Therapeutic effects of DCDDP, a calcium channel blocker, on chronic pulmonary hypertension in rat

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PULMONARY HYPERTENSION (PH) has been widely investigated, but its pathogenesis and therapeutics are still not clear (8). In clinics, Ca^{2+} channel blockers are effective drugs for the acute PH and acute attack of the chronic PH. However, the Ca^{2+} antagonist is not satisfactory in the treatment of chronic PH because of the side effect of decreased systemic blood pressure and the limit for long-term administration (7). The inhalation of nitric oxide (NO) at a lower concentration can selectively relax pulmonary arteries and decrease pulmonary circulation pressure and resistance (9), but special instruments are needed.

Dimethyl 4-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (DCDDP) is one of the dihydropyridine calcium channel blockers. Mahmoudian and Richards (18) reported that DCDDP could relax ileal smooth muscle in preparations. Bossert and Vater (1) found that DCDDP could also relax systemic vascular smooth muscle and depress the systemic circulating pressure. However, the underlying mechanism of DCDDP on the systemic vascular smooth muscle is still unclear. We hypothesize that, during chronic PH, DCDDP could intensify relaxation of pulmonary vascular smooth muscle by blocking Ca^{2+} from entering the pulmonary vascular smooth muscle cells and reducing free radicals, thereby inhibiting the response of the pulmonary artery to the constricting factors. The present study was designed to explore the therapeutic effect and elucidate the mechanism on long-term use of DCDDP on chronic PH induced by monocrotaline (MCT) in the rat.

METHODS

Animals and treatment protocol. The experiments were performed on male Sprague-Dawley rats, weighing between 180 and 220 g at the age of 8 wk (from Laboratory Animal Center of Fourth Military Medical University, Xi'an, China). The animals were randomly divided into six groups. In the control (C) group (n = 10), the rats were treated with saline (single subcutaneous injection). In the MCT group (n = 10), the rats were injected subcutaneously with 60 mg/kg MCT once a day for 28 days. In the three DCDDP groups (n = 8 each), DCDDP was applied in 5 [low (DCDDP{5})], 50 [medium (DCDDP{50})], and 500 [high (DCDDP{500})] μg/kg doses [high (DCDDP{500})], respectively. DCDDP was administered intraperitoneally 30 min before and after MCT injection once a day for 28 days. In the nitrendipine (NIT; Sigma Chemical) group (n = 10), 10 mg/kg NIT, as a positive control drug, which can effectively reduce pulmonary arterial pressure (Ppa), was administered intraperitoneally 30 min before and after MCT injection once a day for 28 days.

To make the MCT solution, 200 mg of MCT were first dissolved in 1.2 ml of 1 M HCl and then diluted with distilled water to ~5 ml and neutralized with 0.5 M NaOH. The total volume was adjusted to 10 ml with distilled water. DCDDP was synthesized by our laboratory. The structure of DCDDP

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was confirmed with mass spectrometry and nuclear magnetic resonance spectrometry, and the purity was measured as >98% by HPLC. For experimental use, DCDDP was dissolved in dimethyl sulfoxide and diluted with a mixture of ethanol, polyethylene glycol, and distilled water (1:5:3.0:5:5).

Measurement of hemodynamics and right ventricular hypertrophy. The animals were anesthetized with urethane (1 g/kg ip) and placed on an animal respirator after 4 wk of MCT injection. Carotid arterial pressure (CAP; stands for systemic arterial pressure), Ppa, and pulmonary blood flow (PBF) were recorded by a microcomputer via transducers. CAP and Ppa were obtained through catheterization of the carotid artery and the main pulmonary artery separately. PBF was recorded at the ascending aorta using a blood flowmeter (MF2100, Japan). Pulmonary vascular resistance (PVR) was calculated using the formula PVR = Ppa/PBF. Right ventricular hypertrophy was estimated by the ratio of the right ventricular (RV) weight to the sum of the left ventricular and septal weights.

Measurement of endothelin-like immunoreactivity and NO. To determine endothelin (ET)-1 of the plasma and pulmonary homogenate, each blood sample was placed in chilled tubes containing 30 μl of 10% EDTA-2Na and 20 μl of aprotinin and centrifuged at 900 g for 10 min at 4°C. The plasma was stored at −40°C. For the measurement of ET-like immunoreactivity (ir-ET), 10 mg of right lower lung tissue were placed in a tube containing 1 ml of 1 M acetic acid, boiled for 10 min, and homogenized for 1 min. The homogenate was then centrifuged at 900 g for 15 min at 4°C. The supernatant was diluted with phosphoric acid buffer and stored at −40°C. ir-ET levels of the plasma and pulmonary homogenate were measured by RIA (10) (RIA kit was purchased from the institute of Eastern Asia, General Hospital of PLA, China).

Each blood sample was placed in heparinized tubes and centrifuged at 900 g for 20 min to obtain the plasma. The 10 mg of lung tissue were homogenized with a homogenizer for 1 min in 1 ml of saline, and the homogenate was centrifuged at 900 g for 20 min. The method of Inoue et al. (13) was employed to measure the contents of NO in the plasma and pulmonary homogenate.

Measurement of superoxide dismutase and malondialdehyde. Venous blood samples anticoagulated with heparin were centrifuged at 900 g for 15 min at 4°C to obtain the plasma. Pulmonary tissue taken from the right lower lung (adding 1 ml of cold saline) was homogenized with a homogenizer in an ice water bath. The homogenate was centrifuged at 900 g for 15 min at 4°C to obtain the supernatant. The method of hydroxylamine hydrochloride (14) and thiobarbituric acid reaction (20) was employed to measure activities of superoxide dismutase (SOD) and concentrations of malondialdehyde (MDA) in plasma and pulmonary homogenate, respectively.

Remodeling of pulmonary small arteries. A block of tissue of the left lower lung was taken and embedded in paraffin wax by the method of Farhat et al. (5). Three sections at 4-μm thickness were prepared. One was stained with hematoxylin and eosin, the second one was stained with Verhoeff’s elastin, and the third one was DNA stained by the method of Feulgen (26). With the use of a color image analysis system (Quantimet 570, Leica), the number and medial thickness of arteries (<100 μm in diameter) in 10 consecutive fields (with 0.0627 mm² on the average) in each animal were determined. The percentage of medial thickness of circular or quasi-circular vessels (10 vessels/each group) was calculated. DNA content and 2c, 3c, 4c, and 5c DNA-ploid were recorded and calculated by an image analysis system.

Labeling of 5-hydroxytryptamine and 5-hydroxytryptamine receptor. With the use of the immunohistochemistry technique, 5-hydroxytryptamine (5-HT) and 5-HT receptor (5-HTR)-positive cells in pulmonary tissue were labeled. The primary antibodies (anti-5-HT antibody and anti-5-HT/anti-5-HTR antibody) were provided by Dr. W. Q. Huang, Department of Histology and Embryology, Fourth Military Medical University, Xi’an, China. Biotin-labeled sheep anti-rabbit antisera and avidin-biotin complex were from Bako. Staining procedures were performed as described by Hsu et al. (12). In the alternative sections, 5-HT (1:5,000) or 5-HT/5-HTR (1:100) were incubated, respectively. In Ctrl subjects, the primary antibody was replaced with normal rabbit serum. By using the image analysis system (Quantisemt 570, Leica), the number of 5-HT and 5-HTR-positive cells was counted in 10 consecutive fields for each slide (1 slide/rat, n = 5). The optical density level was also determined with software of the image analysis system (Quic; at ordinary light and blue light filter) from a total of 150 5-HT and 5-HTR-positive cells (30 cells/rat, n = 5).

Culture of pulmonary arterial smooth muscle cell. Pulmonary arterial smooth muscle cells (PASMCs) of rat were cultured by the method of Chamley-Campbell et al. (3). Male Sprague-Dawley rats weighing between 100 and 110 g (n = 8) were anesthetized with pentobarbital sodium (30 mg/kg, administered ip). The heart and lung were extracted using a sterile technique and put in a culture dish with D-Hanks’ fluid (Hanks’ solution free of calcium and magnesium anion). The extrapulmonary artery was isolated, and the endothelium was removed. After two washes with D-Hanks’ fluid, the middle lamella smooth muscle was cut into 1-mm³ blocks, which were cultured using the method of Chamley-Campbell et al. PASMCs reached confluence in 4–6 days in medium RPMI-1640 with 20% fetal calf serum (FCS). Cells used for these studies were between passages 3 and 6. Unless otherwise noted, the culture medium was replaced every other day with fresh RPMI-1640 containing 10% FCS.

Functional experiment on cultured PASMCs. The passage 3 PASMCs were enzymatically removed from the surface with 0.3% trypsin. The cellular suspension was uniformly placed in 12-well tissue culture clusters with cover glass and cultured for 48 h. The confluent PASMC monolayers (from the same rat) were exposed to a single administration of 5-HT (10 μM) with or without DCDDP (1, 10, or 100 nM) for 30 min. After the medium fluid was absorbed, the adherent cells on the cover glass were stained with 1% of eosin, and the length was measured by the microscope (24).

Proliferation experiment of PASMCs. PASMCs from passages 3–6 were placed with a density of ~5,000 cells/well in 96 well multwell plates and cultured for 24 h. The culture medium was then replaced with fresh RPMI-1640 containing 5% FCS added with 10 μM 5-HT (final concentration). The effect of DCDDP and NIT on the proliferation enhanced by 5-HT was observed using the dosages of 1, 10, and 100 nM with 5-HT. Proliferation of PASMCs was measured by performing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; thiazolyl blue) microculture tetrazolium colorimetric assay based on the ability of viable cells to reduce the MTT compound to a blue formazan product. After 44 h of culturing, the cellular culture medium was added, 20 μl of 0.5% MTT. After 24 h, the cell culturing was continued for an additional 4 h in a new medium with the same concentration of DCDDP, NIT, or 5-HT. Finally, the culture medium was aspirated from each well and vibrated in 150 μl of dimethyl sulfoxide for 10 min. Absorbance of each well was determined by enzyme-linked ELISA at 490 nm of wavelength.
Measurement of free Ca$^{2+}$ in cultured PASMCs. PASMCs from passages 3–6 were placed in six multiwell plates and cultured for 24 h. The culture medium was replaced with fresh RPMI-1640 containing 5% FCS. The effect of 5-HT (10 μM) with and without DCDDP (10 nM) or NIT (10 nM) on the intracellular free Ca$^{2+}$ concentration was assessed. DCDDP or NIT was added and preincubated in cultured PASMCs for 30 min before 5-HT treatment. The cell monolayer was washed twice with sterile Ca$^{2+}$-Mg$^{2+}$-free Hanks’ balanced salt solution to remove nonadherent cells. The adherent cells were enzymatically removed from the surface with 0.3% trypsin and adjusted to the density of ~2 × 10^5 cells/l. The cell survival rate was >90%, which was determined by trypan blue exclusion reaction. One milliliter of cellular suspension was incubated with 10 μM fura 2-acetoxymethyl ester (final concentration) at 37°C for 45 min and then centrifuged at 100 g for 5 min. After the supernatant was abandoned, the cells were rinsed twice with sterile Ca$^{2+}$-Mg$^{2+}$-free Hanks’ balanced salt solution. Finally, 1 ml of cellular suspension was incubated for 10 min, and the concentration of free Ca$^{2+}$ in PASMCs was determined by fluorophotometry (22).

Statistics. All data are expressed as means ± SD. Group means were compared using an unpaired Student’s t-test. A value of P < 0.05 was accepted as statistically significant.

RESULTS

Changes in pulmonary hemodynamics. MCT greatly affected the mean Ppa (MPAP) and PVR in the experimental rats (Table 1). After 28 days of administration of MCT, MPAP was increased by 114% (2.1 ± 0.4 kPa in Ctl vs. 4.5 ± 0.9 kPa in MCT; P < 0.05), whereas PVR was elevated by 162% (45 ± 10 kPa·min⁻¹·l⁻¹ in Ctl to 118 ± 17 kPa·min⁻¹·l⁻¹ in MCT; P < 0.05). DCDDP could effectively block the effect of MCT on the MPAP and PVR. Compared with the MCT group, the MPAP was reduced by 29, 23, and 16% in DCDDP₈, DCDDP₉, and DCDDP₁₀ groups (P < 0.05), respectively. Meanwhile, PVR dropped by 45, 42, and 36%, respectively, in these three DCDDP groups (P < 0.05). NIT had a similar effect on the pulmonary hemodynamics, as DCDDP did. The mean CAP and PBF were not significantly affected by DCDDP and NIT (Table 1).

Effects on contents of ir-ET, MDA, NO, and activity of SOD. In the MCT group, the content of ir-ET and NO was dramatically increased in plasma (P < 0.05) (Fig. 1) but decreased in pulmonary homogenate, compared with that in the Ctl group. In both venous plasma and pulmonary tissue, the content of MDA was significantly increased, whereas the activity of SOD dramatically decreased. DCDDP did not affect the content of ir-ET in either plasma or pulmonary tissue. Compared with that in the MCT group, in all three DCDDP groups, the NO level was decreased in plasma but increased in pulmonary tissue; the content of MDA was reduced to normal levels in pulmonary tissue but remained high in plasma. DCDDP also significantly elevated the activity of SOD in both plasma and pulmonary homogenate. After NIT treatment, the effects on ir-ET, NO, MDA, and SOD were basically similar to those produced by DCDDP (Figs. 1–4).

Table 1. Changes in pulmonary hemodynamics with MCT-induced PH

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCAP, kPa</th>
<th>MPAP, kPa</th>
<th>PBF, ml/min</th>
<th>PVR, kPa·min⁻¹·l⁻¹</th>
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<tr>
<td>Ctl</td>
<td>10</td>
<td>14.5 ± 1.9</td>
<td>2.1 ± 0.4</td>
<td>48 ± 9</td>
<td>45 ± 10</td>
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<tr>
<td>MCT</td>
<td>10</td>
<td>14.1 ± 2.0</td>
<td>4.5 ± 0.9*</td>
<td>39 ± 6</td>
<td>118 ± 17*</td>
</tr>
<tr>
<td>DCDDP₁₈</td>
<td>8</td>
<td>13.9 ± 1.6</td>
<td>3.2 ± 0.2*</td>
<td>52 ± 12</td>
<td>65 ± 16†</td>
</tr>
<tr>
<td>DCDDP₉</td>
<td>8</td>
<td>14.7 ± 1.7</td>
<td>3.5 ± 0.6*</td>
<td>54 ± 14</td>
<td>68 ± 18†</td>
</tr>
<tr>
<td>DCDDP₁₀</td>
<td>8</td>
<td>14.1 ± 1.9</td>
<td>3.8 ± 0.9*</td>
<td>50 ± 9</td>
<td>76 ± 18†</td>
</tr>
<tr>
<td>NIT</td>
<td>8</td>
<td>13.7 ± 2.0</td>
<td>3.6 ± 0.5†</td>
<td>46 ± 5</td>
<td>79 ± 18†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. Ctl, control; MCT, monocrotaline; DCDDP, dimethyl 4-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate; DCDDP₁₈, DCDDP applied with 5 μg/kg (low dose); DCDDP₉, DCDDP applied with 50 μg/kg (medium dose); and DCDDP₁₀, DCDDP applied with 500 μg/kg (high dose); NIT, nitrendipine; PH, pulmonary hypertension; MCAP, mean carotid arterial pressure; MPAP, mean pulmonary arterial pressure; PBF, pulmonary blood flow; PVR, pulmonary vascular resistance. *Significant difference from Ctl group (P < 0.05); † significant difference from MCT group (P < 0.05).

Fig. 1. Effects of dimethyl 4-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (DCDDP) on the contents of endothelin-like immunoreactivity (ir-ET) and nitric oxide (NO) in plasma. Experimental groups: 1, control (Ctl); 2, monocrotaline (MCT); 3, low-dose DCDDP (5 μg/kg; DCDDP₁₈); 4, medium-dose DCDDP (50 μg/kg; DCDDP₉); 5, high-dose DCDDP (500 μg/kg; DCDDP₁₀); 6, nitrendipine (NIT). *Significant difference from Ctl (P < 0.05); #significant difference from MCT (P < 0.05).

Fig. 2. Effects of DCDDP on the contents of superoxide dismutase (SOD) and malondialdehyde (MDA) in plasma. Experimental groups are as described in Fig. 1 legend. *Significant difference from Ctl (P < 0.05); #significant difference from MCT (P < 0.05).
Effects of DCDDP and NIT on the pulmonary artery and DNA in PASMCs. After 4 wk of MCT treatment, there were a number of inflammatory cells in which monocytes and lymphocytes were predominant around vascular vessels. MCT made the walls of the small pulmonary artery (<100 μm in diameter) thicker. The average percent ratio of medial thickness to vessel diameter of 10 circular vessels increased by ~70% in the MCT group (Table 2). DCDDP had an inhibitory effect on the change in thickness produced by MCT. In the MCT group, DNA contents of PASMCs and the percentage of 3c to 4c DNA-ploid were significantly increased, compared with that in the Ctl group. DCDDP eliminated DNA changes in PASMCs produced by MCT (Table 3). NIT also had a similar reverse effect on the thickness of small pulmonary arteries and DNA contents in PASMCs (Tables 2 and 3).

Effects of DCDDP and NIT on proliferation, constriction, and free Ca2+ content of PASMC. MTT reduction assay showed that 5-HT could induce hyperplasia and constriction of PASMCs. After 10 μM 5-HT treatment, the absorbance at 490 nm of wavelength of PASMCs was elevated by 38%, from 0.098 ± 0.021 (in Ctl) to 0.135 ± 0.008 (P < 0.05). DCDDP and NIT had a reverse effect on 5-HT to varying extents, compared with that in the Ctl group (Table 4).

After administration of 10 μM 5-HT, the average length of PASMCs shortened from 184 ± 49 μm (in Ctl) to 106 ± 23 μm (P < 0.05). DCDDP (10 and 100 nM) downregulated pulmonary arterial constriction produced by 5-HT by 25–30% (P < 0.05), compared with the length of 5-HT-treated PASMC (Table 5). However, NIT had only a weak effect (Table 5).

After the treatment with 10 μM 5-HT in cultured PASMCs, the intracellular free Ca2+ concentration increased by ~150%, compared with that in the Ctl group. This effect could be partially blocked by either DCDDP or NIT. DCDDP (10 nM) and NIT (10 nM) reduced the intracellular free Ca2+ concentration by ~50% (Fig. 5).

DISCUSSION

The MCT-induced PH is an ideal animal model for investigating the mechanism on PH (11, 18). PH is
characteristic of the remodeling and abnormal response of pulmonary vessels. In the present study, the dramatic changes in morphology and functional metabolism of pulmonary vessels were observed in MCT-induced PH, such as the increase in MPAP and PVR, the wall thickness of the right ventricle, the thickness of small pulmonary arterial vessels, and DNA contents in PASMCs as well. DCDDP could modify the changes in pulmonary hemodynamics and morphology in MCT-induced PH and restore the content of DNA to the normal level. Basically, NIT had a similar effect as DCDDP did on chronic PH. The therapeutic effects of DCDDP could be related to relaxing the PASMCs, protecting endothelial cells from damage, and inhibiting the DNA synthesis of PASMCs as well.

Vascular endothelial cells play an important role in regulating the vascular smooth muscle tension by releasing NO and ET. Dysfunction of vascular endothelial cells is associated with the development of PH. Changes in ET, NO, MDA, and SOD activity in plasma and pulmonary tissue were observed in MCT-induced PH in the present study. Vascular endothelial cells are the main origin of ET and NO in the normal condition. Our study showed that the content of ir-ET and NO was dramatically increased in plasma but decreased in pulmonary homogenate in the MCT group, compared with that in the Ctl group. We speculate that the differences might be caused by selective roles of MCT, which damage pulmonary vascular endothelial cells but only stimulate systemic vascular endothelial cells. Our experimental data show that damages of pulmonary endothelial cells are more serious than those of systemic endothelial cells, such as abdominal artery and renal artery, which may, at least in part, explain the differences in ir-ET and NO between plasma and pulmonary homogenate. But the detailed causes of these differences in ir-ET and NO between plasma and pulmonary homogenate need to be studied further. The change in ET and NO was out of proportion in pulmonary tissues, and NO content was decreased much more (97%), compared with ET (49%), from control. Functionally, the injured pulmonary vascular endothelial cells can impair the formation and metabolism of NO. The dramatic decrease in activity of SOD and increase in content of MDA in venous plasma and pulmonary homogenate indicated an enhanced production of free radicals [especially superoxide anion (O2•−)] and an intensified lipid peroxidation response. Because SOD is a scavenger of O2•−, the content of O2•− increased, whereas the activity of SOD significantly decreased. The excessive O2•− can not only injure vascular endothelial cells and inhibit NO generation, but also inactive NO (16,21) and produce a more poisonous free radical, peroxynitrite anion (23), by combining with NO. Peroxynitrite anion could weaken vascular relaxation and intensify the injury of endothelial cells. Li et al. (17) also reported that O2•− can cause dose-dependent constriction of the intrapulmonary artery. Because MDA is a product of lipid peroxidation, the increase in MDA indicates intensification of lipid peroxidation and the injury of the cellular membrane. In MCT-induced PH, there were increasing numbers of inflammatory cells, in which monocytes and lympho-

Table 5. Effects of DCDDP on hyperplasia and constriction induced by 5-HT in cultured PASMCs

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>OD at 490 nm</th>
<th>Length of PASMC, μm</th>
</tr>
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<tbody>
<tr>
<td>Ctl</td>
<td>8</td>
<td>0.098 ± 0.021</td>
<td>184 ± 49</td>
</tr>
<tr>
<td>5-HT (10 μM)</td>
<td>8</td>
<td>0.135 ± 0.008*</td>
<td>106 ± 23*</td>
</tr>
<tr>
<td>DCDDP (1 nM) + 5-HT (10 μM)</td>
<td>8</td>
<td>0.055 ± 0.033†</td>
<td>96 ± 24†</td>
</tr>
<tr>
<td>DCDDP (10 nM) + 5-HT (10 μM)</td>
<td>8</td>
<td>0.043 ± 0.024†</td>
<td>137 ± 23†</td>
</tr>
<tr>
<td>DCDDP (100 nM) + 5-HT (10 μM)</td>
<td>8</td>
<td>0.108 ± 0.036†</td>
<td>132 ± 25†</td>
</tr>
<tr>
<td>NIT (1 nM) + 5-HT (10 μM)</td>
<td>8</td>
<td>0.113 ± 0.030</td>
<td>102 ± 24*</td>
</tr>
<tr>
<td>NIT (10 nM) + 5-HT (10 μM)</td>
<td>8</td>
<td>0.092 ± 0.024†</td>
<td>115 ± 26†</td>
</tr>
<tr>
<td>NIT (100 nM) + 5-HT (10 μM)</td>
<td>8</td>
<td>0.055 ± 0.005†</td>
<td>108 ± 22†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. PASMC, pulmonary arterial smooth muscle cell. *Significant difference from Ctl (P < 0.05); †significant difference from MCT (P < 0.05).
cytes were predominant around vascular vessels. These inflammation cells may be the main source of free radicals. DCDDP, a Ca$^{2+}$ channel blocker, can protect the endothelial cells from damage by intensifying the lipid peroxidation of pulmonary tissue, which may be related to the elevated NO and ET in pulmonary homogenate with the treatment of DCDDP in MCT-induced PH.

In 1965, Furner and Lalich (6) proposed that 5-HT released from mast cells could result in PH. However, Kanai et al. (15) reported that the elevation of 5-HT in blood was only a temporary phenomenon, and 5-HT recovered to normal levels within 3 days after subcutaneous injection of MCT. The response to 5-HT in the pulmonary artery was stronger in MCT-induced PH, which suggested that the density of 5-HTRs in the pulmonary artery be upregulated. Our experiment provided further evidence that both 5-HT and 5-HTR-positive cells and their relative contents in lung tissue were significantly increased after 4 wk of treatment with MCT in PH. 5-HT can produce proliferation of pulmonary small vessels, either directly or indirectly, by stimulating alveolar macrophage. In cell culture, 5-HT was also found to enhance the constriction and proliferation of PASMCs, while the level of Ca$^{2+}$ in PASMCs was elevated. All of these results suggest that the increase in the amount of 5-HTRs in pulmonary tissue underlies the mechanism by which the pulmonary artery response to 5-HT was intensified in MCT-induced PH.

It has been reported that NIT could inhibit constriction of sheep pulmonary arterial strip produced by 5-HT (4). Felodipine could increase relaxation of the pulmonary artery in MCT-induced PH in rat (25) and improve pulmonary hemodynamics in chronic obstructive pulmonary disease, at least in part, by decreasing the maximum constriction effect of the human pulmonary artery to 5-HT (19). The present study showed that both DCDDP and NIT can not only reduce pulmonary arterial constriction and proliferation induced by 5-HT at 10 μM, but also decrease 5-HT and 5-HTR-positive cells in lung tissue. The function of DCDDP is attributed to protecting the endothelial cells, in which 5-HT is decomposed and decreasing the Ca$^{2+}$ concentration in PASMCs. These effects are the therapeutic mechanisms of DCDDP in MCT-induced PH.

In summary, as a Ca$^{2+}$ channel blocker, DCDDP is a more effective drug than NIT for PH induced by MCT in rat. The mechanism of DCDDP for treatment is that DCDDP can protect predominantly the endothelial cells from injury by a free radical derived from lipid peroxidation. DCDDP is also related to the alteration of 5-HT metabolism and PASMCs response to 5-HT, as well as the dysfunction of the Ca$^{2+}$ channel.

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