Keratinocyte growth factor attenuates hydrostatic pulmonary edema in an isolated, perfused rat lung model

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Keratinocyte growth factor (KGF) is a mitogen for epithelial cells and fibroblast growth factor family that acts in a paracrine fashion as a mitogen for epithelial cells (13). First characterized in 1989, keratinocyte growth factor (KGF) is a heparin-binding member of the fibroblast growth factor family that acts in a paracrine fashion as a mitogen for epithelial cells (13).

Release of KGF from fibroblasts may be triggered by exposure to serum or cytokines, peaking at 8 h and then tapering to minimal levels after 48–72 h (3). Several types of lung injury have been demonstrated to increase lung levels of KGF, and a role in repair of the epithelial surface injury has been proposed (1, 16).

KGF has been shown to prevent lung injury and pulmonary edema accumulation in numerous models of permeability-type pulmonary edema, including α-naphthylthiourea, acid aspiration, and bleomycin (10, 22, 23). KGF has long been thought to be specific to epithelial cells and has been shown to induce hyperplasia of type II alveolar pneumocytes (17). Type II alveolar epithelial cells (AEC2) are an integral component of the blood-gas barrier in the lung. Greater than 92% of the resistance to macromolecular flux lies in the epithelial layer of the alveolar-capillary membrane (7). This suggests that KGF’s effect on the alveolar epithelium is responsible for the protection seen in models of permeability-type edema.

KGF has not been studied in hydrostatic pulmonary edema. Interestingly, recent evidence has shown that KGF may also affect capillary endothelial cells (6). Although at extremes of transmural vascular pressures, evidence of epithelial and endothelial cell disruption has been described (2, 20), hydrostatic edema is thought to occur as a result of an increased capillary-alveolar pressure gradient, leading to flow of fluid into the lower-pressure alveolar space, as opposed to the disruption of the epithelial layer cell junctions observed with permeability-type edema.

Therefore, we hypothesized that KGF might influence hydrostatic pulmonary edema formation. In the present investigations, we utilized an isolated, perfused rat lung model of hydrostatic vascular stress to determine the effect of pretreatment with KGF on hydrostatic edema formation and to examine KGF’s effect on alveolar-capillary permeability.
METHODS

Animals

Male Sprague-Dawley specific pathogen-free rats (250–274 g; Charles River Breeding Laboratories, Wilmington, MA) were housed in the Louisiana State University Health Science Center Animal Care Facility, with food and water provided ad libitum. Approval by the Louisiana State University Health Science Center Institutional Animal Care and Use Committee was obtained before this work.

Isolated Perfused Lung Preparation

The isolated perfused lung model has been previously described (8, 10). Briefly, animals were anesthetized with an intramuscular injection of ketamine and xylazine (50 and 5 mg/kg, respectively; Fort Dodge Laboratories, Fort Dodge, IA), and after anesthesia was achieved, the trachea was cannulated and the lungs were connected to a small animal ventilator (Harvard Apparatus, South Natick, MA). The lungs were ventilated with a constant tidal volume of 7 ml/kg of 95% O2-5% CO2 at 60 breaths/min, with 2 cmH2O positive end-expiratory pressure. A catheter was inserted into the left carotid artery, and after the administration of 1.0 ml of sodium heparin solution (5,000 U/ml) the animal was exsanguinated. The chest was opened, and the main pulmonary artery was exposed. The pulmonary artery was cannulated through a right ventriculotomy, and the lungs were perfused with Krebs-Henseleit buffer. The left atrium was then cannulated via a left ventriculotomy, and a device allowing application of variable resistance was placed on the left atrial cannula; the device could be adjusted to achieve a preselected left atrial pressure (PLa). The lungs and heart were removed en bloc from the chest and placed in a heated water jacket. The lungs were perfused with a mixture of one part Krebs-Henseleit buffer to three parts heparinized whole blood at a rate of 4.5 ml/min with a Masterflex pump (Cole-Parmer Instrument, Barrington, IL). The perfusate circulated through a 37°C heat exchanger. The perfusate reservoir was constantly stirred with a magnetically driven stir bar to provide adequate mixing. Airway pressure (Paw), pulmonary arterial pressure (Ppa), pulmonary venous pressure (Pve), and the pressure in the blood-buffer reservoir were recorded continuously with an eight-channel recorder (Grass Instruments, Quincy, MA). Dynamic compliance was calculated at 30-min intervals for the length of the ventilatory period. After an initial 15-min period of stabilization, Pia was raised to 3 mmHg. Fluid draining via the left atrial catheter was returned to the perfusate reservoir. Another reservoir was positioned below the isolated lungs and used to collect fluid that effluxed from the surface of the lungs (lymph flow). To quantify lung fluid, changes in the perfusate reservoir pressure, which are recorded continuously during the experiment, were correlated with volume change in the perfusate reservoir, as well as lymph flow. Lung edema was calculated as the difference between lung fluid (the volume lost from the perfusate reservoir) and lung efflux (the volume effluxing from the surface of the lungs) and confirmed by wet-to-dry (WD) lung weight ratios in animals from each group. Microvascular pressures were directly measured at the end of each experimental interval by the double-occlusion technique under conditions of zero flow.

WD Weight Ratios

W/D weight ratios were determined by removing the lungs at the conclusion of the experiment and recording the wet weight. The lungs were then placed in a 37°C incubator for 7 days, at which time the dry weight was recorded. For each pair of lungs, the W/D weight ratio was then calculated.

Microvascular Permeability Measurements

To quantitate capillary-alveolar permeability, leakage of large-molecular-weight FITC-labeled dextran (FITC-dextran, avg 71,200; Sigma Chemical, St. Louis, MO) from the perfusate into the alveolar space was measured spectrophotometrically. Under normal conditions, this tracer is confined to the vascular compartment. One milliliter of a 0.5 mg/ml solution of FITC-dextran was added to the perfusate immediately after ex vivo suspension of the lung. At the end of the experimental period, the lungs were lavaged with 30 ml of PBS with 3 mM EDTA in 5-ml aliquots. The fluid was centrifuged at 200 g for 10 min. The fluorescence was measured in the supernatant with a fluorescence spectrophotometer (model 650-40, Perkin-Elmer, Norwalk, CT) with an excitation wavelength set at 495 nm and an emission wavelength of 517 nm. A standard curve was prepared with known amounts of FITC-dextran to calculate the concentrations and total amounts recovered in the bronchoalveolar lavage fluid (9).

Experimental Protocol

Pretreatment regimens. Intravenous injection of KGF (1 mg/kg in 1 ml; generously provided by Thomas R. Ulich, Amgen, Thousand Oaks, CA) or endotoxin-free PBS daily for 3 days was accomplished via the dorsal penile vein with a 30-gauge needle during anesthesia by methoxyflurane (Metofane) inhalation. Animals were allowed to recover after anesthesia, with prompt return to normal feeding and grooming behavior.

Quantification of the protective effect of KGF. For all groups, n = 6. The model simulated an acute onset of pulmonary edema with rapid alveolar flooding. After pretreatment with PBS, control lungs were maintained at a PLa of 3 mmHg for 3 h. The KGF group was pretreated with intravenous KGF; the hydrostatic group received PBS as described above, and then, after 1 h at a Pia of 3 mmHg, Pia was raised to 18 mmHg for the 2nd h, and the animals were allowed to recover for 1 h with a Pia of 3 mmHg. In all groups, W/D weight ratios and lung edema (lung flux – efflux) were determined.

Permeability effects. To investigate and quantitate changes in microvascular-alveolar permeability, the concentration and total amount of FITC-dextran egressing from the vascular compartment into the alveolar space were determined as described above. Two groups (n = 6 for both groups), KGF-pretreated and PBS control animals, were examined using the acute increase in Pia from 3 to 18 mmHg paradigm without a recovery period.

Histology. Additional animals underwent a protocol of ventilation and perfusion for collection of histological specimens. At the end of the experimental perfusion period, but before the final 60-min recovery period, lungs were gently inflated with 10% formaldehyde and stored in formaldehyde-filled containers. The lungs were then embedded in paraffin, and sagittal sections were cut. Representative histological samples were stained with hematoxylin and eosin. Tissue sections were examined at various magnifications, and photomicrographs were made.

Statistical Analysis

Group mean data were compared with ANOVA to detect differences between the groups, as well as unpaired t-test
comparisons between two groups. Where appropriate, a Bonferroni-Dunn multiple comparison test was performed. \( P \leq 0.05 \) was accepted as statistically significant. Results were reported as group mean data.

RESULTS

Physiological Parameters

**Pulmonary capillary pressure.** The capillary pressures (P\(_{\text{cap}}\)) as determined by the double-occlusion method were not significantly different in the KGF and hydrostatic groups after 1 h at 18 mmHg P\(_{\text{la}}\) (20.5 ± 0.5 and 20.6 ± 0.6 mmHg; Fig. 1). However, during the recovery period at 3 mmHg P\(_{\text{la}}\), the P\(_{\text{cap}}\) was higher in the KGF-treated lungs than in the hydrostatic group (4.5 ± 0.2 and 3.6 ± 0.2 mmHg, \( P < 0.05 \)).

**P\(_{\text{aw}}\).** The initial P\(_{\text{aw}}\) was not significantly different among the three groups (4.6 ± 1.0 to 5.2 ± 0.7 mmHg). The dynamic airway compliance declined in all groups over the duration of the experiment. At a constant tidal volume, the final P\(_{\text{aw}}\) was greater in the hydrostatic group than in the control and KGF groups (21.4 ± 4.4 vs. 6.4 ± 0.9 and 9.5 ± 1.4 mmHg, \( P < 0.05 \)) but the control and KGF groups were not statistically different (Fig. 2).

Lung Edema

Pretreatment with KGF attenuated the hydrostatic pulmonary edema associated with increased P\(_{\text{la}}\) and P\(_{\text{cap}}\) in the isolated, perfused rat lung model. The W/D weight ratios were significantly greater in the untreated lungs exposed to elevated hydrostatic forces than in the KGF group (11.5 ± 0.6 vs. 7.6 ± 0.5, \( P < 0.05 \)). There was no significant difference between the KGF group and controls (Fig. 3).

Microvascular Permeability

**Fluorescence measurements.** The concentration of FITC-dextran recovered in the bronchoalveolar lavage fluid was greater in the hydrostatic group than in the KGF-pretreated animals when lavaged after 1 h at P\(_{\text{la}}\) of 18 mmHg (0.9 ± 0.3 vs. 0.2 ± 0.1 \( \mu \)g/ml, \( P < 0.05 \); Fig. 4). Inasmuch as the initial perfusate concentration of FITC-dextran was equal in all groups and all lungs were lavaged with the same amount of fluid, this is consistent with less leakage of the high-molecular-weight marker into the alveolar space and preservation of the blood-gas barrier (9).

Histology

Lung sections from experimental groups were stained with hematoxylin and eosin and examined by light microscopy at \( \times \)400 magnification. Histological sections from animals pretreated with KGF in the low-P\(_{\text{cap}}\) groups showed areas of mild type II pneumocyte hyperplasia compared with PBS-pretreated animals (Fig. 5). Animals in the hydrostatic injury group without KGF pretreatment demonstrated marked hemorrhagic pulmonary edema and some perivascular and peribronchial infiltrates (Fig. 6A). Histological sec-

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**Fig. 1.** Pulmonary capillary pressure (P\(_{\text{cap}}\)) measured by the double-occlusion method. P\(_{\text{cap}}\) was greater in the hydrostatic and keratinocyte growth factor (KGF) groups than in the control group. There was no difference between hydrostatic and KGF groups. *\( P < 0.05 \) vs. control group.

**Fig. 2.** Airway pressure (P\(_{\text{aw}}\)) at constant tidal volumes at the end of 3 h of ventilation and perfusion. There was a greater increase in P\(_{\text{aw}}\) in the hydrostatic group than in the control or KGF group. Values in control and KGF groups were not statistically different. *\( P < 0.05 \) vs. control group; †\( P < 0.05 \) vs. hydrostatic group.

**Fig. 3.** Wet-to-dry (W/D) lung weight ratios. There was a greater increase in the W/D weight ratio in the hydrostatic group than in the control or KGF group. Control and KGF groups were not statistically different. *\( P < 0.05 \) vs. control group; †\( P < 0.05 \) vs. hydrostatic group.
tions from KGF-pretreated animals subjected to hydrostatic injury showed small amounts of interstitial congestion without significant alveolar edema (Fig. 6B).

DISCUSSION

KGF has previously been shown to attenuate injury in a number of animal models of permeability-type lung injury. Panos and associates (12) found a reduction in hyperoxia-induced mortality after pretreatment with intratracheal instillation of KGF. The improved survival was associated with AEC2 hyperplasia and less alveolar wall widening, intra-alveolar exudate, and hemorrhage. Similarly, KGF pretreatment in in vivo models of acid aspiration and radiation and bleomycin reduced signs of lung injury (22, 23). Our laboratory previously showed a reduction in lung leak and pulmonary edema after α-naphthylthiourea lung in-

Fig. 4. Concentration of FITC-dextran in recovered bronchoalveolar lavage fluid. The concentration of FITC-dextran was greater in the hydrostatic group than the KGF group. *P < 0.05 vs. hydrostatic group.

Fig. 5. Hematoxylin- and-eosin-stained histological section from PBS control (A) and intravenously KGF-pretreated (B) animals. Compared with the normal control alveolar architecture, KGF lungs show mild, diffuse type II alveolar epithelial cell hyperplasia. Magnification ×400.
jury using the isolated, perfused rat lung model (8, 10). However, all these studies demonstrating the protective effect of KGF have been in models of permeability-type lung injury.

The data presented in the present study extend our knowledge of the protective effects of KGF to the area of pulmonary edema induced by hydrostatic forces. A significant increase in the lung W/D weight ratios after exposure to elevated $P_{la}$ validated our ex vivo rat lung model as reflective of hydrostatic pulmonary edema. The diminution of the W/D weight ratios in the KGF group after exposure to a similar elevation in $P_{cap}$ demonstrated the protection afforded by KGF. Likewise, the low final $P_{aw}$ in the KGF-pretreated group is consistent with protection against the reduced pulmonary compliance seen with edema. Although unlikely, intrinsic positive end-expiratory pressure in the hydrostatic group cannot be ruled out. This protective effect was confirmed histologically by the marked reduction in the alveolar edema observed on the photomicrographs of hematoxylin-and-eosin-stained sections.

The pulmonary alveolar-capillary junction is normally a thin barrier that facilitates gas exchange while preventing the egress of fluid from the vasculature and, ultimately, the accumulation of fluid within the alveolar spaces. Increased transvascular pressure results in the formation of hydrostatic pulmonary edema, usually due to increases in $P_{la}$ (the backpressure for the pulmonary circulation). With mild increases in transvascular pressure, interstitial edema occurs initially, followed by alveolar flooding and frank pulmonary edema with greater increases in transvascular pressure. Although the intra-alveolar edema accumulation that occurs with hydrostatic pulmonary edema

Fig. 6. Hematoxylin- and-eosin-stained histological sections ($\times200$ magnification) from lungs exposed to a left atrial pressure of 18 mmHg. A: PBS control lungs showed marked pulmonary edema with intra-alveolar edema (arrowhead) and frank alveolar hemorrhage (arrow). B: KGF pretreatment significantly reduced pulmonary edema.
Initially results from flow of fluid due to the transmural pressure gradient, at higher pressures other mechanisms may be operational.

Extremes of intravascular pressures can disrupt the endothelial and epithelial integrity, allowing the transgression of macromolecules into the alveolar units, thereby exacerbating pulmonary edema. Permanent injury to the blood-gas barrier may not necessarily occur. Elevated vascular pressures may reversibly open “pores” in the endothelial or epithelial layers, allowing egress of large molecules, with rapid restoration of the vascular integrity after the transmural pressures are reduced (4, 15).

Our results showing recovery of high-molecular-weight FITC-dextran from the alveolar space after elevation of microvascular pressure are consistent with previous observations. The protection provided by KGF was accompanied by a reduction in alveolar FITC-dextran. This would suggest a mechanism of action that includes preservation of the alveolar-capillary integrity and impedance to macromolecular flux. Unfortunately, the use of this technique to evaluate the blood-gas barrier does not allow partitioning of the observed effect into epithelial and endothelial components (9).

Human airway epithelial cell monolayers cultured on Transwell membranes in the presence of KGF show significantly less permeability to albumin after exposure to hydrogen peroxide injury than controls. KGF also prevents disruption of actin filaments, suggesting stabilization of the cytoskeleton (19). However, KGF is thought to be specific for AEC2, which cover only 3% of the alveolar surface (11). Therefore, it seems unlikely that AEC2 hyperplasia alone could account for changes in the blood-air membrane permeability unless secondary effects (e.g., changes in extracellular-matrix composition) or alteration in type I pneumocytes were to occur.

Although enhancement of alveolar liquid clearance has been noted after KGF administration, this is less likely to be a major factor in this model. After intracheal KGF pretreatment, the rate of alveolar fluid export as measured by the changing concentration of an instilled alveolar albumin solution is ~0.1 ml/h (18). With the markedly diminished AEC2 hyperplasia observed with intravenous KGF (and probably near-normal numbers of Na\(^+\)-K\(^+\)-ATPase pumps), it seems unlikely that ~1 ml of fluid clearance could account for the significant differences in edema over the 1-h experimental period. However, a minor contribution to the protective effect cannot be ruled out, inasmuch as prevention of edema formation in the alveolar space is likely to be multifactorial.

Recently, a direct KGF effect on microvascular endothelial cells has been reported. Initial investigations found no changes in large vessel endothelial cells, although an induction in vascular endothelial growth factor has been suggested (5, 21). However, Gillis and co-investigators (6) found a mitogenic response and stabilization of the endothelial barrier in adrenal capillary endothelial cells not observed in aortic cells. Whether this effect will be seen in pulmonary capillary endothelial cells and the clinical significance of such a finding are uncertain.

The $P_{\text{cap}}$ during the experimental period of $P_{\text{en}}$ of 18 mmHg was not different between groups, suggesting that our hydrostatic driving pressures were equivalent. However, the $P_{\text{cap}}$ at the conclusion of the experiment, during the recovery period, showed a slightly, although statistically significantly, higher value in the KGF group. Inasmuch as there was less edema in the KGF group, we cannot attribute this to changes in pulmonary compliance due to edema. This might suggest a change in vascular compliance by KGF that is only evident after the induction of hydrostatic edema. However, we believe this finding is of questionable importance. During our preliminary experiments, we found that final $P_{\text{cap}}$ values were not statistically different after perfusion at different levels of hydrostatic pressure (data not shown). Furthermore, we retrospectively examined the change in reservoir height during changes in $P_{\text{en}}$ as a reflection of vascular compliance, but did not find a statistically significant difference between groups. Although it is possible that KGF may induce alterations in the pulmonary endothelium (or epithelium that restricts interstitial edema movement to the alveolar space) that change vascular compliance, we believe this is not evident in our experiments. When a Bayesian approach is taken, the statistically significant difference in final $P_{\text{cap}}$ is of uncertain significance. A determination of KGF’s specific effects on the pulmonary vasculature requires further study.

In conclusion, pretreatment with intravenous KGF attenuates the formation of hydrostatic pulmonary edema in an isolated, perfused rat lung model. This protection afforded by KGF appears to be mediated, in part, by preservation of the alveolar-capillary barrier. The potential for therapy of heart failure with congestive pulmonary edema remains speculative and necessitates further study but may be a unique addition to the presently available pharmacological armamentarium.

REFERENCES