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ORIGINAL ARTICLE

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Contrast-enhanced ultrasonography with Sonazoid for evaluation of renal microcirculation

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Abstract
Purpose. The renal medullary microcirculation plays an important role in regulating sodium and water excretion, and its impairment is closely associated with various renal diseases. Contrast-enhanced ultrasonography (CEUS) using Sonazoid has not yet been reported as a method for evaluating the renal microcirculation; consequently, this study was carried out to reveal the characteristics of renal microcirculation using CEUS with Sonazoid.
Methods. CEUS was performed on three healthy volunteers after they had fasted for at least 6 h. A GE LOGIQ7 ultrasound unit was used with a 2.0- to 5.5-MHz convex probe. Within approximately 1 min of intravenous injection of 0.0050 ml/kg Sonazoid, contrast images of the right kidney were acquired using a coded phase-inversion mode. Time-intensity curves were calculated for the cortex and medulla.
Results. Peak contrast intensity was significantly higher in the cortex (–56.4 ± 1.9 dB) than in the medulla (–66.7 ± 1.7 dB; $P < 0.005$). Peak times were significantly earlier in the cortex (17.4 ± 3.7 ms) than in the medulla (28.8 ± 6.3 ms; $P < 0.05$).
Conclusion. CEUS using Sonazoid enables differentiation between the cortical and medullary microcirculation and is useful in clarifying renal pathophysiology and pharmacology.

Keywords contrast-enhanced ultrasonography · renal microcirculation · cortex · medulla · microbubbles

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Introduction

The exquisite vascular architecture of the kidney allows differential regulation of the cortical microcirculation and the medullary microcirculation. The renal medullary microcirculation plays an important role in the regulation of sodium and water excretion,[1–3] and its impairment is a key mechanism responsible for many renal diseases.[4–9] Analysis of the renal microcirculation, particularly the medullary microcirculation, is thus considered one of the best methods for evaluating severity and prognosis in many renal diseases. However, few modalities for evaluating the renal microcirculation have proven reliable, accurate, and easy to use in clinical settings. Contrast-enhanced ultrasonography (CEUS) using Sonazoid enables real-time evaluation of the microcirculation in several tissues[10–12] and can be safely used in subjects with renal disorders,[13,14] but it has not yet been reported as a method for evaluating the renal microcirculation. The present study was performed to determine the feasibility of CEUS using Sonazoid in evaluating the renal microcirculation and to clarify its characteristics in healthy volunteers.

Materials and methods

Subjects

The present study was approved by the Ethics Committee at Nara Medical University. All subjects provided written informed consent to participate in the study after the nature of the procedures had been fully explained.

Subjects comprised three healthy male volunteers (age 31.0 ± 6.6 years). None of the subjects had a history of smoking or any family history of hypertension in first-degree relatives. In addition, no evidence of previous or current cardiovascular, respiratory, renal, or hepatic disease was obtained from medical histories. No subjects had received any medication for 1 month before starting the study.

CEUS was performed twice, at least 7 days apart. Subjects fasted from 9:00 p.m. on the evening before the beginning of the study and did not have breakfast the following day; they were asked to consume as little water as possible. They were also requested to refrain from consuming alcohol and food and drink containing caffeine from the day before the beginning of the study until the end of the study.

Hemodynamic analysis

An HEM-780 automated sphygmomanometer (Omron, Matsusaka, Japan) was used to record heart rate and blood pressure (BP), and a Sonos 5500 echocardiography unit (Philips, Eindhoven, the Netherlands) was used to evaluate left ventricular ejection fraction (LVEF) and aortic, mitral, pulmonary, and tricuspid function. LVEF was calculated using the modified Simpson method.[15] All measurements were obtained with the subjects in the supine position in a quiet room after the subjects had evacuated their bladders.

Renal and neurohormonal assays

Blood samples were taken with subjects in the supine position immediately after hemodynamic evaluation. Blood urea nitrogen (BUN), serum creatinine (Scr), cystatine C, angiotensin II, and aldosterone concentrations and plasma renin activity (PRA) were measured at a contract laboratory (SRL, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the simplified Modification of Diet in Renal Disease (MDRD) equation: $eGFR\ (ml/min/1.73\ m^2) = 0.741 \times 175 \times age^{-0.203} \times Scr^{-1.154}$ ($\times 0.742$, if subject is female)[16] (age: years; Scr: mg/dl). Urinalysis was performed using a Multistix SG reagent strip (Bayer, Leverkusen, Germany).

Ultrasonography protocol for the kidney

A LOGIQ7 ultrasound unit (GE Medical Systems, Milwaukee, WI, USA) with a 2.0- to 5.5-MHz convex probe (4C) was used in this study. The long-axis view of the right kidney was obtained by placing the probe over the lower back during inspiratory breath-holding with the subject in the lateral position.

Conventional ultrasonography parameters were bilateral renal size and resistance index (RI) in the area of the interlobar artery by the color-duplex technique.[17,18] CEUS was performed using the coded phase-inversion mode at a mechanical index of 0.16–0.20. The depth of focus was approximately 9 cm at the inferior margin of the kidney and the frame rate was 10–12 Hz. Sonazoid (Dai-ichi Sankyo, Tokyo, Japan), which contains microbubbles of perfluorocarbon gas encapsulated in a phospholipid shell, was used as the contrast agent.[13] Sonazoid was administered as a 0.0050 ml/kg bolus together with 10 ml of saline solution over 3–5 s. Perfusion was subsequently quantified using the inbuilt time intensity curve (TIC) software of the ultrasonography system.

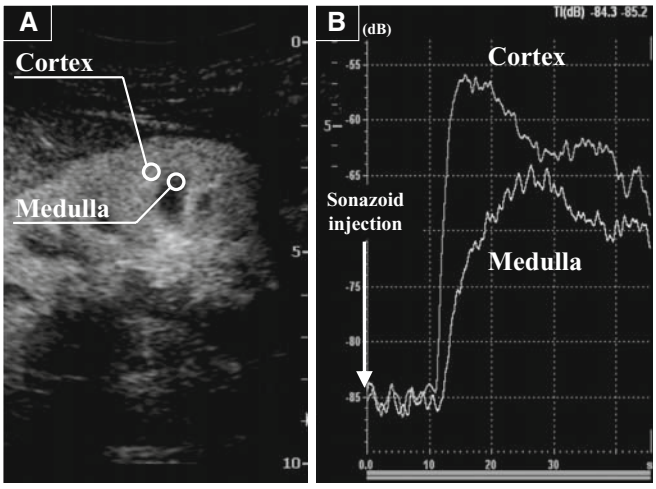

Fig. 1. A Setting regions of interest (ROIs). A circular ROI with a diameter of 5 mm was carefully placed in the area of the *cortex* and *medulla*. **B** Time intensity curve (TIC) showed bimodal patterns in both *cortex* and *medulla*. First peak contrast intensity (CI) and time were significantly higher and earlier in the cortex than in the medulla

The method for setting regions of interest (ROIs) is shown in Fig. 1A. Circular ROIs with a diameter of 5 mm were placed in the area of the cortex and the medulla. ROIs of the medulla were selected in the central, most clearly visualized area, and those of the cortex were carefully selected at an area distant from the arcuate arteries. Both ROIs were placed as close to the same depth as possible. A digital clip was reviewed to identify and correct any misplacement of ROIs and to eliminate minor motion artifacts. Contrast intensity (CI) was defined as the mean value within the ROI. The first peak CI and time were compared between cortical and medullary TICs.

Statistics

Statistical analysis was performed using StatView 4.5 software (Abacus, Berkeley, CA, United States). Data are presented as mean ± SD. An unpaired *t* test was used to check for any differences between two sets of measurements. Linear regression and Bland–Altman analysis were used to examine correlations and agreement between two sets of measurements.[19,20] Values of $P < 0.05$ were considered statistically significant.

Inter- and intraobserver variability

The first observer (S.O.) and the second observer (T.H.), who were both blinded to previous results, randomly repeated TIC analysis to quantify inter- and intraobserver error.

Results

Subject characteristics

Subject characteristics are shown in Table 1. BP, renal function, and the renin–angiotensin–aldosterone system were all normal. No renal disease was present, including renal artery stenosis and primary aldosteronism. Echocardiography showed normal left ventricular wall motion with no valvular diseases.

Ultrasonography

Data from conventional renal ultrasonography are shown in Table 2. Renal size and RI values were within normal ranges and did not differ significantly between right and left kidneys. After injection of the contrast agent, significant enhancement of both the cortex and medulla was observed in all subjects. A typical sequence of CEUS images is shown in Fig. 2. The cortex was quickly enhanced a few seconds after injection, whereas the medulla remained almost hypoechoic. The medulla was slowly and peripherally enhanced. A typical TIC and analysis data are shown in Fig. 1B and Table 3, respectively. The TIC in the cortex showed bimodal patterns, whereas the TIC in the medulla did not clearly show a second peak because of motion artifacts. In the cortex, the first peak CI was higher than the second peak. The first peak CI was significantly higher in the cortex (–56.4 ± 1.9 dB) than in the medulla (–66.7 ± 1.7 dB; $P < 0.005$). The first peak time was significantly earlier in the cortex (17.4 ± 3.7 s) than in the medulla (28.8 ± 6.3 s; $P < 0.05$). The intraobserver repeatability of the first peak CI is shown in Fig. 3. There was a significant positive correlation between the first and second peak CI measurements in the cortex ($r = 1.00$, $P < 0.01$) and medulla ($r = 1.00$, $P < 0.01$), respectively. Bland–Altman analysis showed a bias of 0.05 with 95% limits of agreement of –0.94 to 1.04 in the cortex, and a bias of 0.15 with 95% limits of agreement of –0.71 to 1.01 in the medulla, and thus revealed good intraobserver repeatability. Interobserver repeatability of the first peak CI is shown in Fig. 4. There was a significant positive correlation between observer A and B in the cortex ($r = 1.00$, $P < 0.01$) and medulla ($r = 0.99$, $P < 0.01$), respectively.

Table 1. Characteristics of the volunteer subjects

Age (years)	31.0 ± 6.6	BUN (mg/dl)	15.7 ± 4.7
Male/female	3/0	Scr (mg/dl)	0.79 ± 0.14
Body mass index (kg/m^2)	21.3 ± 1.1	Estimated GFR (ml/min/1.73 m^2)	87.2 ± 13.3
Systolic BP (mmHg)	111.0 ± 9.8	Cystatine C	0.76 ± 0.09
Diastolic BP (mmHg)	66.5 ± 8.3	Plasma renin activity (ng/ml/h)	1.1 ± 0.2
Pulse (beats/min)	65.2 ± 3.8	Plasma angiotensin II level (pg/ml)	6.3 ± 0.6
Echocardiography EF (%)	60.4 ± 5.2	Plasma aldosterone level (pg/ml)	106.4 ± 36.7

Values are mean ± SD
Estimated glomerular filtration rate was calculated using the simplified MDRD equations
BP, blood pressure; EF, ejection fraction; BUN, blood urea nitrogen; Scr, serum creatinine; GFR, glomerular filtration rate

Table 2. Conventional renal ultrasonography

	Right kidney	Left kidney	*P*
Long axis diameter (mm)	104.1 ± 10.4	104.6 ± 12.0	0.96
Short axis diameter (mm)	53.7 ± 4.1	55.9 ± 3.4	0.66
Resistance index	0.63 ± 0.05	0.63 ± 0.02	0.79

Table 3. Contrast-enhanced renal ultrasonography

	Cortex	Medulla	*P*
Peak time (s)	17.4 ± 3.7	28.8 ± 6.3	<0.05
Contrast intensity (dB)	–56.4 ± 1.9	–66.7 ± 1.7	<0.005

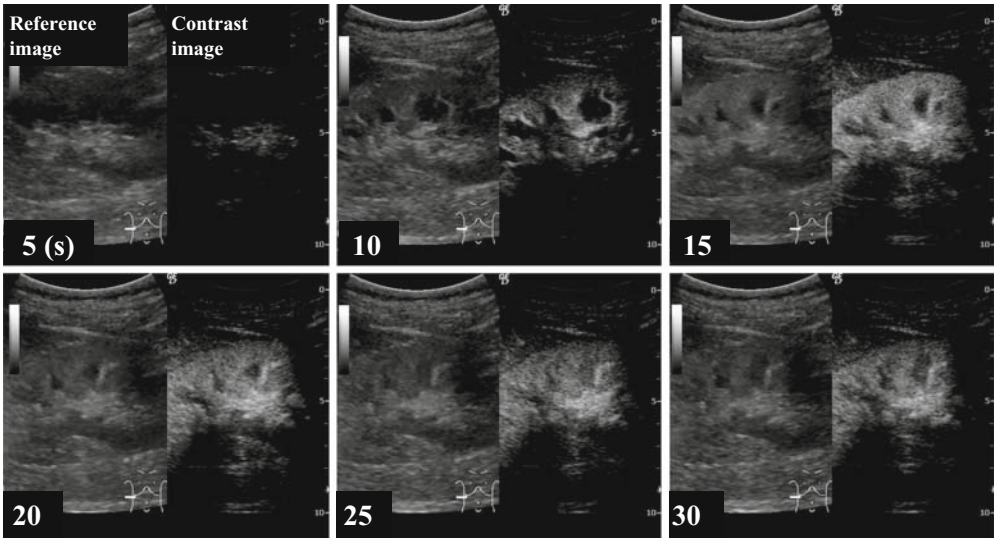

Fig. 2. Temporal course of Sonazoid enhancement. The cortex was quickly enhanced a few seconds after injection, whereas the medulla remained almost hypoechoic. The medulla was slowly and peripherally enhanced

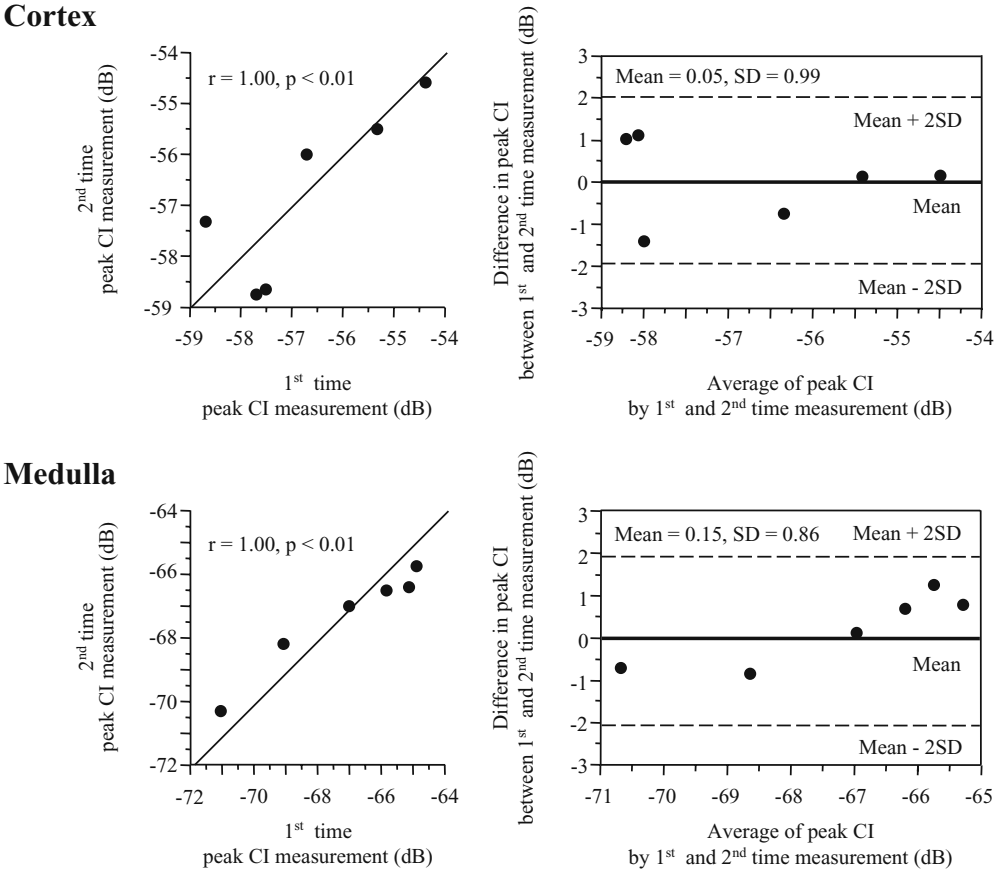

Fig. 3. Intraobserver repeatability of the first peak *CI* in the *cortex* (*upper row*) and *medulla* (*lower row*). Correlation (*left*) and Bland–Altman analysis (*right*) between the first and the second time assessment

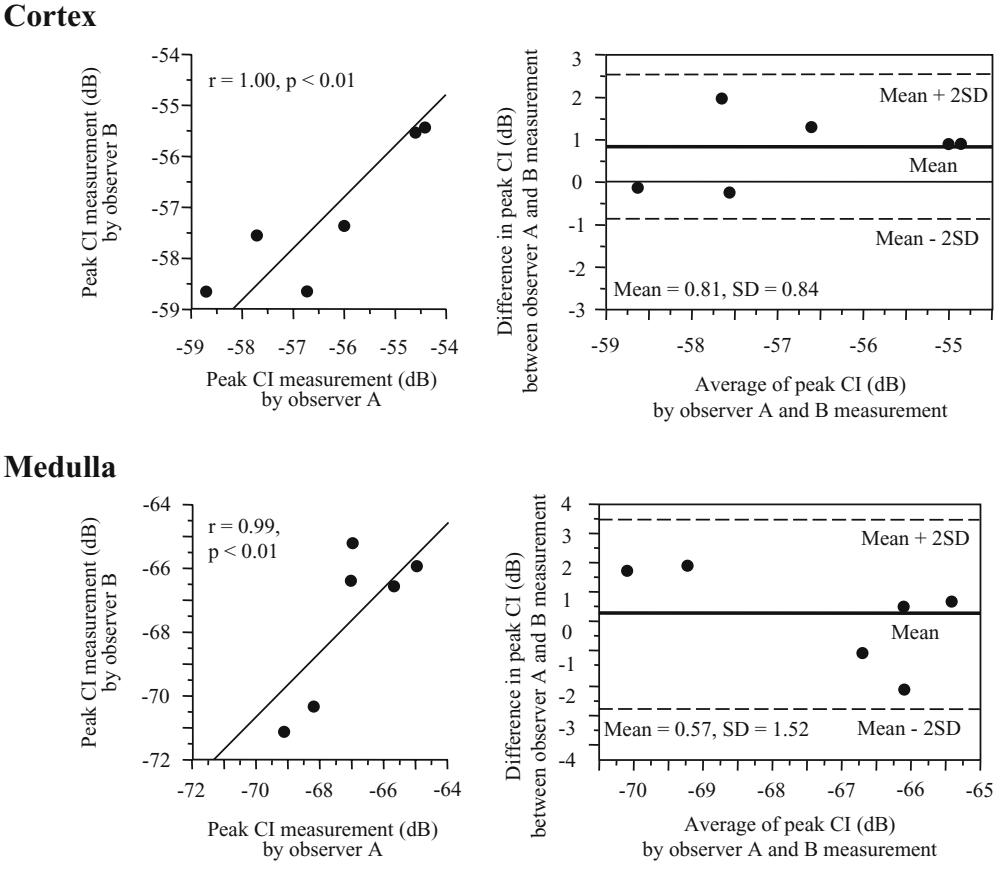

Fig. 4. Interobserver repeatability of the first peak CI in the *cortex* (*upper row*) and *medulla* (*lower row*). Correlation (*left*) and Bland–Altman analysis (*right*) between observers A and B

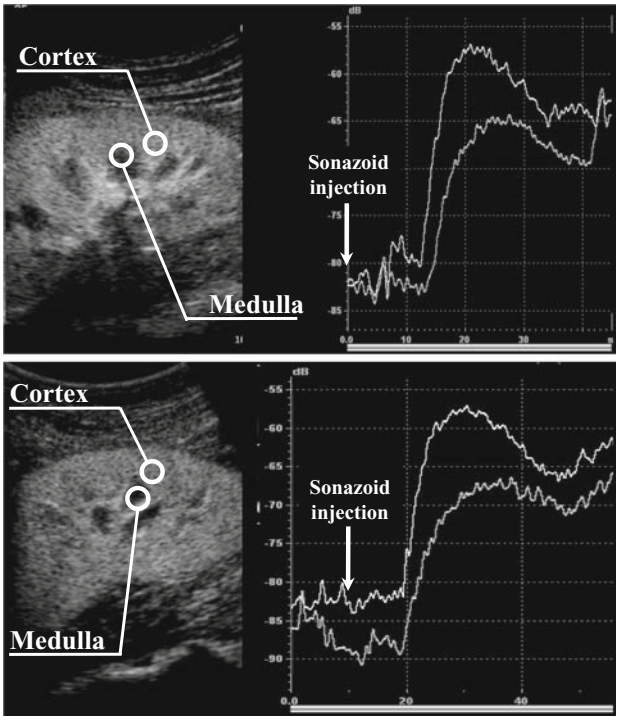

Fig. 5. Typical repeatability of a volunteer

1st time Peak measurement

	Peak CI (dB)	Peak time (s)
Cortex	57.7	21.2
Medulla	65.2	28.0

2nd time Peak measurement

	Peak CI (dB)	Peak time (s)
Cortex	58.7	21.5
Medulla	67.0	36.5

Bland–Altman analysis showed a bias of 0.81 with 95% limits of agreement of –0.03 to 1.65 in the cortex, and a bias of 0.57 with 95% limits of agreement of –0.95 to 2.09 in the medulla, and thus revealed good interobserver repeatability. Typical repeatability of a single volunteer is shown in Fig. 5.

Discussion

The present study showed that CEUS using Sonazoid enables reliable, real-time evaluation of renal microcirculation with differentiation between cortex and medulla, and it shed light on two characteristics of the renal microcirculation.

The first peak CI is significantly lower and the first peak time is significantly more delayed in the medulla than in the cortex, representing lower levels of microcirculation in the medulla than in the cortex. According to several reports,[1–3] approximately 90% of renal blood flow remains in the cortex, with the remaining 10% of blood flow perfusing the medulla through the vasa recta arising from the postglomerular vasculature. Total tissue blood flow is significantly higher in the cortex (700 ml/min/100 g) than in the medulla (50–300 ml/min/100 g). Moreover, blood flow velocity is low in the vasa recta. Our study data were compatible with these previous findings.

In addition, the TIC shows bimodal patterns in the cortex. The first peak is higher than the second peak, representing the first and second passes of Sonazoid, respectively. The second peak is not considered appropriate for evaluation, as macrophages such as Kupffer cells phagocytose Sonazoid,[21] and extended inspiratory breath-holding induces motion artifacts.

Several methods have recently been developed to evaluate the renal microcirculation, such as RI by color-duplex ultrasonography,[17,18] CEUS using microbubble contrast agents other than Sonazoid,[22–28] perfusion computed tomography (CT),[29] and blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI).[30–32] Color-duplex ultrasonography displays difficulty in detecting small lesions and low blood flow. RI is calculated from the velocity in the interlobar artery and cannot differentiate between microcirculations of the cortex and medulla. CEUS using several microbubble contrast agents other than Sonazoid have been attempted to evaluate renal microcirculation. These include Sono Vue (Altana Pharma, Konstanz, Germany), Levovist (Bayer Schering, Berlin, Germany), and Optison (Amersham, Princeton, NJ, USA). In many of these methods, renal microcirculation was quantified by destroying microbubbles and measuring their tissue replenishment with intermittent harmonic imaging during a continuous venous infusion of the microbubbles, which were not phagocytosed by macrophages. This microbubble destruction and replenishment method is hardly affected at all by the infusion procedure and cardiac function and may be superior to our method using Sonazoid. However, these methods reportedly do not differentiate between the microcirculations of the cortex and medulla in humans. Perfusion CT using iodinated contrast media cannot be used in patients with severe renal disease. BOLD-MRI is an attractive method for evaluating tissue oxygenation without contrast agents, but it requires expensive and specialized equipment. Moreover, renal tissue oxygenation as evaluated by BOLD-MRI is affected by oxygen supply, consumption, and clearance,

and it varies independently of renal vascular oxygenation. For these reasons, CEUS using Sonazoid may be more widely applicable for the evaluation of the renal microcirculation compared to other modalities in the future due to its reliability, accuracy, ease of use, safety, and cost-effectiveness.

Study limitations and technical difficulties

The present study displays some potential limitations and a technical difficulty. First, the number of subjects enrolled in the present study was very small. Although we were only able to recruit three healthy volunteers due to the strict limitations on lifestyle, blood and urine collection, echocardiography, and CEUS, these restrictions on the selection of subjects are believed to have increased the quality of the present study. Second, TICs calculated from the first pass data may have been affected by the infusion procedure and cardiac function. However, we found that the infusion procedure and cardiac function affected the time from injection to contrast arrival but did not affect TIC in several organ tissues, which is probably attributable to the low dose of microbubble contrast agent injected. Third, the exact placement of ROIs in the medulla was not easy. Many medullas were difficult to clearly visualize in image sequences because the medulla is relatively small and sterically arranged in the kidney. Moreover, planar correction of misplaced ROIs could be performed to eliminate motion artifacts, but steric correction was not possible. This technical obstacle in CEUS may be overcome by the development of 3-dimensional CEUS and automatic ROI placement. Finally, our results should be confirmed in multicenter studies using a larger number of subjects and patients with various renal diseases.

Conclusion

CEUS using Sonazoid allowed noninvasive evaluation of the renal microcirculation with differentiation between cortex and medulla. The present results were compatible with past findings regarding the renal microcirculation. This method is thus useful for elucidating pathophysiology in many renal diseases and for investigating the effects of drugs on the renal microcirculation.

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