Original Article

Ultrasound Evaluation of Valsartan Therapy for Renal Cortical Perfusion

Noriko KISHIMOTO, Yasukiyo MORI, Takashi NISHIUE, Atsuko NOSE, Yasuaki KIJIMA, Toshiko TOKORO, Hideki YAMAHARA, Mitsuhiko OKIGAKI, Atsushi KOSAKI, and Toshiji IWASAKA

An increase in renal blood flow with a concomitant decrease in filtration fraction at the onset of angiotensin II receptor blocker treatment has been shown to predict a long-term renoprotective effect. However, no studies are available regarding angiotensin receptor blocker-induced changes in renal cortical perfusion observed in the clinical setting. We have recently developed a convenient method of evaluating human renal cortical blood flow with contrast-enhanced harmonic ultrasonography. The goal of this study was to use this method to examine the effect of valsartan, an angiotensin II receptor blocker, on renal cortical perfusion. We performed intermittent second harmonic imaging with venous infusion of a microbubble contrast agent in 7 healthy volunteers. Contrast-enhanced harmonic ultrasonography performed after oral administration of valsartan (80 mg) showed a significant increase in microbubble velocity, which correlated well with the increase in total renal blood flow determined by p-aminohippurate clearance ($r=0.950$, $p<0.001$). Although fractional vascular volume was not significantly increased, alterations in renal cortical blood flow calculated by the product of microbubble velocity and fractional volume were also correlated with the change in total renal blood flow ($r=0.756$, $p<0.05$). These results indicate that valsartan increases the renal cortical blood flow in normal kidneys, mainly by increasing blood flow velocity. Contrast-enhanced harmonic ultrasonography is a promising technique for evaluating the precise effect on renal cortical perfusion and optimal dose of valsartan in diseased kidneys. (Hypertens Res 2004; 27: 345–349)

Key Words: angiotensin antagonist, regional blood flow, renal circulation, renal disease, ultrasonography

Introduction

To date, few methods of determining regional renal blood flow (RBF) have proved reliable, accurate, and easy to use in the clinical setting. It is notable that contrast-enhanced harmonic ultrasonography (CEHU) with the constant infusion of a microbubble contrast agent has been shown to quantitatively measure canine myocardial blood flow at the capillary level ([1]). The intermittent harmonic imaging destroys gas-filled microbubbles in the capillaries and measures their replenishment, thereby generating data for pulse interval (PI) vs. acoustic intensity (AI) plots for evaluation of the regional blood flow. Because these bubbles remain entirely within the intravascular space and have a rheology similar to that of red blood cells, the kinetics of bubbles can provide an accurate quantitative measure of regional tissue perfusion, which is quite difficult to obtain by other methods ([2, 3]). The basis of a new method that can be used to obtain quantitative measurement of regional renal blood flow in humans has just been developed ([4, 5]). In this study, we used CEHU to assess changes induced by valsartan, an angiotensin II type1 receptor blocker (ARB) in the renal cortical blood perfusion.

From the Department of Medicine II, Kansai Medical University, Osaka, Japan.
Address for Reprints: Yasukiyo Mori, M.D., Ph.D., Department of Medicine II, Kansai Medical University, 10–15 Fumizono-cho, Moriguchi 570–8506, Japan. E-mail: moriy@takii.kmu.ac.jp
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Methods

Subjects

We recruited 7 healthy subjects (5 men and 2 women who ranged in age from 24 to 43 years) as study participants. All subjects were free of both personal and family histories of cardiovascular, renal, and endocrine diseases. Results of physical examinations were normal, including blood pressure. Subjects’ median serum creatinine level was 61.9 µmol/l (normal range: 44.2 to 70.7 µmol/l). Prior to the study, subjects were encouraged to continue their usual isocaloric diet without any salt or water restriction. The subjects took no medications during the study. The study protocol was approved by the institutional review board at Kansai Medical University, and written informed consent was obtained from each subject prior to participation.

Contrast-Enhanced Harmonic Ultrasonography

All subjects underwent CEHU as described previously (5). Briefly, intermittent second harmonic imaging was performed with the SONOS-5500 system (Philips Medical Systems, Andover, USA). A long-axis view of the left kidney was obtained by placing the transducer probe over the lower back while the subject was prone. Levovist® (Schering, Berlin, Germany), which contains galactose with a small amount of palmitic acid (0.9%), was used as the contrast agent. Recipients received bolus injections of 2 ml of Levovist® solution (final concentration, 300 mg/ml) at the rate of 2 ml/5 s, followed by continuous infusion of the same suspension at 1.5 ml/min via an intravenous catheter placed in the arm. The ultrasound PI was progressively decreased from 4 s to 0.2 s. Up to 4 ultrasound image data sets were acquired at each PI and stored on a magneto-optical disk. In accordance with the manufacturer’s instructions, we chose to display the ultrasound image data in the intensity units obtained by squaring the voltage acoustic units (V-SQUARE). Regions of interest were selected over the peripheral renal cortex, i.e., the part most distant from the arcuate arteries. The average value of background-subtracted AI in V-SQUARE at each PI was recorded. PI vs. AI plots were generated and fitted as a time-intensity curve with graphing software (Origin 6.1J; OriginLab, Northampton, USA); the slope related to the speed of blood moving into the slice, and the maximum level reached (the asymptote) related to the vascular volume. The equation describing this blood refill is y = A (1 - exp - βt), where y is AI at the PI of t, A is the fractional vascular volume (FVV), and β reflects the microbubble velocity (MV). The product A·β is the volume flow rate and approximates the regional blood perfusion (6).

Study Protocol

CEHU and clearance studies were carried out simultaneously. Baseline CEHU data were obtained before subject tests of p-aminohippurate clearance (C\textsubscript{PAH}) for renal plasma flow (RPF) and thiosulfate clearance (C\textsubscript{thio}) for glomerular filtration rate (GFR), performed with a single-injection technique described previously (7, 8). Briefly, subjects drank 500 ml of water to enhance urinary volume during the test. After 30 min, a solution of 10% p-aminohippurate (0.30 ml/kg) mixed with 10% thiosulfate (60 ml) was injected intravenously over 10 min followed by a 30-min equilibration period. Three 10-min renal clearance tests were then performed. After the completion of the C\textsubscript{PAH} and C\textsubscript{thio} tests to determine the basal level of total RPF and GFR in each subject, valsartan (80 mg, p.o.) was administered immediately. After waiting an additional 4 h to achieve the maximum effect of valsartan (9), CEHU and three additional 10-min clearance tests were performed under conditions identical to those for the basal clearance tests. Blood pressure and pulse rate were monitored every 10 min during the experiment. Total RBF was calculated as RPF/ (1 - hematocrit). All clearance data were corrected for body surface area calculated by body weight and height. Filtration fraction (FF) was determined by calculating the ratio of GFR to RBF. Renal vascular resistance (RVR) (mmHg/ml/min) was determined by calculating the ratio of mean arterial pressure to total RBF.

Statistical Analysis

All values are expressed as the mean ± SD. Statistical analysis of the systemic and intrarenal hemodynamic variables in each participant was performed with nonparametric Wilcoxon rank-sum tests. Linear regression analysis was also performed to compare measures obtained by CEHU against the results of clearance tests. Values of p < 0.05 were considered statistically significant.

Results

Oral administration of valsartan (80 mg) did not significantly affect arterial pressure or pulse rate (in comparison to basal values) at 4 h in the 7 healthy subjects (Table 1). However, significant increases in C\textsubscript{PAH} and total RBF were observed at 4 h. GFR calculated by C\textsubscript{thio} was not increased, resulting in a slight decrease in FF. RVR was decreased significantly.

Up to four AI values from second harmonic imaging were obtained at each PI, and the corresponding PIs vs. AI plots were fitted to a time-intensity curve. Representative data from a participant before and after the administration of valsartan are shown in Fig. 1. When the plots were calculated using the exponential function y = A (1 - exp - βt), β, reflecting the MV, was increased 2.3-fold (from 0.99 to 2.24) at 4 h after the administration of valsartan. However, the changes in A (from 35.5 to 38.7), representing the FVV,
were not as prominent as the changes in $\beta$. The $C_{PAH}$ study in a representative subject showed that valsartan induced a 1.6-fold increase in total RBF (from 64.7 to 111.8 ml/min).

In all participants, the relative changes in CEHU-derived parameters ($A$, $\beta$, and $A \beta$) induced by administration of valsartan were compared with changes in total RBF estimated by $C_{PAH}$ (Fig. 2). The changes in $\beta$ and $A \beta$, reflecting regional blood perfusion, showed a strong positive correlation with changes in total RBF (Fig. 2B and C, $r = 0.950$, $p < 0.001$ and $r = 0.756$, $p < 0.05$). $A$ (FVV) did not correlate with total RBF (Fig. 2A).

**Discussion**

The major finding of this study was that the single administration of an ARB (valsartan) in a healthy human increases renal cortical blood perfusion measured by CEHU, a novel and noninvasive method. The increase in cortical perfusion is mainly caused by the increase in blood flow velocity and correlates with the total RBF as estimated by the traditional clearance method with $p$-aminohippurate ($C_{PAH}$).

Previous large-scale trials of treatment with an angiotensin converting enzyme inhibitor (ACEI) and ARB have clearly shown that blockade of the renin-angiotensin system (RAS) reduces the deterioration in renal function associated with diabetes, non-diabetic nephropathies, and renal sclerosis (10, 11). Strategies to enhance blockade of the tissue RAS may result in greater renoprotective effects, by further reducing intra-glomerular hydrostatic pressure, glomerular hypertrophy, and angiotensin II-mediated effects on mesangium cells and cytokines, as well as by restoring albumin reabsorption in proximal tubules (12, 13). Of note, an increase in RPF
with a concomitant decrease in FF at the onset of ACEI treatment has been shown to predict a long-term protective effect on renal function in patients with chronic renal disease (14). In humans, treatment with ARB represents a more rational approach to blockade of RAS than does treatment with ACEI, because of the presence of non-ACE pathways in angiotensin II formation (10, 15). In addition, ARBs are better tolerated than ACEIs and have fewer adverse effects; therefore, prescribed patients achieve higher levels of compliance with ARBs, making these drugs an increasingly popular treatment option. Butler et al. (16) observed a favorable short-term renal response in patients receiving candesartan cilexetil, an ARB, for more severe renal impairment than those in the study with ACEIs. Clearance tests performed in the healthy volunteers in our study also showed a significant increase in total RBF, whereas mean arterial blood pressure and GFR were not altered, resulting in a significant decrease in FF and RVR. Thus, knowledge of the changes in regional RBF is essential to evaluate the initial effect of ARB and the prognosis of patients undergoing this treatment. Indeed, in certain clinical situations such as preexisting renal injury, dehydration or decreased status of effective circulating volume, which is easily caused by a reduction of renal cortical flow, the use of an ARB may be harmful, leading to the deterioration of renal function. However, the traditional clearance tests are time-consuming, difficult to perform in outpatients, and incapable of evaluating renal tissue perfusion within the kidney. In addition, some recently developed methods rely on imaging modalities, such as nuclear magnetic resonance, that require expensive and specialized equipment (17). Although the application of power Doppler ultrasound is another option (18), it takes skill to obtain reliable data. Our present study indicates that CEHU is a feasible, promising method that will enable more precise clinical decision-making regarding the use of agents which affect renal hemodynamics, such as ARBs.

The PI vs. AI plots derived from CEHU findings provide two important measures, those of MV and FVV (6). In addition, this method permits separate evaluation of each parameter. In the present study, we observed ARB-induced changes in renal cortical blood flow with an increase in MV, which correlated well with the increase in total RBF identified by $C_{\text{PAR}}$. Although FVV did not increase significantly, alterations in CEHU-derived renal cortical perfusion calculated by the product of MV and FVV also correlated with alterations in total RBF. ARBs reportedly achieve vasodilative effects on the renal vasculature without affecting hemodynamic variables such as heart rate or systemic blood pressure (19). Our present findings suggest that the vasodilative effect of valsartan operates mainly on the proximal artery, resulting in increased blood flow velocity in the peripheral artery, arteriole, and glomerulus, at least in a normal kidney.

Although valsartan is widely used as an effective and well-tolerated antihypertensive agent, its responder rate at lowering blood pressure for 8 weeks is still limited to 66.7% (20, 21). Therefore, it may be necessary to determine the optimal dose of valsartan in each patient even in antihypertensive therapy. In addition, the renoprotective effect of valsartan needs to be monitored more carefully. Weinberg et al. (12) showed that a population of patients with heavy proteinuria required a very high dose of ARB (candesartan cilexetil 96mg) to decrease proteinuria significantly. In contrast, Murayama et al. (22) suggested that early kidney damage in type 2 diabetic patients can be prevented by low-dose ARB (candesartan cilexetil 4mg/day), indicating that tailored prescription of ARB in each patient is important. In this context, the observation of the increase in renal cortical blood flow at ARB treatment may be critical to predict a long-term renoprotective effect (16). Because ARB-induced intrarenal hemodynamic alterations have been assumed to occur mainly in the cortical region, a simple, accurate, clinical method of monitoring regional changes within the complex renal structure is still needed to enable evaluation of the efficacy and optimal dose of an ARB in individual patients. Future study of our renal CEHU method and its clinical applications could lead to the individualized, specific use of ARB for patients with kidney diseases such as diabetic nephropathy or chronic glomerulonephritis, in which RAS is activated in renal tissues (23, 24). Furthermore, whether or not the baseline CEHU findings such as the increase in renal cortical blood flow by valsartan predict a long-term renoprotective outcome remains to be clarified.

In summary, the present study demonstrates that the ARB-induced increase in regional blood flow in the human kidney can be measured with CEHU. Because the noninvasive nature of microbubbles as sonographic agents easily allows repetitive measurements, our method of CEHU is suitable for monitoring the effects of ARB treatment on renal hemodynamics.

References

6. Cosgrove D, Eckersley R, Blomley M, Harvey C: Quantifi-


