Comparison of preventive effect of sildenafil and therapeutic effect of sildenafil treatment in rats with monocrotaline-induced pulmonary arterial hypertension

Rieko YOSHIYUKI1)*, Ryuji FUKUSHIMA1), Ryo TANAKA1) and Noboru MACHIDA2)

1)Department of Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu-shi, Tokyo 183–8509, Japan
2)Department of Veterinary Clinical Oncology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu-shi, Tokyo 183–8509, Japan

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ABSTRACT. This study aimed to investigate the potential effects of sildenafil on pulmonary hypertension (PH) in the monocrotaline (MCT)-induced PH rat. Twenty-four, 12-week-old, male Sprague-Dawley rats were injected with MCT or saline solution. After injection of MCT, rats received oral sildenafil immediately (early-phase treatment group: E group), 4 weeks after injection (late-phase treatment group: L group) or no treatment (MCT group) until 6 weeks after injection. Serial echocardiography and right ventricular systolic pressure (RVSP) measurements via a cardiac catheter were performed. RVSP was reduced in the E and L groups compared with the MCT group. Echocardiography indicated that sildenafil therapy prevents an increase in RVSP and preserves diastolic function, and this effect is not dependent on timing of initiation of therapy.

KEYWORDS: echocardiography, monocrotaline, pulmonary hypertension, rat, sildenafil

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Pulmonary hypertension (PH) is defined by a mean pulmonary artery pressure at or above 25 mm Hg based on hemodynamic criteria [11]. Invasive hemodynamic assessment in cardiac catheterization is the gold standard for the measurement of pulmonary artery pressure in the diagnosis of PH and hemodynamic changes following drug administration [18]. Echocardiography is a noninvasive repeatable method for monitoring right ventricular function in patients with PH, and echocardiographic assessment is useful in the diagnosis and evaluation of PH [23].

Sildenafil is a selective inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase-5 (PDE5). PDE5 is an effective vasodilator for reducing right ventricular pressure and improving exercise tolerance and quality of life [10, 21, 24]. The previous studies indicate that an early diagnosis of PH and early therapeutic intervention may result in an improvement in long-term outcomes [7, 14, 15]. Although early inflammatory of right ventricular heart failure in a rat model has contributed to a better understanding of vascular remodeling in PH [4, 25], the optimal time of sildenafil treatment of PH and the utility of echocardiography for indicating therapeutic effect of sildenafil are not fully understood. Therefore, this study aimed to investigate the potential effects of sildenafil on progressive PH. Several echocardiographic parameters were investigated for their prediction of the cardiovascular effects of sildenafil.

This study was approved by the Tokyo University of Agriculture and Technology (Approval number 24–53). Rats were managed and cared for in accordance with the standards established by the Tokyo University of Agriculture and Technology, and described in its Guide for the Care and Use of Laboratory Animals.

A total of 24, male 12-week-old Sprague-Dawley rats were purchased from Charles River Laboratories (Yokohama, Japan). They were kept in a constant temperature room, with a daily constant 12/12-hr light/dark ratio. Monocrotaline (MCT, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 1N HCl, and the pH was adjusted to 7.4 with 1N NaOH [1, 27]. The solution was randomly administered to 18 rats as a single subcutaneous injection (30 mg/kg) at a volume of 3 ml/kg, and an equal volume of saline was administered to another six rats (saline group, n=6).

MCT-induced PH rats (n=18) were randomly assigned to three groups. The MCT-injected group (MCT group, n=6) was the placebo drug group. In the early-phase sildenafil treatment group (E group, n=6), sildenafil (10 mg/kg, Kamagra, Ajanta Pharma India Co., Ltd., Mumbai, India) was initiated immediately after MCT injection, for a maximum duration of 6 weeks. In the late-phase sildenafil treatment group (L group, n=6), sildenafil (10 mg/kg) was initiated 4 weeks after MCT injection, for a maximum duration of 2 weeks. The medication period of the late-phase treatment was determined by an increase in right ventricular systolic pressure (RVSP) after MCT injection at week 4. All of the rats were trained to eat margarine, which served as the vehicle for sildenafil. All of the sildenafil dosage was consumed.

Echocardiographic tests and hemodynamic examinations were performed in each group on day 42 (saline group, n=6; MCT group, n=6; E group, n=6; and L group, n=6).
After the rats were anesthetized (isoflurane 1.0% in 1:1 O2/air mix; DS Pharma Animal Health Co., Ltd., Osaka, Japan), their chests were shaved and transthoracic echocardiography was performed. Two-dimensional echocardiography, pulsed Doppler and tissue Doppler imaging were performed using the ProSound SSD α-10 (Hitachi-Aloka Medical, Ltd., Tokyo, Japan) with a 10.0-MHz sector transducer. Sweep speed during the Doppler and M-mode recordings was set to 150–200 mm/sec. An electrocardiogram was recorded by ecocardiography measurement. All of the echocardiographic recordings were stored on an internal hard drive of the echocardiography unit and transmitted to the DICOM server online (Image ONE Co., Ltd, Tokyo, Japan). The following echocardiographic parameters were measured. The eccentricity index (EI), defined as the ratio of the length of two perpendicular minor-axis diameters, one of which bisects and is perpendicular to the interventricular septum, was obtained at end-systole [17]. Tricuspid annular plane systolic excursion (TAPSE) was measured by M-mode. An M-mode cursor was oriented to the junction of the tricuspid valve plane and the right ventricular free wall using the apical four-chamber view to measure TAPSE [12]. The tricuspid annular systolic velocity (Sm) was acquired in apical four-chamber views at the right ventricular free wall using tissue Doppler imaging [23]. Using the Doppler signals of the tricuspid inflow, peak trans-tricuspid early diastolic wave (E wave) velocity and active filling with atrial systolic (A wave) velocity were measured, and E/A was calculated [8]. The same tricuspid inflow tract view on the four-chamber view was used to evaluate lateral tricuspid annular velocity (Em) with tissue Doppler imaging, using the same sample volume. Using the Doppler signals of tricuspid inflow, E wave velocity was measured, and E/Em was calculated.

After echocardiographic tests, a pressure-sensing catheter (Codman MicroSensor; Codman & Shurtleff, Inc., Raynham, MA, U.S.A.) was inserted from the right jugular vein into the right ventricle under anesthesia for measurement of RVSP. The results are expressed as mean values ± standard deviation (SD). The statistical significance of differences in echocardiographic parameters, heart rate and body weight were estimated using one-way analysis of variance for factorial measures with Tukey’s multiple comparisons for post-hoc analysis. A significant difference was defined as P<0.05. Statistical analyses were performed using statistical software (Prism v. 6.0, GraphPad Software, Inc., La Jolla, CA, U.S.A.).

RVSP in the MCT group was significantly higher than that in the saline group (P<0.001). However, RVSP in the sildenafil-treated groups (P<0.001) was lower than that in the MCT group (Table 1).

The EI in the MCT group was higher than that in the saline group (P<0.001). The EI in the sildenafil-treated groups was reduced compared with that in the MCT group (both P<0.001, Table 1). The EI was not significantly different between the E and L groups (Table 1).

E/A in the MCT group was higher than that in the saline group (P<0.05). E/A in the E group (P<0.01) and the L group (P<0.001) was lower than that in the MCT group (Table 1).

E/Em in the MCT group was higher than that in the saline group (P<0.001). E/Em in the E group (P<0.001) and the L group (P<0.001) was lower than that in the MCT group (Table 1).

TAPSE in the MCT group was lower than that in the saline group (P<0.001). TAPSE in the sildenafil-treated groups was also lower than that in the saline group (both P<0.001, Table 1).

Sm in the MCT group was lower than that in the saline group (P<0.001). Sm in the sildenafil-treated groups was also lower than that in the saline group (both P<0.001, Table 1).

The main finding of the present study was that the reduction in RVSP with PH was not significantly different between early- and late-phase treatment of sildenafil. Early-phase treatment of sildenafil did not show a marked improvement in PH compared with late-phase treatment. Late-phase treatment of sildenafil was able to prevent progression of disease in rats with MCT-induced PH. This was associated with a reduction in right ventricular afterload, accompanied by a marked reduction in RVSP. We also found that EI and E/A were useful parameters for indicating improvement of right ventricular function after administration of sildenafil. Additionally, administration of sildenafil did not preserve systolic

### Table 1. Summary of right ventricle systolic pressure and echocardiographic parameters

| Parameter | Saline group (n=6) | MCT group (n=6) | E group (n=6) | L group (n=6) |
|-----------|--------------------|----------------|---------------|---------------|
| RVSP (mmHg) | 30 ± 3 | 61 ± 16*** | 30 ± 7††† | 27 ± 4††† |
| HR (min) | 335 ± 41 | 341 ± 29 | 282 ± 28*** ††† | 278 ± 45*** ††† |
| BW (g) | 357.8 ± 73.8 | 356.5 ± 96.5 | 248.3 ± 12.8* | 216.5 ± 17.3*** |
| EI | 0.9 ± 0.2 | 1.3 ± 0.4*** | 1.0 ± 0.1††† | 0.9 ± 0.1††† |
| E/A | 0.77 ± 0.39 | 0.96 ± 0.28* | 0.71 ± 0.14††† | 0.68 ± 0.11††† |
| E/Em | 6.53 ± 3.81 | 12.22 ± 7.73*** | 7.24 ± 1.53††† | 6.20 ± 2.08††† |
| TAPSE (mm) | 2.8 ± 1.0 | 1.4 ± 0.4*** | 1.9 ± 0.5*** | 1.8 ± 0.6*** |
| Sm (cm/s) | 7.6 ± 1.6 | 5.0 ± 1.7*** | 5.8 ± 1.0*** | 4.8 ± 1.5*** |

The data represent means ± SD. *P<0.05, **P<0.01, ***P<0.001 vs saline group; †P<0.05, ††P<0.01, †††P<0.001 vs MCT. a) MCT, monocrotaline; b) E, early-phase treatment; c) L, late-phase treatment; d) RVSP, right ventricle systolic pressure; e) HR, heart rate; f) BW, body weight; g) EI, Eccentricity index; h) E/A, the ratio of peak trans-tricuspid early diastolic wave (E wave) velocity to active filling with atrial systolic (A wave) velocity; i) E/Em, the ratio of peak trans-tricuspid early diastolic wave (E wave) velocity to lateral tricuspid annular velocity (Em) with tissue Doppler imaging; j) TAPSE, tricuspid annular plane systolic excursion; k) Sm, lateral tricuspid annulus velocity in the systolic period.
function parameters, such as TAPSE or Sm.

Previous studies have shown that sildenafil reduces RVSP in rats with MCT-induced PH [9, 26]. However, few studies have investigated the utility of echocardiography for evaluating therapeutic improvement and the optimal time for initiation of sildenafil therapy. In the present study, RVSP was significantly decreased in rats that were administered sildenafil. E1 and E/A values indicated that sildenafil prevented right ventricular dysfunction. Sildenafil is an inhibitor of PDE5, which in turn leads to dilatation of pulmonary arteries [5]. Inhibition of PDE5 results in reduction of RVSP, and the beneficial effect of sildenafil is usually attributed to a decrease in pulmonary vascular resistance [3, 16, 20]. Vasodilatory effects of sildenafil have previously been evaluated [16, 22]. Reducing afterload with administration of sildenafil results in a reduction in RVSP and improvement in diastolic function parameters of E/A and EI. Our results suggest that sildenafil has a beneficial effect on RVSP in rats with MCT-induced PH in accordance with previous studies [6, 9, 25]. However, the beneficial effects of sildenafil showed no significant difference between early- and late-phase treatment of sildenafil.

In the E group, RVSP and E/A were significantly lower than those in the MCT group. However, there was no significant difference in RVSP and E/A between early- and late-phase treatment of sildenafil. This result suggested that late-phase treatment of sildenafil improved diastolic function as much as early-phase treatment of sildenafil. Additionally, early-phase treatment did not show any more potential for improvement of PH compared with late-phase treatment. These results are consistent with previous studies [20, 25].

In the present study, the EI showed that sildenafil prevented flattening of the interventricular septum because of right ventricular pressure overload. The EI might be affected by a reduction in afterload following administration of sildenafil. The EI is well correlated with RVSP [17]. Therefore, the EI indicated a reduction in RVSP after administration of sildenafil. The EI is a useful parameter to indicate the vasodilatory effect of sildenafil.

Sildenafil improved diastolic function parameters, such as E/A or E/Em, in the present study. A reduction in right ventricular afterload after administration of sildenafil improves diastolic function parameters [13]. Vasodilatory effects of sildenafil might decrease not only afterload, but also preload. Therefore, diastolic function parameters, such as E/A or E/Em, indicate the therapeutic effect of sildenafil.

TAPSE and Sm are parameters of assessment of right ventricular systolic function [23]. TAPSE and Sm in the MCT/sildenafil groups were not significantly different compared with those in the MCT group. Sildenafil does not increase cyclic AMP and lacks inotropic effects [20]. The results of TAPSE and Sm support the lack of an inotropic effect of sildenafil. TAPSE indicates right ventricular systolic function, which is a load-dependent index and is well correlated with the right ventricular ejection fraction [28]. Sm is an index of right ventricular systolic function and is well correlated with the right ventricular ejection fraction as derived from magnetic resonance imaging [29]. Previous studies have shown that sildenafil has little effect on TAPSE, supporting the notion that sildenafil does not have intrinsic effects on the myocardium [2, 20]. Additionally, systolic function parameters do not indicate any vasodilatory effect of sildenafil, but suggest the lack of an inotropic effect of sildenafil [2, 20]. These findings are consistent with another previous study, which showed that sildenafil has slightly positive intrinsic inotropic effects [19]. An improvement in systolic function parameters after administration of sildenafil was not observed in the present study, which is in agreement with a previous study [20]. Sildenafil showed minimal or no inotropic effects on the right ventricle with regard to improvement of systolic function parameters. In the present study, diastolic parameters were more useful parameter for showing vasodilatory effects of sildenafil compared with systolic function parameters.

There are limitations to the present study. First, there was a small sample size, which might have resulted in a type II error regarding the minimal intrinsic positive inotropic effects of sildenafil. Second, the relationships between echo parameters and biomarkers were not evaluated. Therefore, the neurohormonal responses in right ventricular cardiac function when there is a decrease in blood pressure after administration of sildenafil are unknown.

CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

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