Studies on the Clinical Usefulness of the Evaluation of Cerebral Blood Flow Using Transcranial Doppler Ultrasonography in Dogs

（犬における経頭蓋超音波ドプラ法を用いた
脳血流評価の臨床的有用性に関する研究）

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Contents

Abbreviations

AT	acceleration time
AUC	area under the receiver operating characteristic curve
CSF	cerebrospinal fluid
CV	coefficient of variation
CVCi	cerebral vascular conductance index
Dm	diastolic mean velocity
ECG	electrocardiograms
EDV	end-diastolic velocity
$EtCO_2$	end-tidal partial pressure of carbon dioxide
ICC	intraclass correlation coefficient
IV	intravenous
MAP	mean arterial pressure
MRI	magnetic resonance imaging
$PaCO_2$	partial pressure of arterial carbon dioxide
PaO_2	partial pressure of arterial oxygen
PI	pulsatility index
PSV	peak systolic velocity
RI	resistive index
ROC	receiver operating characteristic
Sm	systolic mean velocity
Sm/Dm	ratio of systolic to diastolic mean velocity
SpO_2	percutaneous oxygen saturation
TCD	transcranial Doppler ultrasonography
Vm	mean velocity

Notes

Contents of the present study were partially published in the following article list. The first article is corresponding to chapter 1 and chapter 3. The second article is corresponding to chapter 2. The present study is reproduced with the permission of the American Veterinary Medical Association.

List of published articles

1. Sasaoka K, Nakamura K, Osuga T, Morita T, Yokoyama N, Morishita K, Sasaki N, Ohta H, Takiguchi M. Transcranial Doppler Ultrasound Examination in Dogs with Suspected Intracranial Hypertension Caused by Neurologic Diseases. J Vet Intern Med 32, 314–323, 2018.
2. Sasaoka K, Ohta H, Ishizuka T, Kojima K, Sasaki N, Takiguchi M. Transcranial Doppler ultrasonography detects the elevation of cerebral bloo

d flow during ictal-phase of pentetrazol-induced seizures in dogs. Am J Vet Res 83, 331–338, 2022.

General Introduction

Cerebral blood flow is essential for maintaining the brain which requires constant oxygen and glucose. Cerebral blood flow is affected by multiple factors, including carbon dioxide concentration, oxygen concentration, hydrogen ion concentration, systemic blood pressure, intracranial pressure, brain metabolism, neural activity, vessel diameter, and blood viscosity.[1] In the physiological range, cerebral blood flow is highly maintained by cerebral autoregulation, which is a proportional change of cerebral vascular dilation or constriction responding to changes in cerebral perfusion pressure.[2] In contrast, pathological factors such as intracranial hypertension and cerebrovascular disease can significantly alter cerebral blood flow.[3] Positron emission tomography, single-photon emission computed tomography, and xenon computed tomography are used as a gold standard for the evaluation of brain metabolism and cerebral blood flow in human medicine.[4] However, the assessment of cerebral blood flow in veterinary medicine is limited due to the paucity of facilities, high cost, and invasiveness caused by radiation exposure.

Transcranial Doppler ultrasonography (TCD) is a rapid, noninvasive modality used to measure cerebral artery flow velocity. In general, air and bone attenuate or reflect ultrasound waves, making it difficult to observe their internal structures by ultrasound examination. Aaslid *et al.* reported that blood flow waveforms in cerebral arteries could be observed through the skull bone using low-frequency ultrasound of 2 MHz, which is less attenuated by bone.[5] This report promoted the clinical application of TCD in human medicine, especially in strokes which causes significant changes in cerebral blood flow.[6,7] In addition, TCD has been reported to be applied in the field of intensive care because ultrasonography has high immediacy and usability at the bedside.[1,8,9]

TCD obtains the real-time blood flow waveforms of cerebral arteries such as the basilar artery, middle cerebral artery, rostral cerebral artery, and caudal cerebral artery based on the Doppler signals penetrating the skull bone.[10,11] Blood flow velocities such as peak systolic velocity (PSV), end-diastolic velocity (EDV), and time-averaged mean velocity (Vm) are obtained by tracing the Doppler waveform. TCD velocity is used as a

non-invasive indicator for the estimation of cerebral blood flow.[12–14] In addition, TCD vascular resistance variables are calculated from the change rate of blood velocity between systolic phase and diastolic phase. Commonly used TCD vascular resistance variables are resistive index (RI) and pulsatility index (PI).[15] These are calculated as follows: RI = (PSV - EDV) / PSV, and PI = (PSV- EDV) / Vm. RI and PI have been shown to correlate with intracranial pressure and are also reported as a non-invasive indicator of intracranial pressure.[16–18]

There are some advanced variables obtained from cerebral blood flow waveform. The ratio of systolic to diastolic mean velocity (Sm/Dm), a limited use vascular resistance index, is a variable calculated by dividing the mean systolic velocity by the mean diastolic velocity. Sm/Dm was reported as a variable to assess atherosclerosis in the ophthalmic artery because Sm/Dm reflects vascular resistance and compliance.[19] Cerebral vascular conductance index (CVCi) is reported as an index of cerebral vasodilation, which is calculated as Vm / mean blood pressure.[20] CVCi was commonly used in the study of cerebral physiology. Acceleration time (AT) was the time from the initial increase in velocity to the time of peak velocity and is reported to be prolonged with severe stenosis of proximal vessels.[21] The pathological factors cause not only total changes in cerebral blood flow but also changes of the blood flow waveform within a single heartbeat.

The studies of TCD in veterinary medicine are limited, and even within the limited reports, only general parameters were considered. Fukushima *et al.* showed the experimental studies, which showed that the TCD velocity, such as Vm, PSV, and EDV, correlates with the directly measured cerebral blood flow[13] and that RI correlates with intracranial pressure.[16] However, the ability of TCD to diagnose intracranial hypertension in dogs with various neurological diseases has not been established. In clinical studies, elevated RI has been reported in dogs with symptomatic hydrocephalus,[22] and TCD findings have been reported in granulomatous encephalitis[23] and hepatic encephalopathy.[24] Epilepsy is the most common chronic neurological disorder in human and dogs.[25,26] Although TCD findings in human patients with epilepsy were inconsistent and needed further studies,[27,28] there are no reports of TCD concerning canine epilepsy.

The purpose of this study was to investigate the usefulness of cerebral blood flow assessment using transcranial Doppler ultrasonography in dogs. In chapter 1, repeatability

of TCD in anesthetized dogs and awake dogs have established. In chapter 2, the ability of TCD to detect electrical neural activity in drug-induced epileptic seizures have investigated, and the relationship between TCD variables and systemic parameters in an experimental setting have assessed. In chapter 3, the ability of TCD to detect intracranial hypertension in dogs with neurological diseases in a clinical setting have evaluated.

Chapter 1

Assessment of Repeatability of Transcranial Doppler Ultrasonography in Dogs

1. Introduction

TCD is a rapid, noninvasive modality used to measure cerebral artery flow velocity. TCD obtains the real-time blood flow waveforms of cerebral arteries based on the Doppler signals penetrating the skull bone. Cerebral blood flow and its waveform are affected by many factors, including carbon dioxide concentration, oxygen concentration, hydrogen ion concentration, systemic blood pressure, intracranial pressure, brain metabolism, neural activity, vessel diameter, and blood viscosity.[1] Therefore, blood flow waveform assessment using TCD provides information about the intracranial environment. The gold standard to assess cerebral blood flow is invasive modalities (e.g., Xenon computed tomography, positron emission tomography, single-photon emission computed tomography).[4] TCD is an indirect method of measuring cerebral blood flow and allows noninvasive, relatively simple, and repeatable measurements.

Blood flow velocities such as PSV, EDV, Vm, systolic mean velocity (Sm), and diastolic mean velocity (Dm) are obtained by tracing the Doppler waveform. TCD velocity is used as a non-invasive indicator for the estimation of cerebral blood flow.[12–14] In addition, TCD vascular resistance variables such as RI, PI, and Sm/Dm[19] are calculated from the change rate of blood velocity between systolic phase and diastolic phase. RI and PI have been shown to correlate with intracranial pressure and are also reported as a non-invasive indicator of intracranial pressure.[16–18]

The studies of TCD in veterinary medicine are limited. Canine repeatability study has been reported in only RI,[29] but the study did not cover other TCD variables. In order to determine whether differences in data reflect clinically important changes resulting from disease in individual patient, it is necessary to evaluate the repeatability of TCD variables. Assessment of cerebral blood flow is expected to be applied in both anesthetized and awake conditions. A previous study showed that general anesthesia improves the repeatability of echocardiography.[30] It is necessary to assess the repeatability under both conditions.

Therefore, the aim of chapter 1 was to evaluate the repeatability of TCD variables in healthy awake and anesthetized dogs.

2. Materials and methods

2.1 Dogs

Six laboratory dogs (sex, 2 males and 4 females; age, 2 to 12 years; body weight, 10.3 to 12.4 kg) were used. All dogs were confirmed as healthy on the basis of physical, neurologic, and blood examinations. The Laboratory Animal Experimentation Committee of the Graduate School of Veterinary Medicine at Hokkaido University approved the procedural design (approval number: 16-0132).

2.2 Study Protocol

Each dog was performed TCD with or without isoflurane anesthesia to assess repeatability. TCD under awake and isoflurane general anesthesia were performed on separate days. A 22-gauge catheter was placed in the right cephalic veins to establish a route for single rapid intravenous (IV) infusion. Dogs were sedated with midazolam (Dormicum Injection; 0.1 mg/kg, IV) and butorphanol tartrate (Vetorphale; 0.2 mg/kg, IV). General anesthesia was induced with propofol (7 mg/kg, IV) and maintained following intratracheal intubation with isoflurane (end-tidal concentration, approximately 1.5%) in 100% oxygen. Each dog was intravenously administered lactated Ringer's solution (Solulact; 10 mL/kg/hr). Body temperature was maintained about 37.0–38.0℃. End-tidal partial pressure of carbon dioxide ($EtCO_2$) was maintained about 35 mmHg using mechanical ventilation. The mean arterial blood pressure (MAP) was kept over 60 mmHg. These physiologic parameters were monitored using Vitals monitor (Life Scope BSM-5192; Nihon Kohden Corporation). Following a stabilization period, each dog underwent nonconsecutive TCD performed by one observer (KS) three times at 15-minute intervals. TCD variables analysis was performed after all procedures.

2.3 Transcranial Doppler Ultrasonography

TCD was performed with an ultrasound machine (Toshiba Aplio XG; Toshiba Medical Systems Corporation [renamed Canon Medical Systems Corporation]) with a 4−11-MHz convex probe (PVT-745BTV; Toshiba Medical Systems Corporation). The position of dogs was held by hand in sitting position in awake dogs and in left lateral recumbency in

anesthetized dogs (Figure 1A). Dogs were positioned with their heads flexed to a 90° angle to visualize the basilar arteries through the transforaminal window. Following B-mode examination with a sagittal view, color flow Doppler was performed to identify the basilar artery (Figure 1B). The basilar arterial Doppler waveforms were obtained with 6.0-kHz pulse repetition frequencies, a 94-Hz wall filter, 2.5-mm sample width, and angular correction at less than 40 degrees.[10,16] The obtained Doppler waveforms were recorded with simultaneous electrocardiograms (ECG), and then manually traced to determine the TCD velocities, including PSV, EDV, Vm, Sm, and Dm. In addition, the TCD vascular resistance variables, including RI, PI, and Sm/Dm were calculated.[16] AT was defined as the time from the initial increase in velocity to the time of peak velocity (Figure 1C). The heart rate was calculated using the R-R intervals on simultaneous ECG. Mean values were calculated for all the TCD variables for five consecutive cardiac cycles.

2.4 Statistical Analysis

Statistical software program (JMP Pro 14.0; SAS Institute Inc, and R; R Core Team 2021; R Foundation for Statistical Computing) were used. Results were summarized as mean and range. A one-way ANOVA was used for comparison between TCD variables in awake dogs and anesthetized dogs. Intraobserver repeatability was assessed by the coefficient of variation (CV) and intraclass correlation coefficient (ICC). Repeatability was considered to be acceptable when the CV was < 15% [31] and the ICC was > 0.70.[32] For all analyses, *P*-values < 0.05 were considered significant.

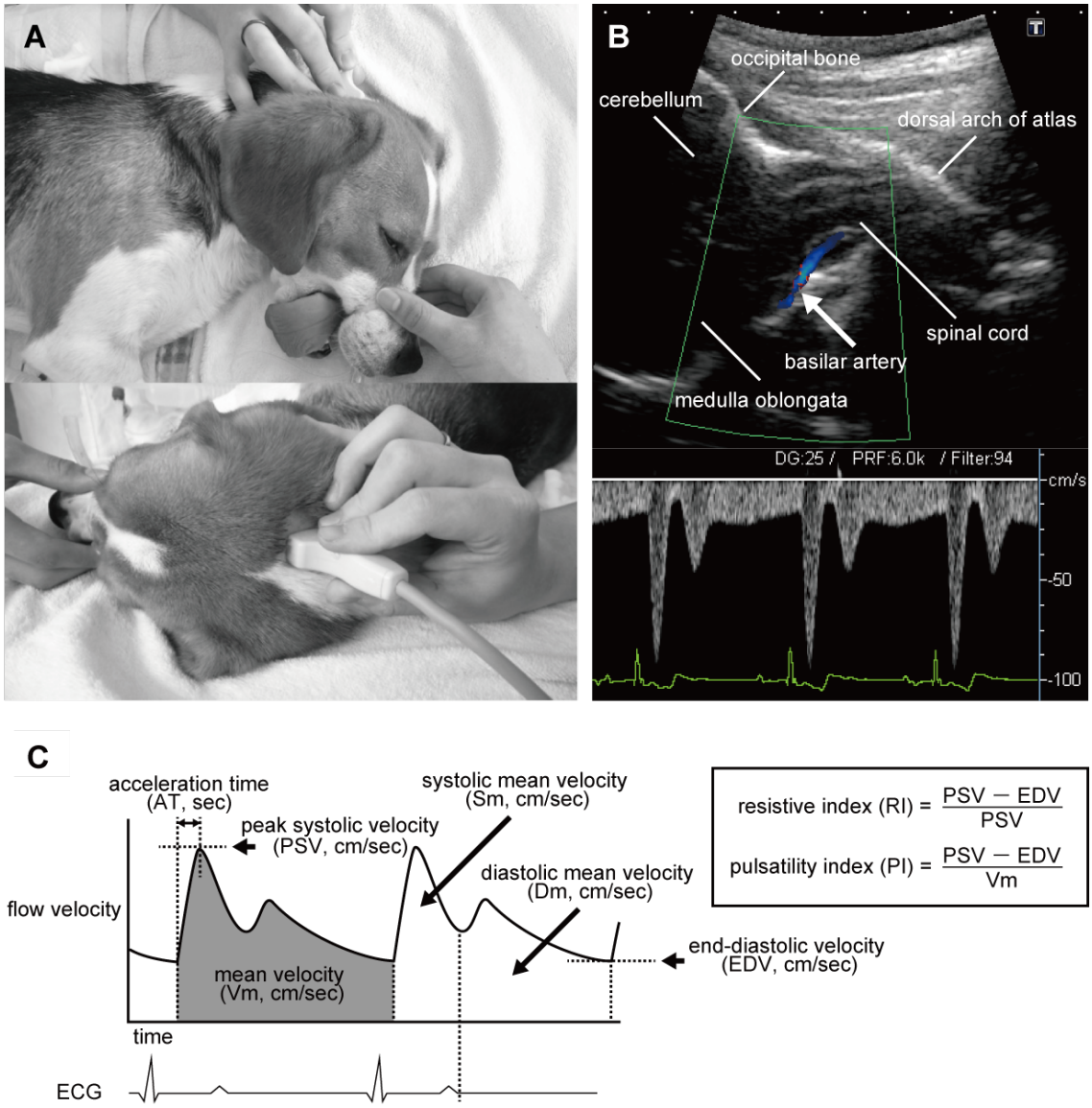

Figure 1. Transcranial Doppler ultrasonography (TCD) images in dogs. Anesthetized dogs were positioned in left lateral recumbency with their head flexed at 90 degrees to visualize the basilar arteries through the transforaminal window (A). Basilar artery flow was identified with a flow moving away from the probe below medulla oblongata on a view of color flow Doppler (B). Schematic representation of TCD waveform with ECG and TCD variables was shown (C).

3. Results

The blood flow waveform of the basilar artery could be obtained in all awake and anesthetized dogs. No difference was identified in TCD velocities, such as PSV, EDV, Vm, Sm, and Dm, between awake and anesthetized dogs. Among TCD vascular resistance variables, RI and PI were significantly increased in anesthetized dogs, however, there was no significant change in Sm/Dm. AT was significantly prolonged and heart rate was significantly decreased in anesthetized dogs. These data were summarized in Table 1.

In the assessment of repeatability, all CV values were < 10%, in addition all ICC values were > 0.70 in anesthetized dogs. In awake dogs, clinically acceptable repeatability was shown in PSV, EDV, Vm, Sm, Dm, RI, and heart rate, while clinically acceptable repeatability was not shown in PI, Sm/Dm, and AT. All CV in anesthetized dogs showed lower values than awake dogs (Table 2).

Table 1. Comparison between Transcranial Doppler ultrasonography variables in awake and anesthetized dogs.

Variable	Mean (range) in awake dogs	Mean (range) in anesthetized dogs	*P*-value
Heart rate (beats/min)	99 (72–132)	79 (69–95)	< 0.001
PSV (cm/sec)	79.0 (35.1–101.0)	79.0 (65.2–113.6)	1.00
EDV (cm/sec)	24.9 (14.0–34.5)	22.5 (14.8–32.8)	0.25
Vm (cm/sec)	37.3 (19.4–49.9)	33.9 (25.1–46.5)	0.22
Sm (cm/sec)	49.9 (24.1–65.1)	44.2 (32.9–60.7)	0.13
Dm (cm/sec)	33.4 (17.4–47.3)	29.0 (21.4–40.3)	0.10
RI	0.68 (0.58–0.73)	0.72 (0.64–0.81)	0.01
PI	1.46 (0.96–1.69)	1.70 (1.29–2.35)	0.006
Sm/Dm	1.51 (1.37–1.71)	1.54 (1.40–1.69)	0.40
AT (sec)	0.050 (0.044–0.064)	0.060 (0.047–0.091)	0.01

AT, acceleration time; Dm, diastolic mean velocity; EDV, end diastolic velocity; PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index; Sm, systolic mean velocity; Sm/Dm, ratio of systolic to diastolic mean velocity; Vm, mean velocity.

Table 2. Repeatability of transcranial Doppler ultrasonography variables in awake and anesthetized dogs.

	Awake dogs		Anesthetized dogs	
Variable	**CV (%)**	**ICC**	**CV (%)**	**ICC**
Heart rate	9.5	0.81	3.0	0.92
PSV	9.0	0.91	9.0	0.74
EDV	8.6	0.89	6.4	0.95
Vm	9.8	0.87	7.3	0.86
Sm	9.3	0.89	8.9	0.79
Dm	10.4	0.87	7.2	0.90
RI	3.3	0.72	2.2	0.90
PI	7.9	0.69	5.8	0.88
Sm/Dm	4.6	0.53	3.2	0.82
AT	10.0	0.17	4.8	0.96

Repeatability was considered to be acceptable when the CV was < 15% [31] and the ICC was > 0.70.[32] AT, acceleration time; CV, coefficient of variation; Dm, diastolic mean velocity; EDV, end diastolic velocity; ICC, intraclass correlation coefficient; PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index; Sm, systolic mean velocity; Sm/Dm, ratio of systolic to diastolic mean velocity; Vm, mean velocity.

4. Discussion

The present results showed that TCD has good repeatability in anesthetized dogs for all TCD variables. However, some variables were shown to have poor repeatability in awake dogs. This result suggested that isoflurane-general anesthesia improves the repeatability of TCD variables. This is the first report to evaluate the repeatability of TCD variables in multiple conditions in dogs.

TCD velocities did not change significantly with anesthesia in this study, however, variables that evaluate the shape of the waveform, such as RI, PI, and AT, changed significantly. This result indicated that cerebral blood flow was highly maintained under anesthesia, while there were changes in cerebral blood flow waveform within a single heartbeat. It remains unclear which effects of the anesthetic altered the blood waveform. The normal waveform in the cerebral arteries is determined by the proximal vessels, cardiac function, and the resistance of the distal vascular bed.[33] Isoflurane is a vasodilator agent,[34] which means it decreases vascular resistance. This pharmacological effect contradicts the results of this study. However, in addition to vasodilation, isoflurane causes decreases in cerebral metabolism, hypotension, and hypothermia.[35,36] Although the effects of isoflurane anesthesia on cerebral blood flow were minimal,[36] the blood flow waveform may have been altered by various pharmacological mechanisms. Further study is needed to determine which factors affected the cerebral blood waveforms. Furthermore, the present results suggest that anesthesia conditions should be taken into account when assessing TCD variables, especially RI, PI and AT.

Especially in anesthetized dogs, the TCD variables showed good repeatability and were shown to be acceptable for clinical use. The blood flow waveform of each beat is changed by respiration and heart rate. It has been mentioned that the values are preferable to be obtained by averaging five heartbeats.[37] In contrast, there are various methods of obtaining TCD values in veterinary medicine, such as obtaining the average of three similar sequential waveforms,[23] selecting one midway waveform between the slowest and fastest heart rate,[22] or selecting one arbitrary waveform.[24] Repeatability and objectivity are important in the assessment of blood flow waveforms, and it was shown

that using the average of multiple waveforms is appropriate.

The CVs tended to be lower in the anesthetized dogs than in the awake dogs. One reason for the decrease was that the physiological parameters were maintained uniformly by the general anesthesia. Meanwhile, it is necessary to consider that the range of variation in awake dogs is larger than that in anesthetized dogs. In a previous study of canine echocardiography, better repeatability has been shown in anesthetized dogs than awake dogs,[30] which is consistent with the present study. In addition, the effect of anesthesia on each variable was different, and it was reaffirmed that assessment of repeatability as a preliminary step to clinical application is important.

This study had several limitations. First, the present study was conducted by a single operator. Repeatability studies with multiple operators have been reported,[38] and further studies would be needed. Second, the anesthesia protocol used only one type of anesthetic. The degree of preservation of cerebrovascular autoregulation differs depending on the anesthetic.[2] The cerebrovascular autoregulation may affect the degree of repeatability. Therefore, it should be noted that the TCD variables and repeatability may vary with each anesthetic.

This study reported here revealed that TCD was a feasible and reliable method for assessment of cerebral blood flow in anesthetized healthy dogs. Some TCD variables were changed between anesthetized dogs and awake dogs. Therefore, TCD variables derived from different conditions should be interpreted with caution.

5. Summary

In this chapter, the repeatability of TCD variables in anesthetized healthy dogs has been established without changes of TCD velocities due to anesthesia. On the other hand, some variables were not shown to have good repeatability in awake dogs. This result suggested that isoflurane-general anesthesia improves the repeatability of TCD variables, and TCD was a feasible and reliable method for assessment of cerebral blood flow especially in anesthetized healthy dogs.

Chapter 2

Cerebral Blood Flow Assessment During Ictal-phase of Pentetrazol-induced Seizures in Dogs Using Transcranial Doppler Ultrasonography

1. Introduction

Epilepsy is the most common chronic neurological disorder in human and dogs.[25,26] Status epilepticus is clinically defined as a continuous seizure lasting for more than 5 minutes.[39,40] 59% of dogs with idiopathic epilepsy had 1 or more episode of status epilepticus.[41] Epileptic seizure suppression is the goal during the treatment of status epilepticus,[42] because a seizure lasting for more than 30 minutes can cause permanent damage to the brain.[43] Therefore, timely and appropriate treatment is necessary for status epilepticus, and this can be achieved by suppressing neuronal excitation and the consequent seizures with antiepileptic drugs and anesthetics.[42,44]

The primary goal of treatment of status epilepticus is to stop both clinical and electrographic seizure activity, and electroencephalography has been used as the reference standard for seizure suppression.[42,44,45] During anesthetic treatment of status epilepticus, non-convulsive status epilepticus may occur, wherein seizures occur as electrical activity without convulsions, and subsequently warrants monitoring of brain activity for appropriate treatment.[46,47] Although techniques such as wireless electroencephalography that can be easily used in veterinary medicine has been reported in recent years,[48] electroencephalography is not commonly used in veterinary medicine currently.

Seizures cause a variety of physiological changes. One such change is an increase in cerebral blood flow,[49] known as the cerebrovascular coupling concept[50,51]: the electrical activity of neurons leads to an increase in metabolic activities, such as oxygen and glucose metabolism, subsequently increasing cerebral blood flow to compensate for the increased demand for oxygen and glucose. Thus, cerebral blood flow reflects brain neural activity.

The increase in cerebral blood flow during seizures has not been established in dogs with noninvasive modalities such as TCD. The hypothesis of this study is that cerebral artery flow velocity measured by TCD could also increase during seizures in dogs. Since the TCD waveform is affected by vascular resistance and the conductance of blood vessels, it is necessary to confirm the changes in the TCD waveform during seizures.

The objective of this chapter was to investigate the association between the

changes in cerebral blood flow and electrographic epileptic seizure in dogs using TCD. Cerebral artery flow velocity using TCD were measured before, during, and after seizures and compared various parameters in dogs with drug-induced seizures. The relationship between systemic parameters and the TCD waveform were also analyzed.

2. Materials and methods

2.1 Animals

Six laboratory Beagle dogs (sexually intact females) were used in this study. Dogs were 2 to 6 years old with body weights ranging from 9.5 to 13.3 kg. All dogs were confirmed to be healthy based on physical, neurologic, and blood examinations. The Laboratory Animal Experimentation Committee of the Graduate School of Veterinary Medicine at Hokkaido University approved the procedural design (approval number: 16-0132).

2.2 Study protocol

Each dog was administered pentetrazol or saline (0.9% NaCl) solution under general anesthesia as described. Pentetrazol is recognized as a noncompetitive antagonist of the γ-aminobutyric acid A receptor ($GABA_A$) complex.[52] Pentetrazol was administered to each dog, and after an interval of at least 28 days, saline solution was administered to the same dog as time-matched control.

A 22-gauge catheter was placed in the right and left cephalic veins to establish a route for single rapid IV infusion and continuous rate infusion. The left cephalic vein route was used for the administration of drugs such as pentetrazol or saline solution, and diazepam, while the right route was used for anesthetics and fluids. General anesthesia was induced in each dog with propofol (2% Propofol Maruishi) 1 mg/kg/min IV using a syringe driver (COOPDECH syringe pomp; Daiken Corporation). After endotracheal intubation, anesthesia was maintained with continuous rate infusion of propofol started at a rate of 0.2 mg/kg/min and adjusted accordingly. At first, the criteria for the optimal depth of anesthesia were the severely absent or diminished palpebral reflexes and absence of purposeful movements. Rocuronium bromide (ESLAX intravenous) was administered continuously at a rate of 2 mg/kg/hr to prevent obvious convulsions. Each dog was connected to a mechanical ventilator system (SERVO-air; Fukuda Denshi Corporation) and ventilated with a fraction of inspired oxygen of 0.4. Mechanical ventilation was performed to maintain an $EtCO_2$ of 35–45 mmHg, a respiratory rate of 10–20 breaths/min, and a tidal volume of 10–15 mL/kg. Lactated Ringer's solution (Solulact) was infused intravenously at a rate of 5 mL/kg/hr. Meloxicam (Metacam injection; 0.2 mg/kg,

subcutaneous) and cefazolin sodium hydrate (Cefamezin α; 20 mg/kg, IV) were administered to each dog. Heart rate, ECG, rectal body temperature, and percutaneous oxygen saturation (SpO_2) were monitored and recorded continuously using a vital monitor (Life Scope BSM-5192, Nihon Kohden Corporation).

Each dog was placed in left lateral recumbency. An 18-gauge central venous catheter was inserted into the right external jugular vein using a 16-gauge trocar. A pulse contour analysis monitor was used to evaluate cardiac output. A 3-Fr arterial catheter (PiCCO catheter PV2013L07-A; PULSION Medical Systems SE) was placed in the left femoral artery. Calibration of cardiac output was performed via aortic transpulmonary thermodilution after hemodynamic stabilization. Cardiac output, direct mean arterial pressure (MAP), systolic arterial pressure, and diastolic arterial pressure were measured and recorded using a circulation monitor (PulsioFlex; PULSION Medical Systems SE). After insertion of catheters, the position of each dog was changed to the sternal recumbency position with their head flexed at an approximately 90-degree angle to visualize the basilar artery through the transforaminal window with the aid of an inverted v-shaped positioning trough. After the head was fixed, 14 electrodes (Natus classic disposable subdermal stainless-steel EEG needle; Natus Medical Incorporated; 12 mm long, 0.4 mm (27-gauge) diameter, 1.5 m cable) for electroencephalogram were placed as described in a previous study by Pellegrino *et al*.[53] Twelve subdermal needle electrodes (frontal [F3, F4], parietal [P3, P4], temporal [T3, T4], occipital [O1, O2], longitudinal midline [Cz, Pz], and auricular [A1, A2]) were placed over the scalp to record the electroencephalogram (sensitivity: 7 μV/mm, time constant: 0.3 sec, notch filter inserted, low cut filter: 0.5 Hz, high cut filter: 50 Hz, impedance: < 2 kΩ). The ground electrode was placed in the neck caudal to the occipital protuberance and the reference electrode was placed in the center of the bridge of the nose. Digital electroencephalogram (Comet-PLUS; Fukuda Denshi Corporation) recordings were performed after complete instrumentation set-up. After electrodes were placed, anesthesia level was checked using an electroencephalogram by detecting the presence of delta and spindle patterns but not burst suppression.

Baseline TCD waveform was recorded after the heart rate, blood pressure, electroencephalogram, cardiac output, and $EtCO_2$ were stabilized, which was about 2 hr

after the infusion of propofol. Pentetrazol (Pentylenetetrazole; Toronto Research Chemicals Inc) was diluted to 50 mg/mL in saline solution and sterilized through a filter (Millex-GS, Merck Millipore Ltd). Pentetrazol was infused at 1.5 mg/kg/min (1.8 mL/kg/hr) and terminated when the generalized electrographic seizure or myoclonic body twitch occurred. The number of spikes were counted manually to monitor the seizure activity. The ictal phase was recorded during the electrographic seizure. Diazepam (Horizon Injection; 1 mg/kg) was then administered intravenously and repeated every 5 min to terminate the electrographic discharges. The after-ictal phase was recorded after terminating the seizure activity. Saline solution was infused at 1.8 mL/kg/hr to measure time-matched controls. Further, when under saline solution infusion, diazepam was administered only once. TCD velocity analysis was performed after all procedures.

2.3 Transcranial Doppler ultrasonography

TCD was performed using an ultrasound machine with a 3–6 MHz sector probe (Artida with PST-50BT; Toshiba Medical Systems Corporation [renamed Canon Medical Systems Corporation]). TCD was performed in the basilar artery same as chapter 1. After measuring basilar arterial blood flow, the left middle cerebral artery was identified through the left temporal window located dorsal to the zygomatic arch. Middle cerebral artery Doppler waveforms were obtained using the same settings. The Doppler waveforms were recorded with ECG and then traced to determine the TCD velocities, which included PSV, EDV, and Vm. In addition, TCD vascular resistance variables such as RI, PI, and Sm/Dm. The cerebral vascular conductance index (CVCi) was calculated as follows: CVCi = Vm/MAP. AT was defined as the time from the initial increase in velocity to the time of peak velocity. Mean values were calculated for all the TCD variables for three consecutive cardiac cycles.

2.4 Statistical analysis

A statistical software program (JMP Pro 14.0; SAS Institute Inc) was used to create a generalized mixed linear model, with the drug (pentetrazol or saline solution), the state (baseline, after drug administration, or after diazepam administration), and the interaction between drug and state indicated as categorical fixed effects while dog identification was

indicated as a random effect. The effects of drug and state on the systemic and TCD variables were assessed using the *F* test. Pairwise comparisons between baseline and time points were performed by obtaining the least squares means. A Bonferroni correction was used to account for multiple comparisons. Partial correlation analysis was performed using R (R Core Team 2021; R Foundation for Statistical Computing) to control for the individual effects of dogs by determining the relationship between TCD velocity variables (outcome variables) and systemic parameters (explanatory variables). For all analyses, *P*-values < 0.05 were considered significant.

3. Results

3.1 Changes in systemic parameters in pentetrazol-induced seizure model

Changes in systemic parameters at baseline, after drug (pentetrazol or saline solution) administration, and after diazepam administration are shown in Table 3 and Figure 2. The seizure, which was electrographic discharge with subtle twitch, was induced with a mean pentetrazol dose of 60.4 mg/kg (range: 45–72 mg/kg) and terminated after approximately 2.5 administrations of diazepam, corresponding to a mean dose of 2.5 mg/kg (range: 2–3 mg/kg). No changes were observed in heart rate, MAP, systemic vascular resistance, cardiac index, $EtCO_2$, SpO_2, and body temperature during pentetrazol-induced seizures. MAP significantly decreased after diazepam administration compared with that of baseline and after drug administration.

3.2 Changes in TCD variables in pentetrazol-induced seizure model

All cerebral artery variables were determined using TCD at baseline, after drug administration, and after diazepam administration. TCD velocities, such as PSV, EDV, and Vm, in the middle cerebral and basilar artery, were significantly increased during pentetrazol-induced seizures (Figures 3A-C). PSV and Vm in both arteries were significantly decreased after diazepam administration, and were close to the baseline values. TCD vascular resistance variables did not significantly change during pentetrazol-induced seizures and after diazepam administration in either artery (Figures 3D-F). CVCi significantly increased after pentetrazol administration but subsequently decreased significantly in the basilar artery after diazepam administration (Figure 3G). AT remained unchanged after pentetrazol administration (Figure 3H). Changes in the TCD variables are summarized in Table 4 and 5.

3.3 Partial correlation analysis between TCD variables and systemic parameters

Controlling for the individual effect of dogs, partial correlation analysis between TCD variables and systemic parameters was performed, as summarized in Table 6. PSV was significantly correlated with cardiac index and body temperature in both the middle cerebral and basilar arteries. EDV was significantly correlated with $EtCO_2$ in both arteries.

Vm was significantly correlated with cardiac index, body temperature, and $EtCO_2$ in both the arteries.

Table 3. Least squares mean values (95% confidence interval) for systemic parameters as determined for 6 healthy adult Beagles at baseline, after continuous administration of pentetrazol or saline solution, and after administration of diazepam.

Variables	Baseline	After drug administration	After diazepam administration	Overall *P*-value
Heart rate (beats/min)				
Pentetrazol	146 (128–181)	167 (140–194)	158 (131–185)	0.64
Saline solution	154 (127–180)	150 (124–177)	146 (119–173)	
MAP (mmHg)				
Pentetrazol	149 (138–160)	153 (142–164)	136 (125–148) *†	0.57
Saline solution	148 (137–159)	148 (137–159)	137 (126–160) *†	
Cardiac index (L/min/m^2)				
Pentetrazol	4.7 (3.7–5.8)	5.2 (4.1–6.3)	5.0 (3.9–6.1)	0.86
Saline solution	5.0 (3.9–6.1)	5.1 (4.0–6.2)	5.0 (3.9–6.2)	
Systemic vascular resistance (dynes·sec/cm^5)				
Pentetrazol	4971 (4084–5859)	4642 (3755–5530)	4321 (3433–5209)	0.85
Saline solution	4529 (3611–5448)	4448 (3530–5367)	4161 (3242–5080)	
$EtCO_2$ (mmHg)				
Pentetrazol	44.3 (42.9–45.8)	45.8 (43.4–47.3)	45.2 (43.7–46.6)	0.35
Saline solution	45.2 (43.7–46.6)	45.2 (43.7–46.6)	44.8 (43.4–46.3)	
SpO_2 (%)				
Pentetrazol	95 (94–97)	96 (94–97)	94 (93–95)	0.58
Saline solution	95 (94–97)	95 (94–97)	95 (94–96)	
Body temperature (°C)				
Pentetrazol	37.0 (36.5–37.4)	37.1 (36.5–37.6)	37.1 (36.7–37.6)	0.23
Saline solution	37.2 (36.7–37.6)	37.2 (36.8–37.7)	37.1 (36.6–37.5)	

* Values differ significantly ($P < 0.05$) from the baseline value. † Values differ significantly ($P < 0.05$) from the values after drug administration. $EtCO_2$, end-tidal partial pressure of carbon dioxide; MAP, mean arterial pressure; SpO_2, percutaneous oxygen saturation.

Table 4. Least squares mean values (95% confidence interval) for cerebral blood flow indices of basilar artery as determined for 6 healthy adult Beagles at baseline, after continuous administration of pentetrazol or saline solution, and after administration of diazepam.

Variables	Baseline	After drug administration	After diazepam administration	Overall *P*-value
PSV (cm/sec)				
Pentetrazol	73.5 (59.5–87.5)	99.5 (85.5–113.5) *	76.9 (62.9–90.8) †	< 0.001
Saline solution	80.5 (66.5–94.5)	76.4 (62.4–90.4)	73.2 (59.2–87.1)	
EDV (cm/sec)				
Pentetrazol	24.8 (19.6–30.0)	38.0 (32.8–43.2) *	25.8 (20.5–31.0) †	0.002
Saline solution	30.4 (25.2–35.7)	28.1 (22.8–33.3)	26.8 (21.6–32.0)	
Vm (cm/sec)				
Pentetrazol	33.5 (27.4–39.7)	49.2 (43.0–55.4) *	36.3 (30.1–42.5) †	< 0.001
Saline solution	40.8 (34.6–46.9)	38.0 (31.8–44.2)	36.3 (30.1–42.5)	
RI				
Pentetrazol	0.66 (0.59–0.73)	0.62 (0.54–0.69)	0.66 (0.59–0.73)	0.55
Saline solution	0.61 (0.54–0.69)	0.62 (0.55–0.70)	0.63 (0.55–0.70)	
PI				
Pentetrazol	1.45 (1.16–1.74)	1.28 (0.99–1.57)	1.48 (1.18–1.77)	0.60
Saline solution	1.23 (0.94–1.52)	1.27 (0.98–1.56)	1.28 (0.99–1.57)	
Sm/Dm				
Pentetrazol	1.51 (1.41–1.61)	1.40 (1.30–1.50)	1.46 (1.36–1.56)	0.57
Saline solution	1.44 (1.34–1.54)	1.43 (1.33–1.53)	1.44 (1.34–1.54)	
AT (sec)				
Pentetrazol	0.042 (0.038–0.046)	0.038 (0.034–0.042)	0.045 (0.041–0.045) †	0.14
Saline solution	0.039 (0.035–0.043)	0.039 (0.035–0.043)	0.040 (0.036–0.044)	
CVCi				
Pentetrazol	0.227 (0.177–0.276)	0.322 (0.273–0.371) *	0.267 (0.218–0.316) †	< 0.001
Saline solution	0.276 (0.226–0.325)	0.259 (0.210–0.308)	0.268 (0.219–0.318)	

* Values differ significantly ($P < 0.05$) from the baseline value. † Value differs significantly ($P < 0.05$) from the value after drug administration. AT, acceleration time; CVCi, cerebral vascular conductance index; EDV, end diastolic velocity; PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity; Vm, mean velocity.

Table 5. Least squares mean values (95% confidence interval) for cerebral blood flow indices of left middle cerebral artery as determined for 6 healthy adult Beagles at baseline, after continuous administration of pentetrazol (1.5 mg/kg/min) or saline solution, and after administration of diazepam.

Variables	Baseline	After drug administration	After diazepam administration	Overall *P*-value
PSV (cm/sec)				
Pentetrazol	33.4 (26.5–40.3)	49.2 (42.3–56.1) *	35.8 (28.9–42.7) †	0.009
Saline solution	42.6 (35.8–49.5)	41.8 (34.9–48.7)	37.5 (30.6–44.3)	
EDV (cm/sec)				
Pentetrazol	18.2 (14.8–21.6)	25.8 (22.3–29.2) *	19.2 (15.8–22.6)	0.03
Saline solution	23.9 (20.5–27.4)	22.8 (19.3–26.2)	19.1 (16.1–23.0)	
Vm (cm/sec)				
Pentetrazol	23.7 (19.1–28.2)	33.8 (29.3–38.3) *	25.1 (20.6–29.7) †	0.02
Saline solution	30.3 (25.8–34.8)	29.6 (25.1–34.1)	25.8 (21.3–30.3)	
RI				
Pentetrazol	0.46 (0.41–0.50)	0.47 (0.43–0.52)	0.46 (0.42–0.51)	0.78
Saline solution	0.44 (0.39–0.49)	0.45 (0.41–0.50)	0.47 (0.42–0.52)	
PI				
Pentetrazol	0.65 (0.55–0.74)	0.69 (0.60–0.78)	0.66 (0.57–0.75)	0.65
Saline solution	0.62 (0.52–0.71)	0.64 (0.55–0.74)	0.69 (0.59–0.78)	
Sm/Dm				
Pentetrazol	1.35 (1.29–1.41)	1.35 (1.29–1.41)	1.37 (1.31–1.43)	0.87
Saline solution	1.33 (1.27–1.39)	1.35 (1.29–1.41)	1.36 (1.30–1.43)	
AT (sec)				
Pentetrazol	0.039 (0.032–0.045)	0.038 (0.032–0.045)	0.044 (0.038–0.051)	0.12
Saline solution	0.044 (0.038–0.051)	0.042 (0.036–0.049)	0.041 (0.034–0.047)	
CVCi				
Pentetrazol	0.160 (0.122–0.198)	0.222 (0.184–0.260) *	0.185 (0.147–0.224)	0.03
Saline solution	0.205 (0.167–0.244)	0.202 (0.164–0.241)	0.191 (0.153–0.230)	

* Values differ significantly ($P < 0.05$) from the baseline value. † Value differs significantly ($P < 0.05$) from the value after drug administration. AT, acceleration time; CVCi, cerebral vascular conductance index; EDV, end diastolic velocity; PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity; Vm, mean velocity.

Table 6. Results of partial correlation analysis of transcranial Doppler ultrasonography (TCD) velocities and systemic parameters controlling for the effect of individual dogs during drug-induced seizures in 6 healthy beagles.

	PSV		EDV		Vm	
	BA	**MCA**	**BA**	**MCA**	**BA**	**MCA**
Heart rate	0.39 *	-0.03	0.002	-0.08	0.14	-0.07
MAP	0.11	-0.003	0.30	0.17	0.06	0.06
Cardiac index	0.61 *	0.41 *	0.20	0.32	0.45 *	0.38 *
Systemic vascular resistance	-0.38 *	-0.27	-0.02	-0.13	-0.31	-0.22
$EtCO_2$	0.22	0.48 *	0.39 *	0.40 *	0.46 *	0.46 *
SpO_2	-0.18	-0.01	0.34	0.14	-0.005	0.04
Body temperature	0.68 *	0.53 *	0.18	0.42	0.56 *	0.50 *

Values in the table were r. * Values significantly ($P < 0.05$) correlated. BA, basilar artery; EDV, end diastolic velocity; $EtCO_2$, end-tidal partial pressure of carbon dioxide; MAP, mean arterial pressure; MCA, middle cerebral artery; PSV, peak systolic velocity; SpO_2, percutaneous oxygen saturation; Vm, mean velocity.

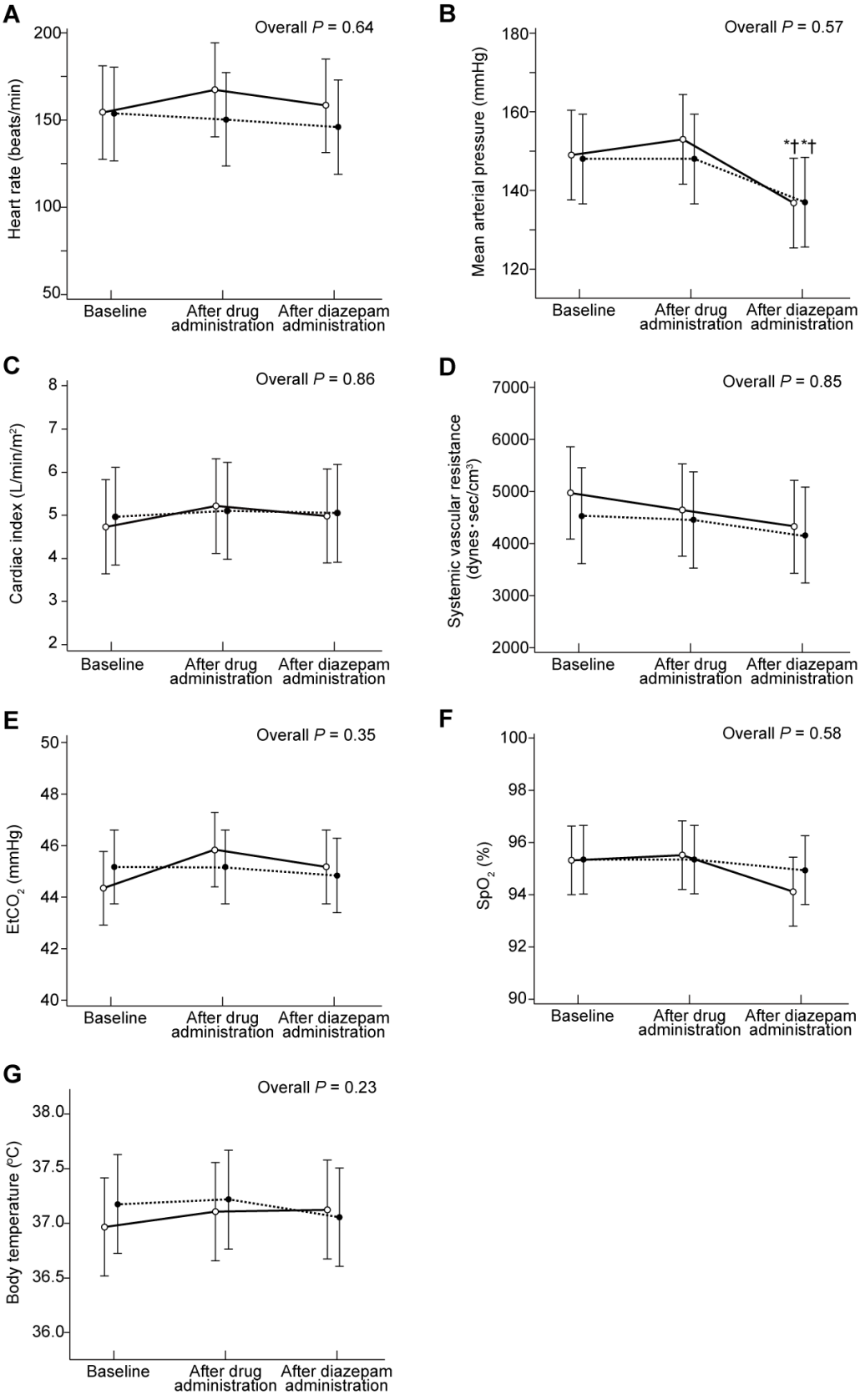

Figure 2. Changes in systemic parameters. Least squares mean and 95% confidence

interval for heart rate (A), mean arterial pressure (B), cardiac index (C), systemic vascular resistance (D), end-tidal partial pressure of carbon dioxide (E), percutaneous oxygen saturation (F), and body temperature (G) were determined at baseline, after drug administration, and after administration of diazepam in 6 healthy adult Beagles. Systemic parameters were obtained in dogs with pentetrazol (white circles and black lines) or saline solution (black circles and dash lines). * Value differs significantly ($P < 0.05$) from the baseline value. † Value differs significantly ($P < 0.05$) from the value after drug administration. $EtCO_2$, end-tidal partial pressure of carbon dioxide; SpO_2, percutaneous oxygen saturation.

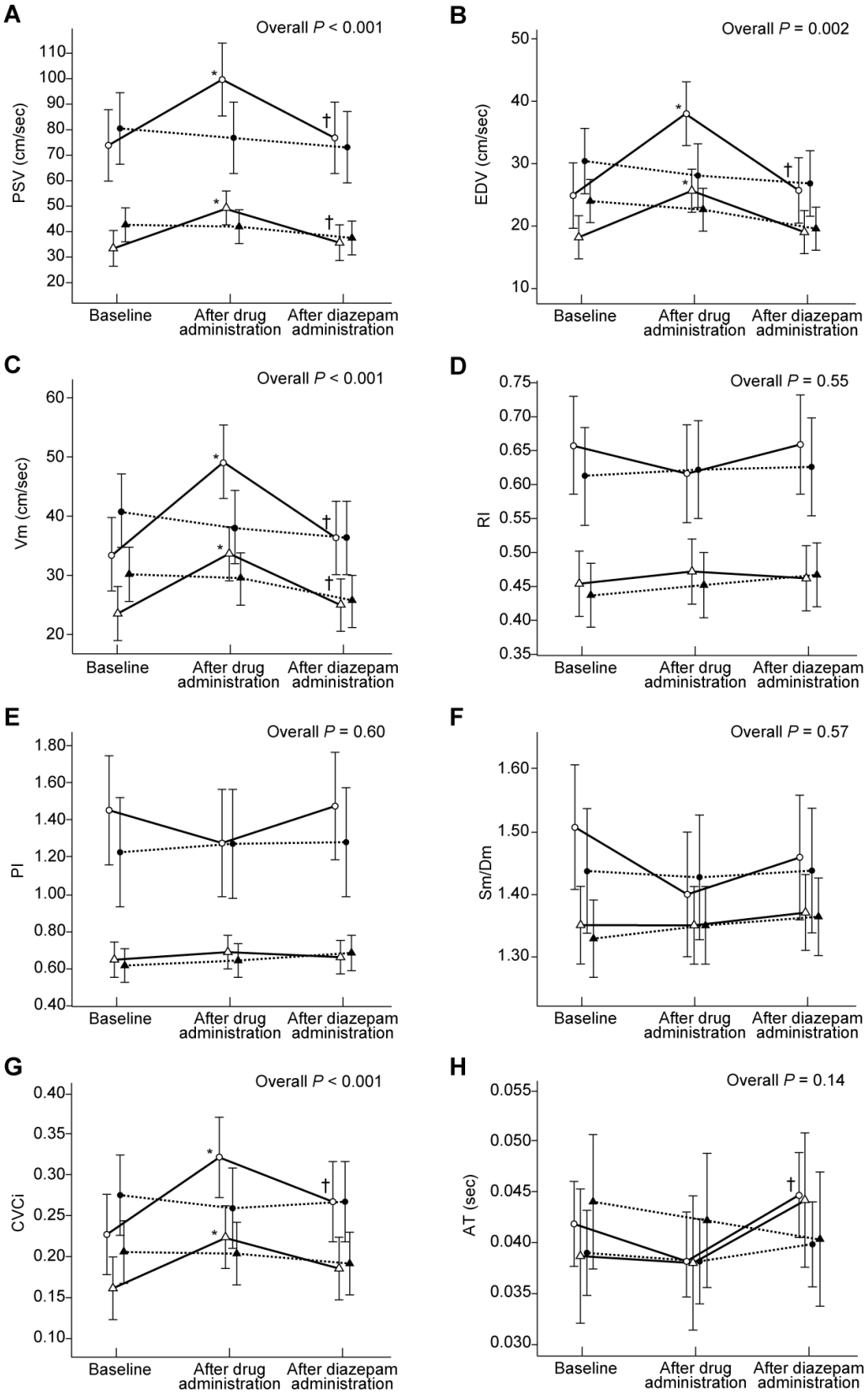

Figure 3. Changes in transcranial Doppler ultrasonography (TCD) variables. Least

squares mean and 95% confidence interval for PSV (A), EDV (B), Vm (C), RI (D), PI (E), Sm/Dm (F), CVCi (G), and AT (H) were determined using TCD at baseline, after drug administration, and after diazepam administration in 6 dogs. Basilar artery (circles) and middle cerebral artery (triangles) TCD variables were obtained in dogs with pentetrazol (white markers and black lines) or saline solution (black markers and dash lines). * Value differs significantly ($P < 0.05$) from the baseline value. † Value differs significantly ($P < 0.05$) from the value after drug administration. AT, acceleration time; CVCi, cerebral vascular conductance index; EDV, end-diastolic velocity; PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity; Vm, mean velocity.

4. Discussion

The present results showed that the TCD velocities of PSV, EDV, and Vm significantly increased in the basilar and middle cerebral arteries during pentetrazol-induced seizures. The TCD velocities significantly decreased after diazepam administration. This result indicates the possible utility of increased TCD velocities in detecting epileptic seizures during the treatment of status epilepticus. In addition, the results showed that TCD has potential efficacy to monitor resolution of seizure activity. The systemic parameters and TCD vascular resistance variables did not change while the vascular conductance index increased. To the best of my knowledge, there is no other study describing the increase in TCD velocity during seizures in dogs, as studied with the conductance indices and systemic parameters.

The cerebral blood flow was estimated from the arterial velocity measured by TCD and increased by 130% in the present study; this is consistent with the values reported in previous studies,[49,54–56] and it is consistent with the cerebrovascular coupling concept.[50,51] TCD velocities are correlated with cerebral blood flow[13] and this correlation is used in clinical and research settings as a noninvasive modality for cerebral blood flow measurement.[12,57] Cerebral blood flow (mL/min) is volumetric data that can be obtained from the product of the blood flow and the square of the vessel diameter. Notably, the artery diameter affects cerebral blood flow, and TCD velocity can only estimate cerebral blood flow under the assumption that the vessel diameter remains unchanged. In the previous human report examining hypercapnia-induced cerebral blood flow changes, cerebral blood flow estimated from phase contrast magnetic resonance imaging (MRI) was 18% greater than that estimated from TCD velocity.[58] Enlargement of the vessel diameter was also observed in MRI, and this was thought to be the cause of the underestimation in the previous study. Although vessel diameter was not measured in the present study, the rate of increase in TCD velocities may be an underestimate of the actual rate of increase in cerebral blood flow because elevated CVCi was an indicator suggestive of vasodilation.[20]

The TCD velocities, which were elevated during electrographic seizures, decreased with diazepam administration and returned to baseline. The decreases of TCD

velocities were assumed to be the result of decreased cerebral electrical activity, which may be used to monitor the termination of epileptic seizure. Electrographic seizures were reported to continue in 48% of human patient with convulsive status epilepticus after convulsion was controlled by medication.[47] In further clinical studies, it will be necessary to evaluate the presence or absence of electrographic seizures after treatment, as well as changes in electroencephalogram and TCD velocities with additional treatment escalation. In addition, TCD waveform recording techniques that continuously measure blood flow and show the trends[59] may be useful to capture changes during treatment. Although assessment of TCD alone to evaluate epileptic seizure activity may be difficult due to the involvement of various factors, which are discussed below, it is expected to be useful as an assistive evaluation tool in combination with electroencephalogram monitoring.

General anesthesia is one of the main factors affecting cerebral blood flow. Therefore, the increase in TCD velocities during seizures in the present study may have been influenced by the use of propofol, which could have preserved cerebral autoregulation. Propofol is used in veterinary and human medicine as a general IV anesthetic drug for the treatment of status epilepticus.[42,44,45] This drug is also known to have a cerebroprotective effect by suppressing brain metabolism[2] and maintaining cerebral autoregulation even when used at high doses compared to inhalational anesthetics.[60] This suggests that the changes in cerebral blood flow and TCD velocities during seizures may differ depending on the anesthetic agent used, and further studies are necessary to elucidate the outcomes when different agents are used.

The cerebral artery blood flow waveform was related to cardiac output, body temperature, and $EtCO_2$, suggesting that the effects of these factors should be taken into consideration when using TCD for clinical monitoring. To understand the cerebral artery blood flow waveform, it is necessary to separate it into the systolic and diastolic phases. Systolic blood flow is related to the proximal blood vessels and cardiac function, while diastolic blood flow is related to the resistance of the distal vascular bed.[33] Cerebral blood flow, defined as blood flow per unit volume per minute, is dependent on cerebral perfusion pressure, which is calculated as MAP minus intracranial pressure. The MAP is obtained from the product of the cardiac output and systemic vascular resistance. In the present study, PSV was found to be related to cardiac output, but not to MAP and systemic

vascular resistance. TCD, which allows us to see changes occurring in a single heartbeat, has clarified the relationship between PSV and cardiac output. This suggests that TCD systolic blood flow reflects the cardiac driving force, making cardiac output an essential factor to consider during acquisition of TCD waveforms. Other factors such as arterial compression can also alter blood flow velocity.[61] Hence, appropriate technique during recording TCD waveforms is necessary.

$EtCO_2$ was associated with EDV and Vm, which may be due to the cerebral vasodilation caused by carbon dioxide. The mechanism of cerebral vasodilation due to carbon dioxide is thought to be due to a decrease in interstitial pH that subsequently lowers Ca^{2+} in vascular smooth muscle, leading to vasodilation. Cerebral vasodilation by carbon dioxide increases cerebral blood flow, and a previous study showed that an increase in arterial carbon dioxide of 1 mmHg causes an increase in cerebral blood flow of 1.56 mL/100 g/min.[62] Cerebral vasodilation decreases vascular resistance and increases diastolic blood flow,[63] which could have resulted in changes in EDV and Vm. Therefore, any changes in diastolic blood flow due to respiratory status or acid-base imbalance must also be considered when interpreting TCD velocities.

Although I expected that the effect of body temperature would be minimal, the present study showed that body temperature was associated with TCD velocities. Body temperature is an essential factor involved in cerebral metabolism, and it has been reported that TCD velocities decrease during hypothermia which may suppress cerebral metabolism.[64] Since hypothermia is also a concern during general anesthesia and sedation,[35] it should be noted that body temperature can alter TCD velocities. It is also possible that body temperature was not directly related to TCD velocities but was involved in systemic circulation, such as cardiac output. In recent years, noninvasive technology has been developed to measure brain temperature using magnetic resonance spectroscopy,[65] enabling further physiological experiments to examine the relationship between temperature and blood flow.

This study had certain limitations. First, all the dogs used in this study were young females. Although some studies suggest that there is no sex difference in vascular reactivity,[66] this study was not able to evaluate the effects of the estrous cycle, hormones, and age on changes in vascular reactivity. Second, cerebral blood flow was not directly

measured. Cerebral blood flow (mL/min) is calculated by multiplying the square of the vessel diameter with the blood flow velocity. The diameter of cerebral arteries was not measured in this study. The assessment of cerebral blood flow was based on the assumption of no change in the cerebral artery diameter. Apart from invasive methods, cerebral blood flow can be measured using positron emission tomography, single-photon emission computed tomography, near-infrared spectroscopy, and functional MRI. Combining these methods may allow us to measure the increase in cerebral blood flow during electrographic seizures with higher accuracy. Further research utilizing more precise modalities for measurement may improve seizure detection.

In conclusion, this study showed that cerebral blood flow, as obtained from TCD velocities, increased by 130% during pentetrazol-induced seizures in dogs suggesting that TCD may be used to detect electrographic seizures during the treatment of status epilepticus in dogs. Additionally, the return of the elevated TCD velocities to normal, pre-seizure levels after seizure suppression suggests that it may be useful for monitoring the success of the seizure treatment. However, further clinical studies in non-convulsive seizure cases are needed to verify the diagnostic accuracy. In addition, cardiac output, $EtCO_2$, and body temperature affected the TCD waveform, and these factors need to be taken into consideration in clinical research and clinical use of TCD.

5. Summary

In this chapter, cerebral blood flow, as obtained from TCD velocities, increased by 130% during pentetrazol-induced seizures in dogs suggesting that TCD may be used to detect electrographic seizures during the treatment of status epilepticus in dogs. Additionally, the return of the elevated TCD velocities to normal, pre-seizure levels after seizure suppression suggests that it may be useful for monitoring the success of the seizure treatment. In addition, cardiac output, $EtCO_2$, and body temperature affected the TCD waveform, and these factors need to be taken into consideration in clinical research and clinical use of TCD.

Chapter 3

Transcranial Doppler Ultrasonography in Dogs with Suspected Intracranial Hypertension Caused by Neurologic Diseases

1. Introduction

Intracranial hypertension is defined as the continuous elevation of intracranial pressure above the normal range.[67] It can be caused by various intracranial diseases (e.g., trauma, hemorrhage, infarction, ischemia, edema, masses, encephalopathy, and status epilepticus). Intracranial hypertension can cause lethal damage to the brain as a result of decreased cerebral blood flow and mechanical compression of the brain structure.[67,68] Therefore, the rapid diagnosis and appropriate treatment of intracranial hypertension are important.[9,69]

The diagnosis of intracranial hypertension has been confirmed by direct intracranial pressure measurement in the neurointensive care of humans, although this method is invasive and can cause complications.[9] Direct intracranial pressure management has rarely been used to diagnose increased intracranial pressure in veterinary medicine as it is sometime considered invasive technique.[70] Although several clinical signs (e.g., low level of consciousness, absence of brainstem reflexes, abnormal motor activity and posture, Cushing response) alert veterinarians to the possibility of intracranial hypertension, these signs are non-specific and can be absent.[71,72] MRI findings are good indicators of intracranial hypertension, however these findings are also non-specific.[71,73] Previously reported MRI findings indicative of intracranial hypertension include effacement of cerebral sulci,[74] brain herniation,[75,76] compression of cerebrospinal fluid (CSF) space,[76] and brain shifting.[73]

Increased intracranial pressure causes the reduction of the cerebral perfusion pressure, which alters the cerebral arterial flow waveforms.[77,78] TCD vascular resistance variables, such as RI and PI, are commonly used to evaluate the altered cerebral waveforms. Those variables reported as a non-invasive indicator of intracranial pressure in human medicine.[16–18] Additionally, Sm/Dm is another vascular resistance parameter, which is reported to be a sensitive index for detection of the changes in arterial compliance and resistance of ophthalmic artery.[19]

In veterinary medicine, the RI was reported to be correlated with intracranial pressures measured directly in experimental intracranial hypertension.[16,78] However, there have been no reports of the clinical usefulness of TCD in the diagnosis of intracranial hypertension in dogs. Previous studies demonstrated alterations of Doppler

waveforms of cerebral arteries in dogs with hydrocephalus, hepatic encephalopathy, and granulomatous meningoencephalitis, however the relationship between the Doppler waveform and intracranial hypertension was not determined in these studies.[22–24] Thus, a clinical study evaluating the ability of TCD to detect suspected intracranial hypertension is needed. In addition, it needs to be clarified whether intracranial structural diseases even without intracranial hypertension affect TCD variables in dogs.

Therefore, the overall aim of this chapter was to evaluate the usefulness of TCD in the diagnosis of intracranial hypertension in dogs with intracranial diseases. Specific aims were to: (1) determine the association between TCD variables and MRI findings of suspected intracranial hypertension, and (2) determine the association between TCD variables and the presence of intracranial structural diseases.

2. Materials and methods

2.1 Study Population

Client-owned dogs were prospectively and consecutively recruited between October 2011 and October 2012 at the Hokkaido University Veterinary Teaching Hospital. Dogs were included in this study: (1) if they were suspected to have intracranial disease and (2) if they underwent MRI under isoflurane general anesthesia. In the present study, all dogs underwent TCD under isoflurane anesthesia after brain MRI. Dogs were excluded if Doppler waveforms could not be obtained from the basilar artery, or if their clinical condition was poor and immobilization required for MRI could be obtained with sedation or without any anesthesia.

Owner interviews and physical examinations were performed to obtain the following data: sex, age, body weight, and clinical history. Following blood and neurologic examinations, dogs underwent MRI under isoflurane general anesthesia. All physical and neurologic examinations were performed by an experienced neurologist in Hokkaido University Veterinary Teaching Hospital (HO), with emphasis on signs suggestive of intracranial hypertension, such as alteration in the level of consciousness, gait abnormalities, postural reaction deficits, pupil size abnormalities, absence of pupillary light reflexes, deficits in the menace response, and Cushing response.[67,71] Following brain MRI, CSF was collected via cisternal puncture if dogs were not suspected to have intracranial hypertension based on the absence of the below-mentioned MRI findings indicative of intracranial hypertension. For each dog, the clinical diagnosis was determined on the basis of clinical data including MRI findings.

2.2 Magnetic resonance imaging

MRI was performed with a single 0.4 Tesla unit (APERTO Eterna; Hitachi Medical Corporation). Prior to MRI examination, all included dogs were sedated with midazolam (Dormicum Injection; 0.1 mg/kg, IV) and butorphanol tartrate (Vetorphale; 0.2 mg/kg, IV). General anesthesia was induced with propofol (7 mg/kg, IV) and maintained following intratracheal intubation with isoflurane (end-tidal concentration, approximately 1.5%) in 100% oxygen. Each dog was intravenously administered lactated Ringer's

solution (Solulact; 10 mL/kg/hr). $EtCO_2$ was maintained between about 25 to 35 mmHg using mechanical ventilation. The mean arterial blood pressure was kept over 60 mmHg. These physiologic parameters were monitored using Vitals monitor (Life Scope BSM-5192; Nihon Kohden Corporation). If dogs were suspected to have intracranial hypertension on the basis of MRI, the dogs were intravenously administered 1 g/kg mannitol for 30 min during MRI. Transverse T1W pre- and post-contrast, T2W, and FLAIR sequences and sagittal T2W sequences were routinely obtained. MRI findings were reviewed by an experienced veterinarian (HO) who was unaware of the results of TCD. The MRI findings indicative of intracranial hypertension were recorded according to the following criteria: (1) effacement of the cerebral sulci,[74] (2) brain herniation (foramen magnum, transtentorial, subfalcine),[75,76] (3) compression of CSF space (third ventricle, fourth ventricle),[76] and (4) displacement of the lamina quadrigemina.[73] Dogs were suspected of having intracranial hypertension if any two or more of the above-mentioned MRI findings were identified.[73,79]

On the basis of the MRI findings, dogs were categorized into three groups to elucidate the relationships among the intracranial structural diseases, suspected intracranial hypertension, and TCD variables. Dogs in group I had no structural disease of the brain. Dogs in group II had intracranial structural diseases (e.g., neoplasia, encephalitis, cerebrovascular brain disease, and hydrocephalus) without suspected intracranial hypertension. Dogs in group III had intracranial structural diseases with suspected intracranial hypertension. In this study, the group I dogs were regarded as controls for group II and III because the group I dogs showed no abnormal findings on MRI.

2.3 Transcranial Doppler Ultrasonography

Following MRI, TCD was performed by one operator (KS) with an ultrasound machine (Toshiba Aplio XG; Toshiba Medical Systems Corporation) with a 4−11-MHz convex probe (PVT-745BTV; Toshiba Medical Systems Corporation) under the same anesthetic conditions as during MRI. The administrations of mannitol were started soon after the detection of the MRI findings indicative of intracranial hypertension, and TCD was performed 0 to 30 minutes after the administration finishing. TCD was performed in the

basilar artery same as chapter 1. The means of five consecutive cardiac cycles were calculated for all TCD variables. During TCD, physiologic parameters including the MAP and $EtCO_2$ were also recorded.

2.4 Statistical Analysis

Statistical analysis was performed with commercially available software (JMP Pro, 12.0.1; SAS Institute Inc. and IBM SPSS Statistic, version 22; IBM Corporation). All continuous variables are expressed as the median (range). Assumption of the normal distribution of the data was evaluated by means of a Shapiro-Wilk test, and the assumption of the homogeneity of variances was determined using Bartlett's test. If the distribution was assumed to be normal and the variance was assumed to be homogeneous, the overall difference among groups was determined using one-way analysis of variance (ANOVA), and then post-hoc multiple comparisons were made using the Tukey-Kramer HSD test. When the distribution was not assumed to be normal or the variance was not assumed to be homogeneous, the overall difference among groups was determined by the Kruskal-Wallis test (nonparametric one-way ANOVA), and then post-hoc multiple comparisons were made using the Steel-Dwass test. Categorical variables were compared by Fisher's exact test. Spearman's rank-correlation test was used to investigate correlations between variables. Receiver operating characteristic (ROC) analysis with the Mann-Whitney U test with Bonferroni correction for multiple testing was used to assess the ability to use the TCD variables to detect dogs with suspected intracranial hypertension among those with neurologic signs. The area under the ROC curve (AUC) and 95% confidence interval were calculated for each variable. Optimal cutoff values were chosen for each TCD variables based on the highest Youden index. For the optimal cutoff value of each variable, sensitivity and specificity were calculated. For all analyses, P-values < 0.05 were considered significant.

3. Results

3.1 Study population

Fifty-nine client-owned dogs with neurologic signs were recruited. Six dogs were excluded because their immobilization required for MRI could be obtained with sedation or without any anesthesia. Of the remaining 53 dogs, three dogs (Golden Retriever, 28.7 kg; French Bulldog, 12.4 kg; and American Cocker Spaniel, 9.7 kg) were excluded because Doppler waveforms could not be obtained from the basilar artery. Consequently, Doppler waveforms could be obtained in 50 dogs (50/53, 94%). Of the 50 finally enrolled dogs, there were 14 sexually intact males, 16 castrated males, 8 sexually intact females, and 12 spayed females. The median age was 7 years (range, 2−13 years), and median body weight was 5.8 kg (range, 1.3−33.3 kg). The most commonly represented breed was Miniature Dachshund (n = 8), followed by Chihuahua (6), mixed breed (5), Toy Poodle (4), Maltese (4), Shetland Sheepdog (3), Miniature Schnauzer (3), Golden Retriever (2), French Bulldog (2), Yorkshire Terrier (2), and 1 each of Australian Shepherd, Bearded Collie, Cavalier King Charles Spaniel, English Cocker Spaniel, Japanese Spitz, Labrador Retriever, Norfolk Terrier, Papillon, Shiba, Shih Tzu, and Welsh Corgi. Clinical diagnoses included idiopathic epilepsy (n = 17), intracranial neoplasia (14), encephalitis (9), cerebrovascular disease (6), and hydrocephalus (4).

On the basis of the MRI findings, 15, 22, and 13 dogs were categorized into groups I, II, and III, respectively. As for MRI findings indicative of intracranial hypertension, the effacement of the cerebral sulci was identified in 7 dogs; brain herniation in 13 dogs (foramen magnum in 10 dogs; transtentorial in 5 dogs; subfalcine in 4 dogs); compression of the CSF space in 16 dogs (third ventricle in 13 dogs; forth ventricle in 9 dogs); and displacement of the lamina quadrigemina in 10 dogs (Table 7). Any one of the MRI findings was recorded in 9 dogs: these dogs were categorized into group II. Meanwhile, among dogs in group III, 6 had any two of the MRI findings; 3 had any three of the MRI findings; and 4 had all of the four findings.

The proportion of the dogs that had abnormalities in neurologic examination in each group was dependent on the distribution of clinical diagnosis in the group. Five dogs (5/22, 23%) in group II and four dogs (4/13, 31%) in group III exhibited neurological

abnormalities suggestive of intracranial hypertension, such as low level of consciousness, pupil size abnormalities, absence of pupillary light reflexes, and deficits in the menace response. There were no dogs showing Cushing response among included dogs. None of the data on study population except for clinical diagnosis were different between groups. All dogs diagnosed with idiopathic epilepsy were categorized into group I (control group).

3.2 Comparison of the TCD variables

Of the TCD vascular resistance variables, only Sm/Dm was significantly different among the groups (Table 8, Figure 4, $P = 0.01$). The Sm/Dm was significantly higher in group III (median, 1.78; range, 1.44–2.58) than in group I (control group: median, 1.63; range, 1.43–1.75; $P = 0.02$) and group II (median, 1.62; range, 1.27–2.10; $P = 0.02$). On the other hand, there was no significant difference between groups I and II ($P = 0.96$). Regarding the other TCD variables including RI and PI, there were no significant differences among groups.

There were no differences in TCD variables between dogs with the neurological abnormalities suggestive of intracranial hypertension (n = 10) and without the neurological abnormalities (n = 40). The medians (ranges) of RI were 0.73 (0.59–0.81) and 0.72 (0.57–0.89) in dogs with and without the neurological abnormalities, respectively. The medians (range) of PI were 1.60 (1.02–2.74) and 1.55 (1.03–4.41) in dogs with and without the neurological abnormalities, respectively. The medians (ranges) Sm/Dm were 1.64 (1.42–2.10) and 1.63 (1.27–2.58) in the dogs with and without the neurological abnormalities, respectively.

3.3 Correlation between the number of identified MRI findings indicative of intracranial hypertension and TCD variables

There was a significant positive correlation between the number of identified MRI findings indicative of intracranial hypertension and Sm/Dm (Spearman's rho = 0.38, $P = 0.007$). No significant correlations were observed between the number of identified MRI findings and other TCD variables. (Figure 5).

3.4 Assessment of the diagnostic ability to detect intracranial hypertension

ROC analysis was performed to evaluate the ability to use the TCD vascular resistance variables to detect dogs with suspected intracranial hypertension (group III) among dogs with neurological diseases (groups I, II, and III). The ROC analysis revealed that only Sm/Dm could be used to detect dogs in group III among all enrolled dogs (Bonferroni-corrected $P < 0.05$). The AUC was 0.66 (95% confidence interval, 0.47–0.85) for RI, 0.61 (95% confidence interval, 0.40–0.82) for PI, and 0.79 (95% confidence interval, 0.63–0.95) for Sm/Dm (Table 9, Figure 6).

Table 7. Demographic data, physiologic variables, neurologic examination findings, clinical diagnoses, and magnetic resonance imaging (MRI) findings of 50 dogs that underwent transcranial Doppler ultrasonography.

Variable	Group I (n = 15)	Group II (n = 22)	Group III (n = 13)	Overall *P*-value
Age (years)	7 (2–13)	6 (3–13)	8 (2–13)	0.88
Body weight (kg)	5.9 (2.4–24.1)	5.5 (1.7–33.3)	5.2 (1.3–20.6)	0.50
Sex				0.43
Male (No. sexually intact)	9 (4)	15 (9)	6 (1)	
Female (No. sexually intact)	6 (2)	7 (2)	7 (3)	
Physiologic variables				
MAP (mmHg)	65 (58–80)	75 (55–98)	70 (56–94)	0.43
$EtCO_2$ (mmHg)	36.0 (32.0–38.0)	34.5 (23.0–43.0)	33.0 (26.0–38.0)	0.09
Heart rate (beats/min)	76.3 (58.3–147.4)	75.6 (48.5–125.0)	82.1 (40.7–158.7)	0.89
Clinical diagnosis				< 0.001
Idiopathic epilepsy	15	2	0	
Intracranial neoplasia	0	7	7	
Encephalitis	0	6	3	
Cerebrovascular disease	0	6	0	
Hydrocephalus	0	1	3	
MRI findings				
Effacement of the cerebral sulci	0	0	7	
Brain herniation	0	3	10	
Foramen magnum	0	2	8	
Transtentorial	0	0	5	
Subfalcine	0	1	3	
Compression of CSF space	0	4	12	
Third ventricle	0	4	9	
Fourth ventricle	0	0	9	
Displacement of the lamina	0	2	8	

quadrigemina

Continuous data were expressed as the median (range). All overall *P*-values were determined by the Kruskal-Wallis test (continuous variables) or Fisher's exact test (categorical variables). CSF, cerebrospinal fluid; $EtCO_2$, end-tidal partial pressure of carbon dioxide; MAP, mean arterial pressure; MRI, magnetic resonance imaging.

Table 8. Transcranial Doppler ultrasonography (TCD) variables for 3 groups: no structural diseases (group I), structural diseases without intracranial hypertension (group II), and structural diseases with intracranial hypertension (group III).

Variable	Group I (n = 15)	Group II (n = 22)	Group III (n = 13)	ANOVA or Kruskal-Wallis	Overall *P*-value
PSV (cm/sec)	76.4 (41.5–97.9) [a]	64.2 (34.3–99.3) [a]	81.7 (46.9–111.3) [a]	A	0.24
EDV (cm/sec)	20.1 (13.4–33.1) [a]	19.9 (8.6–39.4) [a]	17.3 (8.9–39.0) [a]	A	0.84
Vm (cm/sec)	34.2 (19.1–49.9) [a]	33.5 (13.7–56.8) [a]	27.7 (13.9–68.4) [a]	K	0.68
Sm (cm/sec)	47.6 (24.9–64.7) [a]	44.9 (18.4–78.2) [a]	41.5 (22.0–95.8) [a]	A	0.60
Dm (cm/sec)	30.0 (17.5–44.6) [a]	28.0 (11.4–48.2) [a]	23.6 (11.8–50.9) [a]	A	0.79
RI	0.72 (0.62–0.78) [a]	0.71 (0.57–0.80) [a]	0.75 (0.59–0.89) [a]	A	0.16
PI	1.57 (1.09–2.12) [a]	1.47 (1.03–2.41) [a]	1.84 (1.02–4.41) [a]	K	0.47
Sm/Dm	1.63 (1.43–1.75) [a]	1.62 (1.27–2.10) [a]	1.78 (1.44–2.58) [b]	K	0.01

Continuous data were expressed as the median (range). Values with different superscript letters indicate significant ($P < 0.05$) differences among groups. A, ANOVA; Dm, diastolic mean velocity; EDV, end diastolic velocity; K, Kruskal-Wallis test; PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index; Sm, systolic mean velocity; Sm/Dm, ratio of systolic to diastolic mean velocity; Vm, mean velocity.

Table 9. Area under the receiver operating characteristic curve (AUC) and optimal diagnostic cutoffs to detect dogs with suspected intracranial hypertension among dogs with neurological diseases.

Variable	AUC	95% CI	Sensitivity	Specificity	Cutoff	*P*-value
RI	0.66	0.47–0.85	0.38	0.95	0.79	0.09
PI	0.61	0.40–0.82	0.38	0.92	2.21	0.23
Sm/Dm	0.79	0.63–0.95	0.62	0.92	1.78	0.002

AUC, area under the receiver operating characteristic curve; CI, confidence interval; PI, pulsatility index; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity.

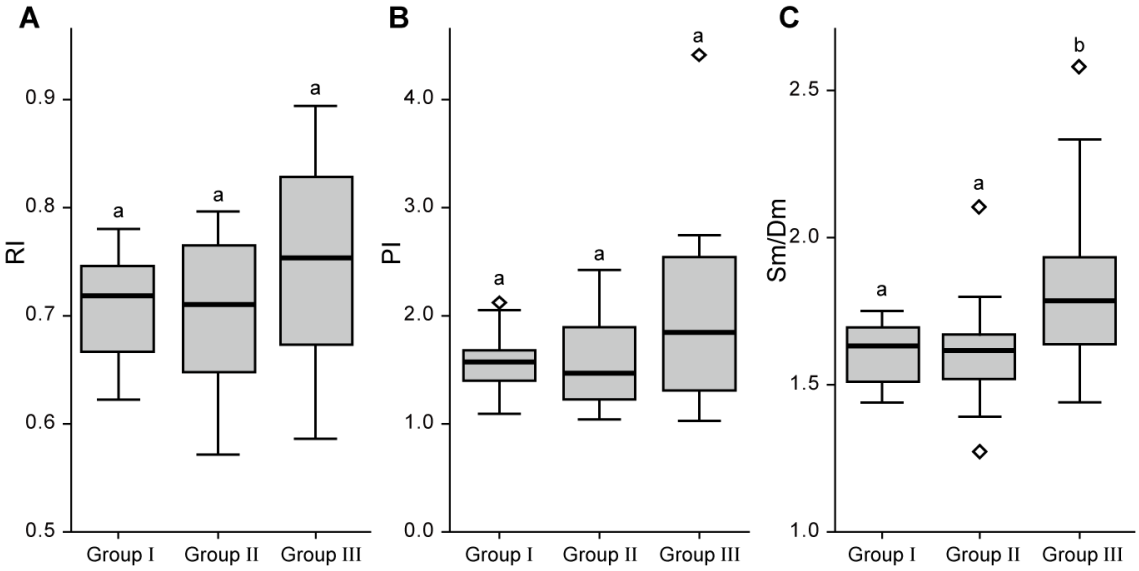

Figure 4. Box and whisker plot of transcranial Doppler ultrasonography (TCD) vascular resistance variables in 3 groups: no structural diseases (group I), structural diseases without intracranial hypertension (group II), and structural diseases with intracranial hypertension (group III). The box represents the interquartile range (IQR) from the 25th to 75th percentile for resistive index (A), pulsatility index (B), and ratio of systolic to diastolic mean velocity (C). The upper and lower whiskers represent the highest datum still within 1.5 IQR of the upper quartile and the lowest datum still within 1.5 IQR of the lower quartile, respectively. The individual dots beyond the whiskers represent outliers. Medians with different letters indicate significant differences among groups. PI, pulsatility index; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity.

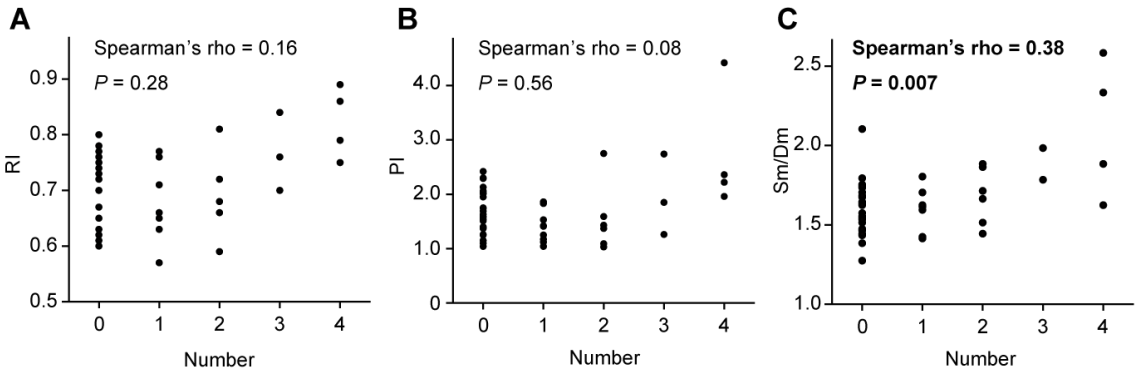

Figure 5. Correlations between the transcranial Doppler ultrasonography (TCD) vascular resistance variables and number of identified magnetic resonance imaging (MRI) findings indicative of intracranial hypertension (horizontal axis). The MRI findings indicative of intracranial hypertension were effacement of the cerebral sulci, brain herniation, compression of cerebrospinal fluid (CSF) space, and displacement of the lamina quadrigemina. Spearman's rho and *P*-value are included for each plot. PI, pulsatility index; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity.

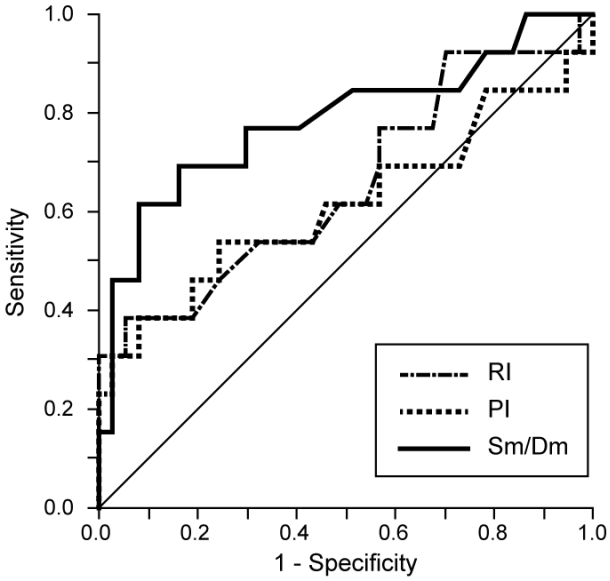

Figure 6. Receiver operating characteristic (ROC) curves of transcranial Doppler ultrasonography (TCD) vascular resistance variables for the detection of dogs with suspected intracranial hypertension among 50 dogs with neurological diseases. PI, pulsatility index; RI, resistive index; Sm/Dm, ratio of systolic to diastolic mean velocity.

4. Discussion

The results of this chapter suggest that the TCD vascular resistance variables can be used to detect dogs with MRI evidence of intracranial hypertension. While no significant differences in the TCD vascular resistance variables were detected between dogs without intracranial structural diseases (group I) and those with intracranial structural diseases without MRI evidence of intracranial hypertension (group II), the present results showed that Sm/Dm was significantly higher in dogs with MRI evidence of intracranial hypertension (group III).

In the present study, the RI, PI and Sm/Dm values tended to increase as the number of identified MRI findings indicative of intracranial hypertension increased. However, RI and PI did not significantly differ between dogs with and without suspected intracranial hypertension. Previous experimental studies in dogs have shown that increased intracranial pressures measured directly are associated with an increase in RI obtained by TCD.[20] In humans, a correlation between directly measured intracranial pressures and the PI has been shown in neurointensive care studies involving patients with intracranial hypertension after traumatic brain injury.[18,80] The discordance between this results and these findings may have been caused by the difference in the study population: dogs with a severe clinical status precluding general anesthesia were excluded in the present study.

The findings of this study indicate that intracranial hypertension may be diagnosed on the basis of an increased Sm/Dm. The Sm/Dm in the ophthalmic artery has been reported as an indicator of systemic atherosclerosis in humans.[19] In human atherosclerosis, high values of Sm/Dm reflect a decrease of the diastolic flow due to diminished arterial compliance with a preserved systolic velocity.[19,81] This study is the first report in both humans and dogs using Sm/Dm as a variable of TCD. Sm/Dm would also reflect the alterations in cerebral blood flow observed in intracranial hypertension: as the intracranial pressure increases, the resultant reduction in cerebral blood flow alters the Doppler waveforms of the cerebral artery so that the diastolic velocity is decreased with the systolic velocity being preserved.[77,78] Considering that there were no significant differences in Dm among the groups, the relative changes in diastolic velocity might

occur in individual Doppler waveforms, and Sm/Dm reflected as a ratio of these relative changes.

The findings of the present study indicate that Sm/Dm may be a more sensitive variable to detect intracranial hypertension compared with RI and PI. Actually, a previous study evaluating the Doppler waveforms of the ophthalmic artery showed that Sm/Dm can be more sensitive for the detection of glaucoma patients than RI.[82] The higher sensitivity of Sm/Dm in the present study might have been because of the difference in calculation methods of each TCD vascular resistance variables. All of these indices are calculated using systolic and diastolic components of arterial waveforms. However, RI and PI are calculated by the use of instantaneous velocities (i.e., PSV and EDV), while Sm/Dm is determined on the basis of mean velocities. Given that the initial alterations of cerebral blood flow in intracranial hypertension are mainly associated with the decreases in diastolic flow,[77,78] the changes in diastolic mean velocities of cerebral arterial waveforms could have been more prominent than those in diastolic instantaneous velocities in dogs with suspected intracranial hypertension in this study.

Interestingly, no significant differences in TCD variables were observed between dogs in groups I (no structural diseases) and II (intracranial structural diseases without intracranial hypertension). According to the volume-pressure curve, the intracranial pressure remains relatively stable against the increase in the intracranial volume when the intracranial volume is mildly increased from the normal volume (i.e., the brain's compensation mechanism). Consequently, the cerebral perfusion pressure is initially maintained despite the mild increase in the intracranial volume.[67] In human hydrocephalus patients, mildly-moderately increased intracranial pressure (i.e., 20 mmHg) did not cause significant changes in cerebral perfusion and PI.[83] Considering this compensation mechanism, the lack of significant differences in the TCD variables between groups I and II might have been because the intracranial structural diseases in group II only caused mild or no increase in the intracranial pressure. Therefore, the possibility that a mild increase in the intracranial pressure cannot be detected by the TCD vascular resistance variables should be considered.

The combination of MRI findings including effacement of cerebral sulci, brain herniation, compression of CSF space, and brain shifting is useful and reliable for the

diagnosis of intracranial hypertension in dogs, and therefore was used as the diagnostic criteria for diagnosing intracranial hypertension in the present study.[73,79] However, each MRI finding indicative of intracranial hypertension is non-specific. To determine the relationship between TCD vascular resistance variables and each MRI finding, multiple linear regression analysis was performed. This statistical analysis showed that only effacement of the cerebral sulci was the predictor of high TCD vascular resistance variables. In the present study, TCD vascular resistance variables may be changed by the influence of the forebrain swelling than local structural influence.

Mannitol administration performed in some cases could have affected the TCD variables in the present study. Due to ethical concerns, TCD was performed only after treatment with mannitol. Previous studies in human patients with intracranial hypertension have shown that PI was decreased by 20% following mannitol administration via the increase in diastolic flow velocity associated with the decrease in intracranial pressure.[84,85] Therefore, in the present study, it is possible that the worsening of the TCD vascular resistance variables associated with intracranial hypertension could have been blunted by mannitol administration.

This study had several limitations. Firstly, the intracranial pressure was not directly measured in the present study. The diagnosis of intracranial hypertension was by direct intracranial pressure measurements, but this was not acceptable in this study because it required invasive techniques. An increased intracranial pressure causes a shift of intracranial structures such as brain herniation and compression of the CSF space, which are observed on MRI.[76] Therefore, the dogs in this study were categorized by the MRI findings of suspected intracranial hypertension. Secondly, the study population was restricted to dogs with clinical conditions acceptable for general anesthesia: and therefore dogs with severe intracranial hypertension that precluded isoflurane general anesthesia were excluded in this study. The dogs enrolled in this study were under isoflurane general anesthesia, because (1) to obtain MRI findings of suspected intracranial hypertension and (2) to abolish the effect of differences in anesthetic/sedation/non-sedation protocols on TCD variables. However, it should be of clinical significance that the present study demonstrated that in dogs, TCD variables can be altered even in relatively mild intracranial hypertension where brain’s compensatory mechanism is preserved. Thirdly,

it was a cross-sectional study, and thus the present study cannot show a causal relationship between the changes in TCD variables and intracranial hypertension. Since TCD can be performed non-invasively and repetitively, it may be possible to confirm the causality between them by serially monitoring the dogs with intracranial hypertension with TCD. Lastly, Doppler waveforms of the basilar artery could not be obtained in three dogs. Furthermore, evaluation of cerebral blood flow in the middle cerebral artery is expected to be more difficult due to skull variations. Since basilar arterial TCD requires neck flexion, which can alter cerebral blood flow, the ability to evaluate in the middle cerebral artery would be highly clinically useful. This problem can occur also in humans, especially in older women due to hyperostosis of the skull.[7] To improve the ability of detecting Doppler signals, the use of ultrasound contrast agents may be useful.[86] However, it will be necessary to study the influence of the use of contrast agents on TCD variables.

In conclusion, the findings of this study may indicate that TCD can be a clinical tool useful for the diagnosis of suspected intracranial hypertension. Additional studies involving dogs with a severer clinical status and conscious dogs are needed to establish the usefulness of TCD as a routine clinical diagnostic test for intracranial hypertension.

5. Summary

In this chapter, TCD vascular resistance variable, especially Sm/Dm, was increased in the dogs with MRI evidence of intracranial hypertension. The findings of this study may indicate that TCD examination can be a clinical tool useful for the diagnosis of intracranial hypertension. Additional studies involving dogs with a severer clinical status and conscious dogs are needed to establish the usefulness of TCD as a routine clinical diagnostic test for intracranial hypertension.

General conclusion

The goal of this study was to investigate the usefulness of cerebral blood flow assessment using transcranial Doppler ultrasonography in small animal veterinary medicine. The findings of the present study indicate that TCD variables are feasible and reliable methods for assessing cerebral blood flow in anesthetized dogs. TCD velocities are increases in the ictal phase of drug-induced epileptic seizures. TCD vascular resistance variables are increases in clinical dogs with presumed intracranial hypertension. These results indicate that TCD has a great ability to assess cerebral blood flow in small animal veterinary medicine.

The first step, chapter 1, was to investigate the applicability of cerebral blood flow assessment using TCD to dogs. TCD was performed in awake dogs and anesthetized dogs, differences in measured values of blood flow waveforms were determined and the repeatability of TCD variables was evaluated. TCD velocities did not change significantly with anesthesia in this study, however, variables that evaluate the shape of the waveform, such as RI, PI, and AT, changed significantly. Although some variables were shown to have poor repeatability in awake dogs, all TCD variables have good repeatability in anesthetized dogs.

In chapter 2, the association between the changes in cerebral blood flow and electrographic epileptic seizure was investigated using TCD in healthy dogs. TCD variables and systemic parameters were measured before, during, and after seizures and compared in dogs with pentetrazol-induced seizures. The results showed that the TCD velocities of PSV, EDV, and Vm significantly increased in the basilar and middle cerebral arteries during pentetrazol-induced seizures. The TCD velocities significantly decreased after diazepam administration. This result indicates the possible utility of increased TCD velocities in detecting epileptic seizures during the anesthetic treatment of status epilepticus. In addition, the results showed that TCD has the potential efficacy to monitor the resolution of seizure activity.

Chapter 3 described the diagnostic performance of TCD in a clinical setting for the diagnosis of intracranial hypertension. The dogs with intracranial diseases underwent TCD of the basilar artery under isoflurane anesthesia following MRI and investigate the

relationship with the MRI findings of suspected intracranial hypertension. The results showed that TCD vascular resistance variable, especially Sm/Dm, was increased in the dogs with MRI evidence of intracranial hypertension. Conventional TCD vascular resistance variables such as RI and PI showed no significant association with the MRI findings of intracranial hypertension. Furthermore, in the absence of intracranial hypertension, TCD variables did not change in the presence or absence of intracranial structural diseases. The findings of this study may indicate that TCD examination can be a clinical tool useful for the diagnosis of intracranial hypertension.

One of the problems to be resolved in the future is to improve the detection of Doppler waveforms. In the clinical study, cerebral blood flow evaluation was impossible in 3 of 53 dogs. One of the reasons may be that the dogs unable to be measured were relatively large, however, reliable acquisition of cerebral blood flow is essential for expanding clinical adoption. One solution is to use ultrasound contrast agents to enhance the Doppler signal. However, it will be necessary to study the influence of the use of contrast agents on TCD variables. In addition, it is necessary to accumulate knowledge of TCD waveforms in other neurological diseases, such as cerebral infarction and brain death, in order to further expand its clinical usefulness.

In conclusion, the applicability of TCD in dogs was able to be established. TCD velocities were increased in ictal-phase of drug-induced epileptic seizure in healthy dogs. TCD vascular resistance variables were increased in clinical dogs with presumed intracranial hypertension. These results indicate that TCD has a great ability to assess cerebral blood flow in small animal veterinary medicine.

Acknowledgement

This PhD study is not one that can be achieved alone. I would like to thank all people who have supported and encouraged me.

First and foremost, I would like to express my gratitude to my supervisor, Dr. Mitsuyoshi Takiguchi for giving me the opportunities to achieve my goals and aspirations, and for his constant encouragement, support, advice, and guidance.

I wish to also thank Dr. Takashi Kimura, Dr. Kensuke Nakamura, Dr. Jun Tamura for their time and effort to provide advice and critical comments in making this dissertation a better one.

My gratitude also extends to Dr. Masahiro Yamasaki (Iwate University), Dr. Hiroshi Ohta (Rakuno Gakuen University), Dr. Tomohito Ishizuka, Dr. Keitaro Morishita, Dr. Noboru Sasaki, Dr. Nozomu Yokoyama, Dr. Tatsuyuki Osuga (Miyazaki University), Dr. Tomoya Morita (Iwate University), Mr. Noriyuki Nagata, Dr. Masahiro Tamura (Rakuno Gakuen University), Dr. Sue Yee Lim, Dr. Khoirun Nisa, Dr. Angkhana Dremlim, Dr. Rommaneeya Leela-Arporn, Dr. Kazuki Kojima, Mr. Takachika Saito, Mr. Yuki Omura, Ms. Kyoko Kuroda, Ms. Yumiko Inoue, and so on. So many kind people in laboratory of veterinary internal medicine and Hokkaido University Teaching Hospital give me a lot of inspiration, advice, comments, support, and courage.

In addition, my graduate school life and research were supported by Hokkaido University, Program for Leading Graduate Schools “Foresting global leaders in veterinary science toward contributing to One Health” (coordinator, Dr Motohiro Horiuchi) and Japan Society for the Promotion of Science “Grand-in-Aid for JSPS fellows (Grant Number 18J11947)”.

Finally, I wish to thank all dogs in the laboratory for contributions to my PhD study.

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Summary in Japanese（和文要旨）

Studies on the Clinical Usefulness of the Evaluation of Cerebral Blood Flow Using Transcranial Doppler Ultrasonography in Dogs
（犬における経頭蓋超音波ドプラ法を用いた
脳血流評価の臨床的有用性に関する研究）

脳血流は酸素とグルコースを常に必要とする脳を維持するために不可欠なものである。脳血流は動脈血中二酸化炭素分圧や全身血圧、血液粘稠度や脳活動などの様々な生理的因子によって変動するが、生理的な範疇では脳血管の自己調節能により維持される特性をもつ。一方で、頭蓋内圧上昇や脳血管障害といった病的要因により脳血流は顕著に変化するため、これらの病態把握を目的として脳血流評価が行われる。脳機能評価や脳血流評価には核医学検査がゴールドスタンダードとして用いられるが、施設的制限が大きく、また被曝による侵襲性が問題となり、獣医学領域での利用は限定的である。

脳血流を非侵襲的に評価する手法として、経頭蓋超音波ドプラ法（Transcranial Doppler ultrasound, TCD）が挙げられる。一般的に超音波検査は空気と骨を不得意とする検査であり、その内部の構造、つまり脳実質を観察することは難しいと考えられていた。Aaslid らは、骨や軟部組織による減衰の少ない 1–2 MHz の低周波超音波を用いて、脳動脈の血流波形を観察できることを報告した。これを契機に、脳血流が大きく変化する脳梗塞を中心として TCD の臨床報告が行われている。また、超音波検査の利点である即時性、ベッドサイドでも使用可能な簡便性、非侵襲性を活用し、神経集中治療の領域において TCD を用いた脳血流モニタリングが報告されている。

TCD では頭蓋骨を透過したドプラシグナルを元に、脳底動脈や中大脳動脈、前大脳動脈などの脳動脈の血流波形を描出する。血流波形から血流速度を得ることができ、特に平均血流速度は脳血流量を推定する指標として知られる。また、収縮期と拡張期の変化率から血管抵抗指標が算出される。血管抵抗指標として、抵抗指数（RI）や拍動指数（PI）があり、これらは頭蓋内圧との相関が示されている。さらに、血流の通りやすさの指標である脳血管コンダクタンス指標（CVCi）や眼動脈において血管硬化性変化を捉える収縮期拡張期平均血流速比

（Sm/Dm）といった応用的な指標も血流波形から算出される。

迅速性、簡便性、非侵襲性という小動物臨床に適した特性を有する TCD ではあるが、獣医学領域においての報告は限られており、限られた報告内でも一般的なパラメータで検討するに留まっている。実験的な研究では、脳血流と TCD の血流速度が相関する報告、頭蓋内圧と RI が相関する報告が挙げられる。また、臨床例では症候性水頭症の犬での RI 高値が報告されているが、脳炎や肝性脳症においては症例報告があるのみである。犬の最も一般的な神経疾患であるてんかんに関して脳血流評価の観点からアプローチした報告はない。また、実験的に示される脳圧亢進との関連についても臨床例においては検証されていない。したがって、本研究の目的を TCD による犬の脳血流評価の臨床的有用性を検証することと定め、3 段階からなる研究を行った。

第一段階として、TCD による脳血流評価の犬への応用可能性を検討した。全身麻酔もしくは覚醒状態の健常犬に対して TCD を実施し、脳底動脈血流波形における測定値の差異を検討するとともに、再現性評価を行った。その結果、全身麻酔と覚醒状態とで脳血流速度では差異を認めないものの、血管抵抗指標などの血流波形の形を評価する指標では有意な差異を認めた。再現性評価では全身麻酔の犬において全ての指標で良好な再現性が認められた。一方で、覚醒状態の犬では全身麻酔に比べて再現性が劣る傾向にあり、一部指標においては再現性が良好ではなかった。以上の結果から、TCD による脳血流評価において特に全身麻酔では良好な再現性を示し、臨床応用可能であることが示された。

続いて第二段階として、てんかん発作における電気的脳活動の検出に対する TCD の応用可能性を検討するための基礎的実験として、健常犬において全身麻酔下でペンテトラゾール投与により誘発された電気的てんかん発作が全身指標および TCD の血流評価指標に与える影響を検討した。結果として、電気的なてんかん発作において全身血圧や心拍出量などの全身指標および TCD の血管抵抗指標には有意な変化が認められなかったが、中大脳動脈と脳底動脈の両方で TCD の血流速度が発作時において有意な上昇を認めた。さらに、上昇した血流速度はジアゼパムによって発作活動が抑制されると発作前の範囲に戻ることが示された。したがって、電気的な脳活動を TCD による脳血流上昇として検出できる可能性が示された。加えて、TCD の血流速度と全身指標の偏相関解析により、収縮期血流と心拍出量および体温との間、拡張期血流と二酸化炭素分圧

との間、平均血流速度と心拍出量・体温・二酸化炭素分圧との間に有意な相関関係を認めた。今後の検討及び臨床応用にあたっては、これらの全身指標が TCD の血流速度に影響を与えうることに留意する必要があることが示された。

第三段階として、臨床的な現場において TCD による脳血流評価が頭蓋内圧亢進を評価可能であるか検討するために、附属動物病院に来院した神経疾患罹患犬に対して TCD による脳血流評価を行い、MRI 検査における頭蓋内圧亢進所見との関連を検討した。結果として、血管抵抗指標の一種である収縮期拡張期平均血流速比（Sm/Dm）が頭蓋内圧亢進所見を有する群において有意に高値であることが示された。既存の血管抵抗指標である RI や PI では頭蓋内圧亢進所見との有意な関連は認めなかった。さらに、頭蓋内圧亢進所見がない場合には器質病変の有無では TCD の血流評価指標は有意に変化しないことも示され、頭蓋内圧亢進が脳血流波形を変化させることが明らかとなった。これらの結果から、TCD が小動物臨床において頭蓋内圧亢進の評価に有用であることが示された。

今後明らかにするべき課題の一つとして、脳血流波形の描出改善が挙げられる。第三段階の臨床症例を対象とした検討において 53 頭中 3 頭で脳血流評価が不可能であった。これらの犬は比較的大型であったことが要因と考えられるが、臨床応用の拡大には脳血流波形の確実な取得が必須である。そのための一つの方策として、超音波造影剤の使用により、ドプラシグナルを増強することが効果的と考えられる。超音波造影剤の使用による描出改善効果の検討および TCD 指標に与える影響を検討する必要がある。また、血流の変化が大きいと想定される脳梗塞や脳死において TCD の血流波形の知見を蓄積し、さらなる臨床的有用性の拡大に向けて検討を行っていく必要がある。

最後に、本研究により TCD による脳血流評価が犬におけるてんかん発作や頭蓋内圧亢進の評価に有用である可能性が示された。今後、TCD による脳血流評価を通して、犬の神経疾患に対する病態把握ならびに適切な治療介入の実現が期待される。