Original Article

A New Method for Evaluation of Split Renal Cortical Blood Flow with Contrast Echography

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The recent development of contrast echography has made renal enhancement possible through an intravenous injection of microbubble-based contrast. In animal models, tissue perfusion can be quantified using contrast echography by measurement of the rate at which microbubbles replenish tissue after their ultrasound-induced destruction. Our purpose in this study was to evaluate renal blood flow with contrast echography in humans. To increase the sensitivity for microbubbles, we used a combination of power Doppler harmonic and intermittent imaging. The pulsing interval (PI) was changed from 10 cardiac cycles to 1 cardiac cycle during an intravenous infusion of the contrast agent, and alterations in the intensity of the renal cortex were represented as a decline ratio (DR). In 24 patients with various renal diseases, we were able to observe all 48 kidneys with adequate enhancement of the renal cortex. At PI of 10 cardiac cycles, the enhancement was homogeneous and strong, while, obviously, changing PI from 10 to 1 cardiac cycles caused a decline of enhancement. An excellent correlation was found between DR using contrast echography and renal plasma flow determined by clearance and radionuclide measurements. An excellent correlation was found between the DR values determined by contrast echography and the renal plasma flow values determined using clearance and radionuclide measurements. These results suggest that DR may be useful for evaluation of both total and split renal blood flow. Thus the contrast echographic method presented here could succeed in assessing renal cortical blood flow less invasively than conventional methods in humans. (Hypertens Res 2002; 25: 77–83)

Key Words: split renal blood flow, microbubble, contrast echography, power Doppler

Introduction

The kidney regulates not only the concentrations of metabolic waste products, but also systemic blood pressure via control of the extracellular fluid (1). These functions are influenced largely by changes in the regional distribution of renal blood flow (2–5). Therefore, frequent renal blood flow measurement is one of the best strategies for preventing the progression of renal disease. Several methods exist for evaluating renal blood flow, including clearance techniques, radionuclide quantitative methods, computed tomography and magnetic resonance imaging (6–9). But these measurements tend to be invasive or to require complicated procedures.

The resistive index measured by Doppler ultrasound has become integral to echographic assessment of renal blood flow, and provides useful information of renovascular resistance in a noninvasive manner (10–13). But conventional Doppler echography has a disadvantage in that it cannot readily visualize renal blood flow in the low-velocity and
low-amplitude flow states. As a countermeasure against this problem, microbubble-based contrast agents have been developed (14–17). Novel contrast agents can enhance the Doppler signal from the blood stream via an intravenous injection of the agents. With the development of contrast agents, imaging technology has been improved. Many studies have shown that contrast agents in conjunction with advanced imaging techniques (harmonic power Doppler imaging and intermittent imaging) allow the detection of small blood flow (18–20). Furthermore, recent studies have shown that microbubbles can be destroyed by ultrasound and that measurements of their kinetics can be used to quantify tissue perfusion (21, 22). During a continuous infusion of microbubbles at steady state, measurement of the rate at which microbubbles replenish tissue after their ultrasound-induced destruction could provide an evaluation of blood flow in the canine kidney (21) and in the heart (22). To our knowledge, however, such a technique has not been applied to evaluation of the human kidney.

Our purpose in this study was to evaluate renal blood flow with contrast echography in humans. For this purpose, we used a combination of harmonic power Doppler and intermittent imaging during a continuous infusion of a contrast agent. We quantitatively compared the results of the renal contrast echography with those of more traditional techniques, i.e., clearance and radionuclide techniques, in a cohort of patients with various diseases.

**Methods**

**Patient Characteristics**

Twenty-four patients with various renal diseases who were admitted to Kagawa Medical University Hospital between September 2000 and January 2001 were enrolled in this study (Table 1). All patients were given a full explanation of the study and gave their informed consent prior to participation according to the Declaration of Helsinki. Seven patients suffered from chronic glomerulonephritis, 6 from diabetic nephropathy, 4 from renovascular hypertension (RVH), 3 from nephrosclerosis, 1 from interstitial nephritis, 1 from systemic lupus erythematosus, and 2 from renal insufficiency of unknown cause. All patients underwent echocardiographic examination, and those suffering from cardiac disease were excluded.

**Clearance Measurements for Assessment of Total Renal Plasma Flow**

In 16 patients, effective renal plasma flow was measured using para-aminohippurate clearance (C-PAH) as described previously (11). Clearance values were adjusted for a body surface area of 1.48 m², which is the mean for Japanese adults.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male 14</th>
<th>Female 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.7 ± 22.5 (16–84)</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>54.4 ± 7.7 (42.2–70.0)</td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td>132 ± 14 / 77 ± 10</td>
<td></td>
</tr>
<tr>
<td>Anti-hypertensive drugs</td>
<td>(+)15 (-) 9</td>
<td></td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>68.4 ± 2.8 (55.0–84.0)</td>
<td></td>
</tr>
<tr>
<td>Serum creatinin (mg/dl)</td>
<td>2.1 ± 0.4 (0.5–8.8)</td>
<td></td>
</tr>
<tr>
<td>Creatinin clearance (ml/min)*</td>
<td>55.8 ± 7.8 (7.3–146.0)</td>
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</table>

* Creatinin clearance was measured by 24-h urine collection.

**Radionuclide Measurements for Assessment of Split Renal Plasma Flow**

In all patients, radionuclide quantitative measurements using technetium-99m mercaptoacetyltriglycine (Tc-99m MAG3) were performed to estimate the split renal plasma flow (split RPF) of each kidney (23, 24).

**Contrast Agent**

We used a galactose- and palmitic acid-based contrast agent, Levovist® (Schering, Berlin, Germany) in this study (15, 25). The solution consisted of air-filled shell-stabilized microbubbles with a median diameter of approximately 1.3 μm and a half-life of 2 min. The agent can enhance kidney imaging via an intravenous infusion because microbubbles can pass through the capillary pulmonary filter to the arterial blood stream. For this study, 2.5 g Levovist® was mixed with 7 ml of sterile water to prepare a 300 mg/ml solution, and was administered continuously at the rate of 2 ml/min (600 mg/min) via an indwelling 20 gauge cannula placed into a cubital vein.

**Image Acquisition**

A Sonos 5500 and an S4 transducer were used for image acquisition (Agilent Technologies, Andover, USA). All examinations were performed with harmonic power Doppler imaging, in which ultrasound was transmitted at 1.8 MHz and received at 3.6 MHz. With the patient in a prone position, the transducer was placed on the lumbar region and the long-axis view of the kidney was displayed. The power gain was set as low as possible so that vascular signal was not visualized before administration of the contrast agent. The transmit focus was set at the renal cortex. The transmit power, focus, overall gain, and image depth were held constant throughout the study. In this study, we used intermittent imaging in which ultrasound pulses were gated to the T wave of the electrocardiogram, meaning the systolic phase.

First, the pulsing interval (PI) was set at 10 cardiac cycles while Levovist® was administered continuously. After enhancement of the renal cortex was plateaued, PI was changed rapidly from 10 to 1 cardiac cycles. The patients...
held their breath during recording of the images at each PI. The obtained images were stored on 2.3 GB MO disk (Hewlett-Packard, Palo Alto, USA) and transferred onto a computer. We extracted color pixels (254 levels) representing the microbubble signals by means of a WinROOF® computed imaging analyzer (Mitani Corp., Fukui, Japan). The region of interest was placed at least 1 cm² over the renal cortex, and the intensity within this region was measured by the Win ROOF® analyzer. We measured the intensity in the same region of 5 images at each PI, and then calculated the average intensity from the 5 images. Alterations in the intensity when PI was changed from 10 to 1 cardiac cycles were represented as a decline ratio (DR). DR was calculated according to the following formula: DR = (intensity at PI of 10 cardiac cycles - intensity at PI of 1 cardiac cycle) / intensity at PI of 10 cardiac cycles / heart rate. We calculated a split DR for each kidney, and also calculated an average DR as the mean of left and right DR.

The results obtained from contrast echography were compared with those of clearance and radionuclide measurements.

**Statistical Analysis**

Results are presented as the means ± SD. The intensities at long and short PIs were compared by paired Student’s t-test. Pearson’s correlation coefficient was used to compare the data. A p value of less than 0.05 was considered to indicate statistical significance.

**Results**

**Clearance and Radionuclide Measurements**

In the 16 patients for whom C-PAH values were determined, the average C-PAH was 252 ± 166 ml/min (Table 2). In all patients, the average split RPF value obtained from radionuclide measurements was 148 ± 64 ml/min for the left kidney, and 133 ± 72 ml/min for the right. There was no significant difference in split RPF between the left and right kidneys. To examine the reliability of results of radionuclide measurements, we compared the sum of the split RPF to the C-PAH value. The sum of split RPF obtained from radionuclide measurements using Tc-99m MAG3 was correlated significantly with C-PAH (r = 0.873, p < 0.001, data not shown).

**Ultrasound Studies**

Power Doppler harmonic imaging was performed in 48 kidneys of 24 patients. Significant enhancement of the renal cortex was observed in all patients at a contrast-agent dose of 600 mg/min. Figure 1 shows a series of contrast echographic images obtained from a representative patient. At basal condition before injection of the contrast agent, we set the power gain as low as possible so that the vascular signal was not visualized (Fig. 1A). Under continuous administration of the agent, the renal cortex turned bright orange and yellow. During PI of 10 cardiac cycles, the enhancement was homogeneous and strong (Fig. 1B). Changing PI from 10 to 1 cardiac cycles caused a decline of enhancement obviously (Fig. 1C). Figure 2 shows typical images from 2 patients, one with normal renal function (Fig. 2A) and the other with...
chronic renal failure (Fig. 2B). The renal cortex with normal renal function was enhanced more strongly than the cortex with chronic renal failure at each PI. The changing of PI from 10 to 1 cardiac cycles caused a significant decline in renal cortical intensity (Fig. 2C). In particular, the reduction of the intensity of the cortex with chronic renal failure was greater than that of the normal renal cortex.

The intensity of the renal cortex decreased significantly due to the change in PI from 10 to 1 cardiac cycles (p < 0.005, Table 2). We calculated split DR values individually for either kidney, and also calculated the average of the left and right renal DR. There was no significant difference be-

Table 2. Intensity and Decline Ratio

<table>
<thead>
<tr>
<th>C-PAH (ml/min)</th>
<th>RPF (ml/min)</th>
<th>Total</th>
<th>Average</th>
<th>Left kidney</th>
<th>Right kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>252 ± 166</td>
<td>289 ± 128</td>
<td>148 ± 64</td>
<td>133 ± 72</td>
</tr>
<tr>
<td>Intensity PI 10</td>
<td>386 ± 38</td>
<td>193 ± 21</td>
<td>195 ± 21</td>
<td>191 ± 22</td>
<td></td>
</tr>
<tr>
<td>Intensity PI 1</td>
<td>299 ± 65</td>
<td>150 ± 36*</td>
<td>153 ± 35*</td>
<td>146 ± 36*</td>
<td></td>
</tr>
<tr>
<td>Decline ratio (× 10⁻³)</td>
<td>3.54 ± 1.99</td>
<td>3.37 ± 2.14</td>
<td>3.71 ± 2.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represents the means ± SD. *, p < 0.005 vs. 10 cardiac cycles.

Fig. 3. Relationship between DR and C-PAH (n = 16). DR is represented as the average value of each kidney.

Fig. 4. Relationship between DR and the results of radionuclide measurements in 48 kidneys of 24 patients.
tween the left and right renal DR in any of the patients.

**Comparisons with Clearance and Radionuclide Measurements**

To evaluate the results of contrast echography, we compared the obtained average DR value with the values obtained from conventional clearance techniques. The average DR was significantly correlated with C-PAH in 16 patients ($r = 0.687$, $p < 0.005$, Fig. 3).

In the 48 kidneys of all 24 patients, split DR was correlated with the split RPF obtained from radionuclide measurements ($r = 0.675$, $p < 0.005$, Fig. 4).

**Evaluation for Renovascular Hypertension**

We examined 4 cases of RVH in this study. Contrast echographic images obtained from a representative case of RVH are shown in Fig. 5. In this case, enhancement of the left kidney was markedly decreased. The DR values in the right and left kidneys were $1.35 \times 10^{-3}$ and $5.33 \times 10^{-3}$, respectively. However, in our 4 cases with RVH, there were no significant differences between the stenotic and non-stenotic kidneys in either split DR or split RPF obtained from the radionuclide measurements.
Discussion

Harmonic power Doppler imaging has advantages over conventional Doppler imaging for the evaluation of tissue blood flow (18, 19). Blooming and flash artifacts are eliminated, shadowing artifacts are lessened, and both spatial and temporal resolutions are improved. This imaging technique also has its intrinsic limitations, including limited signal response from deeper tissues due to the use of higher harmonic frequencies and diminished temporal resolution with intermittent imaging. In the present study, however, the lack of flash artifacts and superior spatial resolution effectively countered the effects of the reduced frame rate such that flow to the kidney could be easily detected with intermittent acquisition. Our study showed that contrast-enhanced harmonic power Doppler imaging succeeded in visualization of renal cortical blood flow in real time, without the artifacts commonly associated with conventional Doppler imaging.

Figure 6 shows models of destruction and reappearance of microbubbles in ultrasound fields. At constant velocity of flow, the number of bubbles within the ultrasound field depends on the length of PI (Fig. 6A and B). If the ultrasound pulse can destroy 60% of microbubbles (hatched small circle), the number of bubbles in the ultrasound field decreases instantly. However, new bubbles enter the field via blood flow, and the bubbles in the field increase without ultrasound pulse, meaning long PI (Fig. 6A). On the other hand, a short PI induces destruction rather than reappearance of microbubbles, resulting in a decrease in the number of microbubbles in the ultrasound field (Fig. 6B). When PI exceeds a certain length, microvasculatures in the field are saturated with bubbles, and then the intensity reaches a plateau. Therefore, the maximum intensity at long PI may reflect microvascular volume. In contrast, assuming that short PI is held constant and the flow velocity is variable (Fig. 6B and C), the number of microbubbles depends on the flow velocity because the microbubbles are destroyed at a constant rate. Thus, the intensity at short PI is considered to reflect the flow velocity. Wei et al. have recently demonstrated that regional blood flow could be quantified with contrast echography utilizing ultrasound-induced destruction of microbubbles in ex vivo, in vitro, and in vivo experiments (21, 22). However, in our study, neither intensity at 10 cardiac cycles (long PI) nor that at 1 cardiac cycle (short PI) correlated with the results of clearance and radionuclide measurements (data not shown). We considered that the intensity at each PI did not reflect the absolute blood flow due to individual difference in cardiac function, circulating volume, or renal disease. Theoretically, high blood flow shows low DR when PI is changed from 10 to 1 cardiac cycles (Fig. 6). We hypothesize that DR reflects their own blood flow. As expected, DR had a significant correlation with C-PAH and split RPF obtained from radionuclide measurements. These data suggest that DR may be useful for evaluation of both total and split renal blood flow.

To the best of our knowledge, there has been no previous report using the method of tissue replenishment of microbubbles after their ultrasound-induced destruction to evaluate renal blood flow in humans. A new index obtained from contrast echography was shown to correlate with the results of clearance and radionuclide measurements, and provided for noninvasive measurement of renal blood flow. The resistive index measured by the conventional Doppler method has been used in modern clinical nephrology to evaluate renovascular resistance (10–13), but such measurement requires a mastery of skills and is limited to detection in the interlobar arteries. In our study, contrast echography was easy to perform, and there was no need to correct the ultrasound beam angle. Compared with clearance and radionuclide measurements, contrast echography was also easier to perform and less invasive. We therefore conclude that this method enables repetitive and real-time assessment of renal perfusion.

Renal function is crucial to the maintenance of systemic blood pressure via control of the body fluids. Further, hypertension per se damages the kidney, and finally, hypertensive nephrosclerosis is a leading cause of end-stage renal disease in humans. There are evidences that relatively small changes in the zonal distribution of renal blood flow may have wide-ranging effects on renal excretory function (1–5). Therefore, to accurately measure renal blood flow is one of the best strategies for preventing the progression of renal disease. In this study, we evaluated only the intensity within the renal cortex. Blood flow of the renal medulla might also be evaluated if the ultrasound focus could be placed at the medulla. However, we could not evaluate medullary blood flow because acoustic attenuation interfered with the imaging. In the future, therefore, more powerful equipment will be needed for detection in deeper sites of the kidney.

In the present series we targeted RVH, which could be diagnosed by Doppler echography. Early detection of impairment in renal blood flow might prevent renal function from end-stage renal disease. We anticipated that contrast echography would be able to detect renal blood flow disorder in the present cohort. However, in our 4 cases with RVH, there were no significant differences between the stenotic and nonstenotic kidneys in either DR or split RPF obtained from radionuclide measurements due to the small number of patients. Further evaluation of this method in a larger cohort with RVH will be needed.

In conclusion, the combination of harmonic power Doppler and intermittent imaging during contrast echography successfully visualized perfusion of the renal cortex and provided a good index of renal blood flow in humans. The new method proposed in this study has great potential for assessing alterations in renal blood flow. Further clinical application should be investigated for special renal diseases such as RVH.
Acknowledgements

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References