J Am Soc Nephrol 12: 1900–1907, 2001

Exacerbated Inflammatory Response Induced by Insulin-Like Growth Factor I Treatment in Rats with Ischemic Acute Renal Failure

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Abstract. In agreement with recent studies showing a deleterious effect of growth hormone treatment in critically ill patients, preliminary data showed that insulin-like growth factor I (IGF-I) administration increased the mortality rate of rats with ischemic acute renal failure (ARF). The present study was designed to investigate the mechanism responsible for this unexpected effect. Male rats with ischemic ARF were given subcutaneous IGF-I, 50 μg/100 g at 0, 8, and 16 h after reperfusion (ARF+IGF-I, $n = 5$) or were untreated (ARF, $n = 5$). A group of 5 sham-operated rats were used as controls. Rats were killed 48 h after declamping, and the following studies were performed: in serum, creatinine and urea nitrogen; and in kidneys, histologic damage score, cellular proliferation by bromodeoxyuridine labeling, apoptosis by morphologic criteria, macrophage infiltration by immunohistochemistry using a specific antibody against ED-1, neutrophil infiltration by naphthol AS-D chloroacetate esterase staining, and levels of IGF-I and IGF-I receptor mRNA by RNase protection assay. ARF and ARF+IGF-I groups had a severe and similar degree of renal failure. Kidney damage was histologically more evident in ARF+IGF-I (1.9 ± 0.1) than in ARF (1.3 ± 0.2) rats, and the number of neutrophils/mm^2 of tissue was significantly greater in ARF+IGF-I than in ARF rats at the corticomedullary junction (52.3 ± 5.2 *versus* 37.2 ± 4.1) as well as at the renal medulla (172.5 ± 30.0 *versus* 42.1 ± 9.6). No other differences between the groups were found. It is concluded that IGF-I treatment enhanced the inflammatory response in rats with ischemic ARF. Cell toxicity derived from increased neutrophil accumulation might play a key role in the greater mortality risk of critically ill patients that are treated with growth hormone.

Acute renal failure (ARF) is a common problem in hospitalized patients and results in significant morbidity and mortality despite advances in treatment. The incidence of ARF has been reported to range from 2 to 5% of hospitalized adults, causing a mortality as high as 50 to 60% (1). Although ARF is less frequent in children, its incidence is increasing in neonatal intensive care units, where it may occur in as many as 10% of newborns (2); the mortality rate of oliguric neonatal ARF ranges from 14 to 63% (3). Therefore, better pharmacologic strategies for the prevention and treatment of ARF are needed (4).

Ischemia remains the leading cause of ARF in adults (5), and prerenal ARF related to perinatal asphyxia accounts for the majority of cases of ARF in newborns (2). Thus, experimental models of ischemic ARF have been used widely to gain an understanding of the cellular consequences of ischemic injury and to test new therapeutic agents.

The hallmark of ischemic cell injury is cellular ATP depletion (6), which rapidly causes marked and complex functional and structural changes in renal epithelial cells, especially those of the proximal tubule (7). As a result, the polarity and physiologic functions of the tubular cell are lost. Depending on the severity and the duration of the ischemic insult, the damaged tubular cell may recover, either directly or through an undifferentiated cell intermediate, or die by necrosis or apoptosis (7).

Theoretically, the therapeutic use of growth factors may exert a beneficial effect on this ischemia-induced chain of cellular events because growth factors are, in general, mitogenic and antiapoptotic. On the basis of this assumption, several studies have reported that exogenous administration of growth factors improves the outcome of animals with ARF (8–11).

The use of insulin-like growth factor I (IGF-I) may have some additional advantages in the treatment of ARF. Besides its mitogenic and antiapoptotic properties, IGF-I mediates most of the systemic anabolic effects of growth hormone (GH), increases renal blood flow and GFR (12), and is widely expressed in kidney (13). Accordingly, IGF-I

Received July 6, 2000. Accepted February 26, 2001.
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1046-6673/1209-1900
Journal of the American Society of Nephrology

has elicited substantial interest as a possible therapeutic agent in ARF. A number of reports, although not all (14), have found that administration of exogenous IGF-I is of some benefit in rats with ischemic ARF (15–19). Despite these promising results in animals, a multicenter clinical trial failed to demonstrate that recombinant human IGF-I administration improves the course of established ARF in humans (20). Likewise, recent clinical studies (21) showed that administration of GH increases the mortality rate of critically ill patients, but the mechanism responsible for this harmful effect remains to be determined.

In light of these clinical reports and of unpublished data from our laboratory indicating that administration of exogenous IGF-I worsened the survival rate of rats with severe ischemic ARF, we designed the study presented here to provide some insight on the mechanism responsible for this potential deleterious effect of IGF-I treatment.

Materials and Methods

Animals and Surgical Procedures

Male Sprague-Dawley rats, weighing approximately 250 ± 10 g at the start of the experiment, were used. Rats were housed in individual cages under controlled conditions of light (12-h light/dark cycle) and temperature (21 to 23°C) and were allowed free access to standard laboratory diet (A03; Panlab, Barcelona, Spain) and tap water. After 3 d of acclimation to the experimental area, and under anesthesia with sodium thiopental (12.5 mg/kg, Tiobarbital 0.5 g; G. Braun Medical, Jaén, Spain) and ketamine chlorhydrate (40 mg/kg, Ketalar 50 mg; Parke Davis, Madrid, Spain), the abdominal cavity was exposed via a midline incision. Both renal pedicles were identified and occluded with microvascular clamps (Biemer, FD 562; Aesculap, Tuttlingen, Germany) for 75 min. Core body temperature was maintained at 37°C using a homeothermic table during surgery. After the period of ischemia, the clamps were removed and blood flow returned to the kidneys. When reperfusion was incomplete, as judged visually, the rat was killed and was not considered for statistical purposes. Finally, the abdominal wall was closed in two layers, and rats were given an intraperitoneal injection of 1 ml/100 g body wt of prewarmed (37°C) saline (0.9% NaCl) to compensate for any fluid loss during surgery. Sham-operated rats underwent a similar surgical procedure but without clamping of the renal pedicle. The day of surgery was considered as day 0 of the experiment.

Preliminary Study

ARF rats were assigned to receive three 0.1-ml doses of 50 μg/100 g body wt of recombinant human IGF-I (Pharmacia Upjohn, Stockholm, Sweden) or an equivalent volume of vehicle (saline) subcutaneously at 0, 8, and 16 h after declamping. A group of sham-operated rats that were treated with vehicle were used as controls. Tail-vein blood samples were obtained daily for 7 d for serum creatinine and serum urea nitrogen measurements, using a Kodak Ektachem DT60 analyzer (Rochester, NY). The mortality rate of the three groups of rats is shown in Figure 1. In the IGF-I–treated ARF group, 89% of rats died (33 of 37), whereas ARF rats that received vehicle had significantly ($P < 0.05$, Kaplan Meier analysis followed by log rank test) less mortality (17 of 27 rats [63%]). Serum creatinine and urea nitrogen profiles in the three groups of rats are shown in Figure 2. An apparent beneficial effect of IGF-I treatment may be observed from day 3 on.

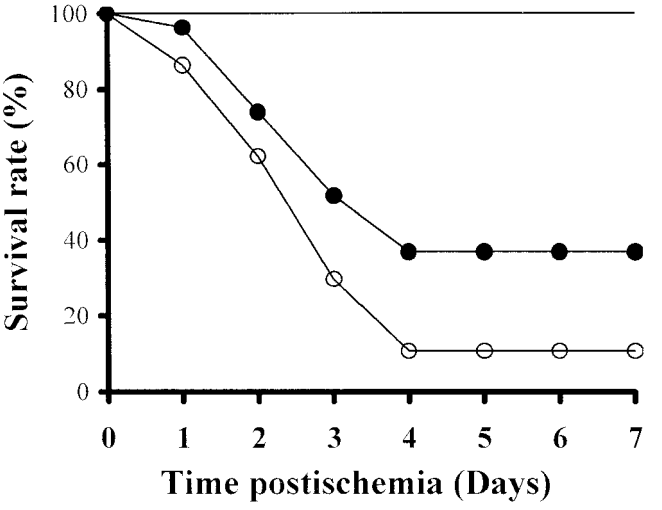

Figure 1. Survival rate as a function of the time in sham-operated rats (—) or rats subjected to 75 min of bilateral clamping of the renal pedicles and treated with vehicle (●) or insulin-like growth factor I (IGF-I; ○).

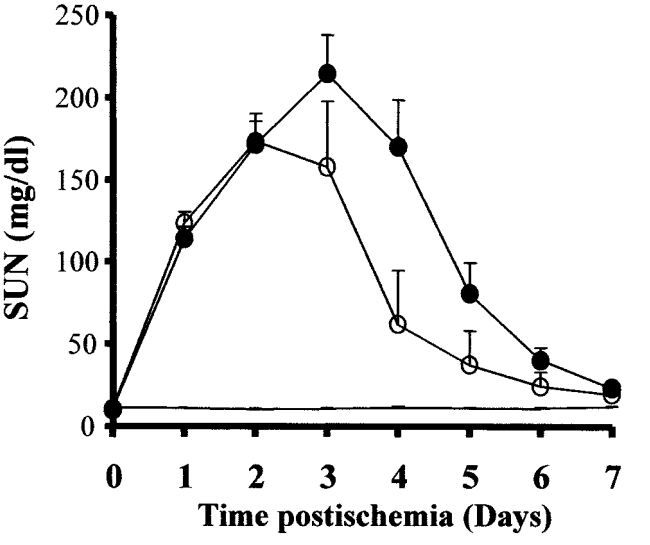

Figure 2. Mean (±SEM) values of daily serum urea nitrogen (SUN) in sham-operated rats (—) or rats subjected to 75 min of bilateral clamping of the renal pedicles and treated with vehicle (●) or IGF-I (○).

Short-Term Study

ARF rats were assigned to receive three 0.1-ml doses of 50 μg/100 g body wt of recombinant human IGF-I (Pharmacia Upjohn, Stockholm, Sweden) or vehicle (saline) subcutaneously at 0, 8, and 16 h postischemia. Sham-operated rats that were treated with vehicle were used as controls. The number of rats per group varied so as to obtain five rats at the moment of killing. Two days after the ischemic insult, rats were anesthetized and killed by exsanguination, via abdominal aorta. Both kidneys were collected for histologic study or RNA analysis. Tail-vein blood samples were drawn daily for determination of serum concentrations of creatinine and urea nitrogen.

Histologic Examination. The kidneys were removed, stripped of their capsules, cut longitudinally into halves, fixed in 10% formalde-

Table 1. Concentrations (±SEM) of serum urea nitrogen and creatinine in the three groups of rats[a]

Group	Serum Urea Nitrogen (mg/dl)			Serum Creatinine (mg/dl)		
	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
Sham	12 ± 1	11 ± 0	10 ± 1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
ARF	11 ± 0	114 ± 7[b]	171 ± 14[b]	0.2 ± 0.0	2.1 ± 0.2[b]	3.5 ± 0.3[b]
ARF+IGF-I	11 ± 0	123 ± 7[b]	173 ± 14[b]	0.2 ± 0.0	2.5 ± 0.2[b]	2.8 ± 0.3[b]

[a] Sham, sham-operated rats; ARF, rats with acute renal failure treated with vehicle; ARF+IGF-I, rats with acute renal failure treated with insulin-like growth factor I (IGF-I).
[b] Significantly different ($P < 0.05$) from the Sham group.

Table 2. Histopathologic scores and BrdU labeling index in the three groups of rats[a]

Group	Score	BrdU-LI (% of Cells)
Sham	0.0 ± 0.0	0.4 ± 1.7
ARF	1.3 ± 0.2[b]	10.3 ± 2.6[b]
ARF+IGF-I	1.9 ± 0.1[b,c]	12.0 ± 1.9[b]

[a] Data are ± SEM from five rats per group. BrdU-LI, 5-bromo-2′-deoxyuridine labeling index.
[b] Significantly different from Sham group.
[c] Significantly different from ARF.

hyde, and embedded in Paraplast (Fluka, Madrid, Spain). Sections of 3 μm were obtained and mounted on glass slides. Different sets of slides were stained with hematoxylin and eosin, periodic acid-Schiff, and naphthol AS-D chloroacetate esterase stains.

Scoring of Renal Damage. Hematoxylin and eosin and periodic acid-Schiff samples were graded by an independent observer using a scoring system that considered the pathologic changes consistent with acute tubular necrosis: peritubular hyperemia at the corticomedullary junction, protein casts, and intratubular cell shedding (22). Grades were added together and divided by 3 to give a final score of 0 to 3. At least 12 microscopic fields per section were chosen randomly to be scored for each parameter.

Neutrophil Infiltration. Neutrophil infiltration was evaluated using naphthol AS-D chloroacetate esterase staining by counting the number of neutrophils present at the corticomedullary junction and at the renal medulla. Sixty microscopic fields per rat were studied. Data are expressed as neutrophils per mm^2 of tissue.

Macrophage Infiltration. Macrophage infiltration was assessed by immunohistochemistry using a mouse monoclonal antibody against ED-1 (Serotec, Oxford, UK; 1:100). After incubation with the primary antibody, sections were incubated with peroxidase-labeled sheep anti-mouse antibody (Amersham, Little Chalfont, Bucks, UK; 1:100). The number of macrophages was counted at the corticomedullary junction and at the renal medulla. Sixty microscopic fields per rat were analyzed. Data are expressed as macrophages per mm^2 of tissue.

Apoptosis. Morphologic criteria were used to identify apoptotic cells, which were counted in 20 high-power fields per section (×400) at the corticomedullary junction. Characteristics of apoptosis included cellular rounding and shrinkage, eosinophilic cytoplasm, nuclear chromatin compaction especially along the nuclear envelope in a crescentic manner, membrane-bound cellular blebbing, and formation of apoptotic bodies (23).

DNA Synthesis. Sixty minutes before killing, rats received an intraperitoneal injection of 100 mg/kg body wt of 5-Bromo-2'-deoxyuridine (BrdU; Sigma, St. Louis, MO) in saline (10 mg/ml). Tissue samples were obtained and processed as stated above. BrdU-labeled nuclei were identified as described elsewhere (24). A labeling index (100 × tubular cells labeled with BrdU/total number of tubular cells) was calculated. At least 1×10^3 cells per section were studied.

RNase Protection Assay. *Probes.* Antisense rat IGF-I receptor RNA probe was synthesized using the *Eco*RI linearized plasmid pGEM-3 (Promega, Madison, WI), which contains a 265-bp *Hin*dIII fragment of the rat IGF-I receptor. Antisense rat IGF-I RNA probe was synthesized from a 376-bp *Eco*RI/*Hin*dIII fragment cloned into the pGEM-3 vector. Both plasmids were used as a template for synthesis of ^{32}P-UTP-labeled RNA probes with SP6 RNA polymerase according to the instructions given by the manufacturer (SP6/T7 Transcription Kit; Boehringer Mannheim GmbH, Mannheim, Germany). A commercially available linearized plasmid into which a cDNA fragment of the rat cyclophilin gene had been subcloned was purchased from Ambion (pTRI-cyclophilin-Rat; Ambion Inc., Austin, TX), and a cyclophilin cRNA was synthesized.

Table 3. Number of neutrophils (neutrophils/mm^2) and macrophages (macrophages/mm^2) at the corticomedullary junction and at the renal medulla in the three groups of rats killed 48 h after the ischemic insult[a]

Group	Corticomedullary Junction		Renal Medulla	
	Neutrophils/mm^2	Macrophages/mm^2	Neutrophils/mm^2	Macrophages/mm^2
Sham	0.2 ± 0.0	0.7 ± 0.6	0.8 ± 0.4	0.3 ± 0.1
ARF	37.2 ± 4.1[b]	28.6 ± 9.4[b]	42.1 ± 9.6[b]	1.4 ± 0.1[b]
ARF+IGF-I	52.3 ± 5.2[b,c]	27.8 ± 5.0[b]	172.5 ± 30.0[b,c]	1.7 ± 0.4[b]

[a] Data are ± SEM from five rats per group.
[b] Significantly different from Sham group.
[c] Significantly different from ARF.

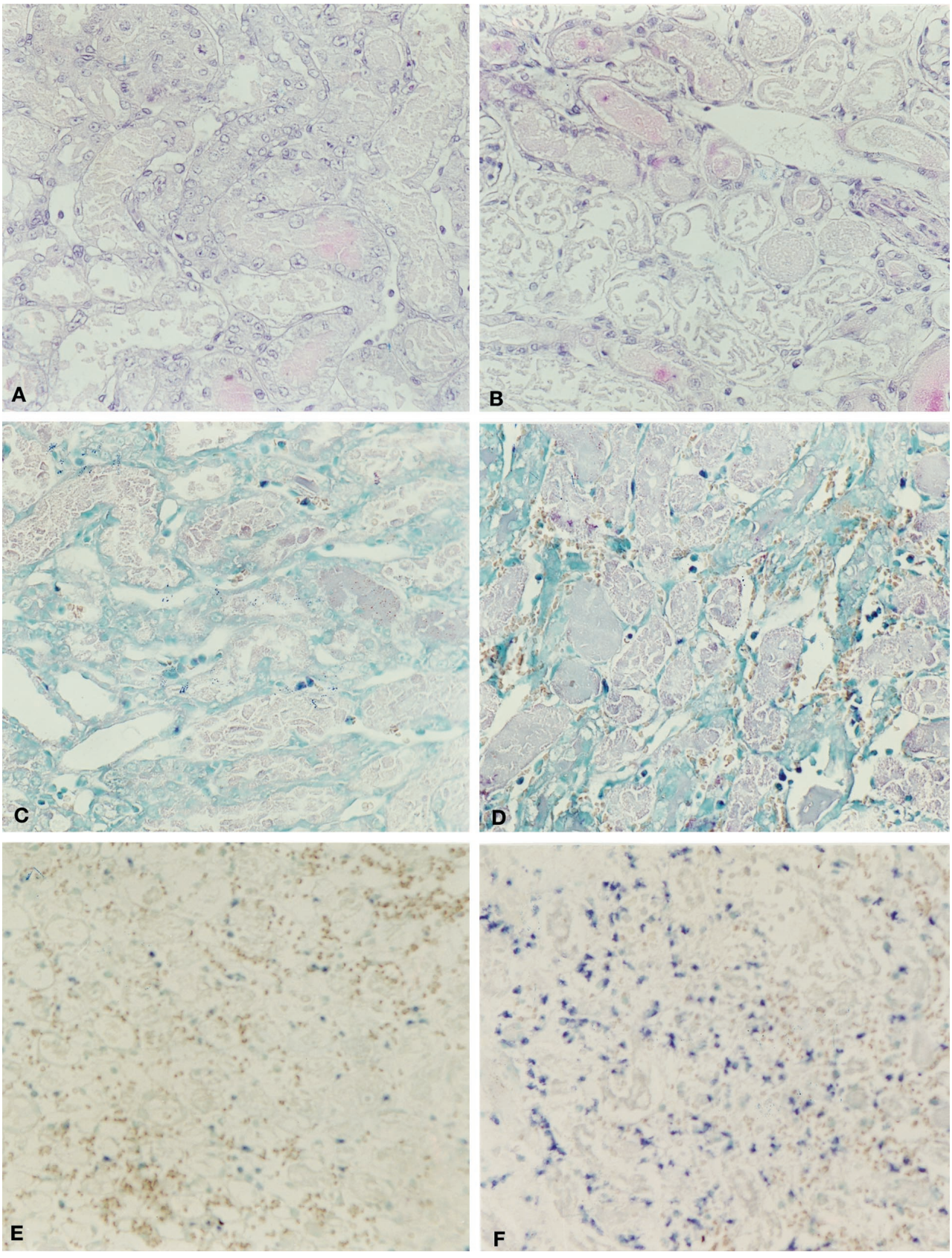

Figure 3. Representative hematoxylin and eosin–stained renal sections from untreated (A) and IGF-I–treated (B) rats, illustrating more severe lesions of acute tubular necrosis in treated rats. Neutrophil infiltration in two representative kidney sections of corticomedullary and medullary areas of untreated (C, E) and IGF-I–treated (D, F) rats, respectively. Infiltration of neutrophils, seen as deep-blue dots, clearly is more prominent in the treated rats, particularly at the medulla. Magnification, ×280.

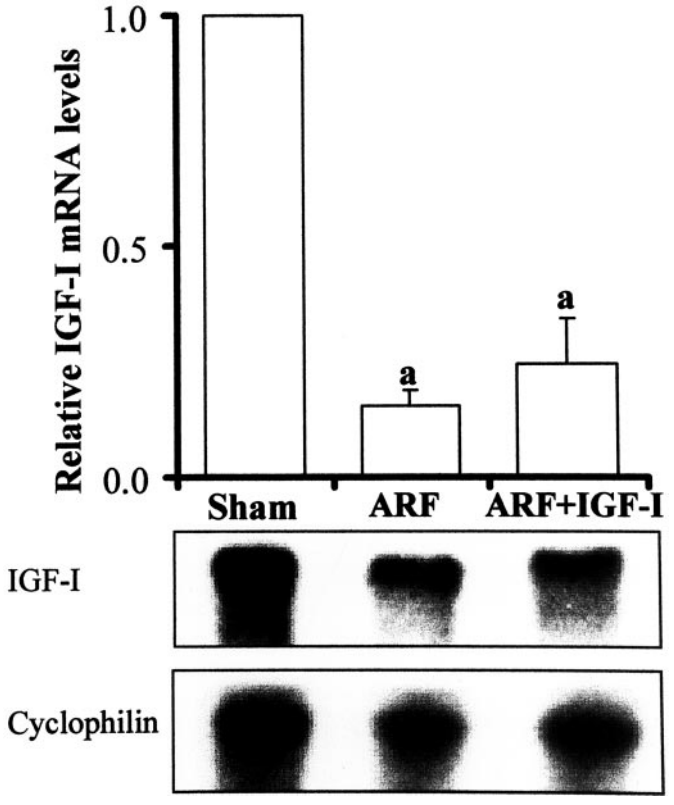

Figure 4. Expression of IGF-I mRNA in kidney tissue of the three groups of rats. Sham, sham-operated rats; ARF, rats with acute renal failure treated with vehicle; ARF+IGF-I, rats with acute renal failure treated with IGF-I; a, significantly different from Sham group. Bar graph shows IGF-I mRNA levels (mean ± SEM) quantified as described in the Materials and Methods section. On the bottom, a representative RNase protection assay experiment is shown.

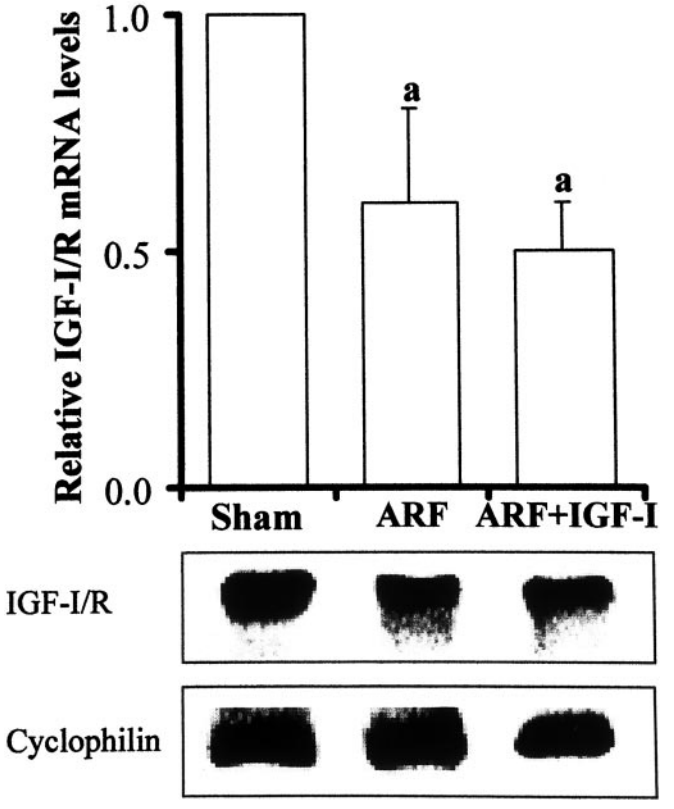

Figure 5. Expression of IGF-I receptor (IGF-I/R) mRNA in kidney tissue of the three groups of rats. Sham, sham-operated rats; ARF, rats with acute renal failure treated with vehicle; ARF+IGF-I, rats with acute renal failure treated with IGF-I; a, significantly different from Sham group. Bar graph shows IGF-I/R mRNA levels (mean ± SEM) quantified as described in the Materials and Methods section. On the bottom, a representative RNase protection assay experiment is shown.

Solution Hybridization. Total RNA was extracted from 50 to 100 mg of kidney tissue of each rat according to the method of Chomczynski and Sacchi (25). A solution hybridization assay was used to quantify IGF-I and IGF-I receptor. In brief, the probes were labeled and hybridized to rat renal RNA using a ribonuclease protection assay kit (RPA II; Ambion) and following the indications given by the manufacturer. The protected fragments were separated on an 8-mM/L urea/6% polyacrylamide denaturing gel. The gels were dried and exposed to Hiperfilm-MP (Amersham Ibérica, Madrid, Spain) with two intensifying screens at −70°C. The radioactivity of the hybridized probes was measured by densitometric scanning of the x-ray films with a Bioimage densitometer (Millipore, Ann Arbor, MI) coupled to a computer with Analysis 1-D program (1D Manager; TDI, Madrid, Spain). Data for IGF-I and IGF-I receptor were adjusted for the corresponding cyclophilin reading and expressed as arbitrary densitometric units. For each gel, the measurement of sham-operated rats was assigned a value of 1 and the results of ARF and ARF+IGF-I groups were estimated in relation to this value.

Statistical Analyses

Data are given as mean ± SEM. Comparisons were made by one-way ANOVA at a level of significance of 95%. Student-Newman-Keuls test was performed for comparisons between two groups.

Results

Renal Function

As shown in Table 1, baseline concentrations of serum urea nitrogen and creatinine were similar in the three groups of rats. Clamping of renal pedicles resulted in a marked degree of renal failure as demonstrated by maximal elevation of basal serum concentrations of urea nitrogen and creatinine up to 17 times, approximately. No difference in the degree of renal failure was found between ARF and ARF+IGF-I rats.

Pathological Changes

Forty-eight hours after reperfusion, widespread necrosis, sloughing of the proximal straight tubules with obstructing casts, different degrees of brush border loss, and detached tubular cells were seen in kidney samples from ARF and ARF+IGF-I rats (Figure 3). As shown in Table 2, IGF-I administration increased ($P < 0.05$) the score of renal damage in comparison with ARF rats.

DNA Synthesis

Proliferative activity of tubular cells, as assessed by the percentage of BrdU-labeled cells, was much higher in ARF and

ARF+IGF-I groups; no difference was found between the two groups of rats with renal failure (Table 2).

Neutrophil Infiltration

Neutrophils accumulated at the corticomedullary junction and the renal medulla in both groups of clamped rats (Table 3, Figure 3). As compared with the ARF group, the number of infiltrating neutrophils was significantly ($P < 0.05$) higher in the IGF-I–treated rats; the difference was even more marked at the renal medulla.

Macrophage Infiltration

The number of macrophages was higher ($P < 0.05$) in ARF and ARF+IGF-I rats at the corticomedullary junction as well as at the medulla (Table 3) in comparison with the controls. There was no difference between the two groups of rats with renal failure.

Apoptosis

Similarly increased numbers of apoptotic cells were found in ARF (42.9 ± 7.0 per mm^2) and ARF+IGF-I (47.6 ± 9.9 per mm^2) as compared with sham-operated rats (8.2 ± 2.9 per mm^2; $P < 0.05$).

Renal Expression of IGF-I mRNA

RNase protection assay for IGF-I mRNA in kidney tissue revealed a band of 376 bp. A representative experiment is shown in Figure 4. IGF-I mRNA levels were significantly ($P < 0.05$) reduced in the two groups of rats with renal failure in comparison with sham-operated rats (ARF, 0.1 ± 0.0; ARF+IGF-I, 0.2 ± 0.1; sham, 1.0 ± 0.0). There was no difference between the ARF and ARF+IGF-I groups.

Renal Expression of IGF-I Receptor mRNA

RNase protection assay for IGF-I receptor mRNA in kidney tissue revealed a band of mRNA of 265 bp. A representative experiment is shown in Figure 5. IGF-I receptor mRNA levels were significantly ($P < 0.05$) reduced in the two groups of rats with renal failure in comparison with sham-operated rats (ARF, 0.6 ± 0.2; ARF+IGF-I, 0.5 ± 0.1; sham, 1.0 ± 0.0). No difference was found between the ARF and ARF+IGF-I groups.

Discussion

Analysis of the results of our preliminary study (Figures 1 and 2) raises the issue that administration of IGF-I seemed to protect the renal function of treated rats and, at the same time, increased the mortality rate of this group of rats. This seeming paradox may be justified by a methodological bias caused by the fact that serum urea nitrogen and creatinine values were calculated on the basis of surviving rats on each day of the study. If, as suggested by our findings, administration of IGF-I was an aggravating factor that increased the risk of death, the treated rats that had a severe degree of renal failure died and only rats with milder renal impairment survived. However, a greater percentage of untreated ARF rats with severe renal failure survived, giving rise to higher concentrations of serum urea nitrogen and creatinine in this group of rats. This distortion of the results may have been overlooked in some of the former reports that analyzed the effect of IGF-I treatment in animals with ischemic ARF. In fact, data on mortality rate frequently have been omitted in these studies (15,16,18). However, a different response to exogenous IGF-I derived from different experimental conditions and/or a different severity of ischemia-induced renal failure cannot be ruled out. It is of note that the doses and timing of administration of IGF-I used in our protocol were similar to those reported in other studies (16,26).

Our short-term study was designed to shed light on the mechanism responsible for the increased mortality induced by IGF-I treatment. In agreement with the findings on day 2 of the preliminary 1-wk experiment, the serum concentrations of urea nitrogen and creatinine were equivalent in the ARF and ARF+IGF-I groups. However, the histologic damage was more marked in the kidneys of rats that were treated with IGF-I (Table 2). Thus, it can be assumed that if the rats had been allowed to live longer, then a greater risk of death, resulting from a more severe renal lesion, had existed in the IGF-I–treated group.

The analysis of cellular proliferation, apoptosis, and local expression of IGF-I and IGF-I receptor mRNA revealed that IGF-I treatment caused no changes beyond those already induced by ischemia. Thus, it seems that some of the main events linked to the reparative process after the ischemic insult were not modified substantially by exogenous administration of IGF-I. By contrast, the administration of IGF-I markedly enhanced the accumulation of neutrophils in the kidney. In comparison with the control rats, the neutrophil count in untreated ARF rats 48 h postischemia increased by a factor of 180 at the corticomedullary junction and by a factor of 50 at the renal medulla. In ARF rats that were treated with IGF-I, the accumulation of neutrophils was markedly higher, reaching values well over 200 times those of untreated ARF rats both at the corticomedullary junction and the renal medulla. This effect of IGF-I administration was in some way specific for neutrophils because it was not observed for macrophages. To determine whether the neutrophil infiltration was resolved with the recovery from acute renal failure and whether massive neutrophil accumulation found in the ARF+IGF-I group was transient and dependent on IGF-I administration, we also investigated neutrophil infiltration in stored kidney samples of rats from the preliminary study, *i.e.*, rats killed 7 d after clamping. In comparison with the neutrophil infiltration observed on the second day postischemia (Table 3), the number of neutrophils per mm^2 of tissue decreased to 7.7 ± 1.4 and 12.7 ± 4.6 at the corticomedullary junction and to 3.2 ± 0.4 and 3.3 ± 0.2 at the renal medulla in ARF ($n = 5$) and ARF+IGF-I ($n = 4$), respectively. These figures, although still higher than those observed in sham-operated rats (0.2 ± 0.1 and 0.3 ± 0.1), were markedly lower than those found 48 h after clamping. Moreover, there was no difference between the ARF and ARF+IGF-I groups. The reduction of neutrophil infiltration found 7 d after clamping clearly is consistent with an inflammatory response related to the acute period of renal failure and markedly exacerbated by IGF-I administration.

With the use of indium-labeled neutrophils, it has been shown that the number of neutrophils retained in the kidney is dependent on the duration of renal ischemia and the activity state of the neutrophil (27). The recruitment, activation, and transendothelial migration of neutrophils after ischemic aggression is a complex process that has been investigated extensively in recent years (5,28,29). It is mediated by a variety of molecules such as selectins, integrins, Ig-like adhesion molecules, and chemokines. Several lines of evidence now indicate an important role of this accumulation of neutrophils in renal ischemia/reperfusion injury (5,28,29).

Despite that inflammation is considered to be the most important cause of tissue injury in organs subjected to ischemia, the effect of IGF-I treatment on the inflammatory response has not been analyzed in rats with ischemic ARF. Miller *et al.* (15) reported the degree of peritubular hyperemia observed in IGF-I treated rats as a criteria of histologic damage. However, specific histochemical staining for neutrophils (chloroacetate esterase reaction) or assays for neutrophil-derived enzymes such as myeloperoxidase were not used. It also is of note that vascular changes found in kidneys of rats with ischemic ARF treated with IGF-I may be the result not only of the effect of ischemia itself but also of the specific renal hemodynamic actions of IGF-I and, therefore, may not be a reliable index of the underlying inflammatory response. Using a murine model, Daemen *et al.* (30) suggested that apoptosis may be the mechanism that triggers inflammation after ischemia and that IGF-I, by means of its antiapoptotic potential, may prevent reperfusion-induced inflammation and subsequent tissue injury. In the same study, it also is shown that administration of IGF-I fails to prevent loss of kidney function and inflammation when given once apoptosis of renal cells has started. Thus, the timing of IGF-I action may be important, and it may be hypothesized that an earlier administration of IGF-I, before the ischemic insult, and/or the use of a route of administration with a faster absorption of the peptide might have led to a favorable response in our treated rats.

The association between increased mortality and enhanced neutrophil accumulation observed in our IGF-I–treated rats with severe renal failure also supports the contention that the greater risk of death caused by treatment with high doses of GH in critically ill patients (21) may be linked to the hypermetabolic and proinflammatory effects of GH (31). Because IGF-I mediates most of GH actions, it is tempting to speculate that an IGF-I–induced exacerbated inflammatory response might play a pivotal role in this increased risk of death.

In conclusion, in the study presented here, IGF-I treatment increased mortality rate of rats with severe ischemic ARF. The higher risk of death was associated with a massive accumulation of neutrophils in kidney tissue, indicating that IGF-I administration may have led to an exacerbation of the inflammatory response caused by the process of ischemia-reperfusion. Our study does not provide any clue to the mechanism that is responsible for this IGF-I–induced proinflammatory effect that, however, might play an important role in the deleterious effect of GH therapy observed in critically ill patients.

Acknowledgments

This work was supported in part by grants PM 96–0110, FIS 97–0556, and FIS 00/0140 from the Spanish Ministries of Science and Education and Health. We are grateful to Pharmacia-Upjohn for supplying the recombinant human IGF-I and to Dr. Derek Le Roith and Dr. Charles Roberts for the gentle provision of the IGF-I and IGF-I receptor DNA probes.

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