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

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ABSTRACT. Recent studies have demonstrated the benefits of nitric oxide (NO) on myocardial hypertrophy and myocardial fibrosis. It was suggested that NO has a protective effect on myocardial cell through the neurohormonal system. This effect serves to highlight the important role of NO in maintaining the function and form of heart with chronic heart failure. However, there are no known reports about on the effect of prolonged administration of nitrate on pressure over-load heart. This study was conducted to examine the long-term effect of oral nitrate therapy in rats with pressure-overloaded heart. An abdominal aorta constricted (AC) model of pressure-overloaded heart was created in male Wistar rats. Sustained release isosorbide dinitrate (sr-ISDN) (5 mg/kg once a daily) was administered to the rats once a daily for 12 weeks. The animals were euthanized during the study period, and the heart was collected and weighed. Histopathological examination was performed to evaluate the effect of sr-ISDN on myocardial hypertrophy and fibrosis. The ratio of heart to body weight increased significantly in AC rat and this increase was significantly prevented by sr-ISDN treatment. Histopathological examination showed significant increase in fibrotic area of AC rat compared to sham rat, this increase was inhibited by sr-ISDN treatment. Cardiomyocyte transverse diameter was significantly increased in AC rat compared to sham rat, but this increase tended to decrease by sr-ISDN treatment. In conclusion, intermittent administration with sr-ISDN has mild effect in inhibiting cardiac hypertrophy and marked effect in inhibiting fibrosis due to pressure-overload.

KEY WORDS: aortic constrict model, cardiac fibrosis, cardiac hypertrophy, isosorbide dinitrate, rat.

Isosorbide dinitrate (ISDN) belongs to a group of compounds called organic nitrates. Organic nitrates are vasodilators which induce vascular smooth muscle relaxation through a pathway involving biotransformation from nitrate to NO [3, 26]. Nitrates works in all vascular smooth muscles, especially in venous and coronal vessels, in decreasing pre-load and preventing ischemic heart disease [12, 22]. For this reason, nitrates have been used in the treatment of angina pectoris [1, 11], ischemic heart disease and chronic heart failure in humans [5, 6, 20, 25]. The effect of nitrates are observed not only in relation to neurohormonal system but also to hemodynamics. Several researchers have demonstrated the regulatory role of NO in modulating extracellular matrix. Hou et al. [7] had shown that chronic NO synthase inhibition induces cardiac fibrosis in rat. They suggested that NO work as a regulator in fibroblast proliferation in cardiomyocytes. NO was also shown to be an important agent in modulating the synthesis of extracellular matrix proteins [16, 30]. The antiproliferative effect of NO in extracellular matrix protein depresses cardiac remodeling due to pressure-overload or myocardial infarction [14]. These reports suggest direct effect of NO on cardiomyocyte protection. Histopathological changes in pressure-overload heart are characterized by concentric hypertrophy with myocardial hypertrophy and fibrosis due to proliferating fibroblasts in the interstitium [18]. Organic changes in the heart, including myocardial fibrosis and myocardial hypertrophy, result in cardiac dysfunction. The inhibitory effect of NO on these changes is thought to be an important factor in heart failure therapy. Previous experiments in vivo have shown that NO suppresses myocardial fibrosis and myocyte hypertrophy [21, 27]. Furthermore, NO has inhibitory effect on cardiac remodeling of angiotensin converting enzyme inhibitor and angiotensin receptor blocker mediated by increasing endogenous NO production intervening kinin [15, 17]. Further studies indicate useful effects of NO on morphological changes in the heart due to pressure-overload. However, there is no known report on the efficacy of long-term administration of nitrate on pressure-overload heart.

In this study, the effect of prolonged administration of nitrate on rats with cardiac pressure-overload was investigated using an aortic constricted model (AC). Intermittent administration of sustained-release ISDN (sr-ISDN), at a dose rate of 5 mg/kg once a day, was used, because previous studies have shown that an intermittent dosing regimen, including a nitrate-free interval of 10 to 12 hr, is an effective therapeutic approach for preventing the development of nitrate tolerance. Myocardial fibrosis and myocardial hypertrophy was examined through histopathological evaluation in rats with pressure-overload treated with sr-ISDN.

MATERIALS AND METHODS

Animals: Twenty-one 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 feed and given tap water ad
**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**: An abdominal aorta constricted (AC) model of pressure-over loaded heart was created in rats according to the procedures described by Morkin et al. [23]. 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. In order to assure a reasonably uniform degree of constriction of abdominal aorta below the renal artery, the constricting tie was placed over a 21-gauge needle which was then removed, leaving a constriction equal to the diameter of the needle. The abdominal musculature and skin incision were closed using standard techniques. Sham-operated animals were subjected to the same surgical procedure without creation of AC. The rats were allowed a seven-day recovery period to adjust before the treatment was started. **Experimental protocol**: The rats were randomly divided into four groups consisting of 7 animals for each group, as follows:1)AC rats given oral sr-ISDN at 5 mg/kg (AC-sr-ISDN); 2) AC rats given oral distilled water as placebo (AC-Placebo); 3) sham-operated rats given oral distilled water as placebo (Sham-Placebo). Distilled water and sr-ISDN were administered via gastric gavage once a day for 12 weeks. To avoid the initial stress of surgery, the treatments started 7 days after AC and sham operations and continued for the entire duration of the experiment. At the end of 12 weeks, the rats were anesthetized with sodium pentobarbital and cardiac arrest was induced in diastole by intravenous injection of 20% KCl solution (0.2 ml/100 g body weight). The rats were then necropsied and the hearts were collected and weighed. **Histopathological analysis**: The hearts were fixed in 10% formalin, dehydrated with ethanol, embedded in paraffin, and sectioned at a thickness of 3 μm. Histopathological examination was performed in a blind fashion by 2 observers for 3 independent samples from each subject. To determine myocardial hypertrophy, the shortest transverse diameter was measured in 100 nucleated transverse section of the cardiomyocytes of the left ventricle (LV) myocardium stained with hematoxylin-eosin, using a microscope (OLYMPAS KS-630 KS: OLYMPAS, Japan) connected to a computer with an image analysis software, as previously described [34]. A minimum of 10 fields from each of four LV sections (inner, mid, outer) was examined using the 200 × magnification. To assess changes in perivascular fibrosis, the ratio of the area of pericascular fibrosis with vessel lumen area was obtained. Collagen volume percent for each animal was expressed as the average of all fields examined. **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 Tukey-Kramer test. Temporal differences among groups were evaluated using repeated measure ANOVA followed by the Tukey-Kramer test. The level of significant differences was set at p<0.05.

**RESULTS**

**Effect of sr-ISDN on heart weight**: The heart weight to body weight ratio was calculated after 12 weeks of treatment either with sr-ISDN or placebo. These results are shown in Fig. 1. The ratio in AC-Placebo group significantly increased compared to the Sham-Placebo group (p<0.05). The increase in AC-sr-ISDN group was lower than with AC-Placebo group (p<0.05). **Effect of sr-ISDN on cardiomyocyte**: Quantitative distribution of the cardiomyocyte transverse diameter was shown in Fig. 2. The cardiomyocyte transverse diameter in AC-Placebo and AC-sr-ISDN groups increased significantly compared with the sham-Placebo group (p<0.05). The diameter in AC-sr-ISDN group was lesser than that of the AC-Placebo group, although no significant difference was
observed between the two groups (p>0.05). Interstitial fibrosis and perivascular fibrosis of the left ventricle were seen in the samples stained using picric acid Sirius red stain (Fig. 3). The quantitative distribution of the volume percentage of interstitial fibrosis and perivascular fibrosis was shown in Fig. 4. Both perivascular and interstitial collagen were increased significantly in AC-Placebo group compared with Sham groups (p<0.05). The volume of both perivascular and interstitial collagen in the AC-sr-ISDN group was significantly lower than that of the AC-Placebo group (p<0.05).

DISCUSSION

The present study demonstrated that mild inhibition of myocardial hypertrophy and significant inhibition of myocardial fibrosis were presented by prolonged intermittent administration of sr-ISDN. Nitrates have been shown to induce immediate tolerance in previous studies conducted in vivo and in vitro [8, 9, 19, 28], although the mechanism of tolerance induction through nitrate administration has been unclear until the present time. However, it has been demonstrated that the intermittent administration of nitrates with a drug-free time of 10 to 12 hours can prevent development of tolerance [10, 24]. In this study, sr-ISDN was administered intermittently with a drug-free time 12 hours of once a day treatment.

After-load of the left ventricle was increased due to abdominal aortic constriction in AC model rats. Increase in after-load made augments myocardial rigidity, resulting in constricted hypertrophy characterized by increasing myocardial transverse diameter. Interstitial myocardial fibrosis due to fibroblast activation occurred with progression of constricted hypertrophy. The AC rat, in the present study, showed an increased heart weight. Furthermore, myocardial transverse diameter increased with development of both myocardial and perivascular fibrosis. These results showed that constricted hypertrophy due to pressure-overload can result in the development of myocardial hypertrophy and myocardial fibrosis.

The inhibitory effect of intermittent administration of sr-ISDN on myocardial hypertrophy was demonstrated in the AC rat. Myocardial transverse diameter in both AC-sr-ISDN and AC-Placebo groups increased significantly com-
pared to the Sham-Placebo group, but the increase in the AC-sr-ISDN group was smaller than the AC-Placebo group. Furthermore, an increase in the ratio of heart weight to body weight due to constricted hypertrophy was inhibited significantly in sr-ISDN administrated animals. These results suggest that sr-ISDN inhibits myocardial hypertrophy. Previous studies have shown that chronic administration of L-arginine, the precursor of NO, attenuated cardiac hypertrophy in spontaneously hypertensive rats [21] and NO/cGMP was shown to be a negative regulator of cardiomyocyte hypertrophy without changing blood pressure [33]. These experiments suggest direct inhibitory effect of NO on myocardial hypertrophy, which is consistent with the results obtained in the present study.

However, significant difference in myocardial transverse diameter was not observed between AC-sr-ISDN and AC-Placebo groups, although the difference in heart weight was significant. This is the reason why myocardial transverse diameter was slightly different in one dimension augmented in three dimensions. It is also possible that increase in myocardial fibrotic area strongly affected heart weight. Whereas L-arginine was used in previous studies in vivo [21], it was suggested that through oral administration of sr-ISDN in the present study, the animal was not able to obtain sufficient dose of nitric oxide to significant suppress myocardial hypertrophy. A significant finding in this study is the suppression of myocardial fibrosis in the AC rat through intermittent administration of sr-ISDN. Essential changes in myocardial fibrosis in pressure-overload heart were observed in perivascular and interstitial fibrosis. At first, myocardial fibrosis occurred around the coronal vessels, and then progressed in the interstitial tissues [4, 29, 32]. Intermittent administration of sr-ISDN significantly suppressed in both the coronal vessels and interstitial tissues. These results suggest marked anti-myocardial fibrosis effect of NO.

The anti-myocardial fibrosis effect of NO has been demonstrated in many experiments in vivo and in vitro [2, 14, 16], and was consistent with the result obtained in the present study. Previous studies and the results of the present study suggest the possibility that sr-ISDN administration improve the diastolic capacity of the left ventricle of pressure-overload heart resulting in the anti-myocardial fibrosis effect. Excessive deposition of fibroblast in the myocardial tissue depresses the rigidity of the heart, leading to decrease in the diastolic capacity. Finally, cardiac dysfunction causes regression of the systolic capacity [31]. Therefore, inhibition of myocardial fibrosis maintains the rigidity of the heart, leading to improve cardiac function. Our observation that the anti-myocardial fibrosis effect of sr-ISDN worked in maintenance of the stiffness of AC rat was showed the long-term effectiveness of sr-ISDN administration to pressure-overload heart. In conclusion, the results of the study suggest that sr-ISDN administration affects pressure-overload heart by inhibition of myocardial fibrosis and myocardial hypertrophy.

REFERENCES


