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Functional magnetic resonance imaging of the kidney using macromolecular contrast agents

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Abstract

Background: Functional magnetic resonance (MR) imaging of the kidney relies on low-molecular-weight contrast agents. These agents are glomerular filtration markers and are neither secreted nor reabsorbed by the tubules but are filtered at the glomerulus. Low-molecular-weight contrast agents provide limited functional information. A new generation of macromolecular magnetic contrast agents is under development for MR angiography. These agents may provide additional renal functional information not provided by low-molecular-weight agents.

Methods: We review the use of macromolecular contrast agents such as gadolinium-bound albumin (Gd-albumin), gadolinium-bound dendrimer (Gd-dendrimer), and ultrasmall particles of iron oxide (USPIO) in specific renal parenchymal diseases. These data are largely derived from animal studies because many of these agents have not been extensively deployed in human populations.

Results: Different specific uses have been documented for macromolecular contrast agents. Gd-albumin appears to detect the source of proteinuria and localize the site of recurrent proteinuria after transplantation. Gd-dendrimer uptake reflects damage to the proximal straight tubule in the outer medulla. USPIO agents demonstrate sites of inflammatory changes within the kidney.

Conclusion: Although not yet in widespread clinical use, macromolecular MR contrast agents may play a role in the evaluation of functional diseases of the kidneys.

Key words: Magnetic resonance—Macromolecular contrast agents—Kidney function—Proteinuria—Cis-platinum nephrotoxicity—Ischemia

Functional magnetic resonance (MR) imaging (MRI) of the kidneys became possible with the introduction of gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), also known as gadopentetate dimeglumine, in the late 1980s [1]. It was quickly realized that, as a molecule smaller than 1 kD, Gd-DTPA was treated by the kidney like inulin: it is filtered at the glomerulus and is neither secreted nor reabsorbed in the tubules or ducts. This made it a suitable agent for studies of glomerular filtration and assessing the concentrating ability of the kidneys [2]. Over the past 20 years other low-molecular-weight gadolinium chelates have been developed but all of them share similar physical properties with Gd-DTPA and therefore provide similar information.

Dynamic contrast-enhanced MRI of the kidneys has produced different methods for evaluating renal function, largely borrowed from the nuclear medicine community. These include the Patlak and Gates methods of estimating renal function [3, 4]. The former provides approximations of glomerular filtration rate (GFR) and the latter provides split function assessment. The slope with which the cortex enhances is roughly proportional to renal blood flow and is decreased when GFR is decreased. In obstruction, the concentrating ability of the kidney is decreased, thus leading to longer transit times and decreased enhancement.

Underlying these methods is an assumption that it is possible to accurately measure the concentration of Gd-DTPA within the kidney [5]. Gd-DTPA causes T1 and T2* shortening, the former causing an increase in signal and the latter a decrease. As a consequence, the relation between signal and concentration is complex and nonlinear. For nuclear medicine studies, the assumption of linearity between signal and concentration is straightforward because the count rate is directly related to the concentration. In MRI the relation between concentration and signal is more complex. The relation is made even more difficult by the large range of concentrations that the human kidney generates depending on the hydration status of the body.

Despite these theoretical advances, low-molecular-weight functional MRI of the kidneys has had little clinical effect thus far. There are several reasons for this,

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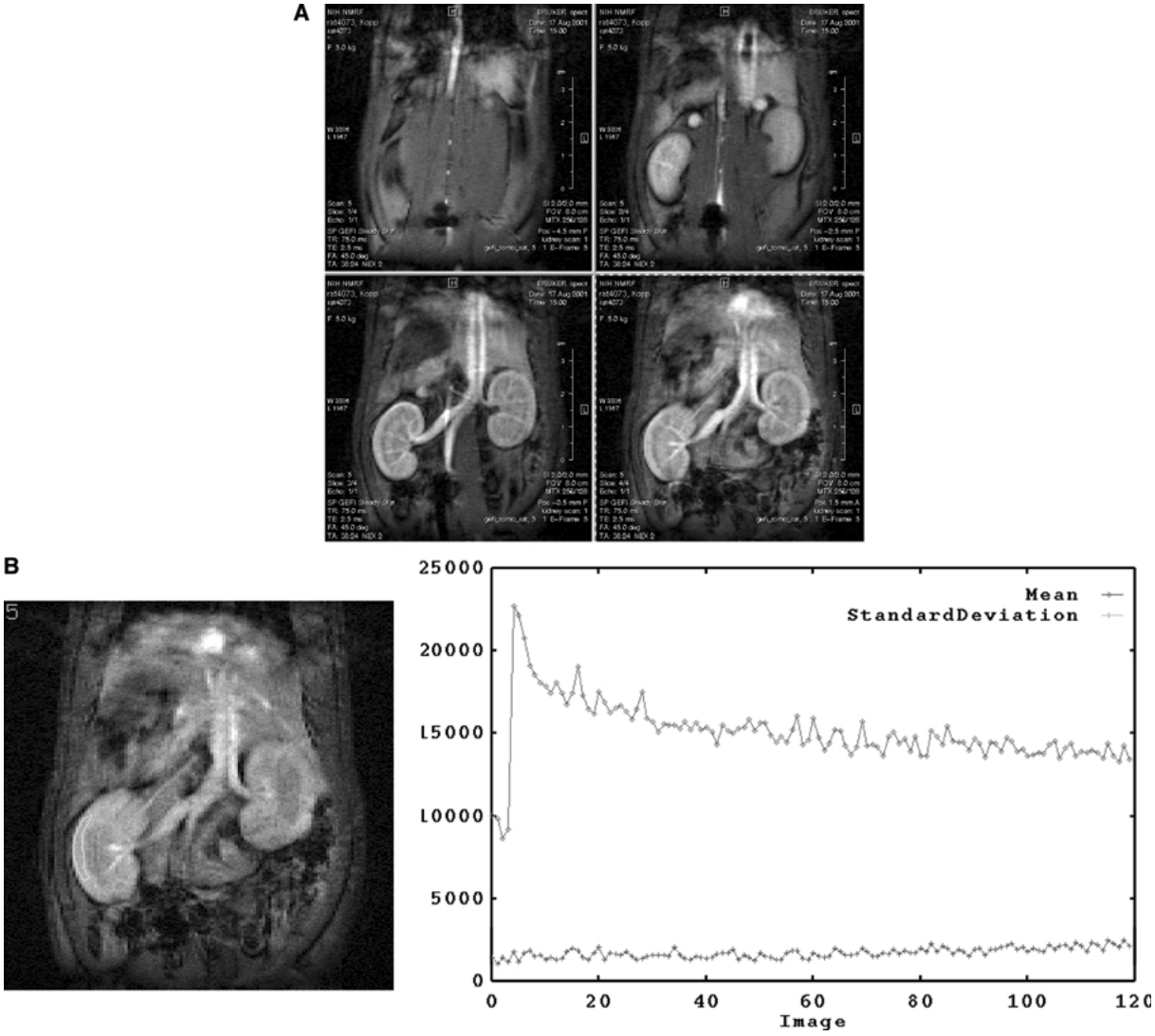

Fig. 1. Gd-albumin in normal (**A, B**) and proteinuric (**C, D**) rabbit kidneys. The normal kidney demonstrates a rapid increase in signal intensity followed by a relatively rapid washout when the signal is measured over the renal cortex. In contrast, the proteinuric kidney demonstrates rapid accumulation but very little washout over the 20-min experiment. This indicates that the albumin leaking into the renal parenchyma has overwhelmed the absorptive capacities of the kidney, thus demonstrating which kidneys are leaking protein. (Continued on page 226)

the most important of which is that analogous radionuclide methods already provide useful information. The requirement for software capable of analyzing serial dynamic data that are still not standardized and the need to calibrate the signal intensity to gadolinium concentration add levels of complexity that have also limited the propagation of these methods into the clinic.

Recently, several more practical functional MRI methods have been successfully reported including accurate assessments of renal blood flow with phase contrast MR angiography and measurements of extraction fraction [6–11]. The effects of these advances have yet to be felt in the wider medical community but remain promising.

The pharmaceutical industry has been developing macromolecular contrast agents [12]. A major motivating force for the development of macromolecules with relatively long intravascular half-lives has been to provide a blood pool agent capable of high-resolution MR angiography. Recent improvements in scanner speed for MR angiographic applications casts some doubt on the rationale for such blood pool agents. Nonetheless, other applications have come to attention that may ultimately surpass the original intention. Ultrasmall particles of iron oxide (USPIO) have found utility in evaluating lymph nodes during cancer staging due to their phagocytosis by macrophages [13, 14]. Other similar agents are being developed as sentinel node imaging agents.

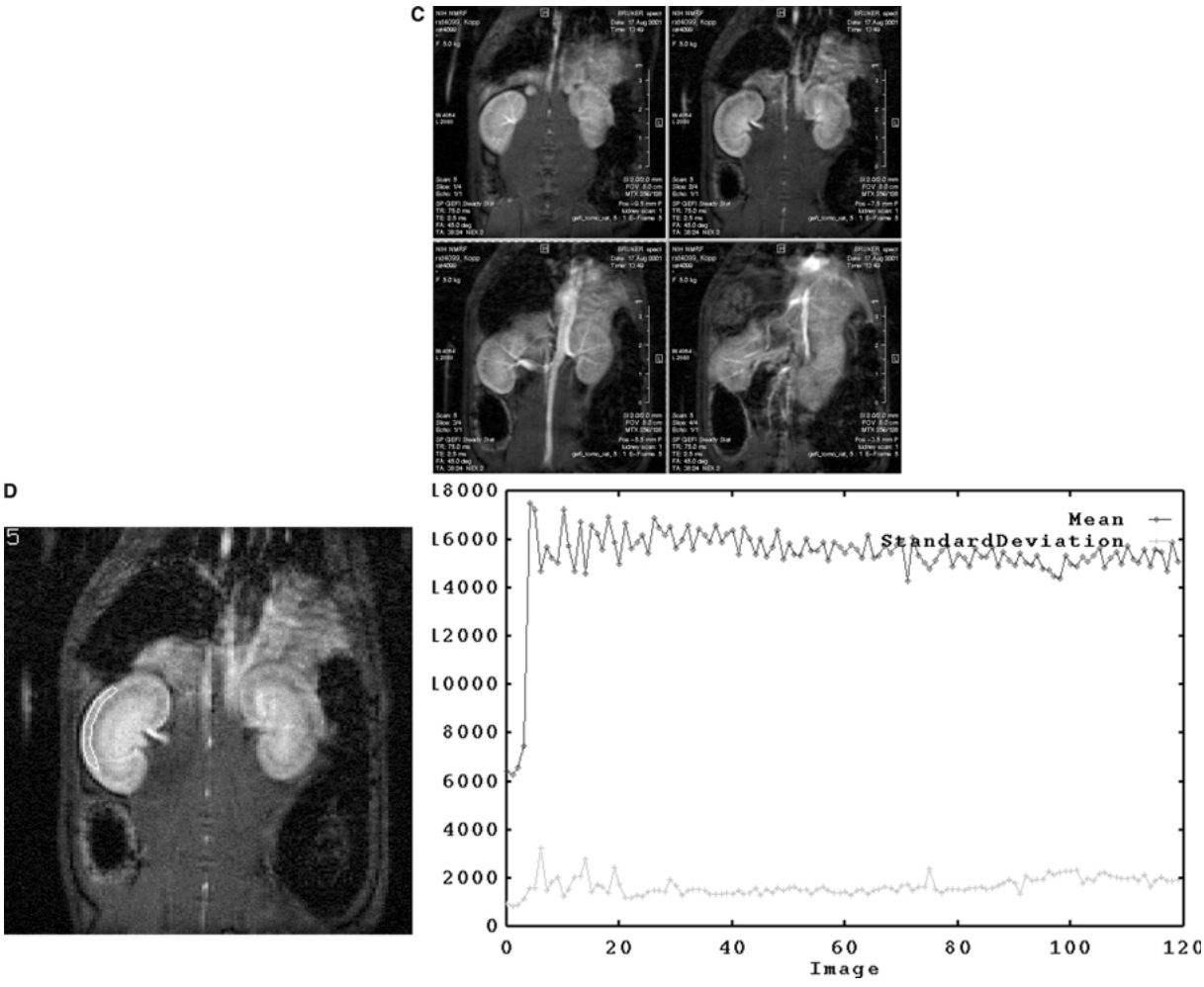

Fig. 1. Continued

All of these agents have potential value for evaluating functional disorders of the kidney. The purpose of this review is to summarize potential applications of the new macromolecular imaging agents for analyzing renal dysfunction based on early experimental data.

Macromolecular contrast agents

There is no universal definition of what constitutes a macromolecular contrast agent. It can be defined by molecular weight (>1 kD), diameter (>1 nm), or pharmacokinetics (half-life in blood > 10 min) [14]. The kidney handles such agents differently depending on the size of the macromolecule. Although filtration through the glomeruli is decreased as the size/weight of the agent increases, it is not altogether eliminated. Moreover, selective leakage through the glomerulus leads to reabsorption in the proximal straight tubules. Nanoparticles smaller than 7 nm will be filtered, albeit at a lower rate than low-molecular-weight contrast agents [14]. Larger particles, larger than 10 nm in diameter, may not be filtered by the glomerulus unless the glomerulus itself is damaged (Fig. 1). In the case of USPIO, which are 20 to 40 nm, only the vessels are opacified after injection. However, because they are internalized by macrophages and monocytes, USPIO-laden cells may accumulate in damaged portions of the kidney where there is inflammation.

Gd-albumin

Gd-albumin (MS-325) has been proposed as a macromolecular contrast agent to facilitate MR angiography. When this agent is injected intravenously, it rapidly binds to circulating albumin, forming a complex that remains largely within the intravascular space for a short period before the Gd-DTPA dissociates again from the albumin. The agent then is excreted via the urine.

Within the kidney, Gd-albumin is treated like albumin, i.e., a portion of it is filtered at the glomerulus and reabsorbed in the outer medulla. In normal renal function, the small "spill over" of albumin is readily accommodated by the proximal straight tubules; how-

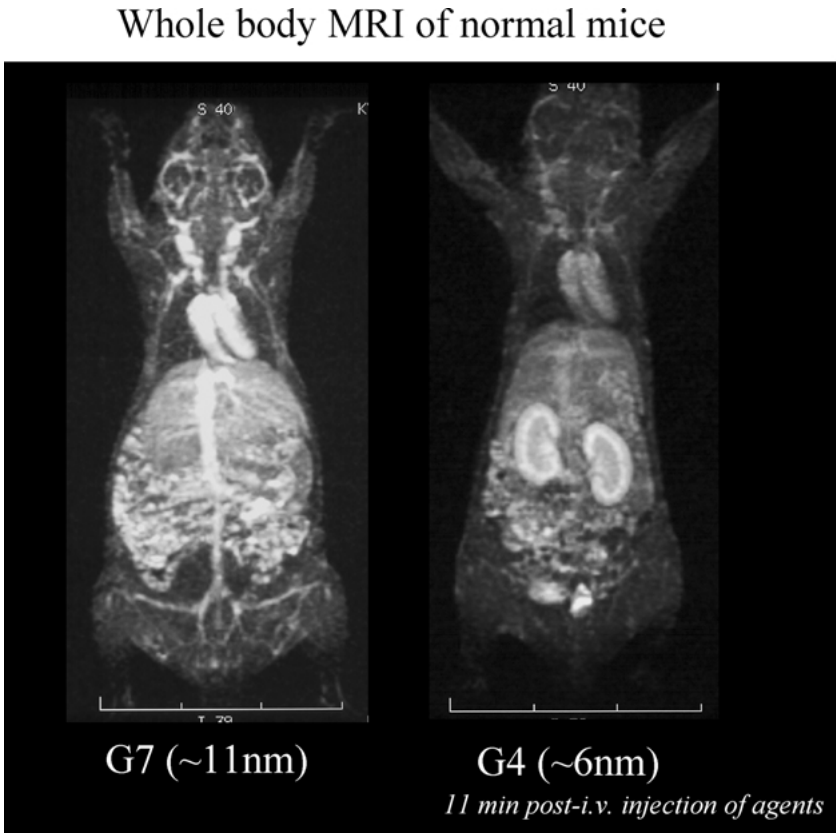

Fig. 2. A comparison of a G-7 (~11 nm in diameter) and a G-4 (~6 nm in diameter) gadolinium-labeled dendrimer. **Left** The G-7 dendrimer is not excreted by the kidney in sufficient concentration to be visualized and, in effect, becomes a blood pool imaging agent. **Right** In contrast, the G-4 dendrimer is excreted by the kidney and leads to a distinctive stripe in the outer medulla where the G-4 dendrimer accumulates.

ever, as proteinuria becomes more severe, the reabsorbing capacity of the tubules is overwhelmed and protein escapes into the urine (Fig. 1).

Proteinuria is a clinical finding that can easily be tested for with dipstick colorimetric tests of the urine. However, it is much more difficult to ascertain the actual origin of proteinuria, which can be unilateral (e.g., Nutcracker syndrome), bilateral, or arising from a renal transplant. Another specific clinical situation when determining the locus of proteinuria is relevant is in patients who develop recrudescence of proteinuria after renal transplantation. Is the transplant the source of the protein leak or is it an exacerbation of the native kidneys? A test that would localize proteinuria could be useful in this setting.

Gd-albumin dynamic renal scans in animal models of proteinuria demonstrated that proteinuric kidneys had distinctive excretion patterns compared with nonproteinuric kidneys (Fig. 1). Normal kidneys demonstrated a rapid increase in signal after injection followed by a relatively rapid washout, whereas proteinuric kidneys demonstrated substantially slower washout with retention of high signal over 10 to 15 min despite the absence of obstruction.

Thus, Gd-albumin functional MRI of the kidney has the potential to demonstrate the site and severity of proteinuria within the urinary tract and may ultimately play a role in the evaluation of this disorder.

Gd-dendrimer

Dendrimers are polymers that have different medical and nonmedical uses. From a chemical perspective they are organic molecules that can be polymerized to form nanoparticles of precise size. Whereas other particles are polydispersed (i.e., they can be produced only in a broad range of sizes), dendrimers are mostly monodispersed (i.e., their size range is uniform for a given synthesis). Because their outer surface is covered with primary amino groups, they can be readily chelated to imaging agents such as gadolinium. These same amino groups can be used to target the nanoparticles to specific receptors [14].

The body is exquisitely sensitive to particle size and handles specific nanoparticles very differently. For instance, it handles 2-nm particles very similarly to low-molecular-weight contrast agents but demonstrates significantly decreased renal excretion when the particle is 6 nm and virtually no renal excretion when the particle reaches 11 nm (Fig. 2). Moreover, the hydrophilicity and the charge of the particle influence how it is taken up by the organs and then eliminated.

Polymerized dendrimers are named by their "generation," which translates to molecular diameter. Thus "G-2 particle" refers to generation 2, G-3 to generation 3, and so on. Polypropylenimine diaminobutane dendrimers are more hydrophobic than polyamidoamine

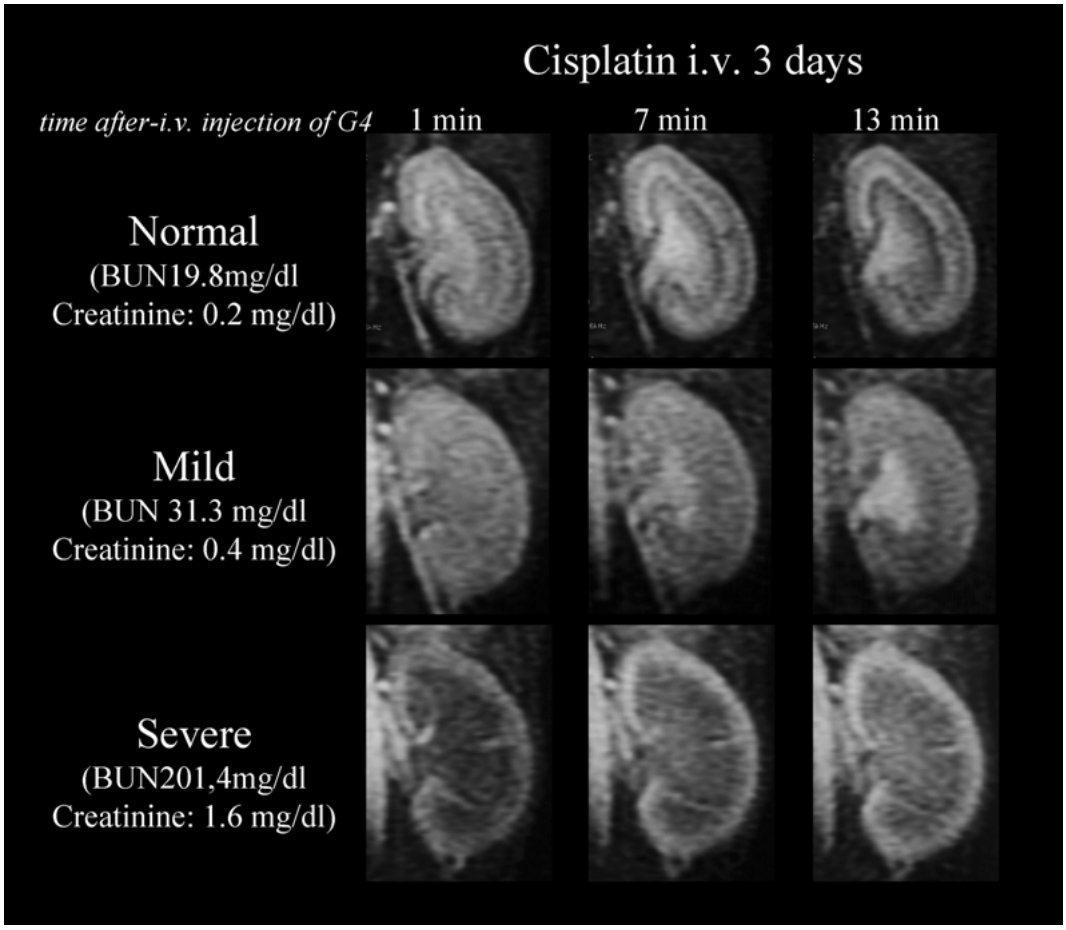

Fig. 3. Comparison of normal, mild, and severe cisplatin nephrotoxicities in an animal model. The normal kidney demonstrates the characteristic outer medullary stripe, which is better seen by 13 min. Even in mild nephrotoxicity, this stripe is no longer seen, although excretion into the renal pelvis can be perceived. In severe nephrotoxicity, only the cortex opacifies with contrast medium due to acute tubular necrosis.

ethylenediamine dendrimers and thus are retained less in the kidneys.

After experimenting with these particles it was found that the G-4 dendrimer, which is approximately 6 nm in diameter, was best suited for renal imaging [15, 16]. In the normal kidney the G-4 dendrimer accumulates in the outer stripe of the medulla, corresponding to the proximal straight tubules (Fig. 3). This is likely due to the reabsorption of the dendrimer at this site in the nephron.

In an animal model of tubular nephrotoxicity caused by cis-platinum, Kobayashi et al. [17] observed loss of the normal outer stripe in the medulla (Fig. 3). The loss of the normal renal architecture after G-4 dendrimer was proportional to the degree of renal damage (Figs. 3, 4). Excellent correlation between renal function as measured by serum urea nitrogen and MRI findings was observed ($r = 0.93$). When G-4 was compared with another macromolecule, gadomer 17, the findings were quite specific for the G-4 dendrimer and relatively unimpressive for gadomer 17 [16]. Moreover, other generations of dendrimers (G-2, G-3, etc.), although comparable to G-4, were less ideal because their pharmacokinetics were quite different.

Thus, precisely sized nanoparticles consisting of dendrimers could be used to monitor specific outer medullary damage caused by nephrotoxic events (e.g., sepsis, drugs, ischemia, infection, and obstruction; Figs. 4, 5). Severe damage to GFR can be seen as markedly delayed excretion of the G-4 dendrimer agent (Fig. 5). In this case the agent acts like a pure blood pool contrast agent and the kidneys are not visualized (Fig. 5).

Ultrasmall particles of iron oxide

Small particles of iron oxide (SPIO) were originally designed as liver contrast agents and were large (200 to 300 nm in diameter). These agents are in commercial use but have not found widespread acceptance because routine agents perform quite well in the liver. USPIO were developed to address the need for a macromolecular agent for MR angiography that was based on iron.

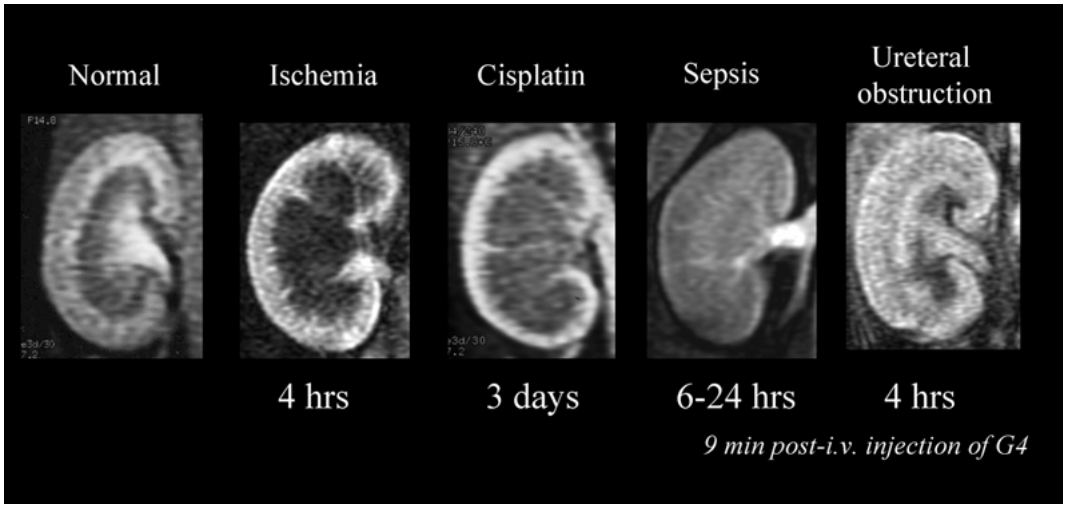

Fig. 4. Comparison of different causes of renal failure and their effects on the appearance of the kidney. **Left to right** An ischemic kidney and severe cisplatin toxicity demonstrate similar acute tubular necrotic features. Sepsis at 6 to 24 h demonstrates loss of the stripe but continued opacification of the renal medulla. There is also loss of the stripe in renal obstruction in addition to delayed excretion.

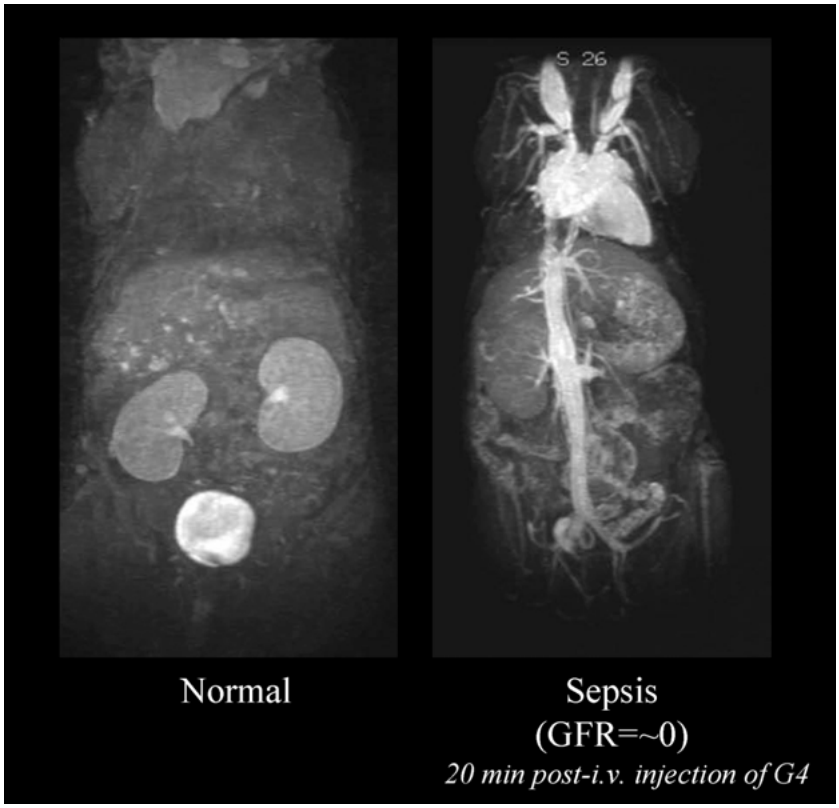

Fig. 5. Severe renal failure induced by sepsis. **Left** Normal excretory pattern. **Right** Pattern from an animal with severe sepsis and no urine output. The contrast agent (G-4 dendrimer) acts as a blood pool agent that opacifies the vessels but not the kidney.

USPIO are dextran coated in a formulation commercially known as Combidex and chemically known as ferumoxtran-10. At 20 to 30 nm they are considerably smaller but still too large to be filtered at the glomerulus. Thus, on first pass they could serve as blood pool agents.

Once injected intravenously, these agents circulate intravascularly and can be used to evaluate renal blood volume and renal blood flow [16]. Unlike SPIO that must be administered slowly, newer versions of USPIO such as ferumoxytol appear to be safe even when injected rapidly [18, 19]. Thus, dynamic intravascular blood flow measurements will be possible with these agents.

However, 24 h after injection almost all circulating USPIO are phagocytized by macrophages and mono-

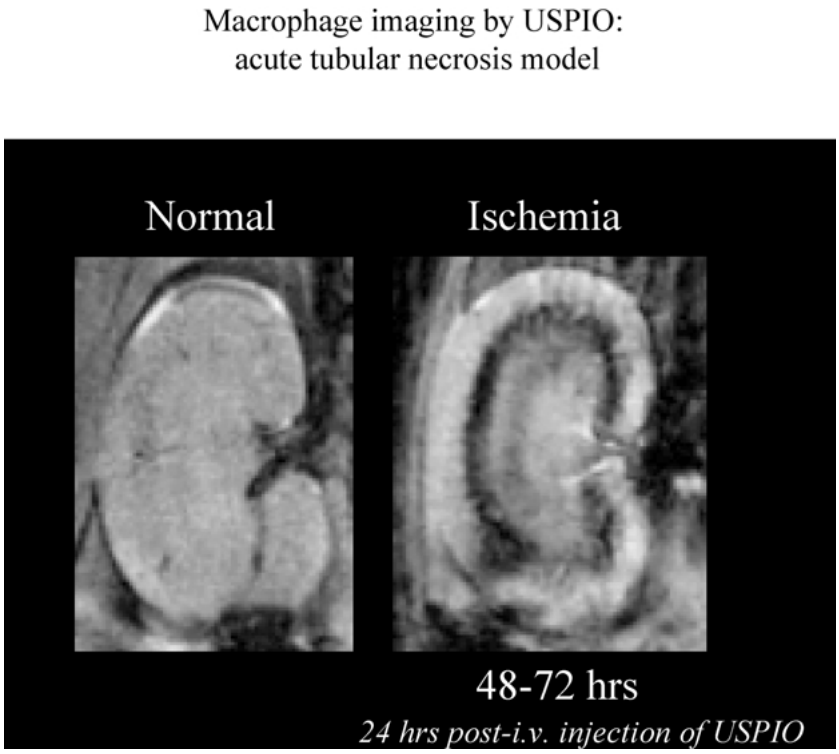

Fig. 6. USPIO renal imaging. **Right** In this model of reversible ischemia, macrophages laden with USPIO accumulate within the damaged portions of the outer medulla, indicating a significant inflammatory response to ischemia. **Left** The normal kidney does not accumulate USPIO.

cytes. These cells will then traffic to lymph nodes and areas of inflammation. Ye et al. [20] demonstrated that, in renal transplant models, allograft rejection could be identified by uptake of USPIO and that the degree of lymphocytic infiltration corresponds with the loss of signal in the renal parenchyma. Others have demonstrated that, in an acute ischemia-reperfusion model, USPIO-laden macrophages accumulate in the outer medulla, corresponding to an inflammatory infiltrate induced by the ischemia (Fig. 6) [21]. Uptake correlated with serum creatinine, implying a ''damage-response'' relation. The USPIO had no deleterious effects on renal function itself. Other groups have documented how focal inflammatory disease including glomerulonephritis can be demonstrated with USPIO agents. Thus, USPIO renal imaging could be used to assess the degree of renal inflammation associated with a renal parenchymal disease. It also serves as a means of monitoring inflammation independent of renal function studies.

Summary

Although macromolecular MR contrast agents are just beginning to emerge from the development phase and are just starting to enter clinical testing, they are demonstrating utility in various animal models of renal dysfunction. Rather than providing nonspecific global functional information as current clinical tests offer, these agents have the potential to provide specific clues to the cause of renal dysfunction. Moreover, because of their specificity, they could become methods of monitoring the response to therapy. It is not hard to imagine that these current ''passive'' macromolecular agents will one day be actively targeted to specific cell surface markers, thus providing even more specificity. This area of imaging will remain fertile for exploration for many years to come.

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