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NOTE

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Inotropic effects of a single intravenous recommended dose of pimobendan in healthy dogs

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ABSTRACT. We investigated the effects of an injectable pimobendan solution (0.15 mg/kg) on cardiac function in healthy dogs. Fifteen dogs were divided into placebo, intravenous pimobendan injection, and subcutaneous pimobendan injection groups. In the placebo, the heart rate, systolic and end-diastolic left ventricular pressure (LVPs and LVEDP), and peak positive (max dP/dt) and negative (min dP/dt) first derivatives of the left ventricular pressure did not change for 60 min. After the intravenous pimobendan injection, LVEDP decreased significantly within 5 min, while the max dP/dt increased, and the effects continued until 60 min. In comparison, there were no hemodynamic changes after the subcutaneous pimobendan injection. This study demonstrates that injectable pimobendan induced a rapid inotropic effect and decreased the LVEDP in dogs.

KEY WORDS: canine, heart failure, phosphodiesterase inhibitor, pimobendan, systolic function

There are eleven families of hydrolytic enzymes called cyclic nucleotide phosphodiesterases (PDE), and PDE3 is a cGMP-inhibited cAMP PDE; hydrolase of cAMP and cGMP phosphodiester bonds [12]. PDE3 is abundant in cardiomyocytes and vascular smooth muscle and regulates cardiac and vascular smooth muscle contractility [8, 12].

Selective inhibitors of PDE3, including milrinone, amrinone, and pimobendan, induce inotropic and vasodilatory actions via an increase in the intracellular cAMP content in cardiomyocytes and vascular smooth muscle [17, 18, 20]. Consequently, PDE3 inhibitors have been used to treat congestive heart failure [12]. The benzimidazole-pyridazinone derivative pimobendan is a non-sympathomimetic, non-glycoside inotropic agent that exhibits vasodilatory action via the inhibition of PDE3 [1, 23]. In addition to inhibiting PDE3, pimobendan has a Ca^{2+}-sensitizing effect and directly increases the affinity of the regulatory site on cardiac troponin C for Ca^{2+}, which contributes to its positive inotropic action [3, 10, 18]. Pimobendan increased the Ca^{2+}-binding affinity of cardiac troponin C in isolated papillary muscles from guinea pigs, whereas milrinone did not [3, 18].

Pimobendan can improve the hemodynamics, morbidity, and physical activity of patients with chronic heart failure [9, 21]. In a dog model of mitral valve regurgitation, pimobendan (0.25 and 0.50 mg/kg, twice daily by mouth) increased cardiac output and decreased systemic vascular resistance and left atrial pressure in a dose-dependent manner [19]. Several clinical studies have shown the beneficial effects of oral pimobendan in dogs with mitral valve disease and dilated cardiomyopathy [2, 5, 6, 11, 13]. A prospective, randomized, placebo-controlled, blinded, multicenter clinical trial demonstrated that oral pimobendan in dogs with mitral valve disease prolonged the preclinical period and survival time compared with a placebo group [2]. Recently, injectable pimobendan has been developed for the treatment of congestive heart failure in dogs. However, little is known about the positive inotropic effects of injectable pimobendan in dogs. Therefore, this study investigated the effects of injectable pimobendan on cardiac function in healthy dogs.

Fifteen beagle dogs were used in this study (10 males and 5 females; aged 3–6 years; weight 10–13 kg). All dogs were determined to be healthy based on the results of complete physical and echocardiographic examinations. The dogs were housed individually in cages and fed commercial dry food with free access to water. This study was approved by the Guidelines for Institutional Laboratory Animal Care and Use of the School of Veterinary Medicine at Rakuno Gakuen University, Japan (approval number VH16B7).

Pimobendan for intravenous (IV) administration was purchased (Vetmedin Injectable Solution for Dog 0.75 mg/m*l*, Boehringer Ingelheim, Ingelheim am Rhein, German).

The dogs were sedated with butorphanol (0.2 mg/kg IV), diazepam (0.5 mg/kg subcutaneously [SC]), and atropine (0.025 mg/kg

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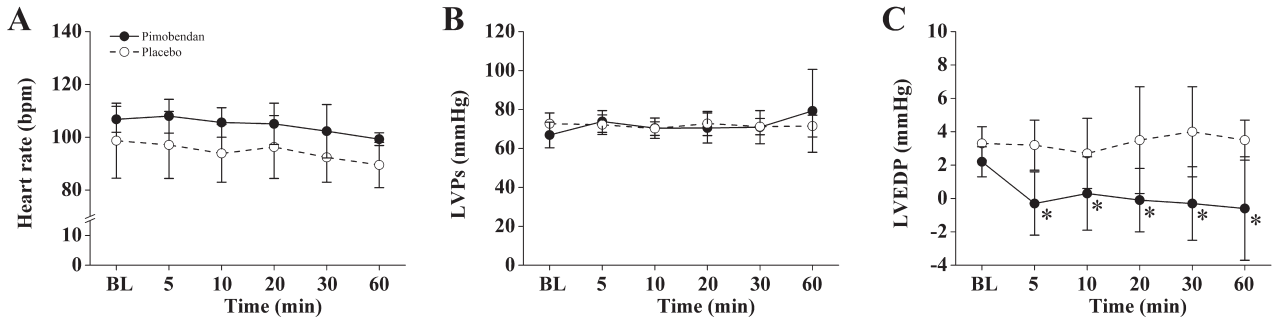

Fig. 1. Changes in heart rate, LVPs, and LVEDP with the intravenous administration of pimobendan. Data are shown as the means ± standard deviation. BL, baseline; LVPs, peak systolic LV pressure; LVEDP, end-diastolic LV pressure. *P<0.05 vs. baseline.

SC); anesthetized with propofol (6.0 mg/kg IV); and intubated. Anesthesia was maintained using a mixture of 2.0–3.0% isoflurane and oxygen. The end-tidal partial pressure of carbon dioxide in arterial blood ($PaCO_2$) and hemoglobin saturation level of oxygen (SPO_2) were monitored and maintained between 35–45 mmHg and 95–100%, respectively. The heart rate, respiratory rate, $PaCO_2$, and SPO_2 were monitored using a biological information monitor (Life scope VS, BSM-3592, Nihon Kohden, Tokyo, Japan). The respiratory rate was maintained with an artificial ventilator (MD-702, SENKO Medical Instrument, Tokyo, Japan).

The dogs were positioned in the right lateral recumbent position. Under fluoroscopic guidance, a high-fidelity 3.5 Fr micromanometer-tipped catheter (Millar Mikro-Tip Catheter Transducers, SPR-524, ADInstruments, Nagoya, Japan) was placed through the left carotid artery into the left ventricle. The catheter was connected to a manometer (PowerLab 2/26 ADInstruments) and the peak systolic left ventricular (LV) pressure (LVPs), end-diastolic LV pressure (LVEDP), and the peak positive (max dP/dt) and negative (min dP/dt) first derivatives of the LV pressure were monitored (LabChart, ADInstruments). After completing the procedures, a 20- to 30-min stabilization period was allowed to establish a stable baseline condition for the hemodynamic measurements.

The dogs were randomly assigned to placebo (n=6), intravenous pimobendan injection (n=6), and subcutaneous pimobendan injection (n=3) groups. As a baseline, all of the measurements were recorded initially. Pimobendan (0.15 mg/kg, 0.2 ml/kg) was injected via the cephalic vein or SC into the lumbar region; the dose was based on the manufacturer's instructions (Boehringer Ingelheim). In the placebo group, sterile saline (0.2 ml/kg) was administered via the cephalic vein. The time-dependent effects of pimobendan and saline were monitored 0 (baseline), 5, 10, 20, 30, and 60 min after administration. Following the examinations, the dogs were allowed to recover from anesthesia.

Normality of data was assessed with the Kolmogorov–Smirnov test. The data are presented as the means ± standard deviation. A one-factor repeated measures analysis of variance (ANOVA) was used to compare the time-dependent changes with the baseline values. A two-way ANOVA was used to compare time-dependent changes between the groups. The significance of the differences between the mean values of the each condition was tested with Tukey's multiple-comparison test. P<0.05 was considered statistically significant.

Figure 1 shows the changes in heart rate, LVPs, and LVEDP. In the placebo group, the heart rate, LVPs, and LVEDP did not change for 60 min compared with baseline. In the intravenous pimobendan group, the heart rate and LVPs did not change compared with baseline, while pimobendan induced an immediate decrease in the LVEDP, which was seen after 5 min (P<0.05). This effect continued until 60 min and was 2.2 ± 0.90, −0.3 ± 1.9, 0.3 ± 2.2, −0.1 ± 1.9, −0.3 ± 2.2, and −0.6 ± 3.1 mmHg at 0, 5, 10, 20, 30, and 60 min, respectively. Intravenous pimobendan significantly decreased the LVEDP compared with placebo (F=53.8, P<0.001).

Figure 2 shows the changes in max and min dP/dt. In the placebo group, neither parameter changed for 60 min compared with the baseline value. In the intravenous pimobendan group, while min dP/dt did not change compared with baseline, intravenous pimobendan induced a significant increase in the max dP/dt within 5 min (P<0.01). This effect continued until 60 min and was 1,072 ± 285, 1,538 ± 217, 1,426 ± 218, 1,528 ± 326, 1,589 ± 396, and 1,929 ± 529 mmHg/sec at 0, 5, 10, 20, 30, and 60 min, respectively. Compared with placebo, intravenous pimobendan increased the max dP/dt significantly (F=61.1, P<0.001).

Next, to determine whether subcutaneous injection of pimobendan affected hemodynamics, the same dose of pimobendan was injected subcutaneously in the lumbar region. After the subcutaneous pimobendan injection, there were no changes in heart rate, LVPs, LVEDP, and max or min dP/dt for 60 min compared with baseline (data not shown).

Pimobendan has positive inotropic effects and vasodilatory properties via the inhibition of PDE3 and increase in Ca^{2+} sensitivity in cardiac myofilaments [3, 10, 18]. Although studies have examined the effects of intravenous pimobendan using research reagent [1, 14–16, 22], the effects of injectable pimobendan are unknown. This study is the first to demonstrate that injectable pimobendan (0.15 mg/kg IV) in dogs induced a rapid inotropic effect and a decrease in LVEDP without altering the heart rate or LVPs.

The increased cAMP induced by PDE3 inhibition enhances the slow Ca^{2+} inward current in cardiomyocytes, producing a larger Ca^{2+} transient [7]. This increased Ca^{2+} transient results in an increased contractile force. A previous study showed that intravenous pimobendan (0.03, 0.1, and 0.3 mg/kg) in normal dogs induced dose-dependent increases in LV max dP/dt and cardiac output, and decreases in the pulmonary capillary wedge pressure [22]. In the present study, injectable pimobendan induced a significant

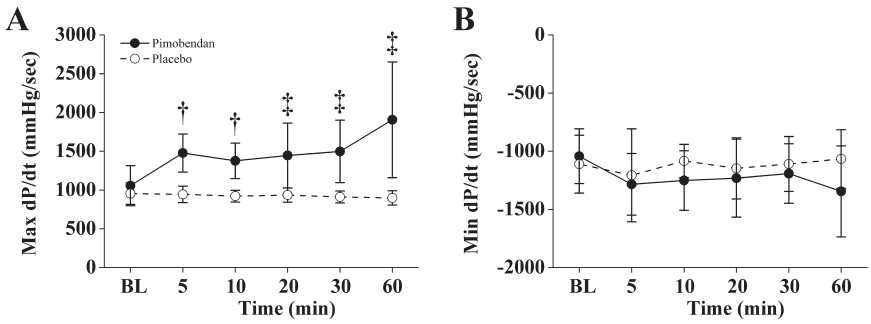

Fig. 2. Changes in max dP/dt and min dP/dt with the intravenous administration of pimobendan. Data are shown as the means ± standard deviation. BL, baseline; max dP/dt, the peak positive first derivatives of the LV pressure; min dP/dt, the peak negative first derivatives of the LV pressure. †P<0.01 vs. baseline, ‡P<0.001 vs. baseline.

increase in max dP/dt within 5 min, and its effect persisted for 60 min. By contrast, oral pimobendan (0.27 mg/kg) induced a significant increase in the LV fractional shortening after 2–4 hr [24]. Oral pimobendan is absorbed and converted into its active metabolite UD-CG 212 by hepatic demethylation [4], and the time taken to reach the maximum concentration in healthy dogs is 1.1 hr [24]. Because intravenous drugs are not affected by absorption in the intestinal tract, intravenous pimobendan induces cardiac effects immediately.

The phosphorylation of phospholamban through PDE3 inhibition via cAMP-dependent phosphokinase in cardiomyocytes enhances Ca^{2+} uptake into the sarcoplasmic reticulum, which accelerates LV relaxation [7]. In dogs, pimobendan (0.25 mg/kg IV) caused a significant decrease in the time constant of LV relaxation in both normal and failing hearts, as well as an increase in max dP/dt [14]. By contrast, our study shows that intravenous pimobendan did not affect the LV min dP/dt compared with baseline. This might reflect differences in the pimobendan dose between studies; the dose used in our study was lower than in the previous study. Our results indicate that intravenous pimobendan (0.15 mg/kg) is less likely to affect LV relaxation.

Intravenously administered pimobendan in dogs has been reported to induce a dose-dependent increase in heart rate [15, 22]. A previous study showed that pimobendan (0.25 mg/kg IV) did not change the heart rate in normal dogs, while it was increased significantly at a dose of 0.5 mg/kg [1]. Even in β-adrenergic antagonist pre-treatment, simultaneous treatment with pimobendan (0.03 and 0.1 mg/kg IV) did not affect the heart rate in dogs, while a positive chronotropic effect was observed with higher dose of pimobendan (0.3 mg/kg) [22]. This result indicates that chronotropic effect of pimobendan is not mediated by adrenergic receptor stimulation. Our study showed that a single pimobendan injection (0.15 mg/kg IV) did not affect the heart rate, but a higher dose may change the heart rate.

Our result shows that pimobendan (0.15 mg/kg IV) immediately and significantly increased max dP/dt and decreased LVEDP, but had less effect on LVPs. The pimobendan-induced positive inotropic effect and vasodilatory effect appear to interact with LV pressure in a dose-dependent manner. A previous study demonstrated that a continuous infusion of pimobendan (10 μg/kg/min) significantly increased the max dP/dt which induced increase in cardiac output and decrease in LVEDP in healthy dogs, whereas LVPs and systemic vascular resistance were unchanged [15]. By contrast, a higher dose of pimobendan (40 μg/kg/min) significantly decreased LVPs and systemic vascular resistance [15]. This result may explained by that pimobendan-induced inotropic effect increased the cardiac output despite the small chronotropic and vasodilating effects, which resulted in the decrease in LVEDP.

This study has several limitations. First, the study protocol was based on the manufacturer's instructions for the drug. Following intravenous administration, the plasma elimination half-life of pimobendan is 0.4 ± 0.1 hr, consistent with the high clearance of 90 ± 19 m//min/kg and short mean residence time of 0.5 ± 0.1 hr described in the manufacturer's instructions. Therefore, the effect of a single dose of pimobendan (0.15 mg/kg IV) was investigated for 60 min. However, the effect of pimobendan likely continues for more than 60 min. In addition, different doses may induce different results [1, 15, 22]. Additional studies should examine the hemodynamic effects of injectable pimobendan in dogs using different doses and longer observation periods. A previous study also reported that pimobendan induced inotropic and lusitropic effects after pacing-induced congestive heart failure was reduced [1]. Because we examined healthy dogs, further study is required to determine the clinical effects of injectable pimobendan in dogs with heart failure.

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