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Integrative Clinical Medicine and Therapeutics

RESEARCH ARTICLE ISSN: 2689-9361

The mechanism of labetalol hydrochloride about fetal lung maturity in preeclampsia

Ruijing Chang and Hong Xin*

Department of Obstetrics, The Second Hospital of Hebei Medical University, Shijiazhuang City, Hebei Province, China

Abstract

Objective: Preeclampsia, is a pregnancy-specific and common syndrome, seriously threatening to the life of pregnant women and the fetus, as iatrogenic premature delivery, fetal lung maturity affects survival ability, fetal lung maturity promotion is often required before termination of pregnancy. In this study, the effect of labetalol hydrochloride on fetal lung structure and lung surfactant protein was studied in rats.

Methods: In this study, we conducted drug experiments in preeclampsia model rats, Lung protein expressions of T1α, SP-A, SP-B, SP-c and SP-D, PCR mRNA and HE staining was observed in four groups of fetal rats, including control group, preeclampsia group, labetalol group, dexamethasone group, labetalol group and dexamethasone combination group. To clarify its affection, further explore the effects of labetalol on fetal lung development, and explore the key molecular pathways of mechanism, so as to solve the current situation of single clinical application of glucocorticoid to promote fetal lung maturation and provide a new theoretical basis in fetal lung maturity.

Results: Labeolol hydrochloride could promote the mRNA and protein generation of lung surface active proteins T1α, SP-A, SP-B, SP-c and SP-D in preeclampsia fetal rats and increases the density blood vessels and development alveoli of pulmonary. The affection of combined hormone therapy was more obvious, and there was statistical significance ($P<0.05$).

Conclusion: labetalol hydrochloride can promote lung surfactant protein in fetal rats with preeclampsia to some extent, and contribute to lung maturation, which needs further clinical verification.

Keywords: *labetalol hydrochloride; preeclampsia; fetal lung maturation; pulmonary surfactant; dexamethasone*

Introduction

Preeclampsia is an idiopathic and common complication during pregnancy. It is a disease that seriously threatens to maternal and infant health. Combined with the injury critical of organ system, it is diagnosed as severe preeclampsia, which can cause damage multiple organs in the body, such as heart, brain, and liver, kidney and placental functions, leading to high morbidity maternal and perinatal babies, which is a important cause of maternal death in developed and developing countries. High-risk factors include obesity, advanced maternal age, maternal diabetes, ethnicity, antiphospholipid antibody syndrome/thromboembolism, family history of preeclampsia, etc. which has been widely concerned in perinatal medical research, but the reason is still unclear.

In recent years, how to minimize the threat to the lives of pregnant women and reduce the perinatal mortality in clinical research has become widely concerned worldwide. Severe preeclampsia before 34 weeks is also called early-onset severe preeclampsia [1]. This complicated problem in clinical practice, which seriously threaten to maternal and fetus, accompanied with high mortality. Due to its pathogenesis is not clear, whether continue or termination of pregnancy, necessary to consider the physical condition of the maternal and the situation of the fetus intrauterine. When a pregnant woman suffers severe damage to essential organs or babies, endangers the safe of life, only termination of pregnancy is the definite treatment method, which will lead to birth rates rise of iatrogenic preterm delivery. Due to the immature respiratory system of premature infants, the active substances of the alveolar surface, construct the structural and functional of the fetal lungs in uterus, useful for gas exchange rapidly and successful after birth, less than normal. If the fetal lung developed abnormally, less lung surfactant, may easily cause insufficiency of ventilation and newborn breathing distress syndrome (NRDS). NRDS is the cause of death in preterm infants, closely related to the lack of synthesis and secretion of pulmonary surfactant (PS) by alveolar type II epithelial cells, even affect the physiological functions of the respiratory system after grow up.

The treatment for preeclampsia is aimed to prevent and control the development of complications in Current studies. The doctors should terminate the pregnancy promptly as severe complications. The unmatured lungs of iatrogenic preterm infants, sensitive to the intrauterine environment, increase the risk of respiratory distress. The study was mainly used to study the effect of labetalol on the structure of rat fetal lung tissue, especially surfactant protein.

There are fewer studies about labetalol hydrochloride promoting fetal lung maturation. The mechanism and molecular pathway of drug hydrochloride promoting fetal lung maturation are still unclear. Possibly because part of labetalol contains β -adrenergic receptor agonists [2], which stimulate the β-adrenergic receptors on alveolar type II cells to play a role. In this study, pregnant rats (SD rats) were used as the object to prepare lung tissue models of preeclampsia. According to different drugs to rats, normal saline (control group), preeclampsia group, labetalol, dexamethasone, labetalol, and dexamethasone, compare the morphological and protein expression of the fetal lung, and evaluate the effects of labetalol hydrochloride in lung structure, physiological function and protein expression in severe preeclampsia for study the molecular mechanism. The comprehensive evaluation of the effect of labetalol is a new idea for the application of drugs to promote fetal lung maturation.

Materials and methods

Establishment of model rat of preeclampsia

The model of preeclampsia was established. The rats were intragastrically administered 0.3 ml of normal saline containing 50 mg/kg L-NAME daily from GD14 to GD17 and measured the artery pressure of tail artery once a day. Performed a caesarean section on the GD17 of pregnancy. Fetus retrieval from the uterus. Measurement method: Preheat the pregnant rats in preheating box to 38-40°C and used AD Instrument MLl25 to measure the blood pressure after the

rats were quiet. Each rat was measured three times and calculated the mean value. The blood pressure was around 150 mmHg, which proved that the modelling was successful [3].

Main instruments and reagents

Paraffin microtome, model RM2235, manufactured in Germany; electric heating constant temperature blast drying oven, model QH01-9030A, manufactured in Shanghai; ultrapure water system, model NW10LVF, manufactured in Hong Kong; microscope, model BX53, manufactured in OLYMPUS, Japan; microscope camera system, model DP73, manufactured in OLYMPUS, Japan. PCR reagents were purchased from BioTeke Company, manufactured in Beijing. The whole protein extraction kit, BCA protein concentration determination kit, goat anti-rabbit IgG-HRP, internal reference antibody β-actin were purchased Wanleibio, protein T1α, SP-B, SP- C purchased from ABclonal Company, and protein SP-A and SP-D were purchased from Affinity Company. Labetalol hydrochloride is produced by Jiangsu Desano Pharmaceutical Co., Ltd.

Experimental methods and testing indicators

In this study, drug experiments were conducted in preeclampsia model rats, Control group, preeclampsia group, labetalol group (preeclampsia + labetalol group), dexamethasone group (preeclampsia + dexamethasone group), labetalol and dexamethasone combined treatment (preeclampsia + dexamethasone + labetalol group) group were used to tested the expression of lung type I cell marker protein T1α and lung type II cell marker protein SP-A, SP-B, SP-C and SP-D, mRNA expression level was detected by RT-PCR, and HE staining on the pulmonary alveolar of fetal rats to observe the alveolar tissue and blood vessels of fetal rat to observed the effect of labetalol.

Experimental method

Sprague-Dawley rats purchased from the Animal Center of Wan Lei Biological Company, Rats were aged 12-14 weeks and weighed 200-300 g. They were kept in clean cages, at temperature (22 ± 1℃) and humidity 45-55% with 12 h/12 h light dark cycle, free to eat and drink. The SD rats were placed in a cage at a ratio of 3:1 to males for mating after one week, the female rats were subjected to vaginal smear observation next day, and the smear field was entirely covered by sperm. The female in the cage were regarded as pregnant rat. The day before the cage was the first day of pregnancy, and the GD14, the pregnant rats were randomly divided into five groups, each group have three rats, the control group, preeclampsia group, labetalol group, dexamethasone group, labetalol+ dexamethasone group. Blank control group: The rats were raised and reproduced commonly, and the lung tissues of the fetus rats for testing. Preeclampsia group: Rats were intragastrically administered 0.3 ml of normal saline containing 50 mg/kg L-NAME daily from GD14 to GD17. After the treatment, a cesarean section was performed on the GD17, and the lung tissue of fetus was taken out for testing. Labetalol group: Rats were intragastrically administered with 0.3 ml of normal saline containing 50 mg/kg L-NAME on the GD14, 4 mg/kg labetalol +0.3 ml of normal saline containing 50 mg/kg L-NAME daily from GD15 to GD17; cesarean section was performed on the GD17, and the lung tissue of the fetus was taken out for testing. Dexamethasone group: Rats were intragastrically administered with 0.3 ml of normal saline containing 50 mg/kg L-NAME on the GD14, 0.3 ml of normal saline containing 50 mg/kg L-NAME, and 0.4 mg/kg of dexamethasone intramuscular injection daily on the GD15 to GD17; after the treatment, cesarean section was performed on the GD17, and the lung tissue of the fetus was taken out for testing. Labetalol + dexamethasone group: Rats were intragastrically administered with 0.3 ml of normal saline containing 50 mg/kg L-NAME on the GD14, and 4 mg/kg Labetalol by intragastric administration on the GD15 to GD17 daily + 0.3 ml of normal saline containing 50 mg/kg L-NAME, and intramuscular injection of 0.4 mg/kg dexamethasone. After the treatment, cesarean section was performed on the GD17, and fetal lung tissue was removed for subsequent detection.

PCR: Primer sequence

T1αF: GGGGAGCGTTTGGTTCTG; T1α R: TCCGTGGTTTCTATTCTGTCTT;
SP-A F: GCTCCAGACTGAACTCTATGA; SP-A R: AATGTTGCCTCCTGCTCT;
SP-B F: AGCCTGGAGCAAGCGATAC; SP-B R: AAGCGCTTCCTTGGTCATC;
SP-C F: CTTGTCGTCGTGGTGATT; SP-C R: GGTAGCGATGGTGTCTGT;
SP-D F: GAGGATGCCAAGGAGATG; SP-D R: AGCCCAGTTAGAATAGACCAG;
β-actin F: GGAGATTACTGCCCTGGCTCCTAGC;
β-actin R: GGCCGGACTCATCGTACTCCTGCTT.

RT-PCR

Total RNA extraction: (1) Add 1ml of TRIpure lysis solution to the sample, fully mixed, and stood for 5 minutes at room temperature; (2) Add 200 μl of chloroform to the solution, mix it upside down, and stand for 3 minutes at room temperature; (3) Centrifugation at 4℃ 10000 g for 10 minutes, the sample were divided into three layers: intermediate layer and colorless water phase, and the water phase transferred to a new centrifuge tube; (4) Add isopropyl alcohol with the same volume as water, mix it upside down, and place it at -20℃ overnight; (5) After 15h, centrifuge at 4℃, 10000 g for 10 minutes, discard the supernatant; (6) Add 1 ml 75% ethanol, centrifuge 3400 g for 3 minutes at 4℃, discard the supernatant (7) Stand at room temperature for 5-6 min to let the residual ethanol volatilize; (8) Add 30 μl RNase-free ddH_2O, place at room temperature for 2 minutes, mix thoroughly, precipitate completely dissolved and obtained total RNA. The concentration of RNA five samples measured by the NANO 2000 UV spectrophotometer.

Reverse Transcription: The RNA obtained was transcribed into cDNA: (1) The following RNA samples were added into the nuclease free centrifuge tube in the ice bath to ensure consistent RNA concentration: Oligo (dT)15 1 μl; The random 1 μl; Add ddH_2O to 12.5 μl; (2) Heated at 70℃ for 5 minutes, cooled on ice for 2 minutes, centrifuged briefly, then added: dNTP(2.5 mM each) 2 μl; 5 x 4 ul Buffer; 0.5 ml Rnase inhibitor; (3)1 μl (200 U) M-MLV, gently mix with pipette; (4) warm bath at 25℃ for 10 minutes, warm bath at 42℃ for 50 minutes; (5) The reaction was terminated by heating at 80℃ for 10 minutes.

Real-time fluorescence quantitative analysis

Reaction system: cDNA template 1 μl; upstream and downstream primers (10 μM) each 0.5 μl; SYBR GREEN mastermix 10 μl; ddH_2O supplement to 20 μl; Reaction conditions: Incubate at 94℃, for 5 minutes; Incubate at 94℃, for 10 s; Incubate at 60℃, for 20 s; Incubate at 72℃, for 30 s; Scan; Go to line 2, Cycle 40; Incubate at 72℃, for 2 minutes 30 s; Incubate at 40℃, for 1 minute 30 s; Melting 60℃ to 94℃, Every 1℃, for 1 s; Incubate at 25℃, for 1-2 minute; Quantitative analysis was performed by Exicycler TM 96 fluorescence quantitative instrument of BIONEER.

Westernblot: Protein extraction; add the lysed sample and let it on ice for 5 minutes. Standard curve preparation for protein quantification: Dispense 0.5 μg/μl BSA protein standard solution was divided into 0, 1, 2, 4, 8, 12, 16, 20 μl into the microtiter plate, and filled with PBS buffer solution to 20 μl. Preparation of protein test solution: mix 1 μl of protein extract with 19 μl PBS buffer. BCA reaction: the volume of BCA working liquid required for this experiment was estimated at 200 μl per well according to the volume ratio of liquid A to liquid B of 50:1, add to each well, mix thoroughly, and react at 37℃ for 20 minutes, the solution changed from green to purple. Data reading: Set the reading wavelength of 570 nm and record the data. The standard curve was drawn with the standard protein

concentration and the corresponding absorbance value, and the protein concentration of the sample was calculated by regression equation, and the protein concentration of the experimental group was calculated. The concentration of SDS-PAGE concentrated gel is 5%, and separation gel is 12%. Connect the electrode, current to maximum, voltage 80 V, constant pressure electrophoresis 2.5 h. Transfer: Voltages 80 V transfer 1.5 h. Blocking: incubate the primary antibody, incubate the secondary antibody (Table 1 and 2), ECL substrate luminescence, antibody stripping, blocking, incubate the secondary antibody, ECL substrate luminescence, film scanning, gel image processing system (Gel-Pro -Analyzer software) to analyze the optical density value of the target band, and analyze the results.

Primary	Secondary antibody	Dilution	Incubation
T1α	Goat Anti-Rabbit IgG-HRP	1:5000	37℃ 45min
SP-A	Goat Anti-Rabbit IgG-HRP	1:5000	37℃ 45min
SP-B	Goat Anti-Rabbit IgG-HRP	1:5000	37℃ 45min
SP-C	Goat Anti-Rabbit IgG-HRP	1:5000	37℃ 45min
SP-D	Goat Anti-Rabbit IgG-HRP	1:5000	37℃ 45min
β-actin	Goat Anti-Rabbit IgG-HRP	1:5000	37℃ 45min

Table 1: Incubation conditions of secondary antibody

Internal	Dilution ratio	Incubation
β-actin antibody	1:1000	4℃ overnight

Table 2 : Incubation conditions of control antibody

Histological analysis: Take lung tissue specimens of fetal rat, cut into 5 μm paraffin sections, stained with hematoxylin and eosin, then perform routine morphological evaluation under an optical microscope (Olympus DP73; Olympus, Tokyo, Japan).

The data were shown as the mean ± standard deviation. Data from the study were analyzed using one–way ANOVA followed by LSD-t test to assess the significance. P-values<0.05 were considered statistically significant. All the analyses were performed using SPSS for software (Version 22.0).

Results

Rat preeclampsia model established. Rats were intragastrically administered 0.3 ml of normal saline containing 50 mg/kg L-NAME daily on the GD14 to GD17, and the tail artery pressure was measured once a day. On the GD17, Caesarean section was performed on the 17th day of gestation. Measurement methods: The quantity of pregnant rats was preheated to 38-40℃ in the preheating box, and then measured with AD Instrument MLl25 after they are quiet. Each rat was measured three times and the average value was recorded. The L-NAME group was significantly higher than the normal pregnancy group (control group). The blood pressures measured was about 150 mmHg, indicating that the model was established successfully established.

Westernblot

The results of westernblot electrophoresis of each group of fetal rats are shown in (Figure 1A). It can be seen that the expression trends of T1α, SP-A, SP-B, SP-C, SP-D in each group of fetal rat lung tissue proteins, and software analysis of gray level, and data analysis show in (Table 3A-3E), expressed by a bar graph, Show in (Figure 1B).

Westernblot results the protein expression levels of T1α, SP-A, SP-B, SP-C, SP-D in the lung tissues of fetal rats in the five groups were analyzed by gray scale analysis, and the levels in the dexamethasone + labetalol group higher than those in the control group. And it is statistically significant. In the preeclampsia group, the protein expression tended to decrease, but there was no statistical significance (P>0.05). Dexamethasone significantly enhanced the protein expression, and the effection was more obvious in the dexamethasone and labetalol group, with statistical significance (P<0.05) (Figure 1B).

Real-time fluorescence quantitative results

Protein primers for T1α, SP-A, SP-B, SP-C, SP-D (see above) were customized for real-time fluorescence quantitative analysis, each group was repeated three times. The measured values of each group were counted and the mean value of blank △Ct was calculated. According to Ct mean and standard deviation results of relative expression level 2-△△ (Table 4A-4E), the changes of protein mRNA content in each group were analyzed (Figure 2).

Results

In the real-time fluorescence quantitative analysis, the relative expression of level decreased in the preeclampsia group, while the mRNA levels of T1α, SP-A, SP-B, SP-C, and SP-D protein increased in the dexamethasone group. and significantly increased in the labelol + dexamethasone group, which was higher than the normal group. And it was statistically significant. The statistical results showed that the P value is 0.000, less than 0.05, and the differences among the groups were statistically significant.

Changes of lung tissue structure in fetal rats

The alveoli represented in the black frame, which separated with blood vessels and connective tissue. The red frame represents the blood vessel; the blue frame represents the trachea, and the arrow show the blood vessel.

The results show: In the normal group, the alveoli were full and well developed on GD17, and with thin interalveoli, while in the preeclampsia group, alveolar septum thickening, and alveolar tissue collapse were observed. No significant changes were observed in the alveolar structure after labetalol treatment, but in the dexamethasone treatment group, pulmonary interstitial tissue blood supply was increased. In the labetalol + dexamethasone group, the alveolar tissue was fuller, the vascular density of alveolar interstitium increased and the blood supply increased. The number of bronchioles increased significantly, the alveolar space expanded, and the alveolar interval is thinner than that in preeclampsia (Figure 3).

Discussion

Preeclampsia is a heterogeneous, multi-system disorder, defined by evidence of new-onset hypertension and proteinuria or organ dysfunction after 20 weeks of gestation, with a worldwide incidence of 2% to 5% [4,5]. Its incidence is associated with iatrogenic premature delivery, early gestational age, placental abruption, and high perinatal mortality, accompanied by significant maternal morbidity and mortality [6]. The survival and mortality rate of hospital premature infants are high by antihypertensive treatment, spasmolysis, sedation, glucocorticoid to promote fetal lung maturation and symptomatic treatment.

At the primal alveolar stage after 24 weeks, the human embryo begins to secrete lung surface active protein. At 26 weeks, type I alveolar cells develop, and the capillary network in the perialveolar mesenchymal hyperplasia occurs. At 28 weeks, the lung is not fully developed, and type I alveolar and type II cells appear. At 31 weeks, lung function was close to maturity and respiratory ability was achieved. At 32-34 weeks, breathing ability reached the peak. Fetal lung development at 35 weeks is almost complete [7]. Respiratory distress syndrome under 32 weeks is common. Then abnormal condition such as asphyxia, hypoxia, acidosis, insufficient lung perfusion, hypothermia, maternal diabetes, thyroid function, and adrenal cortex function, can delays or influence the synthesis, release, and transport of surfactants.

Figure 1A

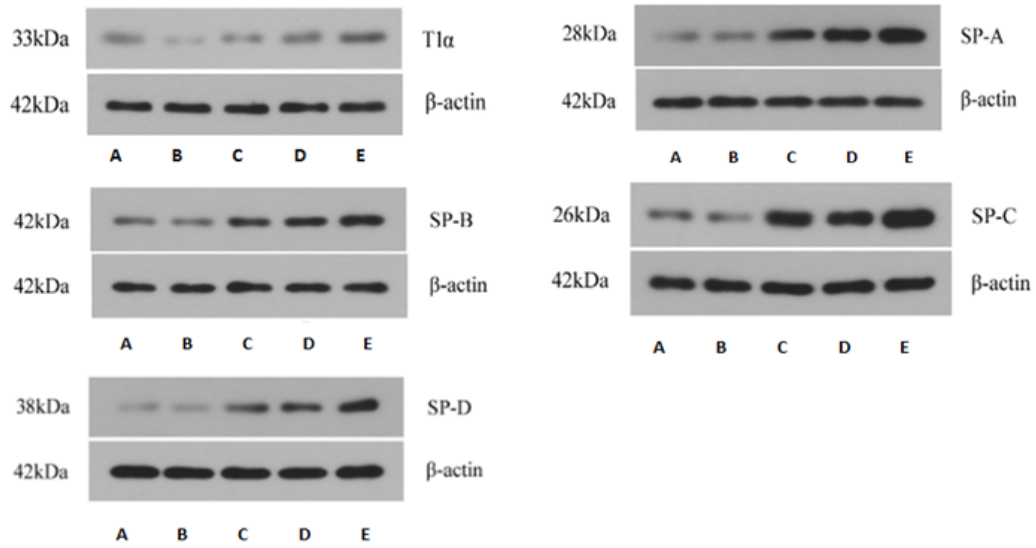

Figure 1B

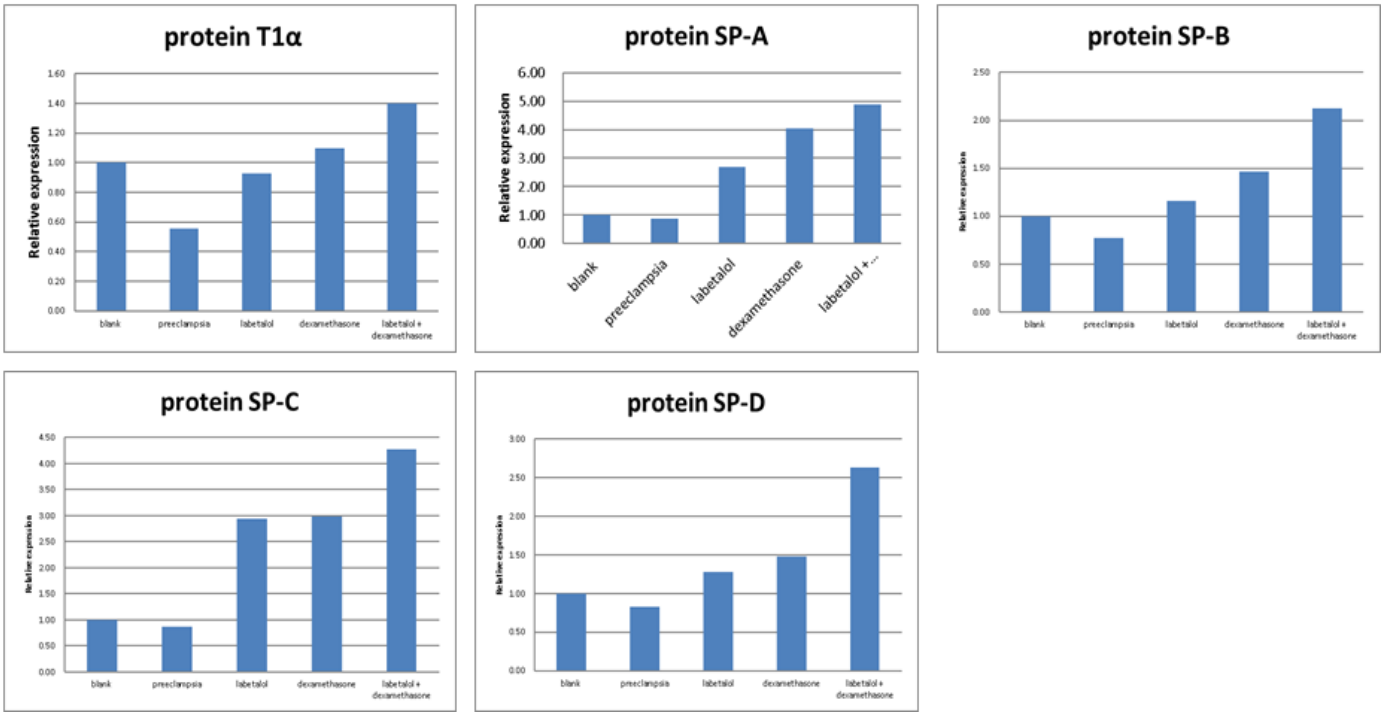

Figure 1: **Figure 1A:** A: Blank group, B: Preeclampsia group, C: Labetalol group, D: Dexamethasone group, E: Labetalol + dexamethasone group, compare the expression level protein T1α in groups, SP-A, SP-B, SP-C, SP-D adjusted β-actin in fetus lung tissue. **Figure 1B:** Westernblot results the protein expression levels of T1α, SP-A, SP-B, SP-C, SP-D in the lung tissues of fetal rats in the five groups were analyzed by gray scale analysis, and the levels in the dexamethasone + labetalol group higher than those in the control group. And it is statistically significant. In the preeclampsia group, the protein expression tended to decrease, but there was no statistical significance ($P>0.05$). Dexamethasone significantly enhanced the protein expression, and the effection was more obvious in the dexamethasone and labetalol group, with statistical significance ($P<0.05$).

Table 3A

Group	T1α	β-actin	ratio	Relative expression
Blank	274.01	494.89	0.55	1.00
Preeclampsia,	145.72	475.54	0.31	0.55
Labetalol	250.45	486.75	0.51	0.93
Dexamethasone	290.83	477.41	0.61	1.10
Labetalol+dexamethasone	366.69	472.71	0.78	1.40

Table 3B

Group	SP-A	β-actin	ratio	Relative expression
Blank	256.21	656.05	0.39	1.00
Preeclampsia,	225.43	654.13	0.34	0.88
Labetalol	655.27	621.71	1.05	2.70
Dexamethasone	978.46	619.04	1.58	4.05
Labetalol+dexamethasone	1167.4	613.51	1.90	4.87

Table 3C

Group	SP-B	β-actin	ratio	Relative expression
Blank	278.73	490.68	0.57	1.00
Preeclampsia,	220.16	501.82	0.44	0.77
Labetalol	347.95	526.61	0.66	1.16
Dexamethasone	432.82	520.65	0.83	1.46
Labetalol	621.99	515.54	1.21	2.12

Table 3D

Group	SP-C	β-actin	ratio	Relative expression
Blank	269.54	721.42	0.37	1.00
Preeclampsia,	234.39	727.29	0.32	0.86
Labetalol	804.91	734.07	1.10	2.93
Dexamethasone	793.19	711.83	1.11	2.98
Labetalol	1154.9	722.07	1.60	4.28

Table 3E

Group	SP-D	β-actin	ratio	Relative expression
Blank	226.75	825.89	0.27	1
Preeclampsia,	185.86	821.82	0.23	0.82
Labetalol	287.05	818.03	0.35	1.28
Dexamethasone	334.98	824.13	0.41	1.48
Labetalol	584.21	807.18	0.72	2.64

Table 3: Expression trends of T1α, SP-A, SP-B, SP-C, SP-D in each group of fetal rat lung tissue proteins, and software analysis of gray level, and data analysis

At present, the treatment methods, and drugs for promoting fetal lung maturation mainly include: 1. Glucocorticoid drugs: dexamethasone and betamethasone，it is widely used in clinical trials and has excellent effect on promoting fetal lung, reducing the incidence and mortality of NRDS（neonatal respiratory distress syndrome，NRDS）in preterm infants. The main mechanism is to bind to specific receptors of alveolar type II cells, produce a variety of glucocorticoid-related proteins, promote the synthesis and release of alveolar surfactant, reduce the osmotic pressure of pulmonary capillaries, reduce pulmonary edema, accelerate the development and maturity of pulmonary antioxidant enzyme system, and improve alveolar function. 2. Ambroxol hydrochloride: Promote the expression of fatty acid synthase gene in alveolar type II epithelial cells [8], increases the production of pulmonary surfactant, reduces alveolar surface tension and prevents alveolar collapse, inhibits the activity of phospholipase A in alveolar macrophages lysozyme, and reduces the decomposition of active substances; Reduces fluid exudation and edema, promote fetal lung maturation and development. Some studies [9] found that medication was significantly superior to dexamethasone in preventing respiratory distress syndrome in premature infants born before 31 weeks. 3. Pulmonary surfactant (PS) preparations: injection of the amniotic fluid around the fetal mouth and nose through amniocentesis, improve the ventilator-ventilation function of fetal lung, so as to prevent the occurrence of post-natal respiratory distress syndrome. In addition, aminophylline can promote fetal lung maturation [10].

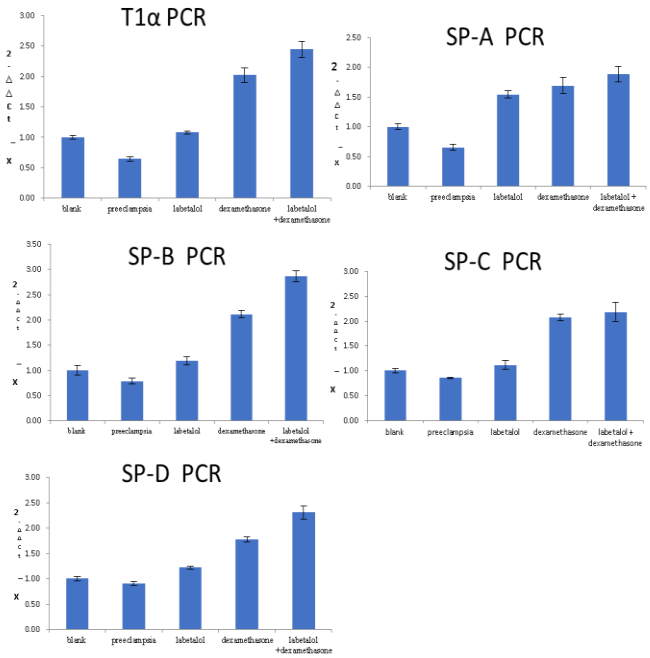

Figure 2: Real-time fluorescence quantitative results of mRNA levels of each protein were compared with the mean values of 2-△△Ct

Table 4A

Group（T1α）	$2^{-\Delta\Delta Ct}$mean value	STDEV
Blank	1.00	0.03
Preeclampsia,	0.65	0.04
Labetalol	1.08	0.02
Dexamethasone	2.02	0.12
Labetalol + dexamethasone	2.44	0.14

Table 4B

Group（SP-A）	$2^{-\Delta\Delta Ct}$mean value	STDEV
Blank	1.00	0.05
Preeclampsia,	0.65	0.05
Labetalol	1.54	0.06
Dexamethasone	1.69	0.14
Labetalol + dexamethasone	1.89	0.13

Table 4C

Group（SP-B）	$2^{-\Delta\Delta Ct}$mean value	STDEV
Blank	1.00	0.10
Preeclampsia,	0.79	0.06
Labetalol	1.19	0.07
Dexamethasone	2.11	0.07
Labetalol + dexamethasone	2.86	0.11

Table 4D

Group（SP-C）	$2^{-\Delta\Delta Ct}$mean value	STDEV
Blank	1.00	0.04
Preeclampsia,	0.86	0.02
Labetalol	1.12	0.09
Dexamethasone	2.07	0.06
Labetalol + dexamethasone	2.18	0.20

Table 4E

Group（SP-D）	$2^{-\Delta\Delta Ct}$mean value	STDEV
Blank	1.00	0.04
Preeclampsia,	0.91	0.04
Labetalol	1.22	0.03
Dexamethasone	1.78	0.05
Labetalol +dexamethasone	2.31	0.13

Table 4: Mean and standard deviation results of relative expression level 2-△△

With the application of glucocorticoid in pregnancy, more and more studies have been conducted on the safety of glucocorticoid and its short-term and long-term effects on neonates. Studies have found that the drug not only affects lung morphology, but also has some side effects: For example (1) Although dexamethasone can promote the maturation of type II alveolar cells, promote the development of alveolar lung, and partially inhibit the formation of the second diaphragm, it also leads to the thinning of the biliary capillary ring in the balloon stage, which accelerates the alveolar differentiation and reduces the final number of alveoli; (2) Increase the growth of the fetal body wall and affect lung growth; (3) The large dose dexamethasone can inhibit the growth of premature infants physical and neurological development, characterized by low birth weight, small head circumference, sepsis, premature adrenal function inhibition, etc., increasing the incidence of maternal with chorioamnionitis endometritis, inhibition of maternal adrenal axis function, leading to elevated blood glucose during pregnancy, etc; (4) There are placental aging, some maternal (such as diabetes, hypertension, maternal infection) use restrictions and other problems; (5) Neves FM [11] reported that dexamethasone may have a quantitative effect on erythrocytes and megakaryocytes and neonatal hematological characteristics of the fetus after maternal dexamethasone treatment. Glucocorticoids inhibit cell growth and DNA replication. Animal

studies have shown that corticosteroids can inhibit fetal growth, increase fetal blood pressure, and improve neurodevelopment at lowest effective dose [12]. Reduce fetal growth and brain development and increase the risk of infants suffering from diabetes and hypertension [13]. The short-term effect of prenatal hormones on the fetus is to reduce the respiration and movement of the fetus and the volume of amniotic fluid [14]. Fetal exposure to cortisol with high levels in the environment comparison normal low levels of cortical, combined with other stress hormones produced by growth restriction, may have an impact on the life of fetus, leading to potential adult fetal diseases, such as hypertension, insulin resistance, diabetes, and metabolic syndrome[15], In addition, maternal corticosteroid administration (oral, intramuscular or intravascular) may produce maternal side effects, such as increased maternal blood pressure and blood glucose concentration, and increase the susceptibility to sepsis [16]. (6) Other influences, etc.

Due to the high mortality rate of premature infants, long-term adverse health effects, increased clinical resources and economic burden. Glucocorticoids promote fetal lung maturation by increasing protein and phospholipid synthesis and increasing the amount of surfactant in the fetal lung, but the short- and long-term safety of treatment in multiple corticosteroids remains controversial.

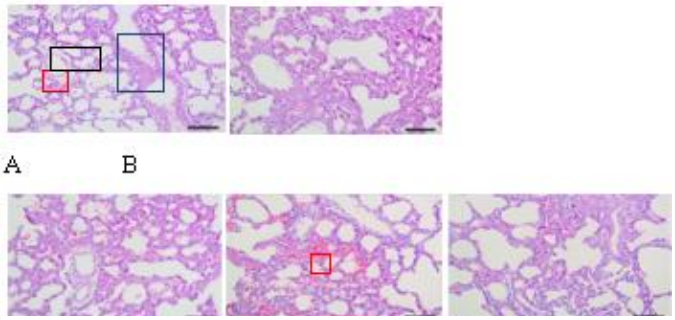

Figure 3: HE, ×200, scale: 100um: A, Blank group; B, Preeclampsia group; C, labetalol group; D, Dexamethasone group; E, Dexamethasone + labetalol group

Pulmonary surfactant is synthesized by alveolar type II epithelial cells. The main surfactant, dipalmitoylphosphatidylcholine, acts synergically with surfactant-related proteins to reduce alveolar surface tension, which Critical for lung structure and respiratory function. There are four subtypes of lung surfactant protein: SP-A, SP-B, SP-C, and SP-D [8]. Synthesis in fetal lung tissue is regulated by a variety of hormones and factors, including glucocorticoids, prolactin, insulin, estrogen, androgens, thyroid hormones and catecholamines. The main chemical components are lecithin, protein, and carbohydrates, the phospholipid content is 85%-90%, and the protein is 8%-10%. There are four kinds of specific proteins, including small hydrophobic LUNG surfactant proteins B and C with molecular weights of 6-8kD and 3-5kD, and large hydrophilic lung surfactant proteins A and D with molecular weights of 28-36kD and 43KD, respectively. The molecular weights of T1α, SP-A, SP-B, SP-C and SP-D antibodies in rats were 33KD, 28KD, 42KD, 26KD and 38KD, respectively. The affection of SP-A include ① It is involved in the formation and metabolism of alveolar surfactant membrane; ② The level of lung surfactant in and outside cells was stable, and the content of lung surfactant in alveoli was stable; ③Increase the antioxidant capacity of alveolar type II epithelial cells; ④ Participate in cellular immunity, the immune cells (especially mononuclear macrophages, neutrophils) have chemotactic and regulation phagocytosis to immune cells; ⑤ Inhibit T cell proliferation, reduce pulmonary allergic reaction or other toxic substances in the lung allergy. SP-B and SP-C play a crucial role in reducing lung surface tension [17-19] Hwang *et al*. The biophysical affect of exogenous surfactant preparation sp-B and SP-C on premature rabbits [20]. In vivo experiments, adding SP-B to natural surfactant preparations containing SP-B further improved its function [21].

The genes encoding SP-A, SP-B and SP-C are independently regulated during fetal lung tissue development. The gene expression of the SP-A occurs entirely in type II cells and was activated after 75% of gestation. The expression of SP-B and SP-C genes was detected earlier than that of SP-A during the development of human fetal differentiated type II cells before emergence. Glucocorticoids and cAMP synergically promoted the transcription of SP-A genes in human fetal lung. In the study of human fetal lung organ culture, glucocorticoids combined with prolactin and/or insulin can increase the synthesis rate of lamellar phosphatidylcholine and change the composition of lamellar glycerol, making it a reactant of surfactant secreted by human fetal lung [22]. T1α protein is expressed in I type cells; expression increase may reflect the increase the number of type I cells and increase in expression per cell.

Labetalol, commonly used as antihypertensive drug in preeclampsia, which is a combination of α-and β-adrenergic receptor antagonists to reduce blood pressure by reducing peripheral vascular resistance. It has been used in clinically many years. Clinical application for many years, the drug in the United States in 1984 was approved, is a kind of alpha and beta adrenergic 1 antagonist, selective and competitive alpha 1-adrenergic antagonism and nonselective, competitive beta adrenergic (beta 1 and beta 2), laboratory analysis estimated after oral and intravenous administration of alpha beta blocker activity than approximately 1:3 and 1:7. It is indicated for the treatment of arterial hypertension, from acute hypertensive crisis to stable chronic hypertension. In clinical practice, it is safe to use during pregnancy in patients with severe hypertension [23]. Eur Heart *et al*. [24] studies have found that there are fewer complications in pregnant women and neonates during its clinical use, and many animal and clinical studies have shown that its application can improve fetal intrauterine growth restriction, resulting in fewer fetal heart abnormalities and other complications. There was no significant change in the mean systolic/diastolic ratio (S/D) of uterine artery, umbilical artery and fetal middle cerebral artery [25]. Labetalol does not affect pregnant women, such as metabolic disorders, makes blood pressure drop dose-related, does not cause reflex tachycardia, decline heart rate. In some studies, the hemodynamic effect of Labetalol has changed to some extent, and cardiac output and total peripheral vascular resistance have not decreased significantly [23].

Labetalol is distributed in vivo. Studies in animals have shown that labeolol is well absorbed by all species by determination of total plasma radioactivity in mice, rats, rabbits, dogs and humans. There have the highest radioactive concentrations in the lung, liver, and kidney. Almost there is no radioactivity in the brain. Metabolized produce inactive glucuronic acid conjugates by the liver. If a large dose of drugs is given to patients with impaired left ventricular function, it may lead to the risk of acute left ventricular failure because of a certain negative inotropic effect [2].

Labetalol has some advantages in patients with preeclampsia, because compared with normal pregnant women, cerebral perfusion pressure in preeclampsia women is increased and brain autoregulation is impaired [26]. After oral labetalol treatment, blood pressure may decrease due to the decrease of peripheral arterial blood pressure, but cerebral perfusion pressure does not change significantly. It becomes an ideal drug for controlling blood pressure in pregnant women with severe hypertension. In animal experimental pregnant ewes, intravenous administration of labetalol improved the effects of circulating norepinephrine on maternal mean arterial pressure MAP,

uterine blood flow, fetal pH, and arterial partial pressure of oxygen (PaO_2), and the degree of adrenergic blockade in the fetus was lower than that in the mother [27]. There was no change in placental blood flow index despite a significant decrease in maternal blood pressure. This showed that vascular resistance in maternal to placental circulation could be reduced [28], fetal to placental vascular hemodynamic was not significantly affected [29]. It has also been studied that labetalol may impair uterine and placental hemodynamic in sheep models of increased placental vascular resistance. However, hypoxia stress can cause different effects of adrenergic receptor antagonists on fetal hemodynamic responses [30], So, in the condition of patients with preeclampsia, labetalol has different effects on the hemodynamic of the fetus. In the application of radioactivity labelling microspheres, the effect of the uterine wall and ovarian perfusion was significantly reduced, and the placental perfusion was not significantly changed after drug treatment [31]. In general, labetalol is an effective drug to blood pressure control which the dose not adversely affect the umbilical arterial blood flow waveform (UAFVW). It can safely prolong the time of patients who complicated with hypertension in pregnancy [32].

Clinically, these advantages can be used to improve the application of preeclampsia, which are safe and effective during pregnancy. Some clinical studies found that labetalol hydrochloride has a certain effect of promoting fetal lung maturity. Michael *et al.* [33] found that oral administration of labetalol can increase the amount of surfactant in fetal lungs from 31 weeks. The concentration of lecithin/sphingomyelin in amniotic fluid was higher than in normal gestational weeks. Nicholas *et al.* [34] found that labetalol increased the fetal pulmonary pressure-volume curve and phospholipid concentration in rabbits. Dai Wenjuan *et al.* found that a specific marker in alveolar type II epithelial cells in the SP-C labetalol intervention group, was significantly reduced in apoptosis of alveolar type II epithelial cells compared with the trauma group. Labetalol hydrochloride can reduce the release of adrenaline by inhibiting sympathetic nerve activity and reducing the apoptosis of alveolar type II epithelial cell to alleviate secondary lung injury caused by mechanical trauma. In addition, the "Obstetrics and Gynecology" [35] also mentioned that labetalol has the effect of promoting fetal lung maturity. It was found that there are few studies on this drug in severe preeclampsia and fetal rat lung maturation, and there are no systematic studies on the mechanism of action and signal pathway. In view of this, considering the effect of drug, combining with the clinical practice, consider whether clinical complications, especially diabetes, gestational diabetes, and the application of glucocorticoid for patients with contraindications, this drug can be used to improve blood pressure and promote fetal lung maturity, or in connection with glucocorticoid synergy is superior to the single application corticosteroids to promote fetal lung maturity, Increased survival of high-risk premature infants.

What is the exact mechanism of fetal lung maturation? What is the relationship with fetal lung maturity during pregnancy? It was found that β receptors are closely related to surfactant during fetal lung maturation closely. Animal studies have shown that the maturation of lung β receptors is consistent with the onset of surfactant (SAM, surface active material) flux in fetal lambs, and the maturation of both is faster in female lambs [36]. Estrogen in pregnancy promote the formation to release of surface-active phospholipids in rat fetal lung tissue [37]. It is suggested that the maturation of lung β -receptor and surfactant flux are regulated by hormonal factors. The rapid maturation of female lung β receptors and SAM fluxes may be one of the factors affecting lung surfactant maturation and premature survival. In vitro antihypertensive drugs have certain effects on the synthesis of placental hormones and angiogenic proteins in preeclampsia. Labetalol has β blockers, antagonistic α- and β-adrenergic receptors, and has direct vasodilatation activity [38]. In some β -blocker studies, glycogen levels in developing type II alveolar epithelial cells were significantly higher in propranolol-treated fetuses. It was found to inhibit the effect of ACTH on fetal lung maturation, suggesting that the mechanism of lung maturation is not only dependent on endogenous cortisol, but also mediated at least in part by adrenergic response [39]. However, some studies found that β-receptor blockers had not significant changes in fetal lung surfactants. It was found that bromopropyl propanol ethane (BrAlp), an irreversible β -blocker, did not affect the phosphatidylcholine composition, phosphatidylcholine synthesis rate, tissue total phosphatidylcholine content or saturated phosphatidylcholine content in lavage fluid. β -adrenergic receptor blockers did not produce significant changes in lung fluid volume at or after birth, regardless of delivery route [40]. The adrenergic system of human fetuses develops morphologically at early stage, And adrenergic receptors develop to a large extent before birth, β-receptor stimulants not only relax uterine smooth muscle, but also regulate the concentration of surfactant in lung fluid [41]. Only a few studies shown that labetalol has β-adrenergic receptor agonist effect on rat uterus in vitro [42]. So why not directly use β adrenergic agonists to promote fetal lung maturation treatment, what is its effect on the fetus? Beta-adrenergic agonists act in conjunction with glucocorticoids in the fetal lung. β -adrenergic receptors have been identified by radioligand binding and are present in the human fetal lung as early as 16 weeks of gestation. The time relationship between the increase of lung β -receptor and plasma free cortisol in pregnant fetal rabbits suggests that endogenous glucocorticoids may lead to the increase of lung β -receptor concentration. Treatment of pregnant rabbits at 24 or 25 days of gestation leads to an increase in precocity of β -receptor and agonist specific high affinity binding in fetal lung. Beta-adrenergic agonists can stimulate at least two developmental responses necessary for the fetal lung as a gas exchange organ, namely the release of surface-active substances and the reabsorption of alveolar liquid. The sensitivity of these reactions increases dramatically in late pregnancy. The increase of glucocorticoid receptor concentration was independent of other endocrine reactions, and 0.1 μM dexamethasone increased the concentration of β receptor at 24 and 48h in lung tissue culture of fetal rabbits. The effect of glucocorticoids on increasing β receptor concentration and high affinity binding may be one of the reasons for the increased lung β-adrenergic response in fetal lung, and it may be part of the reason for the decrease in neonatal respiratory syndrome after prenatal glucocorticoid treatment [43]. Direct application of β-adrenergic agonists may lead to arrhythmia, heart failure, pulmonary edema, and fetal tachycardia and other complications in some pregnant women. Adrenergic receptor agonists have been proven to increase fetal lung (Enhorning *et al.*, 1977), and type II cells haveβ-adrenergic receptors [34]. It was found that the elevation of umbilical cord PI in some fetuses after labetalol was caused by vasoconstriction of fetal placental circulation, suggesting that fetal β -block may occur after maternal treatment with labetalol [44]. The results showed that labetalol could affect the fetus, the uterine activity is decreased in a dose-dependent manner (Tocolytic response), and c-AMP level was increased simultaneously. It reduces uterine blood flow by 18%~30%, while labetalol only reduced uterine blood flow slightly (0~5%) [45]. Animal experiments on promoting fetal lung maturation: Labetalol has bidirectional, partial excitatory effect, and catecholamine effect can promote fetal lung maturation [46].

Labetalol may contain partial β-adrenergic agonists [2], which stimulate the beta-adrenergic receptors on alveolar type II cells to do

their job. Treatment with [47] chronic β-blocker has been affect on the clearance and storage of surfactant fluid in fetal lung, and β-agonists stimulate its secretion. So, is there an effect of the disease itself in people with preeclampsia? [33] Studies found that no neonatal hyaline membrane lesions were found during the treatment of gestational hypertension, which may be caused by stress caused by hypertension or fetal hypoxia (in this study, patients with gestational hypertension took labetalol orally for at least 4 weeks starting from 27 weeks). The effects of fetal labetalol exposure on preterm heart rate, blood pressure and cerebral oxygenation have not been confirmed. Severity of maternal disease appears to play a more important role in neonatal cerebral hemodynamic. Clinically, maternal use of labetalol has no obvious short-term side effects on premature infants [48]. It has also been reported that fetal lung maturity in patients with preeclampsia is not statistically significant with normal gestational age [49], and some studies have found that fetal lung maturity in severe preeclampsia is better than normal gestational age, which may be related to the intrauterine hypoxic environment in severe preeclampsia, and delayed lung maturity is more obvious in fetus with intrauterine growth restriction. Stimac *et al.* [50] found that laminar counts (LBCs) in amniotic fluid were significantly reduced at 31 to 36 weeks of gestation, suggesting delayed fetal lung maturation associated with preeclampsia. In the study of women with preeclampsia at 26-39 weeks of gestation, the median lamellar count was significantly lower at 31-33 weeks of gestation than in the fetal growth restriction group (P=0.022) and the normal group (P=0.031). At 34 to 36 weeks, it was significantly lower than that in the fetal intrauterine growth restriction group (P=0.026, P=0.004). Winn *et al.* studied that the ratio of lecithin to sphingomyelin L/S (lecithin/sphingomyelin) in amniotic fluid significantly decreased in severe preeclampsia at 33-36 weeks of gestation without intrauterine growth restriction, and the ratio was significantly lower than that in premature infants [51].

In this study, animal experiments were found that labetalol had certain effects on the secretion of lung surfactant and the expression of alveolar type I cells and type II cells related proteins in rats. Reduction of plasma and interstitial cells during the cystic phase (E17.5) in rat lung, type I and type II lung cells. According to our research results: In the control group (A), preeclampsia group (B), labetalol (C), dexamethasone (D), labetalol and dexamethasone group (E), the expression of SP-A mRNA level in the labetalol group and the dexamethasone group significantly increased. The mRNA levels of the other proteins were significantly increased in the dexamethasone group. This trend was more significant and statistically significant in the dexamethasone combined with labetalol group. After adjusting the β-actin level, the protein expression of each group showed A decreasing trend in preeclampsia. Compared with the control group, the protein expression of SP-A, SP-B, SP-C and SP-D in labetalol group increased significantly, and the protein expression increased more obviously in labetalol group and dexamethasone group. Animal experiments showed that labetalol can be used together with dexamethasone in preeclampsia pregnant rat and and had synergistic effect on lung maturation in fetal rat.

The results: HE staining showed that the fetal rat of GD17 normal group had well-developed alveolar with thinner interval, and preeclampsia group of alveolar growth visible thickening of alveolar interval, alveolar tissue collapse. The alveolar cavity increases after labetalol treatment of the preeclampsia group, but didn't change alveolar interval, and alveolar cavity fuller after treated with labetalol + dexamethasone. Alveolar septum vascular density and blood supply increased. Results in the study of Haviv *et al.* [52], it was found that glucocorticoids could enter the fetus through the placenta, which could thin the alveolar compartment, promote lung maturation and alveolar differentiation of the fetus, activate endothelium-type chlorosynthase, affect pulmonary blood flow. Increase the number of sodium ion channels in epithelial cells and promote lung maturation.

So, what is the structure of labetalol hydrochloride and how to metabolize in pregnant women and the effect on hemodynamic? Studies have shown that [53] labetalol hydrochloride has been shown to block α and β receptors. It consists of four optical isomers. Labetalol is absorbed quickly after oral administration, and the peak blood concentration is usually reached within 2 hours. Radiochemical analysis of the animals showed high levels of accumulation in the lungs, liver and kidneys and little in brain tissue. It is mainly eliminated by liver metabolism, and about 85% of the blood being eliminated in a single journey through the liver [54]. Labetalol is lipid soluble and has an absolute bioavailability of about 25%. There is no active metabolite, and the half-life of the drug is about 6 hours. Alton *et al.* [55] used improved high performance liquid chromatography to measure the plasma concentration of labetalol and proved that it has a significant impact on the metabolism of labetalol during pregnancy, which is related to the secretion of various hormones during pregnancy. Research [56] showed Labetalol has a fast absorption rate and detected in the fetal umbilical cord and amniotic fluid, and its concentration is 50% and 16% of the maternal vein specimens at the same period respectively. The effects of labetalol on the heart rate, blood pressure, and oxygenation of brain tissue in premature fetuses have not been proven. The severity of maternal disease plays a more important role in neonatal cerebral hemodynamics at a certain level. For example, intravenous administration of labetalol in pregnant ewes can improve circulating norepinephrine, which has certain effects on maternal mean arterial pressure (MAP), uterine blood flow, fetal PH and arterial partial pressure of oxygen (PaO_2). The degree of adrenergic blockade in the fetus is lower than that of mother [27]. In sheep placental vascular resistance increase model, norepinephrine can increase maternal heart rate (HR), mean arterial pressure (MAP) and uterine vascular resistance (RUTA)), and decreased uterine volume blood flow (UTA). Labetalol reduces maternal HR, MAP and R (UTA), but cannot restore Q (UTA). Fetal heart rate and placental volume blood flow (UA) decreased, placental vascular resistance increased, and fetal MAP did not change significantly [30]. In the drug application [45], thermocouple method was used to observe the effect of propanol on uterine blood flow in rats, and the uterine blood flow was reduced by 18%~30%, while the effect of labeolol on uterine blood flow was slightly decreased (0~5%).

At present, there were few studies on labetalol promotion of fetal lung maturation, especially fetal lung maturation. The specific extent, mechanism and molecular pathway of labetalol promoting fetal lung maturation are still unclear, which may be due to the fact that part of labetalol contains β -adrenergic receptor agonists [2], which stimulate β-adrenergic receptors on alveolar type II cells. The β-adrenergic receptors are widely distributed in the lungs. Our results showed have a certain effect on the T1α protein of type I cells. Studies have shown [57] labetalol can promote glycogen decomposition and increase blood sugar through β2 receptor agonists [58]. Alveolar type II cells synthesize and secrete phosphatidylcholine (PC) after β-adrenergic stimulation, a major component of surface-active substances. β1- and β2-adrenergic receptor subtypes (1:3, respectively) are present in this cell type. According to the study of glucocorticoid on fetal lung tissue [59], the density of β -adrenergic receptor in fetal lung tends to increase in late pregnancy. Maternal glucocorticoid treatment also increased the density of this receptor. Glucocorticoids have a direct effect on fetal lung and can increase β -adrenergic receptor density [60].

Dexamethasone increased the number of β-adrenergic receptors and the mRNA expression of β-2-adrenergic receptors in type II cells during the application process [61]. Adrenaline stimulates the secretion of phosphatidylcholine (PC) at all stages, and adrenergic agonists act directly on mammalian fetal and adult type II cells via β2-receptors, stimulating cAMP-dependent pathways. Increase the secretion of surface-active substantial. According to the current results, the effect of labetalol alone on the lung surfactant protein T1α, SP-A, SP-B, SP-C, and SP-D is not obvious, but the effect of combined with dexamethasone is enhanced. At present, the effect of labetalol alone on promoting fetal lung maturation needs further study, and it is suggested that the combination of drugs should be better.

In metabolism [62], significant maternal-fetal metabolic effects were produced on pregnant sheep after maternal administration. The concentration of labetalol in fetal tracheal fluid was consistently higher than that in fetal plasma. Labetalol can quickly cross sheep placenta [63]. The peak plasma concentration was 33.7+/-5.8 ng/ml, the fetal/maternal ratio was 14.37+/-1.54%, and the apparent elimination half-life is 3.71+/-0.5 hours. Labetalol is present in amniotic fluid and fetal tracheal fluid for 24 hours at a concentration 2-4 times in fetal plasma. It may be related to the activity of some β 2 agonists. In addition [64], labetalol exhibited partial agonist activity on β-adrenergic receptors in vitro of rat uterus. Study found those 4 weeks from the 27th weeks of pregnancy, the mother with severe hypertension was given labetalol administration. Maternal and fetal diabetes risk, fetal side effects of the fetus (1250 grams) are manageable. Effective antihypertensive effect was maintained [48]. The results provide a theoretical basis for the prevention of NRDS in preeclampsia patients with labetalol or single dose of dexamethasone before clinical trial. And the effect is equivalent to dexamethasone, with dexamethasone synergistically promote fetal lung maturition, reduce the occurrence of neonatal respiratory distress syndrome, and reduce the mortality of premature infants. This evidence suggest that labetalol has a certain effect on fetal lung and should be studied in the future.

There are few studies on the promotion of fetal lung maturation, For the treatment of preeclampsia, hypertensive pregnant women with systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg should be treated according to the 2020 Guidelines for Hypertensive Diseases in pregnancy; For hypertensive pregnant women with systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, labetalol hydrochloride should be used in antihypertensive treatment, should be started applied as soon as possible in clinical practice, not only decrease blood pressure, but also improve the fetal intrauterine condition and promote fetal lung maturity. Positive assess of maternal and infant risk during the application process if termination of pregnancy can improve the survival rate of premature infants. If the βreceptor agonist is used alone, it may increase blood pressure and aggravate the disease to a certain extent. At present, it is only used as an animal experiment, but the specific duration of medication requires further clinical research. Then we will further develop fetal lung maturation drugs. At the result of view, labetalol had a role to promote fetal lung maturity, but after drug treatment by oral, the metabolism of the liver and placenta, the relatively small impact on the fetus, clinically mainly used corticosteroids such as dexamethasone to promote fetal lung maturity treatment, the synergy is better. Whether labetalol effect the differentiation of alveolar epithelium and the expression of differentiation-related genes such as epidermal growth factor, transforming growth factor β and thyroid transfer factor 1 due to the related signaling mechanism of promoting fetal lung maturation.

But there is some liver damage: several studies have reported that labetalol treatment was associated with mild to moderate increases in serum aminotransferase levels in up to 8% of patients, higher than with other beta-blockers. Clinically, however, severe liver injury is rare, damage is usually temporary, appears 4 to 6 weeks after the start of treatment, and liver cells are usually accompanied by acute hepatitis like disease [65]. Effects on the fetus: Lennestl *et al*. reported that in a cohort study of 1063 238, women treated with β-blockers were compared with those did not take antihypertensive drugs of pregnancy. The probability of cardiovascular malformations is increased (OR 2.8; 95%CI 1.8~4.1). 19 of 1,444 offspring died (RR 1.9; 95% CI, 1.0 to 3.0); 15 of them had a history of exposure to beta blockers during early pregnancy or late pregnancy. Munshi et al. reported a significantly increased incidence of hypoglycemia in the progeny exposed to labetalol compared with the control group ($P<0.01$); An increased risk of perinatal mortality (4.6% versus 0%) was reported associated with labetalol [66], which was found to have a 1.3-fold increased risk of neonatal bradycardia exposure and a 1.8-fold increased risk of hypoglycemia [67]. Some studies have also found that [68] in the California cohort, β-blocker exposure was not found to be associated with an increased risk of congenital heart abnormalities in fetus adjusted for maternal disease. Intravenous labetalol is associated with a higher risk of neonatal bradycardia [69]. Labetalol can metabolize in breast milk, and due to the low levels of labetalol in breast milk, infants are ingested in small amounts, and breastfeeding is not expected to cause any adverse effects on infants at term. Most babies do not need special precautions. No clinical signs of epinephrine blocking were found 24 hours after the application of labetalol，and that drug had no adverse effects on the adaptability of neonates and adverse reactions [70].

In summary, Labetalol is currently safe to be used in hypertensive disorders during pregnancy, with few complications for both mothers and infants, and further research is needed in fetal lung maturation. Now the mechanism of action, such as signaling pathway there is no systematic study, considering the drug effect, consider whether clinically in the face of serious complications, especially diabetes, gestational diabetes, and patients with taboo to glucocorticoids application, can used to administration hypertension, it is also used to promote fetal lung maturation and other considerations, or the synergistic effect of glucocorticoid is better to promote fetal lung maturation and increase the survival of high-risk premature infants, needs further research.

Fund Project

Hospital-level Fund of the Second Hospital of Hebei Medical University (No. 2h201844).

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***Correspondence:** Hong Xin, Department of Obstetrics, The Second Hospital of Hebei Medical University, Shijiazhuang City, Hebei Province, 050000, China, Tel: 0311-66002930, E-mail: xh_hbyd@163.com / angel689bendou@163.com

Received: 24 Jan 2022; **Accepted:** 21 Feb 2022; **Published:** 24 Feb 2022

Citation: Chang R, Xin H. The mechanism of labetalol hydrochloride about fetal lung maturity in preeclampsia. *Int Clin Med Therp*. 2022; 4: 116.