TREATMENT OF NEWBORN RATS WITH A VEGF RECEPTOR INHIBITOR CAUSES PULMONARY HYPERTENSION AND ABNORMAL LUNG STRUCTURE

TIMOTHY D. LE CRAS, NEIL E. MARKHAM, RUBIN M. TUDER, NORBERT F. VOELKEL, AND STEVEN H. ABMAN

Pediatric Heart Lung Center, Departments of Pediatrics and Medicine, University of Colorado School of Medicine, Denver, Colorado 80262; and Department of Pathology, Johns Hopkins Medical Center, Baltimore, Maryland 21205

Received 19 October 2001; accepted in final form 15 April 2002

Le Cras, Timothy D., Neil E. Markham, Rubin M. Tuder, Norbert F. Voelkel, and Steven H. Abman. Treatment of newborn rats with a VEGF receptor inhibitor causes pulmonary hypertension and abnormal lung structure. Am J Physiol Lung Cell Mol Physiol 283: L555–L562, 2002.—To determine whether disruption of vascular endothelial growth factor (VEGF)-VEGF receptor (VEGFR) signaling in the newborn has long-term effects on lung structure and function, we injected 1-day-old newborn rat pups with a single dose of Su-5416, a VEGF inhibitor, or vehicle (controls). Lungs from infant (3-wk-old) and adult (3- to 4-mo-old) rats treated with Su-5416 as newborns showed reductions in arterial density (82 and 31%, respectively) and alveolar counts (45 and 29%) compared with controls. Neonatal treatment with Su-5416 increased right ventricle weight to body wt ratios (4.2-fold and 2.0-fold) and pulmonary arterial wall thickness measurements (2.7-fold and 1.6-fold) in infant and adult rats, respectively, indicating marked pulmonary hypertension. We conclude that treatment of newborn rats with the VEGFR inhibitor Su-5416 impaired pulmonary vascular growth and postnatal alveolarization and caused pulmonary hypertension and that these effects were long term, persisting well into adulthood.

angiogenesis; postnatal lung development; alveogenesis; bronchopulmonary dysplasia; pulmonary vascular development; vascular endothelial growth factor

RECENT EPIDEMIOLOGICAL EVIDENCE has supported the hypothesis that perinatal stress can contribute to adult disease (3). This hypothesis, frequently referred to as “the Barker hypothesis,” is based on the concept that stress or injury during critical periods of organogenesis can permanently alter the normal pattern of development and predispose the individual for disease later in life. Data supporting this hypothesis have been primarily from epidemiological studies, and underlying mechanisms that actually link perinatal stress with adult disease are unknown. Although most studies have focused on systemic hypertension, coronary heart disease, atherosclerosis, and diabetes (3), a few epidemiological studies have suggested that adult respiratory disorders, such as chronic obstructive pulmonary disease (COPD) and asthma, may have origins in the perinatal period (12, 33, 38). However, mechanisms that disrupt early lung development and cause persistent disease later in life are unclear.

In rodents and humans, the alveolar phase of lung development occurs predominantly during early infancy, in the first 2–3 wk after birth in rats, or 1–2 yr of life in humans (5, 7). During this final phase of lung development, lung surface area increases markedly due to the rapid formation of alveoli by the generation of secondary septae (5, 7). Pulmonary vascular growth also undergoes rapid expansion in close coordination with alveolarization (7, 30). Cellular and molecular mechanisms that coordinate alveolar and pulmonary vascular development are unclear, but disruption of postnatal alveolarization and pulmonary vascular growth occurs in a number of neonatal lung diseases, including bronchopulmonary dysplasia (BPD) and congenital diaphragmatic hernia (1, 21, 23).

Critical signals linking airway and vascular growth in the developing lung are not well understood but are known to include vascular endothelial growth factor (VEGF). VEGF-A, a 34- to 46-kDa dimeric glycoprotein, is a mitogen that stimulates endothelial cell proliferation and is believed to play a major role in driving angiogenesis in a number of organs, including the lung (9, 16). VEGF binds to two tyrosine kinase receptors, VEGF receptor (VEGFR)-1 (Flt-1) and VEGFR-2 (also known as Flk-1 in rodents or KDR in humans) (reviewed in Ref. 15). Binding of VEGF to the VEGFR-2, but not to the VEGFR-1, has been shown to be the primary signaling mechanism triggering the proangiogenic activity of VEGF (19, 36). The critical role of VEGF in early vascular development in the embryo has been demonstrated in studies in which knockout of even a single allele of the VEGF gene disrupts vascular growth and is embryonically lethal (9, 16). In addition to its effects on endothelial cell proliferation, VEGF can also have trophic effects on the vascular endothelium and may play an important role in stimulating angiogenesis.
MATERIALS AND METHODS

VEGFR signaling in newborn rats with a single dose of Su-5416 disrupted postnatal alveolarization and pulmonary vascular development (22). The goal of the present study was to determine whether disruption of VEGF signaling has been suggested to play a role in endothelial cell dysfunction and the pathogenesis of pulmonary hypertension (35), we looked for evidence of pulmonary hypertension after treatment with Su-5416. We found that treatment of newborn rats 1 day after birth reduced pulmonary vascular development and postnatal alveolarization and caused severe pulmonary hypertension at 3 wk of age and that these effects persisted into adulthood.

Previously, we have shown that treatment of newborn rats with multiple doses of the VEGFR inhibitor Su-5416 disrupted postnatal alveolarization and pulmonary vascular development (22). The goal of the present study was to determine whether disruption of VEGFR signaling in newborn rats with a single dose of Su-5416 would be sufficient to disrupt postnatal lung development and whether it would have long-term effects on lung structure into adulthood. In addition, because disruption of VEGF signaling has been suggested to play a role in endothelial cell dysfunction and the pathogenesis of pulmonary hypertension (35), we assessed by the radial alveolar count method (10, 13). Radial counts were performed by first identifying respiratory bronchioles. From the center of the bronchiole, a perpendicular line was taken to the edge of the acinus (connective tissue septum or pleura), and the number of alveoli that intersected the line was counted. Ten counts were performed for each animal.

Hematocrits and assessments of pulmonary hypertension: RV hypertrophy and arterial wall thickness measurements. At death, 0.5 ml of blood were removed from the left ventricle (LV) into a heparinized syringe. Capillary tubes were filled with 0.1 ml of blood and centrifuged in a microcapillary centrifuge. Hematocrit was determined by measuring packed red blood cell volume as a percentage of total blood volume using a microcapillary reader. Hearts were removed and dissected to isolate the free wall of the RV from the LV and septum (S). The ratios of RV weight:body wt (RV:BW) and RV weight over LV+S weight (RV:LV+S) were used as indexes of RV hypertrophy resulting from pulmonary hypertension (20). If LV+S weight:BW was different between groups, indicating development of LV hypertrophy, then RV:BW was used instead of RV:LV+S. Vessel wall thickness measurements were performed on heparinized and eosin-stained lung sections by an observer blinded to the identity of the slides. Wall thickness measurements were performed on pulmonary arteries (20–100 μm) associated with terminal bronchioles (~50% measured) and respiratory bronchioles (~50% measured) using Metamorph (Universal Imaging, Downingtown, PA). Six vessels were measured per animal. Wall thickness and external diameter were measured, and percentage of wall thickness was calculated as [2 × wall thickness/external diameter] × 100 (20). Because wall thickness measurements were made on hematoxylin and eosin sections, increases in vessel wall thickness may be due to adventitial as well as medial hypertrophy.
Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed with the Statview software package (Abacus Concepts, Berkeley, CA). Statistical comparisons were made using analysis of variance and Fisher’s protected least significant differences test. *P < 0.05 was considered significant.

RESULTS

BWs. BWs of infant rats treated with Su-5416 1 day after birth were lower than littermate vehicle-treated controls (29.4 ± 1.9 vs. 44.4 ± 0.7 g; P < 0.05). Whereas lung weights of Su-5416-treated infant rats were lower than infant controls (0.36 ± 0.02 vs. 0.45 ± 0.01 g; P < 0.05), lung:BW ratios were slightly higher than controls (1.22 ± 0.09 vs. 1.03 ± 0.02; P < 0.05). BWs of adult rats treated with Su-5416 as newborns were not different from adult controls [328 ± 22 vs. 283 ± 18 g; P = not significant (NS)]. In addition, prior Su-5416 treatment did not reduce BW after exposure to 3 wk of hypoxia in adult rats compared with adult hypoxic controls (349 ± 10 vs. 359 ± 12; P = NS).

Barium arteriograms and arterial density counts. Barium-gelatin arteriograms of the lungs of infant rats showed that Su-5416 treatment caused a decrease in the background filling of small- and medium-sized pulmonary arteries with barium-gelatin (Fig. 1A). These findings were quantitated by vessel density counts, which were 82% lower in the Su-5416 group than in controls (13.5 ± 2.8 vs. 75.3 ± 0.6 vessels/×10 field; P < 0.05; Fig. 2). Barium-gelatin pulmonary arteriograms also showed that adult rats treated with a single dose of Su-5416 1 day after birth had a decrease in background filling of small- and medium-sized pulmo-

nary arteries with barium compared with adult controls (Fig. 1B). Compared with adult controls, pulmonary artery density was 31% lower in adult Su-5416 rats (41 ± 6 vs. 59 ± 5 vessels/×10 field; P < 0.05; Fig. 2). Pulmonary artery density increased threefold in adult Su-5416 rats compared with infant Su-5416-treated rats and fell by 20% in adult controls compared with infant controls (P < 0.05).

Lung histology and radial alveolar counts. Compared with controls, lung histology of Su-5416 infant rats showed enlarged distal air spaces and a 45% reduction in radial alveolar counts compared with in-

Fig. 1. A: effects of neonatal treatment with Su-5416 on lung vascular growth in infant rats. Barium arteriograms of the lungs of Su-5416 (right) and control (left) infant rats demonstrate narrowing of the large conduit arteries and decreased background filling of small pulmonary arteries with barium in infant rats treated with Su-5416 as neonates. Bar = 1 cm. B: barium arteriograms of the lungs of adult rats treated with a single dose of Su-5416 1 day after birth (right) and controls (left). Pulmonary arteriograms of adult rats treated with Su-5416 as neonates showed a decrease in background filling of small pulmonary arteries. Bar = 1 cm.

Fig. 2. Effects of neonatal treatment with Su-5416 on pulmonary artery density in infant and adult rats. Compared with controls, pulmonary arterial density is reduced by 82% in infant rats treated with a single dose of Su-5416 1 day after birth and by 31% in adults treated as newborns with Su-5416. *P < 0.05 vs. controls.
Fig. 3. Effects of neonatal treatment with Su-5416 on lung histology in infant and adult rats. Lung histology of infant rats treated with a single dose of Su-5416 1 day after birth showed alveolar simplification with reduced secondary septation and enlarged distal air spaces. Pulmonary arteries, which appear brown in color due to barium infusion, are markedly reduced after Su-5416 treatment. This pattern of reduced alveolar number and vessel density persisted into adulthood. Micrographs are representative and shown at the same magnification (×80). Bar = 100 μm.

Fig. 4. Effects of neonatal treatment with Su-5416 on radial alveolar counts in infant and adult rats treated with a single dose of Su-5416 1 day after birth. Compared with controls, Su-5416 treatment reduced radial alveolar counts by 45% in infant rats and 29% in adults. *P < 0.05 vs. controls.

Fig. 5. Effects of neonatal treatment with Su-5416 on the development of right ventricular hypertrophy in infant and adult rats (the ratio of right ventricle weight to body wt [RV:BW]). Compared with controls, infant rats treated with a single dose of Su-5416 1 day after birth had a 4.2-fold increase in the ratio of RV:BW. Compared with controls, RV:BW in adult rats treated with neonatal Su-5416 was twofold higher. *P < 0.05 vs. controls. **P < 0.05 vs. infant controls.
with Su-5416 as newborns compared with chronically hypoxic controls (Table 1; \( P < 0.05 \)). RV:LV+S weight ratios in normoxic Su-5416 adult rats and hypoxic controls were similar and increased by 1.7-fold in hypoxic controls vs. normoxic controls (Table 1; \( P < 0.05 \)).

No differences in LV+S:BW ratios were seen between the adult groups (\( P = \text{NS} \)). Compared with normoxic controls, hematocrit increased to a similar extent with exposure to chronic hypoxia in both Su-5416 and control adult groups (normoxic controls 48 ± 2%; normoxic Su-5416 50 ± 3%; hypoxic controls 70 ± 2%; hypoxic Su-5416 69 ± 3%).

DISCUSSION

Mechanisms by which perinatal stress can permanently alter lung structure, such as in acute lung injury in premature newborns, are poorly understood. The aim of this study was to 1) determine whether a single dose of the VEGFR inhibitor Su-5416 would disrupt postnatal lung development and cause pulmonary hypertension and 2) determine whether these effects would be long term, persisting into adulthood. We found reduced pulmonary arterial growth and alveolarization in 3-wk-old infant rats after treatment with a single dose of Su-5416 1 day after birth. Neonatal treatment with Su-5416 also caused severe pulmonary hypertension in infant rats, as demonstrated by increased RV weight and pulmonary arterial wall thickness. In addition, the effects of neonatal treatment with Su-5416 persisted into adulthood, as demonstrated by lower pulmonary artery density, reduced radial alveolar counts, and increased RV weight and pulmonary arterial wall thickness in 3- to 4-mo-old rats.

These findings provide novel data showing the long-term effects of treatment of newborn rats with Su-5416 and also evidence that neonatal Su-5416 treatment causes pulmonary hypertension in infant and adult rats. Previously, we showed that treatment of infant rats with multiple doses of antiangiogenesis agents fumigillin, thalidomide, and Su-5416 reduced postnatal alveolarization and pulmonary vascular development, but long-term effects were not studied (22). In the present study, treatment of neonatal rats with a single dose of Su-5416 reduced pulmonary vascular growth and alveolarization and caused long-term disruption of lung structure persisting into adulthood. Su-5416 treatment 1 day after birth caused a large

![Fig. 6. Histology showing increased wall thickness of small pulmonary arteries (arrows) in infant (top) and adult (bottom) rats. The wall thickness of small pulmonary arteries was increased in both infant and adult rats after treatment with Su-5416 after birth (right) compared with vehicle-treated controls (left). Micrographs are representative and shown at the same magnification (×400). Bar = 50 μm.](http://ajplung.physiology.org/)

![Fig. 7. Effects of neonatal treatment with Su-5416 on percentage wall thickness of small pulmonary arteries in infant and adult rats. Compared with controls, percentage wall thickness of pulmonary arteries (20–100 μM), calculated as [(2 × wall thickness/external diameter) × 100], was increased 2.7-fold in infant rats and 1.6-fold in adult rats after neonatal treatment with Su-5416. *\( P < 0.05 \) vs. controls.](http://ajplung.physiology.org/)
ple doses of Su-5416 over 3 wk increased pulmonary arteriolar density. In the study by Taraseviciene-Stewart et al. (35), treatment of adult rats with multiple doses of Su-5416 over 3 wk increased pulmonary artery pressures but did not cause RV hypertrophy (see Table 2), although they did see more severe RV hypertrophy when Su-5416-treated adults were exposed to chronic hypoxia. The timing of VEGF inhibition, newborn vs. adult (see Table 2 for comparisons), gave distinct effects and may reflect the critical period of lung development just after birth, when effects of VEGF inhibition are more profound. Inhibition of VEGF signaling in this period may affect both endothelial function and the accelerated vascular and alveolar growth. Interestingly, in two recent studies in adult models, VEGF gene transfer inhibited the development of monocrotaline and hypoxia-induced pulmonary hypertension (8, 31). Recent epidemiological studies have shown that adult cardiopulmonary diseases such as COPD and emphysema are associated with abnormal birth weight, suggesting that these disorders may originate as early as the perinatal period (3, 38). We have previously reported that treatment of newborn rats with dexamethasone reduced postnatal alveolarization and pulmonary vascular development and that these effects persisted into adulthood, despite withdrawal of glucocorticoid treatment (27). In addition, adult rats treated with steroids as neonates developed more severe pulmonary hypertension when exposed to chronic hypoxia than did littermate controls (27).

Premature infants frequently require high levels of supplemental oxygen for life support. VEGF expression is known to be regulated by oxygen levels, and a study in newborn rabbits showed that lung VEGF mRNA is reduced with exposure to hypoxia (28). Clinical studies have suggested that reductions in lung VEGF may play a role in acute lung injury in premature newborns and the pathogenesis of BPD. Lassus et al. (26) found that preterm infants with more severe RDS had lower VEGF protein in tracheal lavage sam-

Table 1. Adult rats: comparison of effects of chronic hypoxia on pulmonary arterial density and RV hypertrophy

<table>
<thead>
<tr>
<th></th>
<th>Normoxic</th>
<th>Su-5416</th>
<th>Hypoxic</th>
<th>Su-5416</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary arterial density</td>
<td>59 ± 1</td>
<td>41 ± 6*</td>
<td>36 ± 3†</td>
<td>25 ± 3*†</td>
</tr>
<tr>
<td>RV:LV+S</td>
<td>0.25 ± 0.02</td>
<td>0.46 ± 0.05*</td>
<td>0.46 ± 0.04†</td>
<td>0.64 ± 0.04†</td>
</tr>
<tr>
<td>RV:BW</td>
<td>0.00054 ± 0.00004</td>
<td>0.0011 ± 0.0001*</td>
<td>0.00094 ± 0.0001†</td>
<td>0.0014 ± 0.0001††</td>
</tr>
<tr>
<td>LV+S:BW</td>
<td>0.0022 ± 0.0001</td>
<td>0.0023 ± 0.0001</td>
<td>0.0020 ± 0.0001</td>
<td>0.0021 ± 0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE; *P < 0.05 vs. control; †P < 0.05 vs. normoxic group. RV, right ventricle; LV, left ventricle; S, septum; BW, body weight.

In this study, treatment with Su-5416 caused pulmonary hypertension as early as 3 wk of age. Part of the reduction in arterial density may be due to increases in alveolar size, which would reduce the number of alveoli per unit area and, therefore, the number of arteries per unit area. An earlier study by Gerber et al. (18) showed that Cre-lox knock out of the VEGF gene in neonatal mice caused alveolar simplification, but the effects on pulmonary vascular growth and long-term effects were not studied. Collectively, these studies suggest that VEGF may play an important role in postnatal alveolarization as well as vascular development after birth. Whether this is due to effects on VEGFR on epithelial cells (discussed later and in Refs. 6 and 14) or indirectly through effects on vascular development (22) is unknown.

In this study, treatment with Su-5416 caused pulmonary hypertension as early as 3 wk of age, and the pulmonary hypertension persisted into adulthood. An increase in LV+S:BW and a reduction in BW was seen in the Su-5416 infant rats but not in the Su-5416 adults. This suggests that Su-5416 may have had systemic effects, since increases in systemic vascular resistance can cause LV hypertrophy. Inhibition of VEGF function by Su-5416 may have caused pulmonary hypertension by several routes, including: 1) reduction of vessel growth, which would decrease the cross-sectional area of the arterial system and increase vascular resistance; 2) decreased production or activity of vasodilators, such as NO and prostacyclin (reviewed in Ref. 40), leading to increased vascular tone; 3) induction of endothelial cell dysfunction in addition to potential effects on vasodilator production; and 4) structural vascular remodeling, which increases vessel wall thickness, reduces lumen diameter, and reduces vascular compliance. In the study by Taraseviciene-Stewart et al. (35), treatment of adult rats with multiple doses of Su-5416 over 3 wk increased pulmonary artery pressures but did not cause RV hypertrophy (see Table 2), although they did see more severe RV hypertrophy when Su-5416-treated adults were exposed to chronic hypoxia. The timing of VEGF inhibition, newborn vs. adult (see Table 2 for comparisons), gave distinct effects and may reflect the critical period of lung development just after birth, when effects of VEGF inhibition are more profound. Inhibition of VEGF signaling in this period may affect both endothelial function and the accelerated vascular and alveolar growth. Interestingly, in two recent studies in adult models, VEGF gene transfer inhibited the development of monocrotaline and hypoxia-induced pulmonary hypertension (8, 31).

Recent epidemiological studies have shown that adult cardiopulmonary diseases such as COPD and emphysema are associated with abnormal birth weight, suggesting that these disorders may originate as early as the perinatal period (3, 38). We have previously reported that treatment of newborn rats with dexamethasone reduced postnatal alveolarization and pulmonary vascular development and that these effects persisted into adulthood, despite withdrawal of glucocorticoid treatment (27). In addition, adult rats treated with steroids as neonates developed more severe pulmonary hypertension when exposed to chronic hypoxia than did littermate controls (27).

Premature infants frequently require high levels of supplemental oxygen for life support. VEGF expression is known to be regulated by oxygen levels, and a study in newborn rabbits showed that lung VEGF mRNA is reduced with exposure to hypoxia (28). Clinical studies have suggested that reductions in lung VEGF may play a role in acute lung injury in premature newborns and the pathogenesis of BPD. Lassus et al. (26) found that preterm infants with more severe RDS had lower VEGF protein in tracheal lavage sam-

Table 2. Adult rats: comparison of effects of timing of Su-5416 treatment on arterial density, alveolar counts, and RV weights

<table>
<thead>
<tr>
<th></th>
<th>Neonatal Treatment</th>
<th>Adult Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Su-5416</td>
</tr>
<tr>
<td>Pulmonary arterial density</td>
<td>59 ± 1</td>
<td>41 ± 6*</td>
</tr>
<tr>
<td>Radial alveolar counts</td>
<td>11.8 ± 0.1</td>
<td>8.4 ± 0.8*</td>
</tr>
<tr>
<td>RV:LV+S</td>
<td>0.25 ± 0.02</td>
<td>0.46 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE; *P < 0.05 vs. control. †P from Kasahara et al. (24) and Taraseviciene-Stewart et al. (35).]
ples than those infants with RDS who recovered. Bhatt et al. (4) reported that staining for VEGF and VEGF mRNA levels was reduced in infants dying from BPD. Although these studies raise the possibility that reductions in lung VEGF might contribute to the pathogenesis of BPD, it is difficult to assess whether reductions in VEGF are a cause or effect of the disease. In addition, BPD is a complex disease involving a potentially large number of adverse stimuli (23), many of which may adversely affect postnatal lung development and lung VEGF expression.

Infants with BPD show some improvement in lung function with time, but abnormal lung mechanics persist into infancy and adolescence (1, 2). Our study shows that neonatal treatment with a VEGFR inhibitor disrupts lung growth during infancy and causes pulmonary hypertension and that these effects persist into adulthood. Compared with controls there was significant catch-up growth between 3 wk and 3–4 mo of age after Su-5416 treatment. Pulmonary arterial counts increased threefold between 3 wk and 3–4 mo of age in Su-5416 rats, whereas they decreased by 20% in controls. Radial alveolar counts increased by 50% between 3 wk and 3–4 mo of age in Su-5416 rats, whereas they increased by 20% in controls. The fall in RV:BW between 3 wk and 3–4 mo of age was also much greater in the Su-5416 rats than in the controls.

To determine whether disruption of lung structure in adult rats treated with Su-5416 as newborns would result in more severe pulmonary hypertension with an additional stress, we exposed the adult rats, treated and vehicle controls, to 3 wk of hypoxia. After exposure to chronic hypoxia, adult rats treated with the VEGFR inhibitor as newborns developed more severe right ventricular hypertrophy than controls, although the percentage increase compared with room air (normoxic) groups was similar. Exposure of adult rats to chronic hypoxia also reduced pulmonary arterial density in both Su-5416 and control groups. Reductions in pulmonary arterial density with exposure to chronic hypoxia has previously been reported (20, 32).

A potential limitation of this study is that the duration of effect of Su-5416 given to the 1-day old rats is not known. Although the plasma half-life of Su-5416 in rats is short (30 min), the effects of Su-5416 on VEGFR activity can be long lasting (at least 72 h) (29). Because Su-5416 is lipophilic and rats were treated by subcutaneous administration, VEGFR inhibition may have persisted for several days after absorption. Su-5416 may inhibit VEGFR-1 as well as VEGFR-2 activity (29); however, since a number of studies indicate that VEGFR-1 does not play a role in VEGF-stimulated angiogenesis or production of NO and prostacyclin (15, 19, 36), we suggest that the effects of Su-5416 on lung growth and pulmonary hypertension in this study are most likely due to inhibition of VEGFR-2. In vitro studies have shown that Su-5416 can also inhibit platelet-derived growth factor (PDGF)-β receptor and the stem cell factor receptor kit (17, 19, 34). Although we cannot rule out the possible in vivo effects of Su-5416 on PDGF-β receptor activity, inhibition of PDGF-β receptor activity would be unlikely to account for the effects of Su-5416 in this study, since inhibition of PDGF-β receptor activity reduces smooth muscle cell proliferation and would, therefore, probably decrease arterial wall thickness (37). The kit receptor is expressed by hemopoietic cells, melanocytes, neural crest derivatives, and germ cells as well a variety of solid tumors and cell lines (34). However, to the best of our knowledge, the kit is not expressed on endothelial cells in the lung. Recently, two studies have reported detection of VEGFR-2 expression on epithelial cells in adult rat lung (Clara cells and type II pneumocytes) and distal airway epithelial cells in human fetal lung explants (6, 14). This raises the possibility that VEGF produced by the epithelium may have autocrine and paracrine effects on epithelial proliferation and that the inhibitory effects of Su-5416 on postnatal alveolarization in our study may be due to inhibition of VEGFR-2 on epithelial cells.

We conclude that treatment of 1-day-old rats with a single dose of the VEGFR inhibitor Su-5416 reduced pulmonary vascular growth, impaired postnatal alveolarization, and caused pulmonary hypertension in infant rats. These effects persisted into adulthood, despite catch-up growth. We speculate that the early disruption of VEGFR signaling in the newborn lung might contribute to the pathological sequelae of BPD and a long-term predisposition for adult diseases, such as emphysema, COPD, and pulmonary hypertension.

The authors thank Charles Ahrens for performing radiography of barium-filled lungs, Malathi Jakkula for technical assistance, and Keith Fox for help with data processing.

This work was supported by an American Heart Association Scientist Development Grant (T. D. Le Cras), an American Heart Association Grant-in-Aid (S. H. Abman), National Heart, Lung, and Blood Institute (NHBLI) SCOR Grant HL-57144 (S. H. Abman), a March of Dimes Research Grant (S. H. Abman), and NHBLI Grants HL-6654 and HL-60195 (R. M. Tuder).

REFERENCES


