Imaging of Neuroinflammation with an ICAM-1 Specific Paramagnetic and Fluorescent Nanoparticle

L. H. Deddens[1], M. M. Van Schooneveld[2], S. M. Van der Pol[3], R. Koole[2], H. E. De Vries[3], W. J. Mulder[4], and R. M. Dijkhuizen[1]

[1]Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands, [2]Condensed Matter and Interfaces, Utrecht University, Utrecht, Netherlands, [3]Molecular Cell Biology and Immunology, VU Medical Center, Amsterdam, Netherlands, [4]Translational and Molecular Imaging Institute and Imaging Science Laboratories, Mount Sinai School of Medicine, New York, United States

Introduction:

Neuroinflammation plays a critical role in various brain disorders, but underlying processes are still largely unraveled [1]. Specific multimodal imaging probes may shed new light on the explicit involvement of distinct neuroinflammatory events, which could make way for new or improved anti-inflammatory treatment strategies. This study reports on the assembly and application of a probe consisting of fluorescent quantum dots in silica with a paramagnetic lipid coating. The probe is targeted at intercellular adhesion molecule 1 (ICAM-1), which is highly upregulated on inflamed cerebrovascular endothelium and a potential marker for the sub-acute stage of neuroinflammation. The aim of this study is to investigate the applicability of this bimodal nanoparticulate probe in detecting neuroinflammation with both fluorescence microscopy and MRI.

Methods:

Nanoparticle Synthesis:

Silica particles carrying a quantum dot in the center were prepared as described by Darbandi et al. [2] and analyzed by transmission electron microscopy (TEM). After surface modification with octadecanol, a micellar lipid coating with Gd-DTPA-BSA and (Mal-)PEG-DSPE was applied. Anti ICAM-1 monoclonal antibody (mAb) or irrelevant immunoglobulin G (IgG) Ab was coupled to the nanoparticles by a sulfhydryl-maleimide coupling method described previously [3].

Fluorescence Microscopy:

For fluorescent analysis, mouse cerebrovascular endothelial cells (bEnd3) were grown in a chamber slide and stimulated with tumor necrosis factor-alpha (TNFα) at 37 °C. 48 h after stimulation, cells were left untreated or were incubated with bare, IgG Ab-functionalized or anti ICAM-1 mAb-functionalized nanoparticles as schematically represented in Figure 1A-D. 2'-7'-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) was added for 30 minutes at 51½ h after stimulation to visualize viable cells, after which cells were fixated and analyzed with confocal scanning laser microscopy.

MRI Measurements:

For MRI measurements, $1x10^6$ of bEnd3 cells were grown and subsequently stimulated with TNFα at 37 °C. 48 h after stimulation, cells were left untreated or anti ICAM-1 mAb-functionalized nanoparticles were added at a concentration of 0.05 nmol nanoparticles per ml medium. Cells were incubated for 6 h, washed with PBS and harvested by incubation with EDTA and a mild trypsinization procedure. Cells and medium were collected in tubes and washed again with PBS. The obtained pellets were stored in 40 µl of 4% paraformaldehyde solution. Spin-echo T_1-weighted MRI (TR 1000 ms, TE 3.4 ms, matrix 128 x 128) of these cell pellets was conducted on a 9.4 T horizontal 20-cm bore MR system (Varian Inc., Palo Alto, CA). In addition, for R_1 mapping an inversion recovery Look-Locker sequence (TR 10000 ms, τ 10 ms, number of images 40, TE 4.7 ms, matrix 128 x 64) was used. In all experiments the field-of-view was 2 x 2 cm^2 with a slice thickness of 0.8 mm.

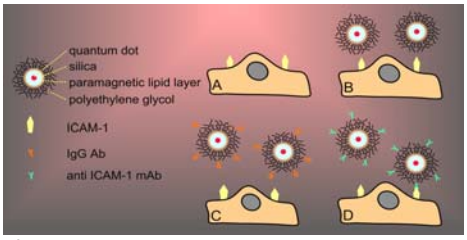

Figure 1

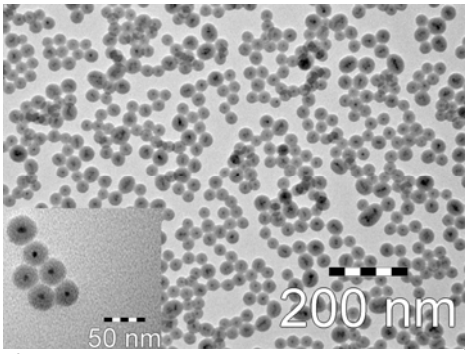

Figure 2

Results:

The incorporation of quantum dots in silica was successfully performed. Most nanoparticles contained one quantum dot in a silica-shell with a diameter of 35 nm (± 12.5%) as determined by TEM (Figure 2).

Fluorescence microscopy showed an equal amount of viable cells in all cell wells, visualized by the green BCECF-AM fluorescence, which underlined the biocompatibility of these nanoparticles [4]. Untreated cells (Figure 3A) or cells incubated with bare (Figure 3B) or IgG Ab-functionalized nanoparticles (Figure 3C) exhibited no significant nanoparticle fluorescence. However, significant quantum dot associated fluorescence was detected in cells incubated with anti ICAM-1 mAb-functionalized nanoparticles (Figure 3D), indicative of specific binding of this probe to the activated cerebrovascular endothelial cells.

The specific binding of the probe was also detected with MRI. T_1-weighted images showed enhanced signal intensity and corresponding higher R_1 in cell pellets of cells that were incubated with anti ICAM-1 mAb-functionalized nanoparticles (Figure 4A; $R_1 = 0.52\ s^{-1}$) as compared to untreated cells (Figure 4B; $R_1 = 0.44\ s^{-1}$).

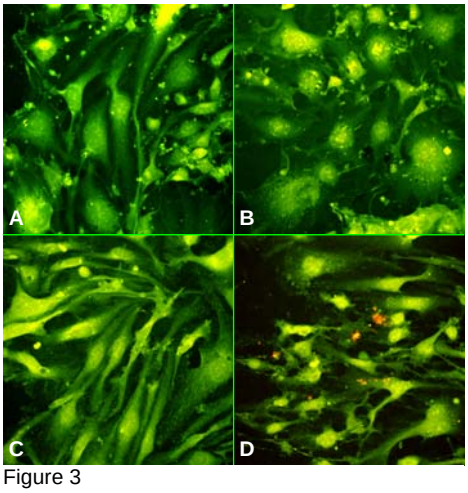

Figure 3

Conclusions:

Our study demonstrates that ICAM-1 targeted fluorescent and paramagnetic quantum dots in silica are biocompatible, specifically taken up by inflamed mouse cerebrovascular endothelial cells and detectable with MRI. This bimodal molecular imaging probe may provide a useful tool for *in vivo* molecular MR and optical imaging of upregulated cell adhesion molecules after neuroinflammation, and thereby could aid in gaining more knowledge on the explicit involvement of distinct inflammatory events in cerebrovascular diseases.

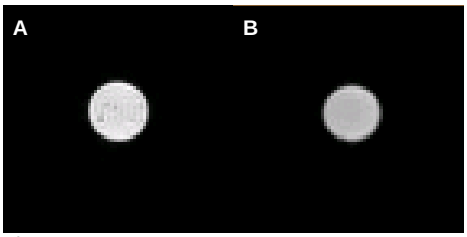

Figure 4

References:

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