Evaluation of Quantitative Magnetic Resonance Imaging as a Noninvasive Technique for Measuring Renal Scarring in a Rabbit Model of Antiglomerular Basement Membrane Disease\textsuperscript{1,2}

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ABSTRACT
Renal function tests are an insensitive means of detecting progressive scarring of the kidney such as occurs in chronic allograft rejection and lupus nephritis. Alternative approaches for the measurement of small progressive changes in renal structure on a repetitive basis are needed. Quantitative magnetic resonance imaging (proton T1 relaxation time) was assessed for this purpose. A rabbit model of antiglomerular basement membrane disease that develops glomerular and interstitial inflammation followed by scarring of the renal cortex was used. Longitudinal studies of cortical T1 showed a marked prolongation of T1 during the initial inflammation and edema associated with glomerular crescent formation (Day 7). The T1 remained prolonged up to Day 120 in animals with significant fibrosis and crescent formation when the wet/dry weight ratio (a measure of edema) had returned to baseline. T1 was a more sensitive index of renal injury than was serum creatinine for all of the end points measured (cortical hydroxyproline per dry weight, percent crescent formation, or histologic fibrosis score). It was concluded from these studies that measurement of renal cortical T1 is quite a sensitive index of renal injury, probably more sensitive than the measurement of serum creatinine, but that this method does not discriminate between edema and scarring.

Key Words: Magnetic resonance imaging, renal imaging, renal scarring, renal function

Renal function measurements are routinely used to assess the well-being of the kidney. Functional measurement as defined by clearance of convenient molecules, such as creatinine from blood by glomerular filtration, are used for this purpose. Problems with such measurements include: (1) routinely used methods for creatinine measurement are subject to interference and inaccuracy, (2) they overestimate glomerular filtration because of secretion of creatinine by the renal tubule \textsuperscript{(1,2)}, (3) the ratio of creatinine clearance to GFR varies with the severity of renal failure \textsuperscript{(3-5)}, and (4) commonly used drugs interfere with tubular creatinine secretion. Formal measurements of GFR with other markers are cumbersome and often inaccurate when used in the clinical setting because of incomplete urine collections. Furthermore, even “accurate” measurements of GFR are not a sensitive measurement of the extent of structural damage to the kidney because of compensatory changes such as increase of single-nephron GFR and RBF that maintain GFR within the normal range in spite of loss of 25 to 30% of nephrons (renal reserve) \textsuperscript{(6)}. Therefore, in an individual patient, it is often difficult to determine whether progressive loss of renal function with replacement by scar tissue is occurring. Once serum creatinine is obviously abnormal, extensive scarring may be present with permanent loss of renal structure and function. This problem is encountered in all forms of renal disease. Thus, for clinical practice, we need a method that could be used as an adjunct to functional measurements that would measure the extent of renal cortical scarring. If such a measurement were available, then scarring could be detected early and progressive scar formation could be measured, so that therapeutic steps could be taken and their outcome could be assessed before large numbers of nephrons are permanently lost. With this alternative approach in mind, we have evaluated structural approaches to measuring scarring in the kidney using a rabbit model of antiglomerular basement membrane disease.
model for which we have extensive knowledge of the time course of events. In this report, we describe results using a quantitative magnetic resonance imaging (MRI) approach where we set out to evaluate the usefulness of the T1 relaxation time for proton nuclear magnetic resonance for the purpose of measuring renal scar formation.

In evaluating this information, the following concepts are important. The proton T1 relaxation time reflects the structure of water in the tissue. Free water has a T1 in the order of seconds. In normal tissues, water molecules are mostly bound to macromolecules, so that the time taken for the average water molecule to relax (recover) back to its equilibrium state of magnetization after a perturbation is relatively short; this corresponds to a short T1 time. If for any reason unbound water accumulates in the tissue, then the relaxation time will become longer. Most water in the normal renal cortex will be intracellular and bound to macromolecules. In contrast, during renal injury, water is present that is not bound to macromolecules. This water may be extracellular, such as in cellular swelling due to ATN, or it may be extracellular, such as accumulates as interstitial edema in the nephrotic syndrome. In both cases, the T1 will be expected to be prolonged. In this report, we suggest that there may be a third situation where the T1 is prolonged in the renal cortex. We suggest that interstitial collagen fibers may hold tubules apart to create a sink of unstructured water between tubules (a collagenous gel), thereby accounting for the prolonged T1 seen in scarred kidneys.

METHODS

Model of Antiglomerular Basement Membrane Disease

The method used to produce crescentic nephritis and renal scarring in rabbits has been previously described in detail (see reference 12). Briefly, New Zealand White rabbits, weighing 2.0 to 3.0 kg, were injected sc with 100 ug of guinea pig immunoglobulin G in complete Freund's adjuvant (Sigma Chemical Co., St. Louis, MO); 5 days later, they were given an iv injection of 25 mg of guinea pig anti-rabbit glomerular basement membrane (anti-GBM) immunoglobulin G. Control animals received no injections. Rabbits were kept in individual cages and were given water and regular rabbit chow ad libitum.

At the indicated times, renal tissue samples were obtained for analysis from these animals. Rabbits were sedated with 50 to 100 mg/kg iv of sodium pentobarbital. The right kidney was harvested, the capsule was removed, and the cortex was trimmed off with scissors. Segments of kidney for light microscopy were placed in 10% formalin.

MRI

MRI evaluation was performed on a clinical 0.5-T whole-body scanner (HP System; Picker Corp. Highland Heights, OH) with the standard head coil. The animals were sedated with im injections of xylazine (20 mg) and ketamine (100 mg).

Imaging commenced with a rapid multiplanar localizing sequence, which was followed by a single, transverse-plane, custom-programmed pulse sequence based on the method of Look and Locker (imaging version of the Look and Locker technique [ILL]) (7). This yielded a set of nine raw images obtained concurrently of the same anatomic section, each displaying a different degree of longitudinal magnetization. The magnetic resonance pulse parameters were TR = 1,000 ms; TE = 13 ms; t0 (time between read pulses) = 104.4 ms; flip angle = 20 degrees; and number of signal averages = 4. The spatial parameters were slice thickness = 7.5 mm; field of view = 20 cm, and matrix size = 256 x 256 or 256 x 196, resulting in imaging times and in-plane pixel sizes of 13 min and 0.78 x 0.78 mm or 17 min and 0.78 x 1.04 mm, respectively.

For the ILL data set, multiple (four to eight) regions of interest were manually drawn in the renal cortex for each T1 measurement. T1 values were calculated from the ILL images with a 9-point fit of the appropriate function to the signal intensity values. Because T1 determination by this technique is sensitive to tip-angle error (7,8), especially for long T1 values, a gel reference standard was placed alongside the rabbit for correcting tip-angle error. (For a tip angle of 20 degrees, this error was found to be less than 3 degrees over 80% of the volume covered by the imaging coil.)

The image intensities used to evaluate T1 under in vivo conditions can be compromised by the following factors: (1) the partial volume and tissue heterogeneity; (2) the body motion of the subject during data acquisition; (3) the tissue absorption or dielectric loss of radiofrequency field in the animals.

Factor (1) is mainly caused by the conflicting requirements of acquiring images with high spatial resolution and high sensitivity. Because, for low-field MRI (e.g., in this case at 0.5 T), sensitivity is normally a high priority, one is forced to compromise on spatial resolution. This presents difficulty for tissues such as the renal cortex in rabbits, which has a small and relatively ill-defined area with good tissue uniformity. Tissue uniformity can be gauged by the ratio of mean to standard deviation of the image intensity in a given region of interest. There are two ways to minimize this source of error. One is to improve sensitivity (and thereby spatial resolution) by using a more sensitive receiver coil or to perform the experiments in a higher-field system. The other way is to upgrade the machine software (which is currently
Histologic Analyses

Formalin-fixed tissue was sectioned and stained by the Masson Trichrome method. The degree of cortical fibrosis was assessed on Masson’s Trichrome-stained sections and graded 0 to 3+ (none, mild, moderate, severe) on the basis of the total amount of blue-stained matrix tissue in the renal cortex (Fibrosis Score). A minimum of 10 × 40 fields were examined per animal (9). The degree of glomerular involvement was also assessed by counting the proportion of glomeruli that contained crescents. For each rabbit section, 100 glomeruli were examined. These measurements were done by a blinded observer.

Collagen Content

Collagen content was measured as total hydroxyproline (OH-Pro) per dry weight by the method of Huszar et al. (10) as modified by Phan et al. (11). Briefly, aliquots of glomeruli or cortex were suspended in 6 N HCl and hydrolyzed overnight at 110°C in tightly capped tubes. The samples were then dried in a Speed Vac concentrator (Savin Inst. Co., Farmingdale, NY) and resuspended in citrate-acetate buffer (pH 6.0). The sample was oxidized by chloramine T (Kodak Co., Rochester, NY) and mixed with p-dimethylaminobenzaldehyde, and absorbance at 550 nm was measured. The OH-Pro content was calculated from a standard curve of purified OH-L-Pro (Sigma Chemical Co., St. Louis, MO). Results were expressed as OH-Pro per milligram of dry weight (for cortex).

Serum Creatinine

Serum creatinine was determined by use of the Jaffe method.

Statistical Evaluation

All data are shown as the mean ± 1 SD. The statistical methods that deal with the accuracy of the T1 measurements have been described in detail elsewhere (7,8).

RESULTS

All T1 measurements were calculated by averaging the results from four to eight oval-shaped regions of interest measured at different sites in the renal cortex. The average of the standard deviations (expressed as a percentage of the mean T1 for each kidney) of 12 normal kidneys was 7.6%. This provides an estimate of the T1 measurement error.

To determine the importance of the particular site at which the T1 measurement was made, we evaluated the T1 across a cross-section of the kidney as shown in Figure 1. The T1 measured in the renal cortex ranged from 404 ± 38 ms in the superficial renal cortex to 496 ± 36 ms in the deep renal cortex. In contrast, the T1 in renal medulla ranged from 820 ± 156 ms in the outer medulla to 942 ± 165 ms in the deep medulla. The T1 in the perirenal fat was low (205 ± 26 ms). We therefore conclude that it is important for the T1 measurements to be made entirely within the renal cortex. If fat or medullary tissue were inadvertently included, we would expect artifactually short or long T1 values to be obtained, respectively.

As an estimate of the range of T1 values expected in normal kidneys, the T1 values in 14 normal kidneys were measured. The average T1 in these 14 kidneys was 499 ± 52 ms. We note that some of the variation may have been a result of systematic shifts in machine performance; for example, 4 of the 14 measurements made during a 1-wk interval were 480 ± 16 ms; for another series of measurements performed during a week-long period several months later, the mean and standard deviation were 561 ± 21 ms. These temporal shifts are substantially smaller than the T1 changes that occurred consequent to injury.

To evaluate the effect of fibrosis on the cortical and scarred kidneys, we measured the T1 across the kidney on two animals with anti-GBM disease induced 3 months previously that were shown histologically to have moderate renal fibrosis. These data are shown in Figure 1 for the individual animals. The T1 in perirenal fat was the same for scarred as for normal rabbits. The T1 for scarred kidneys was higher for both cortex and medulla than for control kidneys. This was especially easily seen for renal cortex.

To assess the changes in T1 at different times of evolution of the scar, a time-course study in two rabbits with anti-GBM disease was performed over
Figure 1. Panel A shows an image of the left kidney in a rabbit with normal renal function. The arrows point to regions where T1 measurements were made to provide the data shown in Panel B. Site A is the perirenal fat (PRF). Site B is the superficial cortex (SC). Site C is the deep cortex (DC). Site D is the outer medulla (OM). Site E is the inner medulla (IM). The values given in Panel B are the mean ± 1 SD for three animals (open squares). The values in the black squares and triangles are for individual animals with scarred kidneys at 120 days after the initiation of anti-GBM disease. The rabbits developed different degrees of severity of injury, as is indicated by the histology shown in Figure 3. Rabbit A had more interstitial scarring and glomerular crescents than did Rabbit B, although the serum creatinine for both animals at the end of the 28-day period was within the normal range (1.2 mg/100 mL for Rabbit A and 0.8 mg/100 mL for Rabbit B). The T1 measurement for Rabbit A at Day 28 was 723 ms, well outside the normal T1 range. Figure 2 also emphasizes that the T1 values peaked at Day 7.

This corresponds with the acute inflammation and associated edema previously documented to be present at Day 7 in this model (9). These data suggested that the T1 measurement in renal cortex is a sensitive index of renal injury, be it inflammatory injury with edema and/or scarring.

A second larger experiment was performed to determine how T1 measurements would compare with measurements of serum creatinine and to make more accurate measurements of renal scarring that could be related to the T1 and serum creatinine values obtained. This study used 13 animals with experimental anti-GBM disease that were euthanized at Day 30, when renal inflammation had subsided. Three different methods of measuring renal scarring were used. These included histologic scoring for percent fibrous crescents, interstitial fibrosis as assessed by Masson Trichrome staining of histologic sections, and a biochemical measurement of cortical collagen content as determined by hydroxyproline content per dry weight. The correlation coefficients for these three measurements of renal scarring were as follows: percent crescents with histologic fibrosis score \( r = 0.89 \); percent crescents with cortical hydroxyproline per dry weight \( r = 0.86 \); and histologic fibrosis score with cortical hydroxyproline per dry weight \( r = 0.86 \). Therefore, these three independently measured parameters of renal scarring correlated well with each other.

We next compared the two measured parameters of renal injury (serum creatinine or T1) with each of the parameters of renal scarring. The data are shown in Figure 4. There was a significant correlation of both T1 and serum creatinine with each measurement of renal scarring. In a side-by-side comparison of T1 and serum creatinine, 6 of 12 animals with

Figure 2. Time course of T1 measurement in two rabbits with anti-GBM disease. Histologic changes in the kidney at Day 28 are shown in Figure 3. Rabbit A had a marked rise in T1 at Day 7 and persistently elevated T1 through Day 28. In contrast, Rabbit B had a small increase at Day 7 and a return to the upper limit of normal at Day 28. The normal range for T1 is 499 ± 52 ms.
variable amounts of scarring had serum creatinine measurements within the normal range (0.5 to 1.25 mg/100 mL representing mean ± 2 SD). In contrast, 2 of 12 animals had a T1 in the normal range (mean ± 2 SD). Thus, in this model, T1 was a somewhat more sensitive index of renal injury than serum creatinine, particularly at low levels of scar formation.

To further address the issue of persistent T1 elevations, long-term (120-day) studies were performed in three animals. Because renal cortical edema could contribute to the long T1 values that we observed at 30 days after injection of anti-GBM antibody, we measured renal cortical wet/dry weight ratios for six normal animals to compare with the long-term group. The results are shown in Table 1. Animal A had a relatively normal kidney histologically and biochemically, a normal wet/dry weight ratio, and a normal T1. Animal B had a large, swollen, and scarred kidney with 50% crescents, edema, a high wet/dry weight ratio, and increased hydroxyproline per dry weight. As expected, this animal had a prolonged T1. Animal C had moderate scarring and, increased hydroxyproline per dry weight but was without swelling evident on gross examination and had a normal wet/dry weight ratio. This animal also had a prolonged T1. These results suggest that the prolonged T1 persisted at 120 days after injury and was not simply a reflection of increased edema, at least as detectable by measuring the wet/dry weight ratio of renal cortex.

DISCUSSION

For this analysis, we used a model of anti-GBM disease in the rabbit that progresses to renal scarring. Prior studies have determined in detail the time course of scarring, the factors driving the scarring process, and the changes in renal function in this model (9,12–18). Measurement of scarring can be performed by both biochemical and histologic criteria, and as confirmed in this report, these measurements correlate well in the model. Under the conditions used, the model was quite variable with respect to the amount of scarring that occurred. Thus, in some animals, scarring was severe, and in some animals, it was quite mild. This is similar to the variation that would be found in humans with lupus nephritis or transplant rejection. This model is therefore a satisfactory system in which to evaluate noninvasive methods of measuring renal scarring.

Much controversy exists in the MRT of fibrosis in different tissues. In a 1-yr time-course study of MRI of irradiated rat lungs, it was concluded that radiation fibrosis shortened T1 in association with increased lung collagen content, although there was no significant correlation between relaxation time changes and water content (19). During the development of liver fibrosis in rats treated with CCl4 and studied over an 8-wk period, it was concluded that the prolonged relaxation time values observed in this model correlated with tissue water content and not with collagen accumulation (20). When T2-weighted images were used to evaluate lymphomas in patients, one group of investigators was surprised to find in lymphomas with histologic evidence of dense fibrosis a high signal intensity (prolonged T2) that would be more compatible with fluid-containing structures (21,22).

Previous studies have evaluated the possibility that MRI could be used as a specific tool for the diagnosis of acute and chronic forms of renal diseases and transplant rejection. Most studies have not been quantitative, but rather have assessed changes in the image between normal and pathologic kidneys (23–29). They did not discriminate between fibrosis and edema, although the MRI findings have been related to biopsy findings in some cases. Most reports...
TABLE I. Outcomes at 120 days after induction of anti-GBM disease

<table>
<thead>
<tr>
<th>Animal</th>
<th>Gross Appearance</th>
<th>Wet/Dry Wt</th>
<th>Hydroxyproline (mg/mg dry Wt.)</th>
<th>% Crescents</th>
<th>Fibrosis Score</th>
<th>T1 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Range</td>
<td>Normal</td>
<td>4.38 ± 0.24</td>
<td>1.72 ± 0.36</td>
<td>0</td>
<td>0</td>
<td>4.99 ± 5.52</td>
</tr>
<tr>
<td>A Normal</td>
<td>4.49</td>
<td>2.65</td>
<td>0</td>
<td>0.5</td>
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<td></td>
</tr>
<tr>
<td>B Swollen</td>
<td>5.75</td>
<td>4.63</td>
<td>60</td>
<td>2</td>
<td>724</td>
<td></td>
</tr>
<tr>
<td>C Normal</td>
<td>4.47</td>
<td>6.31</td>
<td>36</td>
<td>2</td>
<td>627</td>
<td></td>
</tr>
</tbody>
</table>

have focused on loss of the cortical medullary differentiation on the MRI under various circumstances. From our data, we believe that this phenomenon is most likely secondary to a prolongation of renal cortical T1 relaxation time in injury that tends to equalize with the longer renal medullary T1 time. Thus, the difference between cortex and medulla is lost. This phenomenon has been shown to be nonspecific in acute renal injury (e.g., ATN and renal allograft rejection) and can also occur with chronic renal diseases (23,24) and chronic renal allograft rejection (23,25-29). Thus, these initial reports indicate that MRI is not a useful clinical diagnostic tool for discriminating between different types of renal injury. A prior report has also shown that the state of hydration affects the measured T1 of the renal medulla (30).

The T1 of normal human renal cortex has been reported to be 561 ± 57 ms. This is similar to the value we measured in normal rabbit renal cortex (503 ± 57 ms). During acute injury in the rabbit model (Day 7), when edema of the kidneys was present, there was a marked prolongation of the T1 to 900 ms. This result would agree with previous reports that found loss of the corticomedullary junction (which we interpret as due to an equalization of cortical and medullary T1 relaxation times) in a wide range of forms of acute renal injury. However, we also found that the prolongation of the T1 was sustained for up to 120 days in animals with renal scarring. This occurred in spite of the fact that there was no obvious increase in renal water content at the later time. Thus, it seems possible that the prolonged T1 at this time was due to a relative increase in free water in the scarred kidney. We hypothesize that this increase in T1 reflects water trapped between collagen fibers in the interstitial space, which is normally relatively dry in renal cortex. We cannot exclude the possibility that there was mild cell swelling or interstitial edema at this time that accounted for the prolonged T1.

The results from this study support prior conclusions that quantitative proton T1 measurements do not discriminate between scarring and edema. It is possible that other types of nuclear magnetic resonance measurement might allow us to make this discrimination. If so, this would be useful clinically. However, in spite of the lack of specificity in the T1 measurements, they did provide an index of renal injury that seemed to be more sensitive than was serum creatinine. This raises the question of whether making structural measurements of the renal cortex on a repetitive basis might be superior to functional measurements for detecting small changes in renal structure. Further studies are required to evaluate whether quantitative structural approaches can provide useful clinical information.

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REFERENCES

Figure 4. Graphs indicating the relationship between the parameters of renal injury measured indirectly (serum (S.) creatinine on the left set of panels and T1 on the right set of panels) and the actual extent of renal injury as indicated by the percent crescents in the histologic section (upper panels), the fibrosis score on Masson Trichrome-stained sections (middle panels), and the measurement of hydroxyproline content per dry weight of renal cortex (lower panels). The normal ranges (mean ± 2 SD) are shown in boxes. Note that the serum creatinine remains within the normal range in spite of significant injury as measured by all parameters. In contrast, T1 is elevated above the normal range in animals with significant renal injury.


16. Goyal M, Wiggins R: Fibronectin mRNA and protein accumulation, distribution, and breakdown in rabbit anti-glomerular basement mem-


