The Role of Tubular Iron Accumulation in the Remnant Kidney

Brian J. Nankivell, Juchuan Chen, Ross A. Boadie, and David C.H. Harris

ABSTRACT

Iron has been implicated in the pathophysiology of several models of acute and chronic renal disease. In this study, energy-dispersive x-ray spectrometry was used to quantify and localize iron in rat remnant kidneys (RK) and normal kidneys (NK) and to determine its pathophysiologic significance. Substantial iron accumulation occurred in proximal tubular cell secondary lysosomes of RK (P < 0.001 versus NK) and reached a plateau at 8 wk after partial nephrectomy. In NK, minor increases of iron also occurred with aging (P < 0.02). Proximal tubular iron accumulation correlated independently with protein excretion (r = 0.90) and impairment of GFR (r = 0.70) and was associated with tubular damage and phosphate accumulation (both P < 0.001). Iron nitritotriacetate (1 mg/kg ip) increased tubular lysosomal iron accumulation and tubular damage (P < 0.001 versus nitritotriacetate) in NK, comparable to levels seen in untreated RK, and increased cortical cytosolic malaondialdehyde, consistent with reactive oxygen species generation. The iron chelator deferoxamine (30 mg/kg per day ip) significantly reduced iron accumulation and tubular damage in RK at 4 wk, compared with deferoxamine chelated to iron and untreated RK. These results suggest that filtered iron enters the remnant tubular lysosomes across the brush border membrane by endocytosis and may produce tubular damage in chronic renal disease by the generation of reactive oxygen species.

Key Words: Iron, remnant kidney, reactive oxygen species

The rat remnant kidney is a model of progressive chronic renal disease, characterized by glomerulosclerosis and progressive tubulointerstitial damage. Although the mechanisms of tubular injury are undefined in this and other models of renal disease, proteinuria has been implicated as a cause. In partially nephrectomized rats, altered glomerular permselectivity increases the filtration of albumin and other proteins, such as transferrin (1). It has been proposed that filtered iron may have a role in tubular injury associated with proteinuria (2,3).

Iron is present in the tubular urine in proteinuric states (2,3) as a result of the glomerular leak of transferrin and may be released from transferrin in the acid milieu of the tubular lumen (4). Free iron may catalyze the Haber-Weiss reaction (5) and generate the highly reactive hydroxyl radical, which can damage lipids and other biomolecules (5-7). Reactive oxygen species (ROS) have been implicated in the pathogenesis of progressive disease in the remnant kidney (8) and in other types of chronic renal disease. Thus, iron-mediated ROS generation may account, at least in part, for the tubulointerstitial damage that accompanies proteinuria.

Iron has recently been implicated in the pathophysiology of several models of acute renal disease, including hemoglobin- and myoglobin-induced acute renal failure (9) and puromycin nephrotoxicity (10). Renal iron overload has been associated with proximal tubular damage in humans with intravascular hemolysis or transfusion siderosis (11,12). In studies from our laboratory, iron accumulation has been demonstrated in proximal tubular lysosomes in human chronic renal failure, associated with tubular damage and proteinuria (13).

Reduction of iron is renoprotective in some models of chronic renal disease. In nephrotoxic serum nephritis, increased renal parenchymal and urinary iron was associated with tubular damage, and dietary iron depletion delayed the development of tubulointerstitial disease and renal functional deterioration (14). In male Munich Wistar Frater (MWF)/Ztm rats, a low-iron diet reduced proteinuria and glomerular sclerosis (15). Deferoxamine (DFO) was protective in iron loading (16), immunologic (17,18), and toxic (10,19) models of renal injury. Increased iron has been demonstrated in cortical homogenates of diseased kidneys (2,18), although its precise localization, ultrastructural quantification, and pathophysiologic significance in experimental chronic renal disease remain to be defined.
In the following study, elemental iron was localized and quantified at an ultrastructural level by energy-dispersive x-ray microanalysis, electron diffraction, and lysosomal acid-phosphatase histochemistry in the rat remnant kidney model of chronic renal disease. The intracellular distribution of iron, its time course of accumulation, and pathophysiologic relationships were characterized. The role of lysosomal iron in causing tubular damage and ROS generation in partially nephrectomized and sham-operated rats was further investigated by the use of the iron chelator DFO and loading with iron nitritotriacetate (FeNTA).

METHODS

Animals

Male Wistar rats of 300 to 450 g were used in all experiments and were allowed free access to water and standard rat chow containing 20% protein and 150 mg of iron per kilogram of food. Remnant kidneys were produced by unilateral nephrectomy and segmental infarction of two thirds of the contralateral kidney with the rat under anesthesia with ketamine (40 mg/kg) and xylazine (4 mg/kg). For urine collections, animals were fasted while in plastic metabolic cages (Techniplast, Buguggiate, Italy) to reduce contamination with dietary and fecal iron. Tap water (Fe, 20 to 30 μg/dL) was provided ad libitum via plastic dispensers. Experiments were conducted in accordance with principles in the NIH Guide for the Care and Use of Laboratory Animals.

Experimental Protocols

Time Course and Functional Relationships of Iron Accumulation. The intrarenal and intracellular distribution of renal cortical iron was determined by energy-dispersive x-ray spectroscopy (EDS), electron diffraction, and lysosomal acid-phosphatase histochemistry in rats at 4 to 8 wk after partial nephrectomy (N = 13 rats). The time course of iron accumulation was determined at zero (in N = 7 rats), 4 (N = 4), 8 (N = 3), 12 (N = 4), and 16 (N = 3) wk after partial nephrectomy. The relationship of iron accumulation to GFR, proteinuria, urinary iron and transferrin excretion, and tubular damage was determined 6 mo after partial nephrectomy (N = 12).

Tubular Toxicity of Iron. At 4 wk after surgery, partially nephrectomized (N = 8) and sham-operated rats (N = 7) were given either FeNTA (1 mg of Fe per kilogram per day ip) or nitritotriacetate (NTA) for 4 wk. Renal cortical tissue was examined for iron accumulation and tubular damage. Malondialdehyde (MDA), a marker of ROS lipid peroxidation, was measured in lysosomal/mitochondrial and cytosolic fractions of renal cortical homogenates.

DFO and Iron-Mediated Tubular Damage. Partially nephrectomized (N = 18) and sham-operated rats (N = 6) were stratified and paired 10 days after surgery on the basis of a serum creatinine level and were then randomized into groups to receive DFO (30 mg/kg per day; Desferal, Ciba-Geigy, Pendle Hill, Australia) or DFO chelated to equimolar amounts of FeCl₃ (DFO-Fe) delivered by ip osmotic minipumps (2 ml of Azlet; Alza corporation, Palo Alto, CA). The functioning of the osmotic minipumps was verified by weekly analysis of urinary DFO and by ex vivo quantification of DFO output by placement in isotonic saline at 37°C for 24 h. Preliminary experiments confirmed that DFO was able to chelate iron effectively after 1 mo and remained soluble at concentrations ranging from 100 to 400 mg/mL at 37°C for up to 2 mo. After 4 wk, cortical tissue was examined for tubular iron accumulation, damage, and generation of ROS.

Electron Microscopy and EDS

Renal cortical tissue was fixed in modified Karnovsky fixative (2% formalin, 2.5% glutaraldehyde in 0.1 M 3-(N-morpholino)propane sulfonic acid [MOPS] buffer; pH 7.4), embedded in LR white resin (medium grade, London Resin, Bassingstoke, UK), and collected on nickel grids as previously described (13). EDS was performed at 80 keV on sections at ×10,000 magnification and 36-degree specimen tilt angle, for 300 live seconds, at 80- to 100-nA beam current, in point mode with a transmission electron microscope (Philips EM 400; Philips, Eindhoven, Netherlands) equipped with a silicon (lithium) detector and an x-ray analysis system (EDAX 9100/75; EDAX International, Mahwah, NJ).

Quantification of Tubular Iron and Damage

Iron was quantified by the Hall continuum method with a coembedded iron-loaded chelax bead (Polaron, Watford, UK) peripheral standard, validated against an aminoplastic secondary standard, as previously described (13). Results of EDS are expressed as mean lysosomal iron concentration (wt % = mg % = 0.17 mmol of Fe per kilogram of dry tissue). EDS can detect as little as 10⁻¹⁸ g of Fe (20). Major translocation of iron during fixation and processing was excluded by the comparison of EDS spectra from freeze-dried cryosections of normal and remnant kidney tissue with those obtained by in utero perfusion and standard fixation as described above. Unstained iron-containing lysosomes were examined by electron diffraction to define a possible crystalline structure. Other elements that accompany iron within the lysosomes were identified by the characteristic x-ray lines during EDS analysis.

The number of iron-containing lysosomes and the tubular damage score were assessed from up to 10...
tubular cross-sections. Corresponding tubular cross-sectional area was measured from photomicrographs by video imaging and processing (MD 30 Image Analysis System, Version 3.0; Bedford Park, Australia). Results of numerical density are expressed as numbers of iron-containing lysosomes per square micrometer. EDS was performed on a random selection of proximal tubular lysosomes containing electron dense material with the characteristics of iron to determine the mean lysosomal iron concentration (10 lysosomal analyses per rat). The mean lysosomal iron concentration correlated with the number of iron-containing lysosomes in individual tubule cells (r = 0.67; N = 99; P < 0.001). To determine iron uptake by each segment of the nephron, an uptake ratio was calculated. This was defined as the ratio of mean lysosomal iron concentration (by EDS) to the total number of lysosomes observed in the tubular cross-section. Tubular damage was assessed simultaneously on all unstained specimens by a semiquantitative scale based on the extent of damage to the microvillus and the disruption of the tubular cell; it ranged from 0.0 or no damage to 2.0 or generalized (>30%) and severe denudation of brush border membranes and/or disruption of tubular architecture and mitochondria, as previously described (13).

Lyososomal Identification and Morphology

Lysosomes were identified in a separate group of rats (remnant and control) by the presence of acid phosphatase by the use of a modified cerium-based histochemistry method in combination with osmium tetroxide (with omission of potassium ferrocyanide postfixation) (21). Additional morphologic studies to identify the type of iron-containing lysosomes were performed on tissue postfixed in 2% osmium tetroxide, embedded in Spurr’s resin, and poststained with lead citrate and uranyl acetate.

Reactive Oxygen Species

Kidney, urine, and plasma MDA was measured by the thiobarbituric acid reaction modified after Ohkawa et al. (22). Lysosomal/mitochondrial and cytosolic fractions were separated from the cortical homogenate by centrifugation (1,000g for 10 min, then 9000g for 10 min) for an analysis of MDA. Electron microscopy of the lysosomal/mitochondrial fraction demonstrated a subcellular composition, which was similar in all subgroups, of 23.3% lysosomes, 53.3% mitochondria, and the remainder a mixture of peroxisomes and Golgi bodies. Further subcellular differentiation was not undertaken because of insufficient tissue and the artefactual elevation of MDA with excessive manipulation. Cortical tissue supernatant (400 µL; homogenized in 0.003 M EDTA), lysosomal/mitochondrial fraction (20 µL), cytosolic fraction (300 µL), plasma (250 µL), or urine (500 µL) was added to a reaction mixture and placed in a water bath at 70°C for 90 min. The absorbance of fluid after organic extraction by n-butanol-pyridine (15:1 vol/vol) was read at 532 nm (Beckman DU-68 spectrophotometer; Beckman Instruments, Fullerton, CA) with MDA bis (dimethyl acetal) as the standard (Aldrich, Milwaukee, WI). MDA values are expressed per milligram of protein. Tissue protein content was determined by use of the Biuret method. Glutathione was measured on renal cortical tissue by the method of Tietze (23), and the glutathione redox ratio was calculated as the quotient of oxidized and total glutathione. Cytochrome oxidase activity in the lysosomal/mitochondrial fraction was determined by the enzymatic oxidation of reduced cytochrome c.

Other Analytical Techniques

Plasma and urinary iron were determined by flameless atomic absorption spectrophotometry (Perkin-Elmer PE 3030; Perkin-Elmer Corp.). Urinary transferrin was measured by radial immunodiffusion against specific rabbit antiserum to rat transferrin (Nordic Immunology, Netherlands) with transferrin standards and external controls. GFR was measured by the plasma clearance of ipl 99mTcDTPA, which has been shown to correlate very closely with insulin clearance (24). Urinary DFO was determined by the method of Meyer-Brunot and Keberle (25), as modified by Paller and Hedin (26). Briefly, after urinary protein was precipitated by trichloroacetic acid, ferricamine was extracted into benzyl alcohol from aqueous solution saturated with sodium acetate and estimated spectrophotometrically at 429 nm. The difference between the extinction values of samples with and without a slight excess of FeSO₄ was compared with a DFO standard curve.

Statistics

Comparisons between groups were made by an unpaired t test or Wilcoxon test as appropriate. Multiple groups were compared by Tukey’s method after one-way analysis of variance. Correlations were performed by the use of either Pearson’s (r) or Spearman’s (rho) test as appropriate. A x² test was used to examine categorical data. Multiple linear regression was performed to examine the relationship between iron accumulation and GFR and urinary protein, iron, and transferrin excretion (Statistical Package for Interactive Data Analysis, Statistical Computing Laboratory, Eastwood, Australia). A Poisson regression program was also used to compare the frequency distribution of iron-containing lysosomes in remnant and normal kidneys. A P value below 0.05 was considered significant. Descriptive data are presented as mean ± standard error.
RESULTS

Tubular Iron Accumulation

Iron was identified in proximal tubular cells by EDS in organelles, which were identified as lysosomes by the coexistence of cerium (indicating acid phosphatase), and by morphology as secondary lysosomes (Figure 1). Electron-dense material that had the characteristics of iron and that was confirmed as such by EDS in unstained sections was present in 3.8% (31 of 802) of the lysosomes in normal kidneys and 23.7% (550 of 2,319) of the lysosomes in remnant kidneys more than 4 wk after surgery ($\chi^2 = 155.0; P < 0.001$). Diffraction demonstrated that iron was present as amorphous deposits and that there was no evidence of a crystalline lattice structure. Iron deposits were also observed infrequently in proximal tubular cells apparently within the cytoplasm and not surrounded by a limiting membrane. Similarly, iron deposits were also noted within areas of mesangial matrix expansion and sclerosis, although in amounts substantially less than in tubular lysosomes.

The mean lysosomal iron concentration in remnant kidneys was greater in proximal tubules than in distal tubules (275.5 ± 54.0 mg %, $N = 43$ versus 110.4 ± 46.9, $N = 25$; $P = 0.04$). The lysosomal iron concentration decreased along the nephron, with the ratio in pars convoluta:pars descendens:distal tubule being 57:24:19. The number of iron-containing lysosomes per tubule cross-section also decreased, in the ratio of 53:28:19, as did the total number of lysosomes per tubule, in the ratio of 44:36:20. The uptake ratio of the remnant kidney proximal tubular lysosomes was similar to that of distal tubules (19.7 ± 6.0 mg % Fe per lysosome versus 14.9 ± 7.6; $P = 0.048$; $N = 7$). The distribution of iron-containing lysosomes in both normal and remnant proximal tubules was patchy, with large tubule-to-tubule variation, rather than cell-to-cell variation, within a single tubule. The frequency distribution of tubular iron demonstrated increased numbers of iron-containing tubules in remnant kidneys, whereas the majority of tubules in normal kidneys were free of iron ($P < 0.001$ by Poisson regression; Figure 2). In remnant kidneys, iron (three or more iron-containing lysosomes per tubule cross-section) was present in 75 of 109 tubules compared with 8 of 36 tubules in normal rats ($\chi^2 = 23.9; P < 0.001$).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Renal tubular epithelium in the pars convoluta of the proximal tubule showing electron-dense material (arrows) within secondary lysosomes, confirmed as iron by energy-dispersive analysis. Bar = 1 µm. Spur's resin, stained with lead citrate and uranyl acetate.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Frequency distribution of tubules according to the number of iron-containing lysosomes in normal ($N = 34$, solid bars) and remnant ($N = 108$, hatched bars) kidneys. Remnant kidneys contained more iron-laden tubules compared with normal kidneys ($P < 0.001$, by Poisson regression).
Time Course of Iron Accumulation

Iron accumulated progressively in the lysosomes of remnant kidneys, reaching a plateau at 8 wk after surgery (Figure 3). The mean GFR for this group was 0.92 ± 0.13 mL/min and was similar at each time. No significant increase was found in age-matched, sham-operated controls in the number of iron-containing lysosomes per tubule (0.58 ± 0.23; N = 12) or mean lysosomal iron (46.8 ± 31.7 mg%; N = 12).

There was a small but significant increase in the number of iron-containing proximal tubular lysosomes compared with that in younger (10-wk) normal kidneys (2.05 ± 0.46; N = 19 versus 0.73 ± 0.24, N = 30; P < 0.02). The mean lysosomal iron concentration was also fivefold higher in older rats (73.7 ± 37.9 mg%; N = 20 versus 14.6 ± 6.5, N = 30; P = not significant).

In remnant kidneys, iron was accompanied by other elements including phosphorus (in 38% of iron-containing lysosomes), sulfur (25%), silicon (6%), and combinations of these elements (2%). In lysosomes containing larger amounts of iron (upper quartile = 673 mg% iron), phosphorus was found more frequently (69%; χ² = 15.2; P = 0.001). Similarly, lysosomes with phosphorus peaks contained greater amounts of iron than did lysosomes containing sulfur (P = 0.004; Tukey test) or silicon (P = 0.013). Iron may be in the form of hemosiderin, a complex of hydrated phosphates and/or sulfates, or as a stable compound such as ferric hydroxide oxide (20).

Relationships of Iron to Physiologic Parameters

Partially nephrectomized rats had a GFR of 1.3 to 3.0 mL/min 6 mo after surgery and a protein excretion of 12.3 to 107.6 mg/day. Remnant kidneys at 6 mo, when compared with controls, had increased urinary iron excretion (35.6 ± 7.6 μg/24 h, N = 10 versus 1.6 ± 0.5, N = 6; P < 0.001), increased urinary transferrin excretion (2.23 ± 0.91 mg/24 h, N = 10 versus 0.10 ± 0.04, N = 6; P < 0.05), and decreased serum iron (197.5 ± 29.0, N = 10 versus 370.7 ± 21.5, N = 6; P < 0.001). Mean lysosomal iron (by EDS) correlated with 24-h proteinuria (r = 0.90). 24-h urinary transferrin (r = 0.37). 24-h urinary iron (r = 0.76) and correlated inversely with GFR (r = 0.70). By multiple linear regression, however, significant independent contributions to lysosomal iron were shown for 24-h proteinuria (P < 0.001) and impairment of GFR (P = 0.007) only, accounting for variance in urinary iron and transferrin excretion.

Relationship of Iron Accumulation to Renal Damage

Overall tubular damage correlated with mean lysosomal iron (r = 0.39; P < 0.001; N = 99) and with the number of iron-containing lysosomes per tubule (r = 0.43; P < 0.001; N = 106). Tubules with no or minimal damage (score, 0.0 to 0.5) had less lysosomal iron than did more severely damaged tubules (score, 1.0 to 2.0) (52.4 ± 18.2 mg%, N = 49 versus 304.0 ± 51.7 mg%, N = 57; P < 0.001). Iron-containing tubules (two or more lysosomes per tubule cross-section) were more severely damaged (χ² = 21.9; P < 0.001). Within the subset of iron-containing tubules, tubular damage was related to mean lysosomal iron (r = 0.60; P < 0.005; N = 68). In remnant kidneys analyzed by multiple linear regression, significant predictors of histologic tubular damage were tubular iron mass (log [number of iron-containing lysosomes × mean lysosomal iron in mg%]; P = 0.025) and urinary iron excretion (P = 0.003), which was independent of proteinuria.

Ultrastructural Nephrotoxicity of FeNTA

Exogenously administered iron accumulated predominantly within proximal tubular lysosomes in both sham-operated and partially nephrectomized rats (Table 1), in quantities similar to that observed in the untreated remnant kidney. Paradoxically, the efficiency of the tubular uptake of iron was greatest in sham-operated compared with remnant kidneys, when equivalent doses are used. Increased tubular damage occurred in both groups and was associated with iron accumulation.

The Effect of DFO on Tubular Iron Accumulation and Damage

Urinary DFO was detected in all rats throughout the experiment. The average release of DFO, deter-
TABLE 1. Tubular iron accumulation, damage, and ROS generation in renal cortical fractions from partially nephrectomized and sham-operated rats treated with FeNTA and NTA for 4 wk*  

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<th>Sham</th>
<th>Remnant</th>
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<tr>
<td></td>
<td>NTA (N = 15/3)</td>
<td>FeNTA (N = 20/4)</td>
</tr>
<tr>
<td>No. of Iron-Containing Lysosomes per Tubular Cross-Section</td>
<td>0.47 ± 0.22</td>
<td>6.70 ± 1.39*</td>
</tr>
<tr>
<td>Numerical Density of Iron-Containing Lysosomes (number/μm² × 10⁻⁷)</td>
<td>0.52 ± 0.24</td>
<td>9.75 ± 2.18*</td>
</tr>
<tr>
<td>Mean Lysosomal Iron Concentration (mg %)</td>
<td>6.2 ± 1.1</td>
<td>52.0 ± 10.3*</td>
</tr>
<tr>
<td>Tubular Damage (Semiquantitative)</td>
<td>0.30 ± 0.07</td>
<td>0.93 ± 0.08*</td>
</tr>
<tr>
<td>MDA, Cytosolic Fraction (nmol/mg of protein)</td>
<td>1.2 ± 0.1</td>
<td>2.5 ± 0.18*</td>
</tr>
<tr>
<td>MDA, Lysosomal/Mitochondrial Fraction (nmol/mg of protein)</td>
<td>7.3 ± 1.3</td>
<td>9.1 ± 3.6</td>
</tr>
<tr>
<td>Proteinuria (mg/24 h)</td>
<td>16.3 ± 3.5</td>
<td>17.2 ± 4.3</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dL)</td>
<td>0.60 ± 0.01</td>
<td>0.60 ± 0.02</td>
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* Values are mean ± SE. N = tubules per rat.

* P < 0.001 versus NTA.

* P < 0.05 versus NTA.

* P < 0.01 versus NTA.

* P < 0.001 versus sham.

Determined ex vivo, was 6.3 ± 1.1 mg/24 h (mean ± SD), which was similar to the calculated daily release of 7.5 ± 0.8 mg/24 h (r = 0.79; P = 0.007). Postoperative mortality due to bacterial infection was increased in DFO and DFO-Fe groups when compared with that of historical controls (x² = 5.1; P = 0.024). These rats were excluded from further analysis. DFO, when compared with DFO-Fe, did not alter daily food consumption; final body and kidney weights; final blood pressure; serum iron, transferrin, creatinine (Table 2), and urea; or urinary excretion of creatinine, urea, phosphate, and osmoles (data not presented).

DFO reduced the number, numerical density, and mean iron concentration of iron-containing lysosomes in the proximal tubules and tubular damage in comparison to DFO-Fe (Table 3). In comparison with untreated remnant kidneys at 4 to 8 wk after surgery, the number of iron-containing lysosomes

TABLE 2. The effect of DFO treatment on functional parameters and ROS generation in partially nephrectomized and sham-operated rats*  

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<th>Sham</th>
<th>Remnant</th>
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<tr>
<td></td>
<td>DFO (N = 3)</td>
<td>DFO-Fe (N = 3)</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dL)</td>
<td>0.56 ± 0.01</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>Serum Iron (μg/dL)</td>
<td>274 ± 67</td>
<td>182 ± 4</td>
</tr>
<tr>
<td>Serum Transferrin (mg/dL)</td>
<td>498 ± 26</td>
<td>533 ± 53</td>
</tr>
<tr>
<td>Cortical Tissue MDA (nmol/mg of protein)</td>
<td>0.60 ± 0.13</td>
<td>0.62 ± 0.12</td>
</tr>
<tr>
<td>Urinary MDA (nmol/μmol creatinine)</td>
<td>2.3 ± 0.3</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Plasma MDA (nmol/L)</td>
<td>6.5 ± 0.1</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Tissue Redox Ratio (%)</td>
<td>4.6 ± 0.9</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Arterial Blood Pressure (mm Hg)</td>
<td>138 ± 4</td>
<td>147 ± 3</td>
</tr>
<tr>
<td>Kidney Wt (g)</td>
<td>1.65 ± 0.37</td>
<td>1.17 ± 0.08</td>
</tr>
<tr>
<td>Body Wt (g)</td>
<td>340.6 ± 6.9</td>
<td>314.7 ± 31.2</td>
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* Values are mean ± SE. N = rats.

* P < 0.001 versus sham operated.
TABLE 3. Tubular iron accumulation and damage in partially nephrectomized and sham-operated rats treated with ip DFO or DFO chelated to equimolar amounts of iron (DFO-Fe) for 4 wk

<table>
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<th>Sham</th>
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<tr>
<td></td>
<td>DFO (N = 3/15)</td>
<td>DFO-Fe (N = 3/15)</td>
</tr>
<tr>
<td>No. of Iron-Containing Lysosomes per Tubular Cross-Section</td>
<td>0.42 ± 0.16a</td>
<td>3.3 ± 1.07</td>
</tr>
<tr>
<td>Numerical Density of Iron-Containing Lysosomes (number/μm² x 10⁻³)</td>
<td>0.63 ± 0.41b</td>
<td>9.4 ± 2.25</td>
</tr>
<tr>
<td>Mean Lysosomal Iron Concentration (mg %)</td>
<td>6.7 ± 0.99a</td>
<td>70.2 ± 27.8</td>
</tr>
<tr>
<td>Tubular Damage (Semiquantitative Proteinuria (mg/24 h))</td>
<td>0.45 ± 0.04</td>
<td>0.75 ± 0.13</td>
</tr>
<tr>
<td>Urinary Iron Excretion (μmol/24 h)</td>
<td>12.7 ± 3.9</td>
<td>9.4 ± 2.9</td>
</tr>
<tr>
<td>Urinary Transferin Excretion (mg/24 h)</td>
<td>3.8 ± 1.3a</td>
<td>91.2 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>0.52 ± 0.14</td>
<td>0.73 ± 0.10</td>
</tr>
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</table>

* Values are mean ± SE. N = rats per tubule.

a p < 0.05 versus DFO-Fe.
b p < 0.01 versus DFO-Fe.
0.001 versus DFO-Fe.

per tubular cross-section (4.32 ± 1.30; N = 28; P < 0.001 versus DFO), the mean lysosomal iron concentration (211.2 ± 75.6 mg %; N = 27; P < 0.05), and tubular damage scores (0.98 ± 0.08; N = 28; P < 0.001) were also reduced by DFO.

Iron, Tubular Damage, and ROS Generations

FeNTA increased MDA in both cytosolic and lysosomal/mitochondrial fractions, although only in the former was the increase statistically significant (Table 1). Mean lysosomal iron correlated with cytosolic MDA generation (r = 0.60; P < 0.05).

An unexpected result was the reduced MDA production from the lysosomal/mitochondrial fraction of remnant compared with sham-operated kidneys (both NTA treated). No differences were observed by electron microscopy in the subcellular composition of the fraction; however, reduced cytochrome oxidase activity of lysosomal/mitochondrial fraction (0.29 ± 0.03 versus 0.40 ± 0.01 log (cytochrome c)/min/μg of protein; P < 0.05) was demonstrated in remnant compared with control kidneys. These differences between remnant and cortical lysosomal/mitochondrial fractions could be explained by the fractionation process or alternatively because of the impairment of cytochrome electron transport and the "physiologic leak current" from oxidative phosphorylation in remnant mitochondria.

In the DFO experiment, overall tubular damage correlated with the number of iron-containing lysosomes (r = 0.52; P = 0.03), their numerical density (r = 0.388; P = 0.06), and the mean lysosomal iron concentration (r = 0.56; P = 0.04). By multiple linear regression, tubular damage was related both to the redox ratio (P = 0.004) and the mean lysosomal iron concentration (P = 0.005). However, the DFO treatment status accounted for the contribution of tubular iron to tubular damage when incorporated into the model. The generation of ROS, as assessed by whole cortical homogenate, urine, and plasma MDA, was comparable in all treatment groups (Table 2). The effects of iron loading and iron chelation on iron and tubular damage in sham-operated and remnant kidneys are summarized in Figure 4.

DISCUSSION

In this study of partially nephrectomized rats, substantial accumulation of iron was demonstrated by energy-dispersive analysis within the secondary lysosomes of proximal, and to a lesser extent distal, tubules. Iron was present in greater concentrations and within increased numbers of iron-containing lysosomes in remnant kidneys than in controls. This accumulation of iron was a time-dependent phenomenon and reached a plateau within 8 wk of renal ablation. The progressive increase of iron in the hypertrophied portion of the remnant kidney away from the surgical scar and its absence in sham-operated animals suggest that surgical trauma was not a determinant of accumulation. The administration of exogenous FeNTA resulted in lysosomal iron accumulation and damage, whereas both were reduced by the iron chelator DFO.

Iron bound to its transport protein transferrin normally enters tubular cells across the basolateral membrane after receptor binding and is stored in the cytoplasm in a nontoxic form as ferritin. However, in proteinuric renal disease, iron and transferrin may
be filtered along with other proteins and presented to the tubular cell via the tubular lumen. Urinary transferrin is increased in proteinuric states (2,3,18), and iron may dissociate from it in the acidic tubular fluid (3,4). In this study, remnant kidney urinary iron and transferrin excretion increased with proteinuria, consistent with an alteration of glomerular permselectivity, which occurs after renal ablation (1). The mean lysosomal iron concentration in individual rats was proportional to proteinuria and inversely proportional to GFR, reflecting the increased load of iron in the proximal tubular lumen. Furthermore, in remnant kidneys, the lysosomal concentration of iron and the number of iron-containing lysosomes decreased progressively along the nephron. The accumulation of iron, predominantly in the proximal tubule, was associated with a greater total number of lysosomes in proximal versus distal tubular epithelium. These observations and the presence of iron within secondary lysosomes are consistent with the hypothesis that filtered iron enters the remnant kidney tubular cell across the brush border membrane by endocytosis. In contrast, in a study of nephrotoxic serum nephritis, Prussian blue iron staining appeared in the distal tubule (14). This may be explained by differences in the intrinsic pathophysiology of the models, by the preferential detection of hemosiderin rather than other forms of iron, such as ferritin, by this staining method (20), or by the poor sensitivity of the staining method when compared with ultrastructural EDS, which detects elemental iron.

The partial nephrectomy model of progressive chronic renal failure is characterized by progressive glomerular sclerosis, proteinuria, tubular damage, and interstitial fibrosis. Progressive glomerular sclerosis and proteinuria also occur in old Wistar rats (27). The marked accumulation of iron in remnant kidneys and the lesser accumulation seen in aging normal rats in this study was patchy, with variation from tubule to tubule rather than from cell to cell. This is in contrast to studies of proteinuric renal disease with diffuse glomerular damage (puromycin, adriamycin, and diabetic nephropathy) in our laboratory (28), in which the iron uptake and tubular damage were more uniform. It is probable that the leak of iron-laden transferrin, along with other proteins, from an individually damaged or partially sclerosed glomerulus results in increased iron uptake and injury to tubular cells of the same nephron.

Lysosomal iron accumulation was greatest in tubules with the most morphologic damage, and morphologically normal tubules rarely contained iron. These results suggest that lysosomal iron accumulation may have caused the injury. However, tubular damage was also observed unassociated with lysosomal iron, albeit less frequently. Although it is possible that iron was also present within these damaged tubules at a level outside the ultrathin section plane, other factor(s) may have been responsible for the damage.

In this study, iron accumulation was associated with proteinuria, impaired GFR, and lysosomal phosphate accumulation, all factors associated with progressive renal injury. Multiple linear regression revealed that the association of tubular damage with iron accumulation was independent of these other factors. Moreover, in both iron-loading and DFO studies, tissue damage varied directly with iron accumulation.

Iron could exert a deleterious effect via several mechanisms. Iron catalyzes the generation of ROS (5), which cause membrane lipid peroxidation and cellular damage. ROS may also cause functional damage. For example, ROS generated by iron have been shown to reduce the formation of prostaglandins E2 and F2α in rabbit kidney medulla slices (29) and to cause partial or complete loss of renal tubular NaK-ATPase (6). Increased amounts of MDA, a marker of ROS lipid peroxidation, have been demonstrated in remnant nephrons (8). In this study, chronic iron loading increased cortical cytoplasmic and probably lysosomal/mitochondrial MDA. Although the whole cortical homogenate MDA values in the DFO experiment were similar between treatment groups, this does not exclude differences at the subcellular level. Apart from its role in the generation of ROS, iron could cause injury by other mechanisms. Iron in high concentrations has been shown to destabilize lysosomes in a hepatic model of iron overload (30). If this is also the case in the kidney, then iron...
could cause lysosomal disruption with the release of lysosomal enzymes and iron into the cytoplasm. Consistent with these possibilities, iron deposits were also observed in the cytoplasm of proximal tubular cells.

In summary, progressive iron accumulation was demonstrated in the proximal tubular lysosomes of rats after partial nephrectomy and was associated with tubular damage and impairment of GFR. The presence of iron in the secondary lysosomes of the proximal nephron and its correlation with proteinuria suggest that filtered iron enters the tubular lysosomes by endocytosis across the brush border membrane. Exogenously administered FeNTA also accumulated within tubular lysosomes, associated with damage and cystolic ROS generation. Iron chelation by DFO ameliorated iron accumulation and tubular damage. These data suggest that lysosomal iron accumulation causes proteinuric tubular damage in partially nephrectomized rats and may play a role in the progression of chronic renal disease.

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