Effects of Prostaglandins and Leukotrienes on Hypoxic Pulmonary Vasoconstriction in Rats

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Summary: To investigate the effects of prostaglandins (PGs) and leukotrienes (LTs) on hypoxic pulmonary vasoconstriction (HPV), *in vivo* rats experiment and *in vitro* perfused lung experiment were conducted. The effect of hypoxia on hemodynamics, concentrations of TXB_2 and 6-keto-$PGF_{1\alpha}$ in serum and lung tissue during hypoxia and effects of PGs and LTs on HPV were observed. The results showed that pulmonary arterial pressure (P_{pa}) and pulmonary vascular resistance were increased during hypoxia, but cardiac output and systemic arterial pressure were decreased. There were increases of the concentrations of TXB_2 and 6-keto-$PGF_{1\alpha}$ and their ratio in serum and lung tissue during hypoxia. After use of cyclooxygenase inhibitor (indomethacin) *in vivo* and *in vitro*, HPV was augmented respectively, but after use of lipoxygenase inhibitor (diethylcorbamazine) or leukotriene receptor blocker (LY-171883), HPV was attenuated. It was suggested that LTs mediated pulmonary vasoconstriction, PGs inhibited pulmonary vasoconstriction and they played a modulating role during hypoxia.
Key words: hypoxia; pulmonary circulation; prostaglandins; leukotrienes

Alveolar hypoxia can induce pulmonary vasoconstriction, but the mechanism of hypoxic pulmonary vasoconstriction remains unknown. It might relate to arachidonic acid released from endothelium or to the vasoactive substances released from other cells in lung tissue during hypoxia. Furthermore, the extra-pulmonary reflex and the directive effect of hypoxia on pulmonary vascular smooth muscles might also be involved. In this study, we conducted *in vivo* rats experiment and employed *in vitro* perfused lung experiment, in order to exclude the effects of systemic factors, with an attempt to observe effects of PGs and LTs, metabolites of arachidonic acid on HPV.

1 MATERIALS AND METHODS

1.1 *In Vitro* Rats Experiment

1.1.1 Measurement of Hemodynamics

Twenty-eight Wistar rats, weighing 330 ± 70 g, were randomly divided into control group ($n=12$), indomethacin group ($n=7$), and LY group ($n=9$).

After being anesthetized, rats were given artificial respiration through tracheal intubation. Mean pulmonary arterial pressure (P_{pa}) was measured by catheterization through external jugular vein to pulmonary artery and mean systemic arterial pressure (P_{sa}) was assessed by left common carotid artery catheterization. A catheter was inserted into left atrium in order to facilitate administration. Cardiac output (CO) was measured by impedance methods. The following formulations were used to calculate pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR):

PVR ($dyn \cdot sec \cdot cm^{-5}$) = P_{pa} (mmHg) × 80/CO (L/min)

SVR ($dyn \cdot sec \cdot cm^{-5}$) = P_{pa} (mmHg) × 80/ CO (L/min)

The reactivity of hypoxia on pulmonary vessels was expressed as the change of PVR (△PVR) and its change ratio (△PVR%). △PVR % = △PVR/PVR prior to hypoxia × %

Rats in control group were first subjected to normoxia, and then above indexes were assessed. Then mixed gas containing 10 % O_2 and 90 % N_2 was inhaled for 3 min to induce hypoxia.

In indomethacin group, indomethacin (5 mg/kg, cyclooxygenase inhibitor) was injected into right atrium. After 30 min, the same steps similar to control group were taken.

In LY group, LY-171883 (selective LTD_4, LTE_4 receptor blocker) prepared with 0.5 % $NaHCO_3$ was injected into right atrium (26 mg/kg).

1.1.2 Measurement of Prostaglandins in Serum and Lung Tissues

Sixteen Wistar rats, weighing 270 ± 28 g, were divided into two groups with each group having 8 rats. The rats in control group were subjected to the condition of normoxia. Five ml blood was taken from common carotid artery for assessing partial pressure of oxygen in artery and PGs. Thorax was quickly opened and then about 50 mg of right lower lobe tissues was isolated for measuring PGs. The rats in hypoxia group first underwent 3 min hypoxia, and then blood and lung tissue were harvested. Serum was immediately centrifuged from blood. Lung tissues were immersed in heparin-in-

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domethacin solution to remove blood strain, then put into liquid nitrogen for immediate freezing. Lung tissues were homogenized in ice-water bath. Serum and lung tissue homogenization were extracted with ethyl acetate twice and stripped of neutral lipid with petroleum ether. TXB_2, 6-keto-$PGF_{1\alpha}$ (stable metabolite of TXA_2, PGI_2 respectively) were accessed by RIA kit produced by the Institute of Pharmacology, Chinese Academy of Medical Sciences.

1.2 *In Vitro* Perfused Lung Experiment

Thirty-two Wistar rats, weighting 210±48 g, were randomly divided into control group, indomethacin group, diethylcorbamazine group and combined drugs group, with each group having 8 rats. Anesthetized rats were given tracheotomy and thorax was opened. 200 U heparin was injected through left ventricle in order to prevent blood coagulation. 0.3 cm orifice was cut on right ventricle. A plastic catheter was introduced into pulmonary artery, then ligated and fixed. A little orifice was scissored in left atrium for the reflux of perfusate from pulmonary vein. Heart and lung were isolated quickly and suspended on perfused lung equipment. Pulmonary artery was perfused with homologous blood at constant temperature (37 ℃) and constant flow (3 ml·100 g^{-1}·min^{-1}). Simultaneously, artificial positive respiration was applied and P_{pa} was recorded by using 2-channel recording apparatus.

During the experiment, artificial mechanical ventilation was employed, tidal volume being 2 — 3 ml/time, frequency 60/min, positive end expiratory pressure 2 — 3 cm water column, endo-inspiratory pressure 9 — 10 cm water column.

In control group, after isolated lung was installed in perfused lung equipment, it was made to inhale mixed gas containing 20 % O_2, 5 % CO_2 and 75 % N_2, and was perfused for 30 min until arterial pressure became steady (fluctuation below 1 mmHg). Then it was made to inhale mixed gas containing 3 % O_2, 5 % CO_2 and 92 % N_2 for 6 min to induce acute alveolar hypoxia. The change of P_{pa} was recorded. The reactivity of pulmonary vessels was expressed with the change of P_{pa} and its change rate.

In indomethacin group, before experiment, indomethacin was added into perfusion blood, with its concentration in blood being 20 μg/ml. Other steps were the same as those for control group.

With diethylcorbamazine group, before experiment, diethylcorbamazine was added into perfusion blood, with its concentration in blood being 1 mg/ml. Other steps were the same as those for control group.

In combined drugs group, before experiment, the same doses of indomethacin and diethylcorbamazine were added into perfusion blood simultaneously. Other steps were the same as those for control group.

1.3 Statistical Analysis

Self-control paired *t* test was performed in each group before hypoxia and during hypoxia. The *t* test was used between experimental group and control group.

2 RESULTS

2.1 *In Vivo* Experiment

P_{pa} and PVR during hypoxia were higher than those before hypoxia, △PVR was 55 %. But CO and P_{sa} were decreased significantly ($P<0.01$ — 0.001). SVR was reduced with no significant difference found (table 1).

Table 1 Effects of hypoxia on hemodynamics

Indexes	Before hypoxia	During hypoxia
HR (beat/min)	397±11	402±12
CO (ml/min)	52.02±3.86	45.76±3.20△
P_{pa} (mmHg)	17.22±0.59	23.30±2.41△
PVR (dyn·sec·cm^{-5})	28083±3179	42624±5001△
P_{sa} (mmHg)	83.53±4.26	65.64±4.59△△
SVR (dyn·sec·cm^{-5})	131666±10809	118263±8293

△ $P<0.01$ △△ $P<0.001$, as compared with that before hypoxia

PVR in indomethacin group was 25449 dyn·sec·cm^{-5}, significantly higher than that in control group ($P<0.01$). But in LY group, it was 4104 dyn·sec·cm^{-5}, lower than that in control group (table 2). The results showed that indomethacin augmented HPV and LY attenuated HPV.

Table 2 Effects of indomethacin, LY-171883 on the resistance of pulmonary vessels during hypoxia.

Groups	△PVR (dyn·sec·cm^{-5})	△PVR (%)
Control group	14541±3334	55.0±15.6
Indomethacin group	25449±3565△	75.0±9.6△
LY group	4104±2753△	16.7±14.0△

△ $P<0.01$ as compared with control group

TXB concentration in serum and lung tissue homogenization was enhanced significantly during hypoxia. 6-keto-$PG_{1\alpha}$ and TXB/6-keto-$PG_{1\alpha}$ ratio were increased with no significant difference revealed when compared with those before hypoxia ($P>0.05$, table 3).

Table 3 Concentrations of TXB_2 and 6-keto-$PGF_{1\alpha}$ (pg/ml) in serum and lung tissues

Groups	Serum			Lung tissue		
	TXB_2	6-K-$PGF_{1\alpha}$	TXB_2/6-K	TXB_2	6-K-$PGF_{1\alpha}$	TXB_2/6-K
Control	70.6±6.3	555±86	0.15±0.02	12.56±1.60	50.08±7.31	0.25±0.01
Hypoxia	176.0±27.0△	737±101	0.28±0.07	20.10±2.78△	74.20±24.40	0.48±0.13

△ $P<0.05$ as compared with control group

2.2 *In Vitro* Experiment

During hypoxia, P_{pa} in rats was increased. $\triangle P_{pa}$ was 7.62 mmHg, and the change ratio was 40.00 % ($P<0.05$). After administration of indomethacin, P_{pa} was enhanced by 11.50 mmHg during hypoxia, and the change ratio was 60.62 %. Compared with that in control group, HPV was increased with significant difference ($P<0.05$). In diethylcorbamazine group, $\triangle P_{pa}$ and its change ratio were lower than those in control group during hypoxia ($P<0.05$). In combined drugs group, $\triangle P_{pa}$ was lower than that in control group during hypoxia and the difference was significant ($P<0.05$, table 4).

Table 4 The change of P_{pa} during hypoxia after administration of indomethacin and diethylcorbamazine

Groups	Before hypoxia (mmHg)	During hypoxia (mmHg)	Change value (mmHg)	Change ratio (%)
Control	16.13±0.76	23.75±1.29	7.62±0.80	40.00±12.94
Indomethacin	19.25±0.87	30.75±1.26	11.50±1.02$^{\triangle}$	60.62±17.26$^{\triangle}$
Diethylcorbamazine	16.95±2.30	20.69±0.65	3.74±0.39$^{\triangle\triangle}$	22.06±6.50$^{\triangle\triangle}$
Combined drugs	18.44±0.46	22.75±0.73	4.31±0.56$^{\triangle\triangle}$	23.37±8.98$^{\triangle\triangle}$

$^{\triangle}P<0.05$ $^{\triangle\triangle}P<0.005$ as compared with control group

3 DISCUSSION

PGs and LTs, the metabolites of arachidonic acid, possess high biological activity. Under physiological conditions, the vasoconstriction of TXA_2 and LTs and the vasodilating effect of PGI_2 are in balance. Under HPV, those substances will increased and the balance will be upset. Hu[1] reported that hypoxia could augment TXA_2, PGI_2 and its ratio in cultured pulmonary arteriolar smooth muscles of rabbits. In this study, TXB_2, 6-keto-$PGF_{1\alpha}$ and its ratio were increased in serum and lung tissue during hypoxia.

Prostaglandins administered by perfusion[2,3] or inhalation[4,5] could attenuate HPV in some experiments. Gordon[6] proved that HPV was attenuated after administration of cyclooxygenase inhibitor indomethacin in isolated lung experiment. In this study, HPV was augmented after use of indomethacin to inhibit the synthesis of PGs *in vivo* and *in vitro*.

Some researchers[7] reported that LTs mediated HPV, but conflicting results[8] were also reported. In our experiment, after LTs blocker was employed *in vivo* and lipoxygenase inhibitor was applied to prevent the synthesis of LTs, HPV was decreased, suggesting that LTs are involved in HPV responses. After administration of cyclooxygenase inhibitor indomethacin and lipoxygenase inhibitor diethylcorbamazine to block the synthesis of PGs and LTs *in vitro* simultaneously, HPV was also attenuated and the results were similar to those in diethylcorbamazine group.

Our study suggested that LTs could mediate the HPV response and PGs were mainly responsible for the modulation of HPV in Wistar rats and in isolated lung.

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