Keratinocyte Growth Factor Ameliorates Cyclophosphamide-induced Ulcerative Hemorrhagic Cystitis

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ABSTRACT

To determine whether keratinocyte growth factor (KGF), an epithelial and urothelial growth factor, ameliorates cyclophosphamide (CP)-induced cystitis in rats, KGF (5 mg/kg) was injected in rats as a single i.v. injection 24 h prior to i.p. injection of CP (200 mg/kg). Bladders were evaluated histologically 48 h after CP injection, and KGF pretreatment was found to almost completely prevent CP-induced ulcerative hemorrhagic cystitis. Urinary KGF levels were measured by ELISA, and KGF was found to be undetectable in control urine, but it was found to appear in the urine of KGF-treated rats at 8 h, with a peak concentration of approximately 10 ng/ml. Bilateral nephrectomy did not diminish the proliferative effect of KGF on urothelium, suggesting that the contribution of urinary KGF to urothelial proliferation is insignificant. In conclusion, systemic administration of KGF is protective against CP-induced cystitis. Although KGF appears in the urine, urinary KGF is not necessary for the proliferative action of KGF on urothelium.

INTRODUCTION

KGF is a mesenchymally derived epithelial growth factor. KGF in vivo in rats has been shown to induce the proliferation of pulmonary, mammary, hepatic, pancreatic, epidermal, and gastrointestinal epithelium (1–4). KGF has also been shown to cause proliferation of urothelial cells in a basal cell line (5) and urothelial growth factor, ameliorates cyclophosphamide-induced cystitis in rats, KGF (5 mg/kg) was injected in rats as a single i.v. injection 24 h prior to i.p. injection of CP (200 mg/kg). Bladders were evaluated histologically 48 h after CP injection, and KGF pretreatment was found to almost completely prevent CP-induced ulcerative hemorrhagic cystitis. Urinary KGF levels were measured by ELISA, and KGF was found to be undetectable in control urine, but it was found to appear in the urine of KGF-treated rats at 8 h, with a peak concentration of approximately 10 ng/ml. Bilateral nephrectomy did not diminish the proliferative effect of KGF on urothelium, suggesting that the contribution of urinary KGF to urothelial proliferation is insignificant. In conclusion, systemic administration of KGF is protective against CP-induced cystitis. Although KGF appears in the urine, urinary KGF is not necessary for the proliferative action of KGF on urothelium.

MATERIALS AND METHODS

Male Sprague Dawley rats, weighing 250–350 g, were injected via the dorsal penile vein with 5 mg/kg KGF or saline 24 h prior to a 200 mg/kg i.p. injection of CP. Recombinant human KGF derived from Escherichia coli was prepared at Amgen, Inc. (Thousand Oaks, CA). All rats were sacrificed 48 h after CP administration by CO2 inhalation. In some experiments, bilateral nephrectomies were performed immediately prior to KGF administration to determine the potential contribution of urinary KGF to the proliferative effects of systemically delivered KGF. The bladders were removed and fixed in zinc-buffered formalin for 24 h before cross-sectioning and staining with H&E. Immunohistochemistry for PCNA was performed with a primary antiserum to PCNA (clone PC10; DAKO Corp., Carpinteria, CA) followed by a biotinylated horse antimouse antiserum, avidin-biotin complex, and diaminobenzidine as the chromogen (BioTek Solutions, Santa Barbara, CA; Ref. 5).

To study the kinetics of KGF excretion into the urine, rats were placed in metabolic cages immediately following KGF administration. Urine was collected at various intervals following KGF injection. A double-monoclonal antibody sandwich format immunosassay was used, with two monoclonal antibodies raised against alternate epitopes of KGF. The coating antibody was incubated for 24 h before cross-sectioning and staining with H&E. Immunohistochemistry for PCNA was performed with a primary antiserum to PCNA (clone PC10; DAKO Corp., Carpinteria, CA) followed by a biotinylated horse antimouse antiserum, avidin-biotin complex, and diaminobenzidine as the chromogen (BioTek Solutions, Santa Barbara, CA; Ref. 5).

RESULTS

Forty-eight h after CP administration, 12 of 15 rats had severe ulcerative hemorrhagic cystitis (Figs. 1 and 2). The remaining three rats in the CP-treated group exhibited focal microscopic ulcers with submucosal edema and inflammation. All of the rats in this group received i.v. saline 24 h before CP.

Rats receiving i.v. KGF 24 h prior to CP administration (n = 15 rats; 3 experiments with 5 rats/experiment) were almost completely protected from ulcerative cystitis (Fig. 1). Eighty % (12 of 15) of the rats in the KGF-pretreated group did not have ulcerations of the bladder epithelium. Minor histological changes such as edema and acute inflammation of the urothelium and submucosa occurred in a few specimens. The bladders of three rats in the KGF-pretreated group demonstrated focal microscopic ulcers. Rats (n = 6) not receiving CP or KGF had histologically normal bladders.

The trophic action of KGF on urothelium could be the result of urothelial cells responding to either bloodborne KGF reaching urothelial basal cells via the circulation or the topical action of urinary KGF. The hypothesis that topical urinary KGF might contribute to the proliferative effect of KGF on urothelium was considered because urothelium exhibits a very rapid and striking proliferative response to KGF. To address this issue, urine was collected from rats (n = 6) at various times following a single IV injection of KGF. Urinary KGF levels are undetectable in rats prior to KGF administration and in rats not exposed to the growth factor (Fig. 3). Four h following KGF injection, measurable levels of KGF are present in the urine. Thereafter, urinary KGF levels peak at 8 h and then return to near-basal levels by 24 h (Fig. 3).

The appearance of KGF in the urine raised the possibility that KGF excreted in the urine may be responsible for the marked proliferative effect that i.v.-administered KGF has on urothelium. The urothelial proliferative response to i.v. KGF in normal rats as shown by PCNA immunoreactivity involves the transition within 24 h of almost every basal urothelial cell in the bladder from a PCNA-negative to PCNA-positive phenotype (Fig. 4). To test the potential contribution of “topical” urinary KGF to this striking proliferative effect, bilaterally nephrectomized rats (who would not be exposed to urinary KGF) were treated with KGF or saline and sacrificed 24 h later, and their bladders were examined histologically. The bladders of nephrectomized rats receiving i.v. KGF showed a similar number of PCNA-positive urothelial cells as nonnephrectomized rats (Fig. 5). Experi-
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Fig. 1. Representative bladder cross-sections from saline-pretreated CP-challenged rats (left) demonstrate severe ulcerative hemorrhagic lesions (arrows) and extensive submucosal edema (+). The bladders of KGF-pretreated rats were largely free of ulcerations or edema (right).

ments to show directly that intravesicular administration of KGF will not prevent CP-induced cystitis or that there are no trophic effects of urine on the bladder were not performed, although proteins in general do not readily gain access from the urinary space to the basal cells of the urothelium.

DISCUSSION

KGF induces proliferation of epithelial cells in a variety of tissues, including the urothelium (1–5), and has shown epithelium-protective effects in the lung (6, 7). The present study demonstrates that KGF administered systemically 24 h before CP protects against ulcerative cystitis. The rapid proliferative effect of KGF on urothelial cells suggests that KGF may protect against cystitis by maintaining the integrity of the bladder epithelium by inducing proliferation of the urothelial cells during and after chemotoxic injury. Alternately or additionally, KGF may protect the integrity of the urothelium by cytoprotective mechanisms.

KGF may potentially play a role in preventing ulcerative cystitis and hematuria in patients undergoing chemotherapy and irradiation. Hemorrhagic cystitis occurs sporadically after such treatments as irradiation but is most well documented after treatment with CP and ifosfamide. CP and its more urotoxic derivative ifosfamide are thought to induce urothelial injury by multiple mechanisms that may include the high urinary concentrations of the drugs and their highly reactive metabolites (8). The incidence of CP-induced hemorrhagic cystitis varies from 2 to 40%, depending on the dose and duration of therapy (9). Massive hemorrhage has been associated with death in patients treated with high-dose i.v. CP (10). As an aside, hemorrhagic cystitis in bone marrow transplant recipients is associated with persistent BK viruria (11–13), and the potential therapeutic effects of KGF in this setting may be worth exploring.

Study of urinary KGF levels after i.v. injection of KGF showed that immunoreactive KGF appears in the urine shortly after injection. Experiments with nephrectomized, anuric rats, however, showed that “topical” urinary KGF is unlikely to contribute to the trophic action of KGF on urothelium. In contrast, topically delivered KGF does stimulate type II pneumocyte proliferation and differentiation in the lung. This difference between the lung and urinary bladder may be due to the fact that the alveolar lining cell population, unlike the multilayered urothelium, is largely only a single layer thick, allowing for direct cellular access of airway-delivered factors. In the bladder, urothelial multilayering may contribute to the formation of a barrier sufficient to
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Fig. 2. The ulcerations, hemorrhage, and edema caused by CP in the bladders of saline-pretreated rats are illustrated at higher magnification.

Fig. 3. A, the kinetics of urinary KGF after a single, bolus i.v. injection of KGF show a significant accumulation of immunoreactive KGF peaking at approximately 8 h. B, the increase in urinary concentration of KGF is also shown not to be due to any change in urine volume.

Fig. 4. Control rats demonstrate a very low rate of urothelial cell proliferation. The proliferative response to KGF as shown by PCNA immunoreactivity affects almost every basal urothelial cell in the bladder.

Fig. 5. The bladders of bilaterally nephrectomized rats receiving KGF show a similar number of PCNA-positive urothelial cells compared to nonnephrectomized rats (compare also to Fig. 4). Urinary KGF, therefore, is unlikely to contribute significantly to the proliferative response of urothelium to KGF.
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prevent the diffusion of urinary proteins to the basal, proliferative layer. In conclusion, systemically delivered KGF protects rats against the development of CP-induced hemorrhagic cystitis.

REFERENCES


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