Keratinocyte growth factor transiently alters pulmonary function in rats

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Departments of 1Respiratory Medicine and 3Functional and Applied Anatomy, Hannover Medical School, and 2Fraunhofer Institute of Toxicology and Experimental Medicine, D-30625 Hannover; and 4Clinical Research Group “Chronic Airway Diseases,” University of Marburg, D-35032 Marburg, Germany

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Hohlfeld, Jens M., Heinz G. Hoymann, Thomas Tschernig, Antonia Fehrenbach, Norbert Krug, and Heinz Fehrenbach. Keratinocyte growth factor transiently alters pulmonary function in rats. J Appl Physiol 96: 704–710, 2004. First published October 17, 2003; 10.1152/japplphysiol.00783.2003.—Keratinocyte growth factor (KGF) is a mitogen for pulmonary epithelial cells. Intratracheal administration of KGF to adult rats results in alveolar epithelial cell type II and bronchiolar epithelial cell proliferation. While cellular responses to KGF have been intensively studied, functional consequences regarding lung function are unknown. Therefore, in this study, we sought to investigate whether KGF alters pulmonary function variables. Rats received either recombinant human KGF (rHuKGF) (5 mg/kg) or vehicle intratracheally. Before and on days 3 and 7 after treatment, pulmonary function was determined by body plethysmography. Subsequently, lung histological changes were quantified. rHuKGF induced a transient proliferation of alveolar and bronchiolar epithelial cells. The extent of type II cell hyperplasia was significantly correlated with a transient reduction in tidal volume and an increase in breathing frequency. In addition, quasi-static compliance, total lung capacity, and vital capacity were reduced after rHuKGF instillation, suggesting the development of a transitory restrictive lung disorder. Moreover, reduced expiratory flow rates and forced expiratory volumes, as well as increased functional residual capacity after rHuKGF but not vehicle, suggest obstructive lung function changes. In conclusion, the induction of alveolar and bronchiolar epithelial cell proliferation by KGF is paralleled by moderate functional consequences that should be taken into account when the therapeutic potential of KGF is tested.

rodent lung function; plethysmography; epithelial cell proliferation; alveolar epithelial type II cells

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (13), which induces proliferation of adult rat alveolar type II cells and bronchiolar epithelial cells in vivo (4, 5, 16). Alveolar type II cells synthesize, secrete, and metabolize pulmonary surfactant, which covers the inner surface of alveoli and terminal airways. KGF enhances surfactant protein gene expression in vivo and in vitro (19, 20). In the developing lung, KGF did not induce type II cell proliferation but increased surfactant pool sizes, suggesting enhanced phospholipid synthesis in type II cells (11).

In animal models, KGF has protective effects against a variety of pulmonary injurious stimuli when given as a pretreatment (17). It has been demonstrated that KGF protects from oxygen- and ventilator-induced alveolar injury, prevents bleomycin-induced lung fibrosis, and ameliorates acute lung injury in experimental animal models (2, 7, 15, 18). Whether this therapeutic effect could be transmitted into a treatment effect in humans needs to be further investigated. Nonetheless important, potential side effects have to be carefully elucidated. So far, nothing is known about the functional consequences resulting from proliferation of alveolar epithelial cell type II cells and bronchiolar epithelial cells regarding pulmonary function variables. Hyperplasia peaks ~3 days after intratracheal KGF treatment, leading to the formation of monolayers of cuboidal epithelial cells along alveolar septae and mild focal bronchiolar epithelial hyperplasia (16). It is very well conceivable that epithelial cell proliferation may alter alveolar distensibility and airway calibers, resulting in changes in breathing patterns. We, therefore, investigated the effect of recombinant human KGF (rHuKGF) on pulmonary function variables in rats.

Materials and Methods

Animals. Male Brown Norway rats (Charles River Wiga, Sulzfeld, Germany) weighing 200–250 g were used in the experiments. In a supplementary study, Fischer 344 rats (Charles River Wiga) of the same sex and weight were measured. Animals were housed, watered, and fed as described before (8). Care and use of experimental animals was strictly performed, according to the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication 85-23, revised 1985).

Treatment with rHuKGF. rHuKGF prepared at Amgen (Thousand Oaks, CA) was produced in Escherichia coli and purified to homogeneity by conventional methods. KGF was found free of endotoxin and assayed for biological activity with the Balb/MK keratinocyte cell line (14). On day 0, animals were anesthetized with 2% halothane-30% oxygen and intubated orally with a tracheal cannula (Cathlon, Jelco; 52 mm, 1.78 mm ID). They received a single intratracheal bolus of either 5 mg/kg rHuKGF diluted in PBS or PBS alone (1.25 ml/kg) as control.

Study design. Twenty-two Brown Norway rats received either rHuKGF or vehicle on day 0 (n = 11 per group). Pulmonary function testing was performed on eight animals per group before treatment on day 0 and after treatment on day 3. Thereof, five rats per group were killed on day 3 for quantitative histological examination immediately after lung function tests were completed. On day 7, pulmonary function was measured in the remaining three rats per group and in three additional animals per group treated with either rHuKGF or vehicle, respectively, on day 0. On day 7, the three remaining rats per group were killed for quantitative histopathology immediately after lung function analysis.

Pulmonary function measurements. Respiratory function variables, including forced expiratory maneuvers, were measured in anesthetized, intubated, spontaneously breathing rats by body plethysmography, as described before (6, 10). Briefly, animals were anesthetized with 2% halothane/30% oxygen and intubated orally with a tracheal...
cannula (Cathlon, Jelco; 52 mm, 1.78 mm ID). Rats were then placed in a body plethysmograph in the supine position. For measurement of esophageal pressure, an air-filled polyvinyl chloride tube (1.6 mm OD) was inserted into the esophagus, placed in the position of maximal signal, and coupled to a pressure transducer (P75, range ±100 cmH₂O, HSE-Harvard, March-Hugstetten, Germany). Tidal flow was determined by a pneumotachograph attached to the rear wall of the plethysmograph and connected to a differential pressure transducer (MP 45–1, range ±2 cmH₂O, Validyne, Northridge, CA). Airway pressure was measured in a side branch of the breathing port (air-filled P75, range ±100 cmH₂O, HSE-Harvard), and esophageal pressure was subtracted to obtain transpulmonary pressure (Ptp). All signals were amplified, assessed by a pulmonary mechanics analyzer (analyzer model 6, Buxco), monitored continuously as tracings on a chart recorder, digitized, and statistically analyzed by FT-STAT software (Branch Technology; Fraunhofer ITEM, Hannover, Germany).

After placement in the body plethysmograph, anesthesia was maintained with 2% halothane at a constant rate, and animals were allowed to stabilize for 5 min before lung function tests were performed. First, spontaneous pulmonary function variables were determined. Variables measured included tidal volume (VT; in ml), breathing frequency (f; min⁻¹), minute ventilation (VE; in ml/min), dynamic lung compliance (Cdyn; in ml/cmH₂O), and lung resistance (RL; in cmH₂O·ml⁻¹·s). Second, forced lung function maneuvers were performed during temporary apnea induced by hyperventilating the animal with an ambubag. For the forced expiration maneuvers, lungs were inflated up to a Ptp of +30 cmH₂O and then deflated to a Ptp of −50 cmH₂O by applying pressure reservoirs at +40 and −50 cmH₂O, respectively. Forced vital capacity (VC) (ml), forced expiratory volume in 0.1 s (FEV₀.₁; in ml), peak expiratory flow (ml/s), maximum midexpiratory flow (ml/s), and forced expiratory flow at 50, 25% of forced VC (ml/s) were analyzed from the recorded volume-time relations and flow-volume curves (6, 10). In addition, functional residual capacity (FRC) and quasi-static pressure-volume curves were derived during two subsequent apnea periods, as described previously (6, 10), and allowed for calculation of quasi-static compliance (Cqs; in ml/cmH₂O), total lung capacity (TLC; in ml), and residual volume (ml). Animals in all treatment groups were studied on each study day in randomized order.

Fixation. At the appropriate day after treatment, animals were killed by an overdose of intraperitoneal pentobarbital. A cannula was inserted into the trachea in situ via tracheostomy. The chest was opened, and the lungs were removed. For fixation of lungs, 4% buffered paraformaldehyde in PBS was instilled via the trachea at a constant pressure of 20 cmH₂O, and the lungs were immersed in fixation solution overnight at 4°C. According to the principle of systematic uniform random sampling, the lungs were embedded into 2% aqueous agarose and cut into equidistant slices of 3-mm thickness. All slices were then embedded into paraffin.

Immunohistochemistry. Paraffin sections of all slices were stained according to indirect immunohistochemistry, as described earlier (4, Fig. 1. Characteristics of Brown Norway rat lungs fixed 3 days after instillation with PBS (a, d, g) or recombinant human keratinocyte growth factor (rHuKGF) (b, e, h), and 7 days after rHuKGF (c, f, i). Results of indirect immunohistochemistry for nuclear proliferation marker Ki67 (a–c) and surfactant protein D (d–i) demonstrate transient epithelial hyperplasia of bronchiolar (arrowheads) and alveolar epithelial cells (arrows). Hyperplasia is associated with considerable architectural alterations (change in airway epithelial cell shape from cuboidal to columnar with focal papillary cell clusters, alveolar epithelium with large papillae consisting of hypertrophic type II cells and by extended areas of type II cell monolayers), which are largely but not completely resolved at day 7 after rHuKGF. 100 µ = 100 µm.)
After microwave antigen retrieval (2 \times 5 \text{ min at 750 W in 0.01 M citrate buffer, pH 6.0}), dewaxed sections were incubated with hydrogen peroxide and 50% fetal calf serum in PBS for 30 min at room temperature before incubation with primary antibodies against rat surfactant protein D (undiluted, clone S7; courtesy of Dr. S. Albrecht, Dresden, Germany) or against the nuclear protein Ki-67 (diluted 1:5, clone MBB-5; Dianova, Hamburg, Germany), a well-established proliferation marker. After washing with PBS, primary antibodies were detected by using a mouse kit based on the avidin-biotin peroxidase complex technique (Vectastain Elite kit, Vector Laboratories). Specificity of the staining procedure was proved by using dilution buffer and isotype control antibodies instead of the primary antibodies. Counterstaining was done with hematoxylin.

Stereological examinations. The stained sections of all lung slices were analyzed by established stereological methods (9). To obtain a collection of test fields representative of the whole organ, meander scanning was performed, and intersection counting was used to determine the relative alveolar epithelial surface coverage with alveolar epithelial type II cells. Stereological analysis was performed on a computer-based system (Cast-Grid 2.00, Olympus).

Statistics. Results are expressed as mean values ± SE. Statistical differences between mean values of treatment groups were determined by unpaired $t$-test. Differences in changes after treatment compared with baseline within a group were tested for their statistical significance by paired $t$-test. For comparison of days 0 and 7 within groups, a mixed model was used (SAS program PROC MIXED) because unpaired and paired data were to be analyzed. Correlations were assessed by Spearman’s rank test. $P$ values of $<0.05$ were considered significant.

RESULTS

Histological changes. Indirect immunohistochemistry for nuclear proliferation marker Ki-67 and surfactant protein D demonstrated epithelial hyperplasia of bronchiolar and alveolar epithelial cells 3 days after instillation of rHuKGF, but not after PBS treatment (Fig. 1). Hyperplasia was associated with considerable architectural alterations, which were largely but not completely resolved at day 7 after rHuKGF (Fig. 1, c, f, and i). In detail, the airway epithelium exhibited a change in cell shape from cuboidal to columnar, and, focally, papillary cell clusters were observed. In contrast to control lungs, which were characterized by solitary alveolar epithelial type II cells located at the corners of the alveolar walls, the alveolar epithelium of rHuKGF-treated lungs was characterized by large papillae consisting of hypertrophic type II cells and by extended areas of type II cell monolayers.

The fraction of alveolar epithelial surface covered by surfactant protein D immunoreactive type II cells was increased after rHuKGF instillation (30.6 ± 2.2%) compared with PBS-treated controls (8.3 ± 1.3%, $P < 0.0001$). Seven days after rHuKGF instillation, the relative coverage with type II cells was still elevated (15.7 ± 1.3%) compared with controls (6.6 ± 0.2%, $P = 0.003$), but lower than on day 3 after rHuKGF ($P = 0.003$). Thus hyperplasia was associated with architectural alterations in Brown Norway rats comparable to Fischer 344 rats, whereas the resolution at day 7 after rHuKGF was less advanced in Brown Norway compared with Fischer 344 rats (3, 5).

The effect of rHuKGF on respiratory function. Respiratory function variables under spontaneous breathing (f, V T, and V E) were not different at baseline between control animals and rHuKGF-treated rats (Fig. 2). Vehicle instillation to control animals did not change these variables, neither on day 3 nor on day 7 after instillation. In contrast, 3 days after rHuKGF treatment, f increased (Fig. 2A) and V T decreased significantly (Fig. 2B), leading to increased V E 3 days after rHuKGF treatment (Fig. 2C). On day 7, these changes were still signif-

\begin{figure}
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\includegraphics[width=\textwidth]{fig2.png}
\caption{Spontaneous respiratory function variables frequency (A), tidal volume (B), and minute ventilation (C) at baseline (day 0) and at days 3 and 7 after treatment with either vehicle (control) or rHuKGF (KGF). Values are means ± SE ($n = 6–8$). **$P < 0.01$ compared with baseline values on day 0. #$P < 0.05$ and ##$P < 0.01$ compared with the control group.}
\end{figure}
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Fig. 3. Correlation of alveolar epithelial type II cell surface fraction with spontaneous respiratory function variables frequency (A), tidal volume (B), and minute ventilation (C) of rats at days 3 and 7 after treatment with either vehicle or rHuKGF. •, Control rats at day 3; □, control rats at day 7; •, rHuKGF-treated rats at day 3; ◆, rHuKGF-treated rats at day 7. The r values and exact P values are given.

Significantly different from PBS-treated control animals. However, f and V̇e in rHuKGF-treated animals on day 7 did not differ significantly from pretreatment values, whereas V̇t was still reduced compared with pretreatment values.

rHuKGF-induced changes in f, V̇t, and V̇e strongly correlated to the relative coverage of the alveolar surface with type II pneumocytes (Fig. 3, A–C, respectively). With increasing type II cell surface fraction, f increased (Fig. 3A, r = 0.885, P < 0.0001), whereas V̇t decreased (Fig. 3B, r = 0.898, P < 0.0001), resulting in an increase in V̇e (Fig. 3C, r = 0.802, P = 0.0002).

Rt and Cdyn were similar at baseline between treatment groups. rHuKGF, but not PBS, induced a significant fall in Cdyn (P = 0.03) and a trend toward increased Rt (P = 0.07) on day 3 after instillation compared with baseline values (Table 1). On day 7, these changes were back to baseline values. In another study using the same treatment regimen and timing, we additionally measured spontaneous respiratory variables in Fischer-344 rats of the same age and body weight 3 days after instillation of either rHuKGF or PBS (n = 6 per group). Whereas baseline values of Rt and Cdyn were not different between groups and comparable in degree to Brown Norway rats, pooled analysis (Fisher 344 rats, n = 6, plus Brown Norway rats, n = 8, resulting in n = 14 per group) revealed that rHuKGF but not PBS induced an increase in Rt: 3 days after treatment (0.199 ± 0.013 cmH2O·ml−1·s, which was significant compared with baseline values (0.160 ± 0.007 cmH2O·ml−1·s, P = 0.008) and with the control group on day 3 (0.161 ± 0.043 cmH2O·ml−1·s, P = 0.029). Consistently, Cdyn decreased significantly on day 3 (0.284 ± 0.013 ml/cmH2O) compared with baseline (0.348 ± 0.012 ml/cmH2O, P = 0.003) and with control animals on day 3 (0.369 ± 0.099 ml/cmH2O, P = 0.017). The relative coverage of alveolar surface with type II cells determined in Brown Norway rats from the main study again correlated to the increase in Rt (r = 0.646, P = 0.007) and to the reduction in Cdyn (r = −0.530, P = 0.035).

Analysis of Cqs derived from pressure-volume maneuvers revealed a decrease in compliance after rHuKGF treatment, which was significant compared with control Brown Norway rats 7 days after instillation (Table 1). Reduced Cqs was accompanied by a significant loss of TLC in rHuKGF-treated rats on day 7 (Table 1), whereas TLC remained unchanged in control animals. In contrast to the other lung function parameters reported so far, the type II cell surface fraction did not correlate with Cqs (r = −0.105, P = 0.70) and TLC (r = −0.210, P = 0.434). VC (Fig. 4A) was reduced in rHuKGF-treated rats compared with control animals 3 and 7 days after instillation. rHuKGF induced a significant decrease in VC compared with baseline (pretreatment). These changes with reduced Cqs, reduced TLC, and reduced VC suggest a restrictive lung disorder induced by rHuKGF. However, alterations of compliance and TLC did not run parallel to the morphological changes and to the kinetics of the other functional parameters.

Forced expiratory maneuvers with analysis of flow-volume relations and FEV0.1 allowed assessment of obstructive airway alterations. After rHuKGF treatment, FEV0.1 was significantly decreased on both day 3 and day 7 compared with controls (Fig. 4B). Interestingly, FRC was elevated on day 3 after

Table 1. Respiratory function variables

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Rt, cmH2O·ml−1·s</th>
<th>Cdyn, ml/cmH2O</th>
<th>Oqs, ml/cmH2O</th>
<th>TLC, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.170 ± 0.011</td>
<td>0.329 ± 0.008</td>
<td>0.45</td>
<td>13.68 ± 0.35</td>
</tr>
<tr>
<td>Day 3</td>
<td>6</td>
<td>0.175 ± 0.016</td>
<td>0.356 ± 0.053</td>
<td>0.98 ± 0.06</td>
<td>13.95 ± 0.66</td>
</tr>
<tr>
<td>Day 7</td>
<td>6</td>
<td>0.176 ± 0.011</td>
<td>0.360 ± 0.036</td>
<td>1.02 ± 0.03</td>
<td>13.49 ± 0.36</td>
</tr>
<tr>
<td>rHuKGF</td>
<td>6</td>
<td>0.170 ± 0.007</td>
<td>0.329 ± 0.008</td>
<td>0.92 ± 0.06</td>
<td>12.78 ± 0.35</td>
</tr>
<tr>
<td>Day 3</td>
<td>6</td>
<td>0.210 ± 0.021</td>
<td>0.270 ± 0.020</td>
<td>0.84 ± 0.09</td>
<td>13.14 ± 0.45</td>
</tr>
<tr>
<td>Day 7</td>
<td>6</td>
<td>0.176 ± 0.007</td>
<td>0.329 ± 0.026</td>
<td>0.79 ± 0.05</td>
<td>11.68 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Rt, lung resistance; Cdyn, dynamic compliance; Oqs, quasi-static compliance; TLC, total lung capacity; rHuKGF, recombinant human keratinocyte growth factor. *P < 0.05 compared with day 0. †P = 0.07 compared with day 0. ‡P < 0.01 compared with control group.

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rHuKGF compared with baseline, suggesting trapped air due to airway obstruction (Fig. 4C). These changes were resolved on day 7 after rHuKGF treatment. Changes in FRC on the one hand correlated to changes of spontaneous breathing parameters (VT: $r = -0.610$, $P = 0.012$; $f$: $r = 0.599$, $P = 0.014$), and on the other hand were related to the relative coverage of alveolar surface with type II cells ($r = 0.678$, $P = 0.004$).

Finally, comparison of flow-volume relations on day 3 after treatment revealed a significant reduction in forced expiratory flow at 70–55% of VC in rHuKGF-treated rats compared with controls (Fig. 5). These changes with reduced FEV$_{0.1}$ and reduced expiratory flow values, as well as increased FRC after rHuKGF treatment but not PBS treatment, suggest the induction of an obstructive lung disorder.

**DISCUSSION**

KGF has been used extensively in experimental in vivo models to study the effect of this growth factor on cellular and molecular events (17). However, the functional consequences with regard to pulmonary function have not been addressed so far. We here provide evidence that rHuKGF-induced proliferation of pulmonary and bronchiolar epithelial cells in rats is accompanied by a transient deterioration of lung function.

KGF clearly alters lung function parameters that indicate airway obstruction. We observed airflow limitation with reduced forced expiratory flows and a reduction in FEV$_{0.1}$ in rHuKGF-treated rats. Consistently, $R_l$ was increased and $C_{dyn}$ was decreased in response to rHuKGF. In addition, FRC was elevated transiently in rHuKGF-treated animals, which is suggestive of air trapping induced by airflow limitation and expiratory closure of terminal airways. These transient obstructive airway changes were paralleled by a proliferation of airway epithelial cells. Proliferation was particularly present in bronchiolar epithelial cells of the terminal airways. rHuKGF also leads to changes in lung function variables that define restrictive lung disease (TLC and compliance), albeit with different kinetics. In addition, rHuKGF affects the breathing pattern with decreased $V_t$ and increased $f$, resulting in increased $V_E$. Notably, changes in breathing pattern and in parameters indicating airway obstruction are closely related to rHuKGF-induced type II cell hyperplasia, which suggests a direct link between morphological changes and alterations of pulmonary function.

It is not clear how KGF-induced morphological changes like type II cell hyperplasia and epithelial cell shape transformation...
translates into the observed functional consequences. Possibly, proliferation of airway epithelial cells in the terminal airways (Fig. 1) might reduce airway caliber, which results in airflow limitation. According to the law of Hagen-Poiseuille, resistance is dependent on airway radius to the fourth power, so that even very small morphological changes in the epithelial layer might affect airway function. In the alveoli, proliferation of alveolar epithelial type II cells might induce parenchymal alterations with restricted lung volumes. The alveolar epithelium shows large papillae, consisting of hypertrophic type II cells and extended areas of type II cell monolayers. This might explain decreased $V_t$ and a compensatory increase in $f$. Accordingly, the strongest correlation of type II cell surface fraction was seen with $V_t$ (Fig. 2B). In contrast, in a recent study, we did not observe alterations in alveolar and alveolar duct sizes on day 3 after rHuKGF instillation as morphological measures for air space volumes (3). However, the fixation technique of the lungs and the static determination of alveolar volume in contrast to our functional measurement in spontaneously breathing animals might account for the differences observed.

Another likely explanation for the KGF-induced changes in spontaneous breathing pattern relates to the observed increase in FRC from ~4 ml at baseline to 5.7 ml 3 days after rHuKGF instillation. This increase in FRC possibly affects tidal breathing and, because the animals have to breathe at a level of higher elastic recoil, which, in turn, might lead to reduced $V_t$ and a compensatory increase in $f$. In line with this explanation, there was a good correlation between FRC and $V_t$ and FRC and $f$. It is unlikely that changes in breathing pattern are caused by mechanisms usually seen in inhalation toxicity. Inhaled irritants provoke a reduction in $f$ during challenge, preventing the lung from further harm (1). Because rHuKGF was given as a single bolus and lung function tests were performed 3 and 7 days later, it is not likely that rHuKGF affected $f$ directly as do inhaled irritants. It is also unlikely that the observed changes in breathing pattern might relate to volatile anesthesia. Halothane was set at a constant concentration of 2%. Type II cell hyperplasia with increased coverage of the alveolar surface with cuboidal type II cells instead of flat type I cells might have affected systemic absorption of halothane due to differences in diffusion capacity or differences in metabolic activities of alveolar cells. However, it is very unlikely that this mechanism plays a significant role, because potency of halothane and thereby its effect on $f$ is determined by its lipophilicity and not diffusibility (12). Diffusion affects the absorption kinetics and, therefore, the time to reach equal partial pressures in the alveoli and the blood. Because induction and maintenance of anesthesia was achieved by constant halothane concentration and animals were allowed to stabilize 5 min before start of lung function measurements, halothane effects seem negligible. Finally, changes not only in spontaneous breathing pattern but also in lung function parameters that indicate airway obstruction and restrictive lung disorders are additional evidence to favor parenchymal alterations over pharmacological effects as the most likely explanation.

Whereas rHuKGF-induced changes in $V_t$ and $f$ were closely related to type II cell hyperplasia, TLC and lung compliance, the major parameters defining restrictive lung disease, did not run parallel to the morphological changes and to the kinetics of parameters from spontaneous respiration. Methodological aspects, like the small number of paired data sets at day 7, may be responsible for these findings, but, on the other hand, there is also the possibility of delayed parenchymal alterations beside type II cell hyperplasia. However, in a recent study, neither did we observe alterations of alveolar size and alveolar duct size (3) nor did we find any sign of increased fiber content in the parenchyma by electron microscopy. Another explanation for the different kinetics of TLC and Cqs with a significant reduction at day 7 instead of day 3 might relate to nonparenchymal mechanisms, like alteration of pulmonary surfactant function. rHuKGF might have induced a disturbance of intra-alveolar surfactant that could account for the observed kinetics of TLC and compliance. However, data on surfactant function and phospholipid composition from bronchoalveolar lavage after rHuKGF to prove this hypothesis are missing and should be the subject of future studies.

In conclusion, our data on lung function in Brown Norway rats after treatment with rHuKGF demonstrate that instillation of 5 mg rHuKGF/kg induced a transient proliferation of pulmonary epithelial cells that was accompanied by a transient airflow limitation and restrictive breathing pattern. Therefore, the induction of alveolar and bronchiolar epithelial cell proliferation by rHuKGF leads to moderate functional consequences that should be taken into account as potential side effects when planning to use KGF as a treatment option for lung diseases.

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GRANTS

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