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Quantitative estimation of renal blood flow by power Doppler ultrasonography in renovascular hypertensive dogs

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Background. We estimated the value of power Doppler (PD) imaging analysis for the quantitative assessment of renal cortical blood flow (RCBF) in chronic two-kidney, one-clip (2K-1C) hypertensive dogs.

Methods. To evaluate the correlation between RCBF and PD signals, RCBF and the mean pixel intensity (MPI) of PD signals were simultaneously obtained at same region in renal cortex under progressive constriction of left main renal artery in five mongrel dogs. RCBF was measured by electrolytic hydrogen gas clearance method, and PD images were transferred to computer and analyzed by the image-analysis software Openlab®. To assess the value of quantitative PD imaging analysis on RCBF in renovascular hypertension, in six mongrel dogs with chronic 2K-1C hypertension, PD images in both of clipped kidneys (CK) and non-clipped kidneys (NK) were obtained and analyzed before and 60 minutes after the intravenous infusion of captopril or sodium nitroprusside at 10-minute intervals.

Results. There was a linear correlation between RCBF and MPI ($r = 0.878$, $P < 0.0001$). MPI in both CK and NK significantly increased after the infusion of captopril, while no significant change was observed in both CK and NK after the infusion of sodium nitroprusside, despite similar reduction of mean arterial blood pressure.

Conclusion. Our data suggest that the acute inhibition of angiotensin-converting enzyme increased RCBF in both CK and NK of chronic 2K-1C dogs. The quantitative analysis of PD flow signals in kidney is noninvasive and a useful method to evaluate regional changes of renal tissue blood flow in various renal diseases.

In recent advancement of ultrasound (US) technology, Doppler US has facilitated the real-time observation of intrarenal hemodynamics. Doppler US evaluation of intrarenal hemodynamics currently involves the analysis of parameters calculated from Doppler waveforms at the intrarenal arteries [i.e., resistive index (RI), pulsatility index, and acceleration time]. These parameters have been used clinically to diagnose various renal diseases, including renovascular hypertension, ureteral obstruction, and acute rejection in transplanted kidney [1–3]. However, the parameters calculated from the Doppler velocity waveform do not always reflect renal arterial blood flow or renal tissue blood flow directly. Furthermore, it is difficult to obtain the Doppler waveform precisely in a clinical setting from time to time.

Power Doppler (PD)-US enables the depiction of small arterial flow in the renal cortex [4, 5]. Currently, changes in renal perfusion induced by vasoactive drugs are assessed using the PD-US method [6]. Moreover, some investigators have reported quantitative estimation using the intensity of PD signals in the kidney [7, 8].

In the present study, we estimated the direct correlation between renal cortical blood flow and the mean pixel intensity of PD signals in the same region. Furthermore, we evaluated renal hemodynamic changes in two-kidney, one-clip (2K-1C) hypertensive dogs using the PD-US technique.

METHODS

The experimental protocols were approved by the Experimental Animal Care and Use Committee of our institution.

Correlation between renal cortical blood flow and pixel intensity in PD signals

Five mongrel dogs (body weight 9 to 15 kg) were used in this experimental protocol. All procedures were performed under general anesthesia by the inhalation of nitrous oxide, oxygen and 1% to 2% halothane (Takeda, Osaka, Japan) with endotracheal intubation and mechanical respirator after an intravenous bolus injection of sodium pentobarbital (25 mg/kg; Dainippon Pharmaceutical, Osaka, Japan). The animals were put in supine position, given 0.45% sodium chloride and 2.5% glucose in water intravenously at rate of 0.1 mL/kg/min using

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continuous infusion pump, and a 20 G plastic arterial catheter was placed in a femoral artery to monitor mean arterial blood pressure (MABP) throughout the study.

Through a midline abdominal incision, an adjustable screw clamp [9] was placed around the left main renal artery to reduce its lumen. Blood flow in the left main renal artery was measured by the ultrasonic transit time method with a 2 mm flow probe (Transonic System, Inc., Ithaca, NY, USA) placed just distal to the clamp. Renal cortical blood flow in the left kidney was measured by the electrolytic hydrogen gas clearance method with a 0.08 mm wire-type electrode (Unique Medical, Tokyo, Japan). Under progressive constriction of the left main renal artery, we simultaneously measured (*1*) blood flow at the main renal artery, (*2*) renal cortical blood flow, and (*3*) the mean pixel intensity and the power signal ratio of PD flow signals at the same region.

PD scanning method and image analysis. All US examinations were performed using an ALOKA SSD-5500 (Aloka, Tokyo, Japan) with a 5-MHz sector transducer. PD images were obtained using the lowest flow gain. These were oblique longitudinal section and the wire electrode was visualized in each image. All PD images were transferred to a G4 Macintosh computer (Apple Computer, Cupertino, CA, USA) and analyzed by the image-analysis software Openlab® (Improvision, Coventry, UK). In each image, a region of interest (ROI) of 5 mm diameter circle was drawn three times near the electrode. Pixel intensity was measured in equal increments from 0 for black to 31 for maximum brightness in the PD flow signal. The mean pixel intensity and power signal ratio were calculated within the same ROI according to the following formulas:

$$\text{Mean pixel intensity} = \text{total pixel intensity} / \text{total number of pixels}$$

$$\text{Power signal ratio} = (\text{total number of pixels with PD signal} / \text{total number of pixels}) \times 100$$

Statistical analysis. The statistical analysis was performed by SPSS 11.0 (SPSS, Inc., Chicago, IL, USA). The significance of correlations was determined using Pearson's product-moment coefficients. A P value of less than 0.05 was considered to indicate statistical significance.

Quantitative PD imaging of renal cortical perfusion in chronic renovascular hypertensive dogs

In six male mongrel dogs (body weight 13–19 kg), two-kidney, one-clip (2K-1C) hypertension was produced according to the two-step technique described by Masaki et al [10]. Briefly, an adjustable clamp was placed at the left renal artery, and it was screwed to reduce the renal blood flow approximately 50%. After two weeks, the left renal artery was obstructed completely by an adjustable screw clamp using a screwdriver extracorporeally. These six animals were used in our previous study [11]. At least four years after 2K-1C hypertension was created, PD images of both the clipped and non-clipped kidneys were acquired under the same anesthesia and preparation described as the above. PD images were obtained before and after the administration of angiotensin-converting enzyme (ACE) inhibitor (captopril; Sankyo, Tokyo, Japan) or sodium nitroprusside (Maruishi Pharmaceutical, Osaka, Japan) in each kidney. Captopril was infused intravenously at a rate of 0.2 mg/kg/min after bolus injection (2 mg/kg). The dose of sodium nitroprusside was altered to reduce the mean blood pressure to be similar to that with captopril administration.

Conventional gray-scale US was performed to measure the longitudinal diameter of each kidney. PD images were acquired before and 60 minutes after the administration of each drug at 10-minute intervals. At each experimental period, PD images of the kidneys were recorded on VHS videotape.

Before and 60 minutes after administration of each drug, blood samples were obtained from arterial catheter to measure plasma renin activity (PRA). PRA was measured by radioimmunoassay (Dade Behring, Chiba, Japan).

PD scanning method and image analysis. All US examinations were performed using ALOKA SSD-6500 (Aloka, Tokyo, Japan) with a 5-MHz convex transducer. Sagittal PD-US images of each kidney were acquired using the lowest flow gain, and these settings were maintained throughout the entire experiment.

All PD images were transferred from the videotape to a computer at 30 frames per second. PD images were divided into blocks that lasted for the duration of three consecutive heart beats and analyzed using Openlab® (Improvision). A ROI was drawn over the whole renal cortex to quantify flow signal levels. Mean pixel intensity and power signal ratio within the ROI were calculated for each frame during 3 heart beats and averaged.

Statistical analysis. The same analysis software described as the above was used for this protocol. All data were expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) for repeated measures was performed. Differences between groups were analyzed by Student t test. Changes were considered statistically significant at $P < 0.05$.

RESULTS

Correlation between renal blood flow and PD signals

In all five dogs, MABP was stable and no major complications were observed during this experiment.

Measurable changes in renal cortical blood flow did not occur until renal arterial blood flow was reduced by

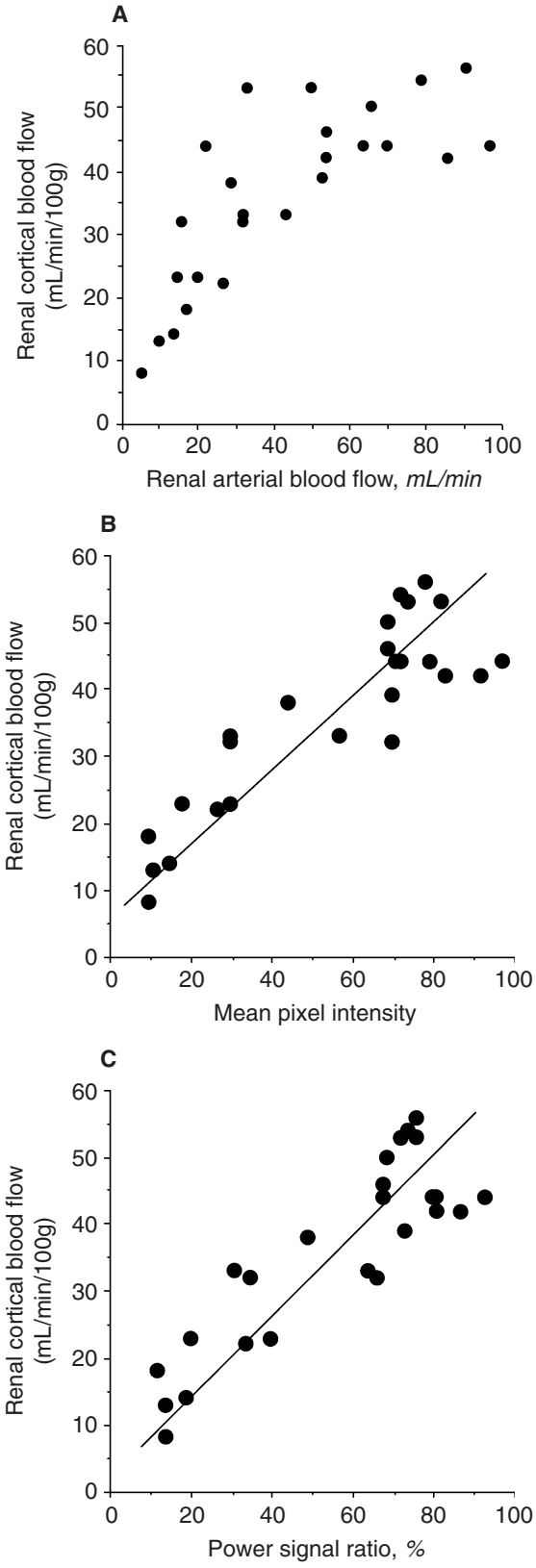

Fig. 1. (*A*) Correlation between renal arterial blood flow and renal cortical blood flow. (*B*) Correlation between renal cortical blood flow and mean pixel intensity ($r = 0.878$, $P < 0.0001$). (*C*) Correlation between renal cortical blood flow and power signal ratio ($r = 0.873$, $P < 0.0001$).

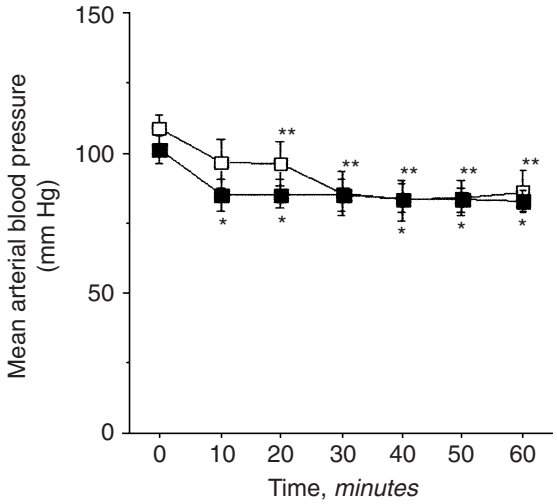

Fig. 2. Effects of captopril (closed square) or sodium nitroprusside (open square) on mean arterial blood pressure. Values are mean ± SEM. $^{*}P < 0.05$ vs. before captopril (expressed as 0 min). $^{**}P < 0.05$ vs. before sodium nitroprusside (expressed as 0 min).

40%. Beyond this point, linear decreases in renal cortical blood flow were observed (Fig. 1A).

The mean pixel intensity decreased linearly with a decrease in renal cortical blood flow. There was a very close correlation ($r = 0.878$, $P < 0.0001$) between renal cortical blood flow and the mean pixel intensity of PD flow signals within the same region (Fig. 1B). Furthermore, a similar linear relationship ($r = 0.873$, $P < 0.0001$) was observed between the power signal ratio and renal cortical blood flow within the same region (Fig. 1C).

Quantitative PD imaging of renal tissue perfusion in chronic renovascular hypertensive dogs

In chronic 2K-1C hypertensive dogs, the longitudinal diameter of the clipped kidney was significantly smaller than that of the non-clipped kidney (48 ± 3.0 mm vs. 60.2 ± 1.6 mm, $P < 0.05$).

Effects of captopril on renal cortical blood flow in 2K-1C hypertensive dogs. During the chronic phase of 2K-1C hypertension, mean pixel intensity and power signal ratio in the clipped kidney were markedly diminished compared to those in the contralateral non-clipped kidneys.

The infusion of captopril significantly reduced MABP (Fig. 2), whereas heart rate did not change. Although MABP was significantly reduced after captopril administration, both mean pixel intensity and power signal ratio were increased in both the clipped and non-clipped kidneys. These changes were statistically significant compared to the values obtained before ACE inhibition ($P < 0.05$, Fig. 3A and B). PRA was significantly increased after captopril administration (Fig. 4).

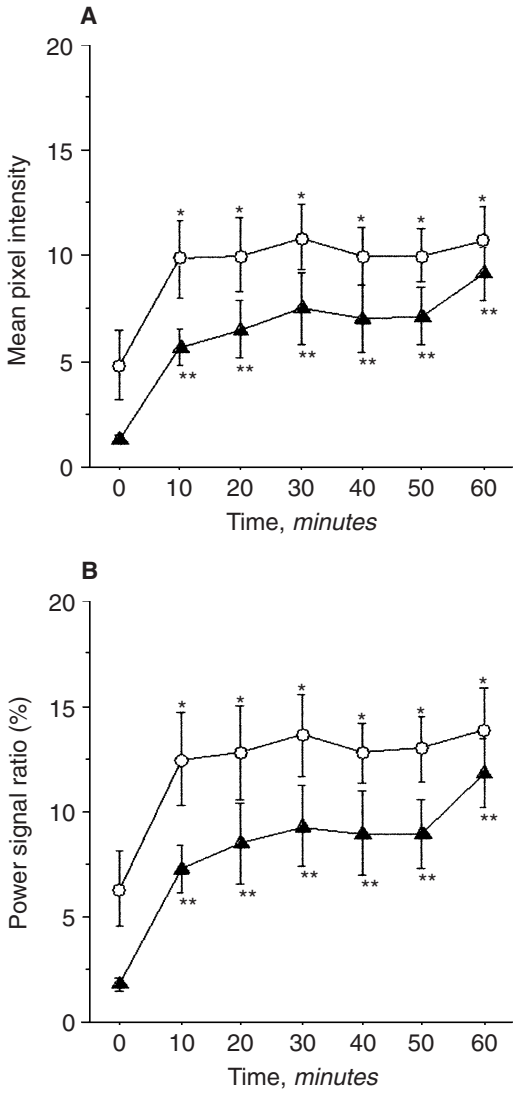

Fig. 3. Effects of captopril on mean pixel intensity (*A*) and power signal ratio (*B*) in clipped (closed triangle) and non-clipped (open circle) kidney. Values are mean ± SEM of data for six dogs. *, **$P < 0.05$ vs. before captopril (expressed as 0 min).

Effects of sodium nitroprusside on renal cortical blood flow in 2K-1C hypertensive dogs. Quantitative PD imaging showed significant differences in the baseline renal cortical perfusion between clipped and non-clipped kidneys. While the infusion of sodium nitroprusside produced significant MABP reduction equivalent to that with the infusion of captopril (Fig. 2), mean pixel intensity and power signal ratio did not change significantly in either clipped or non-clipped kidneys (Fig. 5A and B). Modest rise in PRA was observed after administration of sodium nitroprusside (Fig. 4).

DISCUSSION

With recent advances in Doppler US technology, the evaluation of renal blood flow by Doppler US has become

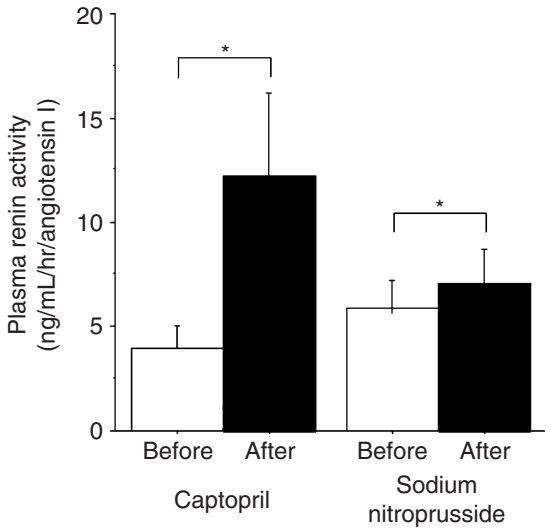

Fig. 4. Effects of captopril or sodium nitroprusside on plasma renin activity before (open bar) and 60 minutes after (closed bar). Values are mean ± SEM. *$P < 0.05$ vs. before captopril or sodium nitroprusside.

an important screening device for patients with suspected renal artery stenosis. The downstream effects of a proximal renal artery are currently analyzed using spectral Doppler waveforms obtained from the intrarenal arteries. We previously reported that an acute reduction in renal blood flow produced a linear decrease in blood flow velocities and RI values in segmental arteries in autotransplanted canine kidney [12]. Furthermore, we also demonstrated that peak systolic velocity and end diastolic velocity at the interlobar arteries in clipped kidneys in chronic 2K-1C hypertensive dogs were significantly diminished compared to those in the contralateral nonclipped kidneys [11]. However, the precise measurement of intrarenal arterial blood flow velocity is sometimes time-consuming, cumbersome, and difficult in a clinical setting, especially in obese patients. Therefore, to achieve wider general acceptance, another noninvasive and simple US technique is expected for the quantitative estimation of renal hemodynamics.

Recently, it has become possible to measure the intensity of US images, and attempts have been made to estimate renal blood flow [7, 8, 13–15]. In the present study, measurable changes in renal cortical blood flow did not occur until a 40% reduction in blood flow at the main renal artery. Beyond this critical point, there was a linear correlation between renal arterial blood flow and renal cortical blood flow. Under these conditions, there was a very close linear correlation between renal cortical blood flow and the mean pixel intensity of PD flow signals in the same region. According to these findings, the quantitative estimation of renal cortical blood flow with PD-US may be practical for depicting changes in renal tissue perfusion.

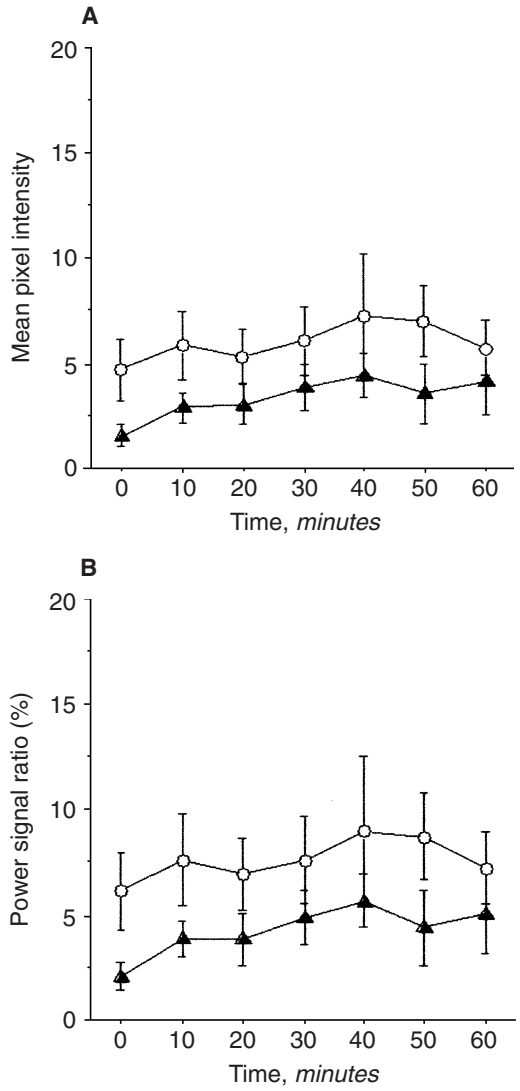

Fig. 5. Effects of sodium nitroprusside on mean pixel intensity (*A*) and power signal ratio (*B*) in clipped (closed triangle) and non-clipped (open circle) kidney. Values are mean ± SEM. No significant change was observed.

Based on the results of our preliminary experiment, we evaluated intrarenal hemodynamic changes in chronic 2K-1C hypertensive dogs using PD-US. During the chronic phase of 2K-1C hypertension, the mean pixel intensity of the clipped kidneys was significantly decreased compared to that in the non-clipped kidneys. Our study clearly demonstrated that the reduction in size and the mean pixel intensity of the ischemic kidney are important diagnostic signs for severe unilateral renal artery stenosis.

In the present study, despite significant systemic blood pressure reduction, significant increases on the mean pixel intensity in both clipped and non-clipped kidneys were observed after ACE ihibition by captopril in chronic 2K-1C hypertensive dogs. These renal hemodynamic effects of ACE inhibition were in agreement with findings from our previous experiments performed in the same 2K-1C hypertensive canine model [16]. In contrast, no significant change in the mean pixel intensity was observed after sodium nitroprusside infusion in 2K-1C hypertensive dogs, while systemic blood pressure was reduced comparable degree to that with captopril administration. These findings suggest that ACE inhibition reduces renal vascular resistance and significantly increases renal tissue perfusion in 2K-1C hypertensive dogs, and that the renin-angiotensin system plays a major role in renal hemodynamics in both clipped and non-clipped kidneys. We also previously demonstrated significant increases in flow velocities at the interlobar artery in clipped kidneys after angiotensin II blockade in chronic 2K-1C dogs [16]. The present study indicated that these velocity changes may result from increases in renal cortical blood flow due to the renal vasodilatory effects of angiotensin II blockade.

The analysis of PD flow signals may represent a noninvasive, real-time method for the quantitative estimation of renal hemodynamics. Scholbach et al [17] and Kuwa et al [18] have reported similar approaches to quantify the renal cortical blood flow by imaging analysis software using color Doppler (CD) or PD image, and their methods enable the noninvasive quantitative estimation of renal blood flow. However, studies including ours unveiled several limitations regarding the clinical application of this technique. While it is relatively easy to acquire CD or PD images of the kidneys, the data must be stored in a video recorder and then transferred to a computer to estimate the intensity of CD or PD signals using imaging-analysis software. However, although this data processing is quite time consuming at this stage, this problem should be able to be resolved by incorporating analysis software in the ultrasound system. Another limitation involves the interpretation of quantitative ultrasound indices. These values extremely depend on the US equipment and the condition of system settings. Therefore, care are must be taken when interpreting and comparing values between individuals or groups because the mean pixel intensity is a relative value. We recommend that renal blood flow indices estimated using PD-US, including mean pixel intensity and power signal ratio, should be assessed by comparing them to the values in the contralateral kidney or by examining the effects of drugs including ACE inhibitor.

CONCLUSION

The mean pixel intensity of PD signals in the kidney is strongly correlated with renal tissue perfusion. Quantitative estimation of renal cortical blood flow with PD-US may be useful for investigating renal physiology and for providing a diagnosis in a variety of renal disorders, including renovascular hypertension.

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