The significance of BOLD MRI in differentiation between renal transplant rejection and acute tubular necrosis

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Abstract

Background. Blood oxygen level-dependent MRI (BOLD MRI) can be used to assess intra-renal oxygen bioavailability by measuring the R2* level, which reflects tissue deoxyhaemoglobin levels. This study was designed to identify the significance of BOLD MRI in differentiation of acute rejection (AR) and acute tubular necrosis (ATN) in patients within 6 months after kidney transplantation.

Methods. Eighty-two patients with normal graft function and 28 patients with biopsy-proven AR (n = 21) or ATN (n = 7) were enrolled. Patients with normal functioning allograft underwent BOLD MRI within 2 to 3 weeks post-transplantation, while patients with AR and ATN underwent BOLD MRI within 6 days before or after kidney transplant biopsy. Cortical R2* (CR2*) and medullary R2* (MR2*) levels were measured.

Results. The mean CR2* level was significantly higher in the ATN group (15.25 ± 1.03/s) compared to the normal group (13.55 ± 2.31/s, P = 0.028) and AR group (12.02 ± 1.72/s, P = 0.001). There was a significant difference also between the AR group and normal group on CR2* levels (P = 0.013). The mean MR2* level was significantly lower in the AR group (14.02 ± 2.68/s) compared to the normal group (16.66 ± 2.82/s, P < 0.001) and ATN group (19.47 ± 1.62/s, P < 0.001). There was also a significant difference between the ATN group and normal group on MR2* levels (P = 0.011).

There were no correlations between characteristics such as patient age, post-operation time, post-biopsy time, Scr level, HB level, urine output volume, MAP level, CNI trough concentration and R2* levels, except between MAP level and CR2* level (P = 0.029).

Conclusions. BOLD MRI could be a valuable method to discriminate between AR and ATN by measuring tissue oxygen bioavailability in early kidney allograft dysfunction.

Keywords: acute rejection; acute tubular necrosis; kidney transplantation; magnetic resonance imaging (MRI); oxygen bioavailability

Introduction

Allograft rejection and acute tubular necrosis (ATN) are two important causes of early kidney allograft dysfunction, and it is difficult to discriminate between them by regular clinical tests. Percutaneous transplant biopsy is the most effective method, but it has risks such as bleeding, kidney rupture, and rarely, graft loss [1,2]. Developing a non-invasive method may be promising.

Blood oxygen level-dependent magnetic resonance imaging (BOLD MRI) is a non-invasive method to assess intra-renal oxygen bioavailability [3]. The technique is based on the paramagnetic properties of deoxyhaemoglobin that generates magnetic moments by its unpaired electrons in a magnetic field. Changes in blood concentration of deoxyhaemoglobin result in increased magnetic spin dephasing of blood water protons and decreased signal intensity on T2*-weighted MR imaging sequences. So the apparent relaxation rate denoted as R2* (1/T2*) is directly proportional to tissue deoxyhaemoglobin levels [4]. The increased R2* level implies an increased deoxyhaemoglobin level and decreased oxygen bioavailability in the tissue.

BOLD MRI has been used to investigate intra-renal oxygen bioavailability in the patients and experimental models of kidney diseases, such as water diuresis [5–7], diabetes [6], acute ischaemic renal dysfunction [8,9] and unilateral ureteral obstruction [10]. These studies provided evidence that BOLD MRI could effectively detect changes of intra-renal oxygen bioavailability in these diseases. Recently, Djamali and Sadowski et al. analysed BOLD MRI data in 23 renal transplant recipients, and found that BOLD MRI had demonstrated significant changes in medullary oxygen availability in allografts with biopsy-proven ATN and acute rejection (AR) [11,12]. In this report, we performed BOLD MRI in 110 kidney transplant recipients. With our analysis,
we plan to further investigate the utility of BOLD MRI and the diagnosis of AR or ATN.

**Subjects and methods**

**Study design**

The ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University approved the protocols, and written informed consents were obtained from all patients. The study was performed between December 2005 and March 2007. All of 110 patients received a BOLD MRI within 6 months after the first deceased donor kidney transplantation. Eighty-two patients with normal functioning grafts (normal group) underwent BOLD MRI within 2 to 3 weeks post-transplantation, while 28 patients with biopsy-proven AR (n = 21) and ATN (n = 7) underwent BOLD MRI within 6 days before or after kidney transplant biopsy. The AR group was subdivided into two groups: acute T-cell-mediated rejection (TMR, n = 13) and acute antibody-mediated rejection (AMR, n = 8) according to Banff’05 criteria [13]. The AMR criteria were defined as positive peritubular capillary (PTC) C4d staining by immunohistochemistry concomitant with polymorphonuclear leukocyte infiltration in PTC and vasculitis or glomerulitis. ATN was diagnosed pathologically as diffuse or multifocal renal tubular epithelium vacuolar degeneration or necrosis, and these patients recovered without intensive immunosuppressive therapies such as intravenous methylprednisolone impulses, antithymocyte antibodies and plasmapheresis.

Within these patients, four patients with AR and four patients with ATN who consented to undergo repeated BOLD MR imaging were enrolled for serial BOLD MRI post-transplantation; the specific time of imaging is displayed in Figure 3.

All patients received triple therapy based on a combination of calcineurin inhibitors (CNIs), mycophenolate mofetil (MMF) and prednisone for maintaining immunosuppression. All patients were refrained from water or intravenous transusion 4 h before BOLD MRI, had no diuretic agents 12 h before BOLD MRI and there was no oxygen inhalation during the BOLD MR imaging. Patients with hypertension usually received calcium-channel blockers (CCBs), β receptor blockers or clonidine; no angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) were used.

**MRI technique**

MR imaging was performed with the 1.5 Tesla system (GE EXITE II, Milwaukee, USA), using a multiple gradient echo sequence to acquire 16 T2* weighted images in coronal planes during breath holds of 15 s. The parameters were as follows: repetition time 100 ms, range of echo time 2.5–77.5 ms (began at 2.5 ms and 5 ms increment each), flip angle 35°, bandwidth ±31.25 kHz, matrix 192 × 128, number of signals acquired 1, field of view 36 cm, thickness 10.0 mm, space 1 mm.

A colour R2* map of the kidney allograft was generated using Functools on the MR working station. On the map, red represents the highest R2* levels, indicating the highest concentration of deoxyhaemoglobin, while blue represents the lowest R2* levels, indicating the lowest concentration of deoxyhaemoglobin. R2* levels were measured using the regions of interest (ROIs) tool. Six ROIs were placed in the cortical region and another six were placed in the medullary region, using regular T2 weighted images as anatomical reference to determine the cortical or medullary region. R2* measurements of all patients were completed by one radiologist who had 15 years of working experience in radiology unaware of the clinical or pathological data.

**Data analysis**

Numerical results were expressed as mean ± standard deviation, and were compared between groups using one-way analysis of variance (ANOVA) with post hoc test to perform pairwise multiple comparisons. Spearman correlation and multinomial linear regression were applied to determine the influence factors of the R2* value. These analyses were performed by SPSS 13.0 software (Spss Inc., USA). Receiver operating characteristic (ROC) curves were performed to determine the sensitivity and specificity of R2* levels for the diagnosis of AR or ATN by MedCalc Statistical Software (MedCalc Software, Belgium).

**Results**

**Clinical data analysis**

Baseline clinical characteristics are displayed in Table 1. Patients with acute rejection were significantly younger compared to patients with normal functioning allograft. The mean time from operation to imaging was longer in the AR group compared to the ATN group and normal group. Patients with ATN had the highest serum creatinine (Scr) level, the lowest urine outputs, haemoglobin (HB) level and blood tacrolimus (FK506) trough concentration compared to the other groups. There was no difference in the time from biopsy to imaging between the AR group and ATN group. As for subgroups, there were no significant differences in age, time from biopsy to imaging, urine outputs and blood FK506 trough concentration between the AMR subgroup and TMR subgroup. But the onset of AMR appeared to be sooner after the operation compared to TMR. Patients with AMR had a significantly higher Scr level and a mean arterial pressure (MAP) level but a lower HB level compared to patients with TMR.

**BOLD MRI data analysis**

The coronal colour R2* maps of kidney allografts are presented in Figure 1. Blue represents the lowest R2* level, indicating the lowest tissue deoxyhaemoglobin concentration, and green and orange distinctly represent increasing R2* levels, showing the increase of tissue deoxyhaemoglobin concentration. In a normal functioning allograft, the cortex is blue with areas of green and the medulla is green and orange reflecting the decrease in tissue oxygen bioavailability from outer medulla to inner medulla. In AR allograft, the
Table 1. The clinical characteristics of the patients receiving BOLD MRI

<table>
<thead>
<tr>
<th>Normal function allograft</th>
<th>AR</th>
<th>Total</th>
<th>TMR</th>
<th>AMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>82</td>
<td>21</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Male-female</td>
<td>58:24</td>
<td>16:5</td>
<td>12:1</td>
<td>4:4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.3 ± 10.9</td>
<td>43.4 ± 9.4</td>
<td>36.6 ± 9.7</td>
<td>18:3</td>
</tr>
<tr>
<td>Time from operation to imaging (days)</td>
<td>17.79 ± 5.17</td>
<td>9.86 ± 5.55</td>
<td>1.29 ± 3.78</td>
<td>1.82 ± 1.03**</td>
</tr>
<tr>
<td>Time from biopsy to imaging (days)</td>
<td>2.59 ± 0.68</td>
<td>1.44 ± 0.85**</td>
<td>2.09 ± 0.79**</td>
<td>1.41 ± 1.26**</td>
</tr>
<tr>
<td>Urine outputs (L/24 h)</td>
<td>98.06 ± 18.27</td>
<td>417.14 ± 64.97**</td>
<td>92.90 ± 19.56</td>
<td>101.15 ± 19.49**</td>
</tr>
<tr>
<td>Scr (μmol/L)</td>
<td>94.55 ± 14.71</td>
<td>75.29 ± 12.46*</td>
<td>104.45 ± 14.17</td>
<td>8.13</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>100.48 ± 12.95</td>
<td>110.14 ± 14.83</td>
<td>104.45 ± 14.17</td>
<td>8.13</td>
</tr>
<tr>
<td>CsA:FK (cases)</td>
<td>56.26</td>
<td>1.6</td>
<td>1.82 ± 0.79**</td>
<td>1.41 ± 1.26**</td>
</tr>
<tr>
<td>CsA trough concentration (ng/mL)</td>
<td>326.51 ± 88.00</td>
<td>270.22 ± 72.99</td>
<td>278.70 ± 74.45</td>
<td>210.80</td>
</tr>
<tr>
<td>FK506 trough concentration (ng/mL)</td>
<td>9.40 ± 2.84</td>
<td>4.38 ± 1.81*</td>
<td>7.15 ± 2.87*</td>
<td>7.82 ± 1.61*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to the normal group; **P < 0.01 compared to the normal group.
***P < 0.001 compared to the ATN group.
#P < 0.05 compared to the AMR group.
##P < 0.01 compared to the AMR group.
###P < 0.001 compared to the AMR group.

CGN: chronic glomerulonephritis.

Fig. 1. The colour BOLD MRI R2* maps of coronal transplant sections and corresponding pathological photos. (A) The colour R2* map of normal functioning transplant: blue represents the lowest R2* level, and green, orange, and red represent increasing R2* levels. Normally, the cortex is blue with areas of green and the medulla is green and orange. (B) The colour R2* map and pathological photo of ATN transplant shows more green areas in the cortex and more orange areas in the medulla in Part B-1, and pathologically tubular epithelial cell necrosis in Part B-2 (×200). (C) The colour R2* map and pathological photo of TMR transplant shows the colour gradient disappears with increased blue areas in the medulla. Conversely, ATN allograft shows increased green areas in the cortex and more orange areas in the medulla.

Reflecting the decrease in tissue oxygen bioavailability both in the cortex and in the medulla.

Statistically, mean cortical R2* (CR2*), mean medullary R2* (MR2*) and medullary over cortical R2* ratio (MCR) were compared between groups, as shown in Figure 2. The mean CR2* level was significantly higher in the ATN group (15.25 ± 1.03/s) compared to the normal group (13.35 ± 2.31/s, P = 0.028) and AR group (12.02 ± 1.72/s, P = 0.001). There was also a significant difference between the AR group and normal group on CR2* levels (P = 0.013). The mean MR2* level was significantly lower in the AR group (14.02 ± 2.68/s) compared to the normal group (16.66 ± 2.82/s, P < 0.001) and ATN group (19.47 ± 1.62/s, P < 0.001). There was also a significant difference between the ATN group and normal group on MR2* levels (P = 0.011). The MCR was significantly higher in the normal group compared to the AR group (1.26 ± 0.18 versus 1.17 ± 0.19, P = 0.043), but there was no significant difference between the normal group and ATN group (1.28 ± 0.09).

Except for the mean CR2* level between the AMR subgroup and normal group (P = 0.184), there were significant differences on mean CR2* and MR2* levels in subgroups compared to the normal group or ATN group. MCR were significantly different only between the AMR subgroup and normal group (1.13 ± 0.17 versus 1.26 ± 0.18, P = 0.049). However, there were no significant differences on CR2* levels, MR2* levels and MCR between the AMR subgroup and TMR subgroup.

Four patients with AR and four patients with ATN received serial BOLD MR imaging post-transplantation. Mean MR2* levels of allografts with ATN displayed obvious reduction following the recovery of allograft function, as shown in Figure 3A. Mean MR2* of allografts with AR displayed lower levels during the rejection episodes and had higher levels before rejection or after recovery from rejection, which also demonstrated the dynamic changes of MR2* levels, as shown in Figure 3B.
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Fig. 2. CR2*, MR2* and MCR values comparing between various groups. *P < 0.05 compared to the normal group; **P < 0.001 compared to the normal group. *P < 0.05 compared to the ATN group; ***P < 0.001 compared to the ATN group.

Influence factors of R2* levels

Spearman correlation analysis revealed that there were no correlations between characteristics such as patient age, time from operation to imaging, time from biopsy to imaging, Scr level, HB level, urine outputs, MAP level, blood CsA/FK506 trough concentration and R2* levels, except between MAP and CR2* levels (correlation coefficient = 0.211, P = 0.029). Stepwise multinomial linear regression analysis indicated that only time from biopsy to imaging could influence R2* levels of medulla and cortex within above characteristics (P = 0.034 for CR2*, P = 0.036 for MR2*).

Diagnostic value of R2* levels

ROC curves revealed that both CR2* (AUC = 0.677, P = 0.0042) and MR2* (AUC = 0.785, P = 0.0001) could discriminate between AR allografts and normal functioning allografts, and MR2* discriminated more accurately than CR2* (P = 0.043). The criterion points of MR2* and CR2* for AR were 14.9/s (sensitivity 80%, specificity 72%) and 11.46/s (sensitivity 55%, specificity 84.1%) respectively, as shown in Figure 4A. As for the ATN group, both CR2*
Fig. 4. ROC curves for the CR2* level and MR2* level. (A) Acute rejection; (B) acute tubular necrosis; (C) acute antibody mediated rejection; (D) acute T-cell mediated rejection. "The criterion point of MR2*, "the criterion point of CR2*."

Discussion

Our study revealed that changes of tissue oxygen bioavailability in kidney transplants existed during the pathophysiological courses of early kidney allograft dysfunction, and BOLD MRI could effectively detect these changes by measuring R2* levels. In AR allografts, tissue oxygen bioavailability (oxyhaemoglobin concentrations) increased significantly, as R2* levels decreased \( (P < 0.001) \) both in the cortex and medulla compared to normal functioning allografts, but oxygen bioavailability in the medulla seemed to change more remarkably. The serial BOLD MR imagings of AR allografts also demonstrated the simultaneous changes of medullary oxygen bioavailability, with decreased MR2* levels during the AR episodes and increased MR2* levels after recovery from AR. Conversely, ATN allografts showed decreased oxygen bioavailability both in the cortex and medulla compared to normal functioning allografts \( (P < 0.05) \), and the serial BOLD MR imagings demonstrated the corresponding changes. We noticed that the similar work by Djamali et al. had found that MR2* levels of ATN allografts were lower than normal functioning allografts [11], reflecting a significant increase in medullary oxygen bioavailability in ATN allografts, which was different from our results.

The previous study showed that in the early stage of acute intra-renal ischaemia, the compromise of blood supply decreased the renal blood flow, which could result in the elevation of CR2* and MR2* levels [8]. The reduced glomerular filtration rate, distal delivery and reabsorption rate under low renal blood flow led to decreased oxygen consumption, which could cause the increased oxygen availability in the allografts during the recovery of ATN [14–16]. We supposed that the time from operation to BOLD MRI in current study \( (9.86 \text{ days in average}) \) was much shorter than the time in the study of Djamali et al. \( (29 \text{ days in average}) \), which represented the different stages of ATN.

As for subgroups, there were no obvious differences between TMR subgroup and AMR subgroup on CR2* or
MR2* levels in our study, which was different from the results of Djamali et al. [11]. They found that AR subtypes manifested different medullary oxygen bioavailability patterns and type IIA rejection (according to Banff’97 criteria) seemed to have the lowest MR2* level, indicating that the R2* levels were related to the severity of pathological presentations [11]. In the current study, the pathological changes of AMR subgroup consisted of four cases of type I and four cases of type II (according to Banff’05 criteria), revealing no severe arteritis. We supposed that the relatively minor pathological changes in AMR allografts lead to no obvious difference of R2* levels compared to TMR allografts.

Some researches indicated that several transplantation-related factors could influence intra-renal oxygen bioavailability. For instance, furosemide [17] and water loading [5–7] inhibited the active reabsorption in medullary tubular epithelial cells, and then significantly improved medullary oxygen bioavailability. ACEIs and ARBs had the pharmacological action of expanding the efferent glomerular arteriole [18], and they increased the blood supply to the medulla and raised the medullary oxygen bioavailability. All the patients in our study were refrained from water intake or intravenous transfusion 4 h before BOLD MRI, and had no diuretic agents, ACEIs or ARBs. In the present study, no correlations between the characteristics such as patient age, time from operation to imaging, time from biopsy to imaging, Scr level, HB level, urine outputs, blood CsA/FK506 trough concentration and R2* levels were observed, except between the MAP and CR2* level, which could be explained by increased blood supply to the cortex following elevated MAP [19]. Multinomial linear regression analysis indicated that the time from biopsy to BOLD MR imaging could influence R2* levels in the medulla or cortex within above characteristics. There were no significant differences on the time from biopsy to BOLD MR imaging between groups in this study. So although there were differences of some clinical characteristics between groups, we concluded that changes in R2* levels may exactly reflect the pathological changes in kidney allografts.

The study had some limitations. Firstly, the number of patients investigated with biopsy-proven AR and ATN were relatively small compared to the number of patients with normal graft function. Secondly, the time from biopsy to BOLD MR imaging was 6 days before or after kidney transplant biopsy. It was a relatively long time interval for certain causes such as severe thrombocytopenia that hindered the timely biopsy after BOLD MR imaging and renal haematoma after biopsy that caused to be unfit for moving. As we had shown that the time from biopsy to BOLD MR imaging could influence R2* levels in the medulla or cortex, it would be more accurate to represent the pathological changes at a narrowed time interval. Lastly, BOLD MRI could not distinguish between the changes of oxygen supply and local oxygen consumption. Yang et al. detected changes of microcirculation in kidney transplants using diffuse-weighted MR imaging (DW MRI) in rat models, and found that the allografts exhibited compromise of microcirculation both in medulla and cortex compared to isografts and native kidneys [20]. It seemed that medullary blood supply could be impaired in rejected allografts. Thoeny et al. also found that in contrast to increased medullary oxygen bioavailability, there was no increased perfusion fraction observed by DW MRI in the cortex or medulla of kidney transplants [21]. So the exact mechanism influencing intra-renal oxygen bioavailability is still unclear; it needs systemic work to determine changes of haemodynamics, active reabsorption rate and tissue oxygen bioavailability simultaneously, which is being carried out in animal models in our lab.

In conclusion, BOLD MRI appeared to be valuable to discriminate between AR and ATN in early kidney allograft dysfunction. The R2* levels were significantly decreased in AR allografts and increased in an early stage of ATN allografts, indicating the simultaneous changes of tissue oxygen bioavailability.

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