

# An Experimental Study on the Establishment of Pulmonary Hypertension Model in Rats induced by Monocrotaline

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**Abstract:** Pulmonary hypertension is called PH for short. It is caused by the pulmonary artery vascular disease leading to pulmonary vascular resistance, and the increase right lung compartment load, which resulting in weakening or even collapse of the right ventricular function. The establishment of rat PH model under the action of monocrotaline is a repeatable, simple and accessible operation technique, which has been widely used in the treatment of pulmonary hypertension. This paper discusses the principle and properties of the PH model on rats under the monocrotaline action.

**Keywords:** Monocrotaline; Induction; Pulmonary Hypertension; Model Construction

## 1. Introduction

Pulmonary hypertension is caused by an abnormal increase in pulmonary blood pressure. The hemodynamic criterion to identify the disease is measuring the average pulmonary artery pressure of the right cardiac catheter based on sea level and it should not be lower than 25mm Hg in a calm state. This disease is generally appeared because of pulmonary vascular lesions, which increase pulmonary vascular pressure and right ventricular after load, resulting in the weakening of the right ventricular or even dysfunction.<sup>[1]</sup> The rats with pulmonary hypertension formed under the monocrotaline action are the ideal animal model for studying this disease so far. Monocrotaline is a kind of bipyrrrole alkaloid. After it enters animal liver, it will undergo certain biological enzyme transformation, flow through with the blood to the lungs in the body, and cause

damage to the blood vessels in the lungs, resulting in inflammation of the blood vessels. This reaction is similar to the clinicopathological mechanism.<sup>[2]</sup>

After years of researches, people have new insights towards the disease mechanism, but it still takes time to fully understand the disease mechanism. A detailed description of the disease's pathology is key to achieving optimal productions, and PH animal models play an important role in this process. Rat PH established by the monocrotaline action under chronic hypoxia can be utilized to study human PH. The mechanism of some vascular lesions provoked by hypoxia is generally mastered, while the circumstances of PH disease patients with vascular blockage have not been found in the rat model. The rat PH model technology developed under the MCT action mechanism is characterized by repeatability, low cost and simple operation, which is the reason it is often applied in PH experiments.

## 2. Pulmonary hypertension model induced by monocrotaline

The rats with pulmonary hypertension formed under the action of monocrotaline are an ideal animal model for the pulmonary hypertension study. Monocrotaline is a kind of bipyrrrole alkaloid, which reacts with oxidase in the liver with the blood flow to the lungs causing damage to the pulmonary artery vessels, and this damage is irreversible. There is a target cell called the endovascular cell in the pulmonary artery, which is very significant for the pulmonary artery. According to rats produced by monocrotaline PAH lung tissue pathology model, monocrotaline injection into animals will lead to the swell phenomenon of pulmonary artery endothelial cells in one week, and under electron microscope it can be observed that the electron density decreases, the nucleus will further increase, the cell membrane become thicker, organelles are swollen again, and all these phenomenon lead to the dysfunction of endothelial cell and it gradually fall off. It can be seen by light microscopy that the endothelial cells of pulmonary artery vessels transform from their original flat state to a stereoscopic state and protrude towards the lumen. After two or three weeks, some of the endothelial cells of the pulmonary artery vessels demonstrated necrosis or even shedding in animals, and the pulmonary artery vessels manifested fibrosis and sclerosis, resulting in narrowing of the pulmonary artery vessels, thrombosis and blockage.

Another consequence is the imbalance between the downstream vasodilatation and constriction of vessels, leading to increased pressure in the pulmonary artery and added pulmonary vascular resistance. In addition, in the process of monocrotaline induced pulmonary artery pressure, inflammation plays an important role and it embodies in the following aspects in particular: firstly, by the study of the monocrotaline pathology model observation it can be found that number of inflammatory cells infiltrate in model lung tissue, which is similar to the vasculitis symptoms and it is mainly around the blood vessels; secondly, the increase of plasma proinflammatory factors in the monocrotaline model was large, of which the tumor necrosis factor was more obvious; thirdly, the utilization of cyclophosphamide for preventive intervention can effectively reduce the level of some inflammatory factors

on the culture medium of alveolar macrophages in the monocrotaline model, such as interleukin 1, tumor necrosis factor and interleukin 6. Finally, the infiltrating and proliferating cells present in the lung tissue of monocrotaline model were mostly mononuclear cells, with a small number of T or B lymphocytes. Therefore, nonspecific inflammation containing macrophages had an important effect, pulmonary arterioles and capillaries will be damaged by monocrotaline, and then monocrotaline infused into alveolar interval induced alveolar macrophage phagocytosis. In the meanwhile the interleukin 1, tumor necrosis factor alpha and interleukin 6 proinflammatory factor used cascade amplification reaction, and make the blood vessel walls and alveolar interval by inflammatory cells infiltration with mononuclear scavenger system activation repeatedly. This increases the thickness of the vessel walls and alveolar septa, and promoted the resistance of the pulmonary vessels. Inflammatory response has important impacts in the pathogenesis of the rat model of pulmonary hypertension induced by monocrotaline. Therefore, this model can be regarded as an animal model closely related to the pathogenesis of connective tissue-related pulmonary hypertension. At present, there is no better model can be put into use, and it is the optimal option based on practical application of the model concerning monocrotaline-induced pulmonary hypertension in rats to study the pathogenesis and drug intervention of connective tissue-related pulmonary hypertension.

## 3. Experimental study on pulmonary hypertension induced by monocrotaline

### 3.1 Experimental methods

(1) Grouping and modeling: 60 rats were randomly divided into the control group (n=12) and the model group (n=48). After 1 week adaptive feeding, 60mg/kg subcutaneous injection of monocrotaline was carried out in the model group, and then the hemodynamic indexes were measured for 1, 2, 3 and 4 weeks with specimens collected. 12 rats were collected at 4 different time points. In the control group, the same amount of normal saline was injected subcutaneously, and the pressure was

measured again after 4 weeks of the injection, and then the data were extracted.<sup>[3]</sup>

(2) Observe the basic conditions of rats: mainly observe the feeding and activity of rats after injection of monocrotaline, as well as the presence or absence of infection and death, and weigh the rats after 4 weeks.

(3) Hemodynamic measurements: mean pulmonary artery pressure was measured at week 1, 2, 3, and 4, respectively, after injection of monocrotaline. Thoracotomy was performed by placing a catheter in the pulmonary artery via the right ventricle.

(4) Detection of right ventricular hypertrophy: the rat heart was completely cut off, and after the atrial tissue was removed, the A ventricle (RV) and the left ventricle (LV) plus ventricular septum (LV+S) were separated, the water was sucked dry with A filter paper, then the weight was weighed and the RV/(LV+S) value was calculated.

(5) Detection of pulmonary vascular micro-morphological indicators: the lung tissues were fixed in negative pressure neutral formaldehyde for 24 hours, paraffin sections were prepared, and routine hematoxylin-eosin staining was performed. Pulmonary vascular morphology was observed under light microscope in both groups. Calculation: 1. CMIAS image processing and analysis system was used to calculate the relative thickness of the medium (external diameter 50 ~ 150  $\mu$ m) and small (external diameter 15 ~ 50  $\mu$ m) muscular arteries. 2. Pulmonary vessel density per unit area (per /mm).<sup>2</sup>

(6) Main observation indicators: changes of right ventricular hypertrophy level, pulmonary vascular micro-morphological indicators and hemodynamic indicators in rats.

(7) Statistical analysis: statistical analysis was carried out with the assistance of SPSS13.0 statistical software, and the data were presented as  $\bar{x} \pm s$ .  $P < 0.05$  was considered significant.

### 3.2 Discussion of experimental results

Inflammatory response plays a key role in the pulmonary hypertension induced by monocrotaline. Observation of the choline induced model pathology showed that there was a large number of inflammatory cell infiltration in the lung tissue of the model, which was similar to the symptoms of vasculitis and mainly distributed around the blood vessels. Plasma

proinflammatory cytokines in the model increased significantly.

After 4 weeks of administration, the right ventricle became hypertrophic and pulmonary artery pressure increased significantly. Pulmonary vascular remodeling, such as thickening of the middle membrane of the pulmonary artery, increased myogenesis degree. And intimal hyperplasia also appeared, which indicated that an effective pulmonary hypertension model could be established by induction of monocrotaline injection.<sup>[4]</sup> Injecting monocrotaline into animals can reduce the pulmonary vascular bed of animals and increase the residual pulmonary blood flow, which is the same as the changes in the pathological morphology of some blood vessels such as pulmonary fibrosis, chronic obstructive pulmonary disease and pulmonary embolism. When the pulmonary vascular endothelium is injured, the monocrotaline is selective and will lead to chronic vascular inflammatory lesions. Compared with the model of chronic hypoxia, this model is closer to the actual clinical causes. In addition, the experimental model of pulmonary hypertension in rats has better control over the pathological process with lower mortality rate, simple operation, and strong repeatability, which has good promotion and application value.

## 4. Conclusion

Inflammatory response plays an important role in the pathogenesis of the rat model of pulmonary hypertension induced by monocrotaline. To some extent, this model can be regarded as an animal model closely associated with the pathogenesis of connective tissue-related pulmonary hypertension. From the practical standpoint, it is a rational choice to study the pathogenesis and drug intervention of connective tissue-related pulmonary hypertension in rats by using monocrotaline model until a more suitable model is developed.

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