Acute and Chronic Effects of T-1032, a Novel Selective Phosphodiesterase Type 5 Inhibitor, on Monocrotaline-Induced Pulmonary Hypertension in Rats

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Pulmonary hypertension (PH) is a fatal disease, characterized by the progressive elevation of pulmonary arterial resistance. Although the causes of this disorder are complicated, abnormal vasoconstriction appears to be a primary factor in the development of PH.1,2)

Although some kinds of vasodilators, such as calcium blockers and prostacyclin, have been used in the treatment of PH,3,4) they cause several side effects due to systemic vasodilation. Recent studies have demonstrated that nitric oxide inhalation produces selective pulmonary vasodilation without affecting systemic blood pressure in patients with PH.5,6) However, since the safety of nitric oxide inhalation has not been established,7) its doses have been restricted.

Phosphodiesterases (PDEs) are enzymes, which break down cAMP and/or cGMP, and they play a key role in the regulation of intracellular cyclic nucleotide levels. A phosphodiesterase type 5 (PDE5) is classified as cGMP-bind, cGMP-specific phosphodiesterase.8,9) PDE5 is abundantly expressed in some specific tissues, including the lung and pulmonary artery.10–12) Therefore, the inhibition of PDE5 is considered to produce an increase in cGMP levels in the lung and pulmonary artery. It has been reported that PDE5 inhibitors such as zaprinast, E-4021 and sildenafil preferably dilate the pulmonary artery in vivo and in vitro experimental conditions.13–18)

T-1032 (methyl 2-(4-amino phenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethyl)oxy)-4-(3,4,5-trimethoxy-phenyl)-3-isoquinoline carboxylate sulfate), a novel selective phosphodiesterase type 5 (PDE5) inhibitor, and evaluated the chronic effect of T-1032 on cardiac remodeling and its related death in monocrotaline (MCT)-induced pulmonary hypertensive rats. T-1032 (1, 10, 100 μg/kg, i.v.) significantly reduced mean arterial pressure (MAP) and right ventricular systolic pressure (RVSP) without a change in heart rate. The change in RVSP was more potent than that in MAP with 1 μg/kg T-1032 treatment (RVSP: −8.2±1.2%, mean arterial pressure: −5.7±1.2%), and reductions in RVSP and MAP reached a peak at doses of 1 and 10 μg/kg, respectively. In contrast, nitroglycerin (0.1, 1, 10 μg/kg, i.v.) and beraprost (0.1, 1 μg/kg, i.v.) did not cause a selective reduction in RVSP at any dose. When T-1032 (300 ppm in diet) was chronically administered, it delayed the death, and significantly suppressed right ventricular remodeling (T-1032-treated: 0.318±0.021 g, control: 0.401±0.013 g, p<0.05). Our present results suggest that T-1032 selectively reduces RVSP, and resulting in the suppression of right ventricular remodeling with a delay of the death in MCT-induced pulmonary hypertensive rats.

Key words phosphodiesterase type 5; monocrotaline rat; pulmonary hypertension

MATERIAL AND METHODS

This study was approved by the Animal Research Committee of Tanabe Seiyaku Co., Ltd.

Animal Preparation Monocrotaline (MCT, 70 mg/kg) was subcutaneously administered in male Wistar rats weighing 49–107 g, as described previously.23)

Acute Hemodynamic Effects in MCT Rats Twenty-six days after MCT (70 mg/kg) or vehicle injection, all survival rats (MCT group: N=41, vehicle group: N=10) were anesthetized with thiobutabarbitual sodium (100 mg/kg, i.p.). After a tracheotomy, the polyethylene tube was inserted into the trachea to facilitate ventilation. A polyethylene tube (PE50) was cannulated into the following vessels: the femoral artery for measuring mean arterial pressure, right ventricular via a right jugular vein for measuring right ventricular systolic pressure, and a femoral vein for the administration of test drugs. The catheters inserted into the femoral artery or jugular vein were filled with heparinized saline. Mean arterial pressure (MAP) and right ventricular systolic pressure (RVSP) were measured by a pressure transducer (TB-400, with PH.15,15,18) However, only a few studies have reported whether the chronic treatment is effective for improving cardiac remodeling and its related death in an animal model with PH.22) In this study, we first examined the hemodynamic properties of T-1032 in MCT-induced PH rats, and we next evaluated the chronic effect of T-1032 on cardiac remodeling and its related death in MCT-induced PH rats.

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Nihon Kohden, Tokyo, Japan). Heart rate (HR) was determined from the recorded arterial pulsation (AT-601G, Nihon Kohden, Tokyo, Japan). These parameters were recorded on a polygraph (WR3300, Graph Tec, Tokyo, Japan).

After recovery from the cannulation procedure, baseline hemodynamics were measured. Then all rats treated with MCT were randomly divided into 4 groups (vehicle-, T-1032-, nitroglycerin-, and beraprost-treated group) by baseline MAP and RVSP values. In the present study, we used 100 μg/kg T-1032, 10 μg/kg nitroglycerin, and 1 μg/kg beraprost as maximal doses. These are comparable doses that caused approximately 15 mmHg reduction in systemic arterial pressure. In addition, therapeutic or pharmacologically intravenous doses of T-1032, nitroglycerin and beraprost were reported to be less than 30 μg/kg (Yano et al., 2000, JJP Suppl.), 32 μg/kg23 and 0.3 μg/kg26 in rats, respectively. Therefore, the doses we used in this study are reasonable to compare the effectiveness in the chronic PH rats. Vehicle, T-1032 (1, 10, 100 μg/kg), nitroglycerin (0.1, 1, 10 μg/kg) and beraprost (0.1, 1 μg/kg) were intravenously administered, with cumulative injections at 10-min intervals. Each rat received one drug with a dose-escalating procedure. After the highest dose of each drug was administered, all rats were sacrificed with high potassium solution under deep anesthesia.

**Chronic Effect of T-1032 on MCT-Induced Right Ventricle Remodeling**

All rats were divided into three groups at random: normal (N=12), MCT-treated control (N=16), and an MCT/T-1032 (N=15) treated group, then they were injected with MCT (70 mg/kg, s.c.). T-1032 (300 ppm) was administered by mixing it with a daily food from the time of MCT injection to 24 h before sacrifice. The calculated dose of T-1032 in this study was 300 ppm (approximately 25 mg/kg/d).

The degree of right ventricular remodeling was assessed as previously described.23 At the end of the experiment, all rats were sacrificed, and the heart and the lung were dissected. Atria were trimmed off, and the right ventricular free wall was carefully separated from the left ventricle plus septum, then the wet weight of right ventricular, left ventricle plus septum and lung were measured.

**Drugs**

MCT (Wako, Tokyo, Japan) was dissolved in 1 M HCl at a concentration of 100 mg/ml, then neutralized with 1 M NaOH and diluted with distilled water.23 T-1032 and beraprost was synthesized at Tanabe Seiyaku Co., Ltd., and nitroglycerin (Milislole injection) was purchased from Nihon Kayaku Co., Ltd. T-1032 was dissolved in 0.001 N HCl/saline, and nitroglycerin and beraprost were dissolved in saline. The vehicle was constructed in 0.001 N HCl/saline. The injection volume of each was 0.05 ml/kg.

**Statistical Analysis**

Data is shown as native values or percent changes from baseline values. Both values represent the mean±S.E.M. Student’s or Welch’s t-tests were performed to compare baseline hemodynamics and heart- and lung weight. The values of hemodynamic parameters from baseline values were analyzed by a randomized complete block design, followed by Dunnett’s method for multiple comparisons. Survival curves were analyzed by a generalized Wilcoxon test. All data were analyzed by Stat View ver 5.0 (SAS Institute, Japan).

**RESULTS**

Acute Effects of T-1032, Nitroglycerin and Beraprost on Hemodynamics in MCT-Induced Pulmonary Hypertensive Rats

Table 1 shows the baseline values of hemodynamics and organ weight in MCT- and normal rats. RVSP, RV weight, RV/LV plus septum (RV/LV+SEP) ratio, and lung weight were increased in MCT-treated rats (p<0.05 vs. vehicle-treated rats, t-test). MCT was decreased modestly in MCT-treated rats (p<0.05 vs. vehicle-treated rats, t-test). There were no significant differences in HR or LV weight between MCT- and vehicle-treated rats.

Figure 1 shows the effects of intravenous administrations of T-1032 on MAP, HR and RVSP in MCT-induced PH rats. T-1032 at doses of 1—100 μg/kg and 10—100 μg/kg reduced RVSP and MAP, respectively. The change in RVSP was more potent than that of MAP at a dose of 1 μg/kg of T-1032. T-1032 did not show any effects on HR at any doses (p>0.05, Dunnett’s test).

Figure 2 shows the dose-response relationships of maximal changes in MAP and RVSP in MCT-induced PH rats. T-1032, nitroglycerin and beraprost all reduced both MAP and RVSP. However, a difference in dose-dependent changes of MAP and RVSP was seen among these compounds. T-1032 at doses of 1—100 μg/kg showed only a slack reduction of MAP and RVSP in a dose-dependent manner. In contrast, both nitroglycerin at a dose of 10 μg/kg and beraprost at a dose of 1 μg/kg showed a sudden reduction of MAP and RVSP. When T-1032 (1 μg/kg), nitroglycerin (10 μg/kg) and beraprost (1 μg/kg) showed a similar reduction of RVSP (T-1032: −8.3±1.2%, nitroglycerin: −6.4±2.0%, beraprost: −8.7±2.0%), the hypotensive effect of T-1032 was relatively weak compared with that of beraprost, but not that of nitroglycerin (T-1032: −5.7±1.2%, nitroglycerin: −13.0±5.5% p=0.07 vs. T-1032, beraprost: −17.9±3.1% p=0.015 vs. T-1032). The vehicle did not show any effects on MAP or RVSP (2.5±1.1% in RVSP and −1.3±0.7% in MAP). T-1032, nitroglycerin and beraprost did not show any effects on HR (p>0.05, Dunnett’s test) at any doses (Table 2).

Chronic Effects of T-1032 on Right Ventricle Hyper-
trophy and Survival in MCT-Induced Pulmonary Hypertensive Rats

Figure 3 shows Kaplan–Meier survival curves in MCT-induced PH rats. When T-1032 was chronically administered by mixing in a daily diet (300 ppm or approximately 25 mg/kg/d, for 26 d), the survival rate (26 d after MCT injection) in normal, MCT-treated and MCT/T-1032-treated rats were 100% (12/12), 43.8% (7/16), 73.3% (10/15), respectively. The final survival rate was higher in the MCT/T-1032-treated rats than in the MCT-treated rats, although it was not a significant increase ($p=0.081$, generalized Wilcoxon test).

Table 3 shows the effect of T-1032 on RV, LV+SEP weight, RV/LV+SEP ratio and lung weight in MCT-induced PH rats. The RV weight, RV/LV+SEP ratio and lung weight were increased in MCT-treated rats as compared with normal rats ($p<0.05$, $t$-test). The RV weight and RV/LV+SEP ratio in MCT/T-1032 treated rats were significantly low compared with those in MCT-treated rats ($p<0.05$, $t$-test).

**DISCUSSION**

In the present study, T-1032 showed a selective reduction of RVSP over MAP compared with nitroglycerin and beraprost in MCT-induced PH rats. Further, the chronic admininistration of T-1032 at 10 μg/kg suspended the pulmonary hypertension.

Table 2. Effect of Intravenously Administered T-1032, NTG and Beraprost on Heart Rate in Monocrotaline-Induced Pulmonary Hypertensive Rats

<table>
<thead>
<tr>
<th>Baseline values (beats/min)</th>
<th>Peak responses</th>
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</thead>
<tbody>
<tr>
<td>T-1032</td>
<td></td>
</tr>
<tr>
<td>100 μg/kg</td>
<td>364±20</td>
</tr>
<tr>
<td>10</td>
<td>365±20</td>
</tr>
<tr>
<td>1</td>
<td>366±20</td>
</tr>
<tr>
<td>NTG</td>
<td>433±16</td>
</tr>
<tr>
<td>0.1</td>
<td>432±15</td>
</tr>
<tr>
<td>1</td>
<td>435±15</td>
</tr>
<tr>
<td>10 μg/kg</td>
<td>438±12</td>
</tr>
<tr>
<td>Beraprost</td>
<td>396±9</td>
</tr>
<tr>
<td>0.1</td>
<td>399±8</td>
</tr>
<tr>
<td>1 μg/kg</td>
<td>399±7</td>
</tr>
</tbody>
</table>

Data represents percent changes from baseline values. There were no significant statistical differences among T-1032, NTG and beraprost ($p>0.05$).

![Fig. 1. Effect of Intravenously Administered T-1032 on MAP, HR and RVSP in MCT-Induced PH Rats](image1)

**Fig. 1. Effect of Intravenously Administered T-1032 on MAP, HR and RVSP in MCT-Induced PH Rats**

T-1032 ($N=15$) was administered cumulatively at 10-min intervals. Data represents native values (mean±S.E.M). Each arrow shows the timing of administration. $\#p<0.05$ vs. baseline values.

![Fig. 2. Dose–Response Relationships of Intravenously Administered T-1032 (Left Panel, $N=15$), Nitroglycerin (Middle Panel, $N=6$) and Beraprost (Right Panel, $N=9$) in MCT-Induced PH Rats](image2)

**Fig. 2. Dose–Response Relationships of Intravenously Administered T-1032 (Left Panel, $N=15$), Nitroglycerin (Middle Panel, $N=6$) and Beraprost (Right Panel, $N=9$) in MCT-Induced PH Rats**

All tested compounds were administered cumulatively at 10-min intervals. Vehicle was constructed from 0.001 N HCl/saline. Data represents percent changes from baseline values (mean±S.E.M). $\#p<0.05$ vs. vehicle in MAP; *$p<0.05$ vs. vehicle in RVSP.
Inhibitors also have pulmonary selectivity in the model of PH. These observations support that selective PDE5 inhibitors such as T-1032 show pulmonary selectivity in PH. When we determined the pulmonary selectivity using RVSP and MAP, other hemodynamic changes such as cardiac output and contraction should be considered, because these parameters influence RVSP. Therefore, further studies on hemodynamics should be required among these three compounds.

A major concern about instituting vasodilator therapy in patients with PH is the risk of severe hypotension due to systemic vasodilation. Therefore, it is useful for vasodilator therapy for PH to reduce PAP without affecting systemic pressure. In this regard, T-1032 has a beneficial profile because it showed a potent reduction in RVSP with a modest reduction of MAP in MCT-induced PH rats.

In the chronic study, the administration of T-1032 tended to decrease mortality compared with the MCT control. Kaplan–Meier survival curves revealed that T-1032 delayed the event (death). Comparative analysis of the survival curves of MCT-treated and MCT/T-1032-treated rats did not show a statistically significant difference \((p=0.081)\). These data suggest that T-1032 delays the development of fatal PH. T-1032 suppressed the MCT-induced increase in RV weight as well as the RV/LV+SEP ratio, which indicated that T-1032 suppressed the RV remodeling. It has been reported that the degree of RV remodeling and the elevation of PAP shows a positive correlation in MCT-induced PH. Therefore, the reduction of PAP by T-1032 treatment resulted in the suppression of RV remodeling. In this study, we used a relatively high dose of MCT (70 mg/kg, s.c.) to induce PH. This dose has been reported to cause a severe lung injury and its related death in a short period. Indeed, several rats died at the early stage (9—13 d after MCT injection), which is in accordance with a previous study.

Nitric oxide inhalation is clinically used to treat PH. The major benefit of nitric oxide inhalation is to reduce PAP without affecting systemic arterial pressure. In a recent study, it has been reported that sildenafil, a potent and selective PDE5 inhibitor, potentiates the reduction of PAP induced by nitric oxide inhalation. However, recent findings have demonstrated that pulmonary vasodilation did not cause the amelioration of chronic PH in animal studies. It has been reported that inhaled nitric oxide did not prevent pulmonary vascular remodeling in MCT-induced PH rats.

Among the three drugs. In our preliminary study, T-1032 reduced PAP more selectively than nitroglycerin and beraprost in hypoxia-induced PH in anesthetized dogs. Other PDE5 inhibitors also have pulmonary selectivity in the model of PH. These observations support that selective PDE5 inhibitors such as T-1032 show pulmonary selectivity in PH. When we determined the pulmonary selectivity using RVSP and MAP, other hemodynamic changes such as cardiac output and contraction should be considered, because these parameters influence RVSP. Therefore, further studies on hemodynamics should be required among these three compounds.

In the acute study, the intravenous administration of T-1032 reduced PAP at doses of 1—100 μg/kg. A sustained and significant reduction of RVSP was apparent at 1 μg/kg, and RVSP reached a maximal reduction at the dose of 10 μg/kg. In contrast, the reduction of MAP was apparent at 10 μg/kg, and it reached almost maximal reduction at a dose of 10 or 100 μg/kg. These results suggest that T-1032 preferentially reduces RVSP compared with MAP.

In the present study, we also examined the effect of nitroglycerin and beraprost, both of which dilate the pulmonary vessels. T-1032 showed a slight reduction of MAP and RVSP. In contrast, nitroglycerin and beraprost showed a sudden reduction of MAP and RVSP. Moreover, when T-1032, nitroglycerin and beraprost showed a similar reduction of RVSP, the hypotensive effect of T-1032 was significantly weak compared with that of beraprost \((p=0.015)\), but not that of nitroglycerin \((p=0.07)\). Therefore, the hemodynamic action of T-1032 in MCT rats seemed to be the most selective for RVSP as the R V/L V ratio.
REFERENCES