Long-term failure of alveologenesis after an early short-term exposure to a PDGF-receptor antagonist

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Lau M, Masood A, Yi M, Belcastro R, Li J, Tanswell AK. Long-term failure of alveologenesis after an early short-term exposure to a PDGF-receptor antagonist. Am J Physiol Lung Cell Mol Physiol 300: L534–L547, 2011. First published January 14, 2011; doi:10.1152/ajplung.00262.2010.—Survivors of moderate-to-severe bronchopulmonary dysplasia (BPD), as seen in the current era generally occurs in infants with BPD, termed classical alveolarization, low ridges containing a double capillary network arise from the primary septa and project into the air spaces. These “secondary septa,” or “secondary crests,” subdivide the precursor saccules and transitory ducts into alveolar sacs and alveolar ducts, respectively (8–10). The initial process, which occurs from postnatal days 4–8 in rats (25), results in a decrease in the diameter of terminal air spaces and an increase in alveolar density. The increase in alveolar number from birth to day 21 in rats is linear (28) but is not associated with any further increase in alveolar density after day 8 (25). Following maturation of the epithelium and the microvasculature, which occurs from day 14 to 21 in rats, there is a reduction to a single capillary network within septa, and a second phase of late alveologenesis occurs (30). In this latter process, new septa are formed by the folding up of the single-capillary networked septum through the duplication of the local capillary network by angiogenesis (30).

Although the mechanisms that regulate alveolar formation remain incompletely understood, it is well-recognized that they involve the concerted actions of various polypeptide growth factors (16), including hepatocyte growth factor (26), fibroblast growth factors (27, 35), vascular endothelial growth factor (19), and platelet-derived growth factor (PDGF)-AA and -BB (5, 7, 23). Growth factor expression is perturbed in BPD and in animal models of neonatal lung injury (31).

We wished to test the hypothesis that early interference in the process of alveologenesis, as observed in infants with BPD, through suppressing growth factor-mediated effects could lead to a permanent inhibition of alveolar formation. We used inhibition of PDGF receptor (PDGF-R) signaling as our approach, based on both our previous study demonstrating that competitive inhibition of the PDGF-Rβ caused an acute failure of alveologenesis (7), and the availability of a receptor tyrosine kinase inhibitor, imatinib mesylate (henceforth called imatinib, which targets PDGF-R and other tyrosine kinase receptors, such as c-abl and c-kit.

MATERIALS AND METHODS

In vivo interventions. Animal experiments complied with Canadian Council on Animal Care guidelines and were approved by the Animal Care Review Committee of the Hospital for Sick Children Research Institute. Newborn rat pups were either uninjected or received daily intraperitoneal injections of PBS (5 μl/g) or imatinib (50 μg/g; LC Laboratories, Woburn, MA) in PBS from day 1–7 of life. For...
assessments of cell DNA synthesis, pups received 20 μg/g ip bromodeoxyuridine (BrdU) 2 h before death. Pups were killed at 8, 14, 28, or 65 days of age by inhalation of ethyl ether. Lung, body, and kidney weights were obtained for eight animals in each group.

Tissue preparation. For protein analyses, the lungs of each individual pup were weighed, and the lungs of four animals in each group were pooled and flash-frozen in liquid nitrogen. Lungs were perfusion-fixed over 12 h using 4% (wt/vol) paraformaldehyde in PBS or, for immunodetection of BrdU, 10% (wt/vol) neutral buffered formalin while inflated with air under a constant airway pressure of 20 cmH₂O. Processing of lung tissue for immunohistochemistry and morphometry was as previously described (7, 24, 26, 27, 39–41). Lung sections were stained with hematoxylin and eosin (Surgipath, Winnipeg, MB, Canada) or with Weigert’s resorcin-fuchsin stain (Rowley Biochemical Institute, Danvers, MA), which were then counterstained with tartrazine (Sigma-Aldrich, St. Louis, MO).

Immunohistochemistry. Immunostaining was performed using an avidin-biotin-peroxidase complex method (18). Lung sections of 5-μm thickness were stained for α-smooth muscle actin (α-SMA) to facilitate identification of secondary crypts, and sections of 10-μm thickness were stained for Clara cell 10 (CC10) to facilitate identification of the distal end of the airway epithelium. Primary antibody dilutions used were 1:800 for mouse anti-α-SMA (Neomarkers, Fremont, CA) and 1:800 for goat anti-CC10 (Santa Cruz Biotechnology, Santa Cruz, CA). The specificity of the antibody used for α-SMA has been previously demonstrated by Western blot (24). Because the antibody to CC10 allowed identification of a clear transition zone from airway epithelium, at an anatomically correct position, further testing of specificity was not undertaken. Vascular endothelial cells were identified using a 1:60 dilution of rabbit polyclonal antibody to von Willebrand factor (Thermo Scientific, Fremont, CA) and a 1:200 dilution of a fluorescent secondary antibody (Alexa Fluor 488, A11008; Invitrogen, Carlsbad, CA) diluted 1:2,000, except for p-c-kit for which a 1:3,000 dilution was used. A11008; Invitrogen, Carlsbad, CA) diluted 1:2,000, except for p-c-kit for which a 1:3,000 dilution was used. Immunoprecipitation. Excised lungs were homogenized and lysed in Nonidet P-40 lysis buffer containing protease and phosphatase inhibitors (Roche Applied Science, Laval, QC, Canada). Protein content was determined according to Bradford (6). Goat anti-PDGF-Rβ antibody or rabbit anti-PDGF-Rα antibody (Santa Cruz Biotechnology) were added to lysates and incubated at 4°C overnight. Packed protein A/G agarose (Santa Cruz Biotechnology) was then added and incubated at 4°C for 2 h with agitation. Supernatants from immunoprecipitated samples were saved for immunoblotting analysis of GAPDH as an immunoprecipitate lysate control.

Immunoblotting. The lungs of four animals in each group were pooled and flash-frozen. Immunoblots of lysates and immunoprecipitated lysates from perfused lung tissue were performed as previously described (40). Protein content was measured as described by Bradford (6). For immunoprecipitated samples, agarose beads were boiled for 5 min in SDS sample buffer before loading on to polyacrylamide gels. For all other immunoblots, protein was normalized, and 40 μg of total protein were loaded on to the gels. Membranes were incubated overnight at 4°C with: 1:500 dilution of mouse monoclonal antibody to phosphotyrosine (Santa Cruz Biotechnology); 1:500 dilution of a rabbit polyclonal antibody to phosphorylated p-c-kit (p-c-kit: Santa Cruz Biotechnology); 1:800 dilution of rabbit polyclonal antibody to tropoelastin (Abcam, Cambridge, MA); 1:1,000 dilution of rabbit polyclonal antibody to GAPDH (Santa Cruz Biotechnology); 1:4,000 dilution of rabbit polyclonal antibody to lysyl oxidase (Lifespan Biosciences, Seattle, WA); and 1:200 dilution of goat polyclonal antibody to fibulin-5 (Santa Cruz Biotechnology). Membranes were then incubated with secondary horseradish peroxidase-conjugated anti-mouse IgG diluted 1:10,000 (Calbiochem-Novabiochem, San Diego, CA), anti-rabbit IgG (Cell Signaling Technology, Beverly, MA) diluted 1:2,000, except for p-c-kit for which a 1:3,000 dilution was used.

Fig. 1. Western blot analysis of platelet-derived growth factor receptor (PDGF-R) α (A) and β (B) phosphorylation and content in day 7 lung homogenates. PDGF-Rα (C and D) and PDGF-Rβ (E and F) were immunoprecipitated, immunoblotted with antibodies against PDGF-Rα (C or β (E) or phosphotyrosine (p-Tyr) (D and F), and normalized to GAPDH content of samples used for immunoprecipitation. *P < 0.05 by 1-way ANOVA compared with no-treatment controls (NT). VT, vehicle-treated (days 1–7) controls; IT, imatinib treated (days 1–7). Values are means ± SE for lung homogenates from 4 different litters. Homogenates were prepared from the lungs of 4 pups within each individual litter.
was used, or anti-donkey IgG diluted 1:2,000 (Santa Cruz Biotechnology) in blocking solution. Signals were visualized by enhanced chemiluminescence (GE Healthcare Amersham, Buckinghamshire, UK) on HyBlot films (Denville Scientific, Metuchen, NJ). Integrated band densities were calculated after subtraction of background values, as previously described (40).

**Hart’s elastin staining.** Tissue sections were stained using Weigert’s resorcin-fuchsin and counterstained with tartarazine, as previously described (24). Elastin fiber density was quantified using threshold analysis of stained images (17). The pixel fractional area for elastin was divided by the pixel fractional area for all tissue to calculate the percent area occupied by elastin fibers (4).

**Morphometric analyses.** All morphometric analyses were performed using four tissue sections per animal and 10 random nonoverlapping images per section. The section average was calculated from the average of the 10 images. The average of the four slides was used to calculate an animal average. All measurements were performed on 5-μm sections, except for radial alveolar counts, which were measured on 10-μm sections stained for CC10. Calculations of tissue fraction, mean linear intercept (14), alveolar surface area per unit lung volume and air space wall surface area (20), secondary crest volume density, and estimated total alveolar number (34) were as previously described (24, 39). Radial alveolar counts were measured as described by Cooney and Thurlbeck (12). A counting grid with 130 points was used to calculate the tissue fraction, as the fraction of total points overlying tissue. Postfixation lung volumes were measured by water displacement. Total tissue volume was obtained by multiplying the tissue fraction and lung volume.

**Data presentation.** All values are presented as means ± SE. Protein measurements for individual groups were obtained from four separate litters. For morphometric analyses, each individual group was made up of four average-sized pups, each from different litters. One-way ANOVA was used to determine statistical significance (P < 0.05), followed by post hoc analyses using Tukey’s test when significant differences were found between groups.

**RESULTS**

The dose of imatinib selected for use was based on the literature (29). To confirm efficacy in our hands, PDGF-Rα and -Rβ were immunoprecipitated from day 7 lung lysates and probed separately with antibodies to the receptors or to phosphotyrosine, to assess receptor activation. Images of the blots are shown in Fig. 1, A and B. As an internal control, results were normalized to the GAPDH content of the samples used for immunoprecipitation. As shown in Fig. 1F, phosphorylation of PDGF-Rβ was significantly decreased by treatment with imatinib. Phosphorylation of PDGF-Rα was also decreased by treatment with imatinib, but this did not quite (P = 0.085) achieve statistical significance (Fig. 1D). There was also a reduction in the contents of both PDGF-Rα (Fig. 1C), which also did not quite achieve statistical significance (P = 0.077), and PDGF-Rβ (Fig. 1E), which was statistically significant. We assumed that this latter finding reflected an effect of receptor activation on receptor expression but wished to exclude any interference in immunodetection induced by imatinib binding to, and masking, the receptor. To this end, an excess of imatinib was added to some samples before immunoprecipitation. Receptor immunodetection was unaffected by the addition of imatinib to the lysates (Fig. 2). Following a 7-day recovery period, PDGF-Rα (Fig. 3A) and -Rβ (Fig. 3B) were again immunoprecipitated from lung lysates and then probed separately with antibodies to the receptors or to phosphotyrosine to assess receptor activation. PDGF-Rβ content (Fig. 3E) and phosphorylation (Fig. 3F) and PDGF-Rα phosphorylation (Fig. 3D) were unaffected by earlier treatment with imatinib, although the content of PDGF-Rα remained significantly decreased (Fig. 3C). Imatinib was also observed to inhibit phosphorylation of another randomly selected tyrosine kinase receptor target, c-kit (Fig. 4).

Lung, body and kidney weights, and postfixation lung volumes were recorded on day 8, 14, 28, and 65 of life (Table 1). None of the measurements differed significantly between the control groups. Body weights were significantly reduced in the pups that had received imatinib from days 1 to 7, on day 8, day 14, and day 28 of life, but had returned to control values by day 65 of life. Lung weights were significantly reduced in the imatinib-treated pups on day 8 and day 14 of life. Lung-to-body weight ratios were significantly reduced in the imatinib-treated pups on day 8, indicating that lung growth had been more severely affected than would have been anticipated from the global impairment of body growth. This was supported by the observation that kidney-to-body weight ratios were normal in the imatinib-treated pups on day 8. Lung volumes in the
pups that had received imatinib from days 1 to 7 of life were significantly increased above control values by day 65 of life. The lung volume-to-body weight ratio was increased at all time points in the imatinib-treated pups but was only statistically significant on days 8, 28, and 65.

On gross inspection of lung sections from pups at days 2, 4, 8, 14, and 28, there appeared to be a sustained thinness of the lung interstitium in imatinib-treated pups from day 8 onward (Fig. 5). Results from tissue fraction analysis (Fig. 6A) confirmed a lesser amount of lung tissue in the imatinib-treated pups, relative to both control values, on days 8, 14, and 28. Tissue fractions were also significantly reduced in the control groups at day 28 relative to their values at day 8. Results from tissue volume analysis (Fig. 6B) confirmed a lesser amount of lung tissue in the imatinib-treated pups, relative to both control values on day 28 and relative to the uninjected controls on days 8 and 14. Lung cell proliferation was assessed using BrdU labeling. Imatinib-treated rats had an apparent decreased lung cell BrdU incorporation at days 8 and 28 and increased BrdU incorporation at day 14, as assessed by immunohistochemistry (Fig. 7). The density of BrdU-positive cells per unit area in the lungs of imatinib-treated animals was significantly decreased relative to both control groups on day 8, and to no-treatment controls on day 28, but was significantly increased relative to no-treatment controls on day 14 (Fig. 6C). After adjustment for differences in the amount of tissue per unit area (Fig. 6D), BrdU incorporation was reduced at day 8, and increased at day 14, relative to both sets of controls. This increase at day 14 was not associated with any increase in PDGF-AA or -BB, as assessed by immunohistochemistry (data not shown). Evaluation of the slides stained for BrdU revealed that the increase could essentially all be accounted for by BrdU-positive endothelial cells of vessels larger than capillaries or small arterioles (Fig. 6E). This was not further pursued. BrdU-positive cells, corrected for tissue, were significantly reduced in both control groups at day 14, relative to their
values at day 8, and there was also a significant reduction in vehicle-treated pups at day 28, relative to day 8 values. An alternative explanation for a sustained thinness of the lung interstitium in imatinib-treated pups was an imatinib-induced increase in lung cell apoptosis, as described in adult lung tissue by others (33). Lung sections from animals at each of days 1–7 of life were examined, but no differences between groups were observed. Exemplars from day 2 and day 4 lungs are shown in Fig. 6F.

Gross inspection of lung sections from pups at days 8, 14, and 28 also showed apparently enlarged distal air sacs in the imatinib-treated pups at all time points (Fig. 5). Morphometric analyses confirmed this qualitative assessment. Mean linear intercepts, which are indexes of alveolar diameter, were significantly increased in imatinib-treated pups, relative to age-matched controls at days 14 and 28 (Fig. 8A), as would be expected if there was a failure of alveolar formation from the larger precursor saccules. Radial alveolar counts, an index of the number of alveolar generations, were significantly reduced in the imatinib-treated pups at days 14 and 28 (Fig. 8B). There was a significant increase in control values at day 28 relative to values at day 8. Secondary crest numbers per unit area, normalized to littermate controls, were significantly decreased by previous imatinib treatment on days 8, 14, and 28 (Fig. 8C), even after adjusting for the amount of tissue (Fig. 8D). As would be expected with a reduced secondary crest density, and the loss of the vessels that they contain, there was a qualitatively obvious loss of small vessels, as assessed by staining for von Willebrand factor on endothelial cells, after imatinib treatment (Fig. 8G), relative to un.injected (Fig. 8E) or vehicle-injected (Fig. 8F) controls. Both estimated alveolar surface area per unit lung volume (Fig. 9A) and estimated total alveolar surface area (Fig. 9B) were decreased by treatment with imatinib, relative to control values, on day 28. The estimated total alveolar surface areas in controls at day 28 were significantly increased over values at day 8. In keeping with the findings above, the estimated alveolar density was significantly reduced, relative to control values at days 8, 14, and 28 (Fig. 9C). The estimated alveolar densities in controls were increased at days 14 and 28 relative to values at day 8. The estimated total alveolar numbers (Fig. 9D) in the imatinib-treated group were decreased at days 8, 14, and 28 relative to control values. There was a significant increase in alveolar number between days 8 and 28 in the control pups but not in the imatinib-treated pups. Consistent with a failure of alveolar formation, BrdU-positive secondary crests per unit area were also decreased in imatinib-treated pups at days 8 and 28 (Fig. 10A). In control tissues, BrdU-positive secondary crests per unit area were significantly reduced at day 14 relative to values at day 8. BrdU-positive secondary crest-to-tissue ratios in imatinib-treated rats were decreased on days 8 and 28, and increased on day 14, relative to control values (Fig. 10B). The BrdU-positive secondary crests-to-total crests ratio was also decreased in imatinib-treated pups at day 8 and increased at day 14, relative to control values (Fig. 10C). To be confident that the decrease in alveolar formation would be longstanding, we also conducted a limited examination of tissue sections at day 65. Obviously enlarged distal air sacs persisted (Fig. 11), and there had been no reduction in mean linear intercepts in the
imatinib-treated pups below that which had been observed at day 28 (Fig. 10D).

To determine if the timing of PDGF-R inhibition was critical for the development of long-standing effects, we undertook a very limited analysis of lung tissue from rat pups that had been treated with imatinib (50 μg·kg⁻¹·day⁻¹) from day 8 to 13, then killed at day 14 or 21. As shown in Fig. 12, treatment with imatinib from day 8 to 13 impaired alveolar formation by day...
14, as reflected in a significantly increased mean linear intercept, but there was complete recovery from this impairment by day 21.

The increased lung volumes at day 65, and the increasing mean linear intercepts from day 14 to 65 in pups treated with imatinib from days 1 to 7 of life, relative to control values, suggested a decreased elastic recoil upon inflation. This led us to examine tissue sections for elastin fiber deposition at days 8, 14, 28, and 65, using Hart’s elastin stain (Fig. 13).

In control lungs, smooth elastin fibers were expressed mainly at the tips of alveolar septa at all the time points, whereas fragmented fibers were prominent throughout the interstitium of lungs from imatinib-treated animals at day 8, and remained unchanged throughout the recovery period. In addition, compared with those found in the control groups (Fig. 14A), elastin fibers found at the tips of secondary crests of imatinib-treated pups appeared abnormal and fragmented (Fig. 14B). There was, however, no apparent difference in the total amount of elastin present in the sections (Fig. 14C). When lung tropoelastin contents at days 7 and 28 were assessed by Western blot, treatment with imatinib had no effect on tropoelastin content (Fig. 14D–G). In view of the apparent fragmentation of elastin observed in pups that had received imatinib, we attempted to examine day 7 lung homogenates for their content of a number of proteins involved in elastin synthesis and assembly. These included lysyl oxidase, a copper-dependent enzyme secreted by fibroblasts, and smooth muscle cells that mediate the cross-linkage of tropoelastin to form elastin fibers, lysyl oxidase-like-1, lysyl oxidase-like-2, fibrillins-1 and -2, and fibulin-5. Despite the use of antibodies from several sources, we were only able to obtain satisfactory Western blots with bands of appropriate sizes for lysyl oxidase and fibulin-5. Treatment with imatinib had no effect on the lung content of lysyl oxidase but did result in a marked increase in the content of fibulin-5 relative to control values (Fig. 15).

DISCUSSION

Our intent was to test the hypothesis that early interference in the process of alveologenesis, as observed in infants with BPD, could lead to a permanent inhibition of alveolar formation. We elected to reversibly inhibit PDGF-R activation during the early classical phase of secondary septation, which is regulated by PDGFs and other growth factors, using imatinib. Imatinib successfully inhibited activation of...
the PDGF-Rβ at day 7, but inhibition of the PDGF-Rα did not quite achieve statistical significance. For our purposes, inhibition of PDGF-Rβ activation alone at day 7 was sufficient in that this arrests alveologenesis without a requirement for concurrent inhibition of PDGF-Rα activation (7). In earlier studies (7, 41), we had used a truncated soluble receptor to inhibit PDGF-Rβ activation, but that was not an economically feasible option for the large number of rat pups used in this study. Our findings were certainly consistent with our hypothesis in that long-term impairment of alveologenesis, as assessed by morphometry, did occur following transient inhibition of early classical secondary septation by imatinib from days 1 to 7 of life. No recovery of either secondary crest formation or alveolar numbers was observed. We had assumed that the major effects of imatinib would be mediated by inhibition of PDGF-R activation. However, imatinib not only effectively blocked PDGF-Rβ phosphorylation but also led to a reduction in total PDGF-Rβ. This is consistent with PDGF-BB activation of PDGF-Rβ upregulating the receptor (15), which is prevented by treatment with imatinib. We currently have no explanation for the sustained reduction in PDGF-Rα evident at day 14.

We had anticipated that antagonism of the PDGF-R would, during the treatment period, impair lung cell DNA synthesis in, and myofibroblast migration to, the tips of secondary crests, resulting in a failure of secondary crest formation and of early alveologenesis (5, 7, 23). Injury/recovery experiments performed on adult animals suggest that recovery is possible following an acute loss of alveoli. Resection of major portions of the lung through unilateral pneumonectomy is an established model for the study of compensatory lung growth (1, 3). Studies in baboons, rats, and mice have demonstrated near complete restoration of
lung alveolar mass. Although the recovery is partly mediated through the dilatation of existing alveoli, much of it is through new alveolar formation that is mediated, at least in part, by the activation of PDGF-R

In contrast to adult animals that have undergone pneumonectomy, the neonatal rats subjected to an early inhibition of alveolar septation in our study had a long-term impairment of alveologenesis. We speculate that the critical difference between the two models is that, in animals subjected to pneumonectomy, the residual lung tissue is structurally normal, whereas the imatinib-treated pups in our study had structurally abnormal lungs at the beginning of the recovery period. Lung tissue sections stained for elastin displayed long-term alterations in elastin deposition and conformation in our imatinib-treated animals at all time points studied. Much of the elastin observed in those animals was deposited in the interstitium of the lung and appeared to be fragmented. Our observation of an imatinib-mediated effect on fibulin-5 content, but not on lysyl oxidase, elastin, or tropoelastin contents, is consistent with dysregulated elastin assembly. Fibulin-5 plays an essential role in tropoelastin assembly into elastin (37) in concert with several other proteins. We speculate that overexpression of fibulin-5 may lead to premature deposition of elastin fragments before full-length fiber assembly. A sustained 4-wk exposure of neonatal rats to either β-alaminopropionitrile, an inhibitor of lysyl oxidase and elastin cross-linking, or elastase has been shown to lead to a loss of elastic recoil and a long-standing reduction in alveolar numbers (21, 22). Our intervention study was of a much briefer duration, but had a similar outcome, and further supports the concept of an intact elastin “fishnet” scaffold being required for alveologenesis (22). Alternatively, fibulin-5 has antiangiogenic properties thought to be due to interference in vascular endothelial growth factor-mediated signaling (37), which is widely believed to be essential for the process of alveologenesis (32).

Our study does have some limitations. Although used both clinically and experimentally as a “specific” PDGF-R antagonist, an action that we were able to confirm in this study, imatinib could be affecting other receptors, such as c-kit, as observed in this study, to a greater or lesser degree, although the changes in alveolar formation that we observed
are all consistent with antagonism of the PDGF-R alone, based on the known properties of the PDGFs. Also, the morphometric techniques that we used lack the elegance and precision of some of the technically sophisticated approaches currently in use (30). The alveolar numbers generated by our methods are estimates, rather than precise counts, but these methods do have the advantage of simplicity and are well suited to conditions in which large differences are anticipated. Last, it became obvious, in retrospect, that the use of a PDGF-R antagonist did not allow us to confirm or refute our original hypothesis. Because of the effect of imatinib on elastin, we could not differentiate between impaired alveologenesis due to a specific effect on elastin cross-linking and that due to a nonspecific effect of targeting early alveologenesis. This would require an intervention against a growth factor pathway, such as the hepatocyte growth factor pathway (26), which should have no effects on elastin.

There are currently no data available on the expression of PDGFs in the lung tissue of infants with, or developing, BPD. However, downregulation of PDGF is observed in animal models of chronic neonatal lung injury (4, 7).
results of our study are consistent with a model in which early disordered elastin assembly leads to a long-term impairment of alveologenesis, due to a failure to form an essential fishnet scaffold. The implication of our findings in the animal model described herein, and the finding that neonatal rats developing a chronic neonatal lung injury have reduced lung contents of PDGF-BB and -AA (7), is that defective elastin assembly as a consequence of altered PDGF signaling could explain, at least in part, the impaired alveolar formation evident during the development of the chronic neonatal lung injury. The other major histological finding in BPD is parenchymal thickening. Whether impaired PDGF signaling contributes to this thickening in the neonatal rat model of chronic neonatal lung injury has not been tested. If impaired elastin assembly, and altered PDGF signaling, are also found in lung tissue from human infants developing BPD, such a model could explain the long-term failure of alveologenesis observed in individuals that have survived moderate-to-severe BPD (13, 36).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


Fig. 13. Images of Hart’s elastin-stained lung sections from no-treatment and vehicle-treated (day 1–7) controls and imatinib-treated (day 1–7) rats at 8, 14, 28, and 65 days of age. Bar = 100 μm.


