

Tetrandrine inhibited chronic "inflammatory" pulmonary hypertension in rats¹

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KEY WORDS tetrandrine; monocrotaline; pulmonary hypertension; vasoconstriction; right ventricular hypertrophy

AIM: To study the effects of tetrandrine (Tet), on pulmonary hypertension. **METHODS:** An "inflammatory" chronic pulmonary hypertension induced by monocrotaline (Mon) in rats was used. **RESULTS:** Tet 50, 100, and 150 mg·kg⁻¹·d⁻¹ ig 3 wk inhibited Mon-induced increase of pulmonary artery pressure (PAP) by 23.8 %, 34.9 % ($P < 0.05$), and 42.0 %, ($P < 0.05$); the right heart index by 2.0 %, 25.0 %, and 30.0 % ($P < 0.05$) respectively compared with those from Mon group, without significant influence on the systemic artery pressure (SAP). Using histological exam by Verhoeff elastic stain and computer scanning analysis, it was found that Tet (100 mg·kg⁻¹·d⁻¹) for 3 wk, inhibited the increase of medial thickness and cross sectional area by 57.8 % ($P < 0.01$) and 54.6 % ($P < 0.01$), respectively vs Mon group. **CONCLUSION:** Tet ameliorated the development of pulmonary vascular and lung tissue injury induced by Mon in rats.

Tetrandrine (Tet), a plant alkaloid isolated from *Stephania tetrandra* S. Moore, has a broad spectrum of pharmacological actions including cardiovascular actions and anti-inflammatory actions^[1]. We have previously reported that Tet reduced pulmonary hypertension caused by acute hypoxia in dogs. It reduced pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR), and increased cardiac output without significant changes in systemic artery pressure (SAP) and blood gas levels^[1]. These findings suggested that Tet might be useful for the treatment of patients with pulmonary hypertension.

The pathology of pulmonary vascular diseases comprises of 2 components, ie pulmonary vasoconstriction and pulmonary vaso-construction^[2,3]. There are 2 widely used animal models of chronic pulmonary hypertension: exposure to chronic hypoxia^[4] and an "inflammatory" model of pulmonary hypertension in rats following monocrotaline (Mon) injection^[5]. That whether Tet may interfere with the development of pulmonary vascular remodeling and right ventricular hypertrophy is not clear. Both platelet activating factor (PAF) and interleukin-1 (IL-1) contribute to the lung vascular remodeling during chronic "inflammatory" pulmonary hypertension induced by Mon^[6,7]. Tet inhibited the generation or the activity of PAF and IL-1^[8], and inhibited mRNA expression in silicosis^[9]. We wonder whether Tet is effective against the pathophysiological process to interrupt pulmonary vasoconstriction and pulmonary vascular remodeling.

This study was to investigate the effects of Tet on the pulmonary hemodynamics, pulmonary vascular remodeling, and right heart hypertrophy in Mon-induced chronic "inflammatory" pulmonary hypertension.

MATERIALS AND METHODS

Wistar rats (48 ♂, weighing 144 ± s 16 g) from Animal Resource Center, China Medical University (Certificate No LSDZ 521), were divided into 6 groups: control, Mon, Mon+ vehicle and Mon+ Tet (50, 100, 150 mg·kg⁻¹·d⁻¹, ig).

Mon model of chronic pulmonary hypertension in rats Mon (Sigma, USA) was dissolved in distilled water and the pH was adjusted to 7.4 with dilute HCl. It was injected sc, 60 mg·kg⁻¹. The rats were housed in an alternating 12 h light/dark cycle under controlled temperature (18 - 23 °C) and humidity (40 % - 50 %). On day 0, rats were sc injected Mon, or saline. Tet was given 1 d after sc Mon for 21 d.

Hemodynamic measurement and right heart index measurement At the end of the 3 wk after Mon or physiological saline injection, the rats were anesthetized with ip sodium pentobarbital 50 mg·kg⁻¹. A pulmonary artery was

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cannulated by a PV-1 polyvinyl tube with a curved tip for the measurement of pulmonary artery pressure. Pressure was measured with a Statham P23ID pressure transducer and recorded on a polyphysiological system (Nihon Konden R6000). The left carotid artery was cannulated with a PE-50 polyvinyl tube for the measurement of systemic artery pressure. The catheters were connected with pressure transducers (TP200P) and the systemic artery pressure was also recorded with the polyphysiological system. Following recording of the arterial pressure, the rats were sacrificed with an over dose of sodium pentobarbital. The right ventricular hypertrophy was assessed as an increase in the ratio of right ventricle weight to the weight of left ventricle plus septum.

Standard lung histology Freshly excised lungs were formalin-fixed via intratracheal installation at a pressure of 250 Pa. Paraffin-embedded blocks were sectioned and then processed by Verhoeff elastic stain procedure^[5]. Three sections per lung were prepared for histological examination. From 6 control and 6 Mon-treated rats, the thickness of arteriole media in the arterioles with external diameters from 65 to 75 μm of approximately 20 pulmonary arterioles per group was measured with the aid of a Computer Scanning Image Analyser (Luzex-F, Japan) attached to a Nihon microscope and the cross sectional area was calculated automatically.

Statistical analysis Data are presented as $\bar{x} \pm s$. Group comparisons were made by *t* test.

RESULTS

PAP and right ventricular hypertrophy

All Mon-treated rats showed progressive tachypnea and dyspnea within 3 wk. The body weight of the Mon-treated group was lower than that of the control group. Increase of PAP and right ventricular hypertrophy were developed in the Mon-treated rats, yet there was no significant changes of the SAP compared to those from control rats. Tet ig 100 and 150 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, rather than 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, inhibited Mon induced increase of PAP and right ventricular hypertrophy

Tab 1. Hemodynamic changes in rats 3 wk after exposure of monocrotaline. $n = 8$, $\bar{x} \pm s$. ^a $P > 0.05$, ^c $P < 0.01$ vs control; ^d $P > 0.05$, ^e $P < 0.05$, ^f $P < 0.01$ vs Mon group.

Treatment/ $\text{mg} \cdot \text{kg}^{-1}$	PAP (kPa)	SAP (kPa)	RV/(LV + S)	RV/BW $\times 10^3$
Control	1.40 \pm 0.25	16.1 \pm 3.1	0.24 \pm 0.02	0.53 \pm 0.03
Mon	2.40 \pm 0.51 ^c	15.9 \pm 2.3 ^a	0.40 \pm 0.08 ^c	0.88 \pm 0.17 ^c
Mon + vehicle	2.60 \pm 0.63 ^c	16.0 \pm 2.1 ^a	0.41 \pm 0.08 ^c	0.89 \pm 0.17 ^c
Mon + Tet 50	2.00 \pm 0.41 ^d	16.5 \pm 2.1 ^d	0.40 \pm 0.09 ^d	0.90 \pm 0.09 ^d
Mon + Tet 100	1.70 \pm 0.21 ^e	15.7 \pm 1.9 ^d	0.31 \pm 0.05 ^e	0.75 \pm 0.11 ^e
Mon + Tet 150	1.50 \pm 0.16 ^f	16.4 \pm 2.1 ^e	0.29 \pm 0.04 ^f	0.68 \pm 0.12 ^f

(Tab 1).

Histopathological changes of lung and pulmonary vasculature Most of the pulmonary arterioles showed increased media thickness due to hypertrophied media smooth muscle cells by Mon sc. Perivascular edema and infiltration of mononuclear cells, medial thickening of pulmonary arterioles and alveolar walls were seen. The vascular changes observed in the lungs of all Mon-treated rats were similar to those reported previously^[6]. Mon produced marked vascular remodeling of media. Tet 100 and 150 $\text{mg} \cdot \text{kg}^{-1}$, but not 50 $\text{mg} \cdot \text{kg}^{-1}$, prevented medial hypertrophy by Mon treatment (Fig 1, Plate 2).

Medial thickening of pulmonary arterioles

Mon produced marked hypertrophy of arteriole smooth muscle cells. The thickness and cross area of arteriole media increased by 422 % and 398 %, respectively, by Mon. Tet 100 and 150 $\text{mg} \cdot \text{kg}^{-1}$ prevented the increase of media thickness and cross sectional area (Tab 2).

Tab 2. Effects of Tet (100 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) on monocrotaline-induced hypertrophy of media smooth muscle. $\bar{x} \pm s$. ^c $P < 0.01$ vs control; ^f $P < 0.01$ vs Mon.

Treatment	Vessels	Media thickness / μm	Media cross area / μm^2
Control	19	3.8 \pm 0.4	719 \pm 78
Mon	19	16.0 \pm 1.7 ^c	2 861 \pm 299 ^c
Mon + Tet	21	6.9 \pm 0.7 ^f	1 299 \pm 105 ^f

DISCUSSION

The " inflammatory " chronic pulmonary hypertensive animal model in rats was successively established, in which the pulmonary hypertension,

pulmonary vascular remodeling characterized by medial thickening of pulmonary artery and right heart hypertrophy were seen as reported by others⁽⁶⁾. The present study demonstrated that oral administration of Tet inhibited the development of pulmonary hypertension and right heart hypertrophy and also produced regression of morphological alterations in the pulmonary vascular bed in the chronic pulmonary hypertension animal model of Mon treated rats. It is the first demonstration that Tet inhibits the elevation in pulmonary artery pressure and vascular structural remodeling of the pulmonary vasculature in pulmonary hypertension model, in which the administration of Tet was associated with a 83 % inhibition of pulmonary hypertension and a 75 % inhibition of medial thickening of small pulmonary arteries to values near to those in the control rats. Results also demonstrate that the right heart hypertrophy caused by Mon was markedly prevented by Tet.

Although, cardiac output of the rat was not measured, in studies of chronic pulmonary hypertension there is generally an excellent correlation between measured pulmonary artery pressure and right heart hypertrophy⁽⁶⁾. This correlation was also found in the present study ($r = 0.9132$, $P < 0.05$, $n = 6$).

Tet has anti-inflammatory effects on pulmonary silicosis and immuno-suppressive activity⁽¹⁰⁾. Tet has been shown to reduce both silica-induced fibrosis and the elevation of lung collagen content in rats⁽⁹⁾. Furthermore, Tet has been shown to exhibit various degrees of effectiveness to prevent silica-induced fibrosis in animal models and has been used as an antifibrotic agent for clinical trials in China⁽¹¹⁾. Tet, a highly effective agent in preventing fibrosis, showed strong binding to both lipid vesicles and alveolar macrophages⁽¹²⁾. Tet could inhibit the development of silicotic nodules and the synthesis of collagen and glycosaminoglycan *in vivo* and *in vitro*⁽⁹⁾. In experiments where thymocytes were directly treated with Tet, a dose-dependent inhibition of thymocyte proliferation was noted with both IL-1 specific and non-specific mitogenic (conavalin A) actions. These results provide a correlation between the antifibrotic effects of Tet and inhibition of macrophage activation⁽¹⁰⁾.

The antifibrotic action of Tet may be mediated in part by direct inhibition of fibroblast proliferation

normally associated with the development and progression of silicosis⁽⁹⁾.

The effects of Tet on gene expression of type I and type III collagen during experimental silicosis. It was found that alpha 1 (I) and alpha 1 (III) mRNA levels increased significantly at 60 and 120 d after the rats exposed to silica dust. The mRNA levels went down at 1 and 3 months after treated by Tet. *In situ* hybridization observation revealed that the silver grains of Type I and Type III collagen were scattered within the fibroblasts in cellular nodules and in thickened interstitial of silicosis tissue. The amount of mRNA silver grains decreased in the lung tissue treated by Tet. It was suggested that Tet may inhibit the gene expression of collagen during silicosis⁽⁹⁾.

In conclusion, Tet has a marked effect to prevent Mon-induced chronic "inflammatory" pulmonary hypertension.

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粉防己碱抑制大鼠慢性“炎症”性肺动脉高压¹

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关键词 粉防己碱; 野百合碱; 肺性高血压;

血管收缩; 右室肥大

肺动脉高压

目的: 观察粉防己碱(Tet)对 monocrotaline (Mon) 引起的肺血管收缩和肺血管增生性改变的影响.

方法: 应用 Mon 引起的炎性肺动脉高压大鼠模型.

结果: 每日 ig Tet 50, 100 及 150 mg·kg⁻¹, 三周后肺动脉压分别比 Mon 组低 23.8 % (P > 0.05),

34.9 % (P < 0.05) 和 42.0 % (P < 0.05); 右心指数分别比 Mon 组低 2.0 % (P > 0.05), 25.0 %

(P > 0.05) 和 30.0 % (P < 0.05). 应用 Verhoeff 弹性染色表明 Tet 明显抑制肺小动脉中膜肥厚.

Tet (100 mg·kg⁻¹) 组肺小动脉中膜厚度及横截面积分别较模型组低 57.8 % 和 54.6 % (P 值均小于

0.05). 结论: Tet 对野百合碱引起的“炎症”肺动脉高压模型的肺血管收缩和肺血管增生性改变均有抑制作用.

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