In vivo assessment of renal blood flow and volume in rat kidney by using a macromolecular MRI contrast agent

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Abstract

No sensitive method is currently available to measure *in vivo* local renal perfusion. A blood pool gadolinium-based agent was selected among various macromolecules for the relative determination of renal blood flow (RBF) and blood volume (RBV) in anesthetized rats. By using fast MRI acquisitions, an index of RBF was calculated from the initial slope of the contrast agent uptake while an index of RBV was estimated from the maximal signal intensity change observed at the steady-state.

Introduction

The kidney plays an important role in the maintenance of body fluid homeostasis through glomerular filtration, tubular reabsorption and tubular secretion. These processes depend on the blood supply to the kidney. The main purpose of the renal blood flow (RBF) is to provide enough plasma so a high glomerular filtration rate (GFR) can be maintained for the effective regulation of body fluid volumes and solute concentrations. Decreased RBF is usually accompanied by a lowering of GFR and urine output (i.e. water and solutes and waste products). Decrease in GFR precedes the onset of kidney failure. RBF is dependent on renal vascular resistance and in spite of the existence of autoregulatory mechanisms for maintaining RBF and GFR (i.e. through local changes in vessel resistance), an abnormality in the balance between vasopressors and vasodepressors in favour of the former can affect RBF and ultimately lead to kidney failure. Moreover RBF is also controlled by the composition and volume of the extracellular fluid which varies drastically depending on the kidney region (i.e. cortex vs. medulla). The measurement of GFR in combination with regional RBF may provide a sensitive approach to test overall efficacy and mechanism of action of new antihypertensive agents for kidney protection. The accepted method to determine RBF applies the Fick principle through clearance of para-aminohippurate (PAH) from the blood by a combination of filtration and, primarily, tubular secretion. Although it uses radioisotope labeling, this method does not provide any insight into regional changes of RBF. Here we present a simple bolus-track method that relies on the use of a pure vascular MRI contrast agent for the *in vivo* determination of cortical and medullar blood flow and volume in rat kidneys.

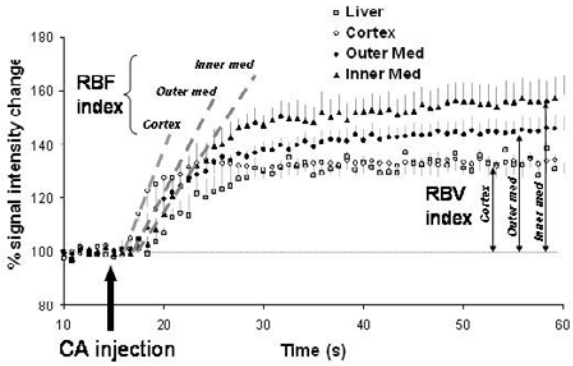
Methods

A number of criteria were retained in selection of an appropriate contrast agent (CA). As a pure vascular agent, the molecule size had to be large enough not to be filtrated through the glomerular membrane (i.e. macromolecular or particle based CA). So a steady-state concentration could be reached in the plasma upon a bolus infusion, the non-toxic molecule also had to be coated with polyethylene glycol (PEG) molecules or dextran to prevent early hepatic metabolism. Finally, it was thought that the CA should (1) contain as many Gd^{III}/molecule as possible to improve density of relaxivity, (2) be relatively cheap and easy to synthesize while (3) showing good solubility and low viscosity to reduce volume and time of injection. A number of dendrimers containing multiple Gadolinium elements were synthesized given these criteria. Assuming that a large concentration of the CA can be reached in the blood pool during the first pass, it was also critical that the imaging pulse sequence allowed for fast acquisitions with 1-2 sec time resolution. Images were obtained using a PharmaScan 4.7-T/16-cm magnet (Bruker Medical Inc., Billerica, MA) equipped with a 90-mm i.d. actively shielded gradient system (maximum strength 300mT/m) and using a 62-mm ^{1}H volume resonator. All experiments were performed in rats anesthetized with 2% isoflurane administered via a facemask. Prior to MRI measurements, the tail vein was cannulated for the administration of the gadolinium-based CA as a bolus infusion. After slice positioning using orthogonal scout scans, 512 consecutive TRUE-FISP images (TR 3 ms, TE 1.5 ms, SW 151KHz, [390□m]^2 in-plane resolution, slice thickness 1.5 mm, flip angle 60°) were collected in the coronal plane. Motion artifacts in images were minimized with sufficient signal averaging (2 averages) resulting in a 0.83 s acquisition time per image. Immediately after acquiring the 15th image of the series, 0.05 mmol Gd/kg of either a pegylated PAMAM G4 (Gd^{III} content 7.66 % w/w) or a pegylated PAMAM G6 dendrimer (Gd^{III} content 6.85 % w/w) was infused in the tail vein at an injection rate of 11 ml/min and then flushed with 0.1-ml of saline within 2s. The rate at which the macromolecular/intravascular CA enters the tissue is proportional to the BF through the tissue. Because of the very fast imaging sequence utilized, the signal intensity-time curve of the CA or transit time could be quantified. An index for RBF was measured in normal rats from the initial slope of the CA uptake in different regions (i.e. cortex, outer and inner medullas) of both kidneys. In addition, an index for renal blood volume (RBV) was estimated from the signal intensity change measured at steady-state. For image analysis, regions of interest (cortex, outer medulla and inner medulla) were manually selected from the second or third image obtained after the bolus infusion. The average signal intensity was plotted against time for each of the selected renal region. Finally, since both CA contained Cy5.5 fluorescent dye, urine was collected pre and post-experiment to verify absence of renal filtration by fluorescence imaging. Data are presented as mean±SEM of the percentage change from the baseline (baseline = 100%).

Results

Of the two CA tested, based on observed changes in signal intensity, the G4 molecule was revealed to be the most efficient relaxation agent (e.g. ΔSI_{cortex}: 55% for G4 vs. 32% for G6, $p<0.05$). For each CA, the initial slope measured from signal intensity-time curves decreased from the cortex to the outer and inner medullas (G4 slopes: 14.1±1.2 > 10.7±1.4 > 8.9±0.7 sec^{-1}; G6 slopes: 17.6±2.9 > 14.4±1.2 > 13.4±1.7 sec^{-1}, respectively), most likely indicative of a lower blood flow in deep (tubular) regions of the kidney (Fig. 1, n=4 rats). A steady-state signal intensity was reached within each region of the kidney and in the liver with the G6 agent, while a slight but gradual decrease in signal intensity was observed in 3 out 4 rats tested with the G4 agent after peak values were reached. In addition, the time to reach the maximum change in signal intensity was delayed with the G4 vs. the G6 agent. This correlated well with data obtained from the fluorescence assay clearly showing presence of the G4 dye in the urine, and hence its filtration. In terms of regional differences in RBV (Fig. 1), for both CAs tested, the deeper in the kidney, the greater the signal intensity change (DSI) observed (G4: ΔSI_{Cortex} 55%, $\Delta SI_{Outer\ med}$ 57%, $\Delta SI_{Inner\ med}$ 71%; G6: ΔSI_{cortex} 32%, $\Delta SI_{Outer\ med}$ 45%, $\Delta SI_{Inner\ med}$ 58%).

Figure 1 – Relative assessment of RBF and RBV using the G6 dendrimer



Discussion

The results of this study showed that an intravascular agent-based method can provide a relative assessment of both RBF and RBV on a regional basis. Of the two CA tested, the G6 molecule was the more promising agent. Most likely attributable to its larger size, there was no visible sign of G6 kidney filtration which could have interfered with the true assessment of kidney perfusion. In addition, solubility of G6 dendrimer appeared to be greater than the G4 dendrimer allowing minimized injection volumes. This method using the G6 blood pool agent could easily be combined with the measurement of glomerular filtration by DCE-MRI as described already [1], thus offering a comprehensive platform for detailed analysis of kidney function.

Reference 1. Laurent *et al* MRM 47(1):127, 2002