容量負荷モデルラットに対する徐放性硝酸イソソルビド(sr-ISDN)の間欠的慢性投与の影響

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The Effect of Intermittent Administration of Sustained Release Isosorbide Dinitrate (sr-ISDN) in Rats with Volume Overload Heart

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ABSTRACT. Recently, it has been reported that intermittent administration of nitrate, with a nitrate-free interval of 10 to 12 hr eliminated expression of tolerance, and maintained its hypotensive effect. In the present study, we evaluated whether nitrate tolerance developed or not with an intermittent administration of sr-ISDN (5 mg/kg/ once a day) in Wistar rats. The effect of this administration protocol for sr-ISDN on the volume overload heart model, aortovenous fistula, was also examined. Furthermore, blood pressure was monitored by radio telemetry during sr-ISDN (5 mg/kg/once a day) administration. Nitrate tolerance did not develop, and eccentric hypertrophy due to volume overload was moderated by sr-ISDN administration. Sr-ISDN administration maintained blood pressure lower level than the placebo group. In conclusion, prolonged intermittent administration of sr-ISDN maintained its hypotensive effect during the entire experiment period, without developing tolerance, and moderated efferent hypertrophy with attenuated volume overload.

KEY WORDS: aortovenous fistura, isosorbide dinitrate, rat, volume overload.

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Organic nitrates, such as isosorbide dinitrate (ISDN), are vasodilators that induce vascular smooth muscle relaxation through a pathway involving biotransformation from nitrate to nitric oxide [3]. Nitrates have been used for more than 100 years in the treatment of angina pectoris [2, 19], ischemic heart disease, and chronic heart failure [8, 9, 24, 30]. Unfortunately, the use of nitrates has the disadvantage of inducing tolerance when administered for long periods [12, 13, 22, 34]. The effect of prolonged use of nitrates on the prognosis for survival and prophylaxis of future cardiac events remains unclear [26]. On the other hand, previous studies have shown that an intermittent dosing regimen, including a nitrate-free interval of 10 to 12 hr, is a useful therapeutic approach for preventing the development of nitrate tolerance [18, 27]. However, cross-tolerance of nitrates with other substances has not been established. In some studies, continuous infusion of isosorbide mononitrate (ISMN) to rats did not affect the decrease in blood pressure caused by bolus injection of glyceryl trinitrate [18, 23, 32, 35]. Also, there are no reports on the efficacy of prolonged intermittent administration of sustained release ISDN (sr-ISDN). In chronic heart failure, such as valvular disease, the prognosis with prolonged use of ISDN would be good, with a decrease in blood pressure and an improvement of coronally circulation [17, 20, 37]. However, there are few reports that have evaluated the long-term effect of intermittent administration of nitrates for chronic heart failure. In the present study, the development of nitrate tolerance and the effect on the volume overloaded heart after long-term intermittent administration of sr-ISDN on rats was investigated. The blood pressure of rats with an aortovenous fistula (AVF) under prolonged intermittent sr-ISDN therapy was observed using a telemetry system.

MATERIALS AND METHODS

Animals: Fifty-two male Wistar rats (Japan Saitama Experimental Animal Supply, Saitama, Japan), weighing approximately 200 g each, were used in the study. The rats were housed under standard environmental conditions and maintained on commercial rat chow and tap water *ad libitum*.

ISDN: The sr-ISDN and ISDN injections were purchased from Eisai Co., Ltd. (Japan). The sr-ISDN was dissolved in distilled water and given orally, while the ISDN was dissolved in physiological saline and administered intravenously.

Methods: The protocol used in the study has been approved by the Laboratory Animal Care Committee of the Tokyo University of Agriculture and Technology.

Experimental model: AVF was created in the rats, as previously reported by Garcia and Diebold [15]. After anesthesia was induced with an intraperitoneal injection of sodium pentobarbital (NEMBUTAL Injection ; Dainippon Pharmaceutical Co., Ltd., Japan), a ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava 1.5 cm below the renal arteries. Both vessels were then occluded proximal and distal to the intended puncture site. An 18-gauge needle was inserted into the exposed abdominal aorta and advanced through the medial wall into the vena cava to create the fistula. The needle was withdrawn and the ventral aortic puncture was sutured with polypropylene (7–0 Prolene; Ethicon, Inc., NJ, U.S.A.). Creation of a successful AVF was visually evident by the pulsatile flow of oxygenated blood into the vena cava. The abdominal musculature and skin incisions were closed using standard techniques. Sham-operated animals were subjected to the

same surgical procedure without creation of an AVF. The rats were allowed a seven-day recovery adjustment period before the treatment was started.

Experiment 1: *Tolerance test*: For the first experiment, the rats were randomly divided into two groups. The first group received distilled water as a placebo, while the second group was given sr-ISDN at 5 mg/kg body weight daily for 30 days. The animals were anesthetized with Nembutal 12 hr after the last treatment with sr-ISDN or distilled water. A 22-gague Teflon catheter was indwelled in the aortic arch through the carotid artery and connected to a transducer (Fukuda Denshi, Japan). Another 22-gague Teflon catheter was inserted into the right jugular vein and used to deliver the ISDN infusion. The ISDN was dissolved in physiological saline. The treatment was started after 10 min of equilibration to steady-state conditions. ISDN was cumulatively given as a bolus into the central vein. Hemodynamic measurements were obtained 5 min after injection. ISDN was cumulatively administered to each rat from 0.1 mg/kg/min to 100 mg/kg/min.

Experiment 2: *Long-term intermittent sr-ISDN administration*: For the second experiment, the rats were randomly divided into four groups consisting of 7 animals in each group, as follows: 1) AVF rats given sr-ISDN orally at 5 mg/kg (AVF-sr-ISDN group); 2) AVF rats given distilled water orally as a placebo (AVF-Placebo group); 3) sham-operated rats given sr-ISDN orally at 5 mg/kg (Sham-sr-ISDN group); and 4) sham-operated rats given distilled water orally as a placebo (Sham-Placebo group). The distilled water and sr-ISDN were administered via gastric gavage once daily for 90 days. To avoid the initial stress of surgery, the treatments were started 7 days after the AVF and sham operations. The treatments were continued for the entire duration of the experiment. At the end of 90 days, the rats were anesthetized with sodium pentobarbital, and cardiac arrest was induced in diastole by intravenous injection of 20% KCl solution (0.2 m*l*/100 g body weight). The rats were then necropsied, and the hearts were collected and weighed. The presence or absence of an aortovenous shunt in the vena cava was comfirmed.

Echocardiography: The echocardiograph system used (ProSound SSD-5000, ALOKA, U.S.A.) employs dynamically focused symmetrical annular array technology for two-dimensional (2D) and M-mode imaging. Echocardiography was utilized to define the temporal pattern of cardiac remodeling after creation of AVF in all the groups. Prior to the examination, the rats were anesthetized with a mixture of ketamine at 10 mg/kg i.p. (Veterinary Ketalar 50; Sankyo, Japan) and xylazine at 15 mg/kg i.p. (Cerakutarl; Bayer Healthcare, Germany). The examinations were performed using a 10-MHz transducer. An M-mode image was obtained and recorded for archiving and analysis of end-diastolic left ventricular internal diameter (LVIDd), end-systolic left ventricular internal diameter (LVIDs), and end-diastolic posterior wall thickness diameter (LVPWd) in accordance to the conventions of the American Society of Echocardiography. These measurements, derived from the short-axis image, were used for offline calculation of ejection fraction (EF) and fraction shortening (FS) using the following formulas.

$$FS\ (\%) = [(LVIDd-LVIDs)/LVIDd] \times 100$$
$$EF\ (\%) = [(LVIDd)^3-(LVIDs)^3]/(LVIDs)^3 \times 100$$

Experiment 3: *Blood pressure monitoring by radio telemetry system*: Radio transmitters (PA-C40, Data Sciences St. Paul, MN, U.S.A.) were used to monitor the daily blood pressure of 10 rats for 37 days after the AVF operation. For the procedure, anesthesia was induced with an intraperitoneal injection of sodium pentobarbital. A ventral abdominal laparotomy was performed to expose the aorta. After creation of the AVF, a calibrated pressure transmitter was implanted nonocclusively through a catheter and inserted in the aorta between the origin of the renal and iliac arteries from the same opening. The catheter was fixed and the opening was closed by suturing with polypropylene. The abdominal musculature and skin incisions were closed using standard techniques. The rats were allowed a 7 day recovery adjustment period before the treatments were started. Blood pressure in systole and diastole were collected for 10 seconds every 5 min and recorded in the data acquisition system (Dataquest LabPRO; Data Sciences International, MN, U.S.A.). The recorded data were analyzed and the averages were calculated daily using a commercially-available software (Dataquest LabPRO; Data Sciences International, MN, U.S.A.). The average obtained from the data for the first seven days was used as the standard value. After the first seven days, the rats were randomly distributed into two groups that consisted of 5 animals each group, as follows: 1) AVF rats given sr-ISDN orally at 5 mg/kg (AVF-sr-ISDN group) and 2) AVF rats given distilled water orally as a placebo (AVF-Placebo group). Changes in blood pressure after treatment were shown as rates of variation from the standard value.

Statistical analysis: Data were expressed as means ± S.E. The differences between the groups were evaluated using one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. Temporal differences among groups were evaluated using repeated measure ANOVA followed by the Bonferroni post hoc test. The Student's *t*-test was used for comparison between two groups. A $p<0.05$ was considered significant.

RESULTS

Effect of sr-ISDN on blood pressure: The effect of ISDN bolus injection on blood pressure after prolonged treatment with sr-ISDN for 30 days is shown in Fig. 1. The values represent the variation from the dose rate of 0 mg/kg/min. A decrease in blood pressure with an increasing dose was observed in both the sr-ISDN and Placebo groups. In dose rates of 10 and 100 mg/kg/min, both the diastolic and systolic blood pressures decreased significantly ($p<0.05$). There were no significant differences between sr-ISDN and the Placebo for all the doses ($p>0.05$).

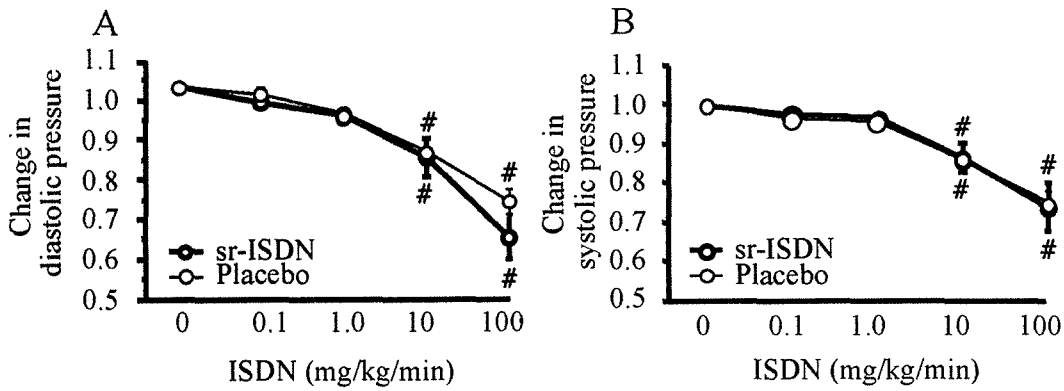

Fig. 1. Effect of ISDN injection intravenously on aortic blood pressure in the diastole (A) and in systole (B) of an anesthetized rat that was administered sr-ISDN (5 mg/kg/once a day) orally for 30 days. An # indicates a significant difference from the 0 mg/kg/min ISDN.

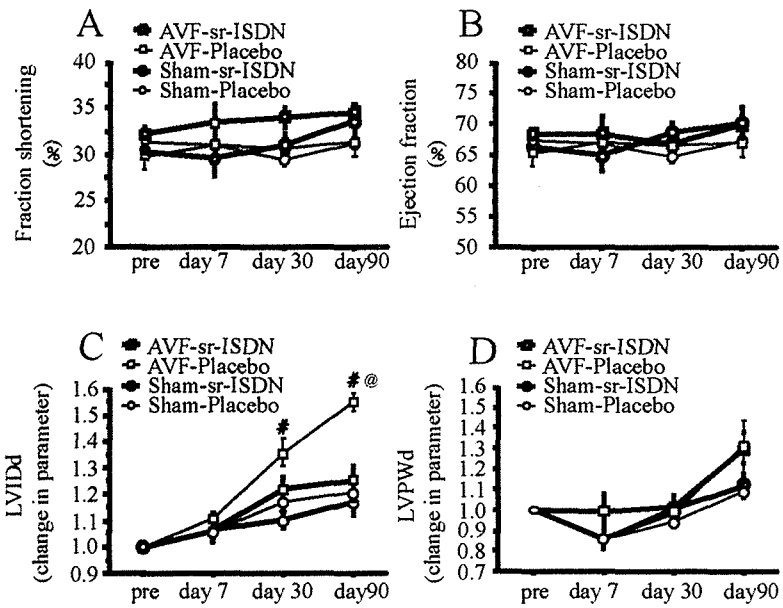

Fig. 2. Effect of oral treatment with sustained-release isosorbide dinitrate on cardiac function and morphology evaluated by echocardiography in anesthetized AVF and sham rats for (A) fraction shortening (FS), (B) ejection fraction (EF), (C) end-diastolic left ventricular internal diameter (LVIDd), and (D) end-diastolic left ventricular posterior wall diameter (LVPWd). C and D show changes in parameter. An # indicates a significant difference between the AVF-Placebo group and Sham-Placebo group. An @ indicates a significant difference between the AVF-Placebo group and AVF-sr-ISDN group.

Effect of sr-ISDN on cardiac function: The temporal changes in cardiac function and form as seen through echocardiography during the sr-ISDN treatment for 90 days are shown in Fig. 2. The examination was performed before the AVF and sham operations and 7, 30, and 90 days after start of the sr-ISDN or Placebo treatment. FS and EF did not show any significant changes throughout the experiment for all groups. No significant differences in FS and EF were observed among the groups (Fig. 2-A, B). LVIDd and LVPWd had the greatest changes from the pre-treatment values (Fig. 2-C, D). An increase in LVIDd with time was observed in all four groups (Fig. 2-C). On day 30, LVIDd was higher in the AVF-Placebo group than in Sham-Placebo group ($p<0.05$ by ANOVA ; AVF-Placeb 1.359 ± 0.51, Sham-Placebo 1.151 ± 0.04). No increase in LVIDd was observed in the AVF-ISDN group compared with the Sham-Placebo group throughout the duration of the experiment. At day 90, the increase in LVIDd in the AVF-sr-ISDN group was smaller compared with the AVF-Placebo group ($p<0.05$ by ANOVA ; AVF-Placebo 1.550 ± 0.32, AVF- sr-

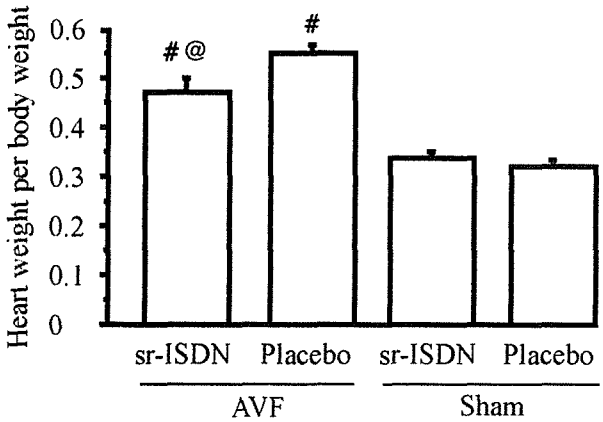

Fig. 3. Effect of oral treatment with sustained-release isosorbide dinitrate on cardiac weight per body weight. An # indicates a significant difference from the Sham-Placebo group. An @ indicates a significant difference between the AVF-sr-ISDN group and AVF-Placebo group.

ISDN 1.256 ± 0.54). No significant changes in LVPWd were observed throughout the entire experiment period for all groups, compared with baseline values. Also, no significant differences in LVPWd were noted among the treatments groups (Fig. 2-D).

Effect of sr-ISDN on heart weight: The heart weight to body weight ratio was calculated after 90 days of treatment either with sr-ISDN or the placebo. The results are shown in Fig. 3. This value was higher in both the AVF-sr-ISDN and AVF-Placebo groups compared to the Sham-sr-ISDN and Sham-Placebo groups ($p<0.05$ by ANOVA ; AVF-sr-ISDN 0.473 ± 0.25, AVF-Placebo 0.550 ± 0.17, Sham-sr-ISDN 0.338 ± 0.13, Sham-Placebo 0.322 ± 0.10). Although the ratio increased in the AVF-sr-ISDN group compared with the Sham groups, the increase was minimal.

Effect of sr-ISDN on spontaneous blood pressure: Spontaneous blood pressure in AVF rats without restraint and anesthesia was monitored using a radio telemetry system. The average of daily blood pressure was calculated, and represented as rate of variation in relation to the average of the seven days of pre-treatment (Fig. 4). An increase in the diastolic blood pressure was observed with the increase in number of days in the placebo group, but not in the sr-ISDN groups. For systolic blood pressure, no clear changes were observed in any of the groups during the course of the treatment. The average blood pressure throughout the entire experiment period for each group was computed from the values shown in Fig. 4 and Fig. 5. For diastolic blood pressure, the mean values for the sr-ISDN groups were compared to the placebo groups ($p<0.05$; sr-ISDN 0.954 ± 0.16, Placebo 1.062 ± 0.42). There were no significant differences in systolic blood pressure between both groups ($p>0.05$).

DISCUSSION

Previous studies regarding the hemodynamic effect of nitrates have focused mostly on the acute responses and effects of high doses of nitrates [23, 35].

In the present study, a clinical dose of ISDN was administered for 90 days and its effect on chronic heart failure was evaluated. Since nitrates are generally used to improve angina pectoris with resultant-induced tolerance, studies on nitrate have focused on its acute effects [13, 28]. Nitrates have been shown to induce immediate tolerance in previous *in vivo* and *in vitro* studies [12, 34]. Whereas the mechanism that induces tolerance through nitrate administration was unclear until recently [14, 16], setting a drug-free time of 10 to 12 hr has been shown to prevent tolerance development [7, 11, 29]. Another study has shown that eccentric long-term nitrate treatment was capable of maintaining the chronic hypotensive effect [27]. In this study, since the sr-ISDN maintained hypotension for 12 hr, it is speculated that setting the drug-time at 12 hr could prevent development of tolerance. This study demonstrated that responses to ISDN injection did not vary after prolonged intermittent treatment of sr-ISDN for 30 days. This result suggests that a long-term intermittent administration protocol can prevent development of nitrate tolerance.

AVF increased blood volume returning to the heart and end-diastolic wall stress, leading to progressive eccentric cardiac hypertrophy [4, 15]. These reactions are similar to those occurring in chronic heart failure, e.g., mitral valve regurgitation, with respect to hemodynamic and hormonal changes [31]. In the present study, the AVF model expressed eccentric cardiac hypertrophy shown as an increasing heart weight and dilation of the left ventricular internal diameter. Since administration of sr-ISDN prevented these changes in volume overload heart, it is suggested that sr-ISDN administration can be effective in this condition. The first reason for the effectiveness of sr-ISDN appears to be its vasodilating effect. A decrease in circulating blood volume results in dilation of the systemic vascular pre-load to the heart to relieve volume overload. The second reason can be the improvement of coronary circulation [1, 25]. Nitrates have a cardioprotection effect in maintaining the coronary circulation volume through dilation of coronary vascular. Thirdly, nitrates may also have an antihypertrophic effect. The antihypertrophic properties of nitric oxide were first recognized in cultured cardiac myocytes [21]. Chronic exposure to nitric oxide inhibited eccentric hypertrophy of the heart [33]. Transforming growth factor-β, an important factor for cardiac remodeling, was shown to be present in cardiomyocyte during shear stress [5]. This is one of the reasons why the expression of this growth factor was inhibited directly by nitric oxide.

Eccentric hypertrophy by volume-overload depresses cardiac function in the end-stage. Although many studies using the AVF model rat have been conducted [4, 6, 36], the severity of the pathology has shown some variations. The model in this study did not exhibit depression of cardiac function. This may be due to the mild volume of load causing minimal shunting of blood between the aorta and vena cava through a compensatory function of the heart. By

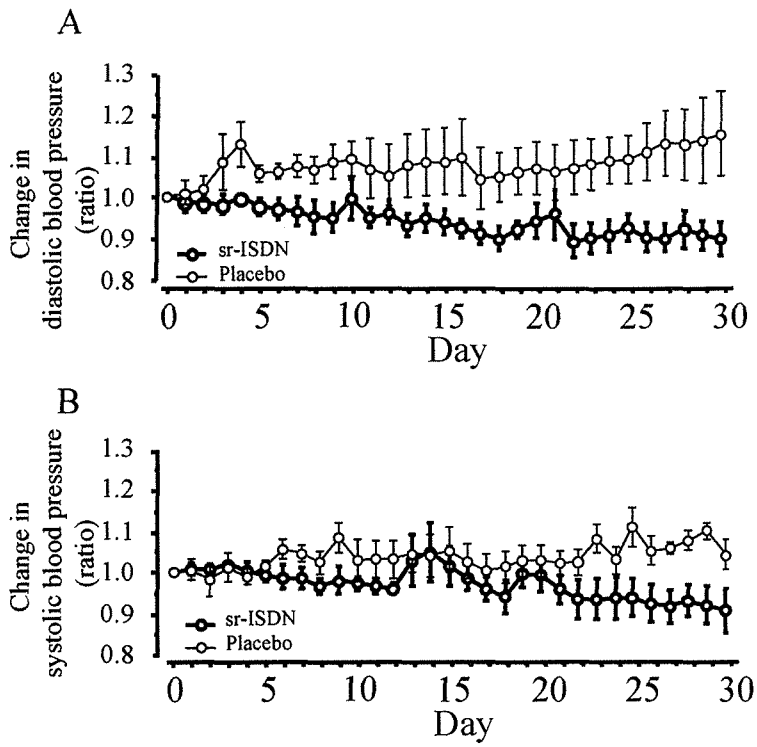

Fig. 4. Effect of oral treatment with sustained-release isosorbide dinitrate on spontaneous aortic blood pressure monitored by telemetry system in AVF rats. (A) The diastolic blood pressure and (B) systolic blood pressure in abdominal aorta showed variations from the original readings.

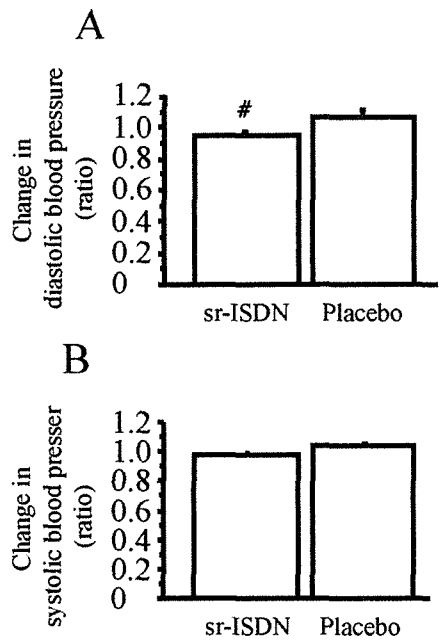

Fig. 5. Effect of oral treatment with sustained-release isosorbide dinitrate on spontaneous aortic blood pressure monitored by telemetry system in AVF rats. (A) Diastolic blood pressure and (B) systolic blood pressure. An # indicates a significant difference between both groups.

using an excessive shunt volume model or by extending the experiment period, the effect of sr-ISDN on cardiac function can be evaluated, but at present, the effect of sr-ISDN on cardiac function is still unknown.

The results of blood pressure monitoring using telemetry system demonstrated the chronic hypotensive effect of long-term intermittent sr-ISDN treatment. In this study using AVF model rats, the control group showed an increase in diastolic blood pressure with increasing time, whereas the intermittent sr-ISDN treatment group did not shown an increase and actually maintained blood pressure reading lower than the baseline value. A previous study using AVF reported an increase in diastolic blood pressure one month after shunt production [10]. In the acute stage, volume overload heart appeared to show dilation morphologically in the left ventricular diameter due to increased blood returning to the heart and excessive constriction. This change in cardiac function appeared to compensate for the increased stroke volume, according to Frank-Starling's law. However, in the end-stage, a decrease in blood pressure was seen in congestive heart failure. There was a depression in cardiac function and a reduction in stroke volume. Volume overload in AVF was sufficiently compensated by an increased cardiac capacity through morphological changes since cardiac hyperfunction was not observed in this study. The AVF model in this study was in the acute stage in which progressive volume-overload and congestion in systemic circulation induced a significantly elevated diastolic blood pressure and mildly elevated systolic blood pressure. Sr-ISDN treatment lowered blood pressure by reducing the circulating blood volume and by preventing an increase in the diastolic blood pressure.

In conclusion, it was shown that intermittent administration of sr-ISDN reduced blood pressure without development of nitrate tolerance. The chronic hypotensive effect of sr-ISDN inhibited eccentric hypertrophy from occurring during volume overload. The present study revealed the efficacy of intermittent administration of sr-ISDN for chronic heart failure with volume overload.

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