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Internal Medicine

Effects of cilostazol on the heart rate in healthy dogs

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ABSTRACT. Appropriate dosages of cilostazol have not been studied in veterinary patients, and the degrees of heart rate (HR) increase have not been studied in dogs administered cilostazol. Therefore, this study aimed to investigate the degrees of HR increase in healthy dogs administered cilostazol. Thirty healthy beagle dogs (15 males and 15 females; age, 5–8 years) were divided into 3 groups of 10 dogs each and orally administered 2.5, 5, or 10 mg/kg cilostazol (twice a day at 8:00 AM and 8:00 PM for 10 days). Higher HR increases were seen in the 5 mg/kg group than in the 2.5 mg/kg group at all time points except 7:00 AM, 9:00 AM, 1:00 PM, and 4:00 PM ($P<0.01$). Higher HR increases were also observed in the 10 mg/kg group than in the 2.5 mg/kg group at all time points except 4:00 PM ($P<0.01$). The 10 mg/kg group showed higher HR increases than the 5 mg/kg group at all time points except 6:00 AM, 7:00 AM, 6:00 PM, and 7:00 PM ($P<0.05$ for 4:00 PM and 5:00 PM; $P<0.01$ for the other time points). These results together show that the HR of healthy dogs increased in a dose-dependent manner after cilostazol administration twice a day at doses of 5 to 10 mg/kg. These results provide a useful basis for choosing cilostazol in the treatment of bradyarrhythmia in dogs.

KEY WORDS: cilostazol, dog, heart rate, Holter electrocardiogram, oral administration

Pacemaker implantation (PMI) is the first-choice treatment for human patients with bradyarrhythmia who frequently develop clinical signs of syncope and collapse [16]. However, PMI must be abandoned for many animal patients, such as cats and dogs, and other medical treatments must be chosen because of various background factors. These include a limited number of facilities that perform PMI in animals, economic constraints of the owner, aggravation of conditions caused by underlying diseases or complicated heart failure, difficulty in obtaining the device, and high risk in the induction of general anesthesia.

Anticholinergic drugs such as atropine sulfate, β-agonists such as isoproterenol, and phosphodiesterase inhibitory and adenosine receptor antagonist agents such as aminophylline and theophylline have been used as therapeutic agents in dogs with bradyarrhythmia [2]. However, these drugs have not yielded completely satisfactory results as alternative therapies to PMI because of their insufficient efficacy and short duration of effectiveness against arrhythmia [6, 16].

Recent studies have reported the effectiveness of orally administered cilostazol, a phosphodiesterase III inhibitor, in treating bradyarrhythmia in humans and animals [9, 11, 12, 14, 17]. Cilostazol is a platelet-aggregation inhibitor and has been administered intrinsically for preventing relapse in patients with cerebral infarction or chronic arterial occlusion. Because the administration of cilostazol often induces side effects including tachycardia, it has been used as a treatment option for bradyarrhythmia based on its pharmaceutical effect.

However, appropriate dosages of cilostazol have not been studied in veterinary patients. Moreover, the degrees of heart rate (HR) increase have not been studied in dogs administered cilostazol. Currently, cilostazol is administered to animal patients according to the judgment of individual veterinary practitioners, depending on the circumstances.

In this study, to reveal the appropriate dosage of cilostazol in dogs, healthy dogs were administered 2.5, 5, or 10 mg/kg cilostazol twice a day. Changes in the HR were monitored for 24 hr by using Holter electrocardiography, and the development of side effects was evaluated. Cardiac functions were determined and compared before and after drug administration by using cardiac ultrasound examination. In addition, electrocardiography and blood pressure measurement were performed.
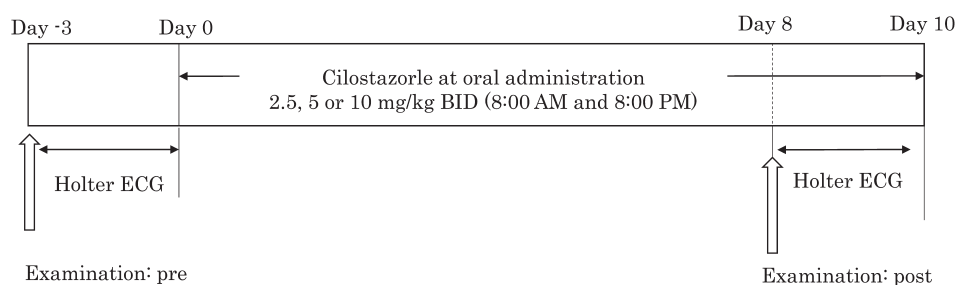
MATERIALS AND METHODS

This study was conducted according to the Ethical Code for Animal Experimentation of the Tokyo University of Agriculture and Technology (29-72).

Fig. 1. Examination schedule. Cardiac ultrasound, chest plain radiography, blood pressure measurement, and blood tests were performed before the administration of cilostazol (Day −3). Holter electrocardiography was carried out from Day −3 to Day 0. The administration of cilostazol was started the day after the completion of Holter electrocardiography (from Day 0 to Day 10). Similar examinations were performed on Day 8 of the administration of cilostazol (all examinations except Holter electrocardiography). Holter electrocardiography was carried out from Day 8 to Day 10.

Animals

Thirty clinically healthy beagle dogs (15 intact males and 15 intact females, aged between 5 and 8 years, and weighing 9.3 to 10.9 kg) were used in this study conducted at the Laboratory of Veterinary Surgery, Tokyo University of Agriculture and Technology. None of the animals showed abnormal findings in the general physical, blood biochemical, cardiac ultrasound, or chest plain radiography examinations, and all were considered suitable for inclusion in this study. The animals were randomly divided into 3 groups of 10 dogs each and administered 2.5, 5, or 10 mg/kg cilostazol (5 intact males and 5 intact females in each group). The dogs were provided drinking water ad libitum and commercially available dog food twice a day at 8:00 AM and 5:00 PM.

Drug administration

Cilostazol (cilostazol tablets 50 mg; Pfizer Japan Inc., Tokyo, Japan) was orally administered as tablets: 0.5 tablet in the 2.5 mg/kg group (2.3 to 2.6 mg/kg), 1 tablet in the 5 mg/kg group (4.7 to 5.3 mg/kg), and 2 tablets in the 10 mg/kg group (9.3 to 10.1 mg/kg), twice a day (every 12 hr) at 8:00 AM and 8:00 PM for 10 days.

Examination schedule

Cardiac ultrasound, chest plain radiography, blood pressure measurement, and blood tests were performed before the administration of cilostazol (Day −3). These tests were conducted between 1:00 PM and 2:00 PM. Holter electrocardiography was carried out from Day −3 to Day 0. The administration of cilostazol was started the day after the completion of Holter electrocardiography (from Day 0 to Day 10). Similar examinations were performed on Day 8 of the administration of cilostazol (all examinations except Holter electrocardiography). Holter electrocardiography was carried out from Day 8 to Day 10 (Fig. 1).

Holter electrocardiography

Dogs were fitted with a data-storage-type Holter electrocardiograph device (QR-2100; Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan) for 3 days before the day of drug administration (baseline) and for 3 days (from days 8 to 10) after drug administration. Data were collected on the 2nd and 3rd days of each Holter electrocardiography, and the mean values obtained from each recording were used as preadministration and postadministration data. Data stored on the device were analyzed using a Holter electrocardiogram analysis software (HS manager V 4.0A; Fukuda M-E Kogyo Co., Ltd.). The total HR, maximum HR, average HR, minimum HR, longest RR interval, and ventricular premature contraction (VPC) events were analyzed at baseline and after drug administration. Changes in the HR were measured every hour for a period of 24 hr (the pre-cilostazol administration value was defined as 100%).

Electrocardiography

Electrocardiography was conducted using a multifunction electrocardiograph for animals (D700; Fukuda M-E Kogyo Co., Ltd.). Animals were held in the right-sided recumbent position and the PQ intervals, QT intervals, corrected QT intervals using Bazett's formula ($QTcB=QT/^3\sqrt{RR}$), and corrected QT intervals using Fridericia's formula ($QTcF=QT/^3\sqrt{RR}$) were obtained [3, 13, 23].

Cardiac ultrasound examination

Cardiac ultrasound examination was conducted using ultrasonic diagnostic equipment (LOGIQ7; GE Healthcare Japan Co., Tokyo, Japan) and a 7S sector probe (variable frequency range: 3.1–8.0 MHz).

The right parasternal left ventricular short-axis–chordae tendineae level view was visualized. The left ventricular inner diameter at end diastole (LVIDd), left ventricular inner diameter at end systole (LVIDs), and fractional shortening (FS) were determined using the M-mode. FS was calculated using the formula [(LVIDd−LVIDs)/LVIDd] × 100.

The right parasternal left ventricular short-axis–basal level view was visualized. The left atrial diameter (LA), aortic diameter (Ao), and the ratio of LA to Ao (LA/Ao) were determined using the B-mode.

The aortic orifice region was visualized using the left parasternal left ventricular outflow tract view, and the pre-ejection period (Ao-PEP), ejection time (Ao-ET), and the ratio of Ao-PEP to Ao-ET (Ao-PEP/ET) were obtained using the aortic orifice blood flow waveform in the Doppler-mode. The Ao-PEP was defined as the time from the Q wave on the electrocardiogram synchronized with echocardiography to the start of blood ejection from the left ventricle. Moreover, the time during which blood was actually ejected from the left ventricle was defined as the Ao-ET [20, 25]. The aortic annulus dimension was measured using the zoom function of the echocardiography instrument, and the velocity–time integral was measured by tracing the aortic orifice blood flow waveform. Thereafter, stroke volume (SV) was calculated as follows: aortic annulus dimension$^2 \times \pi \times 1/4 \times$ velocity–time integral. Furthermore, cardiac output (CO) was calculated using SV $\times$ HR. The mitral orifice region was visualized using the left parasternal apical four-chamber view, and the early inflow wave (E) consisting of the left ventricular inflow wave was determined using the Doppler-mode. The wave height of the protodiastolic component (e') of the mitral valve annulus velocity and the ratio of E to e' (E/e') were determined in the same cross section by using the tissue Doppler method.

Nine consecutive heartbeats were measured, and the mean value was analyzed as the measured value in the aforementioned examinations.

Blood pressure measurement

Blood pressure was measured at the tail head by using the oscillometric method with a cuff width of 40–60% of the periphery of the measured region [5, 22], by using a noninvasive sphygmomanometer for animals (BP-100D; Fukuda ME Kogyo Co., Ltd.). Systolic arterial blood pressure (SAP), mean arterial blood pressure (MAP), and diastolic arterial blood pressure (DAP) were measured in triplicate, and the mean values were obtained.

Systemic vascular resistance (SVR) was calculated according to the formula SVR=(MAP −central venous pressure)/CO × 80 [24], by using the MAP and CO values obtained through echocardiography and indirect blood pressure measurement. Central venous pressure was defined as 5 mmHg attributable to a lack of right-sided heart failure signs, jugular distension, or a positive hepatojugular reflex [26]. None of the dogs used in this study had functional abnormalities of the tricuspid valve; hence, we considered the central venous pressure to be 5 mmHg [21].

Blood tests

Blood samples were collected from the jugular vein and dispensed into containers suitable for each test. Blood dispensed into the tube with ethylene-diamine-tetra-acetic acid was mixed well, and the following tests were carried out using a fully automated hemocytometer suitable for animal measurements (MEK-6450 Celltac α; Nihon Kohden Co., Tokyo, Japan): total leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count. Blood dispensed into the tube with heparin was centrifuged at 1,500 × g for 5 min. Plasma was separated and used for the measurement of blood urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, total protein, and albumin levels by using a biochemical analyzer suitable for animal samples (Fuji DRI-CHEM 7000; FUJIFILM Medical Co., Ltd., Tokyo, Japan). Sodium, potassium, and chlorine concentrations were determined using an ion-selectivity analyzer suitable for animals (Fuji DRI-CHEM 800V; FUJIFILM Medical Co., Ltd.).

Statistical analysis

Measurements are expressed as the mean ± standard deviation. To confirm the normal distribution of measurements at each time point, normal probability plots of the measurements were obtained and the normality of distribution was tested using the Kolmogorov–Smirnov test. Measurements complying with a normal distribution were analyzed using a paired t-test, whereas those not complying with a normal distribution were analyzed using the Wilcoxon signed-rank test. The HR increase rates in the cilostazol-administered animals were analyzed using a two-way analysis of variance and compared using the Tukey–Kramer test. A P-value of <0.05 was considered statistically significant. All statistical analyses were performed using statistical software (BellCurve for Excel, Social Survey Research Information Co., Ltd., Tokyo, Japan).

RESULTS

Holter electrocardiography

No significant differences were observed in the total HR, maximum HR, mean HR, or minimum HR in the animals after the administration of 2.5 mg/kg cilostazol compared to corresponding values at the baseline. In contrast, the total HR, mean HR, and minimum HR increased significantly after the administration of 5 and 10 mg/kg cilostazol (P<0.01). Furthermore, the maximum HR increased significantly after the administration of 10 mg/kg cilostazol compared to the corresponding value at the baseline (P<0.01).

The maximum RR interval decreased significantly after the administration of 5 and 10 mg/kg cilostazol compared to the corresponding value at the baseline (P<0.01). In contrast, no significant difference was observed in the maximum RR interval in the 2.5 mg/kg group.
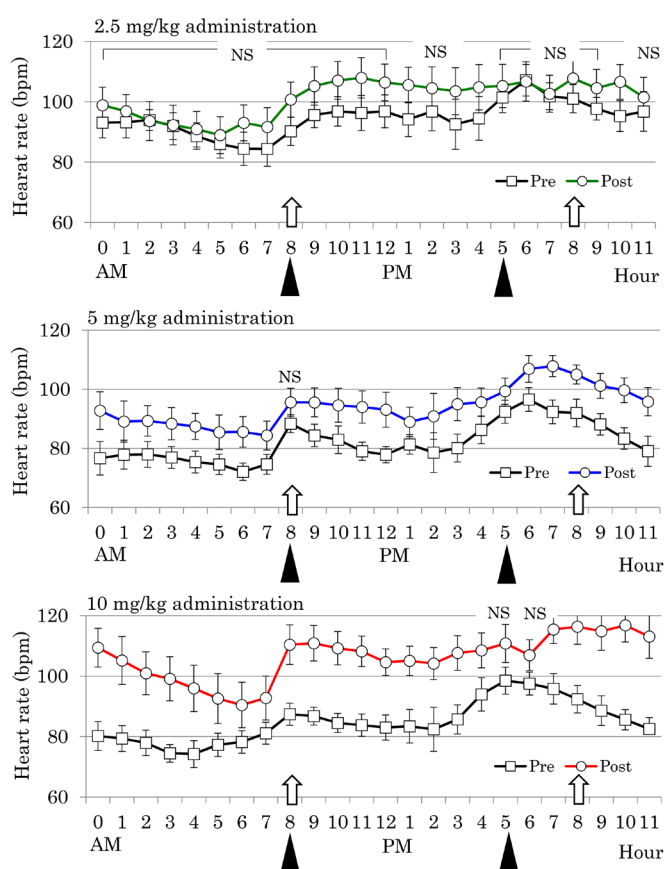
The administration of cilostazol induced no significant differences in the frequency of VPC in any of the groups compared to the corresponding value at the baseline (Table 1).

Dogs administered 2.5 mg/kg cilostazol showed a significant increase in the HR at 1:00 PM, 3:00 PM, 4:00 PM, and 10:00 PM (P<0.05). In the group administered 5 mg/kg cilostazol, significantly increased HR was observed at most time points after

Table 1. Holter Electrocardiogram findings

|		2.5 mg/kg	5 mg/kg	10 mg/kg
Total heart rate (beats/day)	pre	136,024 ± 21,856	116,805 ± 17,185	117,643 ± 21,338
	post	145,672 ± 27,793	135,486 ± 20,512 [a]	152,303 ± 22,064 [a]
Maximum heart rate (beats/min)	pre	215 ± 18	227 ± 27	218 ± 17
	post	219 ± 17	236 ± 24	242 ± 18 [a]
Mean heart rate (beats/min)	pre	93 ± 15	79 ± 12	82 ± 12
	post	99 ± 19	92 ± 14 [a]	104 ± 15 [a]
Minimum heart rate (beats/min)	pre	49 ± 16	39 ± 11	42 ± 8
	post	54 ± 16	47 ± 14 [a]	53 ± 11 [a]
Maximum RR interval (msec)	pre	2.9 ± 1.2	4.1 ± 1.4	3.7 ± 1.3
	post	2.3 ± 0.6	3.1 ± 1.3 [b]	2.9 ± 0.9
Number of occurrences of Ventricularpremature complex (beats/day)	pre	0.3 ± 0.4	0.4 ± 0.9	0.9 ± 1.9
	post	0.3 ± 0.5	0.4 ± 1.0	0.3 ± 0.6

pre: before administration of cilostazol, post: after administration of cilostazol. a) $P<0.01$ vs pre, b) $P<0.05$ vs pre.

Fig. 2. Changes in the heart rate after the administration of 2.5, 5, and 10 mg/kg cilostazol. Expressed as mean ± standard error. An increase in the heart rate is clearer at 5 mg/kg and 10 mg/kg than at 2.5 mg/kg. Times other than those marked NS (no significant difference) show statistically significant differences. Pre: before the administration of cilostazol; Post: after the administration of cilostazol; White arrow: administration time of cilostazol; Black arrow head: meal time; NS: no significant difference.

drug administration ($P<0.05$ for 5:00 PM; $P<0.01$ for the other time points), but no significant difference was found at 8:00 AM. In the group administered 10 mg/kg cilostazol, significantly increased HR was observed at most of the time points after drug administration ($P<0.05$ for 7:00 AM; $P<0.01$ for the other time points), but no significant difference was found at 5:00 PM or 6:00 PM (Fig. 2).

The HR increase rates were compared among the groups administered different doses of cilostazol. The results showed higher increase rates in the 5 mg/kg group than in the 2.5 mg/kg group at all time points except 7:00 AM, 9:00 AM, 1:00 PM, and 4:00 PM ($P<0.01$). Higher increase rates were observed in the 10 mg/kg group than in the 2.5 mg/kg group at all time points except 4:00 PM ($P<0.01$). Higher increase rates were also observed in the 10 mg/kg group than in the 5 mg/kg group at all time points except
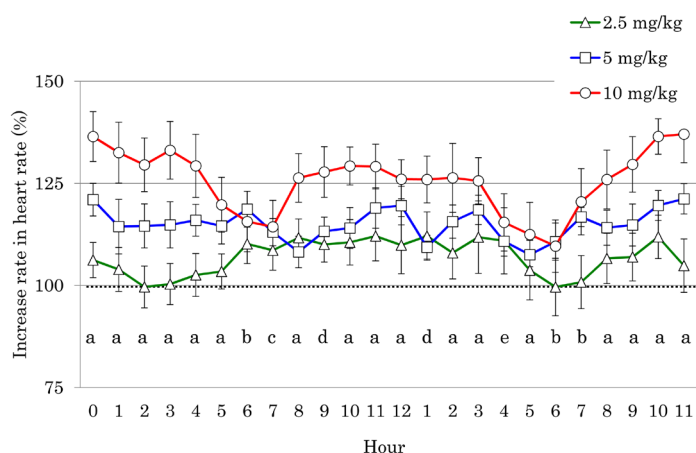
Fig. 3. The rate of change in the heart rate after the administration of cilostazol. The heart rate before the administration of cilostazol is defined as 100% (dotted line). Expressed as mean ± standard error. The heart rate increase was higher as the dose of cilostazol increased. a: $P<0.01$ for 2.5 mg/kg vs 5 mg/kg, 2.5 mg/kg vs 10 mg/kg, and 5 mg/kg vs 10 mg/kg. b: $P<0.01$ for 2.5 mg/kg vs 5 mg/kg, and 2.5 mg/kg vs 10 mg/kg. c: $P<0.01$ for 2.5 mg/kg vs 10 mg/kg. d: $P<0.01$ for 2.5 mg/kg vs 10 mg/kg, and 5 mg/kg vs 10 mg/kg. e: $P<0.05$ for 5 mg/kg vs 10 mg/kg.

Fig. 4. Changes in the PQ interval after the administration of cilostazol. Shortening of the PQ interval was observed in each group after the administration of cilostazol. **: $P<0.01$ vs pre; *: $P<0.05$ vs pre.

Table 2. Electrocardiologic findings

		2.5 mg/kg	5 mg/kg	10 mg/kg
PQ (msec)	pre	83 ± 13	80 ± 10	79 ± 10
	post	78 ± 11 [b]	70 ± 9 [a]	72 ± 10 [a]
QT (msec)	pre	202 ± 21	196 ± 25	194 ± 19
	post	186 ± 16 [b]	188 ± 11	185 ± 20
QTcB	pre	283 ± 25	274 ± 35	273 ± 39
	post	285 ± 21	280 ± 16	286 ± 28
QTcF	pre	252 ± 19	245 ± 29	243 ± 27
	post	247 ± 16	245 ± 11	247 ± 23

PQ: PQ interval, QT: QT interval, QTcB: corrected QT Interval by Bazett formula, QTcF: corrected QT Interval by Fridericia formula. pre: before administration of cilostazole, post: after administration of cilostazole. a) $P<0.01$ vs pre, b) $P<0.05$ vs pre.

Fig. 5. Changes in cardiac output (CO) after the administration of cilostazol. An increase in CO was observed in the group administered 10 mg/kg cilostazol. *: $P<0.05$ vs pre.

6:00 AM, 7:00 AM, 6:00 PM, and 7:00 PM ($P<0.05$ for 4:00 PM and 5:00 PM; $P<0.01$ for the other time points) (Fig. 3).
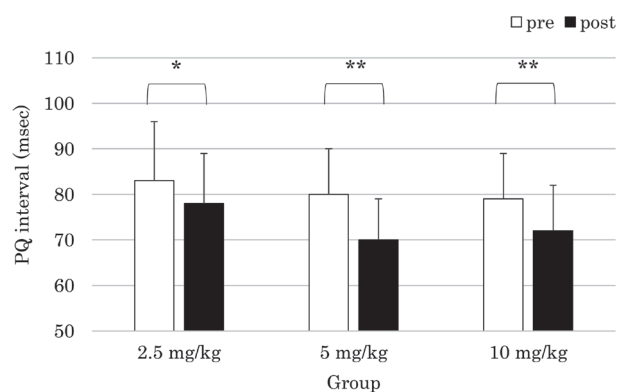
Electrocardiography

The PQ intervals decreased significantly in all of the animals administered 2.5, 5, or 10 mg/kg cilostazol compared to the values determined before drug administration ($P<0.01$). In the 2.5 mg/kg group, the QT intervals decreased significantly compared to the values determined before drug administration ($P<0.05$) (Fig. 4), but no significant difference was observed in the QTcB or QTcF. In the 5 and 10 mg/kg groups, no significant difference was observed in the QT intervals, QTcB, and QTcF (Table 2).

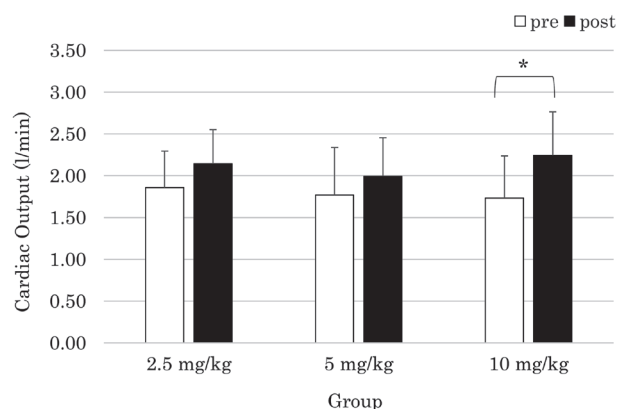*Cardiac ultrasound examination*

Cardiac ultrasound examination revealed no significant changes before and after the administration of 2.5 mg/kg cilostazol.

In the animals administered 5 mg/kg cilostazol, the Ao-ET decreased significantly from 166.3 ± 11.4 msec to 150.6 ± 12.4 msec ($P<0.05$). No significant changes were found in the other examined variables.

In the animals administered 10 mg/kg cilostazol, significant differences were observed in the LVIDd (from 33.0 ± 2.6 mm to 31.6 ± 2.9 mm, $P<0.05$), Ao-PET (from 53.9 ± 6.7 msec to 47.2 ± 5.2 msec, $P<0.05$), and Ao-ET (from 170.5 ± 8.2 msec to 143.6 ± 1 4.6 msec, $P<0.01$). However, no significant changes were found in the Ao-PET/ET before and after drug administration. CO showed a significant increase from 1.7 ± 0.5 *l*/min to 2.2 ± 0.5 *l*/min ($P<0.05$) (Fig. 5). No other variables exhibited significant changes (Table 3). In addition, no abnormal findings, such as endocardial and epicardial echogenic brightness or valve thickening, were observed.

Table 3. Echocardiographic findings

		2.5 mg/kg	5 mg/kg	10 mg/kg
LVIDd (mm)	pre	33.9 ± 2.5	35.0 ± 3.4	33.0 ± 2.6
	post	34.5 ± 3.1	33.7 ± 1.9	31.6 ± 2.9 [b]
LVIDs (mm)	pre	21.6 ± 2.5	21.5 ± 2.2	20.2 ± 1.9
	post	21.6 ± 2.1	20.0 ± 1.4	19.1 ± 1.8
FS (%)	pre	36.5 ± 4.2	38.6 ± 3.8	38.7 ± 4.1
	post	37.4 ± 2.5	40.7 ± 3.4	39.4 ± 3.4
LA/Ao	pre	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
	post	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0
Ao-PEP (msec)	pre	54 ± 5	51 ± 7	54 ± 7
	post	51 ± 3	49 ± 6	47 ± 5 [b]
Ao-ET (msec)	pre	170 ± 17	166 ± 11	171 ± 8
	post	158 ± 10	151 ± 12.4 [b]	144 ± 14.5 [a]
Ao-PEP/ET	pre	0.32 ± 0.05	0.31 ± 0.04	0.32 ± 0.04
	post	0.32 ± 0.02	0.32 ± 0.04	0.33 ± 0.02
SV (m*l*)	pre	17.4 ± 3.3	15.7 ± 3.1	16.0 ± 4.2
	post	16.6 ± 2.5	16.0 ± 4.0	15.9 ± 3.0
CO (*l*/min)	pre	1.86 ± 0.44	1.77 ± 0.57	1.73 ± 0.51
	post	2.14 ± 0.41	1.99 ± 0.46	2.24 ± 0.52 [b]
E Vel (cm/sec)	pre	73.3 ± 6.6	73.1 ± 10.1	68.9 ± 17.7
	post	74.5 ± 8.9	77.5 ± 12.3	72.3 ± 12.3
e' Vel (cm/sec)	pre	7.4 ± 1.3	7.4 ± 1.5	6.5 ± 1.9
	post	7.6 ± 1.2	7.1 ± 1.3	7.0 ± 1.2
E/e'	pre	10.1 ± 2.2	10.1 ± 1.8	10.7 ± 2.0
	post	10.2 ± 1.9	11.2 ± 2.1	10.6 ± 2.1

LVIDd: end-diastolic ventricular inside diameters, LVIDs: end-systolic left ventricular inside diameters, FS: left-ventricular fractional shortening, LA/Ao: left atrium to aortic root ratio, Ao-PEP: pre-ejection period at aortic valve flow, Ao-ET: ejection time at aortic valve flow, Ao-PEP/ET: ratio between pre-ejection period and ejection time at aortic valve flow, SV: left ventricular outflow tract- stroke volume, CO: left ventricular outflow tract-cardiac out put, E vel: peak early diastolic left ventricular filling velocity, e': peak mitral annular velocity during early diastole, E/e': ratio between E and e', pre: before administration of cilostazol, post: after administration of cilostazol. a) $P<0.01$ vs pre, b) $P<0.05$ vs pre.

Table 4. Blood pressure measurements findings

		2.5 mg/kg	5 mg/kg	10 mg/kg
SAP (mmHg)	pre	140 ± 17	145 ± 14	148 ± 6
	post	143 ± 10	145 ± 14	145 ± 12
MAP (mmHg)	pre	101 ± 10	106 ± 13	109 ± 14
	post	110 ± 11 [a]	109 ± 12	109 ± 14
DAP (mmHg)	pre	82 ± 12	88 ± 12	90 ± 19
	post	94 ± 12 [b]	91 ± 12	90 ± 12
SVR (dynes/sec/cm^5)	pre	4,281 ± 792	4,963 ± 1,589	5,088 ± 1,387
	post	4,014 ± 612	4,332 ± 966	3,900 ± 1,073 [b]

SAP: systolic arterial pressure, MAP: mean arterial pressure, DAP: diatolic arterial pressure, SVR: systemic vascular resistance, pre: before administration of cilostazol, post: after administration of cilostazol. a) $P<0.01$ vs pre, b) $P<0.05$ vs pre.

Blood pressure measurement

In the animals administered 2.5 mg/kg cilostazol, significant increases were observed in MAP (101 ± 12.2 mmHg to 110 ± 11.3 mmHg, $P<0.01$) and DAP (82 ± 11.6 mmHg to 94 ± 12.3 mmHg, $P<0.05$). SAP also increased from 140 ± 16.7 mmHg to 143 ± 9.8 mmHg, but the increase was statistically nonsignificant. No significant changes were found in blood pressure in the animals after the administration of 5 and 10 mg/kg cilostazol. SVR showed a significant decrease from 5,088 ± 1,387 dynes × sec/cm^5 to 3,900 ± 1,073 dynes × sec/cm^5 in the animals administered 10 mg/kg cilostazol ($P<0.05$) (Table 4).

Blood tests

No significant change was observed in the platelet count after the administration of cilostazol. Moreover, no significant difference was observed in the measured values of the other parameters (Table 5).

Table 5. Blood test findings

Aitem	Unit	2.5 mg/kg		5 mg/kg		10 mg/kg	
		pre	post	pre	post	pre	post
WBC	×10³/μl	9.85 ± 2.85	12.27 ± 5.82	10.03 ± 1.37	12.05 ± 2.81	11.32 ± 4.71	12.22 ± 2.78
RBC	×10⁶/μl	6.73 ± 0.42	6.26 ± 0.45	6.96 ± 0.63	6.69 ± 0.49	7.05 ± 0.56	6.79 ± 0.33
HGB	g/dl	15.3 ± 1.3	14.2 ± 1	16.2 ± 1.4	15.7 ± 1.1	16.5 ± 1.5	15.9 ± 1.1
HCT	%	43.8 ± 3.4	41.1 ± 3.1	45.2 ± 3.6	43.5 ± 2.8	45.9 ± 3.9	44.2 ± 2.5
MCV	fl	65.2 ± 2.7	65.6 ± 2.4	65.1 ± 2.2	65.1 ± 2.2	65.1 ± 2.7	65.1 ± 2.7
MCH	pg	22.7 ± 0.9	22.6 ± 0.8	23.3 ± 1	23.6 ± 1.2	23.5 ± 1.2	23.5 ± 1.2
MCHC	%	34.9 ± 0.9	34.5 ± 0.4	35.8 ± 0.7	36.1 ± 0.8	36 ± 0.7	36.1 ± 0.8
PLT	×10³/μl	424.1 ± 111.5	422.3 ± 125.6	424.6 ± 123.6	392.2 ± 113.9	431.4 ± 146.8	424.2 ± 118.9
BUN	mg/dl	18 ± 8.3	15 ± 3.3	15 ± 4.9	13.9 ± 4.8	13.6 ± 4.9	12.4 ± 2.5
CRE	mg/dl	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
ALP	U/l	203 ± 88	192 ± 80	242 ± 132	256 ± 139	265 ± 139	257 ± 122
ALT	U/l	51 ± 14	54 ± 15	56 ± 35	47 ± 21	63 ± 65	47 ± 26
TP	g/dl	6.7 ± 0.4	6.6 ± 0.4	6.5 ± 0.4	6.5 ± 0.3	6.7 ± 0.5	6.5 ± 0.3
ALB	g/dl	2.9 ± 0.2	2.9 ± 0.2	3.1 ± 0.3	3.1 ± 0.2	3.1 ± 0.3	3 ± 0.4
Na	mmol/l	148 ± 1	147 ± 2	147 ± 2	147 ± 1	147 ± 2	147 ± 1
K	mmol/l	4.2 ± 0.4	3.9 ± 0.2	4.2 ± 0.3	4 ± 0.2	4.3 ± 0.4	4.1 ± 0.2
Cl	mmol/l	114 ± 3	112 ± 2	113 ± 3	112 ± 4	112 ± 3	111 ± 3

WBC; total leukocyte count, RBC; erythrocyte count, HGB; hemoglobin concentration, HCT; hematocrit, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration, PLT; platelet count, BUN; blood urea nitrogen, CRE; creatinine, ALP: alkaline phosphatase, ALT; alanine aminotransferase, TP; total protein, ALB; albmin, Na; Sodium, K; potassium, Cl; chlorine.

DISCUSSION

In the present study, the cilostazol doses administered to dogs (2.5, 5, and 10 mg/kg) were chosen considering the dose at the start of therapy for patients with bradyarrhythmia (3.33 mg/kg for adults with a body weight of 60 kg) [11, 14]. Dogs were administered 2.5 mg/kg cilostazol as the minimum dose, similar to what is administered in humans. They were also administered 2 and 4 times this minimum dose (5 and 10 mg/kg cilostazol).

The administration of cilostazol increased the HR in a dose-dependent manner in the healthy dogs. Significant increases were observed in the mean HR and total HR after the administration of 5 and 10 mg/kg cilostazol than at the baseline before drug administration. These results suggest the effectiveness of the administration of cilostazol twice a day at a dose of 5 mg/kg to increase the HR at the start of treatment in dogs. They also support the appropriateness of the empirically used dosage of cilostazol. However, an insufficient HR increase rate was observed depending on the time period (6:00 AM to 07:00 AM and 5:00 PM to 6:00 PM) even with drug administration twice a day. These time periods were when cilostazol was administered (8:00 AM and 8:00 PM) or when meals were provided (8:00 AM and 5:00 PM). This is because the HR has already increased before the administration of cilostazol. Alternatively, it may suggest that the HR-increasing effect of cilostazol does not persist for more than 12 hr. The results of electrocardiography showed significant decreases in the PQ intervals in all of the cilostazol-administration groups, suggesting an increase in atrioventricular conduction velocity. In contrast, no significant change was observed in the corrected QT values in any of the groups. Conduction of active potential is generally indicated to correspond to the QRS wave in the atrium, whereas repolarization of the ventricular muscle corresponds to the T wave [3]. Therefore, the QT intervals were considered to coincide with the duration of active potential of the ventricular muscle. Hence, the administration of cilostazol was suggested to induce the activation of atrioventricular conduction of active potential only, without affecting conduction in the ventricle. Therefore, the administration of cilostazol is assumed to induce a strong positive chronotropic response in patients that exhibit incomplete blocking of atrioventricular conduction, such as sinus bradycardia, sinus arrest, and Wenckebach-type 2nd-degree atrioventricular blocking. Cases of dogs with 3rd-degree atrioventricular blocking have also been reported [12]. In these animals, the administration of cilostazol resulted in an improvement of clinical symptoms via an increase in the ventricular rate without an improvement in atrioventricular conduction. These findings suggest the possible involvement of mechanisms other than increased atrioventricular conduction velocity, such as the acceleration of automaticity in cilostazol-induced increases of the HR.

Significant decreases in the Ao-PET were observed in the group administered 10 mg/kg cilostazol and in the Ao-ET in the groups administered 5 and 10 mg/kg cilostazol. The Ao-PEP and Ao-ET were considered susceptible to the effects of the HR, and an increased HR reduced the Ao-PEP and Ao-ET, whereas a decreased HR reduced the Ao-PEP and Ao-ET [20, 25]. Therefore, the ratio of Ao-PEP to Ao-ET (Ao-PEP/ET) has been used as an effective index for the clinical evaluation of ventricular contractility, as with FS [7, 8]. No significant change was observed in the Ao-PEP/ET in this study, suggesting that the decreases in the Ao-PEP and Ao-ET resulted from an increase in the HR. Furthermore, no significant changes were found in FS after the administration of cilostazol. FS shows a high value as the preload increases and/ or a low value as the afterload increases. We speculated that two factors, the decrease in the preload (LVIDd) and the decrease in the afterload (SVR), influenced the phenomenon of FS invariance. Based on the results of PEP / ET and FS in this study, cilostazol may be relatively safe to use for dogs with reduced cardiac contractility.

No significant change was observed in SV after the administration of cilostazol in any of the animal groups. However, an increasing tendency in CO was observed in the animals administered 5 mg/kg cilostazol, and a statistically significant increase in CO was observed in the animals administered 10 mg/kg cilostazol. Because CO was calculated using the formula SV × HR, the increases in CO were considered a result of the increased HR.

Cilostazol is a phosphodiesterase III inhibitor and inhibits the degradation of cyclic adenosine monophosphate (cAMP), resulting in an increase in the intracellular concentration of cAMP [1, 6]. An increase in the intracellular cAMP level has been indicated to inhibit platelet agglutination, thus inducing antiplatelet action [1, 4, 10]. Cilostazol also induces the dilatation of the peripheral blood vessels and coronary blood vessels through the dilatation of vascular smooth muscles, as an effect of cAMP [1, 19, 27]. In the present study, the administration of 5 and 10 mg/kg cilostazol induced no significant decrease in blood pressure. This was presumed to be the result of two factors: not only the occurrence of vasodilation but also the increase in CO. In contrast, increases in MAP and DAP were observed in the animals after the administration of 2.5 mg/kg cilostazol, and blood pressure shifted from a slightly lower value than the reference value to the optimum range [5]. The CO-increasing effect possibly exceeded the vasodilator effect brought about by the administration of 2.5 mg/kg cilostazol.

Cilostazol can be administered at up to 30 mg/kg/day without adverse effects, as demonstrated in a study on healthy dogs orally administered cilostazol for 13 weeks; however, pathological changes were observed in the coronary artery of some of the dogs after drug administration [15]. Nevertheless, because no histopathological examination was performed in the present study, the adverse effects of the administration of cilostazol remain to be elucidated in a future study. Side effects including tachyarrhythmia, such as a marked increase in the HR (sinus tachycardia) and VPC, have been reported in human patients administered cilostazol [5, 11]. In the present study, no statistically significant changes were observed in VPC in any of the animal groups after the administration of cilostazol. Therefore, the possibility of developing side effects such as tachyarrhythmia is small in clinically healthy dogs administered cilostazol for short periods at dosages of 2.5 to 10 mg/kg.

The interpretation of the results of the present study has several limitations. First, the blood pressure of female dogs is known to vary depending on the sex cycle stage [18]. In this study, females in obvious estrus were not used, but their sex hormone levels were not measured. However, to reduce the influence of the estrous cycle on blood pressure, the sex composition of each group was kept identical. Second, a circadian rhythm exists in the HR, and there are time zones wherein the HR is low or high. Depending on the time zone when cilostazol is administered, the results of HR increase may be affected.

In the present study, abnormal findings of general symptoms or abnormal hemostasis and purpura on the body surface at the time of blood sampling were not observed in the healthy dogs throughout the study period. However, in actual clinical settings, cilostazol may be administered to animals with a variety of underlying diseases, which may result in the development of serious or severe arrhythmia and organ derangement requiring treatment. Therefore, the effect of cilostazol on the blood coagulation functions must be tested in detail in future studies. Furthermore, the effects of cilostazol after longer periods of administration than those evaluated in the present study (10 days) must be determined.

PMI is considered the first-choice treatment for patients with clinical symptoms of bradyarrhythmia. To evaluate the effectiveness and safety of cilostazol in the treatment of animals with bradyarrhythmia, the accumulation of clinical data and long-term administration studies are required.

The results acquired in this study show that the HR of healthy dogs increased in a dose−dependent manner after the administration of cilostazol twice a day at doses of 5 to 10 mg/kg. There are various types of clinical bradyarrhythmia cases. Therefore, it may be impossible to directly extrapolate the results of this study to some of bradyarrhythmia. However, these results provide a useful basis for choosing cilostazol in the treatment of bradyarrhythmia in dogs.

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