An IGF-I antagonist does not inhibit renal fibrosis in the rat following subtotal nephrectomy

Simon D. Oldroyd,1 Yohie Miyamoto,2 Arthur Moir,3 Timothy S. Johnson,2 A. Meguid El Nahas,2 and John L. Haylor2

1School of Allied Health Sciences, De Montfort University, Leicester; 2Academic Nephrology Unit, Division of Clinical Sciences North, School of Medicine, and 3Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom

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An IGF-I antagonist does not inhibit renal fibrosis in the rat following subtotal nephrectomy. Am J Physiol Renal Physiol 290: F695–F702, 2006. First published October 4, 2005; doi:10.1152/ajprenal.00058.2005.—Insulin-like growth factor I (IGF-I) has been proposed as a mediator of kidney scarring, although no interventional studies on the role of IGF-I in models of chronic kidney disease have been reported. The effect of a peptide IGF-I receptor antagonist (JB3) has been examined on kidney fibrosis and function in the rat following 5/6 subtotal nephrectomy (SNx). Male Wistar rats were anesthetized and subjected to SNx. JB3 was delivered by subcutaneous infusion using Alzet osmotic minipumps. In vitro studies showed JB3 to displace 125I-IGF-I binding to isolated rat glomeruli and to inhibit IGF-I-induced receptor phosphorylation in renal tubular cells in culture. In the 7-day SNx rats, IGF-I immunostain was present in collecting tubules and JB3 inhibited compensatory renal growth, the maximum effect occurring at 10 μg·kg−1·day−1. After 90 days, the SNx rats developed proteinuria, hypertension, and a fall in glomerular filtration rate. IGF-I immunostain was present in the tubulointerstitial space of the remnant kidney together with marked tubulointerstitial fibrosis. Treatment with JB3 at a dose of 10 μg·kg−1·day−1 had no effect on the renal fibrosis measured by Masson’s trichrome staining or immunostain for collagen III and collagen IV. The proteinuria, hypertension, and lower creatinine clearance all remained unchanged. The remnant kidney was associated with a 50% decrease in renal IGF-I mRNA, which was partially restored by treatment with JB3. Thus an interventional study with an IGF-I receptor antagonist does not support a role for IGF-I in the development of renal fibrosis in the SNx rat, although IGF-I does make an important contribution to compensatory kidney growth.

insulin-like growth factor I; subtotal nephrectomy; compensatory renal growth

INSULIN-LIKE GROWTH factor I (IGF-I) has been proposed as an important mediator of fibrosis in the kidney contributing to the development of chronic kidney disease (CKD) (13, 16). IGF-I increases collagen and fibronectin protein in rat mesangial cells in vitro (38, 39) enhancing collagen transcription (14), whereas in human mesangial cells IGF-I and TGF-β have a synergistic effect on matrix protein accumulation (35). The fibrogenic properties of connective tissue growth factor (CTGF), a downstream mediator for TGF-β, are also IGF-I dependent (41). Inhibition of the response to IGF-I has been proposed to explain the antiproteinuric effects of octreotide, a somatostatin analog (19), and G120-PEG, a growth hormone (GH) receptor antagonist (17) in diabetic nephropathy, although neither drug is selective for the IGF-I system per se. As yet, therefore, no interventional studies have been undertaken to establish a role for IGF-I in experimental models of renal disease.

The 5/6 subtotal nephrectomized (SNx) rat is a model of CKD which develops proteinuria, hypertension, and increased matrix protein deposition leading to a fall in glomerular filtration rate (GFR) (18). In the SNx rat, renal fibrosis is associated with an increase in IGF-I immunostain in the tubulointerstitial space and injured distal tubular cells, together with that for TGF-β and collagen (30). Compensatory renal growth following unilateral nephrectomy is already known to contain an IGF-I-dependent component (20). The present study was undertaken to determine whether renal fibrosis could be reduced by selective IGF-I inhibition in the SNx rat.

An IGF-I receptor antagonist, JB3, was generated by solid phase peptide synthesis (34). IGF-I antagonist activity was confirmed in vitro by measuring receptor binding to isolated rat glomeruli and receptor phosphorylation in OK tubular cells in culture. Functional antagonism of the IGF-I receptor was assessed in vivo, acutely through inhibition of compensatory renal growth and chronically through a reduction in body weight. Dose-response studies on compensatory renal growth were used to determine the dose of JB3 for chronic fibrosis experiments. JB3 was administered to the SNx rat by continuous subcutaneous infusion using Alzet osmotic minipumps. JB3 has been previously used in vivo to provide evidence of a role for IGF-I in the vascular smooth muscle proliferation that follows balloon damage to the rat carotid artery (22).

METHODS AND MATERIALS

Chemical Synthesis of IGF-I Receptor Antagonist (JB3)

A 12-amino acid peptide was generated by solid-phase peptide synthesis (1) using dextro-isomer amino acids in the order Cys-Tyr-Ala-Ala-Pro-Leu-Lys-Pro-Ala-Lys-Ser-Cys and cyclized by oxidation to produce JB3 (34). Cyclization was produced by the generation of a disulfide bond between terminal cysteine residues, resulting in the loss of two hydrogen ions, giving a single peak by mass spectrometry at 1248.

IGF-I Antagonist Activity of JB3: In Vitro

Receptor binding. Glomeruli were isolated from Wistar rat kidneys by sieving (180/100/53 μM) in a HEPES binding buffer and incubated

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with 5 μCi 125I-IGF-I (Amersham) and 1% BSA for 150 min on a rotary shaker. Displacement curves were performed with one of the following ligands: recombinant human IGF-I, 3Arg-IGF-I (Gropep), or JB3.

Receptor phosphorylation. Opossum kidney (OK) cells were serum starved (24 h), incubated with JB3 (3 h) followed by recombinant human IGF-I 50 ng/ml (1 min). Western blots were performed on cellular homogenates using a method described for the EGF receptor (43) employing mouse monoclonal antibodies to the β-subunit of the IGF-I receptor, AB-5 (clone 1–2; Neomarkers, Fremont, CA) and to phosphotyrosine (clone PY20, ICN, Aurora, OH). The intensity of signal bands at 92 kDa was quantified by densitometry (Bio-Rad GS-390).

5/6 Subtotal Nephrectomy

Male Wistar rats (200–300 g) were housed two to three per cage at an ambient temperature of 17°C, humidity of 45%, 12:12-h light-dark cycles and had free access to water and standard animal chow (protein/casein content 18%). All experimental procedures were carried out according to the rules and regulations laid down by the Home Office (Animal Scientific Procedures Act, UK 1986). The experimental protocol for this study was reviewed and approved by the National Kidney Research Fund (Animal Scientific Procedures Act UK 1986). SNx was performed under halothane anesthesia. The right kidney was exposed through a flank incision, the adrenal gland pedicle was ligated, and the right kidney was removed. The left kidney was also exposed through a flank incision, the adrenal gland was separated from the upper pole, and the kidney was decapsulated. The renal pedicle was ligated, and the right kidney was removed. The left kidney was also exposed through a flank incision, the adrenal gland was separated from the upper pole, and the kidney was decapsulated. Ligatures were placed around the upper and lower poles, and the poles were excised. Both the abdominal wall and skin wounds were closed using discontinuous reabsorbable sutures. Alzet osmotic minipumps (Alza, Palo Alto, CA) were loaded, primed for 4 h in isotonic saline at 37°C, and implanted subcutaneously into a dorsal pocket under halothane anesthesia 3 days before nephrectomy.

Inhibition of IGF-I-Dependent Kidney Growth: In Vivo

Six groups of rats (n = 4 per group) were subjected to SNx. JB3 was administered by a subcutaneous minipump (Alzet 2002) over the dose range (3–100 μg·kg⁻¹·day⁻¹) to examine the inhibitory effect on compensatory renal growth. Seven days after the nephrectomy, the kidneys were removed under halothane anesthesia, homogenized in phosphate buffer containing a cocktail of proteolytic enzyme inhibitors, and assayed for protein (27) and DNA (25). Values of remnant kidney weight, DNA, and protein at day 0 were calculated from the degree of excision, and assayed values were obtained from the right kidney.

Effect of 90-Day Treatment with JB3 on Renal Fibrosis and Renal Function

Two groups of rats were subjected to SNx, and pumps containing either JB3 10 μg·kg⁻¹·day⁻¹ (n = 18) or drug vehicle (n = 15) were implanted subcutaneously into a dorsal pocket followed by a subcutaneous loading dose of JB3. Pumps were replaced into a new dorsal pocket every 4 wk. A third group of rats were sham-operated but received no additional treatment. Before the end of each study, rats were placed in individual metabolic cages for 24 h for the collection of urine, with free access to food and water. Urine was assayed for creatinine and protein. Rats were then placed in a restraining perspex tube, and their mean systolic blood pressure was recorded by computerized tail-cuff plethysmography (ITTC Life Science, Woodland Hills, CA). Animals were killed under halothane anesthesia, and a blood sample was taken by cardiac puncture and serum assayed for creatinine. The remnant kidney was removed, weighed, and divided for 1) immediate storage in liquid nitrogen for mRNA analysis and 2) fixation by placement in 10% neutral buffered formalin before being embedded in paraffin wax, sectioned, and mounted on gelatinized slides for matrix staining and immunohistochemical analysis.

Histochemical Analysis

Fibrosis in the SNx kidney was assessed from Masson’s trichrome-stained sections. Staining was quantified (×10 objective) by image analysis (Scion Image for Windows). Randomly selected fields (12 per section) were digitized and subjected to color threshold analysis, (green) giving a final average percentage positive stain per section.

Immunohistochemical Analysis

IGF-I. Dewaxed, hydrated sections were quenched with hydrogen peroxide followed by blocking with 1.5% normal goat serum. The primary antibody was rabbit polyclonal anti-human recombinant IGF-I (Dr. R. Baxter, National Institute of Diabetes and Digestive and Kidney Diseases), 1:1,000 in 0.1% BSA/PBS, and the secondary was biotinylated goat anti-rabbit antibody (Vector), 1:1,200 in 0.1% BSA/PBS.

Collagen type III. Dewaxed, hydrated sections were quenched with hydrogen peroxide, digested with trypsin (Digest All, Zymed Laboratories) followed by blocking with 1% normal horse serum. The primary antibody was goat anti-collagen type III (Southern Biotechnology), 1:50 in 0.1% BSA/PBS, and the secondary was a biotinylated rabbit anti-goat antibody (Dako AS), 1:400 in 0.1% BSA/PBS.

Collagen type IV. A similar procedure was employed to that for collagen type III with the addition of a microwave step following hydration. The primary antibody was rabbit anti-collagen type IV (Euro-Diagnostica) used at 1:40 in 0.1% BSA/PBS.

Antibody binding was revealed by an avidin-biotin-peroxidase procedure (ABC Elite kit, Vector) with 3'-amino-9-ethyl carbazole as the substrate (AEC substrate kit, Vector), counterstained with dilute hematoxylin and mounted in glycergel. Control sections were incubated with nonimmune γ-globulin or the omission of the primary antibody. Staining for both collagen III and collagen IV was quantified (×10 objective) by image analysis (Scion Image for Windows). Randomly selected fields (12 per section) were digitized and subjected to color threshold analysis (red), giving a final average percentage positive stain per section.

Kidney IGF-I Protein

The IGF-I content of renal tissue homogenates was measured by radioimmunoassay as described previously (30) using 125I-IGF-I (Amersham) as the tracer.

mRNA Analysis

Total RNA was extracted using TRIzol (Life Technologies BRL) and quantified by spectrophotometry. Thirty milligrams of total RNA were electrophoresed on a 1.2% (wt/vol) agarose/4-morpholine-propane sulfonic acid/formaldehyde gel and viewed under ultraviolet (UV) light to verify loading and RNA integrity by the presence of intact ribosomal bands. RNA was then transferred to Hybond N nylon membranes (Amersham) by capillary blotting and fixed by UV irradiation (70 mJ/cm²). Filters were prehybridized in a mixture containing 50% (vol/vol) deionized formamide, 5× saline-sodium phosphate-EDTA, 5× Denhardt’s reagent, 1% (wt/vol) SDS, and 200 mg/ml sonicated and denatured salmon sperm DNA at a probe-specific temperature for 1 h. Hybridization was performed under the same conditions with the addition of labeled cDNA probes for rat IGF-I (33) and rat IGF-1 receptor (42). Filters were washed to a stringency of 0.2% at up to 65°C (depending on the probe) for 1 h and exposed to Kodak XOMAT AR film for up to 7 days with intensifying screens. Developed films were analyzed by scanning volume density analysis using the Bio-Rad GS-690 scanning densitometer and Molecular Analyst.
(version 4) software. Loading was corrected by reference to the housekeeping gene cyclophilin. Determination of transcript size was by reference to RNA molecular markers (Promega) and calculated using Molecular Analyst software.

**Statistical Analysis**

Results are expressed as means ± SE. The difference between control and experimental groups was compared using one-way ANOVA or Student’s *t*-test. All tests were performed with Microsoft Excel 97. *P* < 0.05 was taken as significant.

**RESULTS**

**Evidence for IGF-I Antagonist Activity: In Vitro**

The effect of three ligands for the IGF-I receptor, rhIGF-I, 3Arg-IGF-I, and JB3 on the displacement of 125I-IGF-I from isolated rat glomeruli is shown in Fig. 1A. 3Arg-IGF-I, a ligand which does not bind to IGF binding proteins, had a similar ED50 (10^-9 M) to rhIGF-I. JB3 displaced 125I-IGF-I from rat glomeruli with an ED50 of 10^-6 M, indicating a lower affinity for the rat glomerular IGF-I receptor than the recombinant human IGF-I protein. The pharmacological activity of JB3 was demonstrated in OK tubular cells where JB3 reduced the stimulation of tyrosine receptor phosphorylation by rhIGF-I (50 ng/ml) in a dose-dependent manner, with an ED50 of 8 × 10^-8 M (Fig. 1B).

**Fig. 1.** Insulin-like growth factor I (IGF-I) receptor antagonism by JB3: in vitro. A: displacement binding curves for 125I-IGF-I from isolated rat glomeruli in the presence of the following ligands: recombinant human IGF-I (●), 3Arg IGF-I (○), and JB3 (■). B: inhibition of rhIGF-I induced IGF-I receptor phosphorylation by JB3 in opossum kidney (OK) cells. Vertical bars indicate SE, *n* = 4 per concentration.
increased from 220 to 589 ng/kidney (P < 0.001). The DNA content of the remnant kidney increased from 0.9 to 1.32 g (P < 0.005) after 7 days. Treatment with subcutaneous JB3 at a dose of 10 μg·kg⁻¹·day⁻¹ reduced the remnant kidney wet weight to 1.09 ± 0.04 g (P < 0.05, n = 4 per group). Higher doses of JB3 produced no further reduction in kidney wet weight demonstrating that 10 μg·kg⁻¹·day⁻¹ was a maximal dose.

The protein content of the remnant kidney, 7 days after SNx, increased from 77 ± 1 to 248 ± 4 mg (P < 0.0001). The DNA content of the remnant kidney increased from 0.9 ± 0.01 to 2.55 ± 0.15 mg (P < 0.005), with no change in the protein/DNA ratio (n = 4). Treatment with subcutaneous JB3 produced a significant decrease in total protein content (Fig. 3B) and total DNA content (Fig. 3A) of the remnant kidney. The maximal inhibitory effect of JB3 on the protein and DNA content of the remnant kidney also occurred at a dose of 10 μg·kg⁻¹·day⁻¹, being reduced by 51 and by 65%, respectively. This inhibitory response was sustained at higher dose levels of 50–100 μg·kg⁻¹·day⁻¹.

The degree of kidney resection (69.9 ± 4%, n = 24) did not differ significantly between any rat group (n = 4 per dose). JB3 at a dose of 10 μg·kg⁻¹·day⁻¹ was chosen as the systemic dose for chronic 90-day studies on renal fibrosis.

90-Day Treatment with JB3 in the SNx Rat

A representative example of IGF-I immunostain in the remnant kidney, 90 days after SNx, is shown in Fig. 2B. The distribution of IGF-I immunostain changed by day 90, being localized to the thickened tubulointerstitial space and damaged distal tubules. The kidney content of IGF-I showed a second, localized to the thickened tubulointerstitial space and damaged distal tubules.

Renal Function

SNx rats treated with either vehicle (n = 15) or JB3 at a dose of 10 μg·kg⁻¹·day⁻¹ (n = 18) for 90 days had a similar starting body weight and were subjected to the same degree of

Table 1. Renal function, BP, and body weight in the Wistar rat 90 days after SNx

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 6)</th>
<th>SNx + Vehicle (n = 15)</th>
<th>SNx + JB3 10 μg·kg⁻¹·day⁻¹ (n = 18)</th>
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<tr>
<td><strong>Day 0</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Body weight, g</td>
<td>330 ± 6</td>
<td>332 ± 7</td>
<td>324 ± 4</td>
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<td>Systolic BP, mmHg</td>
<td>116 ± 4</td>
<td>118 ± 2</td>
<td>120 ± 2</td>
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<td>Resection, %</td>
<td>67.9 ± 0.5</td>
<td>68.7 ± 0.7</td>
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<td><strong>Day 90</strong></td>
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<td></td>
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<tr>
<td>Body weight, g</td>
<td>480 ± 17</td>
<td>442 ± 11</td>
<td>415 ± 10*</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117 ± 4</td>
<td>144 ± 5</td>
<td>143 ± 10†</td>
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<tr>
<td>Serum creatinine, μmol/l</td>
<td>61.1 ± 6.2</td>
<td>89.1 ± 13.2†</td>
<td>93.5 ± 25.3†</td>
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<td>Urine volume, ml/24 h</td>
<td>9.2 ± 2.1</td>
<td>22.6 ± 3.6†</td>
<td>21.1 ± 2.8‡</td>
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<td>Creatinine clearance, ml/min</td>
<td>2.04 ± 0.25</td>
<td>1.17 ± 0.08†</td>
<td>1.20 ± 0.09†</td>
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<td>Urinary protein, mg/24 h</td>
<td>9.9 ± 1.1</td>
<td>275 ± 46†</td>
<td>328 ± 59‡</td>
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<td>Food consumption, g/day</td>
<td>ND</td>
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<td>23.6 ± 1.5</td>
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<tr>
<td>Water consumption, ml/day</td>
<td>ND</td>
<td>29.8 ± 2.6</td>
<td>31.6 ± 2.7</td>
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</table>

Results are expressed as means ± SE. BP, blood pressure. Subtotal nephrectomy (SNx) rats were treated with either vehicle or JB3 (10 μg·kg⁻¹·day⁻¹ sc), an IGF-I receptor antagonist. *P < 0.05 compared with SNx + vehicle, †P < 0.05, ‡P < 0.01 compared with sham operation.

Inhibition of IGF-I-Dependent Kidney Growth: In Vivo

A representative example of IGF-I immunostain in the remnant kidney, 7 days after SNx, is shown in Fig. 2A where the IGF-I immunostain as in sham-operated animals was confined to the collecting tubules. The kidney content of IGF-I increased from 220 ± 31 to 589 ± 55 ng/kidney (P < 0.01) compared with sham-operated animals. The remnant kidney from the SNx rat showed a significant increase in wet weight from 0.77 ± 0.05 to 1.32 ± 0.05 g (P < 0.005) after 7 days. The degree of kidney resection (69.9 ± 4%, n = 24) did not differ significantly between any rat group (n = 4 per dose). JB3 at a dose of 10 μg·kg⁻¹·day⁻¹ was chosen as the systemic dose for chronic 90-day studies on renal fibrosis.
kidney resection (Table 1). SNx rats (receiving vehicle) developed proteinuria and hypertension with a lower creatinine clearance than sham-operated controls (Table 1). Treatment with subcutaneous JB3 at a dose of 10 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) reduced the increase in rat body weight over 90 days by 20%, in the absence of any change in fluid or food intake, evidence for the effectiveness of chronic drug treatment. However, treatment with JB3 at a dose of 10 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) had no effect on the development of proteinuria or hypertension, while the reduction in creatinine clearance remained unchanged (Table 1).

**Renal Fibrosis**

Representative examples of staining with Masson’s trichrome are shown in Fig. 4 for remnant kidneys obtained from rats following sham operation (Fig. 4A), SNx with drug vehicle (Fig. 4B), and SNx treated with subcutaneous JB3 at a dose of 10 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) (Fig. 4C). Magnification \( \times 40 \).

![Masson's trichrome staining for matrix protein in the remnant kidney 90 days after SNx.](image)

Fig. 4. Masson’s trichrome staining for matrix protein in the remnant kidney 90 days after SNx. Representative examples are shown from rats subject to sham operation (A), SNx treated with sc drug vehicle (×40; B), and SNx treated with sc JB3 at a dose of 10 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) (C). Magnification \( \times 40 \).

![Matrix protein accumulation in the remnant kidney, 90 days after SNx.](image)

Fig. 5. Matrix protein accumulation in the remnant kidney, 90 days after SNx. Kidneys were stained with Masson’s trichrome (A) and immunostained for collagen type III (B) and collagen type IV (C). Staining was quantified by image analysis. Data for sham-operated animals are shown in the open bars (n = 6). SNx rats were treated with either sc drug vehicle (light gray bars, n = 15) or sc JB3 at a dose of 10 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) (dark gray bars, n = 18) for 90 days. Vertical bars indicate SE.
vehicle (Fig. 4B), and SNx following treatment with subcutaneous JB3 at a dose of $10 \mu g \cdot kg^{-1} \cdot day^{-1}$ (Fig. 4C). After 90 days SNx, the remnant kidney showed a significant increase in tubulointerstitial staining for Masson’s trichrome (Fig. 5A) and tubulointerstitial immunostaining for collagen III (Fig. 5B) and collagen IV (Fig. 5C) compared with sham-operated controls. Renal fibrosis in the remnant kidney remained unaffected by treatment with subcutaneous JB3 at a dose of $10 \mu g \cdot kg^{-1} \cdot day^{-1}$. No decrease in the extent of tubulointerstitial staining for Masson’s trichrome or tubulointerstitial immunostaining for collagen III and collagen IV was observed.

**mRNA Analysis**

The remnant kidney, 90 days after SNx, had a significantly lower expression of IGF-I mRNA than sham-operated animals ($P < 0.001$) as shown in Fig. 6A. The expression of IGF-I receptor mRNA was also lower, but the decrease did not reach significance (Fig. 6B). The remnant kidney of SNx rats treated with subcutaneous JB3 at a dose of $10 \mu g \cdot kg^{-1} \cdot day^{-1}$ showed a significantly higher level of mRNA expression for IGF-I at $24.9 \pm 4.4$ compared with vehicle $14.8 \pm 1.1$ VD/cyclophilin ($P < 0.05$) as shown in Fig. 6C. Although IGF-I receptor mRNA (22.6 ± 1.8 vs. 17.4 ± 0.9 VD/cyclophilin) was also higher, the difference did not reach significance (Fig. 6D). Thus in the SNx rat, treatment with JB3 partially restored the expression of IGF-I mRNA back to the levels determined in the kidneys of sham-operated animals.

**DISCUSSION**

IGF-I is an important regulator of matrix deposition in bone (3, 28) and has been suggested to contribute to the development of fibrosis in both conduit arteries (2) and major organs such as the heart, liver, and lung (4, 10, 40). We previously showed IGF-I protein to increase biphasically in the remnant SNx kidney and have suggested it may also be a mediator of renal fibrosis (30). In the present study, 7 days following SNx, collecting tubules showed enhanced immunostain for IGF-I together with the development of compensatory renal growth. By day 90, the distribution of IGF-I immunostain in the remnant kidney had changed to the renal interstitium and injured distal tubules, whereas the SNx rat developed hypertension and proteinuria. The remnant kidney showed a marked increase in tubulointerstitial fibrosis and a decrease in GFR was detected by an elevated serum creatinine and lower creatinine clearance. Treatment with the IGF-I receptor antagonist JB3, 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$, inhibited compensatory kidney growth but, to our surprise, had no effect on the development of renal fibrosis, hypertension, proteinuria, or the fall in creatinine clearance.

A number of reasons may be put forward to explain the lack of an antifibrotic response to an IGF-I receptor antagonist in the SNx rat. First, the sensitivity of the kidney to the profibrotic effects of IGF-I could have decreased due to the development of resistance. In chronic renal failure, IGF-I resistance is a

Fig. 6. Kidney mRNA for IGF-I and IGF-I receptor (IGF-IR) 90 days after surgery measured by Northern blot. Data (left) compare sham-operated rats (lanes 1–5, open bars) to SNx rats (lanes 6–10, light gray bars) for IGF-I mRNA (A) and IGF-I receptor mRNA (B), $n = 5$ per group. Data (right) compare SNx rats treated with drug vehicle (lanes 1–6, light gray bars) compared with SNx rats treated with sc JB3 at a dose of $10 \mu g \cdot kg^{-1} \cdot day^{-1}$ (lanes 7–11, dark gray bars) for IGF-I mRNA (C) and IGF-I receptor mRNA (D). Volume density (VD) was corrected using cyclophilin as the loading control. Vertical bars indicate SE.
well-described phenomenon responsible for growth retardation in childhood (24), which has been attributed to the retention of low-molecular-weight binding proteins, IGFBP-1 and IGFBP-2, normally excreted by the kidney (5). Recently, an additional mechanism of IGF-I resistance has been described through the downstream inhibition of IGF-I-induced tyrosine phosphorylation by cytokines (6) elevated in chronic renal failure. A similar mechanism has been proposed to explain the insulin resistance seen in type II diabetes (36).

Second, IGF-I may not actually be a profibrotic agent in vivo. The profibrotic role for IGF-I was proposed entirely from in vitro studies using either rat or human mesangial cells (14, 35, 38, 39). No evidence of a similar profibrotic response to IGF-I in the kidney has been described based on in vivo studies. In fact, IGF-I attenuated tubulointerstitial injury following ureteral obstruction in the neonatal rat, reducing renal interstitial collagen deposition, tubular vimentin expression, and apoptosis (9). IGF-I has been suggested to reduce fibrosis in the SNx rat by maintaining renal autoregulation (26) while its ability to inhibit apoptosis (37) or vasodilate blood vessels (21) could also inhibit the fibrotic process. Interestingly, antifibrotic effects for IGF-I have also been described in vivo in experimental liver cirrhosis (31). Doi et al. (11) showed that mice transgenic to GH developed glomerulosclerosis and glomerular hypertrophy, whereas mice transgenic to IGF-I only developed hypertrophy. These data are consistent with our own results where renal growth but not renal fibrosis was inhibited by an IGF-I antagonist. The lack of evidence for IGF-I as a mediator of renal fibrosis in the SNx rat does not exclude a role for growth hormone working through an IGF-I-independent pathway (12). It does, however, question the functional significance of measurements of renal IGF-I protein where consistent increases have not been detected even with compensatory renal growth (15, 32). In the remnant kidney, IGF-I mRNA does not increase during compensatory kidney growth and by day 90 in the present study had declined by some 50%, despite the elevated IGF-I immunostain in the fibrosed interstitium. Any increase in IGF-I protein, occurring in the absence of increased transcription, could result from translational changes such as reduced degradation or enhanced sequestration.

Third, the IGF-I antagonist may have been ineffective under the experimental conditions employed; however, we do not believe this to be the case. The antagonist JB3, a 12-amino acid cyclic peptide, was generated by solid-phase peptide synthesis and its structure confirmed by mass spectrometry. IGF-I receptor binding was demonstrated in isolated rat glomeruli where JB3 displaced 125I-IGF-I albeit with a lower affinity than the recombinant human IGF-I peptide. IGF-I antagonist activity was demonstrated using renal tubular cells in culture where JB3 inhibited IGF-I-induced IGF receptor autophosphorylation. We previously reported JB3 to inhibit IGF-I-induced thymidine uptake under similar conditions (20). JB3 has previously been shown to selectively inhibit IGF-I-induced cell growth in vitro without influencing the response to other growth factors such as EGF or PDGF (34). Previous in vivo studies have shown JB3 at a dose of 10 μg·kg⁻¹·day⁻¹ to inhibit vascular smooth muscle proliferation in a rat model of carotid balloon injury (12). We were able to demonstrate the pharmacological effectiveness of JB3 in vivo through its ability to inhibit compensatory renal growth, the maximal effect occurring at the dose of 10 μg·kg⁻¹·day⁻¹, the dose chosen for fibrosis experiments. Stability studies confirmed that the activity of JB3 remained unaffected by storage in Alzet minipumps for a period of up to 4 wk, the replacement period used in 90-day rat studies. The effectiveness of JB3 as an IGF-I antagonist during the 90-day study was supported by a 20% lower increase in body weight of the SNx rat compared with SNx rats treated with vehicle. The partial reversal of the fall in IGF-I mRNA seen in the remnant kidney by JB3 could also be evidence of receptor antagonism, inhibiting the negative feedback influence of IGF-I on the hypothalamic release of GH (8). However, the development of methodologies to monitor IGF-I receptor antagonism in vivo perhaps through agonist stimulation of receptor phosphorylation or transduction signaling could prove extremely useful.

In conclusion, no evidence was obtained with the IGF-I receptor antagonist to support a role for IGF-I as a mediator of renal fibrosis in the SNx rat, although evidence for both IGF-I-dependent and -independent compensatory kidney growth was confirmed. It may now be important to undertake interventional studies in other models of CKD where evidence of a role for IGF-I has also been obtained (16, 23, 29). A highly specific human monoclonal antibody to the IGF receptor has recently been developed which may provide an alternative to cyclic peptides in studying the renal pathophysiology of the IGF-I receptor (7).

GRANTS

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