Intravenous keratinocyte growth factor protects against experimental pulmonary injury

JANE GUO,[1] EUNHEE S. YI,[2] ANDREW M. HAVILL,[1] ILDIKO SAROSI,[1] LANE WHITCOMB,[1] SONGMEI YIN,[1] SCOT C. MIDDLETON,[1] PIERRE PIGUET,[1] AND THOMAS R. ULICH[1]
[1]*Amgen Preclinical Research, Thousand Oaks 91320-1789; and* [2]*San Diego School of Medicine, University of California, San Diego, California 92103*

Guo, Jane, Eunhee S. Yi, Andrew M. Havill, Ildiko Sarosi, Lane Whitcomb, Songmei Yin, Scot C. Middleton, Pierre Piguet, and Thomas R. Ulich. Intravenous keratinocyte growth factor protects against experimental pulmonary injury. *Am. J. Physiol.* 275 (*Lung Cell. Mol. Physiol.* 19): L800–L805, 1998.—Keratinocyte growth factor (KGF) administered by intratracheal instillation is well documented to stimulate the proliferation of alveolar and bronchial cells. In the present study, intravenous KGF was also shown to stimulate the proliferation of alveolar and bronchial cells in mice and rats, although to a lesser degree than intratracheal KGF. Despite the decreased potency of intravenous KGF on pulmonary cell 5-bromo-2′-deoxyuridine incorporation compared with intratracheal KGF, intravenous KGF was very effective in preventing experimental bleomycin-induced pulmonary dysfunction, weight loss, and mortality in either mice or rats and experimental hyperoxia-induced mortality in mice. The effectiveness of intravenous administration of KGF in preventing lung injury suggests that the mechanisms of the protective effect of KGF may involve more than pulmonary cell proliferation and also suggests the potential use of systemic KGF for clinical trials in settings of pulmonary injury.

bleomycin; hyperoxia

KERATINOCYTE GROWTH FACTOR (KGF or fibroblast growth factor-7) is a member of the fibroblast growth factor family. KGF is a fibroblast-derived mitogen that selectively stimulates the proliferation of epithelial cells in vitro (10, 12) and in vivo (7, 16, 17, 21, 22, 24). The potential therapeutic use of KGF has been evaluated in disease models associated with damage to epithelial cells of the skin, digestive tract, and bladder. KGF has shown beneficial effects in models of dermal injury (11, 13), in chemotherapy or irradiation-induced oral and gastrointestinal mucositis (3), and in cyclophosphamide-induced ulcerative hemorrhagic cystitis (15).

KGF instilled intratracheally causes alveolar type II pneumocyte and bronchial cell proliferation (15). In pulmonary disease models, intratracheal instillation of KGF has prevented lung injury caused by radiation and bleomycin (23), hyperoxia (9), acid instillation (20), and α-naphthylthiourea (8).

The purpose of the present study was to compare the effect of KGF on pulmonary epithelium after intravenous and intratracheal administration and to document the protective effect of intravenous KGF in bleomycin- and hyperoxia-induced rodent models of lung injury. Bleomycin, a chemotherapeutic agent used clinically in the treatment of a variety of human malignancies, can cause pulmonary injury and fibrosis both in animal models and in patients (6). Prolonged exposure to elevated levels of O_2 in animal models and patients also damages the alveolar epithelium, resulting in pulmonary fibrosis and mortality (4).

MATERIALS AND METHODS

Animal treatment. The humane care and use of all experimental animals in this study was overseen by the institutional animal use and care committee.

KGF and bleomycin instillation. Male Lewis rats weighing 200–250 g were purchased from Charles River Laboratories (Cambridge, MA). Female CBA/J and BALB/c mice (~25 g) were obtained from Jackson Laboratories (Bar Harbor, ME). Recombinant human KGF was produced at Amgen in *Escherichia coli*. Intratracheal instillation (0.1 ml) of KGF or bleomycin in mice was performed with a 28-gauge needle after blunt dissection of the soft tissues of the neck to expose the trachea. In rats, 0.5 ml of KGF or bleomycin was instilled via intratracheal cannulation with a sterile 18-gauge catheter using a fiber-optic light source. The doses of bleomycin sulfate (Sigma, St. Louis, MO) in Lewis rats (10 U/kg) (23) and CBA/J mice (2 U/kg) were chosen based on previous studies of bleomycin-induced fibrosis.

Immunohistochemical detection of proliferating cells. Animals were injected with 50 mg/kg of 5-bromo-2′-deoxyuridine (BrdU) in 0.2 ml of saline intraperitoneally 2 h before euthanasia. The lungs were excised and fixed in 10% neutral-buffered Formalin at a pressure of 20 cmH_2O. A midsagittal section was taken to detect cells undergoing DNA synthesis. Specific BrdU labeling was detected with monoclonal rat (MAS 250ps, Accurate) or mouse (Bu20a, Dako) anti-BrdU antibody. Rat type II pneumocytes were identified with polyclonal rabbit anti-bovine surfactant protein B (SP-B) antibody (a generous gift from Dr. Jeffrey Whitsett, Cincinnati, OH). Indirect immunohistochemistry was performed with the Vectastain ABC-AP System (Vector Laboratories, Burlingame, CA) for BrdU labeling. Horseradish peroxidase and 3,3′-diaminobenzidene as the chromogen (Vector Laboratories) were used for SP-B staining. Ten to twenty random ×400 microscopic fields of alveolar parenchyma and bronchioles 0.2–0.5 mm in transverse diameter were used for quantification of BrdU-positive cells in a double-blind fashion by a pathologist. Only nucleated cells of the alveolar walls with the exclusion of identifiable alveolar macrophages were considered for the enumeration of SP-B-positive cells.

Pulmonary function tests. A noninvasive, bias-flow ventilated whole body plethysmographic technique was used to quantitate the tidal volume and frequency of breathing in rats placed in an unrestrained chamber (Buxco Electronics, Troy, NY). The changes in chamber pressure represent the difference between thoracic expansion-contraction and tidal volume. The chamber pressure was differentiated to give a pseudoflow signal with a transducer connected to a preamplifier. Flow signals were analyzed with Buxco Biosystem XA software.

Hyperoxia-induced lung injury. Mice were exposed to >95% O_2 at 3.3 l/min in an airtight chamber (Schroer, Kansas City, MO). Animals had free access to water and food and were monitored at least four times daily for respiratory distress.

Statistical analysis. Data are presented as means ± SD. When two groups were compared, the probability value was determined by a two-tailed *t*-test (Systat, Evanston, IL). Comparisons of multiple groups were made with a Newman-Keuls post hoc test after ANOVA. The significance of survival curves was determined by Proc GENMOD with a logit link function and Kaplan-Meier method. A P value ≤0.05 was considered significant.

RESULTS

Kinetics of alveolar and bronchial cell BrdU incorporation after intratracheal vs. intravenous KGF administration in mice and rats. BrdU labeling was compared in mice (Fig. 1) and rats (Fig. 2) receiving intratracheal or intravenous KGF (5 mg/kg). The kinetics and magnitude of the response were dependent on both the route of administration and the species.

In mice, the stimulation of DNA synthesis, as shown by BrdU incorporation in alveolar cells, reached a maximum 2 days after either intratracheal or intravenous delivery of KGF. Bronchial cells displayed much more BrdU incorporation after intratracheal administration of KGF than after intravenous injection. Control mice injected with saline showed negligible staining for BrdU.

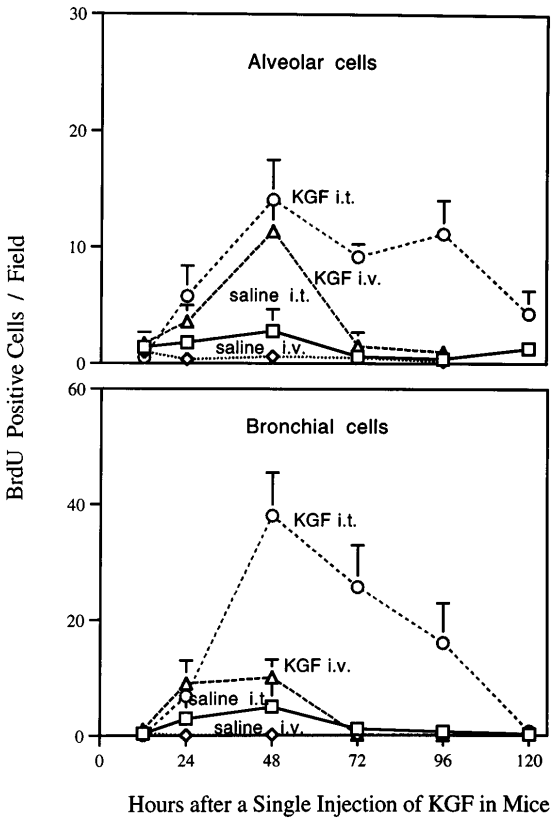

Fig. 1. Kinetics of keratinocyte growth factor (KGF)-induced 5-bromo-2′-deoxyuridine (BrdU) uptake in alveolar and bronchial cells in CBA/J mice (n = 5/group) treated with 5 mg/kg of KGF intratracheally (it; ○) or intravenously (iv; △). Control mice (n = 2/group) received saline it (□) or iv (◇).

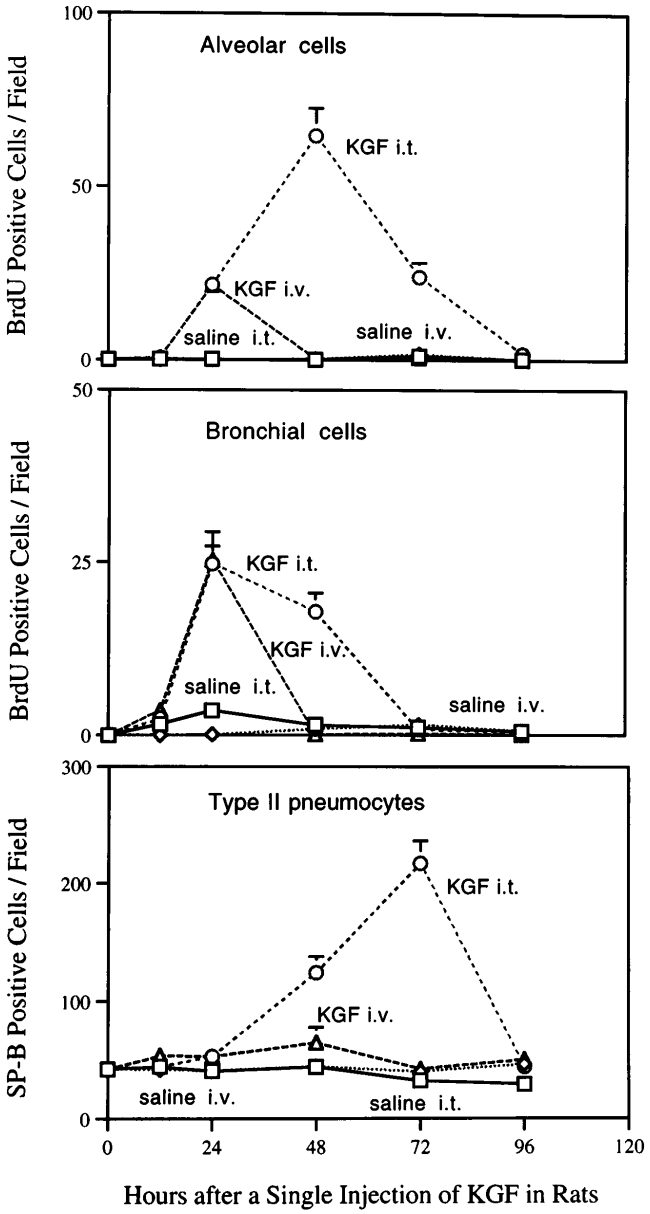

Fig. 2. Kinetics of KGF-induced BrdU uptake and surfactant protein B (SP-B) expression in alveolar and bronchial cells in Lewis rats receiving a single 5 mg/kg treatment of KGF [it (○) or iv (△); n = 5 rats/group] or saline [it (□) or iv (◇); n = 5 rats/group].

In rats, intratracheal KGF caused peak BrdU incorporation in alveolar cells at 2 days, whereas intravenous KGF caused a peak effect at 1 day and no effect at 2 days. The number of BrdU-positive alveolar cells was much lower in the intravenous group than in the intratracheal group. A similar level of BrdU incorporation was seen in bronchial cells after intravenous or intratracheal injection. Intravenous injection of KGF had a marginal effect on the number of type II pneumocytes as quantitated by SP-B-positive cells, whereas a large increase in type II pneumocytes was seen peaking 72 h after intratracheal KGF.

Intravenous KGF prevents bleomycin-induced pulmonary injury. Mice received intravenous KGF or human serum albumin (HSA; 5 mg/kg) on *days −2* and *−3*

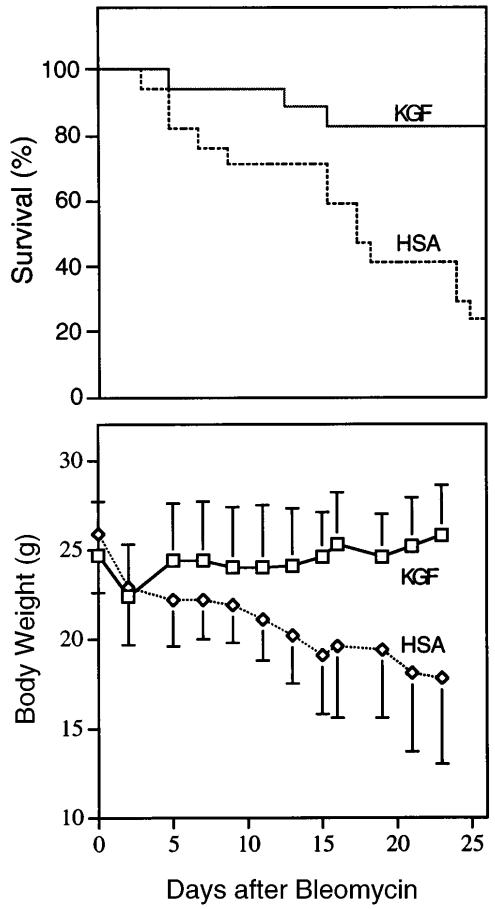

Fig. 3. Intravenous bolus injections of KGF prevent bleomycin-induced mortality and weight loss in mice. CBA/J mice were treated with KGF (5 mg/kg; $n = 18$) or human serum albumin (HSA; 5 mg/kg, $n = 17$) 2 and 3 days before instillation of 0.05 U bleomycin.

before intratracheal bleomycin. Four of seventeen control mice (24%) survived for 26 days after bleomycin administration, whereas 15 of 18 KGF-pretreated mice (83%) survived ($P < 0.001$; Fig. 3). Surviving control mice lost weight progressively, whereas KGF-pretreated mice maintained their weight (Fig. 3).

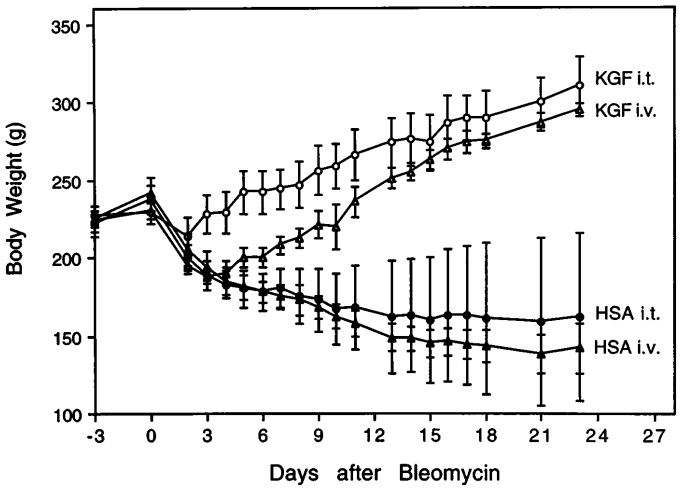

Fig. 4. KGF prevents bleomycin-induced weight loss in rats. Rats ($n = 6$/group) were treated with KGF (5 mg/kg) or HSA (5 mg/kb) 2 and 3 days before instillation of 2.5 U bleomycin it.

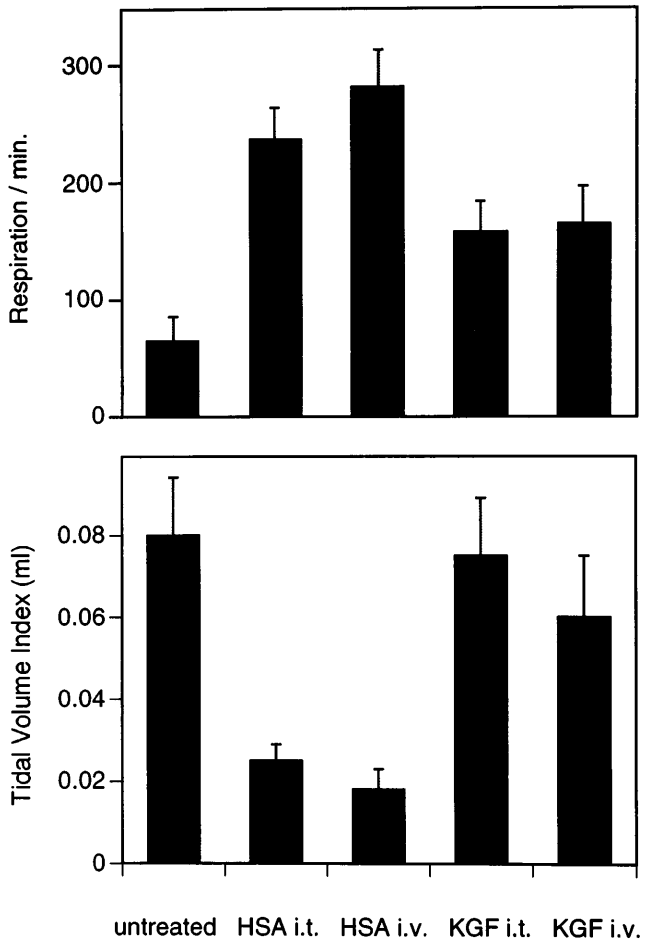

Fig. 5. KGF ameliorates bleomycin-induced loss of pulmonary function as measured by respiration rate and tidal volume. Rats ($n = 6$/group) were treated with KGF (5 mg/kg it or iv) or HSA (5 mg/kg) 2 and 3 days before instillation of 2.5 U bleomycin. Pulmonary function was evaluated 15 days after instillation of bleomycin.

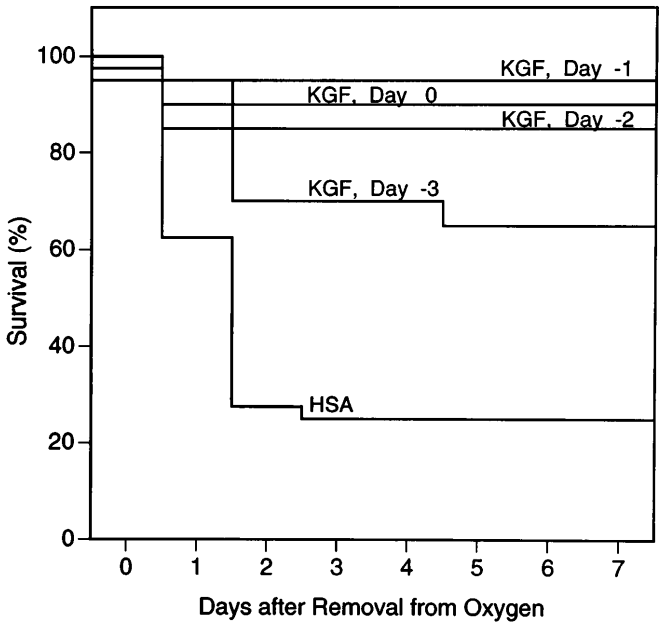

Fig. 6. KGF decreases hyperoxia-induced mortality after 3.5 days of O_2 exposure. CBA/J mice were injected with a single bolus dose of 5 mg/kg of KGF iv ($n = 20$) or HSA ($n = 40$) either on *day −3*, *−2*, or *−1* or immediately before O_2 exposure.

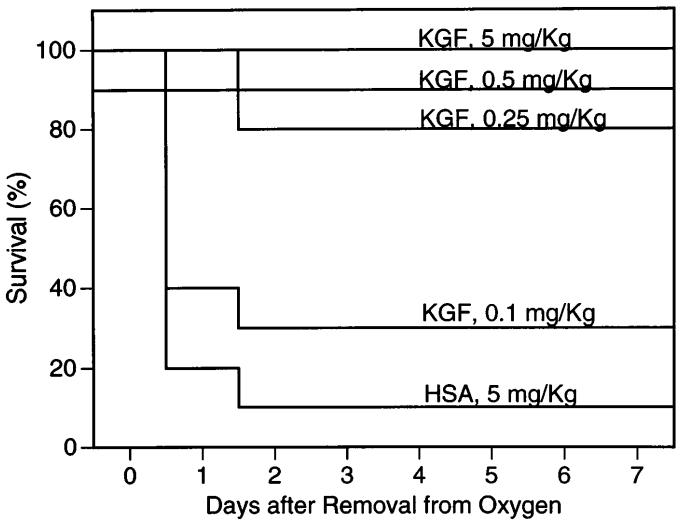

Fig. 7. Dose-response study of KGF shows that a KGF dose of as little as 250 μg/kg iv at *time 0* is effective in preventing hyperoxia-induced mortality. CBA/J mice (n = 10/group) were injected with KGF doses of 0.1–5 mg/kg iv immediately before O_2 exposure.

In the rat bleomycin model at a dose at which mortality was not observed, KGF given intratracheally or intravenously at 2 and 3 days before bleomycin protected against weight loss, although intratracheal KGF was more effective than intravenous KGF for approximately the first 2 wk after bleomycin administration (Fig. 4). The bleomycin-induced deterioration in respiration rate and tidal volume was ameliorated in KGF-pretreated rats (Fig. 5) as shown by the normal tidal volume after intratracheal KGF ($P < 0.001$ compared with intratracheal HSA) and 75% of the normal tidal volume after intravenous KGF ($P < 0.001$ compared with intravenous HSA). The respiratory rate was equally improved, although not completely normalized, after intratracheal or intravenous KGF administration ($P < 0.001$ compared with intratracheal or intravenous HSA).

Intravenous KGF prevents hyperoxia-induced pulmonary injury. CBA/J mice kept in >95% O_2 for 3.5 days and then reexposed to ambient air experienced a mortality of ~75% within the first 2 days after their removal from the hyperoxic environment (Fig. 6). A single intravenous injection of KGF (5 mg/kg) given either on *day −2* or *−1* or immediately before O_2 exposure resulted in nearly complete protection against mortality (~5–15% mortality; $P < 0.001$ vs. control; Fig. 6). Treatment with KGF on *day −3* before O_2 exposure resulted in a moderate protective effect (~30% mortality; $P = 0.003$ vs. control; Fig. 6).

In dose-response experiments, CBA/J mice were treated with 0.1–5 mg/kg iv of KGF immediately before O_2 exposure (Fig. 7). The mice that did not receive KGF experienced a mortality of 90%. KGF at a dose of 5 mg/kg showed complete protection (0% mortality; $P < 0.001$ vs. control). KGF at doses of 0.5 and 0.25 mg/kg was also highly protective ($P \leq 0.001$ vs. control), but the protective effect was lost at a dose of 0.1 mg/kg ($P > 0.05$ vs. control).

To study any variability among mouse strains, we also studied BALB/c mice. BALB/c mice were found to be somewhat more sensitive to O_2, already experiencing a 90% mortality at 2.75 days after exposure to hyperoxia. Nevertheless, KGF at 5 mg/kg iv on *days −1* and *0* before O_2 exposure provided dramatic protection against mortality (Fig. 8). By exposing the mice to varying durations of O_2 exposure, we showed that KGF exhibited a protective effect in mild-to-severe O_2 injury (Fig. 8).

Histological examination of the lungs from both control and KGF mice at the time of removal from the O_2 chamber showed acute bronchiolitis, alveolar edema, hemorrhage, and alveolar neutrophil infiltration. Because most control mice died shortly after their removal from O_2, long-term histological study of significant numbers of these mice was not possible. In KGF-treated mice, histology at 1 wk after removal from the hyperoxic environment showed a focal organizing pneumonia (Fig. 9). By 2 wk, the pneumonia had largely resolved, although focal scanty interstitial deposits of collagen were noted in some mice. By 4 wk, the pulmonary histology of the KGF-treated mice had largely normalized, although focal mild lesions persisted (Fig. 9).

DISCUSSION

Intravenous KGF is nearly as effective as intratracheal KGF in ameliorating bleomycin- and hyperoxia-

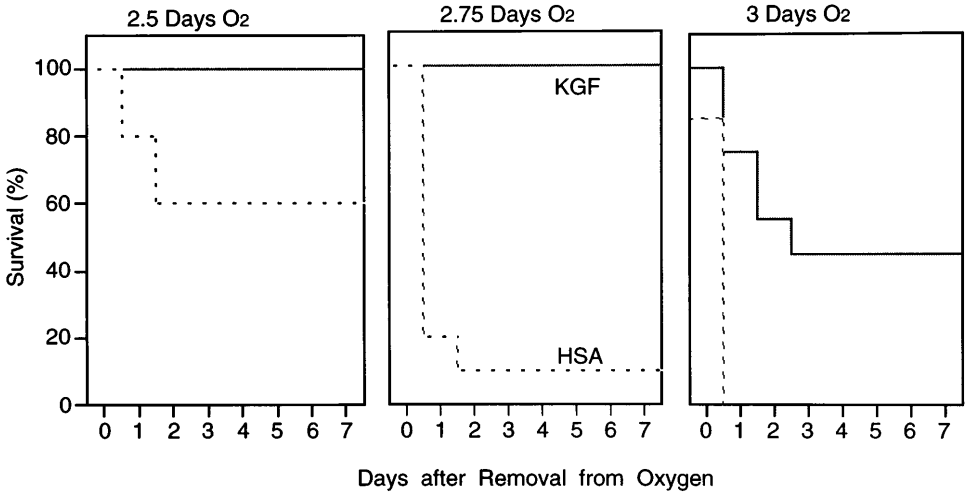

Fig. 8. Magnitude of protective effect of KGF against hyperoxia depends on length of O_2 exposure. BALB/c mice (n = 10/group) were injected with 5 mg/kg of KGF or HSA iv 1 and 0 days before O_2 exposure of 2.5–3 days.

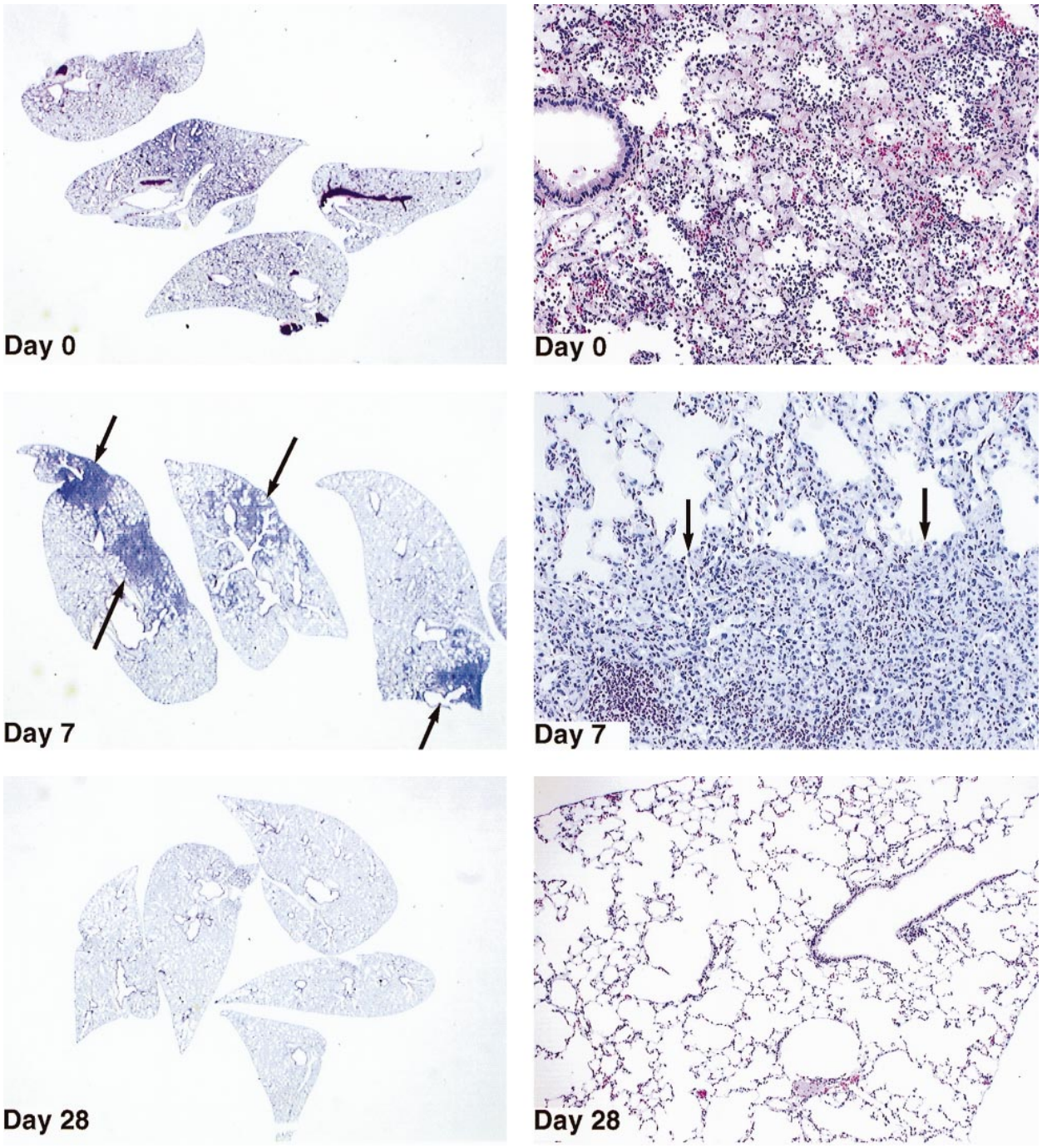

Fig. 9. Lungs of KGF-treated mice show acute neutrophilic and hemorrhagic pneumonitis on day of removal from O_2 chamber (*day 0*), patchy organizing pneumonia (arrows) on *day 7*, and resolution to a nearly normal pulmonary architecture on *day 28*. *Left*, whole mounts; *right*, low-power histology.

induced lung injury in rodents. Although alveolar and bronchial cells in mice and rats incorporate BrdU after intratracheal or intravenous KGF administration, the intravenous route is not nearly as potent in causing BrdU incorporation in pulmonary cells or in causing alveolar cell hyperplasia. Although the mechanism of the protective effect of KGF is not fully understood, mechanisms may be involved that are independent of the proliferative action of KGF on epithelial cells. KGF, for example, has been reported to upregulate functions such as surfactant protein expression (17, 20) and sodium-potassium-adenosinetriphosphatase activity (5) and to decrease permeability between injured epithelial cells (18). Unlike tumor necrosis factor, which was reported to increase expression of the antioxidant enzyme superoxide dismutase (14), Panos et al. (9) did not find an increase in pulmonary superoxide dismutase activity in KGF-treated rats exposed to O_2. Some investigators have proposed effects of KGF on the regulation of apoptosis. In vitro, KGF did not prevent H_2O_2-induced alveolar cell death or alter Bcl-2 expression (19).

The kinetics of BrdU incorporation in mice and rats are not exactly the same, and the alveolar cell hyperplasia noted by routine histology in mice is not as striking as that in rats. It is unclear whether the magnitude of the pulmonary effects of KGF on cellular proliferation in rodents can be readily extrapolated to humans.

Different strains of mice appear to have different sensitivities to O_2. In our experiments, BALB/c mice were more sensitive to O_2 than CBA/J mice, but KGF exerted a protective effect in both strains. In preliminary experiments in our laboratory, the sensitivity of rats to O_2 seems to be greater than that of mice, and we have not yet shown that KGF is protective against O_2 toxicity in rats after intravenous administration. Previous work has shown that the ability of KGF to protect against O_2-induced lung injury is related to the timing of the KGF treatment. In rats, intratracheal KGF was effective if given at 48 and 72 h before O_2 exposure. Treatment at 24 or 0 h before O_2 exposure was ineffective (2). Similar results have been obtained in a rat model of bleomycin-induced fibrosis in which pretreatment with intratracheal KGF was effective in preventing injury but posttreatment with KGF was ineffective (1).

KGF is currently in clinical trials for the prevention of chemotherapy-induced mucositis. The effectiveness of intravenous KGF in preventing lung injury is of importance because intravenous KGF would be easier to administer than intratracheal KGF if KGF were to prove effective in the clinical prevention of lung injury.

We are grateful to Louis Munyakazi and Robyn Murphy-Filkins for performing statistical analysis. We also thank Carol Burgh and Gwyneth Van for their contribution of histological studies.

Address for reprint requests: T. R. Ulich, 1840 DeHavilland Dr., Thousand Oaks, CA 91320.

Received 5 November 1997; accepted in final form 8 June 1998.

REFERENCES

1. **Deterding, R. R., A. M. Havill, T. Yano, S. Middleton, C. R. Jacoby, J. M. Shannon, W. S. Simonet, and R. J. Mason.** Prevention of bleomycin-induced lung injury in rats by keratinocyte growth factor. *Proc. Assoc. Am. Physicians* 109: 254–268, 1997.
2. **Deterding, R. R., C. R. Jacoby, W. S. Simonet, R. J. Mason, and C. W. White.** Keratinocyte growth factor improves survival in rats exposed to hyperoxia (Abstract). *Am. J. Respir. Crit. Care Med*. 151: A646, 1995.
3. **Farrell, C. L., J. V. Bready, K. L. Rex, J. N. Chen, C. R. DiPalma, L. Whitcomb, S. Yin, D. C. Hill, B. Wiemann, C. O. Starnes, A. M. Havill, Z. N. Lu, S. L. Aukerman, G. F. Pierce, A. Thomason, T. R. Ulich, and D. L. Lacey.** KGF protects mice from chemotherapy and radiation-induced gastrointestinal injury and mortality. *Cancer Res*. 58: 933–939, 1998.
4. **Frank, L.** Developmental aspects of experimental pulmonary oxygen toxicity. *Free Radic. Biol. Med*. 11: 463–494, 1991.
5. **Guery, B. P. H., C. M. Mason, E. Dobard, G. Beaucaire, W. R. Summer, and S. Nelson.** Keratinocyte growth factor increases transalveolar sodium reabsorption in normal and injured rat lungs. *Am. J. Respir. Crit. Care Med*. 155: 1777–1784, 1997.
6. **Hay, J., S. Shahzeidi, and G. Laurent.** Mechanisms of bleomycin-induced lung damage. *Arch. Toxicol*. 65: 81–94, 1991.
7. **Housley, R. M., C. F. Morris, W. Boyle, B. Ring, R. Blitz, J. E. Tarpley, S. L. Aukerman, P. L. Devine, R. H. Whitehead, and G. F. Pierce.** Keratinocyte growth factor induces proliferation of hepatocytes and epithelial cells throughout the rat gastrointestinal tract. *J. Clin. Invest*. 94: 1764–1777, 1994.
8. **Mason, C. M., B. P. H. Guery, W. R. Summer, and S. Nelson.** Keratinocyte growth factor attenuates lung leak induced by alpha-naphthylthiourea in rats. *Crit. Care Med*. 24: 925–931, 1996.
9. **Panos, R. J., P. M. Bak, W. S. Simonet, J. S. Rubin, and L. J. Smith.** Intratracheal instillation of keratinocyte growth factor decreases hyperoxia-induced mortality in rats. *J. Clin. Invest*. 96: 2026–2033, 1995.
10. **Panos, R. J., J. S. Rubin, S. A. Aaronson, and R. J. Mason.** Keratinocyte growth factor and hepatocyte growth factor/scatter factor are heparin binding growth factors for alveolar type II cells in conditioned medium. *J. Clin. Invest*. 92: 969–977, 1993.
11. **Pierce, G. F., D. Yanagihara, K. Klopchin, D. M. Danilenko, E. Hsu, W. C. Kenney, and C. F. Morris.** Stimulation of all epithelial elements during skin regeneration by keratinocyte growth factor. *J. Exp. Med*. 179: 831–840, 1994.
12. **Rubin, J. S., H. Osada, P. W. Finch, W. G. Taylor, S. Rudikoff, and S. A. Aaronson.** Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proc. Natl. Acad. Sci. USA* 86: 802–806, 1989.
13. **Staiano-Coico, L., J. G. Krueger, J. S. Rubin, S. D'limi, V. P. Vallat, L. Valentino, T. Fahey III, A. Hawes, G. Kingston, M. R. Madden, M. Mathwich, A. B. Gottlieb, and S. A. Aaronson.** Human keratinocyte growth factor effects in a porcine model of epidermal wound healing. *J. Exp. Med*. 178: 865–878, 1993.
14. **Tsan, M. F., J. E. White, C. Treanor, and J. B. Shaffer.** Molecular basis for tumor necrosis factor-induced increase in pulmonary superoxide dismutase activities. *Am. J. Physiol*. 259 (*Lung Mol. Cell. Physiol*. 3): L506–L512, 1990.
15. **Ulich, T. R., L. Whitcomb, W. Tang, P. O'Connor Tressel, J. Tarpley, E. S. Yi, and D. L. Lacey.** Keratinocyte growth factor ameliorates cyclophosphamide-induced ulcerative hemorrhagic cystitis. *Cancer Res*. 57: 472–475, 1997.
16. **Ulich, T. R., E. S. Yi, R. Cardiff, S. Yin, N. Bikhazi, R. Bilta, C. F. Morris, and G. F. Pierce.** Keratinocyte growth factor is a growth factor for mammary epithelium in vivo. *Am. J. Pathol*. 144: 862–867, 1994.
17. **Ulich, T. R., E. S. Yi, K. Longmuir, S. Yin, R. Blitz, C. F. Morris, R. M. Housley, and G. F. Pierce.** Keratinocyte growth factor is a growth factor for type II pneumocytes in vivo. *J. Clin. Invest*. 93: 1298–1306, 1994.
18. **Waters, C. M., U. Savla, and R. J. Panos.** Keratinocyte growth factor protects airway epithelial cells from a hydrogen peroxide-induced permeability increase (Abstract). *Am. J. Respir. Crit. Care Med*. 155: A173, 1997.
19. **Wu, K., N. Pollack, R. J. Panos, P. Sporn, and D. Kamp.** Keratinocyte growth factor does not attenuate H_2O_2-induced death or alter bcl-2 in alveolar epithelial cells (Abstract). *Am. J. Respir. Crit. Care Med*. 153: A636, 1996.
20. **Yano, T., R. R. Deterding, W. S. Simonet, J. M. Shannon, and R. J. Mason.** Keratinocyte growth factor reduces lung damage due to acid instillation in rats. *Am. J. Respir. Cell Mol. Biol*. 15: 433–442, 1996.
21. **Yi, E. S., A. A. Bedoya, H. Lee, S. Kim, R. M. Housley, S. L. Aukerman, J. E. Tarpley, C. Starnes, S. Yin, G. F. Pierce, and T. R. Ulich.** Keratinocyte growth factor causes cystic dilation of the mammary glands of mice. *Am. J. Pathol*. 145: 1015–1022, 1994.
22. **Yi, E. S., A. S. Shabaik, D. L. Lacey, A. A. Bedoya, S. Yin, R. M. Housley, D. M. Danilenko, W. Benson, A. M. Cohen, G. F. Pierce, A. Thomason, and T. R. Ulich.** Keratinocyte growth factor causes proliferation of urothelium in vivo. *J. Urol*. 154: 1566–1570, 1995.
23. **Yi, E. S., S. T. Williams, H. Lee, D. M. Malicki, E. M. Chin, S. Yin, J. Tarpley, and T. R. Ulich.** Keratinocyte growth factor ameliorates radiation- and bleomycin-induced lung injury and mortality. *Am. J. Pathol*. 149: 1963–1970, 1996.
24. **Yi, E. S., S. Yin, D. L. Harclerode, A. Bedoya, N. B. Bikhazi, R. M. Housley, S. L. Aukerman, C. F. Morris, G. F. Pierce, and T. R. Ulich.** Keratinocyte growth factor induces pancreatic ductal epithelial proliferation. *Am. J. Pathol*. 145: 80–85, 1994.