Vascular and Kupffer Imaging of Canine Liver and Spleen Using the New Contrast Agent Sonazoid

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ABSTRACT. The new ultrasound contrast agent Sonazoid® was injected in 5 healthy dogs, and the time course enhancement in liver parenchyma, portal vein, spleen, and renal cortex was evaluated. In liver parenchyma and spleen, sustained enhancement was observed from at least 8 to 15 min after injection with the peak at 45 sec (liver) and 20 sec (spleen), whereas in the portal vein and renal cortex, the time course enhancement dramatically decreased after the peak enhancement at 30 sec and 20 sec, respectively. No adverse effect was observed after Sonazoid® injection in all the dogs that we examined. Contrast enhanced ultrasound using Sonazoid® is believed to be useful for observing the parenchyma of canine liver and spleen.

KEY WORDS: contrast-enhanced ultrasound, liver, sonazoid®.

Ultrasonography is becoming one of the first-line clinical examinations for visceral organs in both human and veterinary medicine, and the diagnostic ultrasound technology is still progressing. Contrast-enhanced ultrasound (CEU) using microbubble-based contrast agents is one of the methods that have advanced over the past years, and it has started to garner attention as a practical and useful examination in human medicine. The principle of CEU is the visualization of harmonic frequencies generated by microbubbles resonating in an ultrasound beam [2–8, 11, 15]. CEU has been applied to examine solid organs, especially in detection and characterization of liver masses and its vascularity in human medicine. CEU using contrast agents, including SonoVue® or Definity®, has also been reported in dogs for liver investigation, and its safety and usefulness was suggested [3, 5–7, 9, 11, 15]. Levovist® had been the only available CEU agent in Japan since 1999, and it is useful in detecting canine liver tumors [2]. However, Levovist® visualizes the enhanced image by destroying the microbubbles, and hence it is not suitable for continuous examination.

In January 2007, the new contrast agent Sonazoid® was released in Japan. Sonazoid® consists of an aqueous dispersion of lipid-stabilized perfluorobutane-filled gas microbubbles, and it can visualize enhanced images without destroying the contrast media, which enables a continuous observation of the target organs. Furthermore, Sonazoid® has a great advantage as compared to other contrast agents such as SonoVue® or Definity® in that the agent not only has a vascular phase but also a delayed parenchymal or Kupffer phase, which is produced by phagocytosis of the agent by the reticuloendothelial system in the liver or spleen [12–14]. Because of this feature, more accurate and less invasive detection and diagnosis for liver disease is expected in human medicine [1, 13]. The aim of this study is to characterize the image enhancement by Sonazoid in canine liver, spleen and renal cortex and to gather basic information for future clinical applications.

Five adult beagles, weighing 11.5 to 13.3 kg with no evidence of hepatic, splenic, or renal disease, were recruited to this study. For each dog, CEU examinations were done with at least 5-day intervals. Before contrast sonography, conventional B-mode ultrasound examination was carried out for intended organs and confirmed that there was no ultrasound abnormality. Anesthesia was induced by propofol and maintained by isoflurane inhalation after injection of atropine.

We intravenously administered 0.15 ml/head Sonazoid® (Daiichi Sankyo Corporation, Tokyo, Japan) through the cephalic vein as a bolus injection followed by 3 to 5 ml 10% heparin containing saline bolus. The dog was laid in the right recumbent position. The left lateral lobe of the liver and caudal portion of the spleen were demonstrated in each examination. The left kidney was visualized in spleen survey, if they could be drawn in the same cross section. The ultrasound system used was Apio XV (Toshiba Medical Systems Corporation, Tokyo, Japan) and convex transducer with contrast harmonic imaging (CHI) software. All the machine settings were adjusted at the same value in all the surveys. Mechanical Index, an index of acoustic output of ultrasound system, was set at 0.2. Pictures just before injection and at 10, 20, 30, 45 sec, every minute from 1 to 15 min, 20 min and 30 min after injection were saved as JPEG files.

The region of interest (ROI) was set in each JPEG file for the portal vein, hepatic parenchyma in the liver examination, splenic parenchyma in the spleen examination, and renal cortex in spleen examination, if the kidney was...
depicted. All the saved pictures were analyzed using the image analysis software Image J (National Institutes of Health, http://rsb.info.nih.gov/ij/). The mean gray-scale intensity value (MGI) for each part of all dogs examined was calculated, and the time intensity curve was drawn. All of the statistical analysis in this study was done by Wilcoxon test.

Before and after CEU, physical and blood examinations were performed. For each sample, the complete blood count (total white blood cell count, red blood cell count, platelet count, and hemoglobin concentration), C-reactive protein, blood urea nitrogen, creatinine, alanine aminotransferase, alkaline phosphatase, and albumin were assayed.

The time course of MGI was depicted in Fig. 1. Hepatic artery (HA) was enhanced from 10 sec after injection (data not shown), followed by the beginning of hepatic portal vein (PV) and hepatic parenchyma (HP) enhancement. PV enhancement reached a peak at 30 sec (MGI before and 30 sec after injection is 2.22 and 152.63, respectively), and rapidly decreased thereafter; however, the MGI of the PV continued to be significantly higher than that before injection for 10 min (MGI at 10 sec is 8.45). In contrast, the MGI of HP reached a peak at 45 sec (MGI=76.23) and slowly reduced; however, its significant enhancement continued for 15 min (MGIs before and 30 min after the injection are 18.72 and 37.45, respectively). The MGI continued to be higher than that before injection for 30 min (MGI at 30 min is 33.20). The echogenic intensity of PV is significantly higher than that of HP from 20 sec to 1 min, then the intensities reversed, and the MGI of HP was significantly higher from 8 min to 15 min after Sonazoid injection.

The significant enhancement of splenic parenchyma compared with that before Sonazoid injection (MGI=16.45) was observed from 20 sec (MGI=41.10) after injection and lasted for 30 min (MGI at 30 min is 31.45).

MGI of the kidney was calculated from 3 of 5 dogs. MGI of the renal cortex (RC) dramatically increased with a peak at 20 sec (MGIs before and 20 sec after the injection are 3.79 and 122.74, respectively) and rapidly decreased within 2 min after the injection (MGI at 2 min is 35.03). The significant enhancement continued up to 30 min (MGI is 18.07) as determined by the image analysis.

The previous report on CEU using SonoVue® in canine liver demonstrates a different time course in which HP enhancement diminished within 3 min [3], whereas in the present study, HP enhancement continues for longer period.
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(15 min). The discrepancy between these 2 results is attributed to the difference in contrast agents used. Although Sonazoid\textsuperscript{®} has a vascular phase and a delayed parenchymal (Kupffer) phase, SonoVue\textsuperscript{®} is reported not to be phagocytosed by Kupffer cells \cite{14}. It is not clearly understood why certain contrast media are phagocytosed and certain media are not; however, the differences in the shell component of each agent have been reported \cite{14}. Although Levovist\textsuperscript{®} is another agent with the Kupffer phase, the contrast image is acquired by destruction of microbubbles, which results in insufficient examination time and limited frame rate. Furthermore, Yanagisawa \textit{et al.} reported that 99\% of Sonazoid\textsuperscript{®} was phagocytosed in the liver, whereas only 47\% of Levovist\textsuperscript{®} was phagocytosed \cite{14}. As the enhancement of the Kupffer phase should be observed only in tissues with Kupffer or reticuloendothelial cells, malignant tissue is expected to exhibit enhancement defects in this phase \cite{1, 13}. These results indicate that Sonazoid\textsuperscript{®} is a convenient and effective contrast agent, especially in detecting or discriminating malignant tumors, as compared with Levovist\textsuperscript{®} and other vascular phase-specific agents.

Based on the result of the present study, the vascular phase of canine liver is soon after to approximately 1 min after the injection of Sonazoid\textsuperscript{®}, because soon after injection, the vascular shapes, which appear to be arterial vessels, emerged. Further, although the time intensity curve could not be drawn, the gray-scale intensity of PV from 20 sec to 1 min is sufficiently high to distinguish between PV and HP. The parenchymal phase started at 8 min after injection, because from that time, MGI of HP was significantly higher than that before injection and as compared to that of PV at the corresponding time. Although these significances disappear 15 min after injection, the MGI of HP remained higher than that of PV and of HP before injection. There may have been no significance, because the number of dogs examined was not sufficient and microbubbles were partially destroyed by the ultrasound beam resulting from the ultrasound probe maintained in a fixed position in this study. In the spleen, significant parenchymal enhancement was observed from 20 sec to 30 min, which has a nearly similar pattern to that of HP. Although the time-course enhancement of splenic vessels was not monitored due to their small size, the result indicated that the spleen also has the delayed parenchymal phase by Sonazoid\textsuperscript{®} injection.

In the kidney, unlike the liver or spleen, sustained enhancement by Sonazoid\textsuperscript{®} was not observed in this study. Waller \textit{et al.} reported a similar time course enhancement of canine kidney by using SonoVue\textsuperscript{®} \cite{11}, in which the enhancement of the renal cortex was reached at 12.8 sec and rapidly decreased within 3 min. The light enhancement of kidney after 3 min detected by image analysis in this study is probably due to the small amount of Sonazoid\textsuperscript{®} in the blood circulation or due to phagocytosis by macrophages in the renal cortex.

In the present study, none of the dogs displayed signs of adverse reactions and remarkable changes in the blood examination profile during and after the experiment. During the Sonazoid\textsuperscript{®} clinical trial in humans, some minor adverse effects such as headache, diarrhea, and neutropenia had been reported. As Sonazoid\textsuperscript{®} is believed to be excreted mainly from the lung \cite{10}, it should be used carefully in dogs with severe respiratory/lung diseases.

There are some limitations of this study. First, the intensity value of the CEU image is affected by several factors, such as skin thickness, subcutaneous fat, or vessel size of the injection site of the dogs examined. Second, injection of the contrast media was performed by manual bolus; therefore, the time from injection to contrast enhancement had some variations. Further studies are required to investigate the effects of these factors on contrast enhancement by Sonazoid\textsuperscript{®} in dogs and other animals.

In conclusion, the results in the present study revealed that efficient vascular and delayed parenchymal (Kupffer) enhancement of the canine liver can be achieved by CEU using Sonazoid\textsuperscript{®}. The sustained enhancement can also be observed in the spleen, but not in the kidney. The time course of enhancement described in this study will be helpful for the future clinical applications of Sonazoid\textsuperscript{®}.

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REFERENCES


