Published in final edited form as:

Adv Chronic Kidney Dis. 2013 November ; 20(6): . doi:10.1053/j.ackd.2013.06.001.

Nano-sized Contrast Agents to Non-Invasively Detect Renal Inflammation by Magnetic Resonance Imaging

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Abstract

Several molecular imaging methods have been developed that employ nano-sized contrast agents to detect markers of inflammation within tissues. Renal inflammation contributes to disease progression in a wide range of autoimmune and inflammatory diseases, and a biopsy is currently the only method of definitively diagnosing active renal inflammation. However, the development of new molecular imaging methods that employ contrast agents capable of detecting particular immune cells or protein biomarkers will allow clinicians to evaluate inflammation throughout the kidneys, and to assess a patient's response to immunomodulatory drugs. These imaging tools will improve our ability to validate new therapies and to optimize the treatment of individual patients with existing therapies. This review describes the clinical need for new methods of monitoring renal inflammation, and recent advances in the development of nano-sized contrast agents for detection of inflammatory markers of renal disease.

Introduction

Inflammation is central to the pathogenesis of a wide range of acute and chronic kidney diseases. The accurate assessment of inflammatory processes within the kidneys improves our understanding of renal disease pathogenesis, and it also improves our ability to treat individual patients. The treatment of most forms of glomerulonephritis involves immunosuppressive drugs, for example, and there is evidence that other renal diseases may also respond to immunomodulatory drugs. However, all immunosuppressive drugs increase the risk of infection and have to be used with caution. Therefore, the detection of ongoing renal inflammation can guide the use of these medications.

Many new drugs for modulating or blocking the immune response have been developed in recent years, and these new agents have led to significant improvements in outcomes for some renal diseases. Rituximab for example, is effective for the treatment of several types of

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Statement of competing financial interests.

JMT is a paid consultant for Alexion Pharmaceuticals, Inc.

kidney disease (1-4). Some of the newer agents have a narrower range of action and may be less immunosuppressive than older drugs such as cyclophosphamide, but patient selection is very important given the more focused biologic actions of these drugs. Although several tests of the blood and urine can be helpful in diagnosing the underlying disease, nephrologists are still heavily depending upon renal biopsies to determine the etiology and activity of the underlying disease. Conventional radiology does not usually weigh heavily in treatment decisions.

In recent years there have been significant advances in both functional and molecular imaging methods. In addition to anatomic evaluation of the kidneys, these new techniques can provide quantitative evaluation of kidney function [e.g. renal blood flow and glomerular filtration rate (GFR)]. “Molecular imaging” methods can be used to non-invasively detect specific molecules of interest within tissues, and nanoparticles are a useful platform for developing molecular imaging contrast agents. They are small enough to penetrate most tissues, they can be designed for detection by standard radiologic methods, and they can be linked to targeting proteins that direct the nanoparticles to specific molecular markers. Super-paramagnetic iron oxide (SPIO)-based nanoparticles have been used as magnetic resonance imaging (MRI) contrast agents to detect macrophages in animal models of renal ischemia and in renal transplant recipients. More recently, targeted SPIO nanoparticles have been used as molecular imaging contrast agents to detect complement activation in pre-clinical models of glomerulonephritis. In this review we will discuss the use of SPIO-based contrast agents and T2-weighted MRI to detect and monitor renal inflammation.

The Clinical Need for Imaging Biomarkers of Renal Inflammation

Clinicians are typically alerted to the presence of renal disease by the detection of elevations in the serum creatinine or inappropriate substances in the urine (e.g. proteinuria or red blood cells). Patients may develop physical exam findings, such as peripheral edema or signs of uremia, but these are often late-stage manifestations of disease and are non-specific. Once a disease is broadly categorized (e.g. acute kidney injury, nephrotic syndrome, glomerulonephritis), specific blood and urine tests may help to find the etiology of disease. For most types of renal disease, however, the available biomarkers are not sufficient to make an early or definitive diagnosis without performing an invasive biopsy procedure. Improved biomarkers are desperately needed for several different renal diseases and clinical syndromes, and detection of inflammatory markers with nano-sized contrast agents may transform the care of various renal diseases in the near future.

Lupus nephritis—Lupus nephritis is the prototypical immune-complex glomerulonephritis. Renal injury is caused by the deposition of immune-complexes in the glomerulus with activation of the complement system on renal structures. There is uncertainty as to whether the immune system is responding to renal antigens or whether the kidney simply represents the site of immune-complex deposition. It should also be noted that immune-complexes are not prominent in some histologic patterns of lupus nephritis (5). Nevertheless, all forms of lupus nephritis are broadly categorized as “auto-immune” and are treated with immunosuppressive drugs.

Once a definitive diagnosis is made – generally by biopsy – patients are started on courses of therapy that may last several years (6, 7). Lupus is a notoriously heterogeneous disease, and fewer than 50% of patients treated with the standard therapies enter remission within the first 6 months of therapy (7-10). Furthermore, a biopsy samples only a small portion of the kidney. Diseases such as lupus are often focal, and patients can be staged incorrectly due to sampling error of the biopsy. For example, it has been estimated by mathematical modeling that in a biopsy that contains 20 glomeruli, 14 of the glomeruli need to show disease

involvement in order to conclude that there is involvement of more than 50% of the glomeruli (diffuse disease) (11). Obviously, the fewer glomeruli sampled in the biopsy, the greater the risk of misclassifying the disease.

Because of the variable response to treatment in patients with lupus nephritis, clinicians must repeatedly re-evaluate a patient's clinical condition. During a prolonged course of therapy, clinical and laboratory findings are used to determine whether a patient is responding to therapy and the treatment should be continued. For patients who do not respond to treatment, the decision must be made as to whether the treatment intensity should be increased or, conversely, whether damage to the kidney is irreversible and treatment should be discontinued. Common biomarkers of disease activity in lupus nephritis include the degree of proteinuria, the number of red blood cells seen in a spun urine sample, serum anti-double stranded DNA antibodies, and the level of C3 in plasma. All of these biomarkers have limited accuracy for determining the degree of renal disease activity or the degree of irreversible kidney damage (12). In one report, information obtained from a repeat biopsy at the end of induction treatment was predictive of a doubling of serum creatinine whereas no clinical or lab parameters were predictive of this outcome (13). Thus, the biopsy is the currently the best method of judging the severity of a patient's disease and their response to therapy, and the persistence immune deposits in a second biopsy is one of the strongest predictors of disease progression. The ability to non-invasively detect these deposits in tissues could, therefore, provide a powerful method for tailoring a patient's treatment.

Other forms of immune-complex glomerulopathy—Other glomerular diseases associated with immune-complex deposits are frequently treated with immunosuppressive drugs. For example, type 1 membranoproliferative glomerulonephritis (MPGN), IgA nephropathy, and membranous disease are characterized by glomerular deposits of immunoglobulin and complement proteins. The M-type phospholipase A2 receptor was identified as the target antigen for the majority of patients with idiopathic membranous disease (14), raising the possibility that antibodies to this protein can be used as a biomarker of the underlying immune process. The titer of antibody specific to this receptor may be useful for monitoring the response of patients to treatment with immunomodulatory drugs (15), although tests for this antibody are still not widely available. Urinary proteomics has revealed disease biomarkers for other forms of glomerulonephritis, but these test are of limited use and have not entered clinical practice (16, 17). For most forms of glomerulonephritis, therefore, good non-invasive biomarkers of disease activity have not yet been developed.

Other chronic inflammatory diseases of the kidney—Not all chronic inflammatory diseases of the kidney are caused by immune-complexes. C3 glomerulopathy, for example, is a recently described pattern of renal injury defined by the detection of glomerular C3 in the absence of glomerular immunoglobulin deposition (18, 19). The effects of corticosteroids and standard immunosuppressive drugs on this disease are not clear (19). Eculizumab, a therapeutic complement inhibitor, may be effective in some patients (20). C3 glomerulopathy is clinically a very heterogeneous disease. Methods to non-invasively detect immune deposits in the kidney would greatly facilitate the evaluation of new and existing treatments without the need for serial biopsies. Given that this disease is defined by detection of glomerular immune deposits, the detection of these factors by molecular imaging could one day replace the biopsy for disease diagnosis.

Acute kidney injury—Acute kidney injury (AKI) can be caused by a wide range of hemodynamic, toxic, infectious, and metabolic insults to the kidneys (21). In recent years there has been an intensive effort to discover new, early biomarkers of AKI (22-24). AKI is increasingly understood to be an inflammatory disease (25, 26). Some inflammatory

cytokines are detected early in the course of AKI (27), although these markers are not disease or tissue specific. Molecular imaging methods have been developed to detect tissue inflammation in models of AKI (discussed below). Unfortunately, these methods require 24-48 hours, and a key goal of detecting inflammation in patients at risk of AKI is to stratify patients to early interventions. Thus, the role of molecular imaging in the diagnosis and staging of AKI will require the development of more rapid imaging methods.

Transplant rejection—Renal allograft rejection can occur at any time after a renal transplant and is usually detected by a rise in the serum creatinine. Treatment of rejection generally involves escalation of a patient's immunosuppressive treatment. Consequently rejection must be distinguished from non-immunologic causes of injury, such as BK virus nephropathy (28). Several assays show promise as biomarkers for distinguishing rejection from other causes of allograft failure, although none are yet in clinical use (29). Consequently, transplant biopsies are currently necessary for accurately detecting immunologic rejection as a cause of allograft dysfunction.

Functional and Anatomical Renal Imaging

Abnormal renal function is the most common indication for renal imaging. Advances in radiological sciences and nuclear medicine have led to an enhanced repertoire of imaging modalities and end-points which can be applied and observed, respectively, in order to delineate the underlying abnormality. Renal imaging encompasses four main techniques: ultrasound (US), computed tomography (CT), MRI, and nuclear medicine [including positron emission tomography (PET) and single-photon emission tomography (SPECT)] (30-33). Modern anatomical techniques allow for a superb soft-tissue contrast (MRI) and spatial resolution (MRI and CT); while functional (also called “dynamic”) scans allow for precise assessment of excretion rates, glomerular filtration, tubular concentration and transit, blood volume, perfusion and oxygenation (Doppler US, gadolinium-enhanced MRI, BOLD MRI, ^{99m}Tc -MAG3 SPECT, ^{123}I - or ^{131}I -hippuran SPECT). Nevertheless, while various clinical indications can be investigated by a particular imaging protocol (Table 1), there is no existing, validated imaging platform to detect renal inflammation.

Anatomical and Functional MRI—Current MRI protocols are able to display both morphological information on renal parenchyma and vessels as well as functional data, such as perfusion, filtration, diffusion, and oxygenation. MRI has the best soft tissue contrast among all other imaging techniques, even without use of intravenous contrast. Since MRI employs complex physics to generate images (pulse sequences), various parameters can be utilized to optimize assessment of specific anatomic, morphologic, and functional endpoints:

- Renal cell carcinoma, angiomyolipoma and renal cysts can be readily distinguished by anatomical T1- or T2-weighted MRI (31, 34).
- Although the measurement of GFR by MRI is challenging, gadolinium (Gd)-enhanced T1-weighted MRI protocols have been developed for the relative assessment of renal function (Figure 1). These dynamic contrast-enhanced (DCE-MRI) protocols require sampling of the abdominal aorta and both kidneys with a sufficient time resolution (<2sec) to accurately define the arterial input function and to separate the cortical vascular phase (perfusion) and the filtration rate of the Gd contrast (30, 33, 35).
- The blood oxygen level-dependent (BOLD) MRI technique does not measure pO_2 directly but allows for intrarenal R2^* (relaxation rate) measurements, which are closely related to concentration of deoxyhemoglobin (32, 36). Static comparison of R2^* values in both kidneys by BOLD MRI can identify hypoxia in one kidney (e.g. due to renal artery stenosis) (37, 38).

- In acute renal failure, direct ischemic damages to the cells may lead to apoptosis or necrosis of tubular cells. Diffusion-weighted (DW)-MRI is useful to separate cellular edema (reversible damage with decreased apparent diffusion coefficients) from cellular renal necrosis (irreversible damages with increased apparent diffusion coefficients) (36).

- Renal fibrosis involves excess extracellular matrix synthesis accompanied by increased abundance of fibrillar collagens. Fibrosis is generally considered an irreversible process that is unresponsive to treatment with immunosuppression, and portends progression to end-stage renal disease (ESRD). Recent studies have reported on the diagnostic potential of MR elastography, DW and diffusion-tensor MRI as non-invasive methods to detect renal fibrosis in ESRD and transplanted kidneys (39, 40).

MRI Contrast—Diagnostic MRI routinely employs contrast agents to alter the relaxation rate of water protons, as the signal intensity in MRI is dependent on the concentration of water in the area of interest. Effective contrast agents must have a strong local effect on either the T1 or T2 relaxation times, thereby shortening the relaxation time of the water protons. Two commonly employed classes of MR contrast agents include paramagnetic T1-shortening contrast agents (gadolinium, manganese) and superparamagnetic T2-shortening contrast agents (iron oxide). The water molecules bound to these high spin metals relax orders of magnitude faster than free water, resulting in the desired changes in signal intensity. However, Gd and iron oxide differ in their MRI effects; paramagnetic Gd has predominant T1-effects producing a positive contrast/ a bright image on T1-weighted MRI due to shortening of the T1 values; while superparamagnetic Fe has a prevalent T2-effect and produces a negative contrast/ darker signals on T2-weighted MRI due to a reduction in T2 values (Figure 2A).

The most commonly used intravenous MRI contrast agents are Gd-chelates. All FDA-approved Gd-chelates are low-molecular-weight contrast agents. Because they are freely filtered by the glomeruli at first pass without any tubular secretion or reabsorption, they can be utilized as glomerular filtration markers (see above DCE-MRI application description) (41). Unfortunately, free Gd is toxic and the stability of chelated Gd is inadequate in patients with the ESRD due to prolonged circulation times. In such cases, nephrogenic systemic fibrosis (NSF) has been reported in association with Gd-use for MRI, and all Gd-chelates are contraindicated in patients with ESRD, acute kidney injury (AKI) and stage 4-5 chronic kidney disease (42, 43).

Iron oxide (SPIO nanoparticles) decreases spin-spin T2-relaxation times resulting in negative contrast (tissue darkening) on T2-MRI. Nanoparticle imaging agents are small enough to stay in colloidal solution and to penetrate tissues, yet they maintain physical characteristics that make them detectable by standard radiologic methods. In addition, nanoparticles can be easily functionalized (a targeted moiety can be easily added to the iron oxide-containing core). Most importantly, unlike gadolinium, iron is a naturally occurring element in human bodies and is taken up and metabolized by the reticuloendothelial system, Kupfer cells and macrophages (44). Because of their natural metabolic fate, SPIO nanoparticles have been clinically used as liver contrast agents (two FDA-approved agents, Feridex and Resovist) as well as intravenous iron supplement in anemia patients (various FDA-approved agents, including Ferumoxytol which is often used off-label for MRI) (Table 2) (45). A very attractive feature of MRI is its quantitative nature. The quantitative endpoints for T2- based MRI sequences include (but are not limited to) the precise calculations of apparent diffusion coefficients (as mm^2/sec) in diffusion-weighted MRI (by varying b-values), and – significant for nanoparticle applications – T2-relaxation times (in msec) by varying echo times (TE) in T2-based sequences. The following equation is applied for

precise calculations of T2 relaxation time as a function of signal intensity and TE values of each T2-MR image:

$$S = M_0 (1 - e^{-TR/T1} e^{-TE/T2}) \quad (1)$$

$$S = C_2 e^{-TE/T2} \quad (2)$$

where $C_2 = M_0 (1 - e^{-TR/T1})$ is a constant which gets fitted.

Darkening of inflamed tissues (which correlates with macrophage accumulation) after injection of these commercially available SPIO nanoparticles has been observed in animal models of focal ischemia, neuroinflammation, atherosclerotic plaques, heart transplants, renal inflammation and, recently, cancer (46-52). The degree of darkening can be quantitatively assessed by measuring the T2 value within a region of interest (e.g. the renal cortex) before and after injection of the contrast agent (46, 47, 53). The change in T2 value reflects the abundance of SPIO that have accumulated, and thus reflects the abundance of the cells or target to which the SPIO are bound.

Pre-Clinical Studies Using Nanoparticles to Detect Renal Inflammation

Given the great clinical need for methods of non-invasively detecting renal inflammation, several studies have utilized nanoparticles to detect specific immune cells or immune proteins within the kidney. These studies have been successful at detecting inflammatory markers with good sensitivity. Furthermore, they have been used to localize the sites of inflammation within the kidney.

Studies using SPIO-labeled cells—Studies have utilized *in vitro* iron-labeling of various progenitor cells, with subsequent grafting and *in vivo* MRI visualization of labeled cells in the animal. Labeled mesenchymal stem cells (MSC) were observed *in vivo* in the rat kidney cortex as long as 7 days after injection into renal artery of healthy rats at 1.5 Tesla MR field [(54), Figure 2B]. Another study reported the glomerular homing of iron-stained MSC in a rat model of mesangiolysis (55). After intravenous injection of SPIO-MSC, reduced T2 signal intensity was observed in the cortex of pathologic kidneys 6 days after injection. In this study, no loss of T2 signal was seen in the kidneys of control animals. Other groups have reported similar findings using labeled MSCs in rat models of acute ischemia and acute kidney injury caused by glycerol injection (56-58). The persistent loss of T2/T2*-weighted signal was observed up to 14 days after injection of SPIO-labeled MSC (range 72 hrs to 14 days). More recent studies have reported renal localization of SPIO-labeled macrophages in rat renal transplant and mouse ischemia/reperfusion models (59, 60). Animal 4.7 Tesla MR scanners were used in both studies. Negative contrast of the kidneys was observed 24 hours after SPIO-macrophage administration in the rat recipients of allogenic transplants (5-days post-transplant), and the low T2* signal intensity zones corresponded to the distribution of SPIO-labeled macrophages by histopathology. No changes in T2*-weighted MRI were seen in the syngeneic allograft group. Another study labeled macrophages with 150 nm SPIO *ex vivo* (60). The left kidney of Balb/c mice was clamped for 45 minutes, and after 24 hours of reperfusion the mice were injected with 2×10^6 nanoparticle-labeled macrophages or with the nanoparticles. SPIO of this size are primarily taken up by the reticuloendothelial system (not tissue macrophages), so direct injection with the nanoparticles was performed as a control. In the post-ischemic kidneys, the injection of the labeled macrophages caused a discrete darkening between the outer and inner stripe of the outer medulla by T2-weighted images. A negative contrast effect was not seen in the control kidney or in ischemic kidneys injected with the control SPIO.

Studies using untargeted nanoparticles—Studies have also shown that immunocompetent cells (tissue-associated macrophages) can be detected by MRI *in vivo* after SPIO injection without pre-existing *ex vivo* cell labeling. Macrophages, virtually absent in normal kidney, may infiltrate renal tissues in specific nephropathies such as various forms of glomerulonephritis, renal allograft dysfunction (rejection or acute tubular necrosis), and in acute ischemia/ reperfusion injury. As mentioned above, iron oxide nanoparticles are avidly captured by macrophages and induce a significant decrease in T2/ T2* of affected kidneys. Plasma clearance and the route of excretion depends on the particle size. The USPIO probes of 10 nm diameter are removed through extravasation and renal clearance and have shorter plasma half-live times. After intravenous injections of clinically available USPIO (colloidal particle size 15 – 65 nm), the particles stay in the blood until they enter the reticuloendothelial system (macrophages of the liver, spleen and bone marrow); a plasma half-life time of 15 hrs have been reported in humans. Our own data, using a 10 mg/kg iron injection of commercially available Ferumoxylol (USPIO, 18-30 nm), showed that these USPIO rapidly pass through the kidney (4-12 hrs in control mice), with a significant and prolonged T2 decrease in the liver (as expected due to hepatic uptake and metabolism, Figure 2C). However, our data show that in the inflamed kidney (a mouse ischemia/ reperfusion model), the decrease in T2 relaxation times persisted well over 24 hrs after injection of the SPIO, indicating macrophage up-take of iron. Another study using ~20-30 nm dextran coated SPIO nanoparticles examined the contrast effect of the nanoparticles in a rat model of glomerular and tubulointerstitial injury (61). Rats were injected with puromycin, and after two weeks they underwent renal imaging. The kidneys were imaged by MRI using fast low-angle shot (FLASH) gradient-echo sequence, and images were obtained prior to and 24 hours after injection with the nanoparticles. The T2* signal intensity in four different regions of the kidney (cortex, external outer medulla, “deep” outer medulla, and inner medulla) significantly decreased after injection of the SPIO. No significant changes were seen in control rats. In the puromycin-injected rats, there was a strong correlation between the change in signal intensity and the number of macrophages observed by immunohistologic analysis.

The same group applied this method in two additional rat models of renal disease (62). In one of the experiments the investigators induced nephrotoxic serum-mediated glomerulonephritis in rats, a model in which inflammation is restricted to the glomeruli. The authors found that injection with the nanoparticles caused a visible darkening in the renal cortex as well as a significant reduction in the T2* signal in that region. No change in the MRI signal was detected in outer or inner medullae. Interestingly, there was a significant decrease in the signal intensity in the cortexes of rats within two days of injection of the nephrotoxic serum, prior to infiltration of the glomeruli with macrophages. Electron microscopy demonstrated that the SPIO were present within mesangial cells, and the authors posited that this was due to increased endocytic activity of the mesangial cells in this model. Although the endocytosis of the SPIO was performed by mesangial cells, it only occurred after injection of the rats with nephrotoxic serum and still seems to represent a signal of inflammation. In a model of obstructive nephropathy, on the other hand, the authors found that injection of the rats with the nanoparticles caused a reduction in the MRI signal in all regions of the kidney. Based upon their results in different models of renal injury, the authors concluded that enhancement of the kidneys with USPIO can be used both to detect inflammation within the kidney and to localize the macrophage infiltrate to specific regions of the kidney.

Several studies have used SPIO nanoparticles to detect tubulointerstitial inflammation in models of acute kidney injury. Jo et al. used 20-30 nm USPIO to detect tissue inflammation in a rat model of ischemic acute kidney injury (63). In the same study the authors showed that injection of USPIO did not cause a detectable change in the MRI signal of rats with

HgCl₂ induced acute kidney injury. Unfortunately, little information was given regarding the degree of renal injury or macrophage infiltration in the HgCl₂ model.

Studies using targeted nanoparticles—Several studies have used targeted SPIO to detect specific molecular markers of inflammation in models of renal disease. Akhtar et al. conjugated a monoclonal antibody to mouse VCAM-1 to the surface of 1 μ m iron-oxide microparticles (MPIO) (64). They subjected male C57BL/6 mice to 30 minutes of unilateral ischemia. After 16-18 hours of reperfusion they injected the mice with targeted or untargeted MPIO and obtained T2*-weighted images of the kidneys six times within 90 minutes of injection of the MPIO. The VCAM-1-targeted MPIO caused a contrast effect in the cortex and medulla of the ischemic kidneys, and to a lesser extent in the non-ischemic kidneys too. Furthermore, the effect on T2* signal could be blocked by pre-injecting the mice with purified antibody prior to injecting them with antibody-targeted MPIO, confirming the specificity of the contrast effect.

Our group has used C3-targeted SPIO to detect renal inflammation in the MRL/lpr model of lupus nephritis (46, 47). The complement protein C3 is cleaved and fixed to tissues during inflammation (65), and renal biopsies are routinely stained for C3 fragments. We used a recombinant protein that incorporates the C3d binding region of complement-receptor-2 (CR2) in order to target tissue-bound C3d deposits. We conjugated the recombinant protein to the surface of 70 nm SPIO (Figure 3). We then injected MRL/lpr and control mice with targeted or untargeted SPIO, and performed T2-weighted MRI of the kidneys 4, 24, 48, and 72 hours after injection of the SPIO (46). Injection of the diseased mice with the CR2-targeted SPIO caused a significant negative in the kidneys, including cortex (Figure 4), inner medulla, and outer medulla. Injection of control animals with the targeted-SPIO did not decrease the T2-relaxation times (Figure 4), and injection of diseased mice with untargeted SPIO did not affect the T2 intensity of the kidneys.

We next used the same method to determine whether we could assess disease severity in the MRL/lpr model (47). Renal disease becomes progressively more severe as the MRL/lpr mice age, and we confirmed that the abundance of C3 fragments within the glomeruli increases in parallel with the progressive worsening of disease. We imaged the kidneys of MRL/lpr and control mice at 12, 16, 20, and 24 weeks of age. At each time-point we obtained baseline images kidneys, and then injected the mice with CR2-targeted SPIO. Injection of the diseased mice with targeted SPIO caused negative enhancement of the kidneys by T2-weighted images. The magnitude of this change was greatest at 20 weeks of age, the age the greatest abundance of glomerular C3. These studies demonstrate that MRI with CR2-targeted SPIO can be used to identify immune-complex glomerulonephritis and to assess the severity of the disease. The biomarker of disease detected by the CR2-targeted SPIO (C3 activation fragments) is routinely examined in biopsies of patients suspected or known to have immune-complex glomerulonephritis. This molecular imaging method, therefore, can be used to non-invasively monitor a key biomarker that is currently evaluated only by invasive tissue biopsy.

Clinical MRI Studies on Renal Inflammation

In current clinical practice, the degree of renal inflammation can be only determined by renal biopsy. SPIO nanoparticles are taken up by extrahepatic cells with phagocytic activity, including circulating monocytes and resident macrophages present in inflamed tissues. MRI with iron-based nanoparticles has been used to detect renal inflammation in human renal transplant recipients (48). T2*-weighted MRI was performed 72 hours after injection of USPIO nanoparticles. One patient (with biopsy-proven cortical inflammation) showed a significant decrease in T2*-signal intensity. All of the other renal allograft recipients, even

those with chronic and fibrotic disease but with no macrophage infiltration of their biopsies, did not show any changes in T2*-signal intensity after USPIO injection. Although this study by Hauger et al. is the only study utilizing MRI with SPIO in human kidneys, SPIO nanoparticles have been successfully used in humans for detection of liver metastases, islet inflammation, lymph node MRI and, most recently, multiple sclerosis, with no reported toxicities (49, 66-69).

Safety of iron-oxide nanoparticles

Rapid infusions of iron replacement formulations can cause oxidative stress and may be damaging to the kidneys (70, 71). Toxic levels of non-chelated iron can build up, and have the potential to produce radical oxygen species. However, toxic effects were not seen in rats or dogs injected with high doses (3000 $\mu\text{Mol Fe/kg}$) of SPIO (72). The reason for this is that the breakdown of magnetite (or maghemite) in the body forms ferric (and not ferrous) iron release which is then efficiently chelated by endogenous citrate and remains non-toxic. Nanoparticle toxicities are potentially different for each unique particle, however. Surface proteins may be immunogenic, and some surface coatings may cause anaphylaxis.

Ferumoxytol is used as an iron replacement therapy and has been administered to a large number of patients with chronic kidney disease (73). There are reports of anaphylactic reactions and hypotension to ferumoxytol (see www.amagpharma.com/products), but the episodes are usually mild and of short duration. It is not clear whether the lower doses of nanoparticles needed for molecular imaging studies will pose the same risks as the higher doses used for iron replacement.

Future directions

While anatomical and functional imaging remain gold standards for non-invasive assessment of kidney structure and function, recent development in molecular MRI indicate that pathophysiological pathways of renal disease, including inflammation, can be visualized at the tissue level (74, 75). The ultimate goal is the development of molecular imaging methods capable of providing clinicians with the same data that is currently provided by kidney biopsy. Ideally this would include resolution that can approach that obtained with histological examination of tissues. It would also include detection of the same biomarkers that are currently examined by tissues biopsies: immunoglobulins and light chains, complement proteins, inflammatory cells, fibrosis, and other deposits. SPIO-based MRI provides a promising method for macrophage imaging (untargeted nanoparticles) and for non-invasively detecting specific molecular biomarkers of inflammation, such as C3 fragments. In the future, so-called “multifunctional” or “multimodal” nanoparticle probes can be applied for more sensitive detection of inflammatory target proteins using, for example, a PET/MRI approach.

The treatment of autoimmune and inflammatory renal disease will improve in coming years with the development of new immunomodulatory therapies. The concurrent development of molecular imaging methods for monitoring renal inflammation will be critical for the rapid evaluation of these new therapies. Because molecular imaging can be used to detect inflammatory markers throughout both kidneys, such methods will actually provide a much more comprehensive picture of renal inflammation than a renal biopsy. Safe methods capable of reporting the extent and distribution of renal inflammation can be used to rapidly assess the efficacy of new anti-inflammatory therapies without having to conduct long-term clinical studies. Such methods will also be essential for tailoring an individual patient's treatment based upon their response to therapy and the total renal burden of inflammation and/or fibrosis.

Acknowledgments

The original studies reported in this review article were supported by the University of Colorado Cancer Center P30 grant CA046934, and the Colorado Clinical and Translational Sciences Institute UL1 award RR025780. This work was also supported in part by the KIDNEEDS Foundation, the Lupus Research Institute, and the National Institutes of Health Grant R01 DK076690.

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Figure 1. Anatomic and functional magnetic resonance images of the kidney

DCE-MRI of bladder and kidney (images are presented at 0, 2 min, 6 min and 14 min of Gd-bolus injection) of control and 5/6 nephrectomy mice. Decreased enhancement in the bladder and increased enhancement in the kidney are present in nephrectomy animals suggesting decreased filtration and excretion of Gd after surgery (NJS unpublished data).

Figure 2. Contrast enhancement of the kidneys using nano-sized contrast agents
 (A) Effects of T1- and T2-contrast agents on MRI signal intensity. (B) SPIO-labeled mesenchymal stem cell homing in rat kidney [adapted from (54)]. (C) time-course of ferumoxytol accumulation in muscle, kidney, and liver as detected decreased T2-times at various time-points after injection with the agent (NJS unpublished data).

Figure 3. Generation of C3d-targeted SPIO nanoparticles

Iron-oxide crystals can be coated with various organic and inorganic polymers. Targeting molecules, such as recombinant complement receptor-2 (CR2) can be conjugated to the surface of the nanoparticles after they are coated, or incorporated into the polymer prior to encapsulating the SPIO. This is a figurative representation and does not accurately represent the scale of the final targeted SPIO.

Figure 4. T2-mapping in the cortex of MRL/lpr mice injected with t-SPIO

We have mapped T2 values throughout the cortex of imaged kidneys. These T2 maps incorporate the data of the 16 echoes used during image acquisition and allow the quantitative assessment of the T2 throughout a 2-dimensional image of the cortex. The darkening of the cortical region in 20-week old MRL/lpr mice (orange-green → dark blue) represents the decrease in the T2 time after injection with CR2-targeted SPIO. Little change is seen in control mice after injection with the nanoparticles [this analysis was performed on images from previously published experiments (46, 47)].

Table 1

Existing Imaging Modalities and their Advantages and Disadvantages for Renal Imaging

Imaging Modality	Spatial Resolution	Clinical Problem	Advantages	Disadvantages
Ultrasound (US)	5-10 mm	Diffuse renal diseases; Renal mass lesions; Renal cyst; Urinary tract obstruction; Renal stones; Hematuria; Transplanted kidney. <u>Doppler</u> : Vessel patency; Abnormal vascularity; Renal artery stenosis.	Real-time nature of US highly suited for renal biopsy and interventional procedures; High potential for functional Doppler imaging; Microbubbles as new contrast agent; Inexpensive	Low potential for molecular imaging; Sub-optimal image quality in obese patients
Computed Tomography (CT)	5 mm	Renal trauma; Renal cyst; Renal carcinoma; Ureteric calculi	High spatial resolution; Fast (in a single breath-hold) acquisitions of the whole abdomen	Low potential for molecular imaging; Low potential for functional imaging; Contrast is required (toxicity); Radiation exposure
Magnetic Resonance Imaging (MRI)	5 mm	Renal cyst; Renal carcinomas; Renal functions; Renal transplantation (living kidney donors) <u>MRA</u> : Renal artery stenosis; Abnormal vascularity;	High spatial resolution; Superb soft tissue contrast; No ionizing radiation; High potential for functional imaging; Moderate-to-high potential for molecular imaging	Moderate-to-high costs; Prolonged scans; Complex physics; Pacemakers, metal clips are contraindicated;
Nuclear Medicine (PET and SPECT)	10-15 mm	Renal failure; Renal obstruction; ^{99m} Tc-DMSA: renal scarring; ^{99m} Tc-DTPA and MAG3: GFR assessments	Supreme functional (rather than anatomic) imaging; High potential for molecular imaging	Low spatial resolution; High costs; Radiation

The Table is summarized based on previously published radiologically based reviews (32, 33, 76).

Abbreviations: MRA: magnetic resonance angiography; ET, positron emission tomography; SPECT, single-photon emission tomography.

Table 2**Commercial SPIO Nanoparticle Formulations and Their Properties**

Agent	Trade Name	Application	Particle Size
Ferumoxsil	Lumirem, Gastromark	Oral SPIO for MRI	>300 nm
Ferumoxide	Feridex	IV SPIO for MRI	80-150 nm
Ferucarbotran	Resovist	IV SPIO for MRI	62 nm
Ferumoxtran	Sinerem, Combidex	IV USPIO for MRI	20-40 nm
Ferumoxytol	Feraheme	IV USPIO for anemia Rx	18-30 nm

The Table is summarized from (45).

Abbreviations: IV: intravenous; SPIO, superparamagnetic iron oxide; USPIO ultra-small paramagnetic iron oxide.