Detection and Localization of Proteinuria by Dynamic Contrast-Enhanced Magnetic Resonance Imaging Using MS325

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After renal transplantation, persistent glomerular disease affecting the native kidneys typically causes albuminuria, at least for a period of time, making it difficult to determine in a noninvasive fashion whether proteinuria originates in the native kidneys or the renal allograft. To address this problem, dynamic contrast-enhanced magnetic resonance imaging (MRI) using gadolinium (Gd)-based albumin-bound blood pool contrast agent (MS325) to localize proteinuria was investigated. Glomerular proteinuria was induced in Sprague-Dawley rats by intravenous injection of puromycin aminonucleoside (PAN), whereas control rats received physiologic saline vehicle. Both groups of animals underwent a 40-min dynamic contrast-enhanced MRI using radio frequency spoiled gradient echo imaging sequence after injection of Gd-labeled MS325. Contrast uptake and clearance curves for cortex and medulla were determined from acquired MR images. Compared with controls, proteinuric rats exhibited significantly lower elimination rate constants. The use of gadopentetate dimeglumine (Gd-DTPA) as a contrast agent showed smaller and less specific differences between proteinuric and control groups. In rats with one proteinuric kidney (PAN-treated) and one normal kidney (transplanted from a normal rat), MRI using MS325 was able to differentiate between the two kidneys. The results suggest that MRI with an albumin-bound blood pool contrast agent may be a useful noninvasive way to localize proteinuria. If this technique can be successfully applied in human patients, it may allow for the localization of proteinuria after kidney transplant and thereby provide a noninvasive way to detect disease affecting the renal allograft.


The glomerular filtration barrier, composed of the endothelial cells, glomerular basement membrane, and visceral epithelial cells (podocytes), retards the passage of macromolecules from the plasma into the urinary space (1). Size exclusion is a function of molecular radius and molecular charge. The passage of albumin, with a mass of approximately 67 kD and net anionic charge, is effectively retarded by the glomerular filtration barrier. Nevertheless, because of the large amount of albumin present in the plasma, 1 to 2 g of albumin are filtered by the human kidney each day. The proximal renal tubule reabsorbs nearly all this filtered albumin, so that daily normal human urine excretion is <30 mg. Proximal tubular epithelial cells take up albumin from the lumen, mediated by the apical surface receptors megalin and cubulin (2). When the glomerular filtration barrier is compromised because of glomerular disease, plasma albumin passes readily into the proximal tubule. Consequently, glomerular disease is associated with greatly increased quantities of urine albumin.

When ESRD develops, urine production typically continues for a period of months to years, and the urine often contains significant amounts of protein, including albumin. When patients undergo renal allograft transplantation, the urine at first represents the combination of urine production by the native and allograft kidneys. Thus, the presence of proteinuria in renal transplant patients in many cases is an unreliable marker for allograft dysfunction, although a large increase in proteinuria compared with the value obtained before transplantation strongly suggests allograft disease.

A particular clinical problem is recurrent FSGS. Idiopathic FSGS accounts for 2% of ESRD incident cases in the US and is the leading cause of incident ESRD cases among primary glomerular diseases (3). In approximately 30% of FSGS patients who undergo renal transplantation, FSGS recurs in the transplanted kidney (4). The mechanism is not well understood, but there is evidence that a circulating protein acts on the glomerulus as a permeability factor (5). Recurrent FSGS typically manifests within the first 6 mo after transplantation, and many patients lose allograft function because of progressive glomerular scarring. The recurrence of FSGS after renal transplant can

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be detected by an increase in proteinuria and is confirmed on renal biopsy. Therapy with plasma exchange may be effective if instituted early (6–8); therefore, regular screening is desirable to detect recurrent FSGS. Unfortunately, many FSGS patients who undergo renal transplantation have high levels of proteinuria originating from their native kidneys; therefore, urine protein measurements are unreliable in detecting the source of proteinuria. A noninvasive method to detect whether urinary protein, in particular albumin, originates in the native kidney or in the allograft kidney would be of considerable benefit in managing these patients.

MS325 was developed as gadolinium (Gd)-binding blood pool agent for magnetic resonance (MR) angiography. This agent binds strongly but reversibly to human serum albumin in vitro, with an association constant ($K_{A1}$) of 11 mmol (9). As many as 30 MS325 molecules can bind to one albumin molecule at high chelate concentrations. MS325 has a small volume of distribution because of the high protein binding and the low cellular uptake. In primates and rabbits, the elimination half-life is relatively long (2 to 3 h), whereas in rats it is shorter (25 min) (10). Compared with primates and rabbits, rats exhibit lower protein binding of MS325, particularly during the first 5 min after administration, and the agent has a two-fold higher volume of distribution in rats than in humans.

After intravenous administration, equilibrium is reached between free MS325, which is a low-molecular-weight compound, and bound MS325 bound to albumin. This equilibrium has three advantages from a clinical perspective (9). First, albumin binding serves to prolong plasma half-life in vivo by preventing renal excretion of the Gd–MS325 complex, which has a molecular weight of 957 Da and would otherwise be readily filtered at the glomerulus. Second, Gd complexes with high molecular weight or that exhibit plasma protein binding demonstrate higher T1 relaxivities, resulting in higher signal intensity compared with gadopentetate dimeglumine (Gd–DTPA) (11). Third, the reversibility of the interaction between Gd–MS325 and albumin assures the rapid clearance of Gd from the body, whereas the chelation of Gd ensures that this toxic compound is not taken up by tissues (12). MS325 has been used for angiography in rabbits (13), rats (14), and mice (15,16). Recently, clinical studies have demonstrated its usefulness and safety in human subjects (17,18).

In this study, we have evaluated dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) using MS325 for noninvasive detection of glomerular proteinuria in a rat model. We hypothesized that control rats would exhibit prompt excretion of filtered MS-325, because the only unbound MS325 can pass the glomerulus and enter the renal tubule. We hypothesized that MS325 would accumulate in proteinuric kidneys as a consequence of its binding to albumin and consequent uptake by tubular epithelial cells.

**Materials and Methods**

**Puromycin Aminonucleoside Animal Model**

We used a standard rat model of nephrosis induced by puromycin aminonucleoside (PAN) (19). PAN is toxic to the glomerular podocyte, a key component of the glomerular filtration barrier (19). In this model, a single injection of PAN reproducibly induces proteinuria, with a peak at 7 to 10 d after administration.

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200 to 300 g received a single tail vein injection of PAN (Sigma, St. Louis, MO) at a dose of 75 mg/kg, diluted in physiologic saline. Control rats received intravenous injection of an identical volume of physiologic saline. In preliminary studies, the peak of proteinuria was found to occur 7 d after PAN injection. Therefore, at day 7, urine was collected for 24 h using metabolic cages and urine protein was measured using pyrogallol red method performed on a Beckman-Coulter LX20 (Beckman, Fullerton, CA). A total of 30 rats were scanned by DCE-MRI. Two animals died because of technical complications before imaging was complete, and these animals were excluded from further analysis.

Rats were imaged 7 to 10 d after induction of proteinuria. Before imaging, rats were anesthetized by inhalation of 2 to 3% isoflurane and an intravenous catheter was placed in the femoral artery. Respiratory rate was measured using a pneumatic probe and body temperature was measured using a rectal temperature probe. Temperature and respiratory rate were monitored and recorded using a Biopac MP150 system (Biopac Systems, Santa Barbara, CA). The level of anesthesia was adjusted to maintain a respiratory rate of 60 to 80 breaths per minute. Body temperature was maintained near 37°C using a water blanket connected to a circulating warm water system. After imaging, animals were euthanized by injection of potassium chloride via the implanted femoral arterial catheter. Animal care was performed using protocols approved by the National Institute of Diabetes and Digestive and Kidney Diseases Animal Care and Use Committee and was in accordance with National Institutes of Health (NIH) guidelines for animal care.

**Rat Renal Transplant Model**

Renal transplantation was performed as described previously (20) using a modification of the technique of Fabre et al. (21). Kidneys from Lewis rats (Charles River, Cambridge, MA) were transplanted into Lewis recipients that had been treated with PAN 4 d previously. The donor kidney, ureter, and bladder were harvested en bloc, including the renal artery with a 3-mm aortic cuff and the renal vein with a 3-mm vena caval cuff. These vascular cuffs were Anastomosed to the recipient abdominal aorta and vena cava, respectively, using 10.0 nylon monofilament suture. Total ischemic time averaged 10 to 15 min. Donor and recipient bladders were attached dome-to-dome. The right native kidney was removed at the time of transplantation. The left native kidney was left in place. Comparison was between the right normal transplanted kidney and the left native proteinuric kidney. Surgical mortality in recipients was 25%. Surviving rats underwent MR imaging with Gd–albumin 3 d after surgery, which was 7 d after PAN administration.

**MR Renal Functional Imaging and Analysis**

MR images of rat kidneys were acquired on a wide-bore 4.7-T Bruker Biospec MR scanner (Bruker, Billerica, MA) at the NIH Mouse Imaging Facility. A number of gradient echo scout images were acquired first for placement of target slices over kidneys. During dynamic contrast-enhanced imaging, four two-dimensional image slices encompassing both kidneys were repeatedly acquired over 40 min with a spoiled gradient echo imaging sequence. Imaging parameters were: matrix size $256 \times 128$ pixels, field of view $8 \text{ cm}$, slice thickness $2 \text{ mm}$, echo time $2.5 \text{ ms}$, repetition time $75 \text{ ms}$, tip angle $45^\circ$, bandwidth $88 \text{ KHz}$, number of excitations $2$, and repetition number $120$. The total scan time was $40 \text{ min}$. Bolus injection of the contrast was made $1 \text{ min}$ after starting of DCE-MRI image acquisition, followed by the bolus injection of the saline flush.
MS325 (Epix Medical, Cambridge, MA) was administered to 20 rats at a dose of 0.05 mmol/kg, a dose similar to that used in clinical studies. Magnevist (Gd-DTPA; Berlex, Richmond, CA) was administered to an additional 10 rats at a dose of 0.05 mmol/kg. Both agents were injected via femoral artery catheter, followed immediately by a bolus of 1 ml physiologic saline.

Early MR contrast-enhanced images with good cortical medulla image contrast were processed using MEDx software (Sensor Systems, Sterling, VA) to select regions of interest (ROI). ROI over the cortex and medulla were defined with an interactive segmentation tool provided with the software. Time–signal curves for the entire series were generated from the defined ROIs.

For each time–signal curve generated, the peak signal following the rapid contrast uptake was identified. The signal curve subsequent to this time point depicts the contrast clearance through the kidney. These clearance curves were then fit to a single exponential component decay model $Y(t) = A \times \exp(-t/D)$, in which $Y$ is the signal amplitude in arbitrary units, $t$ is time in minutes, and $A$ is a scaling factor in arbitrary units. Decay constants $D$ were estimated from the fitting procedures.

**Statistical Analyses**

Data are presented as mean ± SEM. Statistical tests included Mann–Whitney test for nonparametric data and linear regression. Statistical significance was taken at $P < 0.05$.

**Results**

In the test group ($n = 9$), urine protein reached $487 \pm 164$ mg/d/7 d after PAN injection. In comparison, daily urine protein was $31 \pm 12$ mg/d in the control group ($n = 10$, $P < 0.01$) 7 d after injection of physiologic saline of the same volume. Thus, PAN produced the expected severe proteinuria, consistent with glomerular injury.

Contrast enhancement within cortex and medulla reached a peak approximately 1 to 2 min after administration of MS325. The peak contrast enhancement was similar between control rats and proteinuric rats in cortex ($18,065 \pm 1026$ versus $18,445 \pm 756$ expressed as arbitrary units) and medulla ($19,898 \pm 1023$ and $19,292 \pm 860$). These similarities suggest that peak uptake reflects primarily blood flow, which is likely similar between the two experimental groups. In normal rats, image intensity in cortex and medulla showed subsequent exponential decay caused by renal clearance of MS325 (cortical decay curve shown in Figure 1A). By contrast, nephrotic rats exhibited a flattened decay curve in cortex and medulla and a significantly prolonged excretion time, indicating reduced clearance through the kidney (cortical decay curve shown in Figure 1B). Among proteinuric rats, there was no correlation between proteinuria and MS325 decay constant ($R = 0.02$).

A sample time–signal curve fitted to a single exponential decay model of contrast clearance is illustrated in Figure 2A. For this analysis, we selected cortical and medullary ROI that showed homogenous enhancement and lacked blood vessels. After administration of Gd-DTPA, the decay constants were not significantly different between proteinuric rats and control rats in cortex ($290 \pm 63$ versus $172 \pm 17$) and medulla ($721 \pm 342$ versus $293 \pm 85$) (Figure 2B), although there was a trend toward larger decay constants in proteinuric animals. After administration of MS325, proteinuric rats had larger decay constants than did control rats in both the cortex ($451 \pm 169$ versus $120 \pm 12$; $P < 0.01$) and the medulla ($407 \pm 112$ versus $206 \pm 75$; $P < 0.001$) (Figure 2C).

Figure 3 shows a temporal series of an image slice in the first 3 min after the start of DCE-MRI. Images were acquired at the rate of three per minute. Contrast (MS325) was injected 1 min after start of imaging. Image intensity changes in the kidneys depicted the initial rapid contrast in the renal arteries before contrast filtration and clearance.

Figure 4 shows comparable image slices of the kidneys of control rats during the late vascular phase approximately 1 to 2 min after administration of MS325 (Figure 4A) or Gd-DTPA (Figure 4B). These images demonstrate vascular enhancement, with visualization of the descending aorta, renal arteries, and renal segmental arteries. In addition, at this late phase there is contrast enhancement of both cortex and medulla, with a lesser degree of enhancement of outer medulla; these images likely reflect the filtration of the contrast agent through the glomerulus and into the renal tubule.

In the renal transplant experiment, a PAN-treated rat that had received a normal kidney had $51$ mg/d proteinuria 7 d after PAN administration and 3 d after surgery. By contrast, a saline-treated rat that had received a normal kidney had $6$ mg/d proteinuria 7 d after saline administration and 3 d after surgery. As shown in Figure 5A, the PAN-treated rat with a
proteinuric kidney on the left and the normal (transplanted) kidney on the right had strikingly different MR images. As shown in Figure 5B, the decay curves are markedly flattened in both cortex and medulla for the proteinuric left kidney compared with the normal (transplanted) kidney on the right. The saline-treated rat with the normal kidney transplant exhibited comparable decay curves in cortex and medulla in both kidneys (data not shown).

Figure 2. Exponential fit of the contrast clearance curve and contrast clearance time constants with dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). (A) After administration of MS325 to a normal rat, the time–signal curve of cortical contrast enhancement was fitted to a single parameter exponential model. This model provides a numerical decay constant that is inversely proportional to the clearance rates. This procedure was followed for cortical and medullary ROI for all rats in the study. (B) With gadopentetate dimeglumine (Gd-DTPA) as the contrast agent, cortical and medullary signals were similar between the two groups. (C) Using the contrast agent MS325, increased signal was detected in both renal cortex and medulla of proteinuric rats compared with control rats.

Figure 3. Temporal series of an image slice in the first 3 min after start of DCE-MRI. Images were acquired at the rate of three per minute. MS325 was injected 1 min after the start of imaging. Image intensity changes in the kidneys indicated initial rapid contrast inflow through the renal arteries, accompanied by diffuse cortical enhancement, and followed by medullary enhancement which came to predominate in the later images.

Figure 4. Contrast-enhanced rat kidney images with MS325 and Gd-DTPA. Images were acquired near the peak of contrast enhancement, approximately 1 to 2 min after administration of MS325 (A) or Gd-DTPA (B). These images are comparable, although vascular images are superior with MS325, consistent with blood pool labeling.
Discussion

This study demonstrates that MR imaging of kidney using MS325, a chelate that binds reversibly to albumin, provides a distinctive signal in proteinuric rats compared with controls. Specifically, MR signal decay constants were higher in proteinuric rats, consistent with a markedly slower rate of MS325 renal clearance in proteinuric rats. We interpret this result to mean that the abnormal filtration of the albumin-bound contrast agent at the glomerulus leads to tubular accumulation, which is detectable as a delayed excretion.

MS325 binds reversibly to albumin, which makes it an excellent blood pool imaging agent. MS325 exists in equilibrium between albumin-bound (high-molecular-weight) and unbound (low-molecular-weight) fractions. Albumin binding is lower in rats compared with humans, which in rodents gives MS325 properties of both a macromolecular contrast agent and a low-molecular-weight contrast agent, such as Gd-DTPA. Thus, a component of the injected dose was filtered freely by the glomerulus in both proteinuric rats and control rats. The overall renal enhancement represents the combined signal from the MS325 within the intrarenal vascular pool, filtered albumin-bound MS325, and filtered unbound MS325. After administration of MS325, the decay constant of elimination was increased by three-fold in cortex and medulla in proteinuric animals compared with controls. In normal animals, MS325 is present in the blood pool and is filtered as unbound MS325; thus, clearance from the kidney is relatively rapid. By contrast, MS325 clearance from the kidney is reduced in proteinuric rats, presumably because MS32 bound to albumin has entered the renal tubule because of abnormal glomerular permeability. This albumin-bound MS325 remains within the kidney for a period of time, transiently located within the tubular lumen or taken up by the proximal tubular epithelial cells and present within the cell until degradation occurs. Thus, decreased renal clearance of MS325 can be used to identify and localize proteinuria. In human subjects, with increased MS325 binding to albumin compared with rats, the renal signal differences between normal and proteinuric states should be even more striking.

Gd-DTPA, a low-molecular-weight contrast agent, showed less pronounced differences in clearance between the control and proteinuric states. The decay constants for Gd-DTPA clearance in the cortex and medulla showed differences in proteinuric rats compared with control rats, but these differences did not reach statistical significance. Thus, Gd-DTPA was not as useful as MS325 in differentiating normal and proteinuric kidneys.

Our findings confirmed known characteristics of MS325 as a blood pool imaging agent. MS325 gave excellent enhancement of the rat renal vasculature, as expected for a blood pool agent. Our data are consistent with the kidney as the primary route of excretion for MS325, as is known from biodistribution studies. Finally, MS325 and Gd-DTPA achieved similar peak enhancement levels at equivalent doses.

There are limitations to this study. The images likely represent a composite of MS325–albumin located within the renal vascular blood pool, the tubular lumen, and the renal interstitium. However, we were unable to confirm the exact distribution of the agent in each of these compartments. Future studies will focus on distribution of MS325 within these compartments. Approaches under consideration include ultrahigh resolution, high-field-strength MRI microscopy on tissue slices, or the administration of radio-labeled (or biotinylated) MS325, with analysis of tissue slices by microscopy.

In conclusion, MR imaging with MS325 may be a useful noninvasive method to localize proteinuria. This approach may be clinically relevant in patients with FSGS who manifest proteinuria after transplantation who also still have native kidney function. In these patients, proteinuria may be an early sign of recurrent FSGS affecting the allograft, whereas in others proteinuria may simply reflect pre-existing disease in the native kidneys. In patients with this diagnostic dilemma, MS325 im-

Figure 5. Renal transplantation study using MS325. A rat received PAN and 4 d later underwent renal transplantation, with the right kidney removed and replaced by a kidney from a normal donor rat. (A) The normal (transplanted) kidney is on the right and the proteinuric (native) kidney is on the left. (B) The time constants show a marked slowing of contrast clearance through the left proteinuric kidney, both cortex and medulla, compared with the normal right kidney. Injections were made at 160 s.
aging may offer a rapid, sensitive, and noninvasive method of localizing the source of glomerular proteinuria. We plan studies to address the usefulness of this approach in human subjects.

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