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Quantitative Evaluation of Contrast Enhanced Transcranial Doppler Signal using Galactose Based Echo-Contrast Agent in Dogs

Woojo CHUNG[1)], Hojung CHOI[2)], Kichang LEE[3)], Heechun LEE[4)], Mincheol CHOI[1)] and Junghee YOON[1)]*

[1)]College of Veterinary Medicine, Seoul National University, Seoul 151–742, [2)]College of Veterinary Medicine, Chungnam National University, Daejeon 305–764, [3)]College of Veterinary Medicine, Chonbuk National University, Jeonju 561–756 and [4)]Gyeongsang National University, Jinju 660–701, South Korea

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ABSTRACT. Transcranial Doppler ultrasonography (TCD) is hindered by insufficient ultrasound penetration through the temporal bone. The use of echo-contrast agents to enhance the Doppler signal is an important step toward the solution of this problem. The aim of the present study was to investigate the tolerability and diagnostic value of the intravenous echo-contrast agent, Levovist®. Levovist® was administered intravenously in 8 dogs with two doses (0.2 and 0.3 m*l*/kg) at different concentrations (300 and 400 mg/m*l*). In right middle cerebral artery (RMCA), the duration and degree of the signal enhancement were measured by TCD. All 32 administrations of Levovist® produced an increase in TCD signal of the RMCA without complications. The first assessable pulse curve could be seen on the screen after 4 to 7 seconds after injection. There was no significant difference of latency period between different concentration and dosage. The signal amplitude was increased homogeneously by more than 30 dB when 0.3 m*l*/kg with 300 mg/m*l* concentration of Levovist® and 0.2 and 0.3 m*l*/kg with 400 mg/m*l* concentration were administered. There was no significant difference in the duration of optimal contrasting between 0.3 m*l*/kg with 300 mg/m*l* concentration of Levovist® and 0.2 and 0.3 m*l*/kg with 400 mg/m*l* concentration. The duration of the signal enhancement was 144 to 422 seconds, depending on the degree of concentration and dose of administration. Optimal TCD signal enhancement of RMCA was obtained using 0.3 m*l*/kg with 300 mg/m*l* concentration of Levovist® in dogs, which is considered to provide quality visualization.

KEY WORDS: canine, galactose based echo-contrast agent, right middle cerebral artery, transcranial Doppler.

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Transcranial Doppler sonography (TCD) introduced by Aaslid *et al.* [2] is a non-invasive technique which may be used repeatedly, allowing real-time measurement of blood flow velocity in the major cerebral arteries in humans. TCD can be used to confirm changes in cerebral hemodynamics [5] and has been commonly accepted as a method for evaluation of intracranial arterial stenoses or occlusions and arterial vasospasm after subarachnoid hemorrhage [1, 30]. More recently, ultrasonic improvements have led to evaluation for morphologic imaging of the vessels by color-coded real-time sonography [4]. Preliminary reports suggest that TCD readings in the middle cerebral artery (MCA) also may correlate with intracranial pressure (ICP) elevation in patients with severe brain edema and brain death after head injury, and in hydrocephalic infants [13, 18, 31]. A significant correlation between ICP and resistance index was demonstrated in dogs [11]. Recent report suggested that TCD recording in the basilar artery may be used to predict outcome and the diagnosis of brain damage in dogs [12]. However, there are technical limitations inherent in Doppler imaging methods. Most notably, it is difficult to detect weak signals seen in the case of extremely reduced blood flow and with high-grade stenosis and cerebral vasospasm [20]. Intracranial imaging is further limited by the high degree of ultrasound reflection and absorption by the skull, whereby much of the emitted energy is lost [16]. Due to this limitation, many artifacts are produced as a result of the high ultrasound emission energy needed. This increased the ultrasonic beam scattering with its subsequent large sample volume leads to limited spatial resolution and insufficient signal-to-noise ratio.

The echo-contrast agents have been developed to solve these problems by increasing the reflected ultrasound. Echo-contrast agents were first used in cardiology, to improve ultrasound imaging of the chambers of the heart [15]. The poor capillary penetration of initial substances injected intravenously posed a particular problem in detecting echo-contrast agents in the arterial system as well as disadvantage of a non-standardized, non-reproducible, or even risky application [24]. First results concerning underlying mechanisms and problems in application modalities of industrially manufactured air microbubbles as a contrast medium in TCD have been studied in an animal model [27]. The contrast medium used in that pilot study was galactose based microparticles (Echovist®, Schering AG, Berlin, Germany); it was shown to enhance the TCD signal after intra-arterial injection. This has been somewhat reduced by changing the pharmacologic properties of the echo-contrast agents [26, 27], for increasing its stability and ability for capillary penetration. Currently, there are more than a dozen contrast agents, using different pharmacologic principles like encapsulated gas bubbles, colloidal suspensions, liquid emulsions, and aqueous solutions [14]. Galactose-based microparticles [7, 25–27], albumin-based echo-contrast agents [17], and phospholipids [19] have all been

* CORRESPONDENCE TO: YOON, J., College of Veterinary Medicine, Seoul National University, San 56–1, Sillim-dong, Gwanak-gu, Seoul, 151–742, South Korea.

investigated by transcranial Doppler sonography in animal and human studies, showing increased Doppler intensity.

A galactose based sonographic contrast agent (Levovist®, Schering AG, Berlin, Germany), which produces stable microbubbles capable of traversing the cardiopulmonary circulation, was used to enhance Doppler signal in blood vessels of varying size after intravenous injection in animal model [3]. Previous studies by Bogdahn *et al.* and Ries *et al.* [7, 26, 28] have shown that this galactose based echo-contrast agent has the ability to enhance both color and spectral Doppler signals over a clinically useful period and improves flow sensitivity and resolution of the vessels in question. However, quantitative evaluation of Doppler signal enhancement of intracranial artery after intravenous injection of galactose based echo-contrast agent has not been reported in the veterinary literature. The aim of the present study is to clarify how diagnostic usefulness of galactose based echo-contrast agent is influenced by the dose and concentration of administration in dog.

MATERIALS AND METHODS

Experimental animals: Eight clinically healthy dogs (5 male and 3 female; 4 Yorkshire terriers, 2 Maltese, and 2 mixed breed) weighing 3 to 4.38 kg (age ranged from 1 to 5 years) were used for transcranial Doppler examination of right middle cerebral artery (RMCA). All dogs fulfilled the following inclusion criteria: no arrhythmia, heart rate in the range of 80–160/sec, no pulmonary disease, no hepatic disease, no renal disease, hematocrit values within the range of 35–57%, and normal blood pressure determined indirectly [23]. The dogs were housed indoor and fed a standardized diet. All dogs were screened and considered normal based on complete neurologic and physical examinations prior to use in the study. The animal care and experimentation were carried out in accordance with the Guide for the Care and Use of Laboratory Animals in Seoul National University.

Echo-contrast agent: The sonographic agent used in these experiments was a galactose based microbubble preparation: Levovist® (SH U 508A, Schering AG, Berlin, Germany) injection. Levovist® consists of specially manufactured galactose microparticles with the addition of a small amount of palmitic acid. The microbubbles of transpulmonary stability and reproducible size are formed by creating a suspension of microparticles using sterile water. The resulting increase in acoustic backscatter causes a corresponding increase in the echo-signal intensity obtained from the blood flow. Levovist® has a neutral pH and is biodegradable substance. Following intravenous injection of this substance, the galactose microparticles dissolve in the blood stream. The microparticle sizes in the suspension range from 2 to 8 μm in diameter, with 97% of the microparticles less than 6 μm by microscopic and laser analysis [14]. These microparticles are adsorbed to and stabilized by the saccharide crystals and are capable of traversing the pulmonary capillary bed after passing through the right heart chambers. Galactose is then insulin-independently metabolized, mainly in the liver.

Concentrations of 200, 300 and 400 mg/m*l* were available for intravenous administrations [25]. All the echo-contrast agents were injected through a cephalic vein, and the injection rate of echo-contrast agent was 1 m*l*/sec through a 22-gauge indwelling catheter with a three-way stopcock. The injection was followed by a 5 m*l* flush of physiological saline to clear the injection line.

Transcranial Doppler studies: The transcranial Doppler (TCD) recordings were made with the ultrasound machine (Logiq® 400, GE Medical System, Milwaukee, U.S.A.). The Doppler signals were recorded continuously with the use of a 4 MHz multi-frequency sector probe over the temporal acoustic window. A standard Doppler power level was used with a range gate length of 1 mm, pulse repetition frequency ranged between 3 and 6 kHz.

Dogs remained in a lateral recumbency throughout the trial period. Coupling gel was applied above the zygomatic arch and transverse scan was performed. An ultrasonic window has to be located in each individual by searching this region to obtain maximal amplitude of the Doppler signals. Doppler signals from the RMCA were obtained by imaging the circle of Willis using color mapping Doppler examination. The signal enhancement was quantified with on-line fast Fourier transformation and displayed on the screen in flash color in 3 dB step.

Initially, the Doppler sample volume was centered on the main stem of the RMCA, and the signal amplification was then reduced until the frequency spectrum was no longer discernible on the screen. This adjustment was not altered during the course of the present study. The data were recorded on videotape.

The evaluation was performed off-line by two investigators who determined the time to the appearance of the echo-contrast medium in the RMCA main stem after the start of injection. This latency period is designated as TA. In addition, the maximum signal enhancement was recorded on the basis of the color-coded image, as was the duration of homogeneous representation of the Doppler frequency spectrum. The decline of the effect of the echo-contrast agent was optically recognizable by interruptions in the envelope curve of the Doppler frequency spectrum (Fig. 1). The following parameters were measured after each contrast medium injection: (1) the latency period from the start of injection to the detection of echo-contrast agent in the RMCA (in seconds) and (2) the duration of the optimum/homogeneous enhancement without fragmentation of the Doppler envelope curve (in seconds) and (3) the duration of the Doppler signal enhancement above 3 dB after injections. Doppler envelope curve was represented when signal amplitude was increased by more than 30 dB [21].

Statistical analysis: Statistical analysis was performed using the SPSS statistical computer program (SPSS 11.5.1, SPSS Inc., Chicago, IL, USA). According to property of sample, one-way ANOVA and Scheffe *t*-test were applied to data analysis.

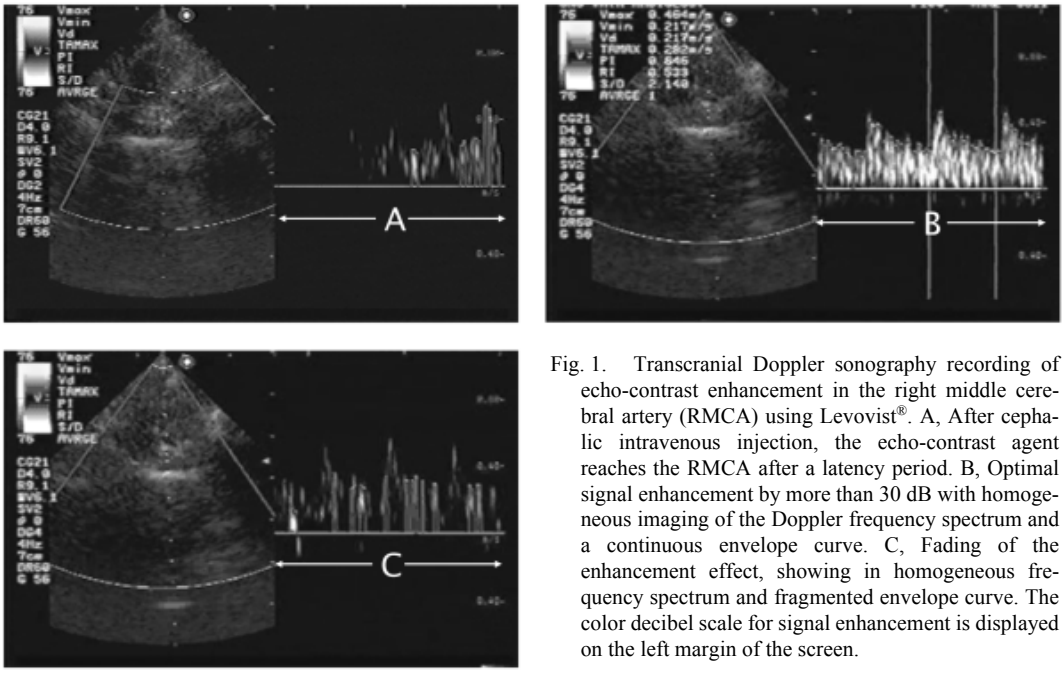

Fig. 1. Transcranial Doppler sonography recording of echo-contrast enhancement in the right middle cerebral artery (RMCA) using Levovist®. A, After cephalic intravenous injection, the echo-contrast agent reaches the RMCA after a latency period. B, Optimal signal enhancement by more than 30 dB with homogeneous imaging of the Doppler frequency spectrum and a continuous envelope curve. C, Fading of the enhancement effect, showing in homogeneous frequency spectrum and fragmented envelope curve. The color decibel scale for signal enhancement is displayed on the left margin of the screen.

RESULTS

Transcranial Doppler studies: Table 1 demonstrates that the latency period (TA) varied only slightly from 4 to 7 sec. There was no significant difference of TA between concentrations and dosages. The signal amplitude was increased, homogeneously by more than 30 dB when 0.3 m*l*/kg with 300 mg/m*l* concentration of echo-contrast agent and 0.2 and 0.3 m*l*/kg with 400 mg/m*l* concentration of that were administered. In most cases, optimal diagnostic image enhancement (homogeneous enhancement without fragmentation of Doppler envelope curve) was not obtained with the 0.2 m*l*/kg of 300 mg/m*l* concentration. The duration of optimal diagnostic enhancement lasted from 4 to 11 sec in other groups (Table 2). There was no significant difference in the duration of optimal diagnostic enhancement between the volume of 0.3 m*l*/kg with the concentration of 300 mg/m*l* and the volume of 0.2 and 0.3 m*l*/kg with the concentration of 400 mg/m*l*.

The duration of the Doppler signal enhancement above 3 dB after injections was dose and concentration dependent and lasted from 144 to 422 seconds (Table 3). The higher dose and concentration of galactose based echo-contrast agent increased the duration of the Doppler signal enhancement above 3 dB.

DISCUSSION

The principle of ultrasound signal enhancement relies on the fact that acoustic properties of physiological media such as blood or soft tissue, differ greatly from those inherent to contrast media such as air bubbles. Dissimilar acoustic impedance values lead to a high degree of reflection [22]. Different substances such as hand-shaken solution, hydrogen peroxide, angiographic contrast media, albumin, and different types of saccharides are used as echo-contrast agent. They vary by their stability characteristics and possible risks [8, 24, 32]. Sonicated albumin microspheres were shown to be sufficiently stable in *in vitro* studies [6]. They were mainly used in enhancing B-mode signals in echocardiography [10]. New studies reported a short-lasting signal-enhancing effect in transcranial Doppler sonography [29]. The substance used in the present study is thus far the only one to have demonstrated a long-lasting stability during cardiopulmonary passage. Contrast-enhancing technique was used first in echocardiography [15] but increasingly has been used in different vascular ultrasound applications.

Feasibility and application modalities of signal enhancement in transcranial Doppler sonography by air microbubbles similar to echo-contrast agent used in the present study were established in an animal model [27]. However, the development of a transpulmonary stable suspension was required for the intracranial use of contrast media in veterinary clinical field.

Contraindications of using the echo-contrast agent in neurosonology are limited and rather infrequent in human. No relevant adverse events were observed in this study, as in all previous studies with galactose based echo-contrast agent [3]. The tolerability even in high doses of up to 0.3 m*l*/kg with 400 mg/m*l* concentration of Levovist® as a bolus injection appears to be good. In particular, there were no clinical central nervous symptoms. One study reported that the intravenous echo-contrast agent enhanced both color and spectral Doppler signals and was nontoxic and effective

Table 1. Latency period of echo-contrast effect

Concentration (mg/m*l*)	Dose (m*l*)	Number	TA[a] (seconds)			
			Mean	SD[b]	Min[c]	Max[d]
300	0.2	8	5.250	0.463	5	6
300	0.3	8	5.000	0.926	4	6
400	0.2	8	5.125	0.991	4	7
400	0.3	8	5.345	0.744	4	6

a) Latency period of echo-contrast effect; b) standard deviation; c) minimum; d) maximum.

Table 2. Duration of optimal diagnostic enhancement

Concentration (mg/m*l*)	Dose (m*l*)	Number	Optimal diagnostic enhancement (seconds)			
			Mean	SD	Min	Max
300	0.2	8	0.625	1.768	0	5
300	0.3	8	5.625	0.916	5	7
400	0.2	8	5.500	0.756	4	6
400	0.3	8	6.625	2.134	4	11

Table 3. Duration of signal enhancement above 3 dB

Concentration (mg/m*l*)	Dose (m*l*)	Number	Signal enhancement above 3 dB (seconds)			
			Mean	SD	Min	Max
300	0.2	8	168.875	16.941	144	191
300	0.3	8	212.625	32.807	170	266
400	0.2	8	253.625	38.243	208	310
400	0.3	8	316.125	70.572	220	422

over a prolonged period, allowing for adequate analysis of the areas of interest in a series of experiments using dogs, rabbits, and woodchucks [14].

Since all of the experimental animals in this study provided good transcranial ultrasound images even without echo-contrast agent, after adjusting the transcranial Doppler sonography measurements in the main stem of the right middle cerebral artery (RMCA) the Doppler gain was reduced until no Doppler signal was detectable. This adjustment was not altered during the course of the study and served as the reference for evaluating the echo contrast effect. As the next step it was performed that envisaging studies under clinical conditions in patients with the problem of poor ultrasound penetration. The results of transcranial Doppler sonography are in agreement regarding the typical course of the echo contrast effect after using galactose based echo-contrast agent [28]. The onset of signal increase depends on the time elapsing between the intravenous injection and the first appearance of air microbubbles in the cerebrovascular bed after cardiac and transpulmonary passage. Thus, decreased cardiac ejection and insufficiency may prolong this silent period, whereas an intracardiac shunt, such as a patent foramen ovale, particularly in case combined with pulmonic stenosis and right heart pressure increases, should shorten it below the minimal time requested for the heart-lung passage [9]. The time for which the envelope curve of the Doppler frequency spectrum remained uninterrupted and free from artifacts was used to quantify the duration of the diagnostic effect. During this time quantitative analysis of the spectrum delivers relevant and useful data. The signal enhancement was quantified with the use of a color scale for relative decibel values. Though individual particles of contrast medium continue to amplify the signal by more than 30 dB after several minutes have elapsed, the Doppler frequency spectrum becomes inhomogeneous in the later phases. Therefore, a dose-effect curve was not constructed in this study.

A study with sonicated albumin contrast medium was reported that a biphasic course of enhancement was observed in human [21]. However, increase and decrease of signal enhancement are linear and there is no plateau or re-increase during the wash-out phase of the galactose based echo-contrast agent in this study.

It is considered that results of the present study contribute to a better understanding of the specific diagnostic properties of galactose based echo-contrast agent, which is a prerequisite for their possible future use in veterinary neurosonology.

In conclusion, this study was carried out to clarify how diagnostic usefulness of galactose based echo-contrast agent is influenced by dose and concentration of administration in dogs. The results were as follows. (1) The first evaluable pulse curve could be seen on screen 4 to 7 seconds after injection. There was no significant difference of the latency

period between different concentrations and dosages in normal cardiovascular condition. (2) The optimal image enhancement was obtained using the 0.3 m*l*/kg with 300mg/m*l* concentration of galactose based echo-contrast agent in dogs. (3) Galactose-based echocontrast agent improves intracranial Doppler imaging while being well tolerated in dogs. Further study might also be required in clinical setting with acoustic window failure in larger breed dogs.

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