Molecular Imaging Using Targeted Nanoparticles for Non-Invasive Detection of Renal Inflammation

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Intorduction

Systemic lupus erythematosus is a complex autoimmune disease with pleiotropic clinical manifestations. One of the great challenges of treating patients with lupus nephritis is assessing the activity of the disease, and tailoring pharmacologic therapy to achieve remission while minimizing toxicity. Recently, macromolecular contrast agents, including superparamagnetic (SPIO, 60 to 150 nm diameter) and ultrasmall SPIO (USPIO, 5 to 40 nm diameter), have been used to detect functional and anatomical renal abnormalities. Furthermore, conjugation of SPIO to targeting proteins permits the detection of specific molecules at the cellular level. CR2 (CD21) is a 140 kD transmembrane protein expressed on B cells, T cells, and dendritic cells, which binds the C3b, iC3b, and C3d cleavage fragments of C3. The goal of the current study was to evaluate whether the binding affinity of CR2 for C3d could be employed to target SPIO to renal C3 deposits in a murine model of lupus-like glomerulonephritis.

Methods

SPIO were synthesized by a solvothermal method using an Iron (III) Acetylacetonate precursor with trioctylamine and heptanoic acid (Sigma-Aldrich) as surfactants, yielding ~10 nm magnetite nanoparticles and then encapsulated using amine-functionalized phospholipids. A recombinant protein containing the first two SCRs of CR2 linked to the Fc portion of a mouse IgG_1 was generated; to conjugate CR2-Fc to the SPIO, 1 mg of the recombinant protein (6.7 nmol) was mixed with 10 mg (1.5 nmol) of the SPIO. 16 week-old MRL/lpr mice (lupus group, n=5) and C57BL/6 wild-type animals (n=6) were assessed by T2-MRI at baseline and 4, 24, 48 and 72 hours after SPIO injection (2 mg/kg, untargeted as well as CR2-Fc conjugated). Anesthetized animals were inserted into a 4.7 Tesla Bruker PharmaScan MRI scanner. A Bruker volume coil (32 mm diameter) tuned to the ^{1}H 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.

Results

At the baseline, T2-weighted signal intensity and corresponding T2-relaxation time values in the cortex and outer medulla of the kidneys of MRL/lpr mice were significantly higher than in wild-type animals (112%, P=0.03, and 120%, n.s., respectively). The higher T2 values in the kidneys of MRL/lpr mice likely represent higher water content, possibly the result of tissue inflammation. Injection of untargeted SPIO does not alter the T2-weighted signal in the kidneys of MRL/lpr mice (Figure 1). Injection of CR2-targeted SPIO into MRL/lpr mice caused a significant decrease in the T2-weighted signal of kidneys in the diseased mice (Figures 1 and 2). Signal in the cortex was reduced from 4 to 48 hours after injection, and signal in the outer and inner medulla was reduced from 4 to 72 hours after injection. The CR2-targeted SPIO caused a significant accumulation of targeted iron oxide with a subsequent decrease in the T2-relaxation times in the kidneys of MRL/lpr but not in control mice. Although the baseline T2-relaxation times in the cortex and outer medulla were higher in MRL/lpr mice, injection of the CR2-targeted SPIO caused a reduction in the T2-relaxation times in the kidneys of MRL/lpr below the values of control mice (Figure 1). The darkening in the MTL/lpr kidneys is clearly visible on the T2-MRI series with increased TE (Figure 2) – these series were used for precise calculations of the T2-relaxation times. No significant reduction in signal was seen in muscle, fat or spleen tissue of either MRL/lpr or wild-type mice (Figure 1). Kidneys were obtained from the mice at the end of the study, and were stained for iron with Perl's Prussian Blue. Iron was still detectable in some glomeruli and in tubules of MRL/lpr mice injected with the targeted SPIO, but was not seen in the kidneys of control mice.

Conclusions

In the current study we used a recombinant form of the complement receptor CR2 to target the SPIO particles to sites of complement activation. In vitro assays confirmed that CR2 linked to the surface of the nanoparticles mediated binding to cells that were opsonized with activated C3 fragments, but the particles did not bind to cells that were not opsonized with C3 fragments. Using the MRL/lpr model of lupus nephritis, we demonstrated that injection of mice with the CR2-conjugated SPIO caused a significant reduction in T2-weighted MRI signal and T2-relaxation time values in nephritic kidneys, whereas injection of the nanoparticles into healthy control mice did not change these quantitative imaging end-points. Furthermore, the injection of untargeted SPIO particles did not affect the T2-weighted signal of the kidneys in MRL/lpr mice. Thus, by conjugating the SPIO nanoparticles with recombinant CR2 protein we have developed a contrast agent that specifically targets the site of complement activation. This method can non-invasively detect active inflammation in immune-complex glomerulonephritis. Molecular imaging of the kidneys by MRI after injection with CR2-conjugated SPIO may provide a quantitative non-invasive method for monitoring disease activity in patients with lupus nephritis.

Figure 1: Changes in T2 relaxation times in MRL/lpr mice

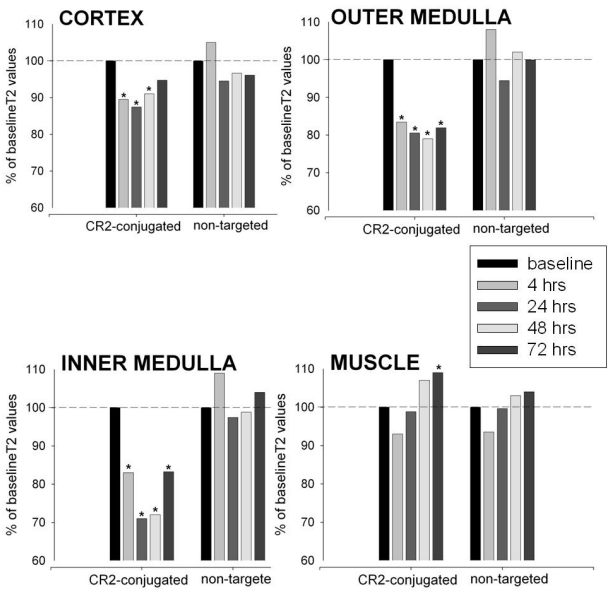

Figure 2: T2-weighted MRI form WT and MRL/lpr mice (TE = 20 and 30 ms)