Effect of Epidermal Growth Factor in the Rat 5/6 Renal Ablation Model

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ABSTRACT

Acute administration of epidermal growth factor (EGF) has been shown to promote recovery from ischemic and nephrotoxic acute renal failure in vivo. The question of whether chronic subcutaneous administration of EGF (19.1 µg/day for 3 or 6 wk) could alter the course of chronic renal failure in rats subjected to 5/6 nephrectomy was studied. By week 6, there was no difference in renal function, as assessed by animal survival, BUN, urea and inulin clearances, proteinuria, renal morphometry, or renal size, between EGF- and vehicle-treated rats. This suggests that chronic renal insufficiency differs from acute tubular injury in its sensitivity to exogenous EGF. Unexpectedly, EGF significantly attenuated the rise in systolic blood pressure that occurred by the fourth week after 5/6 nephrectomy. The antihypertensive effect of EGF was still evident at week 5. Urinary flow rate, free water clearance, and excretion of total solutes, Na, and K, however, were not significantly altered by EGF at weeks 2, 4, 5, or 6, suggesting a mechanism other than increased natriuresis or diuresis for this antihypertensive effect.

Key Words: Epidermal growth factor, chronic renal failure, glomerulosclerosis, hypertension, natriuresis

In humans, the loss of more than 50% of renal parenchyma results in the inexorable progression of chronic renal insufficiency (1,2). In rats, the degree of renal insufficiency correlates with the degree of glomerular (mesangial) sclerosis, which in turn is highly correlated with the degree of glomerular hypertrophy (3–6). The growth factors that cause glomerular growth may be distinct from those causing primarily tubular growth (7). Glomerular hypertrophy, leading to sclerosis, appears to be a maladaptive response to the removal of functioning renal mass (8), whereas tubular hypertrophy and hyperplasia may play a benign, adaptive role (9). If this is the case, then a growth factor that promotes tubular rather than glomerular growth might be expected to ameliorate the course of chronic renal insufficiency.

Epidermal growth factor (EGF) has striking effects on the kidney when administered acutely (see reference 10 for a review). Humes and colleagues have recently shown that a single sc dose of 20 µg of EGF promotes tubular regeneration and recovery from ischemic (11) and nephrotoxic (12) acute renal failure. Within 48 h after ischemic tubular injury, specific binding of EGF is significantly increased (13). Circulating EGF may be necessary for the compensatory tubular hypertrophy seen after contralateral nephrectomy (14). Although EGF is mitogenic for glomerular mesangial cells (15), preliminary reports have suggested that EGF reduces collagen biosynthesis by rat mesangial cells in vitro (16,17). EGF might therefore promote adaptive tubular growth in preference to maladaptive glomerular growth in chronic renal insufficiency.

We asked whether chronic sc administration of EGF could ameliorate the course of chronic renal failure in the rat 5/6 renal ablation model (18–25).

METHODS

Male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 203.4 ± 3.9 g (mean ± SE; N = 36) were anesthetized with ip sodium pentobarbital (50 mg/kg) and subjected to 5/6 nephrectomy (NX) in a one-stage procedure (18–25). Animals were housed singly and were allowed ad libitum access to tap water and standard rodent chow containing 22.8% protein by weight (23) (Ralston Purina Co., St. Louis, MO) to minimize acute surgical mortality. Dietary intake was monitored by 24-h urinary urea nitrogen excretion.

At the time of 5/6 NX, an osmotic pump (Alzet model 2ML4; Alza, Inc., Palo Alto, CA) was implanted...
sc, posterior to the thoracic spine. The osmotic pump was filled with 0.9% (wt/vol) NaCl and 0.1% (wt/vol) rat albumin (vehicle) and was capable of a delivery rate of 2.5 μL/h for 28 days according to the manufacturer. In one group of 5/6 NX rats (Early EGF), the pump also contained recombinant human EGF at a nominal delivery rate of 19.1 μg/24 h. This dose was chosen because of the beneficial effect of a similar dose (20 μg) of EGF given sc at the time of ischemic or nephrototoxic acute renal failure (11, 12).

At the beginning of week 3, the osmotic pumps were removed and a new osmotic pump (Alzet model 2ML4), containing vehicle or vehicle plus EGF (Early EGF and Late EGF groups), at the same nominal delivery rate, was inserted in the same site.

The rats were weighed four to five times a week. Each rat underwent systolic blood pressure measurement four times a week between 9 a.m. and 12 noon, by the tail-cuff method (IITC Life Science, Woodland Hills, CA) and with a physiograph (Beckman Dynograph; Schiller Park, IL). Approximately 10 readings at 28°C were taken from each unanesthetized rat daily.

On weeks 2, 4, 5, and 6, urine was collected for 24 h from each rat kept in a metabolic cage (Nalgene, Rochester, NY). The urinary volume was recorded, and aliquots were frozen at −20°C for subsequent determination of urea nitrogen, NH₄⁺, Na⁺, K⁺, osmolality, total protein, and albumin. Blood was collected from the tail into heparinized tubes at weeks 2 and 4 and centrifuged at 4°C, and the aspirated plasma was frozen at −20°C for later determination of urea nitrogen and osmolality.

At the conclusion of the study (end of week 6), three to four animals from each group underwent measurement of insulin clearance by a standard protocol (23). At the time of death, heparinized plasma was obtained from the aorta. The heart and remnant kidney were quickly removed, trimmed of connective tissue, blotted dry, and weighed, and the kidney was processed for light microscopy by standard techniques (26).

Urea nitrogen was measured by the procedure of Fawcett and Scott (27). NH₄⁺ was measured by the same procedure, omitting the incubation with urease. Plasma and urinary osmolality were determined with a vapor pressure osmometer (model 5100B; Wescor, Inc., Logan, UT). Trichloroacetic acid–precipitable protein was determined by the method of Lowry et al. (28). Urinary albumin was measured by a rat-specific ELISA by the kit protocol (Nephrat, Exocell, Inc., Philadelphia, PA). Urinary Na⁺ and K⁺ concentrations were measured by indirect potentiometry with a Synchron CX3 analyzer (Beckman Instruments, Brea, CA). Glomerulosclerosis was determined semiquantitatively from 4-μm-thick sections of silver-stained renal tissue, by the method of Raij et al. (26). The percentage area of the glomerular profile involved by mesangial sclerosis was scored as follows: 0% (score = 0), 25% (score = 1), 50% (score = 2), 75% (score = 3), 100% (score = 4). A mean of 12.4 ± 1.0 (SE) glomerular profiles was evaluated for each animal.

The proportion of the renal cortex composed of various structures was estimated by determining their volume fractions. Jones' silver-stained sections were examined with a projection microscope at a final magnification of 288 as determined by a stage micrometer. A grid with points 2.0 cm apart was superimposed on the tissue, and the points falling on tubules, interstitium, and other structures were recorded. A total of 300 points were counted per slide. The volume fraction of tubules per volume of cortex (VVTubule/Cortex) was estimated by dividing the points falling on tubules by 300. The volume fraction of interstitium per volume of cortex (VVInterstitium/Cortex) was determined by dividing the points falling on interstitium by 300.

Mean proximal tubular outer diameter was estimated by projecting toluidine blue–stained sections at a final magnification of 288 (29). Cross-sections of periglomerular tubules were examined, and the smallest diameter was measured. Ten diameters were measured per animal in two or three fields of cortex.

Recombinant human EGF, prepared by Carlos George-Nascimento, was the gift of Pablo J. Valenzuela (Chiron Corp., Emeryville, CA). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Calculations and Statistics

Individual values of 24-h urinary volume, urinary sodium excretion (U₅Na-V), and urinary potassium excretion (U₅K-V) were corrected for each rat's 24-h urinary urea excretion, as an index of dietary intake (20, 21). At each week, values for the different groups (Early EGF, Late EGF, Vehicle) were compared by one-way analysis of variance (ANOVA) and the Scheffe F test to identify which groups differed significantly while controlling the experiment-wise error rate. Paired t test was used to compare values within the same group at two different time points. A probability level of ≤0.05 was considered statistically significant.

RESULTS

The Model

Five-sixths NX produced rats with a stable BUN of ≈90 mg/dL over the 6 wk of the study (data not shown), as previously described for this model (19). Acute surgical mortality, defined as death within 72 h of 5/6 NX, occurred in 5 (14%) of 36 rats. Deaths from uremia (Early EGF, 2; Late EGF, 1; Vehicle, 1)
and animals that were euthanized as the result of pump failure (Early EGF, 3; Late EGF, 2; Vehicle, 3) were similar in all three groups. The rats continued to grow at a similar rate in the three groups (Table 1). Urinary urea nitrogen excretion (351.7 ± 17.2 mg/24 h, mean ± SE; N = 93) was in agreement with previously published values (24).

During the course of the study, urea clearances were 0.1 mL/min/100 g body wt (Table 1), indicating stable, severe renal failure (22). Five-sixths Nx resulted in 20 mg of proteinuria/24 h (Table 2, Vehicle group; [23]) and sclerosis of 25% of the glomerular profile (Table 1, Vehicle group; [18,19, 24,25]) by week 6. There was significant systolic hypertension, apparent by week 4 (Figure 1, Vehicle group), as previously reported for this model (19,25). Sham-operated control rats fed ad libitum had a systolic blood pressure of 120 to 125 mm Hg, which did not change during the 6 wk of the study (data not shown), in agreement with previous reports (25,30).

Urinary flow rate (32.4 ± 1.2 mL/24 h, mean ± SE; N = 93; [23,24]), solute excretion (14 mosmol/24 h, mean ± SE; N = 91; [21,23,24]), and UNaV (1.7 ± 0.1 mEq/24 h, mean ± SE; N = 92; [20,23,24]) were consistent with previous reports. Free water clearance (-20.9 ± 2.3 mL/24 h, mean ± SE: N = 70) was negative at weeks 2, 4, and 6, as previously described (9).

Effects of EGF

EGF did not significantly affect BUN or urea clearance throughout the study (data not shown). At the end of the study, there was no difference in inulin clearance between EGF- and vehicle-treated 5/6 Nx rats (Table 1).

EGF tended to delay the onset of heavy proteinuria (Table 2). However, at each time point, the effect on total protein excretion of EGF relative to vehicle was not significant by ANOVA. Likewise, urinary albumin excretion was not significantly affected by EGF at weeks 2, 4, and 6 (data not shown). The degree of glomerulosclerosis was similar in all three groups at the end of the study (Table 1).

EGF had no effect on the wet weight of the remnant kidney, on proximal tubule diameter, or on tubular or interstitial volume fraction (Table 1).

Systolic blood pressure increased significantly in the vehicle-treated group at week 4 (Figure 1). This increase was attenuated by chronic EGF treatment in a time-dependent manner. At week 4, the Late EGF group, which had received EGF for only 1 wk, had an intermediate blood pressure, whereas the Early EGF group, which had received EGF for 4 wk, had the lowest blood pressure. By week 5, the difference between early and late treatment with EGF was no longer apparent but both groups still had a signifi-

### TABLE 1. Values obtained at the end of week 6 of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight Gain (g/week)</th>
<th>Inulin Clearance (μL/min/100 g)</th>
<th>Glomerulosclerosis Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early EGF</td>
<td>14.0 ± 3.5</td>
<td>97.7 ± 28.0 (4)</td>
<td>1.12 ± 0.33 (5)</td>
</tr>
<tr>
<td>Late EGF</td>
<td>14.2 ± 2.7</td>
<td>117.2 ± 34.0 (4)</td>
<td>0.45 ± 0.11 (5)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>13.1 ± 1.9</td>
<td>122.4 ± 56.0 (3)</td>
<td>0.00 ± 0.01 (6)</td>
</tr>
<tr>
<td>Sham operated</td>
<td>13.3 ± 2.7</td>
<td>126.4 ± 58.0 (3)</td>
<td>0.00 ± 0.01 (6)</td>
</tr>
</tbody>
</table>

* Values are mean ± SE; the number of animals is given in parentheses.

* P < 0.001 versus the other three groups by one-way ANOVA.
TABLE 2. Urinary excretion of protein, Na\(^+\), and K\(^+\), and urinary flow rate, corrected for dietary intake

<table>
<thead>
<tr>
<th></th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
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<tr>
<td><strong>U\text{protein} V (mg/24 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early EGF</td>
<td>6.97 ± 3.12</td>
<td>7.96 ± 2.60(^ab)</td>
<td>15.31 ± 4.09</td>
<td></td>
</tr>
<tr>
<td>Late EGF</td>
<td>8.32 ± 1.86(^a)</td>
<td>14.21 ± 2.95</td>
<td>18.57 ± 3.47</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>16.77 ± 5.29</td>
<td>15.31 ± 3.30</td>
<td>23.30 ± 4.51</td>
<td></td>
</tr>
<tr>
<td><strong>V\text{corrected} (\muL/mg of urea·24 h)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Early EGF</td>
<td>265.7 ± 107.1(^c)</td>
<td>77.6 ± 8.7</td>
<td>85.6 ± 11.0</td>
<td>82.0 ± 16.9</td>
</tr>
<tr>
<td>Late EGF</td>
<td>113.9 ± 25.2</td>
<td>76.7 ± 6.8</td>
<td>93.5 ± 10.2</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>262.3 ± 132.3(^c)</td>
<td>130.7 ± 29.9</td>
<td>95.4 ± 12.3</td>
<td>95.7 ± 14.1</td>
</tr>
<tr>
<td><strong>U\text{Na+V corrected} (\muEq/mg of urea·24 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early EGF</td>
<td>13.5 ± 2.8(^c)</td>
<td>5.2 ± 0.8</td>
<td>6.1 ± 1.6</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Late EGF</td>
<td>4.9 ± 0.7</td>
<td>5.5 ± 1.0</td>
<td>3.6 ± 0.5</td>
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</tr>
<tr>
<td>Vehicle</td>
<td>9.8 ± 4.4</td>
<td>4.9 ± 0.9</td>
<td>3.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td><strong>U\text{K+V corrected} (\muEq/mg of urea·24 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early EGF</td>
<td>20.5 ± 3.8(^c)</td>
<td>6.7 ± 0.8</td>
<td>8.0 ± 0.9</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td>Late EGF</td>
<td>7.2 ± 0.9</td>
<td>8.3 ± 0.5</td>
<td>8.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>17.1 ± 6.4(^c)</td>
<td>9.8 ± 1.2</td>
<td>8.6 ± 0.6</td>
<td>8.7 ± 0.9</td>
</tr>
</tbody>
</table>

\(^a\) P < 0.002, Late EGF (week 4) versus Late EGF (week 6).
\(^b\) P ≤ 0.05, Early EGF (week 5) versus Early EGF (week 6).
\(^c\) P < 0.05, week 2 versus the same group at week 6.
\(^d\) P < 0.05, week 2 versus the same group at weeks 4, 5, or 6 by paired t test.

Figure 1. Systolic blood pressure (BP) measured by the tail-cuff method, as described in Methods. The weekly mean blood pressure is presented; the SE is smaller than each symbol. Closed circles, Early EGF; closed triangles, Late EGF; open circles, Vehicle. a, P < 0.05 versus Early EGF or Late EGF; b, P < 0.05 versus Early EGF or Vehicle; c, P < 0.05 versus Late EGF or Vehicle. All comparisons were made by one-way ANOVA and the Scheffe F test.

Effect of EGF in Chronic Renal Failure

...a decrease in the amount of U\text{protein} V and U\text{K+V} between week 2 and subsequent weeks (Table 2). Treatment with EGF did not significantly alter urinary flow rate or the excretion of sodium, potassium, free water, and total solutes (data not shown) relative to vehicle at weeks 2, 4, 5, and 6 (Table 2). Urinary NH\text{4+} excretion, which was similar at weeks 2, 4, 5, and 6, was likewise unaltered by EGF treatment (data not shown).

DISCUSSION

Throughout this study, the urinary excretion of protein and albumin, a sensitive marker of glomerular injury (3), was not significantly affected by EGF treatment. At the end of the study, the degree of glomerulosclerosis was similar in all three groups. BUN and GFR (urea and inulin clearances) were unaffected by EGF throughout the study. Neither remnant kidney wet weight nor tubular size was altered by EGF, despite the hyperplastic tubular response to 5/6 NX (31) and the observed stimulation by EGF of renal tubular \text{\textsuperscript{3}H}thymidine incorporation after ischemic or nephrotoxic insults (11-13). The urinary excretion of NH\text{4+}, recently linked to renal hypertrophy in vitro (32,33), was also unaffected by EGF.

The lack of a beneficial effect of EGF in the 5/6 NX model was disappointing, because other pharmacologic and dietary interventions in this model have been shown to decrease glomerulosclerosis and
improve renal function (3,24,25). It is possible that EGF may produce beneficial effects in less severe renal insufficiency (2). The lack of improvement in response to chronic EGF (19 µg/day) in severe renal insufficiency contrasts with the dramatic regenerative effect of a single 20-µg dose of EGF in acute tubular injury (11,12). The heightened sensitivity to EGF in acute tubular injury appears to be due to an increase in EGF receptor number and/or affinity (13). Unlike acute renal failure, chronic renal insufficiency appears to be characterized by insensitivity to exogenous EGF.

Chronic EGF administration significantly attenuated the hypertension observed in conscious 5/6 NX rats, beginning in week 4. This effect was still present at week 5. Given the acute vasoconstrictive effect of EGF when infused into the rat renal artery in vitro (15) or administered to rat vascular strips in vitro (34,35), our result was surprising, especially because the same species (15) (and strain [34,35]) was used in our study.

An explanation for this apparent paradox may be the difference in the dose of EGF administered. Harris et al. (15) observed vasoconstriction when 500 ng of EGF/kg·min was infused directly into the rat renal artery, a dose calculated to achieve a renal arterial concentration of 3 nM. In vitro, threshold EGF concentrations of 1 and 10 nM were required for vasoconstriction of rat ileocolic and mesenteric arterial strips (34) and rat aortic strips (35), respectively. By comparison, in this study, rats received a dose of EGF that was lower by an order of magnitude (54 ng/kg·min). Because the route of delivery was sc rather than intra-arterial and because the half-life of EGF in the circulation is ≈1.5 min (36), the delivery of EGF to arterial resistance vessels may have been even lower. Arterial vasodilatation, rather than vasoconstriction, has been observed in sheep and dogs when a similar dose of EGF (50 ng/kg·min) was infused either iv (41) or intra-arterially (42,43).

In addition to the dose of EGF administered, differences between this study, showing an antihypertensive effect of EGF in 5/6 NX rats and other studies (15,34,35), showing a vasoconstrictive effect in rats with normal renal function, include the following: the level of consciousness during blood pressure measurement, the presence of uremia, and the chronicity of EGF administration. One (or more) of these variables may also contribute to the apparently conflicting results.

The mechanism of the antihypertensive effect of chronic low-dose EGF in 5/6 NX rats is unknown. The onset of significant hypertension occurred in week 4, when urine flow rate and renal excretory capacity for Na⁺ (and K⁺) were decreased. Indeed, hypertension in ESRD is thought to result from a decline in renal excretory ability (40). We therefore investigated whether the antihypertensive effect of EGF was due perhaps to enhanced natriuresis or diuresis, as has been observed after acute administration of EGF in vitro to rabbit cortical collecting duct (41–43). However, treatment with EGF had no significant effect on the urinary excretion of Na⁺, free water, K⁺, or total solutes.

In dogs with normal renal function, the antihypertensive effect of EGF involves antagonism of agonist-induced vasoconstriction (38,39), presumably by direct interaction of EGF with vascular smooth muscle receptors (44). If the same mechanism underlies our results, then it suggests that agonist-induced vasoconstriction may be significant in the hypertension following subtotal renal ablation in the rat.

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