Effect of Sustained Release Isosorbide Dinitrate (EV151) in Dogs with Experimentally-Induced Mitral Insufficiency

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ABSTRACT. To investigate the hemodynamic effects on seven anesthetized dogs with experimentally-induced mitral insufficiency, isosorbide dinitrate (ISDN) in sustained release form (EV151) was administered at different dosages (0, 2, 8 and 16 mg/kg). The drug administration resulted in altered pulmonary arterial wedge pressure (preload), and cardiac output and total systemic resistance (afterload). Arterial pressure increased in the control group and in animals receiving 2 mg/kg, but decreased in animals 1–2 hr after receiving 8 and 16 mg/kg dosages. Cardiac output increased in animals receiving 2, 8 and 16 mg/kg dosages, with concomitant decreases in total systemic resistance. ISDN caused mild vasodilation at 2 mg/kg and severe vasodilation at 8 and 16 mg/kg. Future experiments on non-anesthetized dogs may be of benefit.

KEY WORDS: canine, isosorbide dinitrate, mitral insufficiency.

Isosorbide dinitrate (ISDN) is widely used in the treatment of angina pectoris and congestive heart failure in humans. ISDN acts by lowering systemic venous pressure, leading to a reduction of the myocardial work-load and oxygen consumption [2, 4, 5, 8, 11]. Administration of the sustained-release form of ISDN (EV151) maintains an effective serum concentration for more than 6 hr [1] but no studies have been conducted to investigate the use of EV151 in dogs with chronic heart failure. In this study, the effect of different dosages of EV151 in dogs with experimentally-induced mitral insufficiency was assessed.

MATERIALS AND METHODS

Induction of mitral insufficiency: This experiment was performed in accordance with the Tokyo University of Agriculture and Technology experimental manual. Seven healthy adult beagle dogs (10.8–13.7 kg) (Table 1), were anesthetized with isoflurane, and the mitral valve chordae tendineae were ruptured with a clamp inserted via the carotid artery. Experimental assessment was carried out to 1 month postoperatively by means of electrocardiography, phonocardiography, radiography and echocardiography.

Preparation of animals: The dogs were injected with atropine sulfate (0.05 mg/kg) and acepromazine maleate (0.03 mg/kg) intramuscularly for sedation, and anesthetized with intravenous thiopental sodium (15 mg/kg) under mechanical ventilation. Anesthesia was maintained with intermittent intravenous pentobarbital infusion (20 mg/kg) and continuous intravenous pancuronium bromide infusion (0.08 mg/kg/hr). The dogs were heparinized (100 U/kg/hr) throughout the experiment to prevent thrombus formation, given intravenous lactated Ringer’s solution (10 ml/kg/hr) and warmed with a mat heater.

A Swan-Ganz catheter was introduced into the pulmonary artery via the jugular vein, and a polyvinyl catheter was introduced into the thoracic aorta through the carotid artery.

Administration of ISDN: EV151 was administered into the duodenum via an endoscope. The dogs were randomly assigned to four groups with seven animals per group as follows: Group 1 (control group, placebo), Group 2 (ISDN, 2 mg/kg), Group 3 (ISDN, 8 mg/kg) and Group 4 (ISDN, 16 mg/kg).

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Measurement of hemodynamics: Body temperature (BT) and heart rate (HR) were determined, and systolic arterial pressure (SAP), diastolic arterial pressure (DAP), pulmonary arterial wedge pressure (PAWP), pulmonary arterial pressure (PAP), right ventricular pressure (RVP), right atrial pressure (RAP) and cardiac output (CO) were measured with a polygraph (DS-5300 system, Fukuda Denshi Co., Ltd.). Cardiac output (CO) was measured by the thermodilution method. Measurements were obtained at: (1) baseline, (2) 15, 30 and 45 min after ISDN administration, and (3) every hr for 10 hr after ISDN administration. Total systemic resistance (TSR) was determined with the following formula:

\[
\text{TSR (dyne/s/cm}^{-5}) = \frac{\text{Mean arterial pressure} - \text{right atrial pressure (mmHg)}}{\text{Cardiac index (m l/s/m}^2)}
\]

Left ventricular fractional shortening (FS) and ejection fraction (EF) were assessed by ultrasonography (EUB-565A Ultrasound scanner, Hitachi Medical Corporation). Measurements were obtained at 1-hr intervals for 10 hr after ISDN administration.

Blood samples were taken at baseline and at 1-hr intervals for 10 hr after ISDN administration and centrifuged immediately for storage at –20°C until analysis could be performed. Plasma concentrations of ISDN, isosorbide-2-mononitrate (2-ISMN) and isosorbide-5-mononitrate (5-ISMN) were measured by gas chromatography.

Statistical analysis: Data are shown as the mean ± standard error. Fisher’s Protected Lead Significant Difference was used to determine the level of statistical significance. A p-value of 0.05 or less was considered statistically significant.

RESULTS

Body temperature was at first low in all groups but gradually increased, and there were no significant differences between the four groups. Systemic hemodynamic changes after administration of EV151 were assessed and are shown in Figs. 1–3.

PAWP increased in the control group shortly after administration of a placebo and was significantly greater 3 hr later. Four hours after receiving ISDN, PAWP was decreased in Group 2 (p<0.05 compared to Group 1). PAWP decreased in Groups 3 and 4 from 15 min to 10 hr after ISDN administration (p<0.001), but there was no significant difference between the two groups (Fig. 1). The lowest values were obtained at 8 hr in Group 2 (–21.4 ± 17.5%; p<0.05), at 45 min in Group 3 (–44.5 ± 15.6%; p=0.001), and at 1 hr in Group 4 (–15.5 ± 8.1%; p=0.001). SAP increased in Groups 1 and 2, but decreased in Groups 3 and 4 (100.0 ± 4.1 mmHg and 101.4 ± 5.1 mmHg at 45 min, respectively) (Group 1 vs. Group 3, p<0.01; Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 3, p<0.001; Group 2 vs. Group 4, p<0.0001). There was no significant difference between Group 3 and Group 4.

DAP increased in Group 1 and Group 2 shortly after
administration of the placebo and ISDN, respectively, but decreased in Group 3 (58.4 ± 3.6 mmHg at 1 hr) and Group 4 (64.3 ± 2.9 mmHg at 45 min) (Group 1 vs. Group 3, p<0.01; Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 3, p<0.001; Group 2 vs. Group 4, p<0.001). There was no significant difference between Group 3 and Group 4.

Although CO gradually decreased in Group 1, it was increased at 30 min in Group 2 (20.31 ± 4.88%, p=0.011), Group 3 (26.69 ± 8.20%, p=0.008), and Group 4 (16.82 ± 7.17%, p=0.025). There was no significant difference between Groups 2, 3 and 4 (Fig. 2).

TSR gradually increased in Group 1, but decreased in Group 2 (–16.63 ± 4.57% at 30 min, p=0.060), Group 3 (–34.51 ± 2.87% at 1 hr, p=0.0001) and Group 4 (–35.56 ± 5.13% at 1 hr, p=0.0012). There was no significant difference between Groups 2, 3 and 4 (Fig. 3).

No significant differences in HR, PAP, RVP or RAP were observed between the four groups.

Echocardiographic findings are shown in Figs. 4 and 5. FS and EF decreased progressively in Group 1, but was maintained in Groups 2 (p<0.0001), 3 (p=0.0002), and 4 (p=0.0002).

The plasma concentration of ISDN peaked 1 hr after administration and remained at a high level for 6–8 hr in Groups 2, 3 and 4.

The plasma concentration of 2-ISMN, a metabolite of ISDN, increased to its maximum level 4 hr after ISDN administration, and this high level was maintained for 4–6 hr. The plasma concentration of 5-ISMN, another metabolite of ISDN, peaked at 8 hr after ISDN administration and remained at a high level for 8–10 hr in the ISDN-treated groups (Figs. 6–8).

DISCUSSION

Previous studies have demonstrated that ISDN administration results in reduced systemic venous pressure, leading to a reduction of the myocardial work load and oxygen consumption [2, 4, 5, 8, 11]. ISDN is therefore a useful agent in the treatment of angina pectoris and congestive heart failure. ISDN acts via dilation of coronary vessels and redistribution of blood flow to the ischemic myocardium in anesthetized dogs [3, 7]. When 2.8 mg/kg of 14C-ISDN was administered to rats, it was distributed to the abdominal aorta, vena thoracica and heart [10].

Previous reports have investigated the pharmacokinetics of ISDN. Metabolites of ISDN in dogs, including 2-ISMN...
and 5-ISMN, can modify vascular activity, and investigators have recorded the relative vasodilatory activities of 2-ISMN and 5-ISMN as 1/6 and 1/50 of ISDN, respectively [10].

The renal clearance of orally-administered radiolabeled ISDN to dogs was 94.7% [10]. Oral administration of ISDN at 1–2 mg/kg also resulted in a dose-dependent decrease in pulse pressure through a reduction in the systolic pressure and a slight increase in the diastolic pressure in conscious dogs [6].

Administration of EV151 has been shown to maintain effective serum concentrations of ISDN for more than 6 hr, but the effect of EV151 on the cardiovascular parameters in dogs with chronic heart failure has not previously been investigated. In this study, PAWP increased after placebo administration in Group 1 and was significantly higher 3 hr later. In contrast, PAWP was decreased in Group 2 at 4 hr after ISDN administration, most likely secondary to systemic venous dilation and a decrease in cardiac preload. SAP and DAP increased progressively in Groups 1 and 2 but were significantly decreased in Groups 3 and 4 at 1–2 hr after ISDN administration. This suggests that oral administration of ISDN at 2 mg/kg causes mild vasodilation, while ISDN at 8 mg/kg and 16 mg/kg causes significant vasodilation. Kogi et al. reported that arterial pressure decreased to a nadir at 4 hr after ISDN administration [6]. But a nadir was reached 1–2 hr after EV151 administration in the present study. The disparity in these results may be secondary to drug formulation or the method of administration.

Systemic vascular resistance decreased significantly after intravenous infusion of ISDN in dogs with congestive heart failure [9]. In the present study, CO increased and TSR decreased immediately after administration of ISDN at all doses, suggesting that EV151 caused peripheral arterial dilation and decreased cardiac afterload.

After administration, plasma concentrations of ISDN peaked at 1 hr after administration and remained at a high level for 6–8 hr thereafter, with concomitant decreases in preload and afterload. Plasma concentrations of 2-ISMN increased to its maximum level 4 hr after administration of ISDN and remained at a high level for 4–6 hr, while concentration of 5-ISMN peaked at 8 hr after administration and remained at a high level for 8–10 hr thereafter, but since ISDN levels remained high, the biological effect of persistent 2-ISMN and 5-ISMN levels could not be determined.

In the present study, there was a slight decrease in afterload and preload at 2 mg/kg of EV151 and a maximal decrease at 8 mg/kg. Therefore, the effective dose-response curve for EV151 in anesthetized dogs with experimentally-induced mitral insufficiency appears to be between 2 and 8 mg/kg. This is in contrast to previous reports, which have recommended ISDN doses of 0.5–2.0 mg/kg.

The laboratory animals used in this study were all subjected to anesthesia, and the use of this medication in a conscious canine model may require different therapeutic concentrations. Future studies to investigate the use of EV151 in non-anesthetized dogs may be of benefit.

REFERENCES