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Budesonide and Poractant Alfa prevent bronchopulmonary dysplasia *via* triggering SIRT1 signaling pathway

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Abstract. – OBJECTIVE: This study aimed to evaluate effect of budesonide combining Poractant Alfa on preventing bronchopulmonary dysplasia (BPD).

PATIENTS AND METHODS: A total of 120 preterm infants were involved. pH value, partial pressure of oxygen (PO_2), and blood gas analysis were evaluated. Peripheral blood was collected and mononuclear cells were isolated. Reactive oxygen species (ROS) in peripheral blood mononuclear cells (PBMCs) were detected with laser confocal. Sirtuin 1 (SIRT1) in PBMCs was detected using immunofluorescence. SIRT1 and small ubiquitin-like modifier (SUMO)-specific protease 1 (SENP1) were detected with Western blot.

RESULTS: Compared with group B, pH value and PO2 were improved significantly in group C and D ($p<0.01$). Compared with group B, oxygen inhalation duration, rate of having a respirator assisted ventilation, and using pulmonary surfactant (PS) again, and BPD incidence were significantly decreased in other groups ($p<0.05$). BPD incidence in group D was less than group C ($\chi^2=4.00$, $p<0.05$). Compared with control group, ROS level of neonatal respiratory distress syndrome (NRDS) group was significantly increased, SENP1 was increased, and SIRT1 was decreased in SIRT1 group. Compared with NRDS, when budesonide combined with Poractant Alfa, ROS decreased, SENP1 decreased, SIRT1 nuclear pulp shuttling rate reduced, nuclear SIRT1 increased ($p<0.01$). Compared with control, ROS level of NRDS group was significantly increased, SENP1 increased, and SIRT1 in nucleus decreased ($p<0.05$). Compared with NRDS group, when treated with budesonide and Poractant Alfa, ROS levels decreased, SENP1 decreased, nuclear SIRT1 increased ($p<0.01$).

CONCLUSIONS: Budesonide combining Poractant Alfa can prevent BPD in preterm infants by activating the SIRT1 signaling pathway.

Key Words:

Bronchopulmonary dysplasia, SIRT1 signaling pathway, Budesonide, Poractant Alfa.

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease caused by oxygen therapy and mechanical ventilation in premature infants with acute respiratory distress[1]. With the continuous improvement of medical technology, survival rate of premature infants increases significantly in recent years. However, at the same time, incidence of BPD, retinopathy, neurological abnormalities, and other diseases also increases gradually, which seriously affects the survival rate and quality of life[2-4]. Authors[5-6] showed that the lower the gestational age, the lower the body weight, and the higher the incidence of BPD.

Presently, there is no systematic way to prevent and cure BPD. Currently, there are many strategies, including oxygen therapy, pulmonary surfactant (PS) alternative treatment, nitric oxide (NO), glucocorticoid (GC), caffeine, vitamin A, bronchial vasodilators, diuretics, antibiotics, and other treatments[7-10]. Using PS to prevent and cure neonatal respiratory distress syndrome (NRDS) for preventing BPD is currently recognized as one of the most important methods. The prevention of GC in BPD has been proved to be effective by many clinical trials, but the early systemic application of GC has many adverse effects[11]. Budesonide is a kind of newly synthesized GC, mainly used for topical use of inhalation[12]. The previous studies[13-16] showed that local use of hormone affects more quickly. Through the mucosal absorption, it can directly reach the target organ, reduce the release of inflammatory mediators and cytokines, and reduce lung injury[17,18].

The mechanism of budesonide combining Poractant Alfa in preventing BPD has not been clarified. Scholars showed that the high oxygen lung injury was significantly associated with sirtuin 1 (SIRT1) signaling pathway. Once high oxygen and

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other factors could lead to the increase in reactive oxygen species (ROS) and small ubiquitin-like modifier (SUMO)-specific protease 1 (SENP1). Meanwhile, the SIRT1 to desumoylation will promote the SIRT1 out of nuclear that triggers nuclear-plasma shuttle, antioxidant capacity decreased, cell apoptosis increased[19-22]. PS can effectively prevent the occurrence of BPD, which may be related to oxidative stress of SIRT1. In addition, the former researches[23,24] showed that SIRT1 also involves in anti-inflammatory reactions. Inflammatory injury is a key link in the development of BPD. GC has the effect of inhibiting inflammatory reaction[25]. We make a bold assumption: SIRT1 signaling pathway also participates in hormone combining pulmonary surfactant to prevent BPD. Therefore, this study would further explore SIRT1 signaling pathway involving in the process of budesonide combining Poractant Alfa to prevent BPD. Meanwhile, this research would provide a novel therapeutic target for preventing and treating BPD.

Patients and Methods

Patients

One hundred and twenty preterm infants 6 h after birth (gestational age ≤ 32 weeks and birth weights ≤ 1500 g) admitted to Department of Newborn Medicine, the Affiliated Hospital, Southwest Medical University from October 2013 to February 2015, were randomly divided into 4 groups (30 cases in each group). Group A was control group, group B was neonatal respiratory distress syndrome (NRDS) group, group C was NRDS with surfactant group, and group D was NRDS with surfactant and budesonide group. Diagnosis of premature infants in NRDS was in line with NRDS diagnostic criteria of "practice of neonatology" according to the previously published study[26]. There were no significant differences for the gender, gestational age, birth weight, and Apgar 10 min scores between four groups (p>0.05).

This investigation has been approved by Medical Ethics Committee of the Affiliated Hospital of Southwest Medical University. All the children who have been brought into laboratory before experiments have consents of the family members and signed the informed consents.

Intervention Measures

Thirty-two weeks following unless the other diseases and without oxygen in preterm infants within 48 h as group A. Group B was treated by continuous up-taking oxygen with continuous positive airway pressure (CPAP; Oxygen lasts more than 48 h and oxygen concentrations more than 40%). Group C was treated with 100 mg/kg pulmonary surfactants (PS, CR Double-Crane Pharmaco. Co. Ltd., Beijing, China) within 48 h on the basis of group B. Group D was treated with 0.25 mg/kg budesonide (CR Double-Crane Pharmaco. Co. Ltd., Beijing, China) suspension for inhalation on basis of group C[14,27].

Record Clinical Indicators

The pH value and partial pressure of oxygen (PO_2), and blood gas analysis were detected in every group before and 1, 6, 12, 24, and 48 h after drug administration. All above groups were also observed whether to have a respirator assisted ventilation, the high oxygen using duration, the total oxygen time, the rate of using PS again, the rate of BPD (the newborn may be diagnosed as the disease by fraction of inspiration O_2 of more than 21% of 28 d[28]), the total hospitalization days and the adverse effects. The adverse effects included high blood pressure, high blood sugar, necrotizing enterocolitis (NEC), and the incidence of nosocomial infection.

Extraction, Cryopreservation, and Resuscitation of Peripheral Blood Mononuclear Cell (PBMCs)

Extraction of PBMCs: after 48 h of oxygen inhalation in premature infants, radial artery blood sampling 3 ml was collected. 2 ml was placed in heparin anticoagulant tube, and Ficoll density gradient centrifugation was used to separate mononuclear cells. The second layer from the top is a white cloud, which is a single nuclear cell layer. In addition, 1 ml blood samples were isolated from serum and stored in –80°C refrigerator.

Cryopreservation of PBMCs: Extraction of PBMCs can be frozen to prepare the back of immune fluorescence and blot western detection. According to 7: 2: 1 ratio, the culture medium, fetal bovine serum, and two methyl sub Maple were prepared into frozen storage solution. The 1 ml cell suspension and 2 ml cryopreservation solution were added to each of sterile frozen storage tubes. And then put the frozen storage tube in 4°C and –20°C refrigerator freezer for 0.5 h and 2 h, respectively, and finally put into –80°C.

Resuscitation of PBMCs: the PBMCs bake in at –80°C of ultra low temperature refrigerator out of 37°C thermostatic water bath box. To dissolve the cells as soon as possible, opening of the frozen

tube can be quickly shaken for 1 min during thawing process. The extracted PBMCs can be used for the detection of the corresponding index.

Laser Confocal Detection of ROS Levels in PBMCs

Red Mitosox can be combined with ROS specificity and emit red fluorescence, and the fluorescence intensity and ROS levels were positively correlated. Therefore, this experiment used Red MitoSOX staining (Cat. No. M36008, Thermo Fisher Scientific, Molecular Probes, Waltham, MA, USA) combining laser-scanning confocal microscope (LSCM: Leica GHBH, Germany) to detect level of ROS in PBMCs. Main steps were listed as the followings: cell images, 4% polyformaldehyde (Beyotime Biotech. Shanghai, China) fixed, prepared concentration for Mitosox 3.85 μg/ml Projekt red (Cat. No. M36008, Thermo Fisher Scientific, Molecular Probes, Waltham, MA, USA) for loading probe, anti fluorescence quencher mounting, laser scanning confocal microscope (LSCM: Leica GHBH, Wetzlar, Germany) to detect red fluorescence (excitation wavelength 510 nm and emission wavelength of 580 nm). Finally, Image Pro Plus 6.0 software (Media Cybernetics, Inc., Bethesda, MD, USA) was used to analyze the average fluorescence intensity of each image.

Detection of SIRT1 Localization in PBMCs Using Immunofluorescence Technique

Immunofluorescence techniques are the antigen antibody specific binding of immunological methods combined with fluorescence labeling technique, due to fluorescein emitted fluorescence can be detected under fluorescent microscope (Mode: BX62, Olympus, Tokyo, Japan). Therefore, the specific protein antigen localization in cells can be studied. Main steps were listed as followings: the cells were fixed with 4% polyformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and blocked with bovine serum albumin (BSA; Beyotime Biotech. Shanghai, China) at concentration of 30 g/l at room temperature for 40 min. Then, cells were incubated with 50 μl mouse anti human SIRT1 antibody (1: 50, Cat. No. ab110304, Abcam Biotech., Cambridge, MA, USA) as the first antibody, and then treated with 50 μl Rhodamine labeled Goat anti mouse IgG (1:50, Cat. No. ab125851, Abcam Biotech., Cambridge, MA, USA) as the secondary antibody. Finally, Pro Plus 6.0 software (Media Cybernetics, Inc., Bethesda, MD, USA) was used to analyze fluorescence distribution of images.

Western Blot Assay Detecting Expression of SIRT1 and SENP1 in PBMCs

The cells were lysed using lysis buffer (Beyotime Biotech. Shanghai, China). Protein lysates were separating with 15% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE; ShineGene, Molecular Biotech. Inc., Shanghai, China), and electrotransferred onto polyvinylidene difluoride (PVDF; Millipore, Billerica, MA, USA). PVDF membranes were blocking with bovine serum albumin (BSA, Beyotime Biotech. Shanghai, China) at concentration of 30 g/l at room temperature for 30 min. The PVDF membranes were treated using mouse anti human SIRT1 antibody (1: 1000, Cat. No. ab110304, Abcam Biotech., Cambridge, MA, USA) and rabbit anti-human SENP1 polyclonal body (1: 1000, Cat No. 1b236094, Abcam Biotech.) at 4°C overnight. Then, the PVDF membranes were incubated using horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (1:500, Cat. No. ab205719, Abcam Biotech., Cambridge, MA, USA) or HRP-labeled goat anti-rabbit IgG (1:500, Cat. No. ab6721, Abcam Biotech., Cambridge, MA, USA) at room temperature for 2 h. Protein bands were visualized using enhanced chemiluminescence kit (ECL; Beyotime Biotech. Shanghai, China). Eventually, Western blot bands were captured and analyzed with the UVP gel image scanning system (version: Labworks 4.6, Bio-Rad Laboratories, Hercules, CA, USA).

Statistical Analysis

Statistical analysis was performed using SPSS17.0 software (SPSS, Inc., Chicago, IL, USA). The measurement data were expressed as mean plus or minus standard deviation, and the comparison between the two groups was statistically analyzed by one factor analysis of variance (ANOVA), which was validated by Tukey's post-hoc test. The statistical analysis was performed by the Chi-square test. $p<0.05$ for the difference was statistically significant.

Results

Changes in Clinical Parameters

Compared with group B, 1 h and 6 h after treatment, pH value was significantly increased in Group C and Group D, and group D increased significantly compared Group C (Table I, all $p<0.01$).

Compared with B group, 1 h, 6 h, and 12 h after treatment, PO_2 was significantly increased in Group C and Group D (Table II, $p<0.01$).

Table I. The pH value of each groups before and after using drugs (mean ± SD).

Group	pH value before treatment	pH value after treatment				
		1 h	6 h	12 h	24 h	48 h
Group A	7.25±0.10	7.32±0.08	7.37±0.06	7.37±0.04	7.38±0.04	7.38±0.03
Group B	7.18±0.08	7.24±0.09	7.27±0.08	7.33±0.04	7.34±0.04	7.36±0.05
Group C	7.18±0.08	7.29±0.06*	7.32±0.05*	7.34±0.04	7.36±0.04	7.37±0.04
Group D	7.19±0.08	7.32±0.07*	7.36±0.05*	7.36±0.05	7.37±0.04	7.37±0.04
F	5.695	8.501	7.895	3.463	2.286	0.253
p	0.002	0	0	0.019	0.082	0.859

Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group B, $p<0.01$.

Table II. The PO_2 of each group before and after using drugs (mean ± SD).

Group	PO_2 value before treatment	PO_2 value after treatment				
		1 h	6 h	12 h	24 h	48 h
Group A	77.40±13.44	83.77±10.15	84.70±8.84	87.17±8.53	88.60±7.77	90.00±5.69
Group B	56.87±9.10	68.00±9.44	73.50±8.87	77.77±8.59	84.93±5.97	85.01±7.24
Group C	57.53±7.37	79.67±8.53*	82.77±6.97*	84.20±7.38*	86.33±5.66	85.40±5.85
Group D	59.93±10.79	80.40±9.26*	85.20±6.87*	85.63±6.54*	86.23±6.30	86.40±6.95
F	30.997	16.31	16.358	9.53	1.582	3.097
p	0	0	0	0	0.197	0.03

Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group B, *$p<0.01$.

Compared with Group B, the duration of high oxygen duration and total oxygen time in Group C and Group D were significantly reduced (Table III, $p<0.01$). However, there was no significant difference in the total length of stay among all groups (Table III, $p>0.05$).

The occurrence of mechanical ventilation and BPD, and the rate of again with PS in Group C and Group D were significantly decreased compared with group B (Table IV, $p<0.05$). The occurrence of BPD in Group D was significantly decreased compared with Group C (Table IV, $p<0.05$).

By comparing the occurrence of hypertension, hyperglycemia, gastrointestinal bleeding, NEC, and hospital infection, there was no significant difference among Group A, B, C, and D (Table V, $p>0.05$).

Changes of ROS Level in PBMCs

In Group A, Group B, Group C, and Group D, the average fluorescence intensity of ROS in PBMCs was 12.20±3.05, 41.59±3.61, 30.38±3.56, 22.95±1.82, respectively. Also, there were no sig-

Table III. The oxygen using duration and the total hospitalization days in each group (mean ± SD).

Group	Duration of high oxygen	Total oxygen inhalation time	Total length of stay
Group A	2.7±0.95*	8.67±6.49*	26.57±7.25
Group B	4.33±1.92	15.8±10.68	28.93±11.47
Group C	2.67±1.06*	10.80±8.89*	26.67±9.29
Group D	2.60±0.97*	9.33±7.28*	27.17±7.30
F	12.432	4.331	0.447
p	0	0.006	0.72

Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group B, *$p<0.01$.

Table IV. The rate of having a respirator assisted ventilation and using PS again, and the occurrence of BPD in each groups (%).

Group	The number of cases (n)	Mechanical ventilation	Occurrence rate (%) again with PS	BPD
Group A	30	6.67	0	0
Group B	30	26.67	30	23.33
Group C	30	16.67*	13.33*	10.00*
Group D	30	6.67*	6.67*	3.33*,#
χ^2		34	30	22
p		0	0	0

Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group B, $p<0.01$. Compared with Group C, $\chi^2=4.00$, $p=0.046$.

nificant differences among these groups (Figure 1, $F=79.951$, $p<0.01$). The average fluorescence intensity of ROS in Group C was in between group B and Group D (Figure 1).

SIRT1 Translocation Rate in PBMCs

The distribution of intracellular fluorescence intensity was determined using the line profile of Image Pro Plus 6.0. The translocation rate of four groups was 3.77%, 51.47%, 33.16%, 12.40%, respectively, and difference was statistically significant (Figure 2, Table VI, $\chi^2= 606.00$, $p<0.01$). In Group A, the red fluorescence was evenly distributed in nucleus, only a very small number in cytoplasm, indicating that SIRT1 mainly distributes in nucleus. In Group B, a large number of SIRT1 was moved from nucleus to cytoplasm, the red fluorescence in cytoplasm was significantly enhanced, and translocation rate increased significantly compared Group B (Figure 2, Table IV, $p<0.01$). In Group C, the fluorescence intensity was between Group B and Group D, with a significant difference (Figure 2, Table VI, $\chi^2= 176.00$, $\chi^2 = 89.00$, $p<0.01$).

Expression Levels of SIRT1 and SENP1 in PBMCs

Western blot assay results showed that SIRT1 relative expression in Group A, Group B, Group C, Group D was 1.212±0.020, 0.429±0.014, 0.705±0.100, 1.058±0.080, respectively. The relative expression of SENP1 was 0.264±0.047, 0.923±0.048, 0.758±0.100, 0.650±0.057, respectively. The statistical analysis showed that SIRT1 was significantly decreased and SENP1 expression was significantly increased in Group B, C, D compared to that in Group A (Figure 3, all $p<0.01$). The relative expression of SENP1 and SIRT1 in Group C and Group D was in between Group A and Group B (Figure 3, all $p<0.01$). The relative expression of SIRT1 in Group D was higher significantly compared to that in Group C, and the relative expression of SENP1 in Group D was lower significantly compared to that in Group C (Figure 3, all $p<0.01$).

Table V. The rate of adverse effects in each group (%).

Group	The number of cases (n)	Occurrence rate (%)				
		Hypertension	Hyperglycemia	Gastrointestinal bleeding	NEC	Hospital infection
Group A	30	0	0	0	0	3.33
Group B	30	0	3.33	0	3.33	6.67
Group C	30	0	0	0	3.33	6.67
Group D	30	0	3.33	0	6.67	6.67
χ^2					4	7
p					0.135	0.072

Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group.

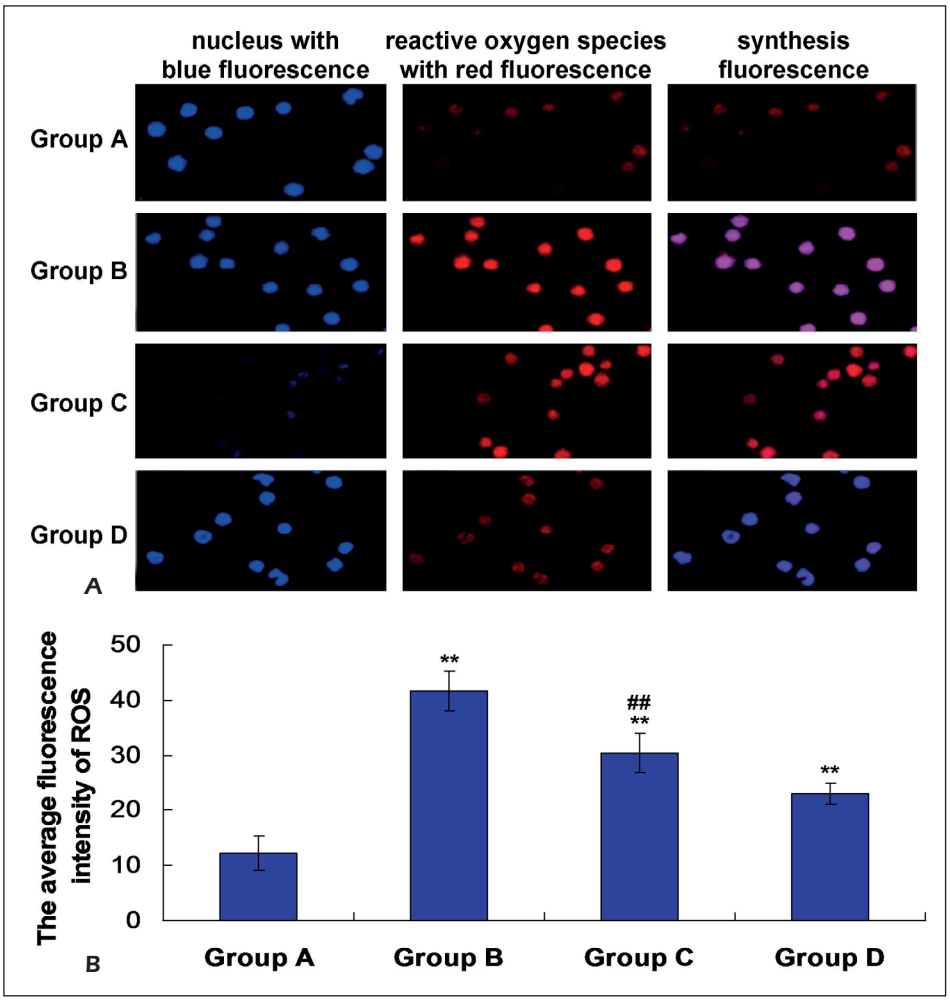

Figure 1. Levels of ROS in PBMCs of each group. **A**, ROS expression examined by laser scanning confocal microscope (magnification 200×). **B**, Statistical analysis for ROS expression. Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group A, $p<0.01$. #Compared with Group B and Group D, $p<0.01$.

Discussion

BPD is one of the severe complications of preterm infants in the neonatal intensive care unit, and its etiology and pathogenesis are complex, without separate prevention and treatment measures[29]. There is a long use of oxygen and length of stay, high mortality, the possibility of sequelae of legacy, family, and social burden in premature infants with BPD. Therefore, prevention and treatment of this disease have become one of the most difficult problems in the Department of Newborn Medicine. PS has been widely used in prevention and treatment of NRDS in clinic. Europe NRDS Prevention Guide (2013) also pointed out that PS replacement therapy plays an important role in the management of NRDS, but for preterm infants of different gestational ages the choice of the optimal formulation, the optimal dose, and the best time is not clear[30]. However, the previous study[31] has pointed out that for very low birth weight infants, PS completely lacks ventilation flow imbalance. Inflammatory injury is a key link in development of the BPD, and GC has the effect of inhibiting inflammatory reaction. GC could reduce bronchial and pulmonary edema, promote lung anti-oxidase and PS production and rapid improvement of lung function, improve extubation success rate and exposed to reduced oxygen, as well as reduce the BPD incidence. Therefore, it is widely used in prevention and treatment of BPD[32]. However, due to the premature lung tissue in a collapsed state, intrapulmonary gas content is less and contains more blood[15]. In this study, the instillation of pulmonary surfactant for carrier drops into hormone not only increases drug utilization and more effective suppression of inflammatory response. In addition, a large number of clinical trials[33] showed that systemic usage of GC may cause infection, hypertension, hyperglycemia, hypertrophic cardiomyopathy, gastrointestinal perforation, necrosis of the small intes-

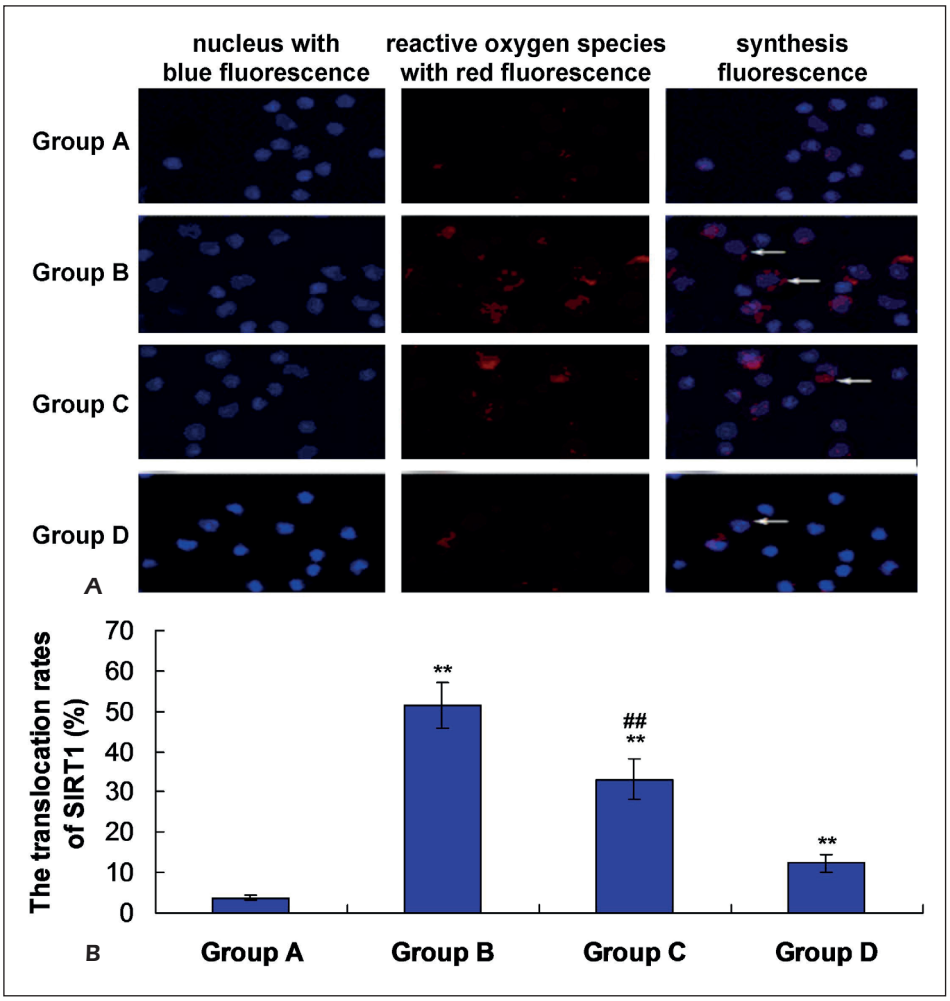

Figure 2. Location of SIRT1 in PBMCs of each group. **A**, SIRT1 location examined by laser scanning confocal microscope (magnification 200×). **B**, Statistical analysis for ROS expression. Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group A, $p<0.01$. #Compared with Group B and Group D, $p<0.01$.

tine colitis, brain development and neurological movement abnormalities, and adverse reactions. Budesonide, as a new synthetic GC, is the only FDA approved atomizing inhaled corticosteroids. Its lipophilicity is stronger than systemic hormone, and the intrapulmonary deposition rate is high. It is conducive to promote the maturity of alveolar type II cells, so as to promote synthesis and release of surfactant. This study proved that the disposable, small dose of endotracheal instillation of the way, not only could reduce the adverse consequences caused by systemic application of GC, but also increase the effective amount of the aerosol inhalation.

Table VI. The rate of adverse effects in each group (%).

Group	The number of cases (n)	Incidence rate (%)		
		Mean number of translocation cells (unit)	Average number of cells (one)	Translocation rates (%)
Group A	5	6.6	180.6	3.77
Group B	5	106.4	205.6	51.47*
Group C	5	69.6	210.8	33.16*#
Group D	5	18.8	154.8	12.4*

Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group A, $p<0.01$. #Compared with Group B and D, $p<0.01$, $\chi^2=176.00$ and $X^2=89.00$, respectively.

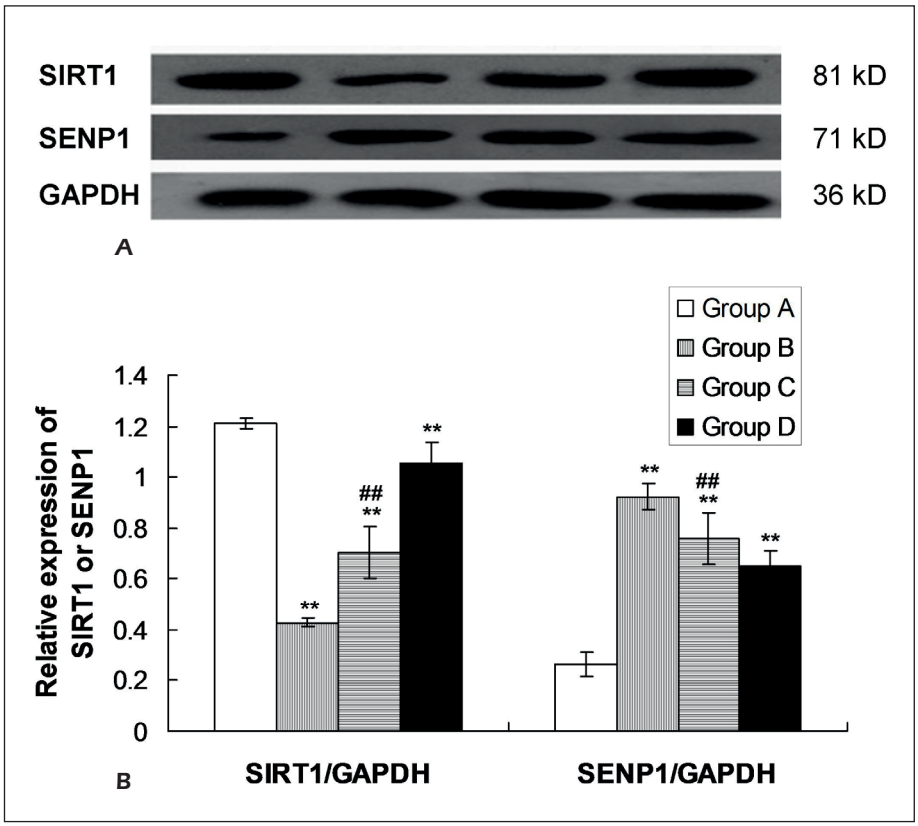

Figure 3. Relative expression of SIRT1 and SENP1 in PBMCs of each group. Note: Group A is control group. Group B is NRDS group. Group C is NRDS with surfactant group. Group D is NRDS with surfactant and budesonide group. *Compared with Group A, $p<0.01$. #Compared with Group B and Group D, $p<0.01$.

The clinical indicators demonstrate the intratracheal instillation of Poractant Alfa within 12 h after medication, the rapid correction of pH and PO_2, as well as the disassembly and reduction of BPD. The usage of budesonide combining Poractant Alfa injection compared with simple instillation of Poractant Alfa, can more effectively prevent BPD, hypertension, hyperglycemia, and gastrointestinal bleeding. However, there is no significant difference in the total length of stay in each group, which is related to the development of various organs in premature infants. In addition, there were no significant differences in the occurrence rate of adverse reactions among

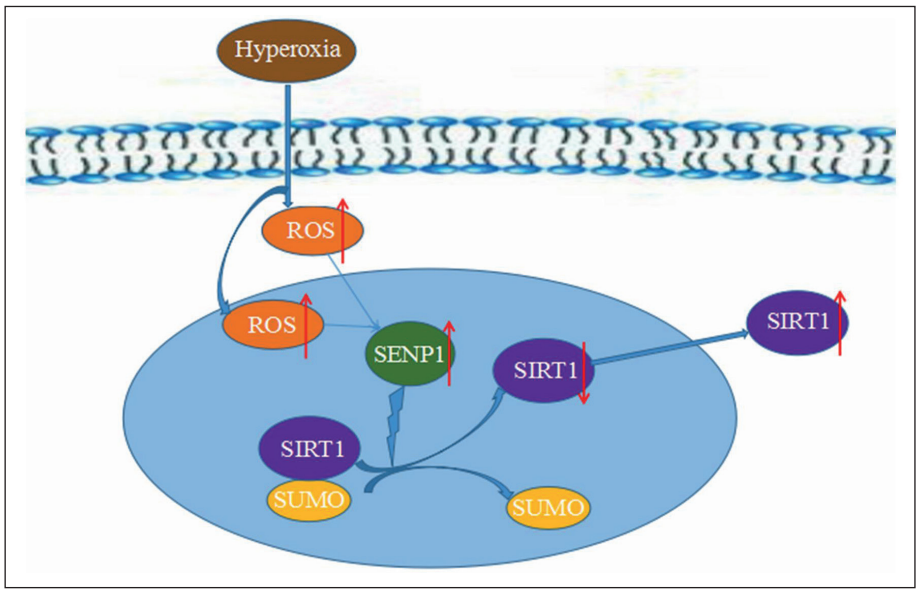

Figure 4. The process of high oxygen lung injury induced SIRT1 signal pathway.

all groups. Also, this study only conducted a single center investigation with a less sample size. Therefore, it also needs long-term follow-up in children with nervous system and growth and development. Moreover, we would amplify the sample size and conduct a multi-center study for further verification. Oxygen therapy is one of the most basic treatment measures for premature infants with dyspnea. It cannot only relieve dyspnea, prevent NRDS, but also reduce occurrence of BPD. However, long time and high concentration of oxygen will make the risk of chronic oxygen dependence, retinopathy of prematurity (ROP), and the other significant increase[34,35]. ROS has strong oxidation ability and is one of the most powerful oxidants[36]. In normal condition, production of ROS and ability of anti-oxidase elimination were in balance, but the premature lung development was not mature, and the activity of antioxidant enzymes was low. When the immature lung is exposed to high oxygen environment, the body produces a large number of ROS, and these ROS can't be cleared in time, resulting in alveolar epithelial cell apoptosis, resulting in lung injury[37]. SIRT1 is a kind of NAD^+ dependent protease, which mainly exists in the nucleus. Its main function is to regulate cellular oxidative stress reaction and regulate life cycle, which can inhibit cell apoptosis. SIRT1 is a key protein in body, which is very important for anti oxidative stress. Under the catalysis of the specific protease SENP1 of small ubiquitin like modifier SIRT1, the 734 lysine of SIRT1 will be modified by SUMO[38]. Tanno et al[39] show that once the high oxygen and other factors lead to increased SENP1 activity, which would promote the ability of SIRT1 out of nucleus that illustrates the nuclear plasma shuttle, antioxidant capacity decreased and increased apoptosis. Therefore, in Figure 4 we demonstrated a conclusion of the previous experiment. Therefore, SIRT1 signaling pathway is an important signaling pathway for the high oxygen lung injury.

Budesonide and Poractant Alfa injection can effectively prevent the occurrence of BPD, but the mechanism can make nothing of it. BPD is often secondary to NRDS, and is closely related to preterm birth and high oxygen lung injury. The former researcher has found that SIRT1 content in nucleus in tracheal aspirate of children with BPD was reduced[40]. PS for the prevention and treatment of BPD has been recognized as effective. However, whether PS can reduce the oxidative stress and reduce mechanism of SIRT1 kernel plasma shuttle and inhibit cell apoptosis has not been clarified. In addition, SIRT1 not only has the role of inhibiting apoptosis, but also has anti-inflammatory effect. Budesonide with strong suppression of the inflammatory response, can promote the anti-oxidase and PS, but whether this process is related with SIRT1 pathway remains unclear. The research results showed that NRDS group compared with control group, the ROS level was increased significantly, the expression of SENP1 was increased, and expression of SIRT1 in nucleus was decreased. These results were consistent with the SIRT1 signaling pathway in high oxygen lung injury. The levels of ROS and SENP1 in NRDS + PS group and NRDS + PS + budesonide group were lower than that of NRDS group. The rate of SIRT1 nucleus plasma shuttle was lower than that of NRDS group, expression of SIRT1 in nucleus was higher than that in NRDS group, and the change of NRDS+PS+budesonide group was more evident than that of NRDS+PS group. The results showed that the hyperoxia lung injury can increase the level of ROS, the expression of SENP1, and the rate of SIRT1 nucleocytoplasmic shuttling, and increase the apoptotic cells. Poractant Alfa or the mixed liquid of budesonide and Poractant Alfa were dripped into inner tracheal in 6 h after birth. The results showed that ROS levels, the expression of SENP1, rate of SIRT1 nucleocytoplasmic shuttling, and apoptotic cells were reduced. Dropping into the mixture of budesonide and Poractant Alfa was better than just Poractant Alfa only. SIRT1 signaling pathways not only involved in Poractant Alfa for prevention of BPD process, but also participated in budesonide combined with Poractant Alfa for prevention of BPD process, and the latter effect is more significant than the former.

Conclusions

Budesonide combining Poractant Alfa can effectively prevent BPD, and better than Poractant Alfa Injection. SIRT1 signaling pathways not only involved in Poractant Alfa for prevention of BPD process, but also participated in budesonide combining Poractant Alfa triggered prevention of BPD process.

Conflict of Interests

The Authors declare that they have no competing financial or commercial interests in this manuscript.

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