Late Consequences of Acute Ischemic Injury to a Solitary Kidney

M aria E nrica P agtalunan, * J ean L. O lson, † N icholas L. T ilney, ‡ and T imothy W. M eyer *

* D epartments of M edicine, V etersan Administration Palo Alto Health Care System and Stanford University, Palo Alto, C alifornia; † D epartment of Pathology, U niversity of C alifornia, San Francisco, C alifornia; and ‡ S urgical Research Laboratory and D epartment of S urgery, Harvard Medical School, Brigham and Women’s H ospital, B oston, M assachusetts.

Abstract. The sequelae of acute ischemic injury to a solitary kidney were assessed in rats subjected to right nephrectomy and transient occlusion of the left renal artery; control rats underwent right nephrectomy alone. Incomplete recovery from ischemic injury at 2 wk (serum creatinine levels of 1.1 \( \pm \) 0.2 \( \mu \)g/dl, \( P < 0.05 \) for ischemia versus control) was followed by deterioration of renal function at 20 wk (serum creatinine levels of 1.7 \( \pm \) 0.4 \( \mu \)g/dl, \( P < 0.05 \) for ischemia versus control). Morphologic studies showed that impairment of function after ischemic injury was associated with widespread tubulointerstitial disease. Some tubule segments were atrophic and others exhibited cystic dilation, so that the tubular cell volume fraction was reduced (37 \( \pm \) 4 versus 53 \( \pm \) 2%, \( P < 0.05 \)), while the tubular lumen and interstitial volume fractions were increased (31 \( \pm \) 4 versus 23 \( \pm \) 2% and 29 \( \pm \) 2 versus 20 \( \pm \) 1%, respectively, both \( P < 0.05 \)). Many glomeruli retained open capillary loops but were no longer connected to normal tubule segments (63 \( \pm \) 8 versus 15 \( \pm \) 7% of glomeruli, \( P < 0.05 \)). There was a strong inverse correlation between the prevalence of such glomeruli and the GFR at 20 wk after ischemia (\( r^2 = 0.79, P < 0.001 \)). Tubulointerstitial disease at that time was accompanied by proteinuria and widespread segmental glomerular tuft injury. The occurrence of similar processes in human patients could contribute to the loss of graft kidneys that suffer ischemic injury during transplantation.

The kidney exhibits a remarkable ability to recover from acute ischemic injury. When ischemic injury is severe, however, kidney function may not completely return to normal (1). Studies in rats have shown that incomplete recovery from acute injury may be followed by progressive renal injury (2,3). Reports that impairment of graft function immediately after transplantation is associated with an increased risk of late graft loss have stimulated renewed interest in this process (4–6). Azuma et al. (3) showed that recovery from acute ischemic injury in rats with a solitary kidney was followed by development of proteinuria and widespread segmental glomerular sclerosis. The study presented here was designed to further assess late changes in renal function and structure in this model. A particular aim was to measure the extent of tubular and interstitial injury. The serial-section technique of Marcussen (7) was used so that the appearance of glomerular and tubular injury in individual nephrons could be related.

Materials and Methods

Animals

Male Sprague Dawley rats weighing 290 to 350 g were anesthetized with thiopental (50 mg/kg, intraperitoneally). One group of rats underwent right nephrectomy and occlusion of the left renal artery for 30 to 35 min (ischemia group, \( n = 12 \)), whereas a second group underwent right nephrectomy alone (control group, \( n = 9 \)). Systolic BP (tail-cuff method), serum creatinine levels (Beckman Creatinine 2 analyzer), and 24-h urine protein excretion (Coomassie blue) were then assessed periodically for 20 wk, during which rats had free access to water and standard laboratory chow. Clearance and morphologic studies were performed at the end of this period. Morphologic studies were also performed at 5 wk after right nephrectomy and occlusion of the left renal artery in a separate group of rats (\( n = 4 \)).

Functional Studies

Rats were anesthetized with Inactin (100 mg/kg, intraperitoneally; A.L.A., Inc., Sturtevant, WI) and placed on a temperature-regulated table. A PE-50 tubing catheter was inserted in the right femoral artery and used for subsequent blood sampling. After tracheotomy, PE-50 catheters were inserted in the right and left jugular veins for infusion of rat plasma, saline solution, and radiolabeled inulin. Plasma was infused in an amount equal to 1% of body weight in 40 to 45 min, followed by reduction of the infusion rate to 0.4 ml/h for the duration of the study. Saline solution was infused at 2.4 ml/h throughout the study. After 60 min, tritiated methoxy-inulin was added to the saline solution to achieve an infusion rate of approximately 7 \( \mu \)Ci/ml, after a loading dose of approximately 6 \( \mu \)Ci. A PE-10 catheter was placed in the left ureter for collection of urine. Clearance measurements were performed for two or three 30-min periods. In each period, an arterial
blood sample was obtained for determination of the plasma inulin concentration.

**Morphologic Studies**

After functional studies, kidneys were fixed by retrograde aortic perfusion with 2.5% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer. Kidneys were sliced perpendicular to the long axis, at approximately 2-mm intervals. Slices from the midportion of the kidney were further fixed in 10% formalin for embedding in paraffin and in 1.25% glutaraldehyde in 0.1 M cacodylate for embedding in Epon. Periodic acid-Schiff reagent-stained sections were prepared from paraffin-embedded slices. For each kidney, a smaller block of tissue, extending from the capsule into the medulla and measuring approximately 1.8 mm on the other sides, was embedded in Epon. This block was sectioned serially (at 3-μm intervals) parallel to its long axis, so that glomeruli at all levels of the cortex were included in the sections. Every other serial section was mounted and stained with toluidine blue, so that the entire block could be examined at 6-μm intervals. One hundred sections were examined for each rat in the ischemia group, and 76 sections were examined for each rat in the control group. Every fourth mounted section was photographed, and a series of prints was prepared for use as maps of the serial sections. These maps served as guides to identification of individual glomeruli, as described previously (8). Only glomeruli contained entirely within the serially sectioned tissue were examined. The average number of glomeruli examined was 23 ± 2 in the ischemia group, 17 ± 2 in the control group, and 27 ± 3 in rats studied at 5 wk. Each glomerulus was first classified as being either connected to a normal proximal tubule, connected to an atrophic proximal tubule, or without any tubular connection, as described by Marcussen (9). The proximal tubule segment connected to a glomerulus was considered atrophic when there was thinning of tubular cells accompanied by loss of the brush border and narrowing of the tubular lumen. Each glomerulus was then further examined at three levels, evenly spaced along the diameter perpendicular to the plane of section, to assess the prevalence of injury to the tuft. The volume of each glomerulus was calculated from the area of its midsection, using the maximal planar area method of Lane et al. (10). The fractional volume of cortical components was determined by point counting, using a 6×6 point eyepiece reticule grid and a magnification of ×400. In each case, a minimum of 500 points in three sections (spaced 60 μm apart) were counted; volume fractions were calculated as the number of points falling on each structural component divided by the number of points evaluated.

**Statistical Analyses**

The unpaired t test was used to assess the significance of differences between groups, and the paired t test was used to assess the significance of differences within each group. Values are expressed as the mean ± SEM throughout.

**Results**

Serum creatinine values are depicted in Figure 1. In rats subjected to right nephrectomy plus left renal ischemia, serum creatinine rose to 5.0 ± 0.4 mg/dl at 1 d after surgery, compared with a value of 0.5 ± 0.1 mg/dl in rats subjected to right nephrectomy alone. The serum creatinine levels in rats subjected to ischemia then declined rapidly but did not completely return to control levels. An increase in creatinine levels from 0.9 ± 0.2 mg/dl to 1.7 ± 0.5 mg/dl between 8 and 20 wk indicated that incomplete recovery from acute ischemia was followed by deterioration of renal function. Examination of values for individual rats revealed that late deterioration of function was most marked in rats in which the initial injury was most severe (Figure 1, inset).

Values for urine protein excretion are depicted in Figure 2. Ischemic injury to a solitary kidney was followed by increasing proteinuria as well as by persistent elevation of the serum concentration.
than the systolic pressure of 131.6 mmHg in the ischemia group was higher than the systolic pressure of 150 mmHg in the control group, so that the systolic pressure 3 to 8 wk after acute ischemia. By 20 wk, protein excretion in rats subjected to ischemic injury to a solitary kidney (●) developed heavy proteinuria. Less proteinuria was observed in uninephrectomized controls (○). *P < 0.05 for ischemia versus control; †P < 0.05 for 8 wk versus 3 wk; ‡P < 0.05 for 20 wk versus 8 wk.

Creatinine levels. By 20 wk, protein excretion in rats subjected to ischemia averaged 275 ± 40 mg/d, compared with a value of 96 ± 39 mg/d for control rats (P < 0.05). Values for systolic BP are depicted in Figure 3. Renal injury reflected by elevated serum creatinine levels and increasing proteinuria was not associated with high BP 3 to 8 wk after acute ischemia. By 20 wk, BP fell slightly in the control group, so that the systolic pressure of 150 ± 6 mmHg in the ischemia group was higher than the systolic pressure of 131 ± 4 mmHg in the control group (P < 0.05). Values for proteinuria and BP did not correlate with the severity of initial injury, as reflected by serum creatinine values.

Clearance studies confirmed that elevation of serum creatinine levels reflected a reduction in GFR at 20 wk after acute ischemia. GFR averaged 1.50 ± 0.25 ml/min in the ischemia group, compared with a value of 2.81 ± 0.10 ml/min for the control group (P < 0.05). Morphologic studies showed that this reduction in GFR was associated with widespread tubulointerstitial injury, as illustrated in Figure 4. Some tubule segments were atrophic, whereas others were dilated. Tubular dilation varied from mild to extreme in degree and was accompanied by epithelial cell attenuation. Hyaline casts were seen in some tubules. These tubular changes were accompanied by patchy interstitial fibrosis, with modest infiltration of mononuclear inflammatory cells and occasional calcifications. Tubulointerstitial disease was prominent throughout the cortex and medulla, whereas the papilla showed relatively less injury. Glomeruli with tuft adhesions to Bowman’s capsule were distributed throughout the cortex. Significant abnormalities were not noted in arteries or arterioles.

Morphometric studies quantifying the extent of injury at 20 wk are summarized in Table 1. Tubulointerstitial injury after recovery from ischemia was reflected by increases in the fractional volumes of the interstitium and tubular lumina and by a reduction in the fractional volume of tubular cells. Examination of serial sections revealed that tubulointerstitial injury was accompanied by the appearance of glomeruli that were not connected to normal proximal tubules, as illustrated in Figure 5. In rats subjected to ischemic injury, only 37 ± 8% of glomeruli were connected to a normal-appearing tubule, whereas 26 ± 4% were connected to an atrophic tubule and 37 ± 5% had no connection to a tubule. In contrast, 85 ± 7% of glomeruli in uninephrectomized control rats were connected to a normal-appearing tubule. Volume measurements showed that glomeruli that lost their connection to normal proximal tubules were significantly reduced in size. In the ischemia group, the volume of glomeruli with normal tubular connections averaged 3.4 ± 0.3 × 10⁶ μm³ and the volume of glomeruli without normal tubular connections averaged 1.9 ± 0.3 × 10⁶ μm³, whereas in the control group the respective values were 3.3 ± 0.1 × 10⁶ μm³ and 2.1 ± 1.0 × 10⁶ μm³.

Recovery from acute ischemia was also followed by structural changes in the glomerular tuft, as illustrated in Figure 6. Most glomeruli without normal tubular connections had a “collapsed” appearance because of reduction of the capillary volume (Figure 6A). In addition, segmental tuft injury was observed in 49 ± 6% of glomeruli at 20 wk after acute ischemia. Of note, segmental injury was observed in 46 ± 7% of glomeruli without normal tubular connections and in 58 ± 7% of glomeruli with normal tubular connections. The features of glomerular injury, however, differed in glomeruli with and without normal tubular connections (Figure 6, B and C). Injury in glomeruli with normal tubules was characterized by the prominence of visceral epithelial cells that had large nuclei and nucleoli and exhibited extensive bleb formation. Epithelial cell injury in these glomeruli was associated with adhesions of the tuft to Bowman’s capsule, usually including the hilus and...
involving <25% of the tuft circumference. Hyalinosis lesions were relatively uncommon. In glomeruli without normal tubular connections, visceral epithelial cell injury was generally less extensive. Injury in these glomeruli was characterized by incorporation of larger portions of the tuft into Bowman's capsule, often with accumulation of mucoid-appearing matrix, splitting of the capsule, and hypertrophy of parietal epithelial cells.

Uninephrectomized control rats examined at 20 wk exhibited much less segmental glomerular injury. Studies in rats euthanized 5 wk after ischemia confirmed that increased segmental glomerular injury was a late consequence of ischemia. These rats, in which the serum creatinine level averaged 0.9 ± 0.1 mg/dl, exhibited tubulointerstitial injury, with an interstitial volume fraction of 24 ± 3% and a tubular cell volume fraction of 39 ± 4%. Serial sections revealed that only 45 ± 11% of glomeruli were connected to a normal tubule, whereas 36 ± 10% were connected to an atrophic tubule segment and 18 ± 6% were atubular. Segmental lesions were seen, however, in only 7 ± 5% of glomeruli with normal tubular connections and in none of the glomeruli without normal tubular connections.

The relationship between renal function and structure at 20 wk after acute ischemia was examined using regression analysis. GFR was closely related to the number of glomeruli that remained connected to normal tubules, as illustrated in Figure 7. GFR was likewise correlated with the tubule cell volume fraction ($r^2 = 0.63, P < 0.004$) and inversely correlated with the sum of the interstitial and tubule volume fractions ($r^2 = 0.58, P < 0.007$). GFR did not, however, exhibit a significant correlation with the volume fraction of the interstitium alone or with the prevalence of sclerotic lesions.

**Discussion**

Ischemic renal failure is usually followed by recovery of renal function. Experimental studies have shown, however, that recovery is incomplete when acute injury is severe (1). Finn (11) and Karlberg et al. (12) found that GFR was reduced by approximately 50% at 4 wk after occlusion of the renal artery in rats. A notable feature of both studies was that single-nephron GFR values returned to control levels while the GFR remained depressed, suggesting that severe ischemia results in complete loss of function in a large portion of the nephron population. The study presented here provides a structural explanation for this finding. In rats examined 5 wk after renal artery clamping, only 45% of glomeruli remained con-

---

**Figure 4.** Tubulointerstitial injury after ischemic injury to a solitary kidney. (A) Prominent structural abnormalities at 20 wk after acute ischemia include cystic dilation of some tubule segments, with atrophy of others, as well as interstitial fibrosis. (B) Less prominent abnormalities are apparent in a control animal subjected only to uninephrectomy (toluidine blue; magnification, ×40).
of tubule injury was proteinuria. Reabsorption of filtered proteins or protein-bound substances has been identified as a cause of tubular injury in a variety of disease states (15–19). We recently found that proteinuria is followed by loss of glomerular connections to tubules in rats subjected to renal ablation (20). The addition of tubular injury caused by proteinuria to tubular injury caused by ischemia could thus account for the progressive loss of renal function after severe ischemic injury.

Interstitial fibrosis accompanied tubular atrophy in the study presented here. These two features of injury are regularly associated in chronic renal disease, but the relationship between them is unclear. Injured tubule cells have been shown to release factors that promote interstitial scarring (16,21). It has been suggested that interstitial scarring can, in turn, cause further tubular injury, so that tubulointerstitial disease becomes self-perpetuating (2,21–23). In this study, however, the interstitial volume fraction was not correlated with the extent of tubular atrophy. This result suggests that interstitial fibrosis was not the chief cause of tubule loss after recovery from acute ischemia.

The appearance of proteinuria and segmental sclerosis after ischemic injury to a solitary kidney was previously described by Azuma et al. (3). They suggested that nephrons that recover function after ischemia become subject to the same injurious processes that cause remnant glomerular sclerosis after partial renal ablation (24). As adherents of this hypothesis, we expected to find that rats that recovered incompletely from acute ischemia became hypertensive and developed sclerotic injury only in glomeruli that remained connected to tubules and became hypertrophic. Our results, however, were contrary to these expectations, indicating that glomerular injury after incomplete recovery from acute ischemia differs in some respects from glomerular injury after renal ablation.

A first unexpected finding was that incomplete recovery of function was not associated with elevation of BP at 3 and 8 wk after ischemic injury. Glomerular injury after renal ablation has been associated with the appearance of both systemic and glomerular hypertension (25–29). Systemic hypertension after ablation has been ascribed to the production of renin by a population of ischemic glomeruli adjacent to areas of scarring (26,30). Glomerular hypertension has, in turn, been ascribed to impaired remnant nephron autoregulation in the presence of systemic hypertension (28,29). Studies showing that glomerular injury is mild when ablation is performed in a way that does not cause hypertension support these hypotheses (26–29). In this study, however, rats subjected to acute ischemia developed proteinuria and glomerular injury in the absence of significant hypertension. Maintenance of normal BP after incomplete recovery from ischemic injury to a solitary kidney was previously observed by Finn (11). Glomerular capillary pressure in this setting has not been assessed.

A second unexpected finding was the absence of hypertrophy in glomeruli that remained connected to normal tubules after recovery from ischemic injury. In rats subjected to renal ablation, sclerotic injury has regularly been associated with glomerular hypertrophy as well as glomerular hypertension.

### Table 1. Morphometric findings at 20 wk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ischemia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney weight (g)</td>
<td>3.46 ± 0.17</td>
<td>3.28 ± 0.10</td>
</tr>
<tr>
<td>Fractional volume of the interstitium (%)</td>
<td>29 ± 2</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Fractional volume of tubular lumina (%)</td>
<td>31 ± 4</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Fractional volume of tubular cells (%)</td>
<td>37 ± 4</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>Glomeruli with normal tubules (%)</td>
<td>37 ± 8</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>Volume of glomeruli with normal tubules (10^6 μm^3)</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>Volume of glomeruli without normal tubules (10^6 μm^3)</td>
<td>1.9 ± 0.3</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>Lesions in glomeruli with normal tubules (%)</td>
<td>58 ± 7</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Lesions in glomeruli without normal tubules (%)</td>
<td>46 ± 7</td>
<td>12 ± 8</td>
</tr>
</tbody>
</table>

* a P < 0.05 versus control.
In the study presented here, however, glomeruli were no larger in rats that had recovered from ischemia than in control animals. Of particular note, glomeruli that remained connected to normal tubules did not increase in size, although their number was reduced. Finn (11) previously found that reduction of the whole-kidney GFR after recovery from ischemia was not accompanied by an increase in the single-nephron GFR of the remaining functioning nephrons. These

Figure 5. Serial sections obtained at 6-μm intervals, illustrating the connection of a glomerulus to an atrophie tubule segment. The arrow marks the vestigial tubule opening into Bowman’s space (toluidine blue, magnification, ×180).

Figure 6. Glomerular tuft injury at 20 wk after acute ischemia. (A) An atubular glomerulus with a “collapsed” appearance, resulting from reduction of the capillary volume, but otherwise normal tuft structure (magnification, ×410). (B) An atubular glomerulus with segmental adhesion of the tuft to a thickened Bowman’s capsule (magnification, ×410). (C) A glomerulus attached to a normal tubule segment, exhibiting visceral epithelial cell injury with attachment of the tuft to Bowman’s capsule in the region of the hilus (magnification, ×290).
results raise the possibility that, after recovery from acute ischemia, residual ischemic injury offsets the hypertrophic effect of reductions in GFR.

An additional unexpected finding was the appearance of segmental injury in glomeruli that were no longer connected to normal tubules. Proteinuria resulting from glomerular injury presumably caused secondary tubular atrophy in some nephrons. This process could not, however, account for the presence of segmental lesions in almost one-half of the glomeruli without normal tubular connections. The appearance of these lesions, moreover, differed somewhat from that of lesions in glomeruli with tubules. We have not developed a hypothesis to account for segmental injury in glomeruli not connected to normal tubules. It is tempting to speculate that tubule loss is followed by gradual incorporation of the tuft into Bowman’s capsule, so that nonfunctioning glomeruli are eventually obliterated. Marcussen and Jacobson (33), however, did not observe this sort of injury in glomeruli that remained atubular for 20 wk after administration of nephrotoxins.

As reviewed by Finn (1), renal function often does not return to normal in human patients who survive acute renal failure. Biopsy studies have shown that impaired function is associated with persistent tubular injury and interstitial fibrosis in such patients. Of note, Price and Palmer (34) also observed segmental glomerular injury, characterized by thickening and splitting of Bowman’s capsule with tuft adhesions, in patients who underwent biopsies ≥6 mo after acute renal failure. Residual impairment of function seems to be most common when severe acute renal failure requires prolonged dialysis support (35). Incomplete recovery, however, is rarely followed by late deterioration of function in patients who suffer acute failure of their native kidneys. The consequences of acute injury to solitary kidneys at the time of transplantation may be more serious. Acute injury in this setting is manifested by delayed graft function, usually defined as the need for dialysis in the first week after implantation. Most studies have shown that delayed graft function predisposes patients to accelerated graft loss, although the magnitude of this effect remains controversial (36–38). Early graft injury may cause late graft loss in part by stimulating recipient immune responses (39). Our experimental findings, however, like those of Fox (2) and Azuma et al. (3), suggest that acute ischemia can cause late loss of function in solitary kidneys independent of any immune response to foreign tissue. It should be emphasized that the effects of ischemic acute renal failure in rats may differ from those of acute renal failure in transplant recipients. In particular, ischemic renal failure in rats is characterized by extensive tubule cell necrosis (40). Tubule cell necrosis is present but is less prominent in the kidneys of patients with delayed graft function (41,42). If the results presented here are applicable to human disease, biopsies from patients recovering from delayed graft function would be expected to reveal glomeruli no longer connected to normal tubule segments. We observed such glomeruli in patients with chronic rejection who underwent biopsies an average of 7 yr after transplantation (8). Studies of biopsies obtained after shorter intervals will be required to determine whether acute injury at implantation contributes to the appearance of glomeruli without normal tubular connections in graft kidneys.

### Acknowledgments

This work was supported by grants from the Baxter Extramural Grant Program and National Institutes of Health (Grant RO1-DK52841) and by the Research Service of the Veterans Administration Palo Alto Health Care System. We thank Miaofen Chou and Helen Kwan for expert technical assistance.

### References

9. Marcussen N: Atubular glomeruli in cisplatin-induced chronic interstitial nephropathy: An experimental stereologic investiga-

![Figure 7. Correlation of GFR with the fraction of glomeruli connected to normal tubule segments at 20 wk after acute ischemic injury \( r^2 = 0.79, P = 0.0002; \) a GFR value was not obtained for one rat.](image-url)


23. Bohle A, Gise H, Mackensen-Haen S, Stark-Jacob B: The oblitera-


