Chronic interstitial fibrosis in the rat kidney induced by long-term (6-mo) exposure to lithium

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LITHIUM IS A COMMON THERAPEUTIC agent used to treat patients with various mood disorders. It does, however, have a narrow therapeutic index (34), plasma levels of between 0.6 and 1.5 mmol/l offering effective treatment, while slightly higher levels are toxic. Grunfeld and Rossier (11) recently reviewed the renal side effects of lithium therapy. These fall into three main categories, Nephrogenic diabetes insipidus (NDI), chronic kidney disease (CKD), and acute lithium intoxication. The most prevalent of these several forms of renal injury (1) is an impaired urinary concentrating ability, present in at least 50% of individuals on chronic lithium therapy (28). Initially, this decreased urinary concentrating ability is largely reversible following cessation of lithium. However, with continued treatment, this defect translates into overt and irreversible NDI in up to 20% of unselected cases (6, 9, 34). Numerous subsequent studies have shown that this defect is the result of downregulation of the water channel aquaporin-2 (AQP2) and loss of the ability to insert this molecule into the apical membrane of the principal cells of the collecting duct (11, 15, 22).

Long-term exposure to lithium in humans has been associated with tubular atrophy and chronic focal interstitial fibrosis predominantly in the cortical region of the kidney (4, 14, 18, 21), changes that may be progressive, leading to end stage renal disease (ESRD). However, lithium-induced ESRD is relatively rare, making up <2% of patients on renal replacement therapy (ANZDATA Registry, www.anzdata.org.au, Annual Report 2010). This complication of lithium treatment was recognized early, and a number of authors reported on the effects of long-term lithium therapy in both humans (2, 9, 12, 14, 18, 21, 28) and rats (8, 19, 20, 23, 25, 26, 27, 32). In general, the CKD that develops from long-term lithium administration is one of progressive renal deterioration involving both chronic tubulointerstitial nephropathy (11) and focal glomerulosclerosis (21, 30). However, our current understanding of the longer term chronic nephrotoxic effects of lithium is limited, as patients on long-term therapy are infrequently biopsied and only a relatively small percentage of patients present with advanced renal disease. In addition, earlier studies of chronic lithium exposure in rats (8, 20, 23, 25, 26, 27, 32) largely concentrated on histological changes only, and the rats were often treated from a very early age, before kidney maturation; plasma lithium levels achieved were higher than those regarded as therapeutic for humans and were sometimes lethal. Many of the studies were carried out when robust immunohistochemical techniques to accurately define the composition of the chronic lesions were not available.

We describe a chronic long-term (6-mo) model of lithium-induced renal fibrosis, with minimal active inflammation, which mimics chronic kidney interstitial fibrosis seen in the human kidney. Rats received lithium via their chow (60 mmol lithium/kg food) daily for 6 mo. No animals died during the exposure. Nephrogenic diabetes insipidus was established by 3 wk and persisted for the 6 mo. Following metabolic studies, the animals were killed at 1, 3, and 6 mo and the kidneys were processed for histological and immunohistochemical studies. Progressive interstitial fibrosis, characterized by increasing numbers of myofibroblasts, enhanced transforming growth factor-β1 expression and interstitial collagen deposition, and a minimal inflammatory cellular response was evident. Elucidation of the underlying mechanisms of injury in this model will provide a greater understanding of chronic interstitial fibrosis and allow the development of intervention strategies to prevent injury.

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At 1, 3, and 6 mo rats were introduced to metabolic cages (on a 12:12-h light-dark cycle. 1 rat per cage) and allowed to settle for 21 h. During the next 24 h, water intake (ml) was recorded, and urine was collected under paraffin and then stored at −20°C for later analysis. The total volume (ml) of the urine was measured, and its composition (osmotic pressure, potassium, sodium, lithium, and chloride concentrations) was determined. Groups of animals were narcotized with carbon dioxide and killed by decapitation at 1, 3, and 6 mo, and the kidneys were removed. Blood was collected and spun immediately, and the plasma was removed. Each kidney was cut in half longitudinally; one-half was put in 10% buffered formalin for immunohistochemistry, and the remaining tissue was snap frozen in liquid nitrogen and stored at −80°C. At the time of decapitation, arterial blood was collected from the neck and spun immediately and the plasma was decanted and frozen for later analysis.

**Blood and urine analysis.** The osmolality of urine and plasma samples was determined using a vapor pressure osmometer (Wescor, Logan, UT). Sodium, potassium, and lithium concentrations in the urine and plasma were determined by flame photometry (Radiometer FLM3). Chloride in the plasma was determined electrometrically with a Collove chloride titrator. Creatinine concentrations in urine and plasma were measured (Randox Laboratories) after appropriate dilution. Urea [blood urea nitrogen (BUN); Pointe Scientific] was also measured in the plasma to further assess renal function.

**Histology and immunohistochemistry.** For histological and immunohistochemical studies, kidneys were wax embedded and sections were mounted in DPX. They were viewed and scanned using an Aperio ScanScope XT Digital Scanner (Aperio Technologies) at the Queensland Institute of Medical Research, and quantitative data were obtained using Scanscope Spectrum software. Some sections were also viewed in an Olympus Provis microscope, and selected regions were digitally recorded using a Spot camera.

Negative controls were carried out either by omitting the primary antibodies or by using appropriate blocking peptides, and positive controls were used appropriately chosen tissues. For the analysis of α-SMA, TGF-β1, ED1, and ApopTag, 10 areas, each of 10,000 μm², were chosen randomly from the renal cortex, outer medulla and inner medulla, and the fraction of positive pixels was determined from scanned sections. For α-SMA, areas incorporating blood vessels were ignored. The results were expressed as the percentage change from control values. For the determination of macrophage numbers by ED1 staining, 10 randomly selected areas were scanned and individual macrophages counted. In the cortex, if a randomly selected area was found to include a glomerulus, the measuring area was moved to the right so as to exclude it from the count. ED1-positive material within blood vessels was ignored.

It was not possible to make quantitative measurements of TGF-β1, since this substance was irregularly distributed. Randomly selected areas were photographed and printed, and observers were blinded to treatments made qualitative judgments.

**Sirius red quantitation of fibrosis.** Estimation of the degree of fibrosis was carried out using sections stained with Picro-Sirius red and viewed using either transmitted or polarized light (33). In brief, dewaxed paraffin sections were hydrated in a series of dilutions of ethanol, immersed in a solution of saturated picric acid with 1% Sirius red for 1 h, and washed in acidified water. After dehydration in a further series of alcohols, sections were cleared in xylene and mounted in DPX.

Sections were viewed in a Nikon inverted microscope (IMS) with a ×20 objective, and grey scale (256 bit) images were captured using a TCA-9.0 camera driven by Zeiss software (AxioVision release 4.8). The sections were scanned visually and images recorded of the entire cortical region, taking care to avoid areas containing either glomeruli or significant blood vessels. Image analysis was carried out using ImageJ (www.rsweb.nih.gov/jj) using a macro based on www.machiophotonics.ca/imagej/.

**Statistics.** Quantitative results are expressed as means ± SE. Differences among the means of multiple parameters were analyzed by ANOVA followed by the Student-Newman-Keuls test (Kaleidagraph Synergy software). Values of P <0.05 were considered statistically significant.

### Table 1. Physiological parameters of control and lithium-treated rats (1, 3, and 6 mo)

<table>
<thead>
<tr>
<th></th>
<th>Control 1 mo</th>
<th>Lithium 1 mo</th>
<th>Control 3 mo</th>
<th>Lithium 3 mo</th>
<th>Control 6 mo</th>
<th>Lithium 6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>134 ± 1</td>
<td>134 ± 1</td>
<td>135 ± 2</td>
<td>137 ± 1</td>
<td>140 ± 2</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>K⁺, mmol/l</td>
<td>56 ± 0.3</td>
<td>50 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Li⁺, mmol/l</td>
<td>0</td>
<td>0.5 ± 0.1†</td>
<td>1.1 ± 0.2†</td>
<td>0.9 ± 0.1†</td>
<td>0.9 ± 0.1†</td>
<td></td>
</tr>
<tr>
<td>Cl⁻, mmol/l</td>
<td>96 ± 6</td>
<td>101 ± 2</td>
<td>93 ± 0.4</td>
<td>96 ± 2</td>
<td>94 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>Osmotic pressure, mosmol/kgH₂O</td>
<td>294 ± 3</td>
<td>296 ± 2</td>
<td>300 ± 2</td>
<td>293 ± 1</td>
<td>299 ± 4</td>
<td>295 ± 3</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>101 ± 30</td>
<td>88 ± 25</td>
<td>90 ± 2</td>
<td>78 ± 12</td>
<td>89 ± 4</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>Urea (BUN), mmol/l</td>
<td>7.0 ± 0.7</td>
<td>6.1 ± 0.2</td>
<td>8.0 ± 0.1</td>
<td>6.9 ± 0.3</td>
<td>8.6 ± 1.2</td>
<td>7.4 ± 0.8</td>
</tr>
<tr>
<td><strong>Water uptake, μl · min⁻¹ · kg⁻¹</strong></td>
<td>34 ± 6</td>
<td>506 ± 35†</td>
<td>67 ± 8</td>
<td>623 ± 62†</td>
<td>48 ± 8</td>
<td>527 ± 68†</td>
</tr>
<tr>
<td><strong>Urine composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine output, μl · min⁻¹ · kg⁻¹</td>
<td>30 ± 5</td>
<td>429 ± 30†</td>
<td>58 ± 4</td>
<td>526 ± 50†</td>
<td>46 ± 9</td>
<td>449 ± 43†</td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>89 ± 9</td>
<td>18 ± 2†</td>
<td>42 ± 12</td>
<td>9 ± 1</td>
<td>52 ± 5</td>
<td>11 ± 1†</td>
</tr>
<tr>
<td>K⁺, mmol/l</td>
<td>188 ± 17</td>
<td>15 ± 1†</td>
<td>86 ± 9</td>
<td>11 ± 1†</td>
<td>130 ± 16</td>
<td>12 ± 1†</td>
</tr>
<tr>
<td>Li⁺, mmol/l</td>
<td>0</td>
<td>3.0 ± 0.3†</td>
<td>0</td>
<td>4.8 ± 0.3†</td>
<td>3.3 ± 0.3†</td>
<td></td>
</tr>
<tr>
<td>Osmotic pressure, mosmol/kgH₂O</td>
<td>2,007 ± 30</td>
<td>168 ± 5†</td>
<td>1,768 ± 144</td>
<td>145 ± 5†</td>
<td>2,550 ± 88</td>
<td>168 ± 4†</td>
</tr>
<tr>
<td>Urinary protein, mg · h⁻¹ · 100 g⁻¹</td>
<td>0.4 ± 0.2</td>
<td>3.9 ± 0.6*</td>
<td>0.7 ± 0.1</td>
<td>1.5 ± 0.1*</td>
<td>0.5 ± 0.1</td>
<td>1.5 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BUN, blood urea nitrogen. *P < 0.001, †P < 0.0001, control compared with lithium for each age group.

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Results

Renal function. Rats fed the lithium diet rapidly developed a copious polydipsia, which peaked after 3 mo, reaching \(623 \pm 62\) μl·min\(^{-1}\)·kg\(^{-1}\) before decreasing slightly to \(527 \pm 68\) μl·min\(^{-1}\)·kg\(^{-1}\), a level that was maintained throughout the experimental period. In contrast, water intake of control rats was much less (34 \(\pm\) 6 to 48 \(\pm\) 8 μl·min\(^{-1}\)·kg\(^{-1}\)). Data collected from metabolic cages at 1, 3, and 6 mo are shown in Table 1. Analyses of plasma showed lithium levels in the experimental groups were initially low at 1 mo (0.47 \(\pm\) 0.08 mmol/l; \(n = 6\)) but rose to the high end of the therapeutic dosage recommended for humans to be 1.10 \(\pm\) 0.16 mmol/l at 3 mo and 0.92 \(\pm\) 0.05 mmol/l at 6 mo. Plasma levels of sodium, potassium, and chloride and osmotic pressure in both experimental and control animals were within the normal range. The rate of urine production of the lithium-treated animals was greatly increased at 1 mo compared with controls, (429 \(\pm\) 30 μl·min\(^{-1}\)·kg\(^{-1}\) against 30 \(\pm\) 5 μl·min\(^{-1}\)·kg\(^{-1}\)), and
Table 2. Extent of renal fibrosis in the kidneys of control rats and rats fed a lithium diet for 1, 3, or 6 mo

<table>
<thead>
<tr>
<th></th>
<th>1 mo</th>
<th>3 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirius red unpolared</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%cortical area)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.2 ± 0.4</td>
<td>3.8 ± 0.5</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>Lithium</td>
<td>9.2 ± 0.9*</td>
<td>12.4 ± 1.0*</td>
<td>15.4 ± 0.6*</td>
</tr>
<tr>
<td>Sirius red polarized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%cortical area)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.025 ± 0.005</td>
<td>0.059 ± 0.019</td>
<td>0.348 ± 0.080</td>
</tr>
<tr>
<td>Lithium</td>
<td>0.681 ± 0.182*</td>
<td>0.484 ± 0.081*‡</td>
<td>3.874 ± 0.960*‡</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE, 60 measurements from 6 sections. Sections were scanned visually and images recorded of the entire cortical region, taking care to avoid areas containing either glomeruli or significant blood vessels. Image analysis was carried out using Image J (www.rsbweb.nih.gov) using a macro based on www.machiophotonics.ca/imagej/). *P < 0.0001, compared with equivalent controls. †P < 0.0001, compared with 1-mo lithium. ‡P < 0.0001, control vs. equivalent lithium for each group.

Table 3. Abundance of interstitial macrophages (number per 10,000 μm²) in the kidneys of rats fed a lithium diet for 1, 3, or 6 mo

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lithium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex</td>
<td>Outer medulla</td>
</tr>
<tr>
<td>1 mo</td>
<td>3.6 ± 0.6</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td>3 mo</td>
<td>2.4 ± 0.5</td>
<td>5.7 ± 1.2</td>
</tr>
<tr>
<td>6 mo</td>
<td>3.8 ± 0.3</td>
<td>7.3 ± 0.6</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE of 25 counts. *P < 0.01, †P < 0.001, control vs. equivalent lithium for each age group. ‡P < 0.0001, control vs. equivalent lithium for each group. Macrophages were stained using antibodies for ectodermal dysplasia 1 (ED1, CD68; AbD Serotec).
Fig. 3. Lithium-induced macrophage accumulation in the renal cortex. Endothelial dysplasia 1 (ED1) immunohistochemistry for macrophages. ED1-positive cells are stained brown. A: control kidney has few macrophages, (arrow) in the cortex. B: rats on a lithium diet for 6 mo have numbers of macrophages (arrows) appearing as flattened cells in the interstitium, adjacent to the basement membrane of the tubules. Magnification: ×200.

Inflammatory changes were minimal, there was a progressive overall increase in macrophage numbers, revealed by staining for the macrophage marker, ED1 (Table 3) and illustrated in Fig. 3. After 1 mo, the number of ED1-positive cells in the inner medulla of the lithium-treated animals was double that of the controls, while there was no significant difference elsewhere in the kidney. By 3 mo, the number of macrophages in the inner medulla had fallen, although it was still greater than in the controls. At the same time, macrophage numbers in the cortex had increased. This pattern of distribution was maintained at 6 mo (Table 3). There was also a steady increase in the number of macrophages within the glomeruli over the 6 mo of treatment (Fig. 3).

Table 4 records apoptosis, identified using ApopTag staining, in renal sections of rats given lithium for 1, 3, and 6 mo, compared with controls. ApopTag-positive nuclei were almost exclusively confined to the collecting ducts. Only when fibrosis was well advanced, at 6 mo, was there an increase in apoptosis in the epithelium of dilated and atrophic tubules in the cortex (Fig. 4).

Progressive exposure to lithium resulted in a profibrotic response that had a differential pattern of distribution throughout the kidney that also varied with time. The abundance of myofibroblasts, as measured by α-SMA staining, increased over time but with a differential pattern between cortical and medullary staining as demonstrated in Table 5 and Fig. 5.

The myofibroblasts were found in the interstitial space and were largely congregated around connecting tubules and collecting ducts. Occasionally, as illustrated in Fig. 5D, α-SMA-positive cells were found within the epithelium of the collecting duct. As fibrosis progressed into the cortex, myofibroblasts were distributed diffusely but largely in foci of fibrosis.

Changes in distribution and amount of TGF-β1 are illustrated in Fig. 6. This was not easily quantified, but it was readily seen in the animals killed at 1 mo where TGF-β1 was largely intracellular and confined to the cells of the collecting duct in the inner medulla. No staining was evident in the outer medulla or the cortex. At 3 mo, some interstitial staining was seen in the medulla, and by 6 mo, there was cortical interstitial staining for TGF-β1 as well as in the inner and outer medulla.

**DISCUSSION**

Long-term exposure to lithium in humans can be associated with the development of chronic tubulointerstitial fibrosis leading to renal insufficiency (2, 12, 14, 15, 18, 34). The exact extent of this in the clinical setting is unknown, as current clinical markers of kidney function (plasma creatinine) are inadequate markers of kidney disease until there has been substantial nephron loss. Likewise, very few patients on lithium therapy undergo a renal biopsy. In this study, we report on the progressive development of renal fibrosis in rats treated with a therapeutic dose of lithium over 6 mo, despite the maintenance of normal renal function. We find that fibrosis develops in the absence of any significant degree of inflammation and has a predominantly cortical distribution. We propose that the chronic lithium-treated rat may be a useful model of human CKD.

Recent animal studies have focused on the renal mechanisms of action of lithium and its influence on the water channel AQP2. A number of studies (13, 24, 29, 35) have confirmed that disruption, by lithium, of the cAMP-protein kinase A (PKA)-dependent transport of AQP2 to the apical membrane of collecting duct cells was an important factor in the loss of renal concentrating ability. However, Li et al. (17) have shown that the lithium-induced development of NDI is dissociated from adenyl cyclase activity. As Bichet (5) pointed out, the cAMP-PKA pathway is closely involved with a number of regulatory functions in cells, and specific activities, such as the mobili-
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zation of AQP2, may be confined to particular intracellular microcompartments.

In addition to its relatively acute effects on urine concentration, lithium has long been recognized as a cause of CKD. Lithium-induced nephropathy is principally characterized by a slowly progressing development of tubulointerstitial fibrosis but may also involve glomerulosclerosis (21). In a study of 74 French patients, Presne et al. (28) found that the mean annual loss of creatinine clearance in patients with lithium-induced nephropathy was 2.29 ml·min⁻¹·yr⁻¹ and the mean latency between onset of lithium therapy and ESRD was 20 yr. A large number of patients have been prescribed lithium when relatively young and thus may be prone to CKD in middle age (11, 18).

The first documentation of chronic tubulointerstitial fibrosis was made by Hestbech et al. (14), who reported focal fibrosis, tubular atrophy, glomerulosclerosis, and a large number of small cysts in renal biopsies from patients on long-term lithium therapy. Their findings were confirmed in a larger study by Hansen et al. (12), and by Aurell et al. (2). Interestingly, a small number of patients, who had been taking lithium as their principal medication for up to 9 yr, when biopsied showed similar morphological changes but serum creatinine and BUN were normal, and there was no proteinuria (3). This is similar to the findings in our animal model. In addition to the cystic dilatation seen in the distal convoluted tubules, there was also a reduction in the size of the glomeruli with a significant number of glomeruli undergoing sclerosis as has previously been reported (14).

After 1 mo on the lithium diet, rats showed a copious NDI but only small morphological changes. There was little evidence of an acute inflammatory response, since there was only a slight increase in the number of macrophages present, in the kidney. On the other hand, there was a significant increase in the abundance of peritubular interstitial myofibroblasts in the inner medulla. At 3 mo, there was little change in the abundance of macrophages, although subjectively there appeared to be an increased number within the glomeruli. However, there was a large increase in the abundance of myofibroblasts throughout the kidney and this persisted in the 6-mo animals. At 3 mo, small foci of fibrosis could be observed in the renal cortex, and by 6 mo, these had become extensive, resulting in compression and atrophy of tubules.

Earlier studies with newborn rats with immature kidneys demonstrated more susceptibility to the toxic effects of lithium than older animals. Christensen and Ottosen (7) showed that newborn rats treated with lithium (plasma levels 0.5–1.6 mmol/l) developed chronic renal failure, associated with a severe interstitial nephropathy, within 16 wk. In spite of a mortality of over 50% during this period, the remaining rats, even with greatly reduced glomerular filtration rate, survived quite well, although their growth rate was diminished and BUN was slightly elevated. The renal cortex of these survivors contained numerous large cysts caused by dilated distal tubules and collecting ducts, and there was widespread interstitial fibrosis (8). Furthermore, Ottosen et al. (27) reported that the morphological and functional changes induced in newborn rats treated with lithium for 6 wk were irreversible, and they proposed that the lithium-induced interstitial fibrosis leads to proximal tubular atrophy and the consequent appearance of atubular glomeruli. Similar changes were seen in adult rats

Table 5. Abundance of α-smooth muscle actin in the kidneys of control rats and rats fed a lithium diet for 1, 3, or 6 mo, expressed as the fraction of pixels stained brown following visualization of the distribution of anti-actin with DAB

<table>
<thead>
<tr>
<th>Control</th>
<th>Lithium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex</td>
</tr>
<tr>
<td>1 mo</td>
<td>0.061 ± 0.041</td>
</tr>
<tr>
<td>3 mo</td>
<td>0.025 ± 0.007</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.020 ± 0.002</td>
</tr>
</tbody>
</table>

Each value is the means ± SE of 25 readings of brown pixel intensity in areas of 100 µm². *P < 0.01, †P < 0.001, values significantly different from corresponding regions in the control sections vs. lithium for each age group.
after 8 wk on lithium, and these changes were found to persist for a further 8 wk after cessation of treatment (27).

Clinically, a significant number of patients presenting with CKD are found to have a chronic interstitial fibrosis with no apparent aetiological event documented. To date, there has not been a good consistent animal model of chronic interstitial fibrosis available. The most studied model is the unilateral ureteric obstruction model (10, 31), which is not really typical of the human clinical CKD scenario. In particular, this model has complete loss of the papillae and medulla, which occurs over a relatively short time course of 1 to 2 wk. We believe that the lithium-treated animal model may more accurately reflect the development of CKD as seen clinically.

We have developed a rat model of chronic progressive interstitial fibrosis. This model is remarkable for its lack of an intense interstitial inflammatory response. Progressive exposure to lithium resulted in a profibrotic response that had a differential pattern of distribution between cortical and medullary regions that varied with time. Elucidation of the underlying mechanisms of injury will provide a greater understand-

Fig. 5. α-Smooth muscle actin (α-SMA) immunohistochemistry for myofibroblasts. α-SMA-positive cells are stained brown. A: control kidney cortex with α-SMA-positive vessel wall (arrow). This is normal for α-SMA and was disregarded in any morphometrical assessment. B: lithium diet for 1 mo is associated with increasing interstitial myofibroblast positivity (arrow). C and D: lithium diet for 3 mo: in C, many positive myofibroblasts (arrows) surround dilated cortical collecting ducts; in D, α-SMA-positive cells (arrows) surround atrophying tubules and are also seen in the tubular epithelium (*). E and F: 6-mo lithium diet is associated with marked myofibroblast accumulation around atrophic tubules in progressive tubulointerstitial fibrosis. Areas are demarcated with arrows. Magnification for A, B, C, and D: ×400 and E and F: ×200.

Fig. 6. Transforming growth factor-β1 (TGF-β1) distribution revealed by immunohistochemistry. A: control rat. In the renal cortex TGF-β1 is absent, while in the rats fed a lithium diet for 6 mo (B) TGF-β1 (brown stain, indicated by arrows) is present in the interstitium, principally near the dilated collecting ducts. Magnification = ×200 (inset). Sections of whole kidneys to illustrate the overall distribution of TGF-β1 (Magnification = ×40).
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...ing of chronic interstitial fibrosis and allow the development of intervention strategies to prevent injury.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES