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# Effects of a Sustained-Release Form of Isosorbide Dinitrate on Left Atrial Pressure in Dogs with Experimentally Induced Mitral Valve Regurgitation

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**Background:** The effects of isosorbide dinitrate (ISDN) have not been sufficiently investigated in conscious dogs with mitral valve regurgitation (MR).

**Objective:** The objective was to investigate the effects of a sustained-release form of ISDN (sr-ISDN) on hemodynamics and the autonomic nervous system in dogs with MR.

**Animals:** Six healthy Beagles weighing 11.2 ± 2.2 kg (2 years of age; 2 males and 4 females) were used.

**Methods:** Experimental, crossover, and interventional study. Dogs with experimentally induced MR were administered placebo, 2, 5, and 10 mg/kg sr-ISDN PO on separate days with a 7-day washout period between randomized dosings. Left atrial pressure (LAP) had been recorded continuously from 30 minutes before administration of sr-ISDN to 12 hours after administration.

**Results:** LAP was significantly decreased after administration in the 5 and 10 mg/kg groups. Significant decrease was observed at 3 and 4 hours after administration in the 5 mg/kg group. In the 10 mg/kg group, significant decrease was observed at 2, 3, 4, 5, 6, 7, 10, and 11 hours after administration. The lowest value was observed at 4 hours after administration in the 5 and 10 mg/kg groups (20.9 ± 4.2 to 15.9 ± 3.9 mmHg, $P < .01$, and 21.3 ± 4.0 to 13.6 ± 4.2 mmHg, $P < .001$).

**Conclusions and Clinical Importance:** Sustained-release form of ISDN showed significant decrease of LAP in the 5 mg/kg and 10 mg/kg groups, and duration of effect was dose related.

**Key words:** Holter monitoring; Nitrates; Radiotelemetry; Vasodilator.

Mitral valve regurgitation (MR) secondary to myxomatous degeneration of mitral valve and chordae tendineae is the most common cardiac disease in dogs. Dogs suffering from MR account for approximately 75% of all dogs with congestive heart failure (CHF).[1,2] MR increases the left atrial pressure (LAP), and this increase in LAP in turn causes dilatation of the left atrium and pulmonary edema. As a result, it leads to the clinical signs of cough, dyspnea, and even death. Therefore, reduction in LAP is an important factor in the treatment of dogs with CHF caused by MR.

Isosorbide dinitrate (ISDN) causes venous vasodilation, and leads to a reduction in preload and cardiac oxygen consumption.[3–6] Furthermore, ISDN causes a decrease in arterial pressure and reduction in afterload.[7] In human medicine, ISDN is widely used for the treatment of ischemic heart disease, including angina pectoris. Moreover, ISDN is also used for congestive heart failure.[8–11] In veterinary medicine, on the other hand, the usage of ISDN for CHF secondary to MR is not yet established.

**Abbreviations:**

| | |
|---|---|
| 2-ISMN | isosorbide-2-mononitrate |
| 5-ISMN | isosorbide-5-mononitrate |
| ANOVA | analysis of variance |
| BP | blood pressure |
| CHF | congestive heart failure |
| DBP | diastolic blood pressure |
| HF | high frequency |
| HR | heart rate |
| HRV | heart rate variability |
| ISDN | isosorbide dinitrate |
| LAP | left atrial pressure |
| LF | low frequency |
| MBP | mean blood pressure |
| MR | mitral valve regurgitation |
| SBP | systolic blood pressure |
| sr-ISDN | sustained-release form of isosorbide dinitrate |
| SVR | systemic vascular resistance |

The sustained-release form of ISDN (sr-ISDN) maintains an effective serum concentration for more than 6 hours. Previous study has shown that the effective dose-response curve for sr-ISDN in anesthetized dogs with experimentally induced MR appeared to be between 2 and 8 mg/kg.[12] However, no study has been conducted to evaluate the effects of oral administration of sr-ISDN on LAP in conscious dogs with MR. In addition, there is no report of the evaluation of the effects of ISDN on the autonomic nervous system. In this study, the effects of different dosages of sr-ISDN on LAP and its duration of action in dogs with

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experimentally induced MR were evaluated. Moreover, the effects of sr-ISDN on hemodynamics and the autonomic nervous system were evaluated by echocardiography and Holter monitoring.

## Materials and Methods

### Animals

Six 2-year-old Beagles (2 males and 4 females) weighing 11.2 ± 2.2 kg were used in this study. Before the study, the dogs were evaluated by general clinical examination, blood and serum biochemical examinations, thoracic radiography, blood pressure (BP), electrocardiography, and echocardiography examinations. All dogs had acclimatized to experimental environment and human handling. During all phases of this study, the dogs were managed and cared for in accordance with the standards established by the Tokyo University of Agriculture and Technology (TUAT) and described in its "Guide for the Care and Use of Laboratory Animals," and this study was approved by TUAT's Animal Experimental Committee (acceptance no. 21-19).

### Induction of MR and Transmitter Implantation

The dogs underwent a surgical procedure to induce MR, and a transmitter catheter[a] was implanted in the left atrium. The surgical procedure and postoperative care were conducted according to our previous report.[13] Thoracic radiography and echocardiography were performed to evaluate pulmonary venous congestion and cardiac dilatation. After radiotelemetry implantation, the dogs were rested for at least 5 weeks, until no major variations were identified in echocardiographic evaluation and LAP.

### Administration of sr-ISDN and Measurements

Sustained-release form of ISDN was administered orally. Before administration of sr-ISDN,[b] LAP recording and Holter monitoring were started. Blood pressure measurements were performed before administration of sr-ISDN, and at 3, 6, and 12 hours post administration. Sustained-release form of ISDN was administered at a dosage of 2, 5, or 10 mg/kg PO or placebo after 30 minutes, LAP recording and Holter monitoring were started. After a 7-day washout period, the other dosage of sr-ISDN was administered using a crossover study. LAP recording and Holter monitoring were continued 12 hours after initiation of the experiment. After LAP recording and Holter monitoring, a washout period was observed, then echocardiographic measurements were performed on a different day. Echocardiographic measurements were performed before administration of sr-ISDN, and at 3, 6, and 12 hours post administration.

### Left Atrial Pressure Measurement

All radio telemetry systems[c] and recording procedures were the same as described in our previous report.[14] The maximum, mean, and minimum LAP were obtained as the averages of 10-second recorded segments from continuous waveform recordings. Left atrial pressure was measured for 12.5 hours from 1930 to 0800 hours. Left atrial pressure measured for 30 minutes from 1930 to 2000 hours was defined as baseline. Left atrial pressure measurements performed at the same time BP measurements were taken were excluded from analysis.

### Echocardiography

Echocardiography measurements were performed by a single operator, and measurements were conducted by means of digital ultrasonographic system.[d] Measurement parameters were left atrial to aortic root ratio (LA/AO), left ventricular end-diastolic diameter (LVEDD), fractional shortening (FS), forward stroke volume (SV), cardiac output (CO), peak transmitral early diastolic wave (E wave) velocity, and mitral annulus velocities (Ea). The procedure of echocardiographic measurements was conducted according to our previous report.[13] All the echocardiography recordings were stored on the internal hard drive of the echocardiograph and transmitted to the DICOM server online.[e]

### Blood Pressure Measurements

All indirect arterial BP measurements were obtained by the oscillometric method.[f] Cuff size appropriate for tail circumference was selected for each dog. Blood pressure measurements were performed 5 consecutive measurements, and these were averaged for each dog for use in our calculation. Systemic vascular resistance (SVR) was calculated as SVR = 80 × (Mean BP − central venous pressure)/CO (80 is a conversion factor to express the results in dyne*s*cm$^{-5}$), and central venous pressure was tentatively defined as 5 mmHg owing to the lack of right heart failure signs.

### Holter Monitoring and Heart Rate Variability Analysis

The dogs wore the Holter monitor[g] for 12.5 hours in the experiment. Electrocardiographic data recorded by Holter recorder were averaged for 5-minute periods by use of an ECG analysis system.[h] A Hamming window function was used to minimize spectral leakage for signal processing. A fast Fourier transform function was used to obtain the power spectrum of the fluctuation. Squared magnitudes and the products of the computed discrete Fourier transform values were averaged to obtain spectral estimates. Values for the low-frequency (LF) and the high-frequency (HF) powers and the LF/HF were obtained. Frequencies ranging from 0.04 to 0.15 Hz were regarded as LF, and those raging from 0.15 to 0.4 Hz were regarded as HF. Sum of all heart beats for 12 hours, from administration of sr-ISDN to 12 hours post administration, was defined as total heart beats.

### Statistical Analysis

Data are shown as the mean ± standard deviation. Statistical significance was defined as $P < .05$. A one-way analysis of variance (ANOVA) in conjunction with a Tukey's multiple comparison test was used for comparing total heart beats and LAP at 4 hours after administration of each dose. A two-way repeated measures ANOVA in conjunction with a Bonferroni's multiple comparison test was used to compare LAP, echocardiographic and hemodynamic parameters, and Holter monitoring parameters before and after each sr-ISDN administrations. Statistical analysis software[i] and spreadsheet software[j] were used to perform these statistical analyses.

## Results

The surgeries to rupture the mitral valve chordae tendineae and to implant the transmitter were successful in all dogs. LAP was 20.6 ± 2.6 mmHg, LVEDD was 39.1 ± 4.0 mm, LA/AO was 1.49 ± 0.05, and E

wave velocity was 0.93 ± 0.08 m/s. All dogs were stage B2 in guidelines for the diagnosis and treatment of canine chronic valvular heart disease of the American College of Veterinary Internal Medicine. Although cough was noted in 1 dog, thoracic radiography indicated that congestive heart failure was not present, and the cough resolved after 3 days without furosemide. No obvious adverse effects were observed during periods of sr-ISDN administration.

### *Left Atrial Pressure*

LAP decreased significantly in the 5 and 10 mg/kg groups, as shown in Figure 1. Significant decrease was observed at 3 and 4 hours after administration in the 5 mg/kg group. In the 10 mg/kg group, significant decrease was observed at 2, 3, 4, 5, 6, 7, 10, and 11 hours after administration. The lowest value was observed at 4 hours after administration in both the 5 and 10 mg/kg groups (20.9 ± 4.2 to 15.9 ± 3.9 mmHg, $P < .01$, and 21.3 ± 4.0 to 13.6 ± 4.2 mmHg, $P < .001$). Statistically significant reduction was not observed in the placebo and 2 mg/kg groups. There was no significant difference among the placebo, 2, and 5 mg/kg groups. There was significant difference between the placebo and 10 mg/kg groups at 3 and 4 hours after administration.

### *Echocardiography and Hemodynamic Parameters*

Peak E and E/Ea decreased significantly in the 5 and 10 mg/kg groups, as shown in Table 1. Cardiac output of the placebo and 10 mg/kg groups at 12 hours after administration decreased significantly when compared with baseline. However, there was no significant difference between any groups. Other parameters did not change significantly.

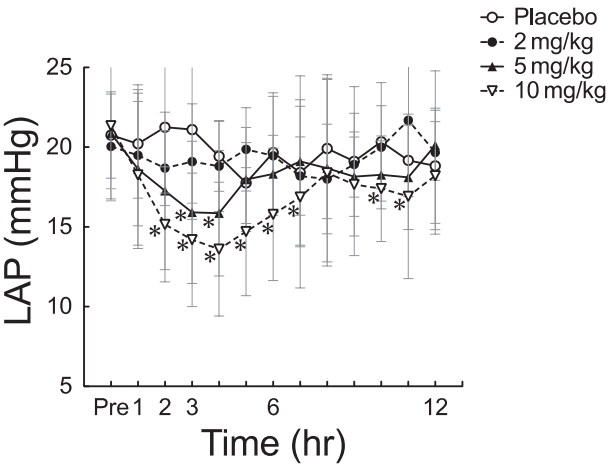


**Fig 1.** Maximal left atrial pressure (LAP) after administration of sustained-release form of isosorbide dinitrate or placebo in the dogs with MR. *Significant differences ($P < .05$) compared with each baseline.

Mean BP and diastolic BP did not decrease significantly compared with baseline in any groups. Systolic BP (SBP) decreased significantly in the 10 mg/kg group, as shown in Table 1. Systemic vascular resistance did not change significantly.

### *Heart Rate and Heart Rate Variability*

Average HR decreased significantly when compared with baseline in all groups. However, there was no significant difference between any groups. There was no significant difference between any groups in total heart beats. HF power of the placebo, 2, and 5 mg/kg groups increased significantly from 2 to 12 hours after administration when compared with baseline. The high frequency power of the 10 mg/kg group did not increase significantly until 5 and 7 hours after administration when compared with baseline. However, there was no significant difference between any groups. LF/HF did not change significantly.

## Discussion

As noted in the introduction, the goal of this study was to investigate the effects of sr-ISDN in dogs with MR. In this study, LAP did not decrease in the 2 mg/kg group. However, significant decrease was observed in the 5 and 10 mg/kg groups. Moreover, duration time of sr-ISDN was dose dependent. Peak E and E/Ea were decreased significantly in the 5 and 10 mg/kg groups. When comparing the different dosage groups, there was no significant difference in HR and HRV. Only in the 10 mg/kg group, SBP decreased significantly.

Previous studies reported that ISDN has beneficial acute hemodynamic effects in humans and dogs with MR because of their preload and afterload reducing properties.[15] Nagasawa et al reported that there was a slight decrease in preload and afterload at 2 mg/kg sr-ISDN.[12] However, in this study, there was no significant change in parameters in the 2 mg/kg group. Compared with the previous study, this study was performed in conscious dogs. This result indicates that 2 mg/kg sr-ISDN does not affect conscious dogs with experimentally induced MR. However, this result also may indicate that 2 mg/kg sr-ISDN assists the work of circulatory system under general anesthesia. In this study, LAP was significantly decreased by administration of 5 and 10 mg/kg sr-ISDN. Compared with our previous study, the effect of high-dose sr-ISDN on reducing the maximum values of LAP was nearly equal to that of diuretic.[13] This result suggests that high dose of sr-ISDN has a significant effect on LAP. However, this result also suggests that high dose of ISDN is required to decrease LAP.

In this study, duration of effect was dose dependent. According to the previous study, the plasma concentration of isosorbide-2-mononitrate (2-ISMN) and isosorbide-5-mononitrate (5-ISMN), which are metabolites of ISDN, peaked at 4 and 8 hours after ISDN administration, respectively.[12] These peaks of

**Table 1.** Comparison of LAP (left atrial pressure), echocardiographic, and hemodynamic parameters at baseline and after sr-ISDN (sustain-release form of isosorbide dinitrate) administration.

| Variables | Dose of sr-ISDN | Baseline | 3 Hours after Administration of sr-ISDN | 6 Hours after Administration of sr-ISDN | 12 Hours after Administration of sr-ISDN |
|---|---|---|---|---|---|
| E wave | Placebo | 0.91 ± 0.11 | 0.90 ± 0.11 | 0.90 ± 0.11 | 0.94 ± 0.08 |
| (m/s) | 2 mg/kg | 0.92 ± 0.08 | 0.86 ± 0.13 | 0.91 ± 0.13 | 0.87 ± 0.13 |
| | 5 mg/kg | 0.93 ± 0.07 | 0.77 ± 0.08*** | 0.78 ± 0.08*** | 0.85 ± 0.11 |
| | 10 mg/kg | 0.96 ± 0.11 | 0.79 ± 0.13*** | 0.80 ± 0.07*** | 0.93 ± 0.14 |
| E/Ea | Placebo | 8.62 ± 0.89 | 8.69 ± 0.73 | 8.73 ± 0.70 | 9.08 ± 0.64 |
| | 2 mg/kg | 8.48 ± 0.76 | 7.92 ± 0.92 | 8.49 ± 1.04 | 7.97 ± 1.09 |
| | 5 mg/kg | 8.66 ± 1.03 | 7.26 ± 0.72* | 7.42 ± 1.05 | 7.72 ± 0.84 |
| | 10 mg/kg | 8.89 ± 1.33 | 7.40 ± 1.43** | 7.66 ± 0.99* | 8.37 ± 1.16 |
| SBP | Placebo | 121.8 ± 6.0 | 120.4 ± 4.3 | 118.9 ± 7.3 | 121.5 ± 7.5 |
| (mmHg) | 2 mg/kg | 119.2 ± 8.9 | 116.7 ± 6.6 | 116.6 ± 7.6 | 124.0 ± 13.1 |
| | 5 mg/kg | 122.8 ± 6.3 | 112.3 ± 7.4 | 117.9 ± 11.4 | 122.3 ± 13.7 |
| | 10 mg/kg | 126.4 ± 9.5 | 112.6 ± 10.6* | 116.6 ± 5.6 | 127.9 ± 9.5 |
| CO | Placebo | 1697.8 ± 334.2 | 1578.3 ± 371.4 | 1325.2 ± 261.0 | 1183.4 ± 179.0* |
| (mL/min) | 2 mg/kg | 1641.8 ± 387.6 | 1492.9 ± 530.7 | 1203.3 ± 278.2 | 1357.0 ± 313.2 |
| | 5 mg/kg | 1669.7 ± 229.5 | 1239.5 ± 327.2 | 1299.6 ± 265.0 | 1373.9 ± 257.9 |
| | 10 mg/kg | 1768.1 ± 540.0 | 1397.1 ± 382.5 | 1427.3 ± 379.9 | 1218.6 ± 356.2** |

E wave, transmitral early diastolic wave velocity; E/Ea, the ratio of transmitral early diastolic wave velocity to lateral mitral annulus velocity in the early diastolic period; SBP, systolic blood pressure; CO, cardiac output.

*$P < .05$, **$P < .01$, and ***$P < .001$ compared with baseline.

the metabolites were later than the peak of ISDN. These metabolites of ISDN are also vasodilators, and previous investigators have reported the relative vasodilatory activities of 2-ISMN and 5-ISMN as 1/6 and 1/50 of ISDN, respectively.[16] In this study, plasma concentrations of ISDN, 2-ISMN, and 5-ISMN were not measured. Therefore, it is not certain that these metabolites show significant effects on hemodynamics. However, it is possible that these metabolites affected duration of effect.

Our previous study reported that E wave and E/Ea can be used for the evaluation of preload and presumption of the reduction in LAP in the short term.[13] Moreover, previous studies have shown that E/Ea is a good index for estimation of LAP and left ventricular filling pressure.[17–19] In this study, E wave and E/Ea of the 5 and 10 mg/kg groups decreased significantly after administration of sr-ISDN. This result was similar to the result of LAP. These results indicate that E wave and E/Ea can be useful for the evaluation of sr-ISDN treatment and the monitoring of reduction in LAP.

Cardiac output decreased significantly in the placebo and 10 mg/kg groups at 12 hours after administration. This result was mainly caused by the reduction in average HR in the placebo and 10 mg/kg groups. In the 10 mg/kg group, reduction in venous return volume may be associated with this result.

There was little measureable effect on afterload, and this means that most of the hemodynamic effect is via preload reduction demonstrated by the decrease in LAP. In the 10 mg/kg group, both SBP and LAP decreased significantly. However, in the 5 mg/kg group, only LAP decreased significantly. These results indicate that it is difficult to evaluate the effects of sr-ISDN treatment by BP measurements. In clinics, BP measurements are suitable ways to monitor serious hypotension rather than to evaluate the effects of sr-ISDN treatment.

Congestive heart failure is not only a series of hemodynamic abnormalities, but also changes to the autonomic nervous system are apparent.[20] Elevated HR are associated with increased mortality in human patients with a history of acute myocardial infarction or heart failure.[21,22] Monitoring of HRV is an effective method to evaluate the autonomic modulation of the heart.[23,24] There is no previously published report on the effects of ISDN on automatic nerves in dogs with MR. In this study, there was no significant difference between any groups in HR and HRV. Activation of the sympathetic nervous system and renin-angiotensin-aldosterone system are known to be two of the causes of nitrate tolerance.[25,26] Although there is no significant difference between any groups in HR, HR tended to increase in the 10 mg/kg group. It is possible that the Holter monitor is not sensitive enough to detect the effect of sr-ISDN on the autonomic nervous system.

In our previous reports, we evaluated the effects of ACE inhibitors, furosemide, and pimobendan on the dogs with experimentally induced MR.[13,27,28] This study and the previous studies indicate that the most effective method to reduce LAP is decreasing the circulating blood volume by diuresis. However, in practice, dogs suffering from severe MR are treated by combination of medicines. Further studies are needed to evaluate these combinations in the treatment of dogs with MR.

## Limitations

In this study, we used six 2-year-old Beagles, and a 5-week period was defined as a subchronic period for experimentally induced MR. Our model dogs may be

close to acute MR. Dogs with chronic MR have cardiac dysfunction and myocardial tissue damage. Therefore, the model dogs in this study may differ from dogs with naturally occurring chronic MR. Isosorbide dinitrate is generally recommended at dosage of 0.5–2.0 mg/kg q12h for dogs with MR. We used high dosage and did not evaluate adverse effects in this study. Prolonged administration of high-dose sr-ISDN on dogs with MR may cause unexpected adverse effects. We performed echocardiography measurements on different days. We cannot easily compare echocardiography measurements with other measurements for that reason.

## Conclusion

Left atrial pressure did not decrease significantly by administration of 2 mg/kg sr-ISDN on dogs with experimentally induced MR. However, LAP decreased significantly by administration of 5 mg/kg and 10 mg/kg sr-ISDN. In addition, sr-ISDN showed dose-dependent duration of effect. Significant decrease in SBP occurred only in the 10 mg/kg group. Sustained-release form of ISDN had no significant effect on HR and HRV. This study did not focus on tolerance of sr-ISDN, and further investigation is needed in this respect.

## Footnotes

[a] TA11PA-D70, Data Sciences International, St. Paul, MN
[b] Nitorol R, Eisai Co, Ltd, Tokyo, Japan
[c] DSI Dataquest; A.R.T 4.1, Data Sciences International
[d] α-10, Aloka Co, Ltd, Tokyo, Japan
[e] DICOM server, ImageONE Co, Ltd, Tokyo, Japan
[f] BP-100D, Fukuda ME, Tokyo, Japan
[g] Holter recorder QR2100, Fukuda ME
[h] ECG analyzing system HS1000V, Fukuda ME
[i] GraphPad Prism version 5.0a, GraphPad, San Diego, CA
[j] EXCEL 2011 for Macintosh, Microsoft, Richmond, WA

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