Keratinocyte Growth Factor Enhances Post-Pneumonectomy Lung Growth by Alveolar Proliferation

Aditya K. Kaza, MD; Irving L. Kron, MD; Shari M. Leuwerke, MS; Curtis G. Tribble, MD; Victor E. Laubach, PhD

Background—Keratinocyte growth factor (KGF) has been shown to play an important role in pneumocyte proliferation and lung development. We hypothesized that exogenous KGF would enhance postpneumonectomy compensatory lung growth through alveolar proliferation.

Methods and Results—Adult Sprague–Dawley rats were used. Left pneumonectomy was performed in group P, sham thoracotomy in group S, and left pneumonectomy with administration of KGF (6.25 mg/week, intraperitoneally) in group PK. Lung weight index (LWI), lung volume index (LVI), and alveolar cell proliferation index (CPI) were measured in the right lung at 10 and 21 days after surgery. Morphometric analysis was used to determine alveolar surface density (Sv) and total volume of respiratory region (TVvr). As expected, LWI, LVI, and CPI were significantly increased after pneumonectomy at both time points in group P. The administration of KGF resulted in further significant enhancements of LWI, LVI, and CPI in group PK. TVvr was significantly increased in group P and further enhanced in group PK. Interestingly, Sv was not altered in group P but was significantly elevated in group PK. Administration of KGF to sham-operated animals did not alter LWI, LVI, or CPI.

Conclusions—KGF enhances compensatory lung growth after pneumonectomy in adult rats as indicated by increased LWI, LVI, and CPI. KGF induces new alveolar formation, as indicated by increases in Sv and TVvr. We believe that this is the first evidence that KGF can induce new alveolar formation in mature lungs. (Circulation. 2002;106[suppl I]:I-120-I-124.)

Key Words: lung growth factors surgery transplantation

A better understanding of the various key modulators of lung growth has important applications in the treatment of various disease processes. Lung disease and injury can potentially be treated by the induction of the growth of healthy, functional lung tissue. Keratinocyte growth factor (KGF), a heparin-binding growth factor, is a 28-kDa protein with potent mitogenic activity for lung epithelial cells. KGF has been shown to greatly increase DNA synthesis in pulmonary epithelial cell lines. This target cell specificity is derived from its binding to the KGF receptor, a variant of fibroblast growth factor receptor 2, which is expressed only in epithelial cells. In vivo studies demonstrate that KGF is a potent mitogen for type II alveolar epithelial cells (pneumocytes) and bronchiolar epithelial cells. These experiments also have shown that intratracheal instillation of KGF shows prominent type II pneumocyte hyperplasia. These data suggest that KGF is involved in regulating both proliferation and differentiation of type II cells and bronchiolar epithelial cells. KGF has also been shown to regulate surfactant protein gene expression in the lung and to play an important role in lung branching and organogenesis.

Postpneumonectomy compensatory lung growth serves as a good model for the study of adult lung growth because of its reproducibility and because it is a well-characterized model. It is a phenomenon that occurs after pneumonectomy or lobectomy whereby the remaining lung exhibits rapid and restorative growth. The process of compensatory lung growth is potentially signaled by the same mechanisms that are involved in healthy, postnatal lung growth. As a result, this model serves as an ideal model for the study of adult lung growth. In this study, we investigated the role of exogenously administered KGF on lung growth using the model of postpneumonectomy compensatory lung growth. We hypothesized that exogenous KGF would enhance postpneumonectomy compensatory lung growth through alveolar proliferation.

Methods

Animals

Adult male Sprague–Dawley rats 7 to 8 weeks of age, which weighed approximately 300 g were used for all experiments. Animal acquisition occurred under the supervision of the Department of...
Comparative Medicine and a licensed veterinarian. Facilities for animal care are accredited by the American Association for Accreditation of Laboratory Animal Care. The facility is approved by the Institutional Animal Care and Use Committee. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and “The Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Science and published by the National Institutes of Health (NIH publication 85 to 23, revised 1985).

Operative Model
Rats were divided into 3 groups (S, P, and PK), and each group was subdivided into 2 groups based on postoperative study time (10 or 21 days). An additional group (SK) was studied at postoperative day 21 to test whether KGF had any effect on sham-operated animals. The groups at 10 days were designated as S10, P10, and PK10; those at 21 days were designated as S21, SK21, P21, and PK21 (n=8/group). All animals were anesthetized with a combination of ketamine and xylazine injected intraperitoneally followed by endotracheal intubation with a 16 French catheter. They were then shaved and prepped in a sterile fashion, and ventilated with room air using a pressure-regulated rodent ventilator (Kent Scientific).

Animals in group S underwent a sham thoracotomy on the left side. Animals in group P underwent a left pneumonectomy. Animals in group PK underwent a left pneumonectomy with the administration of exogenous KGF. Recombinant human KGF (Amgen, Thousand Oaks, CA) was administered intraperitoneally at a dose of 6.25 mg weekly (approximately 21 mg/kg) starting on the day of surgery. This dose was based on previous studies.\(^1\)1,\(^2\) except that we elected to give the rats a higher dose every 7 days because studies have shown little or no changes in alveolar cell proliferation after systemic injection of KGF.\(^3\)\(^,\)\(^4\) Animals in group SK underwent a sham thoracotomy with administration of KGF as described above, and this group was studied 21 days after surgery. After the sham left thoracotomy (groups S and SK), the chest was closed after an expiratory sigh using 3-0 silk suture and the skin closed using surgical staples. Animals undergoing pneumonectomy (groups P and PK) underwent a posterolateral thoracotomy, after which the inferior pulmonary ligament was divided to free the left lung. The lung was then delivered into the surgical wound, the hilum tied with a 4-0 silk ligature, and the lung excised. The chest was closed as described above. Animals were allowed to recover from surgery and extubated after initiation of spontaneous respirations. The animals then received postoperative analgesia in the form of buprenorphine injected intramuscularly every 12 hours for the first 24 hours. The animals were allowed to feed ad libitum and maintained in a controlled environment.

After the designated time interval, the animals were anesthetized, weighed, and intubated via a tracheotomy, and exposure of the environment. The animals were allowed to feed ad libitum and maintained in a controlled environment.

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After the designated time interval, the animals were anesthetized, weighed, and intubated via a tracheotomy, and exposure of the thoracic organs was obtained by a bilateral anterior sternothoracotomy. The animals were rapidly exsanguinated by vena cava division and received intratracheal instillation of 70% ethanol to a ligature, and the lung excised. The chest was closed as described by Davies\(^1\) and Wandel et al.\(^1\)\(^8\).

Lung Morphometry
Lung sections (5-μm thick) were stained with hematoxylin-eosin stained, and lung morphometry was used to measure the total volume of respiratory region (TVr) and alveolar surface density (Sv) as previously described by our laboratory\(^8\)\(^,\)\(^1\)\(^3\) using the point counting technique described by Davieter\(^1\)\(^7\) and Wandel et al.\(^1\)\(^8\).

Percent Gain in Total Body Weight 21 Days Posturgery for the Sham Thoracotomy (S), KGF-Treated Sham Thoracotomy (SK), Untreated Pneumonectomy (P), and KGF-Treated Pneumonectomy (PK) Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Percent Gain in Total Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>23.6±1.7</td>
</tr>
<tr>
<td>SK</td>
<td>29.9±2.3*</td>
</tr>
<tr>
<td>P</td>
<td>21.7±1.5</td>
</tr>
<tr>
<td>PK</td>
<td>30.4±1.8*</td>
</tr>
</tbody>
</table>

*P<0.004 vs. groups S and P.

5-Bromo-2’-deoxyuridine (BrdU) Immunohistochemistry
BrdU, a thymidine analogue, is incorporated into DNA during the S phase (DNA synthesis) of the cell cycle. Cells that have incorporated BrdU can then be detected by immunohistochemistry. The percentage of stained cells is measured to yield the proliferation index. BrdU (50 mg/kg) was injected intraperitoneally 2 hours before lung harvest. For immunohistochemistry, the BrdU in situ detection kit (BD Pharmingen, San Diego, CA) was used as instructed. The alveolar cell proliferation index (CPI) was determined in peripheral alveolar lung tissue using the ratio of the number of labeled nuclei among 1000 total counted nuclei. Endothelial cells and cells from large airways were excluded from this process. This technique allowed us to calculate the percentage of alveolar cells (mostly type II pneumocytes) that exhibit cell division.

Statistical Analysis
Measurements are reported as the mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) was used to determine whether a difference existed between the study groups. A probability value of 0.05 or less is used to indicate significant differences. Bonferroni multiple comparison test was used when appropriate.

Results
Change in Total Body Weight
The initial and final body weights of all animals were recorded after 21 days. We compared the percent change in total body weight for the animals in the different groups. The KGF-treated pneumonectomy (group PK) and KGF-treated sham thoracotomy (group SK) animals both had significant increases in total body weight (P<0.004) when compared with the untreated sham thoracotomy (group S) and pneumonectomy animals (group P) (Table 1).

Lung Weight Index (LWI)
As expected, because of compensatory lung growth, pneumonectomy (group P) resulted in a significant increase in LWI versus the sham thoracotomy (group S) at both 10 and 21 days (Fig. 1). Exogenous KGF resulted in a further, significant enhancement of LWI (group PK) at both 10 and 21 days when compared with pneumonectomy alone (group P) (Fig. 1). Importantly, at 21 days we failed to see any enhancement in LWI by KGF in sham operated animals (group SK) when compared with group S (Fig. 1).

Lung Volume Index (LVI)
As expected, similar to LWI, LVI was significantly elevated at both 10 and 21 days after pneumonectomy (group P) when
compared with the sham thoracotomy animals (group S) as a result of compensatory lung growth (Fig. 2). Analogous to the LWI results, LVI was significantly enhanced in the KGF-treated animals (group PK) at both 10 and 21 days when compared with group P (Fig. 2). In addition, at 21 days we failed to see any increase in LVI in the sham-operated animals that were treated with KGF (group SK) when compared with group S (Fig. 2). Increases in compensatory lung growth are more striking when evaluating LVI because a small increase in LWI can result in a large increase in LVI. These results, along with LWI results, indicate that KGF specifically enhances compensatory lung growth after pneumonectomy.

**Total Volume of Respiratory Region (TVvr)**

Lung alveolar morphometry was analyzed in groups S, P, and PK at 21 days postsurgery because of the maximal changes that are achieved at this endpoint. TVvr is a measure of the total volume of the alveoli and intervening gas exchange tissue of the lung. Pneumonectomy alone (group P) caused a significant increase in TVvr when compared with the sham operated animals (group S) (Fig. 3). The KGF-treated animals (group PK) had a further, significant enhancement in TVvr when compared with pneumonectomy alone (group P) (Fig. 3).

**Alveolar Surface Density (Sv)**

Sv expresses the alveolar surface area per unit volume (cm²/cm³) in the lung. As with TVvr, Sv was calculated in groups S, P, and PK at 21 days postsurgery. Although Sv was not altered after pneumonectomy alone (group P), there was a significant increase of Sv in the KGF treated animals (group PK) (Fig. 4).

**Alveolar CPI**

Alveolar CPI was determined in the various groups at both 10 and 21 days postsurgery using BrdU immunostaining as described in the Methods section. At 10 days pneumonectomy animals (group P10) experienced a significant increase in alveolar CPI when compared with sham thoracotomy animals (group S10) (Fig. 5), which agrees with the historical studies on compensatory lung growth. In addition, KGF
treatment (group PK10) resulted in a significant enhancement in alveolar CPI when compared with pneumonectomy treatment (group P10) (Fig. 5). At 21 days, alveolar CPI remained significantly increased in groups P21 and PK21, although this difference was not as pronounced as was observed on day 10 (Fig. 5). Again, KGF enhanced alveolar CPI as compared with pneumonectomy alone. KGF treatment of sham thoracotomy animals (group SK21) failed to have any effect on the alveolar CPI (Fig. 5).

Discussion

Understanding the various modulators of lung growth has important clinical appeal. Various scenarios such as reduced size lung transplantation and pulmonary insufficiency can benefit from the modulation and induction of lung growth. Our laboratory has been active in determining the role of various growth factors on compensatory lung growth. We have shown in previous studies that both exogenous epidermal growth factor and retinoic acid enhance postpneumonectomy lung growth. However, although those factors modulate lung growth by enhancing the global volume of gas exchange tissue in the lung, we failed to see an increase in the alveolar surface density in those experiments. In this current study, we investigated the role of KGF on lung growth. KGF has been implicated in various aspects of pulmonary morphogenesis. It acts as a mitogen for type II pneumocytes and also as an upregulator of surfactant gene expression.

We measured the percent change in body weight to determine the effect of KGF on the whole animal. At the 21-day time point, we noted that both KGF-treated groups (pneumonectomy and sham thoracotomy) had a significant increase in body weight when compared with the untreated thoracotomy and pneumonectomy groups. However, KGF did not increase LWI or LVI in the sham-operated rats. This suggests that there was a specific effect of KGF on lung growth after pneumonectomy and not on sham animals. Analysis of wet-to-dry weight ratio failed to produce a difference between the various groups (data not shown), thus ruling out pulmonary edema as a contributor to the increase in lung weight index. Thus, we can conclude that this increase in LWI is indeed true growth and not because of edema or interstitial fluid accumulation.

On gross examination of the right lung in animals that had undergone a left pneumonectomy, we noted that the supradiaphragmatic lobe extended across the mediastinum into the left hemithorax. It is interesting, however, that KGF did not enhance lung growth in the sham group. This may be because the postpneumonectomy state most likely results in the upregulation of many genes that trigger and regulate the process of compensatory lung growth. Therefore, it is possible that KGF enhances this growth process through binding with its receptor in the lung epithelia. The postpneumonectomy state is similar to that seen during pulmonary insufficiency secondary to various disease processes and that seen during pediatric lung transplantation. In pediatric lung transplantation, the recipient grows at a normal rate; however, the growth of the transplanted lung lags behind the respiratory needs of the recipient. Thus, we believe that the findings from this study could potentially be applied to those clinical situations.

The role of KGF on the proliferation of pneumocytes was determined using BrdU immunohistochemistry. At the 10-day interval there was a significant increase in alveolar CPI in the pneumonectomy animals when compared with the sham animals, which represents the rapid compensatory growth response of the right lung. We also noted that KGF-treated pneumonectomy animals had a significant increase in alveolar CPI when compared with the untreated pneumonectomy animals. This likely represents the mitogenic role of KGF during compensatory lung growth. Similar results were observed at the 21-day interval; however, the CPI was lower in comparison to the 10-day time interval, agreeing with past studies that describe an early peak of cell division after pneumonectomy, which then declines over time. Although the study by Ulich et al demonstrated enhanced type II pneumocyte proliferation in rats after KGF injection, we did not see an increase in proliferation in our KGF-treated sham animals at day 21. The route of dosage can best explain this apparent discrepancy. We used an intraperitoneal route of injection whereas Ulrich et al used an intratracheal injection, which resulted in a very localized, high dose of KGF directly onto the lung epithelial surface. In addition, Ulrich et al stated that a single intravenous (systemic) injection of KGF did not result in alveolar epithelial cell hyperplasia, which suggests that the intratracheal injection of KGF is a more potent stimulus for type II pneumocyte hyperplasia. A study by Guo et al described alveolar cell proliferation after a single intravenous dose of KGF; however, this peaked at 48 hours and was back to baseline by 72 hours. We measured CPI at day 21 in the sham animals and did not detect any increases, but it is possible that earlier time points in the sham animals could have revealed some increase in alveolar CPI. Despite
this, the conditions after pneumonectomy apparently permitted the enhancement of lung growth by systemic KGF in our study.

The concept of inducing new alveolar formation is a unique finding in this study. It was traditionally believed that alveolar formation reaches completion in early childhood with subsequent hypertrophy during adulthood. Mature alveoli are not present at birth; instead, they begin to appear approximately 5 weeks after birth. In humans, the number of alveoli increase more than 10 fold from 20 million primitive alveolar sacs at birth to about 300 million alveoli by the age of 8 years. Growth of alveoli during aging has been shown to occur mostly by hypertrophy. However, we have shown that we can indeed induce formation of new alveoli by KGF administration in adult lung tissue in this model of compensatory lung growth. This is evident by the finding that KGF enhanced both TVvrs as well as SVv after pneumonectomy, which is most likely a result of alveolar hyperplasia. An increase in SV is likely a result of smaller alveoli, which can most easily be explained by alveolar proliferation (ie, growth of new alveoli) because the lung architecture appears normal by histology in the KGF-treated animals (not shown). This can be of potential value in various arenas of clinical medicine. It can be applied in the treatment of pulmonary insufficiency, during which KGF could possibly induce healthy alveolar formation. KGF could also be used to modulate growth of transplanted lungs to enhance their growth potential. However, further experiments need to be performed that investigate the role of KGF in a disease model and a lung transplantation model. Although KGF has important effects on lung growth, we cannot overlook the concern that exogenous KGF or other growth factors could also play a potential role in enhancing oncogenesis or cancer proliferation.

The rats used in our study were adult rats that were 7 to 8 weeks of age. It is possible that there is a greater effect of pneumonectomy on lung growth in younger animals as compared with older animals. A recent review by Massaro and Massarro shows that the number of alveoli in postnatal lungs of rats increases until about age 44 days and remains constant after that. In addition, the mean volume of individual alveoli decreases dramatically in the first 2 weeks after birth and remains constant after that. Although rats continue to grow throughout life, alveolar formation appears to be complete by age 44 days, and new alveolar growth does not occur thereafter except under special circumstances such as pulmonary fibrosis. Thus, we believe that the lung growth that occurs after pneumonectomy cannot be attributed to normal postnatal lung development.

Most studies performed until now have looked at the role of KGF in the prevention of various forms of pulmonary injury. KGF has been shown to ameliorate lung injury secondary to bleomycin, radiation exposure, and H2O2 instillation. The role of KGF in these lung disease models suggests that KGF must have some sort of restorative function in the lung. We hope to combine these findings with our results to design future experiments to study the role of KGF in various aspects of mature pulmonary growth.

Acknowledgments

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References

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