Paramagnetic micelles targeting VCAM-1 receptors for imaging inflamed endothelium by MRI

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Purpose

The motivation of this work was to design a novel nanosized paramagnetic MRI agent for targeting intravascular epitopes acting as biomarkers of inflammation. Vascular Cell Adhesion Molecules (VCAM-1) are exposed on the activated endothelium with the aim of recruiting and promote the tissue infiltration of cells of the immune system. An intravascular target may overcome the poor sensitivity in the MRI detection of contrast agents, and, in addition, the use of soft lipid-based micellar nanoparticles allows for the delivery of a high number of paramagnetic centers at the target site.

Methods

A nonapeptide specific for VCAM-1 (CNNSKSHTC)[1] was synthesized using Solid-Phase Peptide Synthesis, purified by LC-MS and characterized by conventional 1D and 2D 1H-NMR. Then, peptide was conjugated with DSPE-PEG(2000)-Amine using Disuccinimidyl Glutarate as cross-linker. The same procedure was followed for preparing a scrambled peptide (HSCNKNSCT) used as control. Micelles (diameter 20 nm) containing DSPE-PEG2000 (57.5% in moles), Gd-DOTAMA(C16)$_2$ (40%), DSPE-PEG(2000)-peptide (2%), and Rhodamine-labeled DSPE (0.5%) were prepared. Targeted and control nanoparticles were injected (16 µmol Gd/kg bw) into the tail vein of C57Bl/6J mice bearing LPS-induced peripheral or brain inflammation. MRI experiments were performed at 1 T on Bruker Icon scanner.

Results

Peptide-conjugated phospholipids were obtained with yields around 30% and purity around 70%. Vectorized micelles displayed longitudinal relaxivity (0.5 T, 25°C) of 35.0 $s^{-1}mmol_{Gd}^{-1}$, and displayed good stability (> 2 weeks) in serum at 37°C. First, the micellar aggregates were tested on a model of peripheral inflammation induced by LPS injection. MRI experiment was performed on mice (n=6) 48h after LPS injection, corresponding to the strongest inflammation response.[2] 4 h after the injection of the targeted micelles, a T_1 contrast enhancement of around 35% was observed on the inflamed thigh. This value was ca. 4-fold higher than the enhancement measured in the contralateral healthy leg.

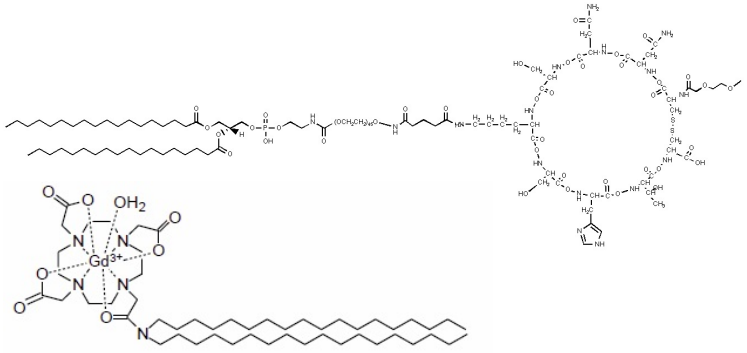

Fig.1: Top: DSPE-PEG-2000 phospholipid conjugated with the cyclic nonapeptide targeting VCAM-1. Bottom: Gd-DOTAMA(C16)$_2$.

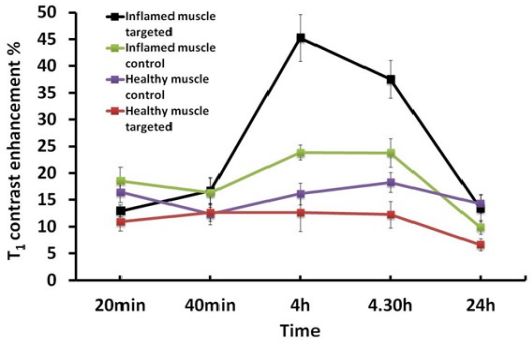

Fig. 2 % T_1 contrast enhancement measured after the injection of targeted or control micelles in C57BL/6J mice bearing peripheral LPS-induced inflammation.

Discussion

The restricted rotational motion of the Gd(III) complex embedded in the micelles makes its relaxivity strongly dependent on the magnetic field strength. The maximum value (35.0 $s^{-1}mmol_{Gd}^{-1}$) was observed around 0.2 T. To exploit better this property, MRI experiments were carried out at 1 T. In the peripheral inflammation model, the T_1 contrast enhancement detected in the diseased region after the injection of the targeted micelles was four-fold higher than the contralateral healthy leg, and ca. two-fold higher than the control experiment performed with a scrambled peptide. The higher contrast observed for the control probe in the inflamed area with respect to the contralateral healthy is the result of the increased permeability of the endothelium associated with the lesion. The effective presence of acute inflammation in the leg 48 h post injection of LPS was confirmed by histology.

Conclusions

Gd-loaded micelles vectorized with a peptide recognizing the endothelial inflammation marker VCAM-1 were prepared and characterized both *in vitro* and *in vivo*. Such a system displayed a good potential for detecting inflammation by MRI.

References

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