Molecular Magnetic Resonance Imaging of the Kidney

Importance of imaging in renal diagnosis

Kidney diseases affect a large portion of the population worldwide. Of all chronic diseases, chronic kidney disease (CKD) and end-stage renal failure are strikingly common. Chronic kidney disease of clinical stage 3 or higher affects about 11 percent of adults over 65, and stages 1-4 affect about 13 percent of the population over 20 (1). Several factors contribute to the prevalence of renal disease, including genetic, environmental and dietary conditions, and the presence of autoimmune disease. CKD has a strong correlation with the development of cardiovascular and heart disease. Unfortunately, CKD is clinically silent at its early stages. As early detection of CKD can lead to greatly improved patient outcomes with treatment, there is a vital need for novel and sensitive molecular diagnostic probes (2). Since the early onset of CKD can start with a focal lesion or pathology in the kidney, molecular imaging will play a role in biomarker discovery to detect early development of CKD.

Severe CKD is characterized by reduced glomerular filtration rate (GFR), indicating a failure of the kidney to sufficiently excrete or reabsorb dissolved solutes in the blood. Thus the total GFR is a standard clinical measurement in patients with suspected CKD or liver disease. Elevated serum creatinine, a derivative of liver creatinine, is another routine blood biomarker for CKD. Both of these clinical biomarkers are highly insensitive to early development of CKD such as polycystic kidney disease, diabetic nephropathy, and nephrotic syndrome, because of a combination of compensatory increases in glomerular filtration in uninvolved nephrons and because of variations in the background of blood creatinine. These variations are exacerbated by liver and kidney disease, confounding clinical measurements of renal disease in these patients. There are several promising blood biomarkers of kidney disease in preclinical and clinical trials, but a large number of renal diseases are detected early only through biopsy.

Imaging techniques in general, and molecular imaging techniques in particular, are just recently being applied to problems in diagnosis and detection of CKD. The most prevalent imaging technologies applied to image the kidney are x-ray computed tomography (CT), ultrasound imaging, and magnetic resonance imaging (MRI). Positron emission tomography (PET) and single-photon emission tomography (SPECT) techniques for renal imaging are important, though targeted molecular imaging probes are relatively new. Each of these imaging modalities has distinct advantages and disadvantages in sensitivity, resolution, contrast formation, level of invasiveness, and cost. A further major consideration in the future role of these

Molecular Imaging in Surgery

While the nuclear imaging community has traditionally focused on the development of radiopharmaceuticals for diagnostic gamma scintigraphy, positron emission tomography (PET), and single photon emission computer tomography (SPECT) imaging, a new imaging modality may bring molecular imaging into surgery and surgical pathology. A plethora of preclinical studies have shown the utility of molecularly targeted, near-infrared fluorescence imaging agents for intraoperative detection of cancer-positive lymph nodes in head and neck, breast, and prostate cancers, as well as in delineation of tumor margins. Indeed, several groups have developed and demonstrated the feasibility of peptide and antibody-based targeting agents that are dual-labeled for non-invasive PET

IN THIS ISSUE

New Editor for Molecular Imaging 5
Tech Corner 6
Industry Partners Circle Meeting 7
MI in the News 7
New Board Members 8
CMIIT Awards 8
Continued on page 2. See Kidney MRI

Continued on page 5. See Surgery.
techniques is how readily the developed probes can receive regulatory approval for clinical use. This ease of regulatory approval can in fact drive the focus of technology development in specific modalities, apart from any advantages or disadvantages in diagnostic ability. To its advantage, the combination of PET or SPECT detection with other imaging modalities will make it possible to coregister detected agents with high-resolution anatomical images. The challenge for MR based molecular imaging is that new agents are typically independent of previously approved compounds and are therefore subject to lengthy toxicity and distribution studies prior to clinical trial. However, there are several examples of sensitive MRI contrast agents, particularly in the area of MRI-based cell tracking, that are being currently investigated in clinical trials (3).

**Endogenous MRI contrast techniques to measure renal function**

MRI is a highly flexible imaging technology that uses radiofrequency (RF) electromagnetic pulses to detect and spatially localize magnetic nuclei and the local magnetic environment of the nuclei in three dimensions. Most commonly in clinical scanners, water $^1$H nuclei are detected because of their high natural abundance. A major advantage of MRI as compared to other imaging modalities is its ability to provide rich soft-tissue contrast, even in the absence of a contrast agent. These contrast mechanisms can be exploited to generate an unprecedented, noninvasive view of renal function. As an example, functional MRI is an important imaging technique that takes advantage of a change in hemoglobin from the diamagnetic to paramagnetic state when it is converted from oxygenated to deoxygenated form. This so-called blood-oxygenation level dependent (BOLD) contrast has been used to understand tubule dysfunction due to changes in oxygen extraction throughout the kidney (4). Renal functional imaging (5), is now being developed to extract quantitative information about renal blood flow. Renal perfusion can also be assessed by noninvasive arterial spin labeling, whereby blood is labeled by an RF pulse and tracked dynamically as it moves into the kidney. This can be used to generate quantitative maps of local renal tissue perfusion (6). Another example of an endogenous MRI technique that has been applied to study renal function is diffusion-weighted MRI (DWI) (7, 8). In DWI, applied magnetic field gradients are used to sensitize the MRI signal to the random motion of water inside the tissue. Because water diffuses throughout the cellular and extracellular microstructure during the MRI scan, DWI provides an indirect view of the tissue microenvironment. The source of changes in apparent diffusion coefficient (ADC), as measured by MRI, with changes in tissue microstructure is an active area of investigation in the kidney and other organs. Importantly, ADC may be correlated with glomerular filtration rate (9). DWI has been further developed to assess allograft viability after transplantation (10).

**Passive MRI contrast agents**

Paramagnetic ions, typically lanthanides and transition metals, have long been used as MRI contrast agents because of their effect on the MR relaxation times of the surrounding water. FDA approved MRI contrast agents include both gadolinium chelates (e.g. Gd-dota, Gd-dtpa, Gd-do3a) and iron oxides. These approved agents include small molecules and larger macromolecules, each with a unique biodistribution and clearance. Other macro-molecular agents are in clinical trials or preclinical research, and are developed specifically to reduce interstitial diffusion while allowing for glomerular filtration, as described in the literature (11). Contrast-enhanced MRI techniques are being developed to measure glomerular filtration rate to eventually provide an accurate imaging surrogate or replacement for standard GFR measurement techniques (12). To date, all clinically approved contrast agents for MRI are “passive” rather than targeted, and are typically used by either nonspecific uptake into tissues with disrupted vasculature or through measurements of dynamic changes in the uptake or excretion rates. Because imaging agents of small molecular weight, such as the lanthanide chelates, are typically rapidly excreted and freely diffusing, the rate of excretion can be used as an indicator of glomerular function. However, the lack of molecular specificity of these agents may be a disadvantage from the standpoint of identifying specific aspects of renal dysfunction. These issues are shared by freely diffusible iodinated tracers. Paramagnetic MRI contrast agents are typically detected by $T_1$-weighting the MRI pulse sequence. $T_1$ is a measured relaxation time that is shortened by the presence of the contrast agent. Fast $T_1$- weighted sequences can provide dynamic measurements of separate phases of contrast agent excretion and can be used in conjunction with mathematical modeling to map agent pharmacokinetics (13 14).

**Targeted MRI contrast agents**

In addition to the clinically approved agents, there is a wide range of innovative, targeted molecular imaging probes for preclinical MRI (16, 17). Many are available commercially and can be bought pre-functionalized for ready attachment to targeting ligands, antibody fragments, fluorophores or specific chemical functional groups. Compared to other techniques, MRI is relatively insensitive to the presence of the contrast agents. For example, typical PET/SPECT agents are detected in pM-fM concentrations. In contrast, many MRI contrast agents are detected in ~μM-mM concentrations. This problem is being overcome through the development of high-relaxivity agents, more sensitive acquisition strategies, and novel hyperpolarized agents with nuclear polarization 1,000-fold greater than traditional imaging agents (18). Many of these probes are being used to assess excretion rates and dynamic changes in renal function. Novel paramagnetic chemical exchange saturation transfer agents (PARACEST) have been developed with specific off-resonance frequency spectra so that individual agents can be distinguished. Recently, PARACEST imaging was used to detect TmDOTA-4AmC(-) accumulation and clearance in the mouse kidney (19). Paramagnetic agents sensitive to renal pH have also been synthesized (20).

Targeted contrast agents have been developed for molecular MRI of the kidney. These targeted agents are typically paramagnetic or superparamagnetic contrast agents with a binding moiety
on the agent surface. When the agents are intravenously injected they are targeted to the glomerulus or must pass through the glomerulus and bind to the tubule. For example, 13-nm nanoparticle contrast agents can be targeted specifically to the glomerular basement membrane because they pass through ~80 nm fenestrations in the glomerular endothelium. We have recently demonstrated that cationic ferritin, a 13 nm superparamagnetic nanoparticle, accumulates in the glomerular basement membrane due to electrostatic binding to anionic proteoglycans. The accumulation of these nanoparticles allows each glomerulus and nephron to be detected with MRI (21). We have recently used this technique to measure whole-kidney nephron endowment (22), as shown in Figure 1. The in vivo application of these techniques will rely on advanced image processing in order to overcome inherent limitations in resolution, as well as the development of a fuller understanding of binding kinetics of cationic nanoparticles as they attach to the glomerulus.

Kidney transplants are commonly required for patients with end-stage renal disease. The causes of transplant rejection are poorly defined and are under active investigation. There is a vital need to assess the structure and function of transplanted kidneys before and after they are surgically implanted, both to ensure sufficient function to provide glomerular filtration and to quickly detect the early onset of rejection. As an example, macrophage infiltration has been identified during transplant rejection by detecting macrophages labeled with an MRI-detectable contrast agent (23). Furthermore, there is a need for noninvasive techniques to study the mechanisms of transplant rejection in large preclinical studies and in patient populations. Cellular imaging techniques have also been used to detect inflammation and macrophage infiltration during development of renal disease (24, 25).

Assessment of renal function is crucial to understanding the metabolism and excretion of new therapeutic agents. Preclinical toxicity screening is a major, costly requirement for regulatory approval of new compounds, and a majority of kidney screens are performed by histological assessment and blood markers. Noninvasive molecular imaging could play a major role in accurate, rapid, inexpensive measurements of toxicology and pharmacodynamics (26) and represents an opportunity to develop both functional MRI techniques and novel molecular probes to assess renal viability.

Regulatory approval of new MRI contrast agents is relatively slow compared to new compounds conjugated to radioligands. Thus, PET/SPECT techniques are advantageous in that preclinical studies can be rapidly translated to the clinic. The development of novel radioactive agents for PET/SPECT imaging of kidney function is a major area of research, as illustrated in Figure 2 (unpublished figure, reproduced by permission from Drs. C. Chad Quarles and Takamune Takahashi, Vanderbilt University Institute of Imaging Science and the Vanderbilt O’Brien Kidney Disease Center). Here, a high molecular weight dextran, tagged with $^{99m}$Tc, is imaged after it accumulates in the mouse glomerulus following retroorbital injection. Microscopic imaging of the optical analog to this agent revealed that it is preferentially localized within the mesangium. Such an agent could potentially be used, both pre-clinically and clinically, to quantitatively assess total nephron endowment in vivo. A similar technique using high-molecular weight dendrimer contrast agents has been used to specifically enhance the outer stripe of the medulla (27). The modulation of contrast agent size and surface charge may be an important way to provide specificity of contrast agents to kidney structures, and may be a window into localized function in these areas.

Conclusions

As the field of molecular MRI develops, a wide range of highly sensitive agents will be available to investigate renal function, with sensitivity to scientifically and clinically useful parameters such as local pH, oxygenation, temperature, and metal ion content. Furthermore, the development of novel switchable agents such as those being currently developed (28, 29), with on-off or one-way contrast changes, will allow for detection levels on the order of...
those observed in PET/SPECT probes. Chemical and enzymatic amplification techniques may be used to enhance the sensitivity of MRI contrast agents. The outlook for MRI of the kidney will depend to a large extent on the development of tools such as these and an investment by both academic and industry partnerships in moving these agents through regulatory approval. Nonetheless, MRI holds a great deal of promise for highly sensitive measurement of renal function.

REFERENCES
14. Mandy, D. et al. Renal functional contrast-enhanced magnetic resonance imaging: evaluation of a new rapid-clearance blood pool agent (p792) in sprague-

By Kevin M. Bennett, PhD, School of Biological and Health Systems Engineering, Arizona State University