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Original Article

Effect of fasudil on hypoxic pulmonary hypertension and right ventricular hypertrophy in rats

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Abstract: Objective: To investigate whether right ventricular hypertrophy in hypoxic pulmonary hypertension (HPH) rats could be prevented by treatment with Rho kinase inhibitor fasudil. Methods: The rat model of pulmonary hypertension was established by exposing rats to normobaric intermitent hypoxia [(10±0.5)% O_2]. Twenty-four Spraque-Dawley male rats were randomly divided into control group, hypoxic model group and hypoxia with fasudil groups (n=8 each). The mean pulmonary arterial pressure (mPAP), and right ventricle hypertrophy index (RVHI) were measured. Ultrastructure of the right ventricular myocardial cells was observed under transmission electron microscope (TEM). –Results: The level of mPAP (31.38±1.98) mmHg and RVHI (0.47±0.03) were significantly higher in the hypoxic model group than (15.25±0.91) mmHg and (0.25±0.02) in control group respectively (P<0.01). Transmission electron microscope (TEM) revealed the model group right ventricular mitochondria increased significantly, swelling, cristae blurred, lost, heart muscle Siming dark band was not clear. The level of mPAP (16.63±1.53) mmHg and RVHI (0.27±0.02) were significantly lower in fasudil treatment group than in model group respectively (P<0.01). After the intervention of fasudil right ventricular myocardial injury was significantly reduced. Conclusions: Fasudil may partly prevent and reverse the development of pulmonary hypertension and right ventricular hypertrophy and myocardial cell injury.

Keywords: Fasudil, pulmonary hypertension, right ventricular hypertrophy, rat

Introduction

Hypoxic pulmonary hypertension (HPH) is the most common complication in cardiovascular diseases characterized by increase of pulmonary arterial pressure, pulmonary vascular structure remodeling and right ventricular hypertrophy. The disease will become worse and lead to right heart failure and death without intervention [1]. Currently, its pathogenesis is not entirely clear and it is short of ideal therapeutic drugs in clinic. In recent years, animal experiments *in vitro and vivo* [2-7] showed that fasudil is a small G-protein Rho kinase inhibitor and plays an important role in relaxing pulmonary vascular and decreasing pulmonary artery pressure. However, there are no intensive studies to investigate whether it has blocking and improvement effects on right ventricular hypertrophy and myocardial ultrastructure. In this study, a rat model of HPH was established to investigate the effect of fasudil on hypoxic pulmonary hypertension and right ventricular hypertrophy in rats.

Materials and methods

Animal grouping and establishment of HPH model

24 healthy male rats at the age of 3 months (equivalent to 10-12 years old in human beings) with the weight of 250~300 g were supplied by Experimental Animal Center of Nanjing Military Region General Hospital. The rat model of HPH was established by exposing rats to a concentration of (10±0.5)% O_2 for 8 h every day, 6 d a week with a total of 21 d in accordance with reference [8]. 24 rats were randomly and equally divided into three groups (n=8 each): (1) Rats

Table 1. Results of mPAP, mCAP, mRV, LV+IVS and RVHI in each group ($\bar{x}\pm s$)

Groups	n	mPAP (mmHg)	mCAP (mmHg)	RV (mg)	LV+IVS (mg)	RVHI
Control group	8	15.25±0.91	121.13±9.80	165.13±12.71	667.83±41.15	0.25±0.02
Model group	8	31.38±1.98*	113.54±14.84#	304.97±15.10*	647.84±32.72#	0.47±0.03*
Fasudil group	8	16.63±1.53▲	110.04±12.24Δ	177.79±16.20▲	655.85±41.80Δ	0.27±0.02▲
F		202.95	1.239	164.769	0.404	67.376
P		0.000	0.318	0.000	0.675	0.000

Note: *Compared with control group $P<0.01$, ▲Compared with model group $P<0.01$; #Compared with control group $P>0.05$, ΔCompared with model group $P>0.05$; 1 mmHg =0.133 kPa.

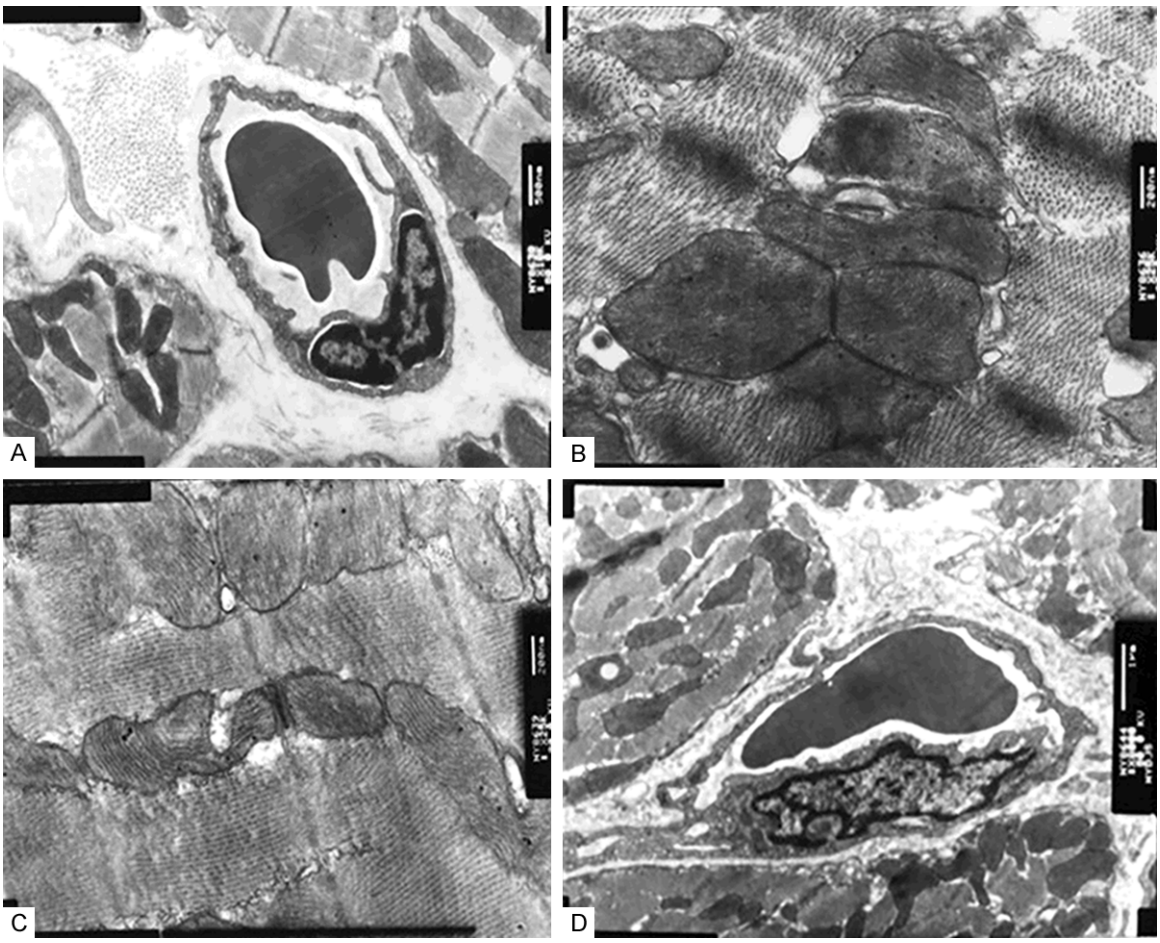

Figure 1. Ultrastructural of RV myocardium observed under transmission electron microsope. A: In control group, the structures of myocardial and capillary endothelial cells are normal (transmission electron microscope, ×10 k); B: In model group, mitochondria in myocardial cell were significantly increased and swelling, crista blurred and disappeared, and bright and dark bands of myocardial myofilament were unclear (transmission electron microscope, ×25 k); C: In fasudil treated group, the structures of myocardial and myofilament membrane had basically returned to normal, and bright and dark bands of myocardial myofilament were clear (transmission electron microscope, ×25 k); D: In fasudil treated group, myocardial and capillary endothelial cells appear essentially normal (transmission electron microscope, ×8 k).

in model group were intraperitoneally injected 2 ml/kg normal saline daily before exposing the hypoxia; (2) Rats in fasudil treated group were intraperitoneally injected 15 mg/kg fasudil (Manufacturer: Asahi Kasei Nagoya Pharmaceutical Factory; specification: 30 mg/2 ml/vial; batch No.: ERS11KM) daily before exposing the hypoxia (Fasudil was diluted with normal

saline by dose of 2 ml/kg; (3) Rats in control group were exposed to the normoxia and intraperitoneally injected the same volume of normal saline at the same time daily as model group. After that, rats were anesthetized with 2% sodium pentobarbital powder for injection (40 mg/Kg) by intraperitoneal injection. Right-heart catheterization [9] was adopted to determine the mean pulmonary arterial pressure (mPAP) and carotid artery was cannulated to determine the mean carotid arterial pressure (mCAP). After determination, the heart was removed. Right ventricle (RV) as well as left ventricle and interventricular septum (LV+IVS) were isolated and weighted. The ratio of [RV/(LV+IVS)] (i.e. RVHI) was calculated to determine whether right ventricular hypertrophy occurred.

Preparation of electron microscope specimens of RV myocardium

RV myocardium was removed. Electron microscope specimens were routinely prepared and stained with uranium acetate and lead citrate. All the slices were sectioned by LKB-V type ultramicrotome. Ultrastructural of RV myocardium was observed under JEM- 1010 type (Japan) transmission electron microscope.

Statistical treatment

SPSS 11.0 statistical software was adopted. Data were expressed as $\bar{x} \pm s$. Comparison among multiple groups was analyzed using analysis of variance and between groups using LSD method. The difference was statistically significant if $P<0.05$.

Results

Effect of fasudil on mPAP, mCAP and RVHI in HPH rats

Results (**Table 1**).

Ultrastructural of RV myocardium observed under transmission electron microscope (**Figure 1**).

Discussion

This study found a significant increase of pulmonary arterial pressure and right ventricular hypertrophy in rats 3 weeks after hypoxia, indicating that rat model of HPH was successfully established. The formation of HPH is a complex process which involves genetic, cellular, humoral and other multifactor comprehensive regulations. Its exact mechanism is not fully understood. Recently, the relationship between Rho/Rho kinase signal transduction pathway and HPH has attracted scholars' attention. Some studies showed [3-6, 10-16] that hypoxia activates Rho/Rho kinase signal pathway, which is one of important parts for inducing the occurrence of HPH. Abnormal activation of Rho kinase signal pathway damages the vascular wall of peripheral pulmonary artery, breaks the balance between proliferation and apoptosis of vascular smooth muscle cells, fibroblasts and endothelial cell and stimulates the excessive proliferation and hypertrophy of cells. Whereas, the excessive proliferation and hypertrophy of endotheliocyte can further exacerbate the luminal stenosis and occlusion. Terminal pulmonary artery with continued contraction and reconstruction of vascular wall can cause the increase of peripheral pulmonary vascular resistance and reducing of terminal pulmonary arterial compliance, eventually leading to pulmonary hypertension and right ventricular hypertrophy.

Rho kinase inhibitors as a new class of antihypertensive drugs have good curative effect [2-6] in the treatment of HPH. However, there are no intensive studies to investigate their effects on the prevention and treatment of pulmonary hypertension, right ventricular hypertrophy and injured right ventricular myocardial ultrastructure. Fasudil, one of the representative drugs of Rho kinase inhibitors, is a novel isoquinoline sulfonamide derivative, which blocks the activity of Rho kinase through competition with ATP to the ATP-binding site of Rho kinase catalytic domain and reduces the level of phosphorylation of myosin light chain (MLC) through regulating the level of phosphorylation of myosin-binding subunit (MBS) and MLC to mediate vasoconstriction. Recent studies showed [4, 17-21] that fasudil improves the balance between endothelin and nitric oxide (NO) and enhances the endothelium-mediated vasodilator response by inhibiting the synthesis and secretion of endothelin-1 (ET-1) and promoting the synthesis and secretion of NO in endothelial cells. Results in this study showed that pulmonary arterial pressure and right ventricular hypertrophy index were significantly reduced

and the injury on right ventricular myocardial cells were significantly improved by administration of Rho kinase inhibitor fasudil during the formation of pulmonary hypertension, indicating fasudil can reduce the pulmonary arterial pressure, improves the right ventricular hypertrophy and relieves the injury on right ventricular myocardial cell; also inhibits the progression of pulmonary hypertension and relieves right ventricular hypertrophy and the injury on right ventricular myocardial cell possibly by directly affecting the contraction of smooth muscle cell, altering the balance between endothelium-derived relaxing factor (NO as a typical representative) and constriction factor (ET-1 as a typical representative), regulating the expression of cell growth gene and other mechanisms.

In conclusion, with the further illumination of the pathogenesis of pulmonary hypertension, the focus on drug therapy has also changed from simply using vasodilators to seek a drug having effect on pulmonary vascular cell function and right ventricular structural remodeling. In this study, the results suggested that Rho kinase inhibitor fasudil can effectively reduce pulmonary arterial pressure, improve right ventricular hypertrophy and alleviate the hypoxia-induced injury on right ventricular myocardial cell. Meanwhile, no side effects such as hypotension were observed during the experiment. The above outcomes indicate that fasudil may play a better role in the prevention and treatment of pulmonary hypertension and right ventricular hypertrophy in clinic. A further intensive clinical study in this field is still in need.

Disclosure of conflict of interest

None.

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