Insulin-Like Growth Factor-1 (IGF-1) Enhances Recovery From HgCl₂-Induced Acute Renal Failure: The Effects on Renal IGF-1, IGF-1 Receptor, and IGF-Binding Protein-1 mRNA

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ABSTRACT
Several growth factors have been found to play an important role in the recovery from acute renal failure (ARF). The effect of the continuous subcutaneous infusion of human recombinant insulin-like growth factor (IGF)-1 (125 μg daily by osmotic minipumps) in a rat model of mercuric chloride (HgCl₂)-induced ARF was examined. HgCl₂ (4 mg/kg) induced ARF with a mortality that was unaffected by IGF-1. However, IGF-1 significantly enhanced functional and histologic recovery in the survivors, as measured by serum creatinine and creatinine clearance and by histologic scoring. Solution hybridization RNAase protection assays showed that renal IGF-1 mRNA, IGF-1 receptor (IGF-1R) mRNA, and IGF-binding protein-1 (IGFBP-1) mRNA were unaffected by exogenous IGF-1, but this treatment significantly increased renal IGF-1 in ARF rats compared with normal rats and ARF rats not receiving IGF-1. After ARF renal mRNA for IGF-1 was decreased, IGF-1R was unchanged and IGFBP-1 was increased. Similar changes occurred in IGF-1-infused ARF rats. Thus, (1) IGF-1 enhances recovery from nephrotoxic ARF both functionally and histologically; (2) in nephrotoxic ARF, there is (a) a reduction in IGF-1 mRNA expression that is not prevented by IGF-1 infusion, and (b) an increase in renal IGFBP-1 mRNA. This may allow a significant increase in renal IGF-1 levels in IGF-1-infused ARF rats, despite the decrease in renal IGF-1 mRNA. A local increase in renal IGFBP-1 and IGF-1 may explain the accelerated recovery from ATN in this model. It was concluded that HgCl₂-induced ARF is amenable to improvement by IGF-1 infusion and that the increase in renal IGFBP-1 mRNA may be an important modulator in the recovery of the kidney.

Key Words: Mercuric chloride nephrotoxicity, insulin-like growth factor-1 (IGF-1) treatment, IGF-1 mRNA, IGF-1R mRNA, IGF-binding protein-1 mRNA

Insulin-like growth factor-1 (IGF-1) is a peptide of 70 amino acids synthesized in the liver and also in many other tissues (1,2). In the kidneys, the peptide and its mRNA are found in the medullary collecting duct epithelial cells and lesser amounts are also found in distal convoluted tubules, thick ascending loop of Henle, the straight S3 portion of the proximal tubule, and mesangial cells (3–7). Isolated tubule segment and renal cell cultures have also shown the collecting ducts to be the main source of renal IGF-1 (8,9). Receptors for IGF-1 are found both in the medulla and at the basolateral surface of the proximal tubule epithelium (10), where IGF-1 has been shown to promote phosphate and sodium transport and gluconeogenesis (11–13). Exogenous IGF-1 has been shown to increase GFR and RBF (14,15). Renal growth in the developing kidney and after the loss of renal tissue has been found to be associated with an increase in renal IGF-1 and IGF-1 mRNA content (10).

Recent reports have suggested a role for several growth factors in the recovery of renal function after acute renal failure (ARF) (16–23). However, the mechanism by which this occurs is far from clear. Some workers have found an increase in renal IGF-1 and IGF-1 mRNA expression during recovery from postischemic ARF, with more widespread distribution along the nephron, especially in the regenerating cells of the outer stripe of the outer medulla (21,24). IGF-1 binding was also increased in these areas. Others have observed a decrease in renal IGF-1 mRNA after nephrotoxic gentamicin-induced ARF (25). The binding of labeled IGF-1 under these conditions appears to be increased in the regenerating nephron (24), but it is unclear if this is by a change in receptor number or affinity or by other mechanisms such as a local increase in IGF-binding proteins (IGFBP). Tissue IG-
FBP-1 is an important modulator of the biologic actions of IGF-1, affecting the binding of IGF-1 to its receptor, its localization within different tissues, and its biologic activity (26,27). It is of interest that others have noted a decrease in renal epithelial growth factor mRNA after ARF, whereas radioactive epithelial growth factor binding increased two- to threefold, suggesting decreased gene expression but increased affinity for this growth factor in the surviving cells (18).

To further examine the role of IGF-1 in the recovery of the kidney after ARF, we have studied the effect of the continuous administration of exogenous IGF-1 to a model of nephrotoxic ARF in rats. We have also studied the concomitant changes in the renal gene expression of IGF-1, IGF-1 receptor (IGF-1R), and IGFBP-1. Our observation that IGF-1 enhances the recovery from ARF is the first report of this action of IGF-1 in a nephrotoxic model of ARF.

METHODS

Male Sabra rats weighing 170 to 200 g were placed in individual metabolic cages with free access to tap water and standard rat chow. After 7 days of acclimatization, ARF was induced by the sc injection of mercuric chloride (HgCl₂). Two hours later, unprimed sc osmotic minipumps (Model 2001; Alzet Corp., Palo Alto, CA) were implanted with the rats under light ether anesthesia to deliver 125 µg daily of recombinant human IGF-1 (donated by Ciba-Geigy, Basel, Switzerland) or its vehicle (0.1% acetic acid). Body weight and food and water intake were recorded daily. Blood obtained from tail veins and 24-h urine samples were analyzed for creatinine and sodium concentration by automated analysis and flame photometry, respectively.

Histologic examination and RNA extraction were performed on kidneys removed from other rats treated similarly but housed in regular cages. Kidneys were also obtained from control groups of rats that had received sc saline injections before the implantation of IGF-1- or vehicle-containing minipumps. The rats were euthanized by aortic puncture desanguination under ether anesthesia. The kidneys were decapsulated, and equal quadrants were immediately frozen in liquid nitrogen for RNA extraction or placed in buffered 4% gluteraldehyde for simple histology.

Tissue mRNA Assay

These assays for IGF-1, IGF-1R, and IGFBP-1 were performed by a solution hybridization–RNAase protection assay as follows: Total RNA was prepared from the renal tissue of individual rats by Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the method of Chomczynski (28) and quantified by absorbance at 260 nm. The integrity of the RNA and the accuracy of the spectrophotometric determinations were assessed by visual inspection of the ethidium bromide–stained 28S and 18S ribosomal RNA bands after agarose formaldehyde gel electrophoresis of 10-µg aliquots as described previously (29). The IGF-1, IGF-1R, and IGFBP-1 riboprobe were generous gifts from Derek LeRoith (NIH, Bethesda, MD).

The antisense RNA probe used to detect IGF-1R mRNA has been previously described (30). This transcript contains 40 bases of vector sequence and 265 bases complementary to 15 bases of 5'-untranslated sequence and to the region encoding the signal peptide and the first 53 amino acids of the IGF-1R α-subunit. On hybridization of this RNA probe with IGF-1R RNA and subsequent RNAase digestion, a protected band of 265 bases was obtained.

The riboprobe used to measure the levels of IGF-1 mRNA was described previously (31). This probe allows the detection of both IGF-1 mRNA species encoding the IGF-1a and IGF-1b prohormones. Only the levels of IGF-1a mRNA, which constitute >90% of the total IGF-1 message and correlate with the levels of IGF-1b mRNA, were measured in this study.

The IGFBP-1 mRNA was measured with an antisense probe derived from a rat cDNA clone isolated from a dexamethasone-treated H-4-11-E-C3 hepatoma cell library (G.T. Ori, unpublished observations). The size of the protected band obtained by hybridizing this antisense RNA probe with IGFBP-1 mRNA was 203 bases.

Solution hybridization–RNAase protection assays were performed as described (32). Briefly, 20 µg of total RNA were hybridized with 10⁶ dpm 32P-labeled antisense RNA probes. The hybridization was carried out at 45°C for 16 h in a buffer containing 80% formamide. After hybridization, RNA samples were digested with RNAase A and T₁ and the protected

Figure 1. Serum creatinine (CREAT) (a) and creatinine clearance (b) in rats after the sc injection of HgCl₂ (5 mg/kg) with or without the subsequent continuous sc administration of IGF-1 (125 µg daily).
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Figure 2. Histology (hematoxylin and eosin, x200) of rat kidneys 3 days after the sc injection of HgCl₂ (5 mg/kg). (a) Outer medullary stripe showing exposed tubular basement membrane, necrotic material in lumen, and little or no regeneration. (b) Outer medullary stripe from IGF-1-treated rat showing some regeneration with occasional mitoses and a thin cellular lining on the tubular basement membrane.

Serum Concentration of IGF-1

These concentrations were quantitated by first dissociating circulating IGF-1 from its carrier protein by treatment with 0.5 N HCl, followed by chromatography through a Sep-Pak column (Waters Associates, Milford, MA). IGF-1 radioimmunoassays were performed with an IGF-1¹²⁵I RIA Kit (Incastar Corp., Stillwater, MN).

Tissue Concentration of IGF-1

These concentrations were quantitated after homogenization of whole frozen kidneys in 1 M acetic acid. After centrifugation, the supernatant was lyophilized, reconstituted in bovine serum albumin-borate buffer, and assayed as described above.

Histologic Scoring

This was performed in a blinded fashion by one of us (J.K.) according to a modified version of the method of Weinberg et al. (33). In brief, 100 tubules in the outer stripe of the renal medulla were scored from each kidney: 1, normal tubule; 2, flattening of the tubular epithelium with some cytoplasmic extrusion into the lumen, which contains a small amount of cellular debris; 3, patchy necrosis of the tubular epithelial cells with necrotic debris in the lumen; and 4, complete tubular epithelial cell necrosis with necrotic debris in the tubular lumen, some of which is calcified. Statistical analyses were by paired or unpaired t test with the Bonferroni correction for multiple comparisons where indicated.

RESULTS

In an initial pilot study with 5 mg/kg sc HgCl₂ in eight rats receiving IGF-1 and seven rats receiving vehicle, three rats in each group did not survive. Serum creatinine and creatinine clearance of the survivors are shown in Figure 1. Worsening in renal...
Renal histology showed marked recovery in IGF-1-infused animals compared to vehicle-treated controls (Figure 5). Quantitative morphologic scoring (Table 2) revealed significantly less tubular damage in IGF-1-treated animals. Normal tubules (Grade 1) were not observed in any sections. Mild (Grade 2) damage was more frequent in IGF-1-treated animals, whereas Grade 4 damaged tubules were only seen in sections from animals that did not receive exogenous IGF-1.

Renal mRNA of IGF-1, IGF-1R, and IGFBP-1

In control rats without renal failure, a 3-day infusion of IGF-1 caused no change in the renal expression of IGF-1 mRNA, IGF-1R mRNA, or IGFBP-1 mRNA (Figures 6 and 7). In rats with ARF for 3 days, the expression of IGF-1 mRNA was decreased by 34% (P < 0.01) as compared with normal controls (Figure 6). A decrease also occurred in ARF rats receiving exogenous IGF-1 infusion, but the decrease just failed to reach significance as compared with normal controls (Figure 8). IGF-1R mRNA expression was unchanged in ARF rats, and 3 days of IGF-1 infusion did not appear to affect this (Figure 8). The expression of IGFBP-1 mRNA was increased three- to fourfold (P < 0.01) in ARF rats (Figures 7 and 9), and exogenous IGF-1 infusion slightly blunted this increase (P = 0.05) (Figure 9). In rats with ARF for 5 days, IGF-1 mRNA was still decreased (P < 0.05) and IGFBP-1 mRNA was increased (P < 0.05), whereas IGF-1R mRNA was unchanged from normal controls (data not shown). A schematic summary of these findings is shown in Figure 10.

Treatment with HgCl₂ resulted in a significant reduction in serum IGF-1 (162 ± 5.6 versus 198 ± 7.2 nM; P < 0.05), whereas supplementation with exogenous IGF-1 to these rats resulted in a significant increase in serum IGF-1 levels as compared with control rats (270 ± 12.4 versus 198 ± 7.2 nM; P < 0.05). Compared with normal rat kidney, renal IGF-1 levels were minimally increased in HgCl₂-induced ARF kidneys not treated with IGF-1 (176 ± 9.9 versus 158.2 ± 5.8 pg/mg tissue; P = not significant [NS]) in normal kidneys treated with exogenous IGF-1 (166.8 ± 3.8 versus 154.0 ± 6.6 pg/mg tissue; P = NS). However, exogenous IGF-1 caused a significant increase in renal IGF-1 levels in HgCl₂-induced ARF kidneys (242.4 ± 16.8 versus 158.2 ± 5.8 pg/mg tissue; P < 0.01). Serum insulin levels were not af-

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TABLE 1. Changes in food and water intake, weight, and renal function in surviving control (N = 8) and IGF-1-infused (N = 8) rats after HgCl2-induced ARF

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IGF-1 Baseline</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (change) (g)</td>
<td>+ 204 ± 6.5</td>
<td>-7.4 ± 4.9</td>
<td>6.0 ± 5.5</td>
<td>17.6 ± 5.7</td>
</tr>
<tr>
<td>Food Intake (g/24 h)</td>
<td>- 212 ± 5.9</td>
<td>-13.1 ± 1.6</td>
<td>-9.8 ± 3.8</td>
<td>0.4 ± 3.1</td>
</tr>
<tr>
<td>Water Intake (mL/24 h)</td>
<td>+ 164 ± 1.5</td>
<td>10.5 ± 1.5</td>
<td>15.7 ± 1.3</td>
<td>21.2 ± 1.6</td>
</tr>
<tr>
<td>Urine Volume (mL/24 h)</td>
<td>- 181 ± 1.2</td>
<td>10.4 ± 0.9</td>
<td>11.4 ± 1.8</td>
<td>13.0 ± 2.1</td>
</tr>
<tr>
<td>Plasma Creatinine (μmol/L)</td>
<td>- 24.6 ± 1.6</td>
<td>37.6 ± 3.2</td>
<td>31.4 ± 3.7</td>
<td>29.0 ± 4.3</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min)</td>
<td>+ 9.8 ± 2.9</td>
<td>26.6 ± 1.9</td>
<td>17.1 ± 2.6</td>
<td>13.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>- 11.2 ± 1.2</td>
<td>32.6 ± 2.3</td>
<td>20.0 ± 3.1</td>
<td>16.3 ± 3.6</td>
</tr>
</tbody>
</table>

a P < 0.05 versus controls.
b P < 0.01 versus controls.
c P < 0.01 versus baseline (see Text).

Figure 4. Weight change from baseline (a) and food intake (b) in rats after the sc injection of HgCl2 (4 mg/kg) with or without the subsequent continuous sc administration of IGF-1 (125 μg daily).

DISCUSSION

This study shows that recovery from HgCl2 ARF in rats is enhanced by the exogenous administration of IGF-1. This enhanced recovery has previously been reported in ischemic models of ARF (22,23). The IGF-1 infusion, which was initiated after the induction of the ARF with two different doses of HgCl2, showed no protective effect against the early functional severity of the renal insult or the mortality. However, subsequent functional and histologic recovery was significantly improved.

There are several possible mechanisms that might explain this effect of exogenous IGF-1. In normal rats and humans and in both acute and chronic renal failure, IGF-1 has been shown to acutely increase GFR and RBF (10,14,15,34,35), and this could reduce the severity of ARF, perhaps by washing out debris from injured nephrons. It should be noted that, in our model, both water intake and urine flow were not affected by IGF-1 administration, and thus, this mechanism is unlikely to explain all of our findings. Furthermore, the mortality and initial renal dysfunction were similar in control and IGF-1-treated rats, suggesting that the HgCl2 had similar toxic effects in both groups. It is known that maximal intracellular mercury concentrations occur within 1 to 3 h after HgCl2 administration (33). Thus, it is unlikely that IGF-1 treatment, which was initiated with unprimed minipumps 2 h after HgCl2 injection, would have affected renal mercury concentrations. Food intake and weight gain were significantly improved, and others have noted improved protein anabolism and reduced net catabolism in rats with ischemic ARF treated with IGF-1 (23). These changes may them-

versus 95 ± 11 μU/mL in control rats). Blood glucose levels were also similar in controls, HgCl2-treated, and HgCl2 + IGF-1-treated rats (11.3 ± 2, 12 ± 3.1, and 10 ± 1.2 mM, respectively; P = NS).

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Figure 5. Histology (hematoxylin and eosin, ×100) of outer stripe of rat kidneys 5 days after the sc injection of HgCl₂ (4 mg/kg). (a) In vehicle-treated animals, there is extensive necrosis with tubular casts of necrotic material and some intraluminal calcifications. (b) In IGF-1-treated animals, the tubules are slightly dilated but necrotic material is almost absent and the tubular epithelium is flattened with a few mitotic figures.

TABLE 2. Renal histologic scores (see Methods) and serum creatinine in rats 5 days after HgCl₂-induced ARF

<table>
<thead>
<tr>
<th>Group</th>
<th>Scoring Grade</th>
<th>Serum Creatinine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>HgCl₂ (N = 4)</td>
<td>37.3 ± 3.7³</td>
<td>34.3 ± 6.1</td>
</tr>
<tr>
<td>HgCl₂ + IGF-1 (N = 4)</td>
<td>52.5 ± 4.9</td>
<td>48.5 ± 4.7</td>
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³ A total of 100 high-power fields were scored from each kidney, and numbers shown indicate the mean number of fields ± SE in each scoring grade.
⁴ P < 0.05 (HgCl₂ versus HgCl₂ + IGF-1).

selves have short-term secondary beneficial effects on renal function (36), although detrimental effects of increased protein intake are recognized both in ARF (37) and in chronic renal failure (38,39). IGF-1 may cause renal hypertrophy in normal rats, although not if factored by the increase in body weight (15). Our histologic data and the complete functional recovery at 6 days in IGF-1-treated animals suggest that simple renal hypertrophy was not the sole cause of the enhanced recovery from the ARF. IGF-1 stimulates regenerative processes at a cellular level and acts on renal cells to stimulate exit from the premitotic or resting (Go/Gi) phase of the cell cycle (40). DNA synthesis is increased in murine proximal tubule cells (41) and in renal tissue of rats receiving IGF-1 after ARF (23), and there are numerous other cellular actions of IGF-1 (11-13) that may improve the well-being of injured cells.

It has been reported that, after ischemic ARF in rats, immunohistologic staining shows more widespread
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Figure 6. Expression of IGF-1 and IGF-1R genes in rat kidneys removed from controls (C), 3 days after the sc injection of HgCl₂ (4 mg/kg) (HgCl₂) and after 3 days of continuous sc IGF-1 infusion (IGF-1). Renal levels of IGF-1 and IGF-1R mRNA were measured by solution hybridization-RNAase protection assays. Constructs used and further details of the assay are described in Methods. (a) Autoradiograph obtained from three to four representative animals. The arrows on the left denote the position and the size of the protected probe bands corresponding to IGF-1 and IGF-1R mRNA. (b) This panel shows the relative density of the bands as a percentage of control (Cont) (*P < 0.01).

Figure 7. Expression of IGFBP-1 gene in rat kidneys: Control (C), HgCl₂, and IGF as in Figure 6. Renal levels of IGFBP-1 mRNA were measured by solution hybridization-RNAase protection assays as described in Methods. (a) Autoradiograph from three representative animals. The arrow on the left denotes the position and the size of the protected probe band corresponding to IGFBP-1 mRNA. (b) Densitometry as in Figure 6. (*P < 0.01).

IGFBP-1 is able to bind IGF-1 and influence its bioavailability and activity (26,27). IGFBP-1 has a diurnal rhythm in the circulation and is dynamically regulated by nutritional and hormonal status (46). Part of the physiologic role of IGFBP-1 in the serum might be to counterregulate the availability of serum IGF-1 to peripheral tissue. The physiologic role of IGFBP-1 in the kidney is not known. In several studies, IGFBP-1 has been shown to inhibit the effect of IGF-1 (27), whereas other studies demonstrated that IGFBP-1 enhances IGF-1 action (26). In the model of streptozotocin-induced diabetes in rats, the increase in IGFBP-1 mRNA appears to be the main reason for increased IGF-1 binding. The correlation between the increase of IGFBP-1 mRNA and IGF-1 binding has been demonstrated in the proximal tubules of diabetic rats (43). Furthermore, it is known that there is a very sensitive correlation between IGFBP-1 production and IGFBP-1 mRNA, the regulation of which being mainly genomic rather than altered mRNA or protein stability (44,45).

IGFBP-1 mRNA is reduced both at 3 and at 5 days after HgCl₂ ARF. We found no changes in IGF-1R mRNA. However, there was a marked increase in IGFBP-1 mRNA in this nephrotoxic model of ARF. None of these changes were prevented by the concomitant infusion of exogenous IGF-1. Increased binding of radiolabeled IGF-1 in ARF has been reported (23). Because we found an increase in whole-kidney IGF-1 levels in HgCl₂ ARF rats treated with exogenous IGF-1 but no increase in HgCl₂ ARF rats not receiving exogenous IGF-1 or in normal rats receiving exogenous IGF-1, we suggest that an increase in IGFBP-1 secondary to the increased expression of IGFBP-1 mRNA caused increased IGF-1 binding. The correlation between the increase of IGFBP-1 mRNA and IGF-1 binding has been demonstrated in the proximal tubules of diabetic rats (43). Furthermore, it is known that there is a very sensitive correlation between IGFBP-1 production and IGFBP-1 mRNA, the regulation of which being mainly genomic rather than altered mRNA or protein stability (44,45).
the increase in renal IGF-1 content because, in this model, IGF-1 mRNA tends to decrease and IGF-1R mRNA is unchanged where IGFBP-1 mRNA is increased (47). Thus, in the diabetic rat, the increase in renal IGF-1 content and the physiologic changes in the kidney are mainly attributed to the increase in IGFBP-1. In our study, the changes in the IGF system expression were similar to those seen in the kidney of the streptozotocin-induced diabetic rat. The increase in the renal IGF-1 concentration after ARF reported by others (23) may therefore reflect the increase in IGFBP mRNA in the renal tissue. It might be that the increase in kidney IGFBP-1 also increases the bioavailability and activity of IGF-1 in the injured kidney, thus causing hypertrophy in diabetes and acceleration of recovery in ARF models.

The infusion of exogenous IGF-1 increases its concentration in the serum and presumably bypasses the physiologic autocrine/paracrine role of endogenous IGF-1. The increased food intake of these rats also might decrease the concentration of IGFBP-1 in the serum (46), making IGF-1 more available for kidney entrapment. The treatment of HgCl2 ARF in rats by the administration of exogenous IGF-1 results in a higher IGF-1 concentration in the kidney, not only because of the increased serum IGF-1 but also secondary to
decreased serum IGFBP-1 and probably to increased IGFBP-1 in the kidney. The decrease in serum IGF-1 level that we observed after ARF might be secondary to increased entrapment of IGF-1 by the injured kidney. However, it may also be that HgCl₂ caused a decrease in hepatic IGF-1 production or that the decrease in food intake was the major cause for the decrease in serum IGF-1 levels (47). Further studies with strict pair feeding in both groups are needed to answer this question. Whatever the underlying mechanisms, HgCl₂ ARF should be added to the growing list of renal functional disturbances that are amenable to improvement by the exogenous infusion of IGF-1.

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REFERENCES


