

Non-Invasive Assessment of Disease Activity in Lupus Nephritis by MRI-Based Molecular Imaging

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Introduction

Previously, we have shown that T2-MRI mapping after injection of targeted superparamagnetic iron-oxide (SPIO) nanoparticles is a useful non-invasive method to detect renal inflammation in a lupus mouse model at late stages of disease (1). Lupus nephritis is characterized by immune-complex deposition as well as complement C3 activation within the glomeruli of the kidney. Currently, percutaneous renal biopsy is the gold standard for monitoring disease activity in patients with lupus nephritis. We have developed a non-invasive method for detecting tissue-bound iC3b/C3d using SPIO nanoparticles linked to the iC3b/C3d binding region of complement receptor type 2 (CR2). Systemically administered CR2-targeted SPIO bind to sites of iC3b/C3d deposition and cause reduction of T2 relaxation times of tissues in proportion to the abundance of iC3b/C3d. In the present work we test whether the CR2-targeted SPIO nanoparticles could monitor disease activity in a murine model of lupus nephritis.

Methods

SPIO were synthesized by a solvothermal method yielding ~10 nm magnetite nanoparticles that were then encapsulated using amine-functionalized phospholipids as ~75 nm aggregates. The particles were conjugated to a recombinant protein that contains the SCR1-2 iC3b/C3d binding region of CR2, generating CR2-targeted SPIO nanoparticles. MRL/lpr mice spontaneously develop a progressive complement-dependent lupus-like glomerulonephritis as they age. MRL/lpr mice and control MRL/Mpj mice were injected with CR2-targeted SPIO nanoparticles at 12, 16, 20 and 24 weeks of age. T2-relaxation times were acquired prior to (baseline) and at 48 hours post injection (maximum of T2 reduction based on our previous work, 1). Anesthetized animals were inserted into a 4.7 Tesla Bruker PharmaScan MRI scanner. A Bruker volume coil (32 mm diameter) tuned to the ¹H frequency of 200 MHz, was used for radiofrequency (RF) transmission and reception. A series of multi slice multi echo (MSME) T2-weighted pulses with 16 various echo times was applied for precise T2 mapping and calculation of T2 relaxation times. The scan parameters were as followed: FOV=4.00 cm; slice thickness 1.50 mm; inter-slice distance 1.80 mm; repetition time TR=2,650 ms; echo time TE1=10 ms; TE2=20 ms (followed by 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160 ms); slice orientation axial; number of slices 16; number of averages 2; matrix size 128x256; total acquisition time 11 min. Kidney volume, as well as T2 relaxation times of kidney cortex, inner and outer medulla were calculated using Bruker ParaVision software. Differences in T2-relaxation times (Δ T2-relaxation times) between pre- and post-SPIO injections for control and MRL/lpr mice were compared.

Results

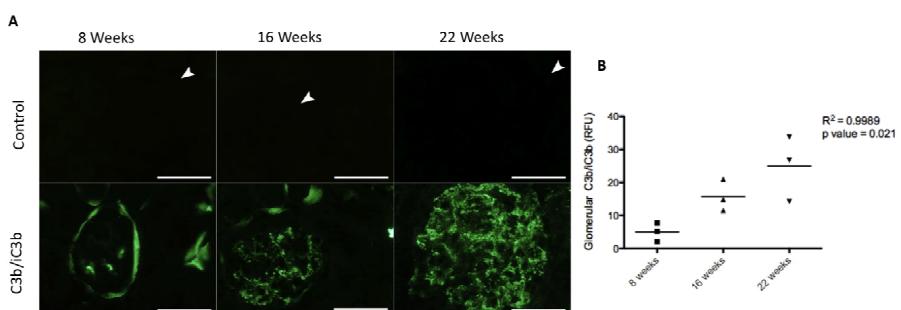
Histological examination demonstrated that degree of iC3b/C3d deposition in the glomeruli of the diseased kidneys positively correlated with disease severity (Figure 1). We found that CR2-targeted SPIO nanoparticles caused a reduction in the T2-relaxation time in the cortex and outer medullas of the kidneys of MRL/lpr mice, but did not affect the T2-relaxation time in the kidneys of control mice. Δ T2-relaxation times for left kidney cortex of MRL/lpr mice at 12 versus 20 weeks were -0.17 ± 0.89 vs. -3.9 ± 1.04 ms ($p = 0.0136$) and at 16 versus 20 weeks were -0.80 ± 1.04 vs. -3.9 ± 1.04 ms ($p = 0.0397$). Δ T2-relaxation times for left kidney outer medulla of MRL/lpr mice at 12 versus 20 weeks were -3.09 ± 0.93 vs. -5.50 ± 1.06 ms ($p = 0.0486$). Therefore, negative enhancement of the renal cortex and outer medulla after injection with CR2-SPIO was strongest in the 20 week-old MRL/lpr mice, corresponding to the peak glomerular iC3b/C3d deposition (Figure 2) and disease severity.

Figure 1:

Increase in iC3b deposition with disease progression. A - immunostaining for iC3b in 8, 16, and 22 weeks old MRL/lpr mouse kidneys. Arrowheads point to glomeruli. B - relative fluorescence units (RFU) of iC3b at same ages and positive correlation of iC3b RFU increase with age.

Figure 2:

T2-weighted MRI images of 20 week old MRL/lpr and control MRL/Mpj mouse kidneys at pre- and 48h post CR2-targeted SPIO injection. Arrowheads point to left kidneys; insert – enlarged image of the left kidney.



Conclusions

Our study demonstrated that CR2-targeted SPIO nanoparticles, directed to kidney-bound iC3b/C3d, showed significant reduction in T2-relaxation times of cortex and outer medulla regions of MRL/lpr mouse kidneys by MRI. This reduction matched with disease progression and peak iC3b/C3d deposition on the diseased kidneys. Therefore, molecular imaging of the kidneys by MRI after injection with CR2-targeted SPIO nanoparticles may provide a quantitative non-invasive alternative for monitoring disease activity in patients with lupus nephritis.

References

1. Serkova NJ, Renner B, Larsen B, Stoldt C, Hasebroock K, Bradshaw-Pierce EL, Holers VM, Thurman JM: Renal inflammation: Targeted iron oxide nanoparticles for molecular MR imaging in mice. *Radiology* 255: 517-526, 2010 [PMID 20332377].