Institutional report - Experimental

Oral sildenafil prevents and reverses the development of pulmonary hypertension in monocrotaline-treated rats

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Abstract

The endothelin system plays an important role in the development of pulmonary hypertension. Several studies have suggested that interfering with the function of the endothelin system will be helpful in pulmonary hypertension treatment. In the present study, we investigated the preventive and therapeutic effects of sildenafil on pulmonary hypertension in monocrotaline-treated rats. In the preventive study, the level of mean pulmonary arterial pressure, right ventricular divide, left ventricular and septum, small pulmonary arterial morphologic and elastic fiber changes were highly improved in the treated group (P<0.05). The expressions of endothelin-1 A type receptors on small pulmonary arterial hypertension were significantly reduced in the sildenafil-treated group (P<0.05). The ET-1 level in plasma was increased in the sildenafil-treated group, but did not reach significance. Emphysema, interstitial pneumonia were significantly improved in the sildenafil-treated group. The same findings were also observed in the therapeutic study. The present results suggest that sildenafil can prevent and reverse the development of pulmonary hypertension in monocrotaline-treated rats by improving the function of endothelin system in pulmonary arteries.

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Keywords: Endothelium; Experimental surgery; Myocardial remodeling; Pulmonary arteries; Vascular tone and reactivity

1. Introduction

Pulmonary arterial hypertension (PH) is a severe disease characterized by vasoconstriction, intimal lesions, medial hypertrophy, and adventitial thickening of the precapillary pulmonary arteries. The progressive pulmonary hypertension leads to increased afterload, right ventricular hypertrophy, right heart failure and death. In primary pulmonary arterial hypertension the median survival is considered to be 2.8 years from the time of diagnosis. Therefore, a novel therapeutic strategy for pulmonary hypertension is desirable [1].

Treatment for pulmonary arterial hypertension was revolutionized in 1996 with the widespread introduction of prostacyclin, but some patients still progress to lung transplant or death despite state-of-the-art medical management. This monograph highlights some of the recent pre-clinical work that holds exciting potential for PH treatment [2].

Although the precise mechanism of pulmonary arterial hypertension is unclear, release by endothelial cells of an endogenous substance that acts on a target receptor has gained acceptance. Endothelin, a product of endothelial cells, has been reported to contribute to pulmonary arterial hypertension. Endothelial cells of the pulmonary microvasculature are frequently exposed to extraneous substances such as drugs, toxins or abnormal metabolic products, and injury to these cells can result in pathological changes in the alveoli [3]. Monocrotaline injures the endothelial cells of the pulmonary blood vessels, causing pulmonary hypertension and interstitial pulmonary fibrosis [4]. This substance also induces the proliferation of muscular intimal cells in arterioles and fibroblasts in alveolar walls at the capillary level. Various growth factors are considered to be involved in cell proliferation following injury to endothelial cells. Among them, the potent vasoconstrictor peptide endothelin-1, comprising 21 amino acids, induces the proliferation and migration of smooth muscle cells and fibroblasts [5] and its association with pulmonary hypertension has been indicated. Endothelin-1 was discovered in a culture medium of endothelial cells from porcine aorta [6]. It is known to act through two types of receptors, ETα-R and endothelin B type receptors [7]. Plasma endothelin-1 levels are reportedly high in pulmonary hypertension patients [8]. These data suggest that endogenous endothelin-1 is involved in the progression of pulmonary hypertension, and reduction of endothelin-1 or downregulation of endothelin-1 A type receptors are expected to prove effective in the treatment of this condition.

In the present study, we investigated the effects of endothelin-1 on pulmonary arterial hypertension and the possible protective role of sildenafil. The study was firstly undertaken to determine the ability of pulmonary arterial hypertension on pulmonary and heart remodeling. Secondly, the study was designed to determine the preventive and therapeutic effects of sildenafil by measuring not only...
pulmonary arterial pressure and right ventricular hypertrophy but also histopathological changes and endothelin-1 level in plasma.

2. Methods

2.1. Animals and materials

Male Sprague Dawley rats (eight weeks old) were obtained from the Experimental Animal Center Medical College of Southeast University (Nanjing, China). Sildenafil was purchased from Pfizer Inc. (Pfizer, USA). Sildenafil was dissolved or suspended in 1% sodium chloride. Monocrotaline was purchased from Sigma (Sigma, USA). An ET RIA Kit was purchased from Beijing Huaying Biotechnology Institute. All other chemicals were of analytical grade. Monocrotaline was dissolved in alcohol and distilled water (1:5). Pulmonary hypertensive rats were prepared by injecting the monocrotaline at a subcutaneous dose of 60 mg·kg⁻¹ into the dorsum of the neck.

2.2. Experimental design

2.2.1. Protocol 1: Drug efficacy on pulmonary hypertension: preventive study

In the preventive study, 20 monocrotaline-treated rats were used. One day after the injection of monocrotaline, the animals were randomly divided into two sub-groups (n=10 each), and given a daily administration of either sodium chloride as the control group, or sildenafil (1.7 mg·kg⁻¹·d⁻¹) as the treated group for three weeks. The other ten rats were not injected monocrotaline and were given a daily administration of sodium chloride for three weeks as sham group. One day after the final drug administration, hemodynamic variables were measured in the same way as in the preventive study. Histopathological examinations were done in all of the rats which had been pre-treated in the same manner as in the preventive study. Two milliliters of blood were conserved (−70 °C) to detect plasma ET-1 levels.

2.3. Histopathological examination

Histopathological examination of tissue slides was performed at the Pathology Department of Zhongda Hospital (Nanjing, China). From the left lobe of lung, five regions, including the hilus and the two anterior and two posterior regions thereto, were cut in cross-section and stained with hematoxylin and eosin in the usual manner. Small pulmonary arterioles were found (diameter <100 μm) by an observer blinded to the experimental group. The percent wall thickness of muscular arteries was calculated for each category using the following formula and the mean value was obtained for each animal. Percent wall thickness (%) = [(cross-sectional area–luminal area)/cross-sectional area] × 100.

2.4. Immunohistochemistry examination

Immunohistochemical staining by streptavidin biotin complex method was performed for the sections of the left lung. We used the following commercially available antibodies at dilutions of 1:50 rabbit polyclonal anti-rat endothelin-1 A type receptors antibody (endothelin-1 A type receptors, lot AN-05; Peptide Peptide (C) NHNT ERSSH KDSMN, Alomone Labs, Israel). Control slides were treated with normal diluted rabbit serum. The slides were reviewed by a pathologist and were semiquantitatively graded according to the degrees of positivity of the immunoreactivity as 0 for absence of staining and 1+ for weak, 2+ for moderate, and 3+ for strong staining. The slides were compared with the respective control slides to exclude non-specific staining. The degrees of positivity were assessed for endothelia of the same peripheral pulmonary arteries in which the percent medial thickness was determined.

2.5. Plasma ET-1 level

We took blood samples from inferior vena cava immediately after measuring pulmonary artery pressure. The samples were put into Eppendorf tubes containing a final concentration of 300 kIU/ml of aprotinin and 2 mg/ml of EDTA·Na2. The samples were centrifuged for 15 min at 3000 rpm at 4 °C. The plasma was stored at −80 °C. Radioimmunology was performed for the determination of plasma ET-1 levels.
2.6. Data analysis

Data are expressed as the means ± S.D. Data were analyzed by the one-way analysis of variance (ANOVA) and Scheffe F-test. A P-value <0.05 was considered to be statistically significant.

3. Results

3.1. Protocol 1: Preventive effects of sildenafil in monocrotaline-treated rats: effect on hemodynamic variables, right ventricular hypertrophy, pulmonary vascular remodeling and histopathological changes in the lung

Compared to the sham group, the rats in the control group showed a marked increase in mean pulmonary arterial pressure at three weeks after monocrotaline injection, demonstrating the presence of pulmonary hypertension. These rats also showed increases in RV and RV/(LV+S) values, indicating marked right ventricular hypertrophy. Three-week administration of sildenafil (1.7 mg·kg⁻¹·d⁻¹) significantly suppressed the increases in mean pulmonary arterial pressure, RV and RV/(LV+S) (Table 1, Fig. 1).

The percent wall thickness (WT%) of small arteries in the control group rats receiving vehicle was greater than that in the sham group in all diameter categories, demonstrating the progression of pulmonary vascular remodeling. Administration of sildenafil significantly prevented these monocrotaline-induced increases in arterial medial thickness (Fig. 1).

On histopathological examination, emphysema was observed in six out of ten of the control group, whereas no such findings were noted in the sham or treated group (Table 2). Interstitial pneumonia, characterized by thickening of the alveolar septum and inflammatory cell infiltration into the thickened area, was observed more obviously in the control group than in the sham group (Table 2). The incidence of interstitial pneumonia in the treated group was significantly lower than those in the control group (Fig. 2).

3.1.1. Preventive effects of sildenafil in monocrotaline-treated rats: endothelin-1 A type receptors and plasma ET-1 levels

On immunohistochemical examination, the expression of endothelin-1 A type receptors on pulmonary small artery (diameter <100 μm) smooth muscular cell was upregulated in the control group. Three weeks after the administration of sildenafil, the expression was significantly downregulated. In the treated group, the plasma ET-1 levels were higher than those in the control group, but did not reach significance (Table 1, Fig. 1).

3.2. Protocol 2: Therapeutic effects of sildenafil in pulmonary hypertension rats: effect on hemodynamic variables, right ventricular hypertrophy, pulmonary vascular remodeling and histopathological changes in the lung

Mean pulmonary arterial pressure was also observed in the control group three weeks after injection. In compari-

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>RV (mg)</th>
<th>LV+S (mg)</th>
<th>Mean arterial blood pressure (mmHg)</th>
<th>ET-1 (pg·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>140±4</td>
<td>519±11</td>
<td>6.17±0.95</td>
<td>52.55±4.35</td>
</tr>
<tr>
<td>Control group</td>
<td>198±7*</td>
<td>484±10*</td>
<td>15.03±0.13</td>
<td>71.56±1.03*</td>
</tr>
<tr>
<td>Treated group</td>
<td>162±10**</td>
<td>492±10**</td>
<td>8.18±1.61**</td>
<td>83.87±5.99***</td>
</tr>
</tbody>
</table>

Following subcutaneous administration of monocrotaline, sildenafil was administered once daily for three weeks. Values represent the mean ± S.E.M. (n=10). RV = right ventricle; LV+S = left ventricle with septum. The doses of sildenafil were 1.7 mg·kg⁻¹·d⁻¹. *P<0.01 compared with the sham group. **P<0.05 compared with the control group. ***P<0.01 compared with the sham group.
Table 2
Histopathological findings in the lung in monocrotaline-treated rats in the preventive study

<table>
<thead>
<tr>
<th>Findings</th>
<th>Group grade</th>
<th>Sham (n = 10)</th>
<th>Control group (n = 10)</th>
<th>Treated group (n = 10)</th>
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<td></td>
<td></td>
<td>10</td>
<td>4*</td>
<td>10*</td>
</tr>
<tr>
<td>Emphysema</td>
<td>1+</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2+</td>
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<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>1+</td>
<td>10</td>
<td>7**</td>
<td>7**</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

(–) Negative, (1+) mild, (2+) moderate, (3+) severe. *P < 0.01 compared with the sham group. **P < 0.05 compared with the control group by χ² test.

Fig. 2. Effects of sildenafil on lung vascular morphology in monocrotaline-treated rats in preventive study. Three weeks after subcutaneous administration of monocrotaline, sildenafil was administered once daily for three weeks. Lung and pulmonary arterioles were stained with hematoxylin and eosin (HE × 50). (a) Sham rats, (b) control rats, (c) treated rats.

3.2.1. Therapeutic effects of sildenafil in pulmonary hypertension rats: endothelin-1 A type receptors and plasma ET-1 levels

On immunohistochemical examination, the expression of endothelin-1 A type receptors on pulmonary small artery (diameter < 100 μm) smooth muscular cell was upregulated in the control group. Three weeks after administration of sildenafil, the expression of endothelin-1 A type receptors was significantly downregulated in the treated group, the plasma ET-1 levels were higher than those in the control group, but did not reach significance (Table 3, Fig. 3).

Table 3
The results monocrotaline-treated rats administered sildenafil in the therapeutic study (X ± S)

<table>
<thead>
<tr>
<th></th>
<th>RV (mg)</th>
<th>LV + S (mg)</th>
<th>Mean arterial blood pressure (mmHg)</th>
<th>ET-1 (pg·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>140 ± 4</td>
<td>519 ± 11</td>
<td>6.17 ± 2.24</td>
<td>52.55 ± 4.35</td>
</tr>
<tr>
<td>Control group</td>
<td>242 ± 6*</td>
<td>457 ± 13*</td>
<td>24.01 ± 1.96*</td>
<td>74.88 ± 2.17*</td>
</tr>
<tr>
<td>Treated group</td>
<td>207 ± 8**</td>
<td>493 ± 10**</td>
<td>8.03 ± 2.03**</td>
<td>85.62 ± 4.35***</td>
</tr>
</tbody>
</table>

Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for four weeks. Values represent the mean ± S.E.M. (n = 9–10). RV = right ventricle; LV + S = left ventricle with septum; the doses of sildenafil were 1.7 mg·kg⁻¹·d⁻¹. *P < 0.01 compared with the sham group. **P < 0.05 compared with the control group. ***P < 0.01 compared with the sham group.
In the present study, we investigated in detail the preventive and therapeutic effects of sildenafil on the development of pulmonary hypertension. A marked increase in pulmonary arterial pressure and right ventricular hypertrophy were observed in rats three weeks after administration of monocrotaline. These conditions closely resemble the corruption changes of pulmonary hypertension patients. Histopathological examination indicated the existence of arterial medial thickening in the small pulmonary artery and alveolar disorders such as emphysema or interstitial pneumonia, suggesting that, in addition to disorders in pulmonary circulation, respiratory function was also aggravated in this model.

In the preventive study, the arterial medial thickening in the pulmonary microvasculature and alveolar disorders were significantly suppressed in the sildenafil-treated rats as well as the increases in pulmonary arterial pressure and right ventricular hypertrophy. In addition to these preventive effects, the same improvements were also seen in the therapeutic study. These findings indicate that sildenafil prevented and deferred the development of pulmonary hypertension in monocrotaline-induced pulmonary hypertensive rats, and strongly suggest that sildenafil may ameliorate the condition of patients with pulmonary hypertension and cor pulmonale in clinical use.

In the preventive study, the plasma ET-1 levels in the control group were significantly higher than those in the sham group. At the same time, they were higher in the treated group than those in the control group, but did not reach significance (P > 0.05). Despite higher ET-1 in these rats, mPAP remained lower after sildenafil administration. It is likely that sildenafil's effect on vasodilatation is enough to overpower the vasoconstrictive signal elicited by high levels of ET-1. The same findings were also found in the therapeutic study. This finding suggests that sildenafil contributes to the increase in ET-1. Jefferson et al. [9] found that sildenafil has the effect to elevate intracellular cyclic guanosine monophosphate. The mechanism behind the increase in ET-1 is that sildenafil may upregulate expression of both inducible and endothelial isoforms of NO synthase. Salloum et al. [10] also described that sildenafil may upregulate expression of both inducible and endothelial isoforms of NO synthase. Sildenafil’s mechanism of vasodilatation attributes to the NO pathway (Pearl et al. [11]). Wiley and Davenport [12] reported that NO has been shown to compete with ET-1 for the endothelin receptor, which may cause signal termination and increase ET-1 levels.

Table 4
Histopathological findings in the lung in monocrotaline-treated rats in the therapeutic study

<table>
<thead>
<tr>
<th>Findings</th>
<th>Group grade</th>
<th>Sham (n=10)</th>
<th>Control group (n=8)</th>
<th>Treated group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emphysema</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>–</td>
<td></td>
<td>10</td>
<td>3</td>
<td>10*</td>
</tr>
<tr>
<td>1+</td>
<td></td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td></td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td></td>
<td>10</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>1+</td>
<td></td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2+</td>
<td></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3+</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(−) Negative, (1+) mild, (2+) moderate, (3+) severe. *P < 0.01 compared with the sham group.
by displacing off its receptor. NO has also been suggested to terminate the vasoconstrictive signal of ET-1 by interruption of downstream signaling processes not yet defined (Goligorsky et al. [13]). So, we can easily draw the conclusion that sildenafil may upregulate expression of NO, then induces the effects of vasodilatation.

In the preventive and therapeutic study, the expression of endothelin-1 A type receptors on pulmonary small artery (diameter < 100 μm) smooth muscular cell was upregulated in the control group by immunohistochemical examination. Three weeks after administration of sildenafil, the expression of endothelin-1 A type receptors was significantly down regulated. It indicates that sildenafil has the effect to downregulate expression of NO, another mechanism behind vasodilatation is that sildenafil may downregulate expression of endothelin-1 A type receptors.

Gillespie et al. [14] described pulmonary function in monocrotaline-treated rats. They demonstrated decreases in tidal volume, lung compliance and respiratory frequency. They also demonstrated decreased diffusion capacity of the lung. These changes indicate the impairment of gas exchange in the lungs and suggest the existence of interstitial and parenchymatous injury in the lung. Chen et al. [15] also described the impairment of endothelial cell and muscularization in pulmonary artery in monocrotaline-treated rats. In the present study, we demonstrated the ameliorative effects of sildenafil on lung interstitial and parenchyma by effecting the endothelin system. As far as we are aware, this is the first report to demonstrate the effectiveness of sildenafil on lung interstitial and parenchyma in monocrotaline-treated rats. The mechanism of preventive and therapeutic effect is that sildenafil inhibits the endothelin system and downregulates expression of endothelin-1 A type receptors.

5. Conclusion and perspectives

In conclusion, we have demonstrated that sildenafil effectively prevented and reversed the development of pulmonary hypertension, and reduced the pulmonary vascular remodeling and parenchymal injury in monocrotaline-treated rats. This effect was associated with a mild improvement in pulmonary arterial remodeling. These data strongly suggest that sildenafil may be clinically useful in patients with both primary and secondary pulmonary hypertension.

References