Evolution of renal oxygen content measured by BOLD MRI downstream a chronic renal artery stenosis

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Abstract

Background. A decrease in renal oxygen content can be measured non-invasively by the increase of the R2* value derived from blood oxygen level-dependent magnetic resonance imaging (BOLD MRI). The aim of this study was to test if renal hypoxia occurs in kidneys downstream a chronic and unilateral renal artery stenosis.

Methods. Chronic renal ischaemia was induced in rats using a calibrated clip inserted on the right renal artery. R2* was determined, using a multiple recalled gradient-echo sequence, before and once a week after a clip insertion over 4 weeks, in a group of clipped (n = 8) and sham-operated (n = 7) rats.

Results. At baseline, in stenotic kidneys, R2* was higher in the outer stripe of outer medulla (105 ± 4.6) and the outer medulla (99 ± 2.5) than in the cortex (84 ± 2.5; P < 0.002 for comparison with both areas). R2* was unchanged in the cortex, the outer stripe of outer medulla and the outer medulla in stenotic kidneys, sham-operated kidneys and contralateral kidneys during the 4 weeks. Mean blood pressure was higher in rats with clipped kidney than in sham-operated rats from Day 11 and remained increased thereafter. The renal volume increased progressively in sham-operated kidneys and contralateral kidneys, whereas it slightly decreased in stenotic kidneys.

Conclusions. Our study shows that after 4 weeks, no renal hypoxia can be detected in the kidney downstream to a renal artery stenosis, suggesting that atrophy could be induced by other factors.

Keywords: Magnetic resonance imaging; Blood level oxygen dependent; Renal artery stenosis; Renal atrophy; Outer medulla

Introduction

Chronic renal artery stenosis (RAS) is a common cause of progressive renal atrophy and chronic kidney disease [1–3]. RAS is responsible for a decrease in renal blood flow (RBF), for an activation of the renin–angiotensin system and finally a decrease in glomerular filtration rate (GFR) [2]. However, unlike in other organs, a decrease in RBF may not induce a decrease in renal oxygen content (renal O2) because of a parallel decrease in GFR and sodium re-absorption resulting in a decrease in O2 consumption. Conflicting results exist regarding the occurrence of renal hypoxia in kidneys downstream to chronic RAS. This question is relevant because it can induce several renal injury pathways leading to tubular atrophy, interstitial fibrosis and ultimately to renal dysfunction. So far, renal O2 was measured invasively by histologic staining and oxygen electrodes [4], methods not allowing continuous monitoring of renal O2. Using a direct and invasive measurement with pO2 electrodes, Welch et al. found that, after 3 weeks, a RAS induced a decreased renal cortical pO2 in rats comparatively to a control group and that this renal cortical pO2 was also decreased comparatively to contralateral kidney [5,6]. Blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) allows the non-invasive measurement of renal O2 in humans and animals [7–10]. Recently, Textor et al. found that BOLD MRI (inversely related to renal oxygen content) in chronically stenotic kidneys without renal artery occlusion was unchanged, suggesting that these kidneys were not hypoxic [11]. Moreover, after 12 weeks, Chade et al. and Zhu et al. found that protein expression of HIF-1α was decreased in kidney downstream RAS in pigs, also suggesting that no
hypoxia occurs because HIF is up-regulated by hypoxia as shown previously [12,13].

The principle of BOLD is that magnetic field perturbations caused by paramagnetic molecules such as desoxyhaemoglobin lead to signal attenuation in gradient-echo T2*-weighted MRI images. Changes in renal O2 lead to a modification of tissue R2* (1/T2*) [14]. R2* measured by BOLD MRI is inversely related to renal O2. Previous studies demonstrated that progressive constriction of renal artery in pigs was associated with progressive decrease of renal O2 measured by BOLD MRI [7]. However, data are lacking concerning renal O2 measured by BOLD MRI in chronic experimental RAS.

The aim of this study was to test if renal O2, evaluated non-invasively by BOLD MRI, is decreased in kidney downstream to an experimental chronic RAS, comparatively to the control kidneys in a rat model.

Materials and methods

Animals

The protocol was approved by the local Institution of Animal Care and Use Committee (number of authorisation #2008-0249). Before MRI study, male Sprague–Dawley rats (Charles River Laboratory, L’Arbresle, France) were kept for a 7-day period of habituation. They received standard food and had free access to tap water.

Furosemide administration study

For validating the measurement of BOLD MRI at 7 Tesla, nine 8-week-old rats, weighing 250 to 300 g, were anaesthetized with Isoflurane 3% (Abbott France, St Remy sur Avre, France). Then, they were placed in the MRI gantry in left supine position with continuous anesthesia of Isoflurane 1.5%. R2* was measured before, then 5, 10 and 15 min after an injection of furosemide (10 mg/kg as previously reported) [8,10] performed through a venous tail catheter (24G).

Chronic RAS study

Fifteen 10- to 11-week-old rats, weighing 300 to 400 g, were assigned randomly to a renal artery stenosis (RAS) group or to sham-operated (SHAM) group. Under anaesthesia (described earlier), the right renal artery was dissected through a right lombotomy in all animals, and a calibrated plastic clip (0.2 mm), made in house, was placed on the artery in order to induce a reproducible renal artery stenosis. Post-operative analgesia was performed by an i.p. injection of Xylazine (10 mg/kg).

In 12-h fasted rats, R2* was measured 3 days before, then 4, 11, 18 and 25 days after surgery. No injection of furosemide was made in this study. Before each MRI acquisition, weight and blood pressure were measured on conscious rats using a blood pressure monitor (Harvard Apparatus France, Les Ulis, France). Six to eight measurements were made and averaged.

On Day 28, animals were weighted then sacrificed. The two kidneys were harvested and weighed without specific drainage.

MRI acquisition

R2* measurements were performed with a Bruker Biospec 70/20 MRI device (Bruker BioSpin MRI GmbH, Ettlingen, Germany) with magnetic field of 7 Tesla. The BOLD MRI pulse sequence was a multiple gradient-recalled echo sequence of 12 images weighted in T2* with echo times (ET) of 4 ms (from 4 to 48 ms), repetition time (RT) of 200 ms, flip angle (FA) of 20°, bandwidth (BW) of 60 kHz, field of view (FOV) of 8 cm, matrix of acquisition of 128 × 196 interpolated to 256 × 256, slice thickness of 2 mm and a number of four repetitions. The acquisition time was 1 min and 45 sec. During acquisitions, animals were free breathing.

In the furosemide administration study, the images of both kidneys were made on a single hilar level during the same acquisition. In the chronic RAS study, the images of each kidney were acquired sequentially, on three transversal sections (hilar level and levels 1 mm above and below the hilar level). The hilar level was determined using an anatomical sequence in frontal and para-sagittal views.

An additional acquisition (covering the entire kidney with slice thickness 1 mm) was performed for measuring the kidney volume. The sequence was the following: Spin Echo RARE sequence with ET 8.9 ms; RT 796.2 ms; FA 180°; BW 50 kHz; FOV 8 × 6 cm; matrix of acquisition of 256 × 256 and four repetitions.

Image analysis

Images were analysed with ImageJ (NIH, Bethesda, MD, USA). Three regions of interest (ROI) were drawn on the cortex (CO), on the outer medulla (OM, region located between the cortex and the inner medulla) and on the outer stripe of outer medulla (OSOM, external part of the OM) (Figure 1). The ROI were determined on the fourth image of the set of images. Despite identical settlement of the animals in the MRI device, ROI were not superimposable and, consequently, delineated at each acquisition.

Fig. 1. Transverse images of the right kidney obtained with multiple echo gradient sequence weighted 0 in T2* at different echo times (ET) (1, cortex; 2, outer medulla; 3, outer stripe of outer medulla). Orientation is up = anterior; right = left.
R2* was measured as the slope of the logarithm of MR signals as a function of the echo time [7]. Only the first six images were used for calculating R2* because the MR signal decreased dramatically at 7 T. For furosemide injection study, the MR signal value was the mean of MR signal measured in the two kidneys, weighted by the number of pixels in the ROI. For chronic RAS study, the MR signal value was the mean of MR signal measured in the three ROI delineated on the three transverse slices, weighted by the number of pixels of each ROI.

Signal to noise ratio (SNR) was calculated as the ratio of mean signal in the studied ROI on the standard deviation of signal in the background of the image [18].

Renal volume was measured using stereology® (BIR, Mayo Clinic, Rochester, MN, USA) as reported earlier [19].

Calculations and statistics
Values were given as mean ± SEM. Kolmogorov Smirnov test was used to test the normal distribution of the data. For the detection of longitudinal changes, the comparison of variances with repeated measure ANOVA and Bonferroni post hoc test were used when distribution was normal; Friedman test and Wilcoxon test were used when data were not distributed normally. For inter-group comparisons, Student t-test was used when data were distributed normally. If not, Mann–Whitney test was used. Differences were considered significant if P ≤ 0.05. Statistical analysis was performed using Statview® (Abacus Concepts, Berkeley, CA, USA).

Results

Furosemide injection study
The weight was 325 ± 16 g. In order to assess the variability of BOLD MRI measurement, we performed two baseline measurements with BOLD MRI, using different ROI drawing, before furosemide injection. For each area of the kidney, the mean values of BOLD MRI were not different. At baseline (Figure 2), R2* was higher in the OSOM (103 ± 4; P < 0.001) and in the OM (98 ± 4; P < 0.001) than in the CO (78 ± 2).

R2* decreased after furosemide injection in the OSOM, the OM and the CO (P < 0.002 for each zone).

Chronic RAS study
At baseline, R2* was higher in the OSOM and in the OM than in the CO for both kidneys in the two groups (P < 0.05). There was no change in R2* in the stenotic kidneys for OM and OSOM, but the change was significant in the CO (Figure 3a). However, post hoc test failed to show differences between mean R2* at the different times.

In the Sham-operated kidneys (Figure 3b) and in the contralateral kidneys of both groups (Figure 4), there was no change in R2* in all zones.
Cortical and medullary SNR ranged between 29 and 30 depending on the studied area.

The volume of all kidneys increased significantly during experimentation (from +9% to +22%; P < 0.05 for each kidney) except for stenotic kidneys (−7%; P = NS). There was a significant difference between volume of stenotic kidneys and those of contralateral kidneys from Day 11 until the end of the study (Figure 5).

After sacrifice, the weight of stenotic kidneys was lower than the weight of contralateral kidneys of the RAS group but not lower than those of the sham-operated kidneys (Table 1).

There was a strong correlation between the renal volume measured by MRI on Day 25 and the renal weight at sacrifice on Day 28 (r = 0.734, P < 0.002).

There was no difference in blood pressure at baseline between the SHAM and RAS groups. From Day 11, mean arterial blood pressure was increased in RAS group (94.4 ± 3.4 mmHg versus 110.6 ± 2.2 mmHg; P < 0.002 on Day 11) and remained higher thereafter (Table 1).

The animal body weights were not different in the two groups at baseline and during the experimentation; no animal died during the RAS protocol.

Discussion

This study shows that renal O\textsubscript{2} as measured by BOLD MRI is unchanged downstream a chronic experimental RAS over 4 weeks despite a significant renal atrophy of the clipped kidneys and the occurrence of hypertension in the RAS group.

This is the first study describing the time-course changes of renal O\textsubscript{2} in chronic experimental RAS. These findings are important for patients with RAS because chronic hypoxia is recognized as a factor leading to renal injury in chronic nephropathies and could have represented a possible therapeutic target for renal protection [20,21].

Juillard \textit{et al.} showed that an acute decrease in renal blood flow induced by an acute and graded renal artery stenosis was associated with a decrease in cortical and medullary renal O\textsubscript{2} measured by BOLD MRI, despite significant decrease in renal oxygen consumption [7]. Renal O\textsubscript{2} was not measured before Day 4 in this study, and the renal O\textsubscript{2} if initially changed, was restored at this time. It can be assumed that the discrepancies between findings during acute and chronic RAS studies may be the consequences of chronic adaptive mechanisms resulting in the restoration of tissue oxygenation. In the study by Juillard \textit{et al.}, the degree of RAS was high, leading to an intense and rapid drop of RBF, preventing the occurrence of any adaptation [7]. Restoration of normal renal O\textsubscript{2} may be due to a decrease in functional oxygen consumption. Indeed, according to a currently accepted model of renal O\textsubscript{2} regulation [22], a decrease in the GFR (as observed by many authors in stenotic kidneys in humans and animals when RAS was critical) [12,13,23] induces a proportional decrease in filtered sodium load, and consequently a lower quantity of sodium has to be reabsorbed by the proximal convoluted tubule (PCT) and the medullary thick ascending limb (mTAL). This decrease leads potentially to a decrease in renal O\textsubscript{2} consumption. Moreover, a decrease in O\textsubscript{2} consumption due to structural modifications of atrophic kidneys is another possible adaptive mechanism. Indeed, atrophic kidneys downstream a chronic RAS show ultrastructural changes in cells of PCT such as cell simplification, disappearance of brush border and decreased number

**Table 1.** Summary of data at baseline and on Day 25 (renal volume, renal weight at sacrifice, mean blood pressure and body weight). All values are given as mean ± SEM

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<th>Mean renal volume at baseline (mm\textsuperscript{3})</th>
<th>Mean renal volume on Day 25 (mm\textsuperscript{3})</th>
<th>Mean renal weight at sacrifice (mg)</th>
<th>Mean blood pressure at baseline (mmHg)</th>
<th>Mean blood pressure on Day 25 (mmHg)</th>
<th>Mean body weight at baseline (g)</th>
<th>Mean body weight on Day 25 (g)</th>
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<td><strong>Control Group</strong></td>
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<td>Sham-operated kidneys</td>
<td>1219 ± 30</td>
<td>1365 ± 76</td>
<td>1372 ± 107</td>
<td>103 ± 2.8</td>
<td>86 ± 1.2</td>
<td>348 ± 15</td>
<td>427 ± 23</td>
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<td>Contralateral kidneys</td>
<td>1227 ± 61</td>
<td>1342 ± 71</td>
<td>1309 ± 65</td>
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<td><strong>Renal artery stenosis Groups</strong></td>
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<td>Stenotic kidneys</td>
<td>1170 ± 37</td>
<td>1084 ± 113</td>
<td>1162 ± 73</td>
<td>107 ± 2.9</td>
<td>114 ± 3.6</td>
<td>338 ± 10</td>
<td>398 ± 19</td>
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<tr>
<td>Contralateral kidneys</td>
<td>1174 ± 57</td>
<td>1434 ± 53</td>
<td>1429 ± 99</td>
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of mitochondria that may occur quickly [24,25]. Interestingly, our results also show a trend to a decrease of R2* (meaning better oxygenation) until Day 18 (however, not statistically significant). This tendency could be explained by a greater decrease of oxygen consumption comparatively to those of oxygen supply, resulting in an increase in renal O2.

Supporting our results, Textor et al. showed that in kidneys with high grade and chronic renal artery stenosis, cortical and medullary R2* measured by BOLD MRI were not different as compared with normal kidneys without vascular lesion, except in atrophic kidneys with total occlusion of the artery [11]. Our results are also supported by the indirect measurement of renal oxygen content. The expression of HIF1α is increased in the presence of hypoxia, whereas under normoxia this subunit is quickly degraded by oxygen-sensitive prolyl hydroxylases [4]. Chade et al. and Zhu et al. showed that HIF1α subunit was decreased in chronic RAS in pigs [12,13,26], suggesting that no chronic hypoxia occurs during chronic RAS. Finally, Wiecek et al. showed that in humans plasma erythropoietin levels in the renal vein of the stenotic kidney were not different from those of the contralateral kidney. Jensen et al. also found no differences in patients with unilateral RAS between the arterio-venous gradient of erythropoietin in the stenotic kidney compared with the contralateral kidney [27,28]. Because hypoxia is the main factor that stimulates erythropoietin production, these results strongly suggest that no renal hypoxia occurs in kidney downstream a chronic RAS, thus supporting our findings.

However, Welch et al. evaluated the cortical oxygenation of stenotic kidneys in Sprague–Dawley rats, using a silver clip with the same size over 3 weeks. They found that cortical pO2 was reduced as compared with sham-operated rats [6]. Using the same protocol, they also found that cortical pO2 was reduced in clipped kidney as compared with the contralateral kidney [5].

Although the reasons for these conflicting results are not clear, they could be due to differences in the models. Indeed, although the size of the clip were the same, animals were of different ages and weights, resulting in significant different body and renal growth. In the study by Welch et al., animal weight increased by more than 100%, whereas weight gain was of 22% in our study. It cannot be excluded that these differences contribute to the different findings.

Renal O2 was studied with BOLD MRI because this is a non-invasive method allowing dynamic and reproducible evaluation of renal oxygenation in vivo [29] that has now been studied extensively. Indeed, several studies using BOLD MRI have demonstrated changes of renal O2 in hypertensive [30] and diabetic rats [17], in older [31] and diabetic [32] patients, and after furosemide administration [10,15]. It has to be emphasized that R2* provides renal O2 measurement without providing information on O2 supply and consumption that are the parameters influencing renal O2.

Renal O2 was studied in the CO, OM and in the OSOM. Indeed, OSOM is particularly sensitive to hypoxia due to important tubular workload poorly matched with oxygen supply [33]. We did not study R2* evolution in the inner medulla because R2* depends on a factor other than oxygen content in this zone. Indeed, several authors [8,17] have found that R2* values in the inner medulla were lower than in CO and OM, whereas oxygen tension is known to be lower in the kidney in this zone [33].

We have not performed simultaneous measurement of renal O2 by oxygen electrodes for several reasons, such as the strong likelihood to induce artefacts with exogenous material and the risk of modifying renal O2 because of the invasiveness of the surgical procedure. Moreover, Pedersen et al. found a significant correlation between renal O2 measured by electrodes method and by BOLD MRI method, in the cortex and the medulla of animals by changing the arterial pO2. In this study, authors also found similar results to ours after furosemide injection [14].

In our study, hypertension and renal atrophy of the clipped kidney occurred in the RAS group. After 4 weeks, we found a similar range of atrophy and increased blood pressure as reported earlier, demonstrating the activation of the renin–angiotensin system induced by RAS [34–36]. An important strength of our study is the monitoring of the renal volume with non-invasive MRI measurements. Kidney volume measurements showed that RAS inhibited renal growth initially, and secondly induced atrophy, concomitantly excluding the occurrence of renal infarction. RBF was not measured because the accuracy of RBF measurement by MRI remains controversial. We also did not measure plasmatic renin activity because it is inconstantly correlated to the development of renal atrophy [12,13,26,37].

It is also the first time that BOLD MRI was performed using a MRI device with a high magnetic field of 7 Tesla, a device specifically developed for studies in small animals. High field devices have the main advantage to increase the SNR [38]. Indeed, in our study, the increase in SNR was greater than expected and was 6 to 12 times higher than observed at 1.5 T in humans for the cortex in our centre (data not shown). Moreover, after a semi-logarithmic transformation, a strong linear relationship was found between the MR signal and the echo time, allowing the measurement of accurate R2* values (data not shown).

In order to validate renal R2* measurement at 7 Tesla, we studied the evolution of R2* after furosemide intravenous injection. Furosemide injection effect on BOLD MRI was studied extensively by several authors in humans and animals at lower magnetic field [8,10,15,17,38]. We found a similar decrease in R2* in CO, OM and OSOM after furosemide injection at the same dose as compared with previous studies at 1.5 or 3 T [8,10,17].

A limitation of our study is the absence of R2* measurement after furosemide administration in stenotic and sham-operated kidneys. It would have been interesting to evaluate the effect of furosemide injection on R2* in stenotic kidneys in order to determine if the decrease in R2* remains as previously described in normal kidneys in rats. Indeed, Textor et al. have shown in humans that ‘viable’ kidneys downstream to an atherosclerotic artery stenosis have the same response as that of non-stenotic kidneys [11].

Another limitation is the absence of data about the inter-observer variability in our study and the bias potentially induced by the manual drawing of the regions of interest.
To our knowledge, this variability has not been evaluated in previous studies using renal BOLD MRI, and this point remains to be evaluated.

Our findings suggest that a global atrophy of the kidney downstream a chronic RAS occurs independently of a chronic renal hypoxia. Nevertheless, our study shows that BOLD MRI allows the non-invasive monitoring of renal $O_2$ during chronic ischaemia. Therefore, BOLD MRI could be an interesting tool to improve the understanding of the regulation of renal $O_2$ under pathologic situations or during therapeutic intervention, such as renal artery revascularisation.

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Conflict of interest statement. None declared.

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