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REVIEW ARTICLE

Mechanism of Oligohydramnios-induced Pulmonary Hypoplasia

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Pulmonary hypoplasia is common in the perinatal period and is a significant cause of death in newborn infants, and oligohydramnios is one of the most commonly associated abnormalities. Neonates exposed to oligohydramnios caused by premature rupture of membranes have an increased risk of acute respiratory morbidity. The exact mechanism by which oligohydramnios alters the respiratory system remains unknown. We herein report the effects of experimental oligohydramnios on lung growth and the expressions of growth factors and extracellular matrix in fetal rats on days 19 and 21 of gestation by producing oligohydramnios from days 16 to 21 of gestation in Sprague-Dawley dams. Rats exposed to oligohydramnios exhibited lung hypoplasia and significantly decreased expressions of extracellular matrix, transforming growth factor-β1 and platelet-derived growth factor on days 19 and 21 of gestation. Concomitant maternal retinoic acid treatment at a dose of 10 mg/kg increased platelet-derived growth factor expression but did not enhance fetal lung development. These results suggest that there is a stage-specific requirement for retinoic acid during lung development, and retinoic acid treatment should be applied with caution.

1. Introduction

Pulmonary hypoplasia is common in the perinatal period and is a significant cause of death in newborn infants, and oligohydramnios is one of the most commonly associated abnormalities.[1] Oligohydramnios may retard fetal lung growth and can result in pulmonary hypoplasia in experimental animals and human fetuses with prolonged rupture of membranes.[2,3] Neonates exposed to oligohydramnios caused by premature rupture of membranes have an increased risk of acute respiratory morbidity, including higher ventilator settings, increased incidences of hypoxemia and hypercapnia, and pulmonary hypertension, and a trend toward more air leaks.[4] That study indicated that oligohydramnios has considerable impact on short-term respiratory morbidity in infants. Physical forces produced by fetal breathing movements and lung fluid in the airspaces play important roles in regulating fetal lung growth and maturation.[5,6] The fluid maintains the lungs in an expanded state and provides the tissue with the mechanical stretching necessary for normal lung development.[7] Mechanical stretching increases growth factor expression and collagen synthesis and secretion in lung

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fibroblast culture.[8–10] However, the exact mechanism by which oligohydramnios induces lung hypoplasia and alters the respiratory system remains unknown. We herein report the results of inducing experimental oligohydramnios on the expressions of growth factors and collagen and elastin in fetal rat lungs on days 19 and 21 of gestation and discuss the effects of maternal retinoic acid (RA) treatment on lung development in Sprague-Dawley rats.[11–13]

2. Oligohydramnios Produces Pulmonary Hypoplasia

Experimental oligohydramnios was produced by puncturing the uterine wall and fetal membrane of each amniotic sac on day 16 of gestation in timed pregnant Sprague-Dawley dams. Fetuses in the opposite uterine horn served as controls. On days 19 and 21 of gestation, fetuses were delivered by cesarean section. Rats exposed to oligohydramnios exhibited significantly lower lung weight/body weight ratios on days 19 and 21 of gestation than did control rats (Table 1). Alveolarization predominantly occurs within the first 2 weeks of postnatal life in rats. The term "saccules" was used instead of "alveoli" for the radial alveolar count because alveolarization was measured in fetal rats on day 21 of gestation during the saccular stage. Maternal oligohydramnios created on day 16 of gestation produced fetal lung hypoplasia as indicated by fewer epithelial tubules on day 19 and fewer saccules on day 21 of gestation (Figure 1A). The radial saccular count was significantly lower in fetal rats exposed to oligohydramnios than in control rats on day 21 of gestation (Figure 1B).

3. Oligohydramnios Decreases Collagen and Elastin Expressions

Collagen is vital for maintaining the normal lung architecture. Collagen fibrils are widely distributed in the interstitium of the bronchial tree, the interlobular septa, the bronchial lamina propria, and the alveolar interstitium. Elastin is an important structural component of

Table 1 Body weight, lung weight, and lung/body weight ratio in control rats and rats exposed to oligohydramnios

Treatment	*n*	Gestational age (d)	Body weight (g)	Lung weight (g)	Lung/body weight (%)
Control	16	19	2.02±0.13	0.08±0.01	3.71±0.10
Oligohydramnios	23	19	2.08±0.11	0.07±0.00	3.18±0.10*
Control	27	21	4.10±0.12	0.15±0.01	3.65±0.11
Oligohydramnios	23	21	3.88±0.20	0.12±0.01†	3.04±0.08*

*$p < 0.001$ and †$p < 0.01$ *vs.* control rats at each gestational age.

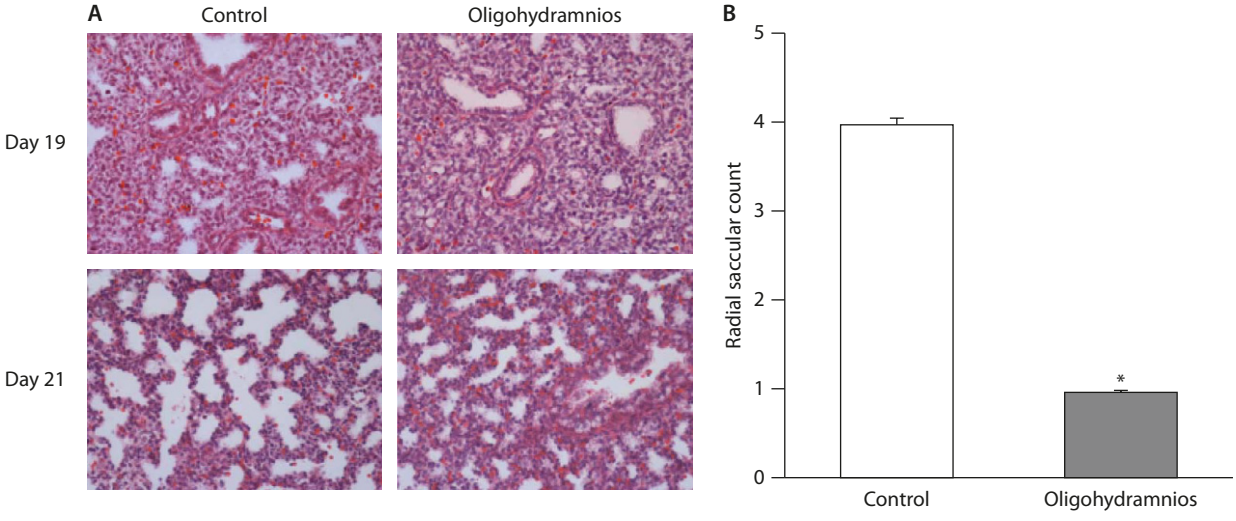

Figure 1 (A) Examination of random lung fields under a light microscope (200×) reveals fewer epithelial tubules in rats exposed to oligohydramnios compared to control rats on day 19 of gestation. The lung section depicts fewer saccules in oligohydramnios rats on day 21 of gestation. (B) The radial saccular count was significantly lower in rats exposed to oligohydramnios than in control rats on day 21 of gestation. *$p < 0.001$.

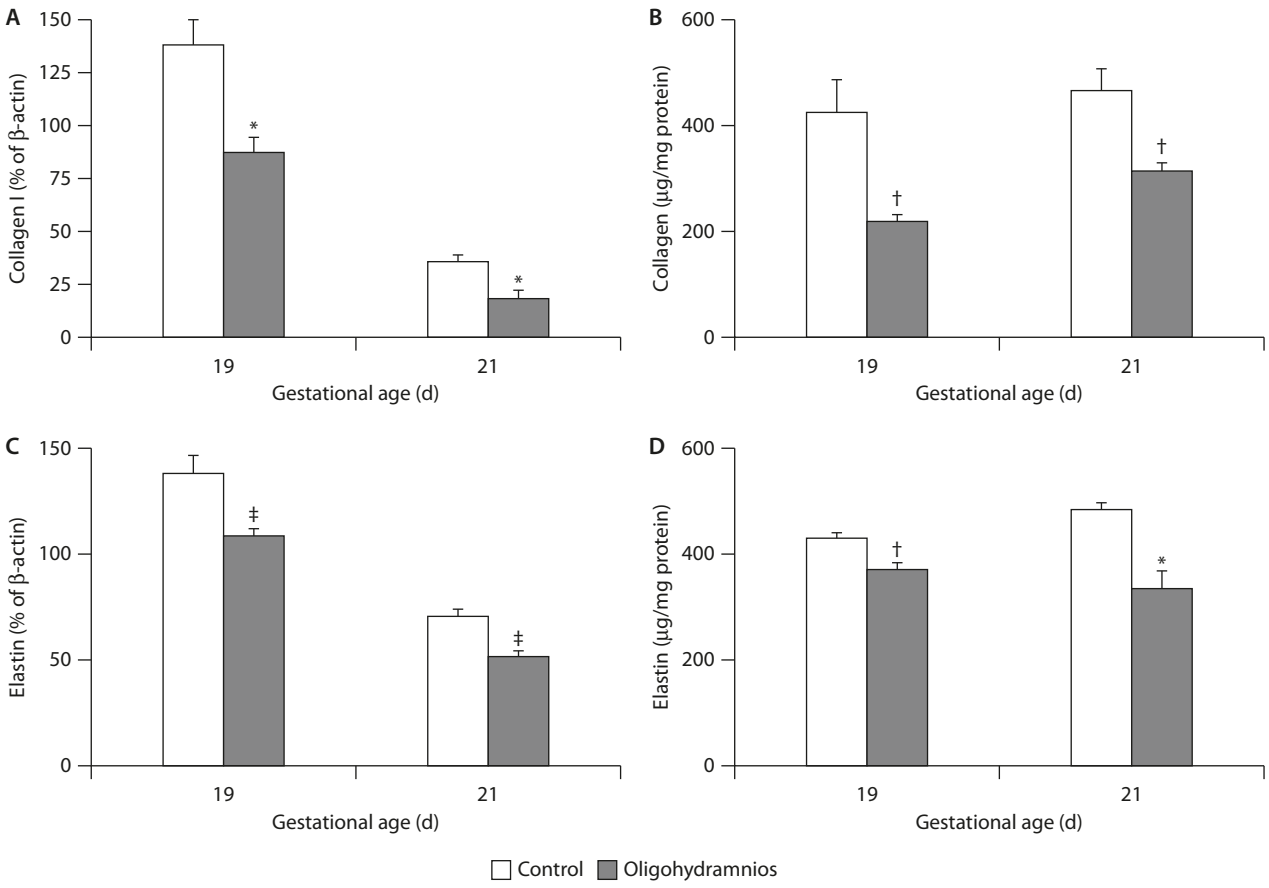

Figure 2 Effects of oligohydramnios on (A, B) type I collagen mRNA expression and total collagen and (C, D) elastin mRNA expression and elastin levels in fetal rat lungs on days 19 and 21 of gestation. $^{*}p<0.01$, $^{\dagger}p<0.05$ and $^{\ddagger}p<0.001$ *vs.* control group on each gestational day.

the alveolar wall and conducting airways and allows the expansion and recoil of the lung that are essential to its mechanical performance.[14] There are strong temporal and spatial relationships between the expression of elastin and the development of terminal airspaces. In the developing lung, elastin is deposited in the mesenchyme surrounding the developing distal airways before alveolarization and at the apex of the secondary septal crests during the process of alveolarization.[15] However, little is known about the effects of oligohydramnios on the lung collagen and elastin status *in vivo*.

Oligohydramnios-exposed rats exhibited significantly decreased collagen I and elastin messenger (m) RNA expressions on days 19 and 21 of gestation (Figures 2A and 2C). Total collagen and elastin levels in fetal rat lungs were also significantly lower in oligohydramnios-exposed rats on days 19 and 21 of gestation (Figures 2B and 2D). This result is consistent with the observations of Haider et al, who found that elastic tissue was missing in hypoplastic human fetal lungs associated with oligohydramnios.[16] Joyce et al found sustained reductions in lung expansion by tracheal drainage-decreased elastin expression.[17] These studies further support pulmonary hypoplasia being associated with oligohydramnios resulting from a reduction in fetal lung expansion. The accumulation of collagen in lung tissues is controlled by various growth factors, and a dynamic equilibrium between the synthesis and degradation of collagen is maintained. This balance is controlled by *de novo* synthesis of collagen, proteolytic degradation by matrix metalloproteinases (MMPs), and inhibition of MMP activity by tissue inhibitors of metalloproteinases (TIMPs).[18] MMP-1 is an interstitial collagenase that specifically degrades type I collagen, and TIMP-1 is a specific inhibitor of MMP-1. MMP-1 protein increased and TIMP-1 protein decreased in oligohydramnios-exposed rats compared to control rats on days 19 (Figure 3A) and 21 (Figure 3B) of gestation. An imbalance in MMP and TIMP can result in metalloproteinase activation, and a relatively higher level of MMP than TIMP may stimulate the degradation of collagen in interstitial spaces. During the pathogenesis of oligohydramnios, homeostasis deteriorates, resulting in a net decrease in the deposited collagen content of the lung. This altered extracellular matrix (ECM) might be responsible for the alterations in respiratory function associated with oligohydramnios.

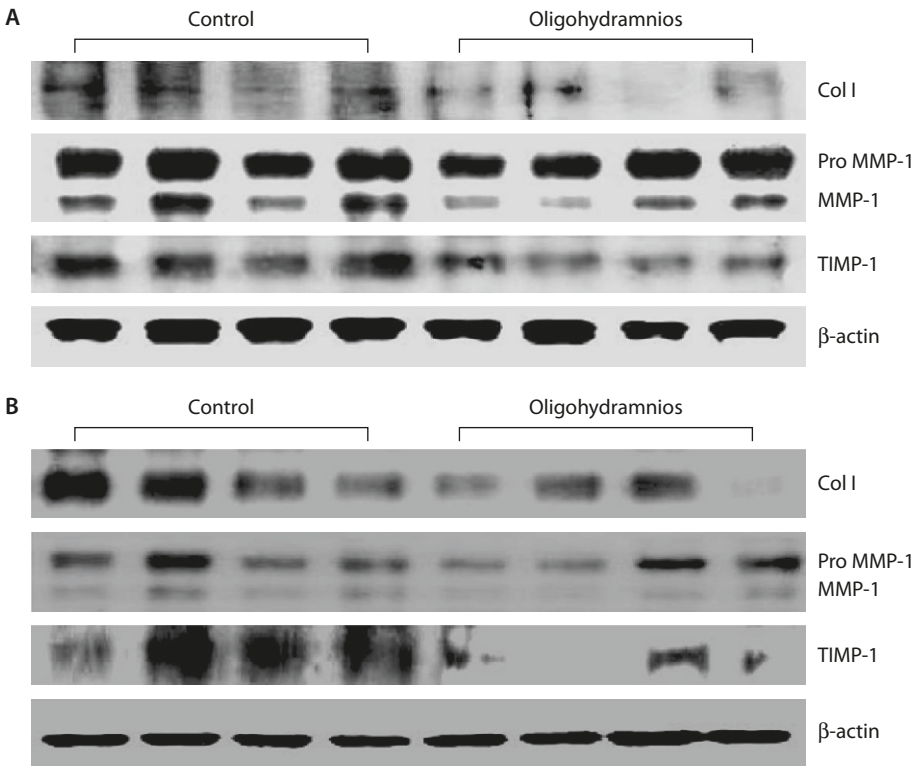

Figure 3 Representative Western blots for type I collagen (Col I), matrix metalloproteinase (MMP)-1, and tissue inhibitors of metalloproteinases (TIMP)-1 proteins in fetal rat lungs exposed to oligohydramnios on days (A) 19 and (B) 21 of gestation.

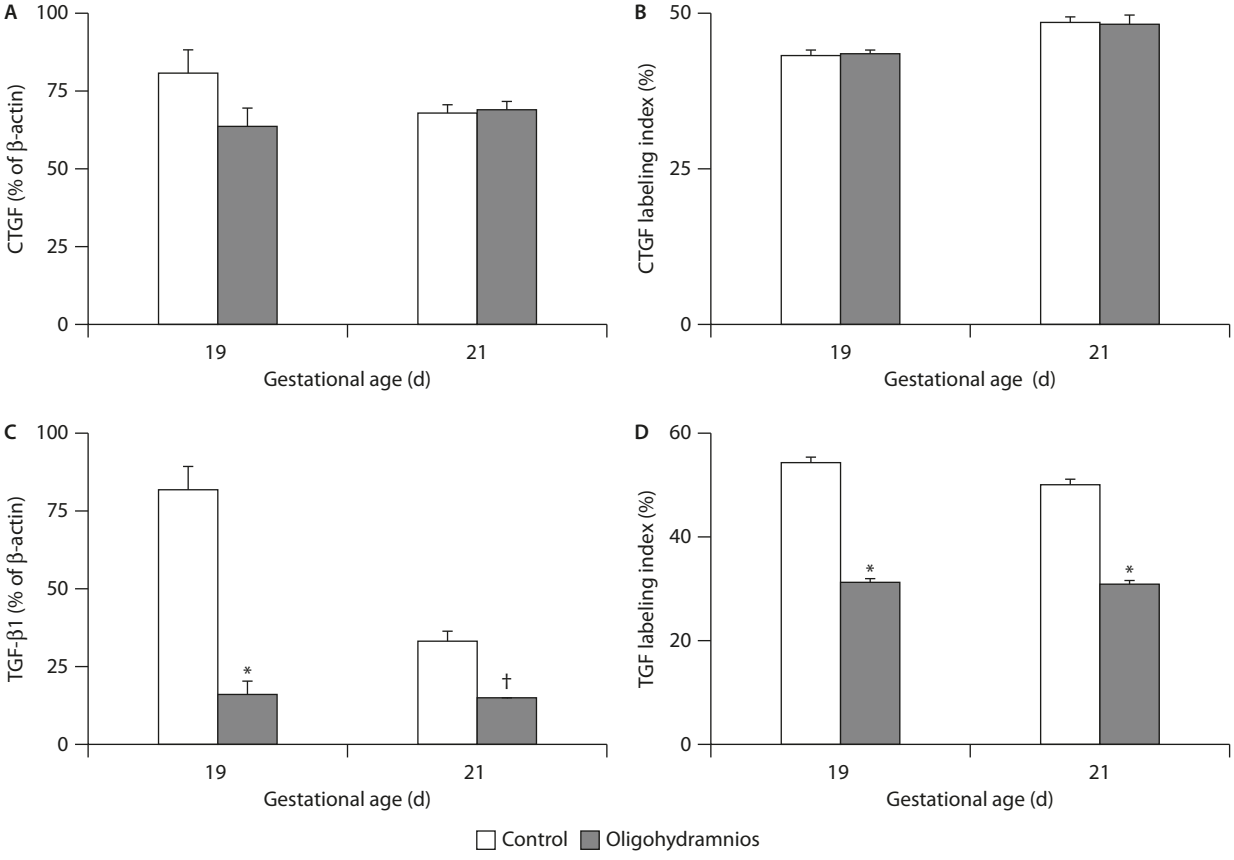

Figure 4 Effects of oligohydramnios on (A, B) connective tissue growth factor (CTGF) mRNA expression and immunoreactivity (labeling index) and (C, D) tumor growth factor (TGF)-β1 mRNA expression and immunoreactivity (labeling index) in fetal rat lungs on days 19 and 21 of gestation. *$p < 0.001$ and †$p < 0.01$ *vs.* control group on each gestational day.

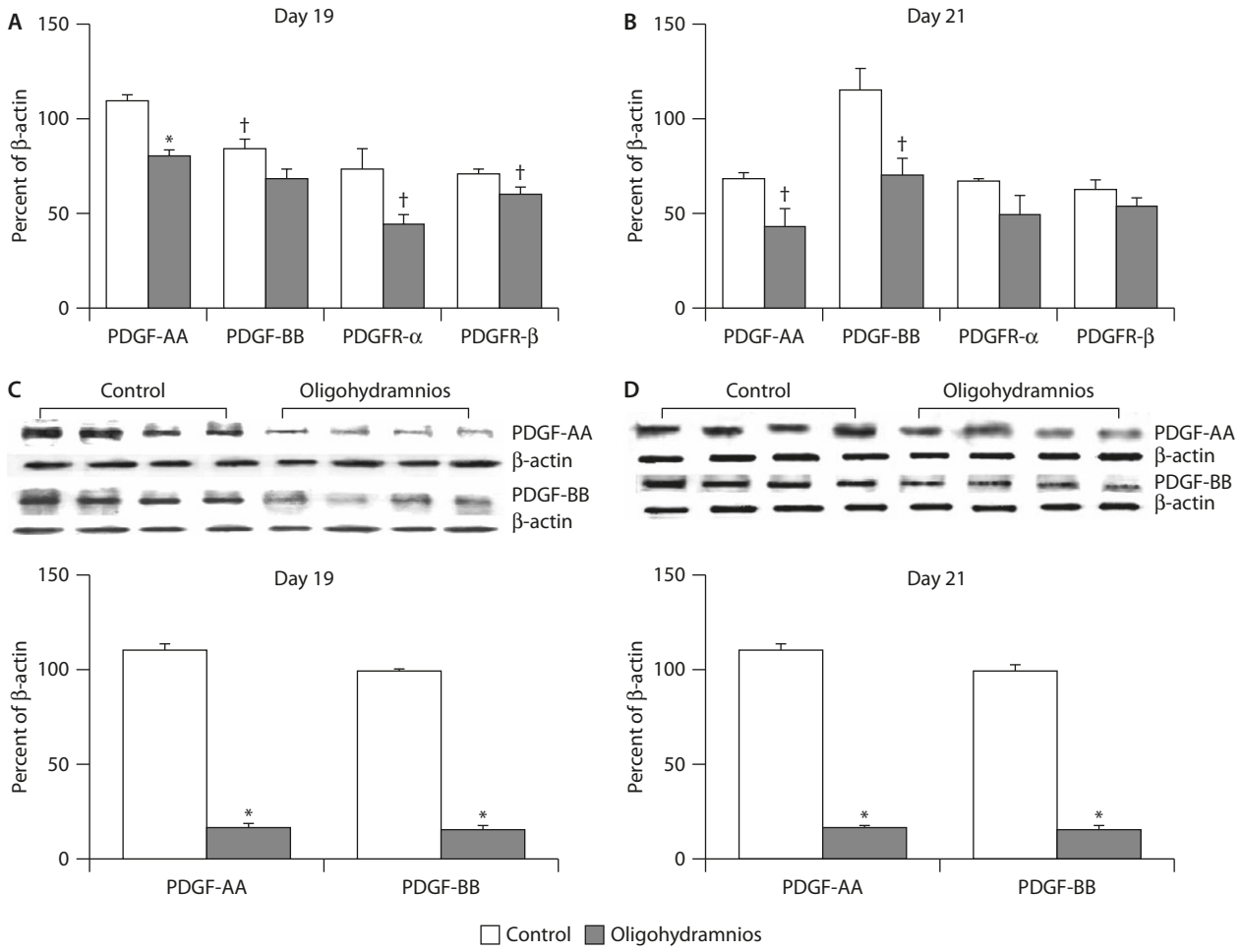

Figure 5 Effects of oligohydramnios on (A, B) platelet-derived growth factor (PDGF) and PDGF receptor (PDGFR) mRNA expressions and (C, D) PDGF protein in fetal rat lungs on days 19 and 21 of gestation. $^{*}p < 0.001$ and $^{\dagger}p < 0.05$ *vs.* control group on each gestational day.

4. Effects of Oligohydramnios on Growth Factor Expression

Transforming growth factor (TGF)-β1 is a potent stimulant of collagen synthesis in fibroblast cultures; it activates collagen promoters and increases the production of type I and III collagen.[19,20] Connective tissue growth factor (CTGF) is a promoter of collagen deposition that acts downstream of TGF-β1, particularly with respect to its profibrotic effects.[21] CTGF was implicated in fibroblast proliferation, cellular adhesion, and ECM synthesis.[22] CTGF mRNA expression and immunoreactivity (labeling index) were comparable between oligohydramnios-exposed and control rats, whereas TGF-β1 mRNA expression and immunoreactivity (labeling index) were significantly lower in oligohydramnios-exposed rats compared to control rats on days 19 and 21 of gestation (Figure 4). These data are compatible with the results of Kessler et al, who found that TGF-β did not stimulate CTGF expression when added to relaxed cells, and no temporal correlation was noted between the expressions of CTGF and TGF-β in stressed or relaxed fibroblasts.[23] These results suggest that the signaling cascade activated by oligohydramnios does not involve an autocrine loop of CTGF. Platelet-derived growth factor (PDGF) is important for alveolarization of the normally developing lung.[24] PDGFs are homodimers or heterodimers consisting of two distinct polypeptide chains (A and B), which can be dimerized via sulfhydryl bridges to form three bioactive isoforms (AA, BB, and AB).[25] PDGF is a powerful stimulator of fibroblast chemotaxis and proliferation.[26,27] Han et al reported that both PDGF and its receptors are present in the fetal rat lung.[28,29] Rats exposed to oligohydramnios exhibited downregulation of the PDGF and PDGF receptor (PDGFR)-α and -β (Figure 5). These results are compatible with analyses by Souza et al who used antisense oligonucleotides in an embryonic rat lung explant culture and suggested that PDGF plays critical roles in early lung growth and branching morphogenesis.[30,31]

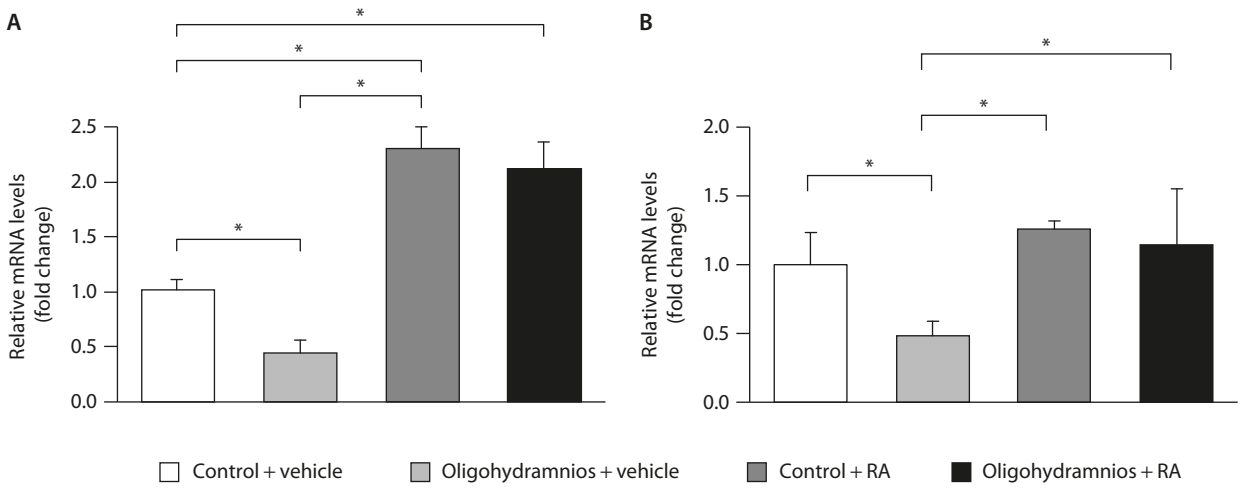

Figure 6 Effects of maternal retinoic acid (RA) treatment on (A) platelet-derived growth factor-A and (B) platelet-derived growth factor-B mRNA expressions in fetal rat lungs on day 21 of gestation. *$p < 0.05$.

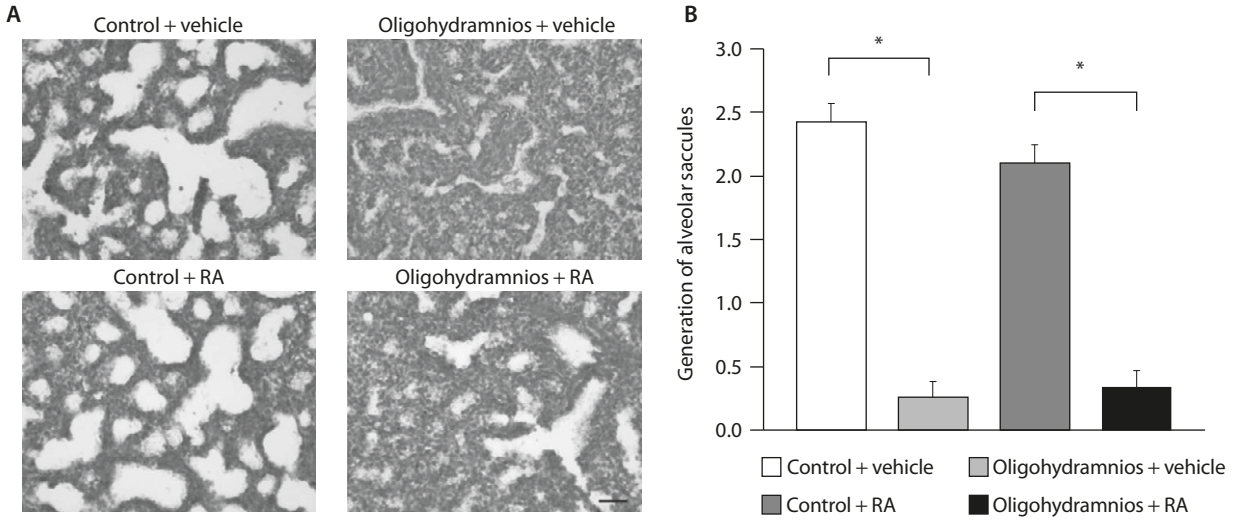

Figure 7 (A) Histological analysis of maternal retinoic acid (RA) treatment on fetal lung development on day 21 of gestation (200×). (B) The generation of alveolar saccules on day 21 of gestation. *$p < 0.001$.

5. Effects of Maternal RA Treatment on Oligohydramnios-induced Lung Hypoplasia

Liebeskind et al noted that all-*trans* RA (ATRA) can stimulate PDGF-A mRNA expression in cultured fetal and postnatal rat lung fibroblasts and in newborn rat lungs.[32] ATRA was also reported to induce septation in a rat model of pharmacologically caused failure of septation.[33] These studies suggest that ATRA may influence alveolarization through a PDGF-mediated mechanism. Maternal oligohydramnios was created from days 16 to 21 of gestation, and maternal RA was concomitantly administered at a dose of 10 mg/kg. This increased PDGF-A and -B mRNA expressions (Figure 6) but did not enhance fetal lung development as evidenced by the generation of alveolar saccules (Figure 7). These contrasting outcomes are likely to be related to differences in the stage of lung development and the timing and duration of the administration of ATRA, and suggest that PDGF might not be the major determinant of lung development in experimental oligohydramnios.

6. Conclusions

Rats exposed to oligohydramnios exhibited lung hypoplasia and significantly decreased expressions of ECM, TGF-β1 and PDGF on days 19 and 21 of gestation. Concomitant maternal RA treatment at a dose of 10 mg/kg increased PDGF expression but did not enhance fetal lung development. RA influences cell programming and differentiation during early lung development, and its activity is reduced during airway branching.[34] Branching morphogenesis is inhibited when RA signaling is activated by exogenous RA *in vitro* and in RA receptor

transgenic mice.[35,36] Blocking RA signaling by a pan-RA receptor antagonist increases explant bud formation, while treatment with RA reduces explant bud formation of fetal mice lung in the pseudoglandular stage.[37] These and our studies indicate that there is a stage-specific requirement for RA during lung development, and RA treatment should be applied with caution.

Acknowledgments

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