A DISSERTATION FOR THE DEGREE OF MASTER

Effect of Catheter Size and Saline Flush Injection Rate on Renal Contrast-Enhanced Ultrasonography with Sonazoid® in Dogs

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Abstract

The aim of this study was to evaluate effects of catheter size and saline flush injection rate on several renal perfusion parameters and variation of parameters obtained with contrast-enhanced ultrasonography (CEUS) in 5 healthy dogs. In 5 beagles, CEUS was performed with intravenous injection of contrast medium (perflubutane microbubble, Sonazoid®)

followed by the injection 5 mL of 0.9 % saline at different rates of 1, 3 and 5 mL/sec through a 20-gauge (G) catheter. After 7 days, CEUS was performed by the same procedure described, except that a 24 G catheter was placed. The CEUS using a 20 or 24 G catheter was repeated three times. Time-intensity curves (TIC) were created, and perfusion parameters were calculated by using off-line software. Repeatability was determined by calculating the coefficient of variation (CV). The perfusion parameter showed no differences between catheter sizes. For the cortex, time to peak (TTP) was affected by the injection rate, and there was a tendency toward lower TTPs at higher injection rates. The CEUS parameters with the lowest CVs between the injection rates were TTPs obtained from the cortex. Higher repeatability was present for perfusion parameters related to the slope and blood volume compared with TTPs. There was a significant difference in CVs obtained from the cortex between injection rates of 1 and 5 mL/sec, and CVs tended to decrease as the injection rate increased. Other parameters were not correlated with injection rates. The results show that the use of a 24-G catheter does not alter CEUS with Sonazoid® renal perfusion parameters and can be applied in small dogs. Moreover, for accuracy, a rate of 5 mL/s is recommended when injecting saline for canine renal CEUS.

Keywords: Contrast-enhanced ultrasound (CEUS), renal perfusion, catheter size, saline flush injection, repeatability

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Abbreviations

AUC	Area under curve
CEUS	Contrast-enhanced ultrasonography
CV	Coefficient of variation
G	Gauge
MI	Mechanical index
PE	Peak enhancement
ROI	Regions of interests
TIC	Time-Intensity curve
TTP	Time to peak
W_{in}	The slope of ascending curve
W_{out}	The slope of descending curve

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Introduction

Contrast-enhanced ultrasonography is an ultrasonographic technique that is used to examine organ microcirculation and applied in veterinary medicine to detect lesions or pathological conditions within organs (Dietrich et al. 2012; Haers and Saunders 2009; Chung and Kim 2015). Ultrasound contrast agents, which consist of encapsulated microbubbles stabilized by an outer shell, remain in the intravascular space and do not cross the vascular endothelium, allowing for dynamic visualization of microvasculature and quantification of tissue perfusion (Tang et al. 2011; Hyvelin et al. 2013; Quaia 2007). Commercially available ultrasound contrast agents, such as Levovist®, Definity®, or SonoVue®, have been used in canine CEUS, and the safety and usefulness of such agents have been assessed (Yanagisawa et al. 2007; Haers and Saunders 2009; Kanemoto et al. 2009). Sonazoid®, which consists of perfluorobutane gas microspheres stabilized by a hydrogenated egg phosphatidyl serine membrane, has higher and longer stability than other contrast agents as well as a delayed parenchymal (Kupffer) phase produced by agent phagocytosis within the reticuloendothelial system of the liver and spleen (Yanagisawa et al. 2007; Nihonmatsu et al. 2016; Tsuruoka et al. 2010; Sontum 2008; Tang and Eckersley 2007). Due to these features, Sonazoid® has been applied to many organs, including the canine spleen and liver (Matsuzawa et al. 2015; Kanemoto et al. 2009; Lim et al. 2015; Hong et al. 2018;

Kanemoto et al. 2008).

Compared to Doppler ultrasonography, CEUS has higher sensitivity to slow blood circulation or microvascular structures within the kidney, and thus, in human and veterinary medicine, it is a promising technique for diagnosing several kidney diseases or detecting renal lesions (Sidhu et al. 2018; Seiler et al. 2013; Haers and Saunders 2009). Since renal CEUS is a functional imaging technique permitting the evaluation of renal tissue perfusion and function, it can be applied to diffuse renal disorders involving blood flow or structural changes, such as chronic kidney disease or ischemic renal injury (Sidhu et al. 2018; Haers and Saunders 2009; Stock, Paepe, Daminet, Vandermeulen, et al. 2018; Lee et al. 2017; Fang et al. 2017; Haers et al. 2013). CEUS with Sonazoid® enables real-time evaluation and prolonged visualization of the microcirculation in renal cortex and medulla without nephrotoxicity in human medicine (Tsuruoka et al. 2010; Okayama et al. 2008), but it has not yet been reported as the renal CEUS using Sonazoid® in canine veterinary medicine.

The clinical value of CEUS is complicated by relatively large variation in several factors, including the animal physiological condition, contrast agent used, and ultrasound platform imaging and quantification settings (Hyvelin et al. 2013; Tang et al. 2011). Of these factors, ultrasound contrast agent injection variables, such as catheter diameter or injection rate, may affect CEUS perfusion parameters (Hyvelin et al. 2013; Tang et al. 2011; Eisenbrey et al. 2015; Dizeux et al.

2016; Palmowski et al. 2010). In human medicine, the bolus injection method performed with a saline flush after injection of the contrast agent is used widely in CEUS for the organ perfusion and a 20-gauge (G) catheter and quick saline flush injection are recommended (Dietrich et al. 2018; Dietrich et al. 2012). However, achieving intravenous access with a 20-G catheter may not be feasible in some small animals. Thus, when using renal CEUS for evaluating microvascular flow in a small animal, uncontrolled saline flush injection may be a strong source of variability due to the animal's smaller blood volume compared to that of humans (Dizeux et al. 2016).

The aim of this study was to evaluate effects of catheter size and saline flush injection rate on several renal perfusion parameters and variation of parameters obtained CEUS in 5 healthy dogs. The hypothesis was that a 24-G catheter would not significantly affect renal perfusion parameters and the saline flush injection rate would be associated with changes in renal perfusion parameters.

Materials and Methods

1. Animals

Five adult beagle dogs including two females and three males, each weighting 8.4 to 14.3 kg (mean, 11.6 kg; SD, ±2.74) were used. The animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee, Seoul National University, Seoul(SNU-180517-4). All dogs were clinically healthy based on physical examination, systolic blood pressure, complete blood count, and serum biochemistry without any evidence of clinical signs of renal disorders. The median body condition score was 6/9 (range, 4/9-7/9).

2. Ultrasonographic examinations

Dogs were fasted for at least 12 hrs before ultrasonographic examination. The hair was clipped over the ventrolateral portion of the abdomen and coupling gel was applied to the skin. A 20-G indwelling catheter was placed in the cephalic vein (20-G catheter group). Sedation was administered intravenously with acepromazine (0.01 mg/kg, Sedaject®, Samu Median Co., Seoul, South Korea) and contrast

agent (Sonazoid®, Daiichi Sankyo Corporation, Tokyo, Japan), 0.0125 mL/kg, was injected intravenously followed by the injection of a 5.0 mL saline bolus. This injection was not used for further analysis, because it results in lower enhancement compared to that of the subsequent injection (Stock et al. 2017). After 15 min, physical exam parameters including respiratory and heart rates were checked and systolic blood pressure was measured using the oscillometric technique (Suntech Vet20; Sun Tech Medical, North Carolina, USA). Following this, CEUS was performed using the same transducer after reducing the transmitted energy to a magnitude of 8 % via a mechanical index (MI) setting of 0.17−0.19 and settings of 30 Hz and 62 % gain for the pulse repetition frequency. Sequential CEUS images of the left kidney were continuously stored for 90 sec after initiation of three bolus injections of contrast agent, 0.0125 mL/kg, into the cephalic vein via a three−way stopcock with an intravenous catheter, immediately followed by the injection 5mL of 0.9% saline at different rates of 1, 3 and 5 mL/sec with a power injector (Stellant; MedRAD, Pittsburgh, USA). The peak injection pressure of the venous catheter was measured by a power injector. After saline injection was over, physical exam parameters and systolic blood pressure were measured again. To avoid artifacts, between subsequent injections, remnant microbubbles were destroyed by setting the acoustic power to the highest level and scanning the caudal abdominal aorta for 2 minutes. Following this process, the next injection proceeded after confirming that there were no remnant microbubbles for 5 to 10

mins. After 7 days, CEUS images were obtained by repeating the same procedure as described, using a 24-G indwelling catheter was placed (24-G catheter group). The CEUS procedure for each catheter size was repeated three times.

3. Image analyses

The acquired dynamic cine loops were analyzed at 30 frames/sec by using integrated software (SOP-ALPHA 7-14, Hitachi-Aloka). For each dog, three circular-shaped regions of interests (ROIs; an area of 0.11 cm^2) located and two ROIs were placed over the renal medulla at the same level (Figure1). Depth of the ROIs was located and calculated as the distance between the body wall and left kidney, was approximately 2.5 cm. During ROI selection, vascular structures such as the arcuate, interlobar, and interlobular arteries and surrounding tissues were excluded. For every ROI, the software created a time-intensity curve (TIC). TICs were analyzed for peak enhancement (PE), time to peak (TTP), total area under the curve (AUC), the slope of ascending curve (W_{in}) and the slope of descending curve (W_{out}). The values for the three ROIs for the cortex and two ROIs for the medulla were averaged. The coefficient of variation (CV), defined as the ratio of the standard deviation over the mean, was determined for the repeated CEUS procedures.

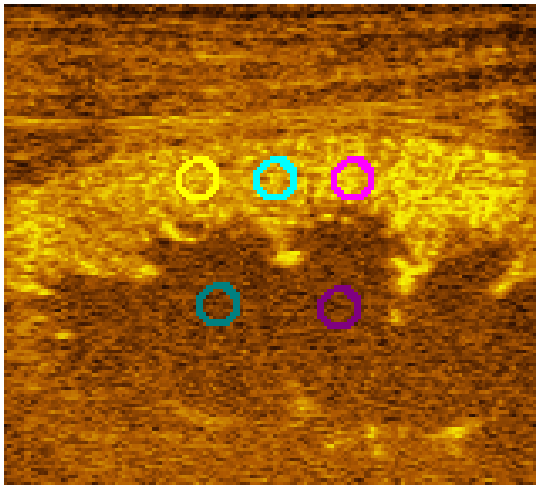

Figure 1. Contrast-enhanced ultrasound image of the left kidney of a dog obtained at peak enhancement, illustrating placement of the regions of interest (ROI). The three ROIs in the renal cortex and the two ROIs in the medulla are located.

4. Statistical analyses

Statistical analyses were performed using statistical software (SPSS statistical program, IBM SPSS Statistics 23, IBM Corporation, NY). Repeated measures ANOVA with Tukey's test and Jonckheere-Terpstra test were used to compare CEUS parameters using 20- and 24-G catheters at 1, 3, and 5 mL/s injection rates. All statistical analysis of the CV data was performed using Microsoft Excel. The CVs of CEUS parameters obtained from each CEUS procedure were calculated for each injection rate. Repeated measures ANOVA with Tukey's test was used to evaluate the effect of the injection rates and catheter sizes on CV. Mann-Whitney U test were used to compare CV values between parameters or renal cortex and medulla. A difference was considered statistically significant at $P < 0.05$.

Results

1. Physical exam parameters, systolic blood pressure, and venous catheter pressure

Pre- and post-CEUS scan respiratory rate, pulse, and indirect systolic blood pressure measurements are summarized in Table 1. There was no significant difference between pre- and post-scan parameters.

Contrast enhancement was clearly visible in the 20- and 24-G catheter groups. The measured venous catheter pressure ranged from 0–26 psi, with a mean pressure of 11.2 psi in the 24-G group. In the 20-G group, venous catheter pressure ranged from 0–15 psi, with a mean pressure of 3 psi. For both catheter groups, all pressure values measured at an injection rate of 1 mL/s were zero.

Table1. Physical exam parameters and systolic blood pressure at the point of pre- and post-scans

(Mean ±SD)

Parameter	Point	Dog number				
		1	2	3	4	5
Systolic blood pressure, mmHg	Pre-scan	120.5±13.6	116.9±12.3	119.3±18.7	136.9±15.6	127.5±3.0
	Post-scan	119.4±8.1	112.7±15.7	117.7±20.5	135.5±14.5	138.9±19.1
Pulse, beats per minute	Pre-scan	86.5±24.4	103.7±14.4	125.0±31.7	101.9±19.5	107.1±21.2
	Post-scan	69.7±15.8	105.2±25.0	123.8±24.1	100.5±21.5	100.6±18.7
Respiratory rate, breaths per minute	Pre-scan	27.4±5.6	27.3±3.8	30.8±5.1	28.4±4.8	28.0±3.7
	Post-scan	26.8±5.8	26.6±6.0	29.3±3.0	28.0±4.3	26.8±3.9

2. Renal CEUS perfusion parameters

During CEUS, the initial contrast enhancement of the renal cortex was homogeneous and intense. Moreover, the initial contrast enhancement of the renal medulla was invariably more delayed than that of the renal cortex. The enhancement pattern of the renal medulla was usually coarser, variably heterogeneous, and mildly hypoechoic compared to that of the renal cortex.

Quantitative CEUS parameters for the renal cortex and medulla at different injection rates with 20- and 24-G catheters are summarized in Tables 2 and 3. The CEUS parameters had no significant differences associated with catheter size. In the renal cortex, TTPs at an injection rate of 1 mL/s were significantly different from 3 and 5 mL/s ($P < 0.01$), whereas there was no difference between renal medulla parameters and injection rate. In the renal cortex, there was a tendency toward lower TTPs with higher injection rates ($P < 0.05$).

Table 2. Renal cortical perfusion parameters based on the saline flush injection rate and catheter size

(Mean ±SD)

	Injection Rate with a 24−gauge catheter			Injection Rate with a 20−gauge catheter		
Variable	1 mL/sec	3 mL/sec	5 mL/sec	1 mL/sec	3 mL/sec	5 mL/sec
TTP	9.67±1.25	8.20±1.25[a]	7.43±1.51[a]	9.83±1.36	8.09±0.76[a]	7.05±0.80[a]
PE	27.48±4.94	28.85±9.17	32.07±6.60	20.29±4.44	26.36±6.17	32.09±8.87
AUC	12492.39 ±856.39	12428.28 ±1211.87	13026.70 ±838.08	11240.72 ±879.27	12067.86 ±1229.15	13314.52 ±1354.67
W_{in}	42.90±7.83	44.99±12.30	45.39±13.34	36.01±9.66	44.34±7.52	50.54±6.16
W_{out}	−0.93±0.04	−1.02±0.12	−1.01±0.15	−0.88±0.10	−0.99±0.06	−0.95±0.10

Abbreviations: TTP, time to peak; PE, peak enhancement; AUC, area under curve; W_{in}, slope of ascending curve; W_{out}, slope of descending curve

[a] Post hoc test comparison significantly different ($P < 0.01$) from an injection rate of 1 mL/sec

Table 3. Renal medulla perfusion parameters based on the saline flush injection rate and catheter size

(Mean ±SD)

Variable	Injection Rate with a 24-gauge catheter			Injection Rate with a 20-gauge catheter		
	1 mL/sec	3 mL/sec	5 mL/sec	1 mL/sec	3 mL/sec	5 mL/sec
TTP	15.00±1.77	12.93±1.86	12.98±3.37	16.77±1.40	14.32±1.31	13.36±1.28
PE	11.78±1.21	11.11±0.90	13.79±2.05	9.52±1.44	11.91±2.65	14.44±2.16
AUC	12299.47 ±551.07	12059.75 ±424.99	12312.64 ±556.02	11286.30 ±827.26	11595.88 ±622.15	12092.12 ±974.48
W_{in}	3.35±0.69	4.12±1.46	5.50±2.56	3.37±1.20	4.01±1.81	6.32±0.97
W_{out}	−0.27±0.14	−0.24±0.04	−0.27±0.09	−0.22±0.08	−0.32±0.17	−0.42±0.12

Abbreviations: TTP, time to peak; PE, peak enhancement; AUC, area under curve; W_{in}, slope of ascending curve; W_{out}, slope of descending curve

3. CVs of renal CEUS perfusion parameters

The CVs for the CEUS parameters are presented in Tables 4 and 5. There were no significant differences in CVs associated with catheter size. The CEUS parameters with the lowest CVs between injection rates were TTPs obtained from the renal cortex. Although no significant differences in CVs between the injection rates of 1 and 3 mL/s or 3 and 5 mL/s were observed, there was a significant difference in CVs between the injection rates of 1 and 5 mL/s ($P < 0.05$). Moreover, in both catheter sizes, the CV of TTP obtained from the renal cortex tended to decrease as the injection rate increased ($P < 0.01$). The W_{in} of the TIC measured from the renal cortex was higher than cortical TTP, PE, and AUC ($P < 0.05$). The W_{in} and W_{out} of the TIC measured from the renal medulla were higher than medulla TTP, PE, and AUC ($P < 0.05$). The TIC slope parameters were significantly higher in the renal medulla compared to the renal cortex ($P < 0.05$). Moreover, no significant differences were found between the renal cortex and medulla for the other parameters.

Table 4. CV values for perfusion parameters assessed in the renal cortex after three repeated renal CEUS based on the saline flush injection rate and catheter size (Mean ±SD)

		Injection Rate with a 24−gauge catheter			Injection Rate with a 20−gauge catheter		
	Variable	1 mL/sec	3 mL/sec	5 mL/sec	1 mL/sec	3 mL/sec	5 mL/sec
CV (%)	TTP	8.8±1.8	5.3±2.2	4.7±1.9[a]	11.4±9.3	5.8±4.0	3.5±1.5[a]
	PE	14.3±5.7	11.9±6.8	10.6±9.5	12.7±8.0	12.6±4.2	12.2±6.7
	AUC	16.6±10.8	18.0±4.3	10.8±6.8	14.1±8.4	18.7±8.6	20.2±7.3
	W_{in}*	39.8±16.9	35.1±23.7	16.9±5.6	20.9±8.8	31.0±12.3	26.6±7.5
	W_{out}	16.0±6.9	25.5±24.8	13.3±13.0	14.1±9.4	12.3±9.3	16.9±10.1

Abbreviations: TTP, time to peak; PE, peak enhancement; AUC, area under curve; W_{in}, slope of ascending curve; W_{out}, slope of descending curve

[a] Post hoc test comparison significantly different ($P < 0.05$) from an injection rate of 1 mL/sec

* Mann−Whitney test comparison significantly different ($P < 0.05$) from TTP, PE and AUC

Table 5. CV values for perfusion parameters assessed in the renal medulla after three repeated renal CEUS based on the saline flush injection rate and catheter size (Mean ±SD)

		Injection Rate with a 24-gauge catheter			Injection Rate with a 20-gauge catheter		
	Variable	1 mL/sec	3 mL/sec	5 mL/sec	1 mL/sec	3 mL/sec	5 mL/sec
CV (%)	TTP	15.9±5.2	†16.6±6.4	†18.7±15.1	17.0±11.5	10.3±6.2	†13.5±4.7
	PE	13.9±5.5	14.4±6.3	14.2±7.3	7.5±4.3	12.4±6.4	12.0±5.7
	AUC	11.8±7.3	13.5±3.4	9.4±6.5	7.8±4.7	12.0±5.1	14.4±6.9
	W_{in}*	†55.1±14.1	†56.0±17.6	†46.7±32.9	†59.0±21.3	†40.2±17.3	†56.0±30.8
	W_{out}*	†57.7±18.8	†62.1±53.9	†45.2±26.5	†45.7±32.0	†33.0±14.8	†41.3±19.1

Abbreviations: TTP, time to peak; PE, peak enhancement; AUC, area under curve; W_{in}, slope of ascending curve; W_{out}, slope of descending curve

* Mann-Whitney test comparison significantly different ($P < 0.05$) from TTP, PE and AUC

† Within an injection condition, parameter differs significantly ($P < 0.05$) from the parameter obtained from the renal cortex

Discussion

In this study, I demonstrated CEUS imaging with Sonazoid® in normal canine kidneys. To my knowledge, this is the first report of renal CEUS study with Sonazoid® in normal canine kidneys. The second-generation agent Sonazoid® has a phospholipid shell in each microbubble as well as stable long-term contrast enhancement compared to first-generation agents (Tsuruoka et al. 2010). The Sonazoid®, unlike other second-generation agents such as Sonovue® or Definity®, has a delayed parenchyma phase caused by the Kupffer cell uptake of agents (Nolsoe and Lorentzen 2016; Yanagisawa et al. 2007). The contrast enhancement pattern was similar to that in previous CEUS reports of normal canine kidneys using other second-generation contrast medium (Waller, O'Brien, and Zagzebski 2007; Macrì et al. 2016; Choi et al. 2016). In human medicine, there are many methods for assessment of the perfusion (Frohlich et al. 2015; Quaia 2011). One method, in which microbubbles are destroyed and tissue replenishment is measured during continuous venous infusion of other non-phagocytosed second-generation agents, is used for renal perfusion since it is less influenced by physiological factors (Okayama et al. 2008; Palmowski et al. 2010). However, this method reportedly can only assess the wash in phase and do not differentiate between the arterial and portal flow or the perfusions of the cortex and medulla (Okayama et al. 2008;

Claudon et al. 2013; Dietrich et al. 2012). The bolus injection method is preferred in clinical medicine due to the convenience of operation and evaluation and the ability to evaluate perfusion of multiple tissue regions, and this method is also used in CEUS for assessment of the renal perfusion in veterinary medicine (Okayama et al. 2008; Dietrich et al. 2012; Haers and Saunders 2009). The renal perfusion using dynamic contrast-enhanced US after bolus injection of Sonazoid® enables prolonged visualization of the renal microcirculation in human medicine (Okayama et al. 2008; Tsuruoka et al. 2010). In special situations such as deep lesions, obese patients and cirrhotic lesion, a relatively higher MI value is required (Dietrich et al. 2011). Sonazoid® is better tolerated by pressure stress and have a higher stability than other second-generation agents (Nihonmatsu et al. 2016; Tang and Eckersley 2007; Sontum 2008). For these reasons, Sonazoid® in renal CEUS imaging may be more applicable for the evaluation of the renal perfusion in veterinary medicine.

Hydrostatic pressure during administration is an important factor involved in microbubbles destruction and this factor is dependent on catheter or needle size, injection rate, concentration of agent, etc (Eisenbrey et al. 2015; Talu et al. 2008; Barrack and Stride 2009). In human medicine, a 20-G catheter is recommended to minimize microbubble destruction during passage through the cannula, and a catheter size smaller than 22-G is contraindicated (Dietrich et al. 2018). In previous canine or feline studies about renal perfusion, 20- or 22-G catheters lager than 24-G were used in CEUS renal perfusion

to avoid microbubble destruction (Stock, Paepe, Daminet, Vandermeulen, et al. 2018; Stock, Paepe, Daminet, Duchateau, et al. 2018; Lee et al. 2017; Macrì et al. 2016; Stock et al. 2017; Haers et al. 2013; Stock et al. 2016). However, these recommended catheter sizes are not likely to be applicable in small sized animal clinics and the minimally required administration route size should allow easier and less painful intravenous access in small animals. In several canine and feline studies, CEUS renal perfusions were performed using 24- or 25-G catheters smaller than the inner diameters at which microbubble destruction occurred in other in vivo or in vitro studies (Eisenbrey et al. 2015; Barrack and Stride 2009; Leinonen et al. 2010; Talu et al. 2008; Choi et al. 2016). However, the catheter size suggested in these in vivo or in vitro studies can be varied by the injection method, the type of contrast agent and imaging techniques (Eisenbrey et al. 2015; Talu et al. 2008; Barrack and Stride 2009). Additionally, the canine and feline renal CEUS studies using 24- or 25-G catheters did not prove whether microbubbles were destroyed (Choi et al. 2016; Leinonen et al. 2010). The TIC parameters derived from the bolus technique allows for the estimation of blood flow and volume of organs (Dietrich et al. 2012). Since it is assumed that the signal intensity in CEUS is proportional to the amount of microbubbles which remain strictly intra-vasal, the PE and AUC correlated to the blood volume of the ROI can evaluate whether there is microbubbles destruction (Dietrich et al. 2012; Eisenbrey et al. 2015). In this study, there were no significant differences in PE and the AUC between the 20- and

24-G sizes, indicating that catheter size did not affect microbubble destruction. . Moreover, in human medicine, saline flush injection is recommended as either a manual bolus injection or at a rate of 2 mL/s (Dietrich et al. 2018; Claudon et al. 2013; Dietrich et al. 2012; Piscaglia et al. 2012). However, the correlation of injection rate with microbubble destruction has been controversial and there is a possibility of the microbubble destruction at either high or low injection rates with small size of catheters (Talu et al. 2008; Barrack and Stride 2009). In present study, since there is no difference in the PE and AUC among saline flush injection rates. This result shows that the microbubble destruction is not affected by saline flush injection rates applied in this study.

Although several studies through experimental animal models show that the injection rate can affect perfusion parameters (Dizeux et al. 2016; Palmowski et al. 2010; Hyvelin et al. 2013), the manual bolus injection of contrast agents and saline in clinical human medicine (Dietrich et al. 2012; Dietrich et al. 2018). However, this injection procedure can be a factor in increasing the variation in small animal veterinary clinics with relatively less weight (Tang et al. 2011; Sidhu et al. 2018; Dietrich et al. 2018). TTPs were observed to be decreased significantly as a function of saline flush injection rate from 1 to 5 mL/sec in this study. This correlation between the injection rate and TTP is in agreement with previous studies of CEUS in experimental animal models but other parameters including PE are in disagreement (Hyvelin et al. 2013; Palmowski et al. 2010). Although the reason of this discrepancy is unknown

precisely, the experimental animal models in previous studies are very small in body weight and may be more sensitive to the amount of contrast agent and may be expected to be due to physiological differences between species or body weights. There are several studies on significant changes in TTP of the renal cortex associated with renal disease such as chronic kidney disease, acute kidney failure or ischemic kidney injury (Dong et al. 2013; Choi et al. 2016; Fang et al. 2017; Stock, Paepe, Daminet, Vandermeulen, et al. 2018). The previous study on renal CEUS of healthy dogs in the long term suggested that TTP may be useful in detecting early changes in renal perfusion related to the development of chronic kidney disease (Liu et al. 2018). Therefore, considering the importance of TTP shown in several studies, my results would expect to play an important role in future studies on renal CEUS for the diagnosis of kidney disease.

CEUS is associated with a relatively high degree of variability, the minimization of the variability and improvement of the reproducibility are important issues in the recognized added value of CEUS in clinical research (Tang et al. 2011; Hyvelin et al. 2013; Dietrich et al. 2012). The highest variation in this study was related to slope parameters and these parameters showed relatively higher variation than TTP, PE and AUC. The CV for PE or AUC for the renal cortex and medulla was lower compared with those previously reported in cat kidneys, ranging about 41–67% for manual injections for the renal cortex and medulla (Stock, Duchateau, et al. 2018). Compared with another study in dog kidneys, our variability for

PE or AUC for the renal cortex and medulla was lower (Liu et al. 2018). The CV for PE or AUC for the renal cortex was similar to the study on mice for the cortex using controlled injection method, ranging about 8–16% (Dizeux et al. 2016). The previous study on feline and canine kidneys showed that the variation of the all perfusion parameters was higher in the renal medulla than in the renal cortex, but no significant increase is observed in this study except for the parameters related to the slope (Liu et al. 2018; Stock, Duchateau, et al. 2018). The correlation between the values related to the slope for cortex and medulla is agreement with previous study and likely to be related to the anatomic and physiologic features of the medullary blood (Stock, Duchateau, et al. 2018; Liu et al. 2018), but the reason about no change of parameters including TTP, PE and AUC between cortex and medulla was unknown. In mice and rat, the manual injection of the contrast agent increases variations of the renal CEUS perfusion parameters and this measurement bias in CEUS could be overcome by the controlled injection (Hyvelin et al. 2013; Dizeux et al. 2016). The study on mice kidneys reported that the controlled injection method reduced the variation of all perfusion parameters (Dizeux et al. 2016). Therefore, although it is not clear, there is a possibility that the controlled injection method influences the results of this study.

Perfusion of the renal cortex, which is the main structure of blood flow fed into the kidney, is related to the renal dysfunction which causes changes in the cortical perfusion (Wang and Mohan 2016; Stock, Duchateau, et al. 2018; Liu et

al. 2018). It is important to improve the reproducibility of TTP of the renal cortex because this parameter was delayed compared to healthy individuals in several studies on dogs and cats with nephropathy such as chronic kidney disease, and has advantages in the detection for early changes of renal perfusion related to the nephropathy (Dong et al. 2013; Choi et al. 2016; Fang et al. 2017; Stock, Paepe, Daminet, Vandermeulen, et al. 2018; Liu et al. 2018). CVs of TTP for the renal cortex, shown to be influenced by the injection rate in this study, at a saline flush injection rate of 5 mL/sec are observed to result in less variation compared to the result at a rate of 1 mL/sec in this present study. TTP and PE of TIC depend on the injection rate of the contrast agent, and the slow injection rate of the contrast agent induces a low bubble concentration during perfusion, and this phenomenon creates the heterogeneous and inadequate enhancement, and this error can be corrected at a rapid injection rate (Tang et al. 2011; Palmowski et al. 2010; Feingold et al. 2010). Therefore, our findings indicate that the injection rate of 5 mL/sec is sufficient to reduce variations of the TTP in renal CEUS in dogs.

As previous described, patient factors such as temperature, pulse, respiratory rate and blood pressure can increase variability of CEUS perfusion parameters and these factors are very difficult to control. To minimize the variation related to physiologic factors, all scans were performed in the room maintained at the constant temperature and humidity. In addition, dogs in this study were sedated by acepromazine, and which of the concentration in the plasma is maintained (half-

life 7.1h) for consecutive scan procedures (Hashem, Kietzmann, and Scherkl 1992). On the other hand, excessive pressure on the venous catheter cause complications such as extravasation, air embolism or pain (Indrajit et al. 2015; Amaral et al. 2006). The pressure of the intravenous catheter measured in this study was measured to be lower than the pressure that could cause side effect (Amaral et al. 2006; Indrajit et al. 2015). In addition, there was no evidence of the vascular rupture after CEUS procedure, and it was confirmed that dogs in this study were uncomfortable or irritated considering no significant change of blood pressure, respiratory rate and pulse before and after scan. Therefore, the catheter size and injection rate used in this study do not cause complications.

This study has some limitations. First, this study included a small number of beagle dogs, which weigh more than many small dog breeds. Therefore, the study results cannot confirm that there is an absence of bubble destruction in small breed dogs using a 24-G catheter. It is also unclear whether the rate of saline injection affects renal CEUS perfusion. Although the main mechanisms of bubble destruction are injection rate and catheter inner diameter, it is thought that differences in body size do not significantly effect bubble destruction (Talu et al. 2008; Eisenbrey et al. 2015). It is not shown how the saline flush injection rate in small breed dogs smaller than the beagle breed affects renal CEUS perfusion parameters, but this effect would be expected to be present in small sized breed dogs because they have the smaller blood volume. Further studies

may find the clear correlation between the injection rate and parameters.

In conclusion, this study demonstrated that CEUS with Sonazoid® permits non-invasive evaluation of renal microcirculation that differentiates between the renal cortex and medulla. The results show that a CEUS procedure using a 24-G catheter does not alter renal perfusion parameters in dogs. Our results also indicate that the use of a saline flush injection rate of 5 mL/sec enhances the reproducibility of the perfusion parameters. Therefore, a 24-G catheter can be applied in small dogs, and a 5 mL/sec saline flush injection rate is recommended to provide better precision.

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국문초록

본 연구 목적은 2세대 초음파 조영제 소나조이드®(perflubutane microbubble, Sonazoid®)를 활용한 개의 정상 신장 조영증강 초음파시 카테터 크기와 생리 식염수 주입 속도가 결과 어떠한 영향을 끼치는 지에 대해 평가하고자 함이다.

다섯 마리의 건강한 비글견을 활용하여 20 G 카테터 장착 및 초음파 조영제 주입 이후 생리식염수를 1,3 그리고 5 mL/sec의 속도로 주입하였다. 7일 이후에 동일한 과정을 24G 카테터 장착 이후 진행하였으며 20 G와 24 G를 장착하여 진행한 CEUS 과정을 총 3번씩 반복 진행하였다. 피질과 수질에서 초음파 프로그램을 이용하여 TIC를 형성하였고 perfusion 수치들을 도출하였다. 반복 측정한 수치들에 대해 평균과 표준편차의 비율인 CV 값을 도출하였다.

실험 결과 20 G와 24 G를 장착하였을 때의 수치들 사이에 명확한 차이점은 확인되지 않았다. 피질의 TTP 수치는 생리 식염수 주입속도가 1 mL/sec 보다 빠를 시에 감소하는 것이 확인되었다. 반복 진행된 초음파 영상 탐색 결과, 가장 낮은 CV 값을 보이는 수치는 피질의 TTP 였으며 높은 CV 값을 보이는 수치는 기울기와 관련된 수치들로 확인되었다. 생리 식염수 주입속도 1 와 5 mL/sec 간에 피질 TTP의 CV 값의 명확한 차이가 있었으며 속도가 빠를수록 CV가 낮게 측정되는 경향이 확인되었다.

본 실험의 결과들을 통해 소나조이드® 를 활용한 신장 CEUS

perfusion 평가 시, 24 G 카테터의 장착이 가능할 것으로 예상되며 생리 식염수 주입속도에 따라 피질의 TTP 수치가 변화할 수 있고 5 mL/sec의 속도 시 변동성이 가장 낮게 확인되는 바, 임상에서 적용시 적합한 속도로 고려된다.

주요어: 조영 증강 초음파, 신장 관류, 카테터 크기, 생리식염수 주입, 재현성

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