Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11876–11880, December 1992
Physiology

Insulin-like growth factor I accelerates recovery from ischemic acute tubular necrosis in the rat

(epidermal growth factor/growth hormone/renal failure/renal regeneration)

STEVEN B. MILLER*, DANIEL R. MARTIN*, JOHN KISSANE†, AND MARC R. HAMMERMAN*‡

Departments of *Internal Medicine, ‡Cell Biology and Physiology, and †Pathology, George M. O'Brien Kidney and Urological Diseases Center, Renal Division, Washington University School of Medicine, St. Louis, MO 63110

Communicated by David M. Kipnis, September 11, 1992

ABSTRACT **The effects of administering insulin-like growth factor I (IGF-I) were examined in a model of ischemic acute tubular necrosis in rats. Injury was induced by 75 min of bilateral renal artery occlusion. Compared to rats administered vehicle, rats administered IGF-I (100 μg/day via continuous subcutaneous infusion) had significantly lower serum creatinine and blood urea nitrogen levels over the course of 7 days postocclusion. Glomerular filtration rate as determined by inulin clearance was examined on day 2 postocclusion and was significantly increased in IGF-I-treated animals (0.16 ± 0.02 ml per min per 100 g of body weight) compared to vehicle-treated controls (0.08 ± 0.02 ml per min per 100 g of body weight). The weight loss that occurred during the course of acute tubular necrosis was ameliorated by IGF-I. Mortality was reduced from 36.7% in vehicle-treated rats to 7.1% in rats administered IGF-I. Histologically, there was much less renal injury evident at day 7 postocclusion in the IGF-I-treated rats compared to vehicle-treated controls. In contrast, growth hormone (200 μg administered subcutaneously for 4 days) did not affect recovery of renal function or reduce mortality postreperfusion. This report demonstrates a beneficial effect of IGF-I administration in the setting of acute tubular necrosis. Several properties of IGF-I render it a pharmacological agent with excellent potential for treatment of this condition in humans.**

Damaged portions of the nephron undergo regeneration after acute ischemic injury. Strategies for treatment of acute renal failure in humans are directed toward supportive care to permit such regeneration to occur and toward acceleration of the pace of regeneration by maintenance of good nutritional status (1). Recently, the use of growth-promoting agents has been advocated to enhance recovery from acute renal failure (1–4). The rationale for this advocacy is based, in part, on the fact that certain of these agents are anabolic and renotropic. For example, both growth hormone (GH) and insulin-like growth factor I (IGF-I) promote whole body and organ growth (5–7), reduce protein breakdown (8), enhance glomerular filtration rate and renal plasma flow (9, 10), and induce hypertrophy of glomeruli and proximal tubules in rats (11). The actions of GH are thought to be exerted by IGF-I (11, 12). Additional rationale for the use of growth factors in the setting of acute renal failure is provided by studies demonstrating changes in renal growth factor synthesis following ischemic injury. For example, expression of IGF-I within kidney postinjury presages repair of proximal tubular epithelium, suggesting that this peptide plays a major role in the regenerative process (13). Production of another renal growth factor, epidermal growth factor (EGF), decreases after ischemic injury and binding sites for EGF in kidney are increased, consistent with a relative deficiency state (4, 14). Administration of EGF to rats shortens the course of kidney dysfunction following acute renal injury, suggesting that correction of the renal EGF deficiency may hasten kidney regeneration (2–4). No data exist regarding effects of exogenous IGF-I administration in this setting.

Because of the potential for use of IGF-I as a pharmacological agent to treat acute renal failure, we carried out studies to determine whether IGF-I might ameliorate this condition. We administered IGF-I, GH, EGF (as a positive control), or a vehicle to rats after induction of ischemic acute tubular necrosis (ATN); measured levels of serum creatinine and blood urea nitrogen (BUN) for 7 days postreperfusion; measured glomerular filtration rate on day 2 postreperfusion; and evaluated kidney histology at 7 days. While GH did not affect recovery from ATN, IGF-I, like EGF, accelerated this process and reduced mortality. In addition, IGF-I hastened regaining of body weight that was reduced in the setting of acute renal disease. Our findings suggest that IGF-I may be useful as a pharmacological agent in the treatment of ATN.

METHODS

Induction of ATN. Male Sprague–Dawley rats weighing 225–250 g were obtained from Harlan. Rats were fed ad libitum and housed in an animal facility at 21°C ± 2°C, with a 12-hr light/12-hr dark cycle. Under anesthesia induced by a combination of ketamine and pentobarbital, the abdominal cavity was exposed via a midline incision. Both renal arteries were identified and freed by blunt dissection. Microvascular clamps were placed on both renal arteries to effect complete cessation of blood flow. Core body temperature was maintained at 37°C ± 1°C by placing the animal on a homeothermic table and monitoring with a temperature-sensing rectal probe. After 75 min, the clamps were removed with return of blood flow to the kidneys. If reperfusion was incomplete, as judged visually, the experiment was terminated and the animal was killed.

Administration of Growth Factors. Animals were randomly assigned to receive either GH, IGF-I, EGF, or vehicle. Recombinant bovine GH (a gift from Gary F. Hartnell, Monsanto) was administered daily over 4 days by a single subcutaneous injection at a dose of 200 μg beginning 30 min postreperfusion. This dose was chosen because we have shown previously that it induces hypersomatotropism in rats as manifested by an increased glomerular filtration rate (10). Recombinant human IGF-I (kindly provided by Genentech) was given by constant infusion with an Alzet pump implanted subcutaneously (Alza) at a dose of 100 μg daily (24-μl/day) for the full course of the experiment. The pump was implanted 30 min postreperfusion. This dose was chosen be-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ATN, acute tubular necrosis; BUN, blood urea nitrogen; EGF, epidermal growth factor; GH, growth hormone; IGF-I, insulin-like growth factor I; PAH, *p*-aminohippurate; H&E, hematoxylin/eosin; PAS, periodic acid/Schiff.

cause we have shown previously that it enhances glomerular filtration rate in rats (10). Administration of IGF-I at this dosage did not result in hypoglycemia as determined by measurement of blood glucose with an Accu-check IIm monitor (Boehringer Mannheim).

EGF has been shown to hasten recovery of renal function postischemic renal injury in rats (3, 4). Therefore, it was administered to rats as a positive control. Rats were given recombinant human EGF (Upstate Biotechnology, Lake Placid, NY) as a one-time dose of 20 μg subcutaneously 30 min postreperfusion exactly as in the studies of Humes *et al.* (3). Rats were administered vehicle in parallel to each of the GH and growth factor treatment groups.

Measurement of Renal Function. Animals were weighed daily. Tail vein blood was obtained before induction of ATN and at 10:00 a.m. daily for 7 days postsurgery for measurement of hematocrit, serum creatinine, BUN, and potassium. Measurements of glomerular filtration rate (inulin clearance) and renal plasma flow [*p*-aminohippurate (PAH) clearance] were performed on day 2 postocclusion in a separate group of vehicle-treated and IGF-I-treated animals. Day 2 was chosen because this was the time of peak BUN and creatinine. Under light ether anesthesia, left femoral arterial (PE-10) and venous catheters (PE-50), along with a suprapubic bladder catheter (PE-50), were inserted. Rats were placed in a Plexiglas holder and allowed to recover from the effects of anesthesia for 1 hr. A priming dose of inulin and PAH designed to produce plasma levels of 50–100 mg/dl and 1–2 mg/dl, respectively, was administered in 0.6 ml of normal saline over 3 min. Saline containing inulin and PAH in amounts to maintain these plasma levels was then infused at a rate of 40 μl/min. After a 60-min equilibration period, four consecutive 20-min collections of urine and blood were obtained for estimation of inulin and PAH clearances. Inulin and PAH in blood and urine were measured by standard techniques (15, 16). Clearances were calculated and expressed per 100 g of body weight. Data reflect the mean of four calculated clearances per rat.

Histopathologic Assessment. All surviving rats on day 7 postsurgery were reanesthetized and the kidneys were exposed through a midline incision. The kidneys were perfused *in vivo* with Hepes buffer warmed to 37°C as described (17). The kidneys were removed, stripped of their capsules, cut longitudinally into two halves, and placed in phosphate-buffered formalin for fixation. The right kidneys from five animals in each group were paraffin embedded and sliced at 5 μm. Slides were stained with hematoxylin/eosin (H&E) and periodic acid/Schiff (PAS). A pathologist (J.K.) blinded to the treatments examined and graded all the specimens by utilizing a scoring system that considered the pathologic changes consistent with ischemic injury and recovery (18, 19). The pathologic scoring system grades four features consistent with ATN and recovery: epithelial calcification, tubular dilatation, proximal tubular papillary proliferation, and interstitial infiltration. Twelve microscopic fields of sections originating from five vehicle-, GH-, and growth factor-treated rats were chosen randomly to be scored for each parameter (240 fields per treatment group). The details of the pathologic scoring system are as follows. (*i*) Epithelial calcification: 0, no epithelial calcifications; 1, 1–4 fields with epithelial calcifications; 2, 5–8 fields with epithelial calcifications; 3, 9–12 fields with epithelial calcifications. (*ii*) Tubular dilatation: 0, normal by H&E and PAS; 1, suggestive tubular dilatation by H&E with partial loss of PAS staining along the brush border; 2, unequivocal tubular dilatation by H&E with widespread loss of PAS staining along the brush border; 3, unequivocal tubular dilatation by H&E with loss of tubular specificity and total absence of PAS staining along the brush border. (*iii*) Proximal tubular papillary proliferation: 0, no proliferation; 1, 1–4 fields with proliferative changes; 2, 5–8 fields with proliferative changes; 3, 9–12 fields with proliferative changes. (*iv*) Interstitial infiltration: 0, no infiltrate; 1, 1–4 fields with an infiltrate; 2, 5–8 fields with an infiltrate; 3, 9–12 fields with an infiltrate. The total score for each kidney examined was divided by 4 to give a final score of 0–3.

Statistics. Rats were randomized to the various test groups after surgery. There was no difference between levels of creatinine and BUN measured postischemic injury in any of the vehicle-treated groups. Therefore, data originating from vehicle-treated groups enrolled in the 7-day protocol were combined. Levels of creatinine and BUN in GH- and growth factor-treated groups were compared with those in the vehicle-treated group by Student's *t* test. Differences in mortality rates between the vehicle-treated animals and those that received GH or growth factors were analyzed by χ^2 analysis. Differences in pathological scoring between sections originating from vehicle-treated rats and GH- or growth factor-treated rats were analyzed by Dunnett's multiple comparison procedure (20). Differences were considered to be significant if $P < 0.05$. Because of mortality, the number of rats (n) used to generate data characterizing creatinine, BUN, and body weight changed during experiments. The n in vehicle-treated controls was 30, 27, 23, and 20 on days 0–3, respectively. The n in the control group stabilized on day 4 at 19 animals and remained at that number until termination of the experiment on day 7. The n in the GH-treated group was 12 on days 0–2, 11 on days 3 and 4, and 10 on days 5–7. The group receiving IGF-I started with 14 animals. There was one death on day 2. Therefore, the n was 13 on days 2–7. The EGF-treated group experienced no mortality and the n was 9 for the entire experiment. The animals that underwent clearance studies and were sacrificed on day 2 were analyzed separately. Values for creatinine, BUN, inulin, and PAH clearance in vehicle- (n = 9) and IGF-I-treated (n = 7) groups were compared by Student's *t* test.

Because of the different dosages and routes of administration for IGF-I and EGF, no attempt was made to compare their relative efficacies in enhancing recovery from ATN.

RESULTS

Bilateral renal artery occlusion for 75 min produced severe ATN. In rats receiving vehicle, serum creatinine increased rapidly and peaked 2 days postreperfusion at a level (±SE) of 3.81±0.31 mg/dl, ≈8-fold above the baseline level of 0.49 ± 0.02 mg/dl. Over the course of the next 7 days, the creatinine decreased to 1.04 ± 0.14 mg/dl in vehicle-treated rats, which is 2 times the baseline level. When the rats received GH, there was no alteration in renal function compared to vehicle-treated rats as reflected by creatinine levels (Fig. 1*A*). In contrast, there was an improvement in renal function as assessed by serum creatinine when animals received IGF-I. On each day postocclusion, there was a significantly lower value for serum creatinine in the IGF-I-treated animals compared to vehicle-treated rats (Fig. 1*B*). IGF-I-treated animals had a peak serum creatinine on day 1 of 2.84 ± 0.27 mg/dl, which returned to baseline on day 7. Similarly, the administration of EGF resulted in more rapid improvement in serum creatinine (Fig. 1*C*) as has been demonstrated by Humes *et al.* (3).

The patterns of change in urea nitrogen levels after bilateral renal artery occlusion were similar to those of serum creatinine. Vehicle-treated rats had a baseline BUN of 19.6 ± 0.8 mg/dl, which peaked 48 hr postreperfusion at 158.3 ± 11.8 mg/dl. Administration of GH resulted in a significant reduction of BUN 24 hr postreperfusion. However, that difference was not detected during the remainder of the experiment (Fig. 2*A*). The administration of IGF-I resulted in a marked reduction in levels of BUN on days 2–7 (Fig. 2*B*). Similarly,

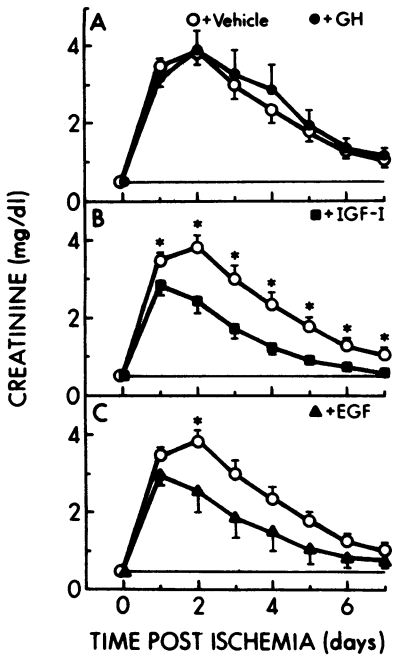

FIG. 1. Levels of serum creatinine in rats measured over time. Shown are levels in vehicle-treated and GH-treated rats (*A*), vehicle-treated and IGF-I-treated rats (*B*), and vehicle-treated and EGF-treated rats (*C*). Data are expressed as means ± SE. Significant differences between vehicle-treated and GH- or growth factor-treated groups ($P < 0.05$) are indicated by asterisks.

the animals that received EGF had a significant reduction in BUN when compared to vehicle-treated rats (Fig. 2*C*).

To ascertain whether the changes in levels of creatinine and BUN shown in Figs. 1*B* and 2*B* were reflective of changes in glomerular filtration rate, we measured inulin clearances in vehicle and IGF-I-treated rats 2 days postischemic injury. This time point was chosen because levels of creatinine and BUN were highest in vehicle-treated rats on this day. Inulin clearance was twice as high in the IGF-I-treated rats compared to animals that received vehicle. PAH clearance was not significantly different between IGF-I and vehicle-treated rats (Table 1).

Table 1. Renal function on day 2 in rats with ATN treated with vehicle or IGF-I

Treatment	Vehicle (n = 9)	IGF-I (n = 7)	P
Serum creatinine, mg/dl	3.46 ± 0.54	1.84 ± 0.22	<0.025
BUN, mg/dl	161.8 ± 13.3	106.1 ± 10.2	<0.007
Inulin clearance, ml per min per 100 g of body wt	0.08 ± 0.02	0.16 ± 0.02	<0.022
PAH clearance, ml per min per 100 g of body wt	0.44 ± 0.14	0.78 ± 0.12	NS

Data shown are means ± SE. NS, not significant.

Associated with the induction of ATN was a loss of body weight. Rats that received vehicle experienced a 15% reduction in body weight over the first 5 days postocclusion. This effect was unaltered by the administration of GH (Fig. 3*A*). In contrast, animals that received IGF-I lost only 8% of their body weight and, by the conclusion of the experiment on day 7, were back to baseline weight (Fig. 3*B*). Their body weights were significantly different on days 4–7 compared to those of animals that received vehicle. The animals treated with EGF after induction of ATN lost 10% of their body weight and their weights were not significantly different from vehicle-treated animals at any time point (Fig. 3*C*).

Acute renal failure of the severity induced in this study was associated with significant mortality. Of the 30 animals that received vehicle, there were 11 deaths, resulting in a mortality rate of 36.7%. The mortality rates for GH-, IGF-I-, and EGF-treated rats were 16.7%, 7.1%, and 0%, respectively (Table 2).

Morphologic analysis performed 7 days postischemia demonstrated remarkable differences between the kidneys of rats treated with vehicle and those treated with IGF-I. Similarly, treatment of rats with EGF resulted in improved renal histology (Fig. 4). Sections from vehicle-treated animals

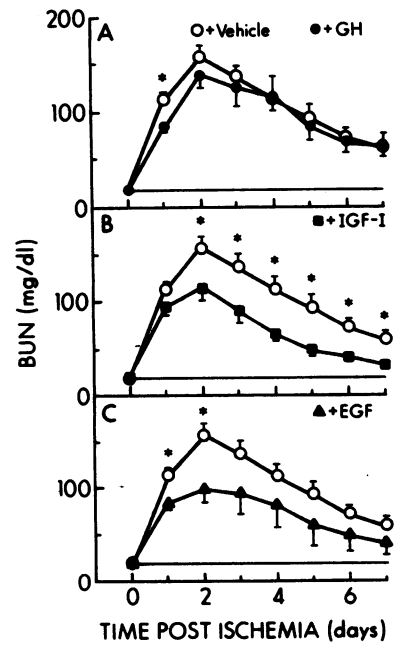

FIG. 2. Levels of serum BUN in rats measured over time. Shown are levels in groups described in Fig. 1. Data are expressed as in Fig. 1.

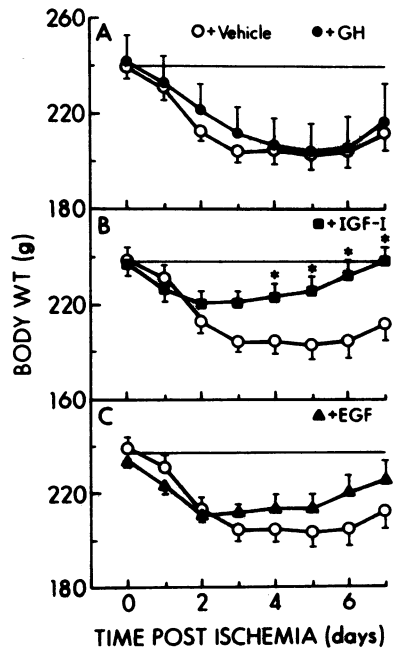

FIG. 3. Body weights of rats measured over time. Shown are weights in groups described in Fig. 1. Data are expressed as in Fig. 1.

exhibited more tubular calcification and dilatation, interstitial infiltration, and papillary proliferation of proximal tubules than did sections originating from growth factor-treated rats. To quantitate these differences, histologic sections originating from five animals from vehicle-treated, GH-treated, and growth factor-treated groups were compared. To ensure that the five animals chosen from each group were representative of the entire group, values of serum creatinine from the five animals measured on day 7 (Table 3) were compared to values from all animals in the group (Fig. 1). There were no significant differences between mean creatinine values in the groups of five animals and creatinine values of the respective entire groups. Kidneys of animals that received vehicle had a histopathologic score of 2.35 ± 0.06. The improved renal function seen with both IGF-I and EGF treatment was reflected by significantly lower scores—0.90 ± 0.17 ($P < 0.01$) and 1.30 ± 0.22 ($P < 0.01$) in IGF-I- and EGF-treated rats, respectively. The histopathologic score of kidneys from animals that received GH (1.80 ± 0.18) was lower than that of vehicle-treated rats ($P < 0.05$) and significantly higher than that of IGF-I ($P < 0.01$) but not of EGF-treated animals (not significant) (Table 3).

DISCUSSION

Acute renal failure in humans is the most costly kidney-related disease requiring hospitalization. The incidence of this condition is increasing (1). Despite many advances, the mortality rate for patients with acute renal failure has not changed in the past 40 years (21). There exists a need for therapeutic approaches that can speed recovery and reduce mortality. For this reason, the use of growth-promoting agents as therapeutic modalities has been proposed, and the actions of one these agents, EGF, have been delineated following administration to animals with experimental acute renal disease (2–4). The present studies were performed to ascertain whether a second agent with therapeutic potential, IGF-I, can accelerate recovery and reduce mortality in a model of ischemic ATN in rats.

Ischemic renal injury in rats results in damage to the most distal (S_3) segment of the proximal tubule and, in some instances, the medullary thick ascending limbs of the loop of Henle (22, 23). Recovery is dependent on the ability of the tubular cells to regenerate and reline the damaged areas along the nephron. One rationale for use of IGF-I in the treatment of acute renal failure is that there is increased immunoreactive IGF-I in cells localized to the proximal tubule associated with the natural regenerative process, consistent with a role for the peptide in recovery (13). A second rationale is that IGF-I has been shown to acutely increase glomerular filtration rate and renal plasma flow (9). Other agents with this property, such as atrial natriuretic peptide (24), have been shown previously to alter the course of acute renal failure, probably by limiting the extent of injury. Additional rationale is provided by the fact that IGF-I is a renotropic agent. It can induce hypertrophy of proximal tubule cells and is postulated to be causative of the renal growth that accompanies hypersomatotropism (11, 16), compensatory hypertrophy (11, 25), and diabetes mellitus (24, 26). Furthermore, IGF-I reduces protein breakdown and exerts a generalized anabolic action that results in reduction of weight loss during starvation (27). Such actions may be beneficial in the setting of the catabolism that accompanies ATN (Fig. 3).

Table 2. Mortality in rats with ATN treated with vehicle, GH, or growth factors

Treatment	Rats, no.	Deaths	% mortality	*P*
Vehicle	30	11	36.7	
GH	12	2	16.7	NS
IGF-I	14	1	7.1	<0.041
EGF	9	0	0	<0.032

GH-, IGF-I-, and EGF-treated groups are compared to vehicle-treated group. *P* values were determined by χ^2 analysis. NS, not significant.

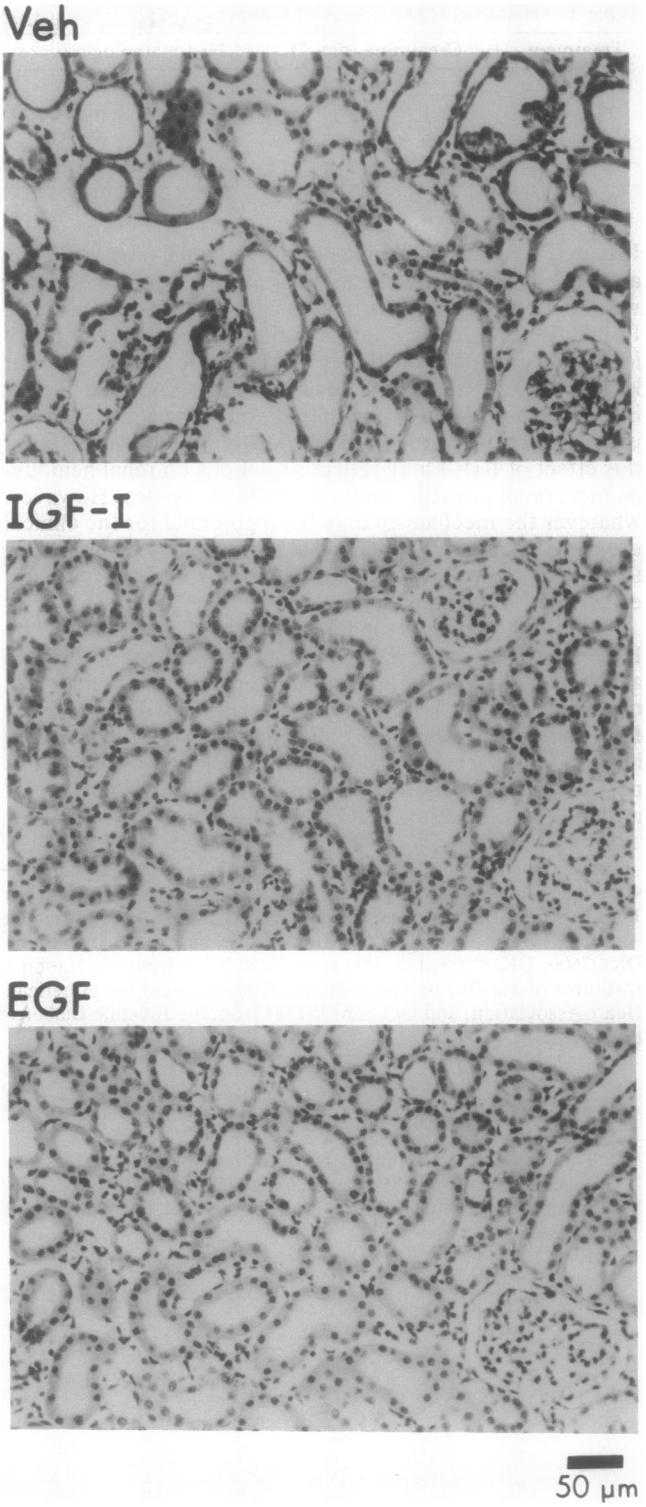

FIG. 4. Photomicrographs of histologic sections stained with H&E originating from kidneys of rats administered vehicle (Veh), IGF-I, or EGF for 7 days. Sections are representative of those originating from five rats in each group.

The results of the present experiments demonstrate that IGF-I can favorably alter the course of ischemic ATN in rats. When compared to animals that received vehicle, the IGF-

Table 3. Histopathologic scores of kidneys

Treatment	Creatinine (day 7)	Histopathologic score
Vehicle	1.08 ± 0.15	2.35 ± 0.06
GH	1.02 ± 0.35	1.80 ± 0.18
IGF-I	0.60 ± 0.05	0.90 ± 0.17
EGF	0.62 ± 0.02	1.30 ± 0.22

Data shown are means ± SE. $n = 5$ rats in each group.

I-treated rats had significantly improved renal function. They also exhibited decreased weight loss and regained their weight faster than animals treated with vehicle, GH, or EGF. In addition, there were fewer pathologic changes on histological examination of kidneys 7 days after injury. To our knowledge, a histological demonstration of growth factor-induced acceleration of renal repair following acute ischemic injury has not been reported previously. The mechanism for this effect of IGF-I may reflect its actions on renal hemodynamics, renal growth, and its anabolic properties. However, whatever the mechanism may be, a potential for the clinical use of IGF-I, a growth factor that can be safely administered to humans (28), as a therapeutic modality in ATN is clearly established by our findings.

Our results using a model very similar to that used by Humes and co-workers (2, 3) demonstrate that IGF-I can accelerate recovery after ischemic acute renal failure in a manner similar to EGF. EGF has been shown to stimulate IGF-I production in renal collecting duct (29), in cultured human fibroblasts (30), and in rat hepatocytes (31). It is possible that the action of EGF in kidney is mediated, at least in part, by stimulation of IGF-I production.

We acknowledge the administrative assistance of Ms. Lynn Wesselmann. S.B.M. was supported by National Institutes of Health Training Grant DK07126 and by a Clinician Scientist Award from the American Heart Association. M.R.H. was supported by Grants DK-27600, DK-42958, DK-45181, and DK-20579 from the National Institutes of Health, by Grant-in-Aid 91006660 from the American Heart Association, and by Grant 191541 from the Juvenile Diabetes Foundation.

1. Toback, F. G. (1991) *Kidney Int.* **41**, 226–246.
2. Coimbra, T. M., Cieslinski, D. A. & Humes, H. D. (1990) *Am. J. Physiol.* **259**, F438–F443.
3. Humes, H. D., Cieslinski, D. A., Coimbra, T. M., Messana, J. M. & Galvao, C. (1989) *J. Clin. Invest.* **84**, 1757–1761.
4. Norman, J., Tsau, Y., Bacay, A. & Fine, L. G. (1990) *Clin. Sci.* **78**, 445–450.
5. Bier, D. B. (1991) *Acta Paediatr. Scand. Suppl.* **374**, 119–128.
6. Guler, H., Zapf, J., Scheiwiller, E. & Froesch, E. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4889–4893.
7. Hizuka, N., Takano, K., Shizume, K., Asakawa, K., Miyakawa, M., Tanaka, I. & Horikawa, R. (1986) *Eur. J. Pharmacol.* **125**, 143–146.
8. Jacob, R., Barrett, E., Plewe, G., Fagin, K. D. & Sherwin, R. S. (1989) *J. Clin. Invest.* **83**, 1717–1723.
9. Hirschberg, R., Kopple, J. D., Blantz, R. C. & Tucker, B. J. (1991) *J. Clin. Invest.* **87**, 1200–1206.
10. Miller, S. B., Hansen, V. A. & Hammerman, M. R. (1990) *Am. J. Physiol.* **259**, F747–F751.
11. Hammerman, M. R. (1989) *Am. J. Physiol.* **257**, F503–F514.
12. Behringer, R. R., Lewis, T. M., Quaife, C. J., Palmiter, R. D., Brinster, R. L. & D'Ercole, A. J. (1990) *Endocrinology* **127**, 1033–1040.
13. Andersson, G. & Jennische, E. (1988) *Acta Physiol. Scand.* **132**, 453–457.
14. Safirstein, R., Zelent, A. Z. & Price, P. M. (1989) *Kidney Int.* **36**, 810–815.
15. Führ, J., Kaczmarczyk, J. & Krüttgen, C. D. (1955) *Klin. Wochenschr.* **33**, 729–730.
16. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B. & Graber, M. (1945) *J. Clin. Invest.* **24**, 388–404.
17. Miller, S. B., Rotwein, P., Bortz, J. D., Bechtel, P. J., Hansen, V. A., Rogers, S. A. & Hammerman, M. R. (1990) *Am. J. Physiol.* **259**, F251–F257.
18. Kelleher, S. P., Robinette, J. B., Miller, F. & Conger, J. D. (1987) *Kidney Int.* **31**, 725–730.
19. Ogata, K. (1990) *Kidney Int.* **37**, 1333–1340.
20. Dunnett, C. W. (1955) *J. Am. Stat. Assoc.* **50**, 1096–1121.
21. Butkus, D. E. (1983) *Arch. Intern. Med.* **143**, 209–212.
22. Brezis, M., Rosen, S., Silva, P. & Epstein, F. H. (1984) *Kidney Int.* **26**, 375–383.
23. Venkatachalam, M. A., Berrnard, D. B., Donohoe, J. F. & Levinsky, N. G. (1978) *Kidney Int.* **14**, 31–49.
24. Shaw, S. G., Weidmann, P., Holder, J., Zimmermann, A. & Paternostro, A. (1987) *J. Clin. Invest.* **80**, 1232–1237.
25. Flyvbjerg, A., Thorlacius-Ussing, O., Naeraa, R., Ingerslev, J. & Orskov, H. (1988) *Diabetologia* **31**, 310–314.
26. Flyvbjerg, A. K. E., Bornfeldt, K. E., Marshall, S. M., Arnqvist, H. J. & Orskov, H. (1990) *Diabetologia* **33**, 334–338.
27. O'Sullivan, U., Gluckman, P. D., Breier, B. H., Woodall, S., Siddiqui, R. A. & McCutcheonn, S. N. (1989) *Endocrinology* **125**, 2793–2794.
28. Guler, H. P., Schmid, C., Zapf, J. & Froesch, E. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2868–2872.
29. Rogers, S. A., Miller, S. B. & Hammerman, M. R. (1991) *J. Clin. Invest.* **87**, 347–351.
30. Clemmons, D. R. (1984) *J. Clin. Endocrinol. Metab.* **58**, 850–856.
31. Barreca, A., Voci, A., Minute, F., de Marchis, M., Cecchelli, E., Fugassa, E., Giordano, G. & Gallo, G. (1992) *Mol. Cell. Endocrinol.* **84**, 119–126.