Consecutive Daily Administration of Intratracheal Surfactant and Human Mesenchymal Stem Cells Attenuates Hyperoxia-induced Lung Injury in Neonatal Rats

Hsiu-Chu Chou
Taipei Medical University

Chien-Hsiang Chang
National Cheng Kung University

Chien-Han Chen
Meridigen Biotech Co. Ltd.

Willie Lin
Meridigen Biotech Co. Ltd.

Chung-Ming Chen (cmchen@tmu.edu.tw)
Taipei Medical University Hospital  https://orcid.org/0000-0001-8762-8957

Research

Keywords: Hyperoxia, Surfactant, Mesenchymal stem cells, Mean linear intercept, Vascular endothelial growth factor

DOI: https://doi.org/10.21203/rs.3.rs-294984/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Abstract

**Background:** Surfactant therapy is a standard of care for preterm infants with respiratory distress and reduces the incidence of death and bronchopulmonary dysplasia in these patients. Mesenchymal stem cells (MSCs) attenuated hyperoxia-induced lung injury. Surfactant reduced the in vitro viability of human MSCs, and the combination therapy of surfactant and MSCs did not have additive effects on hyperoxia-induced lung injury in neonatal rats. The effects of 2 consecutive days of intratracheal administration of surfactant and MSCs on hyperoxia-induced lung injury were undetermined.

**Methods:** Neonatal Sprague Dawley rats were reared in either room air (RA) or hyperoxia (85% O₂) from postnatal days 1 to 14. On postnatal day 4, the rats received intratracheal injections of either 20 µL of normal saline (NS) or 20 µL of surfactant. On postnatal day 5, the rats reared in RA received intratracheal NS, and the rats reared in O₂ received intratracheal NS or human MSCs (3 × 10⁴ or 3 × 10⁵ cells). Six study groups were examined: RA + NS + NS, RA + surfactant + NS, O₂ + NS + NS, O₂ + surfactant + NS, O₂ + surfactant + MSCs (3 × 10⁴ cells), and O₂ + surfactant + MSCs (3 × 10⁵ cells). The lungs were excised for analysis on postnatal day 14.

**Results:** The rats reared in hyperoxia and treated with NS yielded significantly higher mean linear intercepts (MLIs) and cytokine levels and significantly lower vascular endothelial growth factors (VEGFs), platelet-derived growth factor protein expression, and vascular density than did those reared in RA and treated with NS or surfactant. The lowered MLIs and cytokine levels and the increased VEGF expression and vascular density indicated that the surfactant and surfactant + MSCs (3 × 10⁴ cells) treatment attenuated hyperoxia-induced lung injury. The surfactant + MSCs (3 × 10⁵ cells) group exhibited a significantly lower MLI and significantly higher VEGF expression and vascular density than the surfactant + MSCs (3 × 10⁴ cells) group did.

**Conclusions:** Consecutive daily administration of intratracheal surfactant and MSCs can be an effective regimen for treating hyperoxia-induced lung injury in neonates.

**Background**

Supraphysiological oxygen is often required to treat newborns with respiratory disorders. However, administering supplemental oxygen to newborn infants with respiratory failure can lead to lung injury. Term-born rat models are appropriate for studying the effects of hyperoxia on preterm infants with respiratory distress because rats are born at the saccular stage, which is approximately equivalent to a human gestational age of 30 weeks [1]. The prolonged exposure of neonatal rats to hyperoxia results in a decrease in alveolarization and vascularization similar to human bronchopulmonary dysplasia (BPD) [2, 3]. The pathogenesis of BPD is multifactorial, and oxygen toxicity plays a crucial role in the process of lung injury leading to BPD [4, 5].
Surfactant therapy is a standard of care for preterm infants with respiratory distress syndrome and can reduce the incidence of death and BPD [6]. Mesenchymal stem cells (MSCs) are multipotent stromal cells that have immunomodulatory, anti-inflammatory, and regenerative properties and have been demonstrated to treat hyperoxia-induced lung injury in newborn animals [7–14]. In this study, we demonstrated that the addition of surfactant reduced the in vitro viability of human MSCs through mitochondrial dysfunction and that a combination therapy of surfactant and MSCs had no additive effects on lung development in neonatal rats exposed to hyperoxia [14]. The effects of 2 consecutive days of intratracheal administration of surfactant and MSCs on hyperoxia-induced lung injury were undetermined. We hypothesized that consecutive daily administration of intratracheal surfactant and MSCs improves lung development and that high doses of MSCs enhance this effect on experimental BPD in neonatal rats. The aim of this study was to investigate the effects of an animal-derived surfactant (Survanta) and consecutive daily administration of human MSCs on hyperoxia-induced lung injury in neonatal rats.

**Methods**

**Surface tension**

Survanta (AbbVie Inc., North Chicago, IL, USA) was prepared through lipid extraction from minced bovine lungs and contained approximately 84% phospholipids, 1% hydrophobic surfactant proteins (SP-B and SP-C), and 6% free fatty acids. The solution was supplied at a 25 mg/mL concentration of phospholipids suspended in a 0.9% sodium chloride solution. The surface tension of Survanta (25 mg/mL), Survanta (12.5 mg/mL), and Survanta (25 mg/mL) and the human MSCs (1.5 × 10^6 cells/mL) was determined at a volume of 2 mL by using a surfactometer (Amherst Electronics, Buffalo, NY, USA). Each sample was measured three times.

**Isolation of human mesenchymal stem cells**

The MSCs were isolated from human umbilical cords, as previously described [12]. The MSCs were characterized by analyzing the expression of cluster of differentiation (CD) markers (CD44, CD73, CD90, CD105, CD11b, CD19, CD34, and CD45) and the human leukocyte antigen D-related complex through flow cytometry (BD Stemflow hMSC Analysis Kit, BD, Franklin Lakes, NJ, USA). An examination of trilineage differentiation capability (osteocytes, chondrocytes, and adipocytes) and a karyotyping revealed positive results.

**Animal model and experimental groups**

This study was approved by the Animal Care Use Committee of Taipei Medical University (LAC-2019-0396). Time-dated pregnant Sprague Dawley rats were housed in individual cages with ad libitum access to laboratory food and water, kept on a 12:12-h light–dark cycle, and allowed to deliver vaginally at term. Within 12 h of birth, the litters were pooled and randomly redistributed to the newly delivered mothers; the pups were then randomly assigned to room air (RA) or oxygen-enriched atmosphere (85% O_2_) groups for
postnatal days 1–14. The nursing mothers were rotated between the 85% O₂ and the RA groups every 24 h to prevent oxygen toxicity in the mothers and to eliminate differing maternal effects between the groups. An oxygen-rich atmosphere was maintained in a transparent 40 × 50 × 60-cm³ plexiglass chamber receiving continuous O₂ at 4 L/min. The oxygen concentration inside the hyperoxic plexiglass chamber was continuously monitored using an oxygen sensor (Coy Laboratory Products, Grass Lake, MI, USA). On postnatal day 4, the rats received intratracheal injections of either 20 μL of normal saline (NS) or 20 μL of surfactant (Survanta, AbbVie Inc.), corresponding to approximately 50 mg/kg of phospholipids (Fig. 1). On postnatal day 5, the rats reared in RA were treated with NS and those reared in O₂ received intratracheal injections of 20 μL of NS, human MSCs (3 × 10⁴ cells), or human MSCs (3 × 10⁵ cells) in 20 μL of NS. Six study groups were examined: RA + NS + NS, RA + surfactant + NS, O₂ + NS + NS, O₂ + surfactant + NS, O₂ + surfactant + MSCs (3 × 10⁴ cells), and O₂ + surfactant + MSCs (3 × 10⁵ cells). The lungs were excised for histological, western blot, and cytokine analyses on postnatal day 14.

**Intratracheal administration of surfactant and human MSCs**

For intratracheal transplantation, the rats were anesthetized with isoflurane and restrained on a board at a fixed angle as described by Chen et al. [15]. On postnatal day 4, the rats received intratracheal injections of either 20 μL of normal saline (NS) or 20 μL of surfactant. On postnatal day 5, the rats reared in RA received intratracheal NS, and the rats reared in O₂ received intratracheal NS or human MSCs (3 × 10⁴ or 3 × 10⁵ cells).

**Lung histology**

The lungs were placed in 4% paraformaldehyde, washed with phosphate-buffered saline, and then serially dehydrated in increasing concentrations of ethanol before being embedded in paraffin. To standardize the analyses, lung sections were taken from the right middle lobe. Sections of tissue weighing 5 μm were stained with hematoxylin and eosin, examined using light microscopy, and assessed for lung histology. The mean linear intercept (MLI), an indicator of the mean alveolar diameter, was assessed in 10 nonoverlapping fields [14].

**Immunohistochemistry of lung vascular endothelial growth factor and von Willebrand factor**

Immunohistochemical staining was performed on the 5-μm paraffin sections through immunoperoxidase visualization. After routine deparaffinization, heat-induced epitope retrieval was performed by immersing the slides in 0.01 M sodium citrate buffer (pH 6.0). To block the endogenous peroxidase activity and the nonspecific binding of antibodies, the sections were preincubated for 1 h at room temperature in 0.1 M phosphate-buffered saline containing 10% normal goat serum and 0.3% H₂O₂. The sections were then incubated for 20 h at 4°C with rabbit polyclonal anti–von Willebrand factor (vWF) antibodies (1:100; Abcam, Cambridge, MA, USA) or rabbit polyclonal anti–vascular endothelial growth factor (VEGF) antibodies (1:50; Santa Cruz Biotechnology, Inc., CA, USA) as primary antibodies. The sections were then treated for 1 h at 37°C with biotinylated goat antimouse or antirabbit IgG (1:200, Jackson
ImmunoResearch Laboratories Inc., West Grove, PA, USA). After the reagents from an avidin–biotin complex kit (Vector Laboratories, Inc., CA, USA) produced a reaction, the reaction products were visualized with a diaminobenzidine substrate kit (Vector Laboratories Inc.) in accordance with the recommendations of the manufacturer. Pulmonary vessel density was determined by counting the number of vessels with positive vWFs stained in an unbiased manner by using a minimum of four random lung fields at ×400 magnification [16].

**Western blot analysis of growth factors**

The lung tissues were homogenized in ice-cold buffer containing 50 mM Tris-HCl (pH 7.5), 1 mM EGTA, 1 mM EDTA, and a protease inhibitor cocktail (complete minitablets; Roche, Mannheim, Germany). The samples were sonicated and then centrifuged at 500 g for 20 min at 4°C to remove cellular debris. Proteins (30 μg) were resolved on 12% SDS-PAGE gels under reducing conditions and electrophoresed to a polyvinylidene fluoride membrane (ImmobilonP, Millipore, Bedford, MA, USA). After blocking with 5% nonfat dry milk, the membranes were incubated with antibodies against VEGF (1:1000; Santa Cruz Biotechnology, Inc.), platelet-derived growth factor subunit B (PDGF-B; 1:1000; Santa Cruz Biotechnology, Inc.), or anti–β-actin (1:20,000; Sigma-Aldrich, St. Louis, MO, USA) and subsequently with horseradish peroxidase-conjugated goat antirabbit IgG or antimouse IgG (Pierce Biotechnology, Rockford, IL, USA). Densitometric analysis was performed with AIDA software to measure the intensity of VEGF, PDGF-B, and β-actin bands.

**Lung cytokine levels**

The lung tissue was homogenized in 1 mL of ice-cold lysis buffer containing 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 0.01 M deoxycholic acid, and a complete protease cocktail inhibitor. Cell extracts were centrifuged, and the levels of interleukin (IL)-1β and IL-6 in the supernatants were measured with an enzyme-linked immunosorbent assay kit (Cloud-Clone Corp., Houston, TX, USA).

**Statistical analysis**

The data are presented as means ± standard deviations. Statistical analyses were performed using one-way ANOVA with the Bonferroni post hoc test for the multiple-group comparisons. The survival rate was evaluated by using the Kaplan–Meier method, and the log-rank test was used for the intergroup comparisons. Differences were considered statistically significant when \( P < 0.05 \).

**Results**

**Surface tension**

The surface tensions for the 25 mg/mL Survanta, the 12.5 mg/mL Survanta, and the 25 mg/mL Survanta with the human MSCs (1.5 \( \times 10^6 \) cells/mL) were 24.7 ± 1.7, 26.7 ± 3.8, and 34.9 ± 3.5 mN/m, respectively.
The 25 mg/mL Survanta with the human MSCs exhibited a significantly higher surface tension than did the 25 mg/mL Survanta ($P = 0.005$) and the 12.5 mg/mL Survanta ($P = 0.011$).

**Survival rate**

All the rats reared in RA and treated with NS or surfactant survived (Fig. 2). In the $O_2 + NS + NS$ group, one, one, three, and one rats died on postnatal days 5, 9, 10, and 11, respectively. In the $O_2 + surfactant + NS$ and $O_2 + surfactant + MSCs (3 \times 10^4$ cells) groups, one rat each died on postnatal days 6 and 8. In the $O_2 + surfactant + MSCs (3 \times 10^5$ cells) groups, one rat each died on postnatal days 9 and 10. The rats reared in hyperoxia and treated with NS exhibited a significantly lower survival rate than did those reared in RA and treated with NS or surfactant ($P < 0.05$). Treatment with surfactant and treatment with surfactant and MSCs augmented the hyperoxia-induced decrease in the survival rate, but the differences in survival rate were not statistically significant. The survival rates were comparable among the RA + NS, RA + surfactant + NS, $O_2 + surfactant + NS$, $O_2 + surfactant + MSCs (3 \times 10^4$ cells), and $O_2 + surfactant + MSCs (3 \times 10^5$ cells) groups.

**Body and lung weight and lung-to-body-weight ratio**

The body and lung weights and the lung-to-body-weight ratios on postnatal day 14 were comparable among the six study groups (Table 1).

**Histology results**

Fig. 3 presents the lung tissue sections stained with hematoxylin and eosin on postnatal day 14. The rats reared in hyperoxia and treated with NS exhibited large thin-walled air spaces and yielded a significantly higher MLI than did those reared in RA and treated with NS or surfactant (Fig. 3a). Treatment with surfactant and treatment with surfactant and MSCs ($3 \times 10^4$ cells) significantly diminished the hyperoxia-induced increase in the MLI. The $O_2 + surfactant + MSCs (3 \times 10^5$ cells) group exhibited a significantly lower MLI than did the $O_2 + surfactant + NS$ and the $O_2 + surfactant + MSCs (3 \times 10^4$ cells) groups (Fig. 3b). Fig. 4 shows representative lung sections stained for vWF on postnatal day 14. The rats reared in hyperoxia and treated with NS yielded a significantly lower vascular density than did those reared in RA and treated with NS or surfactant. Treatment with surfactant and MSCs ($3 \times 10^4$ or $3 \times 10^5$ cells) significantly augmented the hyperoxia-induced decrease in vascular density. The surfactant + MSCs ($3 \times 10^5$ cells) treatment more significantly increased vascular density compared with the surfactant + MSCs ($3 \times 10^4$ cells) treatment.

**Immunohistochemistry and western blotting of VEGF**

The VEGF immunoreactivities were primarily detected in the endothelial cells (Fig. 5a). The rats reared in hyperoxia and treated with NS exhibited significantly lower VEGF protein expression than did those reared in RA and treated with NS or surfactant (Fig. 5b). Treatment with surfactant and MSCs ($3 \times 10^4$ or $3 \times 10^5$ cells)
cells) significantly augmented the hyperoxia-induced decrease in VEGF protein expression compared with treatment with NS.

**Western blot analysis of PDGF**

Fig. 6 shows the representative western blot of PDGF-A and PDGF-B. The rats reared in hyperoxia and treated with NS exhibited significantly lower PDGF-A and PDGF-B protein expression than did those reared in RA and treated with NS or surfactant. Treatment with surfactant and MSCs ($3 \times 10^5$ cells) significantly augmented the hyperoxia-induced decrease in the PDGF-A and PDGF-B protein expression compared with treatment with NS.

**Lung cytokine levels**

The rats reared in hyperoxia and treated with NS yielded significantly higher IL-1β and IL-6 levels on postnatal day 14 than did those reared in RA and treated with NS or surfactant on postnatal (Fig. 7). The treatment with surfactant and the treatment with surfactant and MSCs significantly diminished the hyperoxia-induced increase in IL-1β and IL-6 levels.

**Discussion**

On postnatal day 14, our in vivo neonatal rat model demonstrated that neonatal hyperoxia exposure during the first 2 weeks of life impaired alveolarization and angiogenesis. The intratracheal administration of surfactant on postnatal day 4 and MSCs ($3 \times 10^4$ or $3 \times 10^5$ cells) on postnatal day 5 improved alveolarization and angiogenesis in the neonatal rats exposed to hyperoxia. The surfactant and MSCs ($3 \times 10^5$ cells) treatment was more successful in improving alveolarization and angiogenesis than the surfactant and MSCs ($3 \times 10^4$ cells) treatment was. The main findings of this study were that the intratracheal administration of MSCs on the day following administration of surfactant improve lung development and that high doses of MSCs amplify the therapeutic effects on experimental BPD in neonatal rats compared with the low doses of MSCs.

Although term-born rats have structurally immature lungs, they are functionally mature and require no surfactant treatment. In one study, neonatal mice exposed to hyperoxia for 4 days exhibited disruptions to type II cell proliferation, which produced pulmonary surfactant [17]. In another study, hyperoxia during the first 3 days of life induced inflammatory cell infiltration in alveolar spaces and increased the wet-to-dry lung weight ratio in neonatal Sprague Dawley rats [18]. The results of these studies have suggested that hyperoxia reduces surfactant production and induces lung inflammation in newborn animals. Pulmonary surfactant is a mixture of phospholipids, surfactant-associated proteins, and neutral lipids, which modulate pulmonary inflammation and stabilize the alveoli by reducing surface tension [19]. In our study, the administration of surfactant on postnatal day 4 diminished the hyperoxia-induced increase in MLI and lung cytokines in the neonatal rats. The surfactant treatment did not augment the hyperoxia-induced decrease in pulmonary vascular density. These results support the idea that pulmonary
Surfactant fulfills an essential role in the lungs for both host defense mechanisms, such as modulating pulmonary inflammation, and for improving alveolarization [14, 20].

Surfactant therapy has become the standard of care for preterm infants with respiratory distress syndrome and can reduce the combined outcomes of death and BPD [6]. Compared with delayed surfactant treatment, early surfactant treatment was more effective in reducing mortality, air leak, BPD, and BPD or death in preterm infants [21]. Although surfactant has a unique spreading property and can reduce surface tension. The addition of a surfactant reduced the in vitro viability of human MSCs, and the combination therapy of surfactant and MSCs did not exhibit any additional benefits to lung development in neonatal rats exposed to hyperoxia [14]. For this reason, we administered intratracheal surfactant and MSCs on 2 consecutive days and found that the intratracheal administration of surfactant on postnatal day 4 and MSCs on postnatal day 5 improved alveolarization and angiogenesis in the neonatal rats exposed to hyperoxia. The time interval between the administration of surfactant and the MSCs for achieving optimal therapeutic effects was not determined. Future studies are required to evaluate the effects of different time intervals on hyperoxia-induced lung injury.

In this study, the administration of surfactant and the administration of surfactant with human MSCs to the hyperoxia-exposed rats significantly improved lung development in the surviving animals, although the survival rate did not significantly improve. The differences in the survival rates between rats treated with surfactant and those treated with surfactant and MSCs were not significant on postnatal day 14. The rats reared in hyperoxia and treated with NS exhibited a low survival rate after postnatal day 5. The treatment with surfactant and treatment with surfactant and human MSCs (3 × 10^5 cells) maintained the survival rate from postnatal days 5 to 9. These results suggest that an additional dose of MSCs is required to maintain the survival rate.

In this study, we determined the levels of VEGF, PDGF-A, and PDGF-B expression and elucidated the mechanisms that mediate the MSCs’ effects because their mRNA and protein expression decreased in the lungs of newborn animals exposed to 14 days of hyperoxia [22–24]. VEGF is a potent endothelial cell mitogen that regulates angiogenesis and alveolar development [25]. PDGF is crucial to alveolarization of normally developing lungs [26]. We demonstrated that the rats reared in hyperoxia and treated with NS exhibited significantly lower levels of VEGF, PDGF-A, and PDGF-B protein expression than did those reared in RA and treated with NS or surfactant. Treatment with surfactant and MSCs augmented the hyperoxia-induced decrease in the VEGF, PDGF-A, and PDGF-B protein expression levels. These results suggest that treatment with MSCs enhanced vascular and alveolar development in the neonatal rats through the induction of growth factors.

**Conclusions**

Consecutive daily administration of intratracheal surfactant and human MSCs likely attenuated hyperoxia-induced defective alveolarization and angiogenesis by increasing VEGF expression. High doses of MSCs enhanced the therapeutic effects more effectively than the low doses of MSCs. Consecutive
daily administration of intratracheal surfactant and MSCs can be an effective regimen for treating hyperoxia-induced lung injury in neonates.

Abbreviations

BPD: bronchopulmonary dysplasia; IL: interleukin; MLI: mean linear intercept; MSCs: mesenchymal stem cells; NS: normal saline; O₂: oxygen-enriched atmosphere; PDGF: platelet-derived growth factor, RA: room air; VEGF: vascular endothelial growth factor; vWF: von Willebrand factor

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Care and Use Committee at Taipei Medical University (LAC-2019-0396).

Consent for publication

All authors consent to publication.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by an agreement between Taipei Medical University and Meridigen Biotech Co., Ltd. Taipei, Taiwan (A-109-008).

Authors’ contributions

CMC and HCC contributed to the study design, data collection, execution of the study, data analysis and interpretation, and preparation of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Authors details
References

1. O’Reilly M, Thébaud B. Animal models of bronchopulmonary dysplasia. The term rat models. Am J Physiol Lung Cell Mol Physiol. 2014;307:L948–58.

2. Manji JS, O’Kelly CJ, Leung WI, Olson DM. Timing of hyperoxic exposure during alveolarization influences damage mediated by leukotrienes. Am J Physiol Lung Cell Mol Physiol. 2001;281:L799–806.

3. Chen CM, Hwang J, Chou HC. Maternal Tn immunization attenuates hyperoxia-induced lung injury in neonatal rats through suppression of oxidative stress and inflammation. Front Immunol. 2019;10:681.

4. Gien J, Kinsella JP. Pathogenesis and treatment of bronchopulmonary dysplasia. Curr Opin Pediatr. 2011;23:305–13.

5. Laughon M, Allred EN, Bose C, O’Shea TM, Van Marter LJ, Ehrenkranz RA, et al. ELGAN Study Investigators. Patterns of respiratory disease during the first 2 postnatal weeks in extremely premature infants. Pediatrics. 2009;123:1124–31.

6. Polin RA, Carlo WA, Committee on Fetus and Newborn, American Academy of Pediatrics. Surfactant replacement therapy for preterm and term neonates with respiratory distress. Pediatrics. 2014;133:156–63.

7. Chang YS, Choi SJ, Sung DK, Kim SY, Oh W, Yang YS and Park WS. Intratracheal transplantation of human umbilical cord blood-derived mesenchymal stem cells dose-dependently attenuates hyperoxia-induced lung injury in neonatal rats. Cell Transplant. 2011;20:1843–54.

8. Waszak P, Alphonse R, Vadivel A, Ionescu L, Eaton F, Thébaud B. Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats. Stem Cells Dev. 2012;21:2789–97.

9. Tropea KA, Leder E, Aslam M, Lau AN, Raiser DM, Lee JH, et al. Bronchioalveolar stem cells increase after mesenchymal stromal cell treatment in a mouse model of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol. 2012;302:L829–37.

10. Pierro M, Ionescu L, Montemurro T, Vadivel A, Weissmann G, Oudit G, et al. Short-term, long-term and paracrine effect of human umbilical cord-derived stem cells in lung injury prevention and repair in experimental bronchopulmonary dysplasia. Thorax. 2013;68:475–84.

11. Sutsko RP, Young KC, Ribeiro A, Torres E, Rodriguez M, Hehre D, et al. Long-term reparative effects of mesenchymal stem cell therapy following neonatal hyperoxia-induced lung injury. Pediatr Res. 2013;73:46–53.
12. Chou HC, Li YT, Chen CM. Human mesenchymal stem cells attenuate experimental bronchopulmonary dysplasia induced by perinatal inflammation and hyperoxia. Am J Transl Res. 2016;8:342–53.

13. Chen CM, Chou HC. Human mesenchymal stem cells attenuate hyperoxia-induced lung injury through inhibition of the renin-angiotensin system in newborn rats. Am J Transl Res. 2018;10:2628–35.

14. Chen CM, Chou HC, Lin W, Tseng C. Surfactant effects on the viability and function of human mesenchymal stem cells: in vitro and in vivo assessment. Stem Cell Res Ther. 2017;8:180.

15. Chen CM, Chen YJ, Huang ZH. Intratracheal instillation of stem cells in term neonatal rats. J Vis Exp. 2020;159:e61117.

16. Irwin D, Helm K, Campbell N, Imamura M, Fagan K, Harral J, et al. Neonatal lung side population cells demonstrate endothelial potential and are altered in response to hyperoxia-induced lung simplification. Am J Physiol Lung Cell Mol Physiol. 2007;293:L941–51.

17. Yee M, Vitiello PF, Roper JM, Staversky RJ, Wright TW, McGrath-Morrow SA, Maniscalco WM, Finkelstein JN, O'Reilly MA. Type II epithelial cells are critical target for hyperoxia-mediated impairment of postnatal lung development. Am J Physiol Lung Cell Mol Physiol. 2006;291:L1101–1110.

18. Wang Y, Yue S, Luo Z, Cao C, Yu X, Liao Z, et al. N-methyl-D-aspartate receptor activation mediates lung fibroblast proliferation and differentiation in hyperoxia-induced chronic lung disease in newborn rats. Respir Res. 2016;17:136.

19. Goerke J. Pulmonary surfactant: functions and molecular composition. Biochim Biophys Acta. 1998;1408:79–89.

20. Reid KB, Clark H, Palaniyar N. Surfactant and lung inflammation. Thorax. 2005;60:620–

21. Bahadue FL, Soll R. Early versus delayed selective surfactant treatment for neonatal respiratory distress syndrome. Cochrane Database Syst Rev. 2012;11:CD001456.

22. Perveen S, Patel H, Arif A, Younis S, Codipilly CN, Ahmed M. Role of EC-SOD overexpression in preserving pulmonary angiogenesis inhibited by oxidative stress. PLoS ONE. 2012;7:e51945.

23. Chen CM, Hwang J, Chou HC. Maternal Tn immunization attenuates hyperoxia-induced lung injury in neonatal rats through suppression of oxidative stress and inflammation. Front Immunol. 2019;10:681.

24. Zhang X, Reinsvold P, Thibeault DW, Ekekezie II, Rezaiekhahigh M, Mabry SM, et al. Responses of pulmonary platelet-derived growth factor peptides and receptors to hyperoxia and nitric oxide in piglet lungs. Pediatr Res. 2005;57:523–

25. Yun EJ, Lorizio W, Seedorf G, Abman SH, Vu TH. VEGF and endothelium-derived retinoic acid regulate lung vascular and alveolar development. Am J Physiol Lung Cell Mol Physiol. 2016;310:L287–98.

26. Lindahl P, Boström H, Karlsson L, Hellström M, Kalen M, Betsholtz C. Role of platelet-derived growth factors in angiogenesis and alveogenesis. Curr Top Pathol. 1999;93:27–33.
Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.