke pathophysiology, but underlying processes are still largely unclear. 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 compared the potential of gadolinium-containing liposomes (Gd-liposomes) and micron-sized particles of iron oxide (MPIO) for target-specific MRI of neuroinflammation after experimental stroke. We focused on intercellular adhesion molecule 1 (ICAM-1), which is upregulated on inflamed cerebrovascular endothelium during the first days post-stroke and therefore may serve as a marker of (sub)acute neuroinflammation. All scans were conducted on a 9.4 T horizontal 20 cm bore MR system (Varian Inc., CA).

METHODS & RESULTS

Liposomes of 200 nm containing 25 mole% Gd-DTPA-DSA and 0.2 mole% rhodamine-PE were synthesized and anti-ICAM-1 antibody (αICAM-1) or irrelevant immunoglobulin G antibody (IgG) were coupled as described previously [3]. αICAM-1 or IgG were also coupled to 1.05 µm MPIO (ProMag™ 1 Series Bind-IT™, Bangs Laboratories, Inc., IN) as described by the manufacturer. Gd-liposomes displayed an r_1 of 1.5 $mM^{-1}s^{-1}$ and r_2 of 18 $mM^{-1}s^{-1}$, representative for T_1 contrast agent. 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 these two contrast agent platforms in vitro, murine cerebrovascular bEnd5 cells were stimulated with TNFα for 24 h and incubated without, or with IgG-functionalized or ICAM-1-targeted Gd-liposomes (4 h) or MPIO (1 h), after which cells were thoroughly washed and pelleted for MRI analysis. A small amount of cells was used to prepare cytospins for microscopic visualization. Cellular uptake of αICAM-1-Gd-liposomes and αICAM-1-MPIO was significant ($p<0.01$, Figure 1; B and D, respectively) when compared to IgG analogues (Figure 1; A en C). MRI of cell pellets showed a significant decrease ($p<0.01$) in T_1 and T_2 for α-ICAM-1-Gd-liposomes (T_1=0.76±0.12 s, T_2=16±2 ms) when compared to control cells without contrast agent (T_1=1.90±0.01 s, T_2=55±2 ms). IgG-Gd-liposomes (T_1=1.81±0.01 s, T_2=54±0 ms) induced no significant relaxation differences compared to control cells. No significant change in T_1 was seen for αICAM-1-MPIO-(T_1=3.2±1.9 s) or IgG-MPIO-incubated cells (T_1=2.4±0.2 s) when compared to control cells (T_1=2.4±0.1 s). Due to strong T_2-effects, a quantitative T_2 value for cells incubated with αICAM-1-MPIO could not be calculated. No significant difference in T_2 was measured between IgG-MPIO-incubated cells (T_2=12±17 ms) and control cells (T_2=21±15 ms).

To evaluate the use of these molecular MR contrast agent platforms for in vivo purposes, adult mice (C57Bl6) underwent a 30 min transient intraluminal occlusion of the right middle cerebral artery. Mice underwent MRI 24 h after stroke onset. Spin-echo MRI was applied for T_2-mapping (TR/TE 2300/12-96 ms, NE 8, NA 4, 100x200x400 $µm^3$, 21 slices) to detect the lesion. All groups had comparable lesion volumes (data not shown). T_1-maps (inversion recovery Look-Locker: TR/TE 9000/4.5 ms, 40 images, α 10°, NA 2, 200x300x400 $µm^3$, 21 slices) were acquired before and up to 3 h after i.v. injection of IgG- or αICAM-1-Gd-liposomes (5 µmol lipid, n=7 per group). T_2*-weighted images (TR/TE 35/15 ms, NA 8, α 10°, 125x125x125 $µm^3$) were acquired before and up to 2 h after i.v. injection of IgG-MPIO or αICAM-1-MPIO (100 µg Fe, n=5 per group). Some mice were subjected to a follow-up scan at 48 h after stroke, i.e. 24 h after contrast agent injection (n=6 for Gd-liposomes groups; n=2 for MPIO groups). No significant difference was found on T_1-maps of mice that received αICAM-1-Gd-liposomes compared to IgG-Gd-liposomes injection (Figure 2, upper graph) at any of the measured time points, although a significant decrease ($p<0.05$) of total brain signal intensity was measured during the first hours due to lasting circulation of Gd-liposomes and a significant increase in T_1 ($p<0.001$) ipsilesional compared to contralesional at 24 h due to intrinsic T_1 changes. After injection of αICAM-1-MPIO, however, a significant acute decrease ($p<0.01$) in ipsilesional T_2*-weighted signal intensity was measured compared to IgG-MPIO injection (Figure 2, lower graph). This difference was present in the first hours, but absent at 24 h after injection.

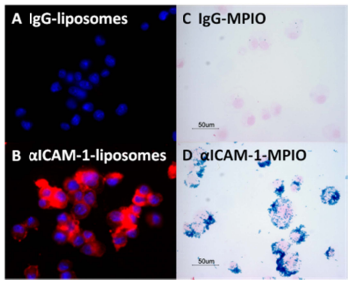
10 µm cryo sections of sacrificed mice were co-stained with αPECAM-1 for fluorescent microscopy (Gd-liposomes) and with αICAM-1 for light microscopy (MPIO). Contrast agent was detected in all tissue sections of mice that received ICAM-1-targeted Gd-liposomes or MPIO (Figure 3; E and J), but not in tissue sections of mice that received IgG-functionalized contrast agent.

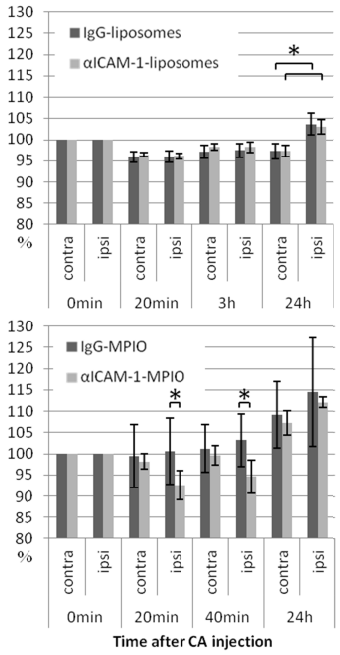
CONCLUSION

We conclude that both αICAM-1-Gd-liposomes and αICAM-1-MPIO specifically target ICAM-1 expressed on inflamed vasculature in mice after stroke, but that αICAM-1-MPIO provide a significantly higher level of contrast necessary for detection with in vivo MRI under conditions of disease-associated changes in intrinsic tissue relaxation times.

REFERENCES

[1] L.C. Hoyte et al, *J Cereb Blood Flow Metab* (2010) **30**, 1178-1187 [2] A.Y. Jin et al, *Contrast Media Mol Imaging* (2009) **4**, 305-311 [3] W.J.M. Mulder et al, *Bioconj Chem* (2004) **15**, 799-806





Microscopic images of cells incubated with contrast agent. **Figure 1** ↑↑

Relative changes in MRI signal intensity in ipsi- and contralesional tissue after contrast agent injection. **Figure 2** ↑

In vivo T_2 (A, F), pre- (B (T_1), G (T_2*w)), 20 min (C (T_1), H (T_2*w)) and 24 h (D (T_1), I (T_2*w)) post-contrast MR images and ex vivo microscopic images (E, J) of post-stroke mice brain injected with ICAM-1-targeted Gd-liposomes (A-E) or MPIO (F-J). **Figure 3** ↓