Contrast-enhanced ultrasonography of the hepatic vein in normal dogs

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ABSTRACT. Contrast-enhanced ultrasonography (CEUS) of the hepatic vein can assess intrahepatic hemodynamic changes and has been studied as a noninvasive method to assess the severity of portal hypertension and hepatic fibrosis in humans. However, few reports have described its usefulness in veterinary medicine. The purpose of this study was to characterize CEUS findings of the hepatic vein in normal dogs and assess the repeatability of this method both in a conscious group (n=6) and a sedated group (n=6). Sonazoid® (0.01 ml/kg) was used as a contrast agent, and scanning of the hepatic vein was performed for 2 min. Time-intensity curves were generated from regions of interest in the hepatic vein. Four perfusion parameters were measured for quantitative analysis: hepatic vein arrival time (HVAT), time to peak (TTP), time to peak phase (TTPP) and wash-out ratio (WR). CEUS examinations were performed three times in each dog. The median (range) values of HVAT, TTP, TTPP and WR in the conscious group were 13.5 sec (9–22 sec), 12.5 sec (6–24 sec), 8 sec (6–13 sec) and 78.0% (60.7–91.7%), respectively. Median (range) values of HVAT, TTP, TTPP and WR in the sedated group were 12 sec (8–17 sec), 12.5 sec (9–17 sec), 9 sec (7–13 sec) and 84.1% (63.0–94.4%), respectively. The coefficients of variation of these parameters in the conscious and sedated groups were 7.6–29.7% and 11.8–14.8%, respectively.
KEY WORDS: contrast ultrasound, dog, hepatic vein

Some canine patients show consistently elevated liver enzyme levels without clinical signs. Elevations in hepatic enzyme levels can be caused by conditions other than liver disease. For example, 79.3% of dogs with pancreatitis show elevated liver enzyme levels [6]. Liver disease cannot be fully assessed from blood tests alone. Conventional B-mode ultrasonography is useful for detecting focal hepatic abnormalities, such as hepatic tumors and biliary disorders, but is less valuable for recognizing and differentiating diffuse liver disease [5]. Therefore, a liver biopsy is almost always required for an accurate diagnosis. However, some owners decline biopsy due to its invasiveness, and an alternative noninvasive method of assessing the severity of hepatic disease is needed.

In human medicine, assessing the severity of hepatic fibrosis provides important information that predicts patient prognosis and supports clinical management [4, 17]. Although a liver biopsy remains the gold standard for evaluating the severity of hepatic fibrosis [3], alternative noninvasive and repeatable methods have been extensively studied in recent years. One of these methods is ultrasonic elasticity imaging, which measures the propagation velocity of shear waves [18]. Since the velocity of tissue correlates with its elasticity, hepatic stiffness measurements have been widely investigated in human medicine. The other method is contrast-enhanced ultrasonography (CEUS) using microbubbles as a contrast agent, which enables real-time noninvasive assessment of intrahepatic perfusion. Albrecht *et al.* first reported that microbubbles injected peripherally arrived at the hepatic vein (HV) much earlier in cirrhotic patients than in normal volunteers [1]. Sugimoto *et al.* reported that the earlier arrival time in patients with cirrhosis was due to intrahepatic hemodynamic changes, such as arterialization of the liver and the development of intrahepatic shunts. The arrival time of the microbubbles to the portal vein (PV) and hepatic artery (HA) was not significantly different among control subjects, non-cirrhotic patients and cirrhotic patients [19]. Thus far, several studies have suggested that the severity of hepatic fibrosis in patients with chronic liver disease is strongly correlated with early HV arrival time assessed by CEUS [2, 11]. Moreover, estimating portal pressure by using CEUS parameters, such as the HV arrival time or intrahepatic transit time, has been reported [8, 10].

In veterinary medicine, CEUS has been used mainly to characterize the vascularity of focal liver lesions, which can lead to another set of differential diagnoses. Sonazoid®, a second-generation contrast agent, is suitable for parenchymal imaging, because it is phagocytized by Kupffer cells [20–22]. Because of this characteristic, Sonazoid® is used

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to differentiate canine hepatic malignant tumors and benign nodules [9, 14]. However, the assessment of canine hepatic perfusion using Sonazoid® has never been reported in dogs.

The aim of this study was to characterize image enhancement of the normal canine HV using Sonazoid® and to establish quantitative parameters from a time-intensity curve (TIC) both in conscious and sedated dogs. The repeatability of this examination was also evaluated. The results will be a valuable reference for evaluating intrahepatic hemodynamic changes associated with canine chronic hepatic disease.

MATERIALS AND METHODS

Twelve adult beagle dogs, 1–10 years old and weighing 9.5–15.8 kg, were used in this study. Dogs were divided into a conscious group (n=6) and a sedated group (n=6). All dogs were healthy based on physical examination and normal CBC and serum biochemistry including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltranspeptidase, ammonia, and fasting and post-prandial total bile acid levels. Prior to the CEUS study, B-mode US was performed on all the dogs, and no focal or diffuse hepatic abnormalities were noted. All B-mode US and CEUS examinations were performed by the same sonographer (KM) with 10 years of experience in performing liver ultrasound. All procedures were approved by Hokkaido University Animal Care and Use Committee.

An ultrasound scanner (Aplio XG, Toshiba Medical Systems, Otawara, Japan) with a 5–11 MHz broadband linear probe (PLT-704 AT, Toshiba Medical Systems) suitable for pulse subtracting imaging was used for CEUS. Imaging was performed with a low mechanical index of 0.21 and a frame rate of 23 frames per sec. The contrast imaging gain was set at 80 dB, and the focus was set at a depth of 4 cm.

Scanning in the conscious group was performed with only manual restraint. Scanning in the sedated group was performed under anesthesia with propofol at an induction dosage of 6 mg/kg and a maintenance rate of 0.4–0.6 mg/kg/min [7]. The dogs were positioned in left lateral recumbency, and the right HV was identified using an intercostal approach (Fig. 1A). The right HV was imaged to maintain clear visualization of the confluence with the caudal vena cava (CVC) as much as possible. Perfusion of the HV was evaluated after intravenous bolus injection of microbubble contrast agent (Sonazoid®, Daiichi Sankyo, Tokyo, Japan). According to our previous report [12], we administered 0.01 ml/kg Sonazoid® through a 21 G butterfly catheter attached to a 22 G intravenous catheter placed in the cephalic vein, flushed by 2 ml of heparinized saline. Immediately after bolus injection, continuous scanning of the right HV was performed for 2 min. The images were recorded in 40-sec cine-loops to a hard disk for further off-line analysis. CEUS examinations were performed three times in each dog by using the same scanning plan, with a period of more than 48-hr between examinations.

Two operators (KM and AH) performed the quantitative analysis of the CEUS images by using an off-line image analysis system (ImageJ, US National Institutes of Health,

Bethesda, MD, U.S.A.). This system measures intensity using a gray-scale level ranging from 0 to 255 mean pixel value (MPV). One image per sec for the first 60 sec followed by 1 image at an interval of every 5 sec until 120 sec from the start of microbubble contrast agent infusion was analyzed. The region of interest (ROI) was drawn in the right HV within a 1.5-cm distance from the confluence with the CVC as large as possible without including adjacent structures (Fig. 1B), and a TIC was generated for each injection. Four perfusion parameters were measured from each TIC (Fig. 2). The hepatic vein arrival time (HVAT) was the time from contrast agent injection to 20% of peak intensity (PI). Time to peak (TTP) was the time taken from 20% of PI to PI. Time to peak phase (TTPP) was the time taken from 20% to 90% of PI, which reflects the initial upslope of TIC better than TTP. Wash-out ratio (WR) was defined as the attenuation rate from PI to the intensity at the end of a CEUS study.

All data were expressed as the median and range. Statistical comparisons and calculations of coefficient of variation (CV) of each parameter were performed using computer software (JMP11, SAS Institute Inc., Cary, NC, U.S.A.). Normal distribution of the data was assessed using the Shapiro-Wilk test. When distribution approached normality, measured parameters from the conscious and sedated groups were compared using Student's *t*-test. Non-normally distributed data were compared using the Wilcoxon rank sum test. Values of $P<0.05$ were accepted as significant.

RESULTS

The examinations were successfully performed, and the TIC of the HV could be measured clearly in both groups. No adverse events were noted during or after injection of the microbubble contrast agent.

After contrast agent administration, the microbubbles first reached the HA and then the PV. The HV was not enhanced during this period (Fig. 1B). The microbubbles reached the HV after a delay of several seconds. The contrast effect of the HV developed more gradually than that of the PV; it took over 10 sec to reach PI. During the PI phase, the HV was enhanced similar to the liver parenchyma (Fig. 1C) and was followed by a gradual wash-out of the contrast agent with gradual loss of enhancement (Fig. 1D). The intensity of the contrast agent in the HV dropped to almost 20% of PI at the end of the examination, while the contrast agent in the PV retained its intensity.

The TICs derived from the median pixel intensity in the HV were similar in the two groups, but PI was higher in the conscious group (Fig. 3A and 3B).

The measured parameters and CVs for each variable are summarized in Table 1. Not all parameters were significantly different between the two groups. CVs were <20% (range, 11.8 to 14.8%) for all parameters in the sedated group. On the other hand, CVs for HVAT, TTP and TTPP in the conscious group were >20% (range, 25.3 to 29.7%) and were higher than sedated group with the exception of WR (7.6%).
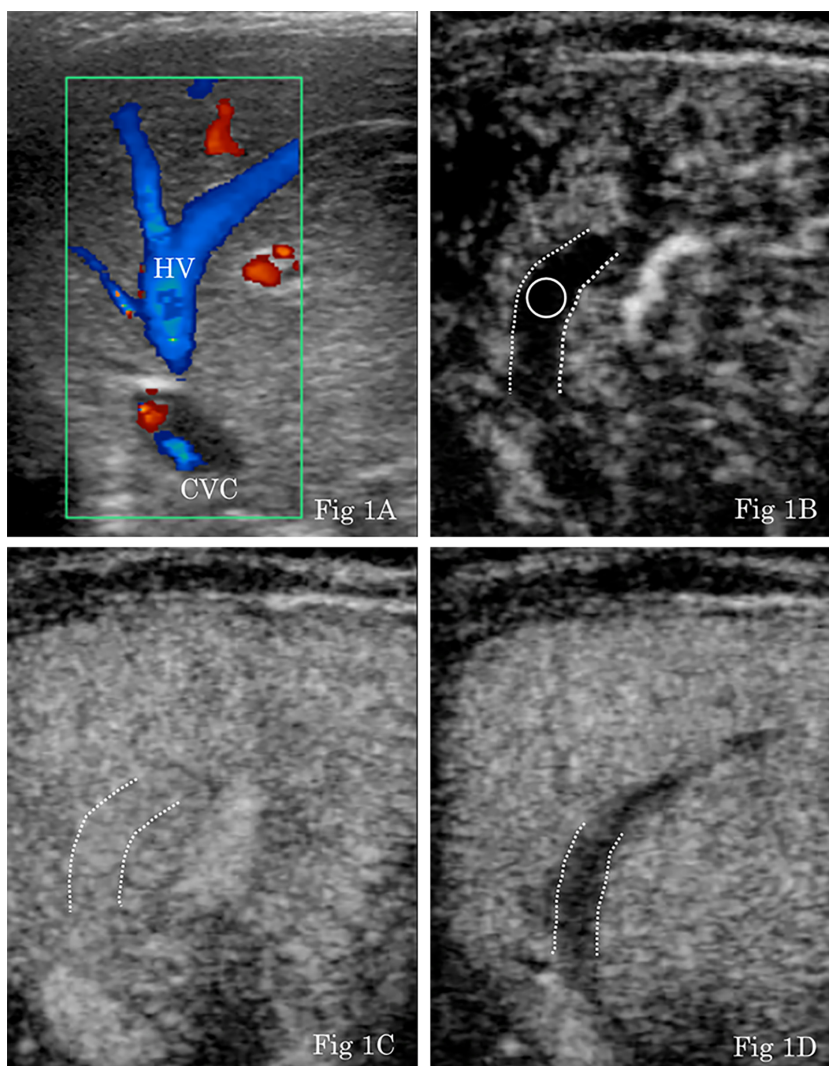
Fig. 1. Color Doppler and CEUS images of the right HV obtained using a right intercostal approach. (A) Right HV displayed in blue with color Doppler flowed into the caudal vena cava (CVC). The operator visualized this transverse image before the CEUS study. (B) At 8 sec, the HV, outlined by a dashed line, was not enhanced, although surrounding liver parenchyma was slightly enhanced. Region of interest (ROI) was manually placed in the HV (circle) to measure the tissue intensity. (C) HV reached its PI, which was similar in intensity to the liver parenchyma (shown here 23 sec after bolus injection of contrast agent). (D) At the end of the examination (120 sec), the contrast agent in the HV appeared washed-out and hypoechoic compared to the liver parenchyma.

DISCUSSION

Our goal was to develop a new, non-invasive method that could assess intrahepatic hemodynamic changes related to underlying progressive hepatic disease. In this study, we characterized contrast-enhanced ultrasonography findings of the HV in normal dogs and evaluated the repeatability of this examination. We chose the right HV for analysis, because it can be imaged constantly while using an intercostal approach without compressing the upper abdomen which can affect the hepatic hemodynamics.

The contrast agent first arrived at the HA followed by the PV. There was a delay of several seconds between these two vessels, and the TIC of the HV rose gradually. This delayed and gradual enhancement of the HV was similar to that observed in the previous human study [1, 10, 19]. Because the HA carries a small amount of microbubbles, the HV is enhanced only after the microbubbles reach the sinusoid from the PV (which has more blood flow than the HA). Since the Sonazoid® used in this study is phagocytosed by Kupffer cells when it passes through the sinusoid, it takes longer for the HV to reach the PI, due to the escape of microbubbles via
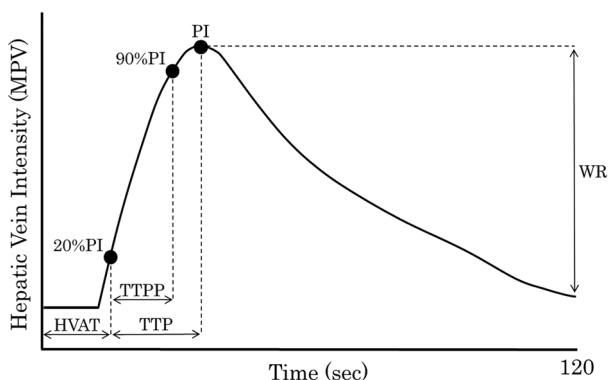
Fig. 2. Schematic illustration of the TIC and measured parameters. The TIC remained unchanged until the contrast agent flowed into the HV. Hepatic vein arrival time (HVAT) was the time when the TIC first reached 20% of peak intensity (PI). Time to peak (TTP) and time to peak phase (TTPP) were defined as the time when TIC reached to PI and to 90% PI, respectively. Wash-out ratio (WR) indicated the attenuation rate from PI to the end of the study.

phagocytosis. After the PI, there is a gradual wash-out of the contrast agent in the HV, while the contrast effect in the PV is still present at the end of the examination. The number of microbubbles that re-enter the sinusoid through the general circulation decreases as time goes on due to phagocytosis by Kupffer cells, which could contribute to the gradual loss of enhancement of the HV.

We established reference values both in conscious and sedated dogs, and also evaluated the repeatability of this examination. Although there was no statistically significant difference in each parameter between the two groups, median value of HVAT was slightly longer in the conscious group. Nyman *et al.* reported that the time to peak enhancement of the hepatic parenchyma, calculated from the time of injection, was significantly shorter in dogs anesthetized with propofol, as compared to that in non-anesthetized dogs [15]. Propofol has been found to increase hepatic arterial blood flow despite having no effect on portal venous flow [23], and the authors speculated that shortened time to peak enhancement was related to the effect of propofol on the vascular system. Although this was observed in the hepatic parenchyma, it is possible that using propofol also affected the HVAT results in the current study. However, statistical differences were not detected between the two groups, possibly because of the relatively low repeatability in the conscious group (CV; 25.3%). Obtaining stable images was slightly difficult in the conscious group compared with the sedated group, and this could have led to the low repeatability. In addition, changes in cardiac output and blood pressure related to the dog's excitation level may have affected the hepatic circulation.

Recent research demonstrated shortening of the HVAT with the development of liver fibrosis in a CCl4-induced canine liver fibrosis model [13]. However, the HVAT was much shorter in the current study compared with that of the baseline value (18.22 ± 0.82 sec) reported in the CCl4-

induced canine liver fibrosis model [13]. We speculate that the difference between our value and the previously reported value is due to differences in contrast agent, the volume of saline flush and/or how rapidly it was administered, and the method used for quantitative analysis. Therefore, the reference values should be applied cautiously, and the methods used to obtain these values should be considered.

TTP and TTPP, which also showed low repeatability in conscious dogs, were not different between the two groups. Even if propofol increases arterial blood flow, the main blood supply associated with the initial upslope is presumably portal blood flow. In addition, because these parameters reflect only the intrahepatic circulation, they may be less vulnerable than the HVAT to extra hepatic factors. Although these parameters are not as common as HVAT in human medicine, Sugimoto *et al.* reported that the HV rising rates in cirrhotic patients were significantly higher than those in the control group and in non-cirrhotic patients [19]. Therefore, the TTP and TTPP, as a reflection of the HV rising rates, could be useful to assess the arterialization of the HV in dogs.

In contrast to the HVAT, TTP and TTPP, WR showed favorable repeatability in both groups. WR can be measured with only two values (the peak intensity and the intensity at the end of the examination), and this simple calculation could contribute to good repeatability. WR may also be less affected by systemic hemodynamic changes, because it is not a time-related parameter, which might have contributed to the lack of difference in this parameter between the two groups.

To the author's knowledge, WR of the HV has never been measured in human studies. In our preliminary research, dogs with chronic hepatitis tend to have a lower WR than normal dogs. Although multiple factors could affect WR, reduced Kupffer cell phagocytic function may be one of the most conceivable reasons. It was reported that nonalcoholic steatohepatitis in a rat model showed reduced Kupffer cell phagocytic function, with no changes in the numbers of Kupffer cells [24]. More recently, Park *et al.* reported that elimination of Sonazoid® from the hepatic parenchyma became slower with progression of cirrhotic disease [16]. The application of CEUS to assess hemodynamic changes, as well as liver function, should be investigated in the future. Additional research on the application of the WR is warranted.

Quantitative assessment of portal pressure by using CEUS was performed in a CCl4-induced canine liver fibrosis model [25]. In that study, the ROI was set on the hepatic parenchyma, and modified parameters based on the area under the curve of the TIC were generated. Compared with this previous report, the perfusion parameters utilized in the current study can be measured more simply. In addition, the ability of CEUS of the HV to detect intrahepatic shunt flow that directly bypassed the sinusoid may be superior to that of CEUS of the liver parenchyma. On the other hand, because a large ROI cannot be drawn on the HV, the repeatability of CEUS of the HV might be inferior to that of parenchyma-targeted CEUS analysis. This could be a major limitation of the current method, especially if adequate imaging of the HV
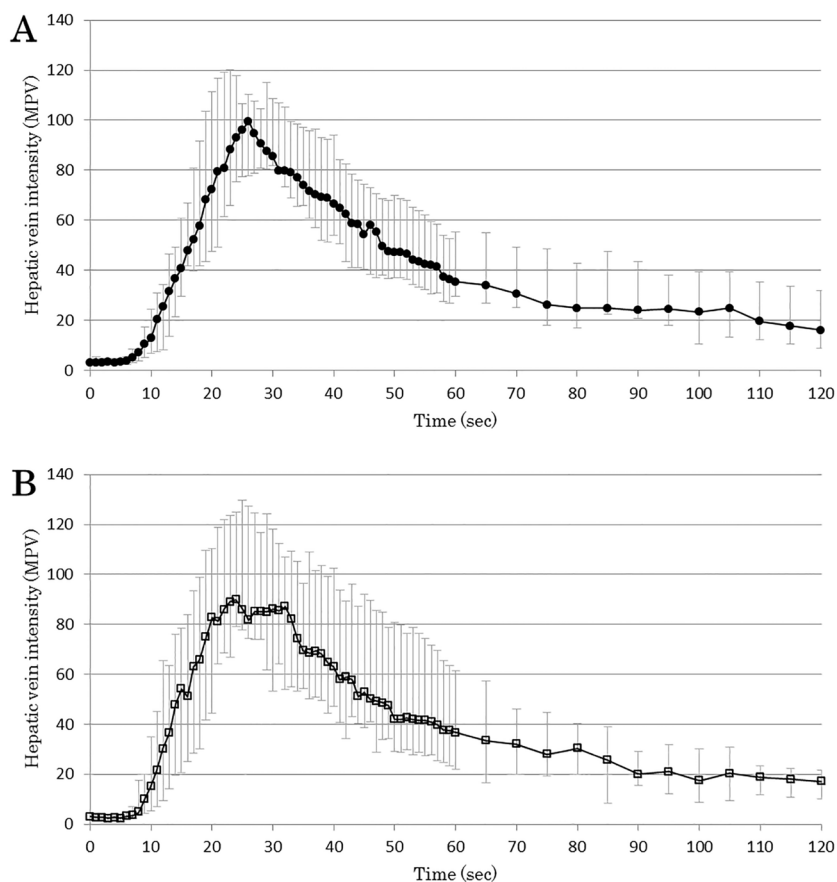
Fig. 3. TIC showing the median pixel intensity in the HV. (A) Conscious group (●, n=6). (B) Sedated group (□, n=6). The vertical bars represent the range of the values. The line graphs in the two groups are nearly identical, but the peak intensity was higher in the conscious group.

Table 1. Median values and CVs of the characteristic parameters of the time intensity curve in six healthy dogs

	Conscious group			Sedated group			P-value
	Median	Range	CV (%)	Median	Range	CV (%)	
HVAT (sec)	13.5	(9–22)	25.3	12	(8–17)	11.8	0.242
TTP (sec)	12.5	(6–24)	29.7	12.5	(9–17)	14.8	0.854
TTPP (sec)	8	(6–13)	27.1	9	(7–13)	12.4	0.377
WR (%)	78.0	(60.7–91.7)	7.6	84.1	(63.0–94.4)	12.3	0.689

cannot be maintained.

This study had several additional limitations. First, the number of animals used in this study was small. Second, the dogs enrolled in this research were all beagles, and therefore, we did not evaluate the influence of body size on hepatic hemodynamics. The differences in body size may affect the repeatability of each measured parameter, because ROI depends on the diameter of the HV, which is associated with the dog's size. Additional studies are needed to investigate the effect of body size. Finally, we used Sonazoid®, because it is the only second-generation contrast agent available in

Japan. However, other vascular-specific contrast agent might be better for assessing time-related parameters, because they would purely reflect hemodynamic changes related to liver disease.

In conclusion, this research characterized image enhancement of the normal canine HV using Sonazoid®. Established quantitative parameters may serve as a reference in the future assessment of liver function as related to hemodynamics. A further study into the application of this technique to evaluate intrahepatic hemodynamic changes associated with canine chronic hepatic disease is warranted.

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