Mechanisms of Hypertension in Renal Radiation

Luis Juncos, Juan Carlos Cornejo, Hugo Cejas, and Carlos Broglia

This study was undertaken to investigate the role played by renal functional and structural changes in the development of radiation-induced hypertension. Four groups of rats were studied: 1) left kidney radiated, 2) sham procedure, 3) uninephrectomy followed 3 weeks later by radiation of the contralateral kidney, and 4) uninephrectomy followed by sham procedure 3 weeks later. All radiated rats became hypertensive at 12 weeks (p<0.05) and had higher protein excretion (p<0.05). In the presence of an intact contralateral kidney, radiation causes mild-to-moderate histological abnormalities, and therefore, creatinine clearance and water and sodium handling do not change. Plasma renin activity increased in this group (p<0.05). Radiated uninephrectomized rats showed decreased creatinine clearance (p<0.05), but renin activity remained unchanged. These rats developed severe histological abnormalities in glomeruli, interstitia, tubuli, and vessels resulting in increased sodium and water output. The average of individual tubular and interstitial scores correlated significantly with both water intake and output but not with sodium excretion. These studies suggest that in the presence of an intact kidney, renin is an important determinant in the development or maintenance of radiation hypertension, whereas in the absence of the contralateral kidney, severe histological changes and renal failure are prominent despite increased water intake and output. The more severe glomerular sclerosis and proteinuria in the latter model could be related to diminished renal mass. (Hypertension 1990;15(suppl I):I-132-I-136)

Renal radiation affects all structures in the kidney.1-2 Hence, regulatory mechanisms such as glomerular filtration rate, urine concentration, and renin release might be altered and thereby contribute to the development of hypertension.

Little information is available in this regard and most of it involves uncontrolled clinical reports. Some studies suggest a role for the renin-angiotensin system,3 whereas others point to the development of renal failure and sodium retention as responsible for the hypertensive process.4-5

Because these hypotheses cannot be tested in an adequate number of humans, we have used experimental models. We studied the effects of radiation on renal histology, plasma renin activity (PRA), glomerular filtration, and sodium and fluid handling in rats subjected to unilateral radiation. Because the responses in the presence of some normal renal mass could differ from those observed with reduced renal mass, we studied rats with and without unilateral nephrectomy.

Methods

Long Evans rats (M. & M. Ferreyra Institute, Córdoba, Argentina), weighing 200–250 g, were used in these studies. Four groups of rats were studied. The 2K-1R group (n=13) underwent radiation to the left kidney; the 2K-S group (n=13) underwent flank incision and manipulation of the renal pedicles; the 1K-R group (n=14) underwent right nephrectomy and, 3 weeks later, radiation to the contralateral kidney; and the 1K-S group (n=9) underwent right nephrectomy and, 3 weeks later, sham surgery as did the 2K-S group.

All procedures were done through a flank incision, under sodium pentabarbital anesthesia (4.5 mg/100 g body wt i.p.). Radiation was provided by an x-ray generator (Style 592-SN113, Pickers X-Ray Corporation, Cleveland, Ohio) at approximately 200 rad/min at the TSD of 15 cm. A localizer, 2 cm in diameter, was used and the total dose per kidney was 3,000 rad. The rats were kept on a standard Purina laboratory chow containing 0.46% sodium and 0.72% potassium and tap water ad libitum. The systolic blood pressure was measured by the tail-cuff method. (Narco BioSystems, Houston, Texas). All those rats with average values above 110 mm Hg from three preexperimental readings were rejected. Blood pressure was again measured 12 weeks after radiation.

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FIGURE 1. Light photomicrographs of glomerular capillary tuft. Panel A: Glomerulus from a rat that underwent flank incision and manipulation of the renal pedicles shows no abnormality. Panel B: Glomerulus from a rat that underwent right nephrectomy and, 3 weeks later, sham surgery shows minimal mesangial expansion. Panel C: Mild segmental sclerosis in glomerulus from a rat that underwent radiation to the left kidney. Panel D: Severe glomerular sclerosis in a rat that underwent right nephrectomy and, 3 weeks later, radiation to the contralateral kidney.

Saralasin Infusion

Twelve weeks after radiation, seven rats in each radiation group (2K-1R and 1K-R), six from the 2K-S group, and four from the 1K-S group were anesthetized as described. The carotid artery was cannulated, and mean blood pressure was monitored through a pressure transducer (P 1000-B, Narco BioSystems) attached to a strain gauge coupler and a multichannel recorder (Physiograph Mark IV, Narco BioSystems). Blood pressure was allowed to stabilize, and then measurements were taken for 10 minutes. Only rats showing variations not greater than 2 mm Hg were continued into the next stage. Saralasin in a saline vehicle was then infused in the jugular vein for 10 minutes at a constant rate of 5 ng/kg/min (Harvard infusion-withdrawal pump, Harvard Apparatus, South Natick, Massachusetts). Blood pressure was simultaneously drawn from the right femoral artery at the same rate of infusion to avoid any influence of the injected volume on blood pressure.

Saralasin was then infused at a rate of 10 ng/kg/min for 10 minutes, again matching the infusion volume with equal rate of withdrawal. At the end of this period, the infusion was stopped, and the blood pressure was continuously measured for another 10 minutes.

Renal Function Studies

Eleven weeks after radiation or sham surgery, the rats were weighed and then placed in individual metabolic cages. The intakes and urinary outputs from 3 consecutive days were averaged. Creatinine, sodium, and protein were measured in the urine collected the third day. Creatinine was also measured in blood drawn from the tail. Twelve weeks after radiation, the rats were decapitated, and during the first 3 seconds after decapitation, blood was collected for determination of PRA by radioimmunoassay. The serum and urine creatinines were determined by the alkaline picrate reaction (Spectrophotometer Metrolab 325, Bernal, Argentina) and urine protein concentration by the method of Saifer and Gerstenfeld. Sodium and potassium in urine and blood were measured by flame photometry (ENIGE, Buenos Aires, Argentina). The creatinine clearances were determined by standard formula.

Histology

At decapitation, the kidneys were removed, and two midcoronal sections were placed in Bowie's
solution and then embedded in paraffin for light microscopy. Sections were cut 4-μm thick and stained with hematoxylin-eosin and by the periodic-acid Schiff technique. In 2K-1R rats, both radiated (RK) and nonradiated kidney (NRK) were evaluated. All histological scores were determined by a blinded pathologist. At least 30 glomeruli in each kidney were evaluated. Glomerular sclerosis was defined as areas of the tuft showing collapse of the capillaries often accompanied by hyaline deposit, adhesion to Bowman’s capsule, or both. Glomeruli were divided into four quadrants by two perpendicular intersecting lines. Because randomly placed lines could cross small lesions and thereby determine two-quadrant involvement in an otherwise normal glomerulus, each dividing line was always placed at the edge of a lesion or group of lesions. Every affected quadrant was considered as 25% involvement. Each glomerulus was then assigned to one of five categories based on percentage involvement: 0, no lesion; 1+, mild; 2+, moderate; 3+, severe; and 4+, complete. Interstitia were evaluated in a low-power field as follows: 0, normal; 1+, mild focal cellular infiltration, no fibrosis; 2+, focal cellular infiltrates, mild fibrosis; 3+, diffuse infiltrates and fibrosis; and 4+, complete infarction. For vessels, 0+ was designated normal; 1+ was minimal wall thickening; 2+ was moderate thickening but lumen diameter larger than wall thickness; 3+ was severe thickening, wall thickness larger than lumen diameter; and 4+ was complete occlusion.

**Statistical Analysis**

Comparisons among sham and radiated groups were made by the Student’s unpaired t test. All results are expressed as the mean±SEM. The averaged histological scores for tubuli and interstitia were correlated separately with water intake, urinary volume, and sodium excretion rates. Significance of differences in blood pressure response to saralasin and in histological scores between the RK-NRK and 2K-S groups was determined by one-way analysis of variance followed by modified t statistic. Critical values were calculated by Bonferroni’s procedure. Significance was defined as p values less than 0.05.

**Results**

As shown in Table 1, both radiation groups developed hypertension, and in both, urinary protein excretion rose significantly as follows: 8.0±0.78 mg/day in the 2K-S group, 12.83±1.40 mg/day in the 2K-1R group (p<0.05), 10.2±1.14 mg/day in the 1K-S group, and 35.33±5.94 mg/day in the 1K-R group (p<0.05).

**2K-1R Model**

Both the RK and NRK groups developed glomerular sclerosis, although as expected, the lesions were milder in the latter (Table 1). Mean body weight, creatinine clearance, and serum creatinine were not different from control, although PRA was significantly higher in this group when compared with all other groups (p<0.05). Table 2 shows a statistically significant fall in mean blood pressure during Saralasin infusion (p<0.05).

As shown in Table 3, the histological scores for tubuli, interstitia, and vessels in RK were higher than in the 2K-S group (p<0.05). The contralateral NRK group showed mild arteriolar thickening (p<0.05) but no significant tubular or interstitial changes. This was accompanied by unchanged intake and urinary water and sodium excretion.

**1K-R Model**

This group developed severe glomerular sclerosis (p<0.05), diminished mean creatinine clearance, and increased serum creatinine (p<0.05), whereas PRA and body weights remained unchanged (Table 1). Mean blood pressure was unaffected by Saralasin infusion (Table 2). Also, 1K-R rats showed the
TABLE 3. Sodium-Fluid Handling and Tubular, Interstitial, and Vascular Histological Scores in Rats After Unilateral Kidney Radiation

<table>
<thead>
<tr>
<th>Variable</th>
<th>2K-S (n=6)</th>
<th>2K-1R (n=7)</th>
<th>1K-S (n=4)</th>
<th>1K-R (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological scores (0-4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubules</td>
<td>0.17±0.15</td>
<td>2.14±0.13*</td>
<td>0.22±0.17</td>
<td>0.40±0.18</td>
</tr>
<tr>
<td>Interstitialia</td>
<td>0.33±0.19</td>
<td>2.00±0.00*</td>
<td>0.43±0.19</td>
<td>0.60±0.18</td>
</tr>
<tr>
<td>Vessels</td>
<td>0.15±0.03</td>
<td>2.18±0.13*</td>
<td>1.43±0.19</td>
<td>0.26±0.05</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>7.50±1.0</td>
<td>...</td>
<td>11.67±1.90</td>
<td>10.40±1.71</td>
</tr>
<tr>
<td>Urinary volume (ml/day)</td>
<td>7.30±0.46</td>
<td>...</td>
<td>8.08±1.17</td>
<td>7.88±0.86</td>
</tr>
<tr>
<td>U_{Na}V (meq/day)</td>
<td>0.847±0.06</td>
<td>...</td>
<td>0.58±0.61</td>
<td>0.87±0.07</td>
</tr>
</tbody>
</table>

Values are given as the mean±SEM. 2K-S, two-kidney, sham operated; 2K-1R, two-kidney, one radiated; RK, radiated kidney; NRK, nonradiated kidney; 1K-S, one-kidney, sham operated; 1K-R, one-kidney, radiated; U_{Na}V, sodium excretion rates.

*Values significantly different from sham-operated group (p<0.05).

Discussion

Our study shows that unilateral renal radiation causes hypertension and high PRA if the contralateral kidney is undisturbed. In previously uninephrectomized rats, however, renal radiation causes hyperfiltration and renal failure without changes in PRA.

Because radiation produces diffuse kidney damage, it could alter mechanisms involved in the regulation of extracellular fluid volume and vascular tone (e.g., filtration rate, PRA, and sodium and fluid conservation).

In our studies, the radiated kidney underwent only mild-to-moderate histological changes when the contralateral kidney was undisturbed. Wachtel et al. also reported minimal glomerular lesions in 2K-1R rats after 2,000 rad of radiation. Possibly, these mild changes preclude a drop in glomerular filtration rate and allow for pressure diuresis in the contralateral kidney such as that observed in the Goldblatt two-kidney, one clip model.9 The latter phenomenon was not studied but, if present, would help explain the elevation of PRA. Saralasin infusion lowered blood pressure in this model. Thus, the evidence suggests that renin and not renal failure is involved in the development of hypertension in the two-kidney model.

Hypertension could be responsible for the mild glomerular sclerosis and thickening of the vessels in the contralateral NRK. So, it would seem that radiation alone causes little damage when some renal tissue is undisturbed. It is no surprise, then, that water intake, urinary output, and sodium excretion rate are unaffected in this group.

In the uninephrectomized rats, the glomerular sclerosis was most severe. Because the radiation dose applied was the same as in 2K-1R rats, it is possible that the combination of hypertension, radiation injury, reduced renal mass, and unrestricted food intake could be responsible for the more severe histological changes.8 Undoubtedly, kidneys irradiated with 3,000 rad are stimulated to grow by removal of the other kidney and, perhaps by these means, develop glomerular sclerosis and secondary impairment of the filtration rate.6-10 A rise of PRA could have been prevented in 1K-R rats by volume expansion secondary to the decreased glomerular filtration rate. The higher sodium excretion rate, however, seems contradictory, but at 12 weeks, a new steady state should have been reached, thus balancing salt intake and output. We did not measure blood or extracellular fluid volume, and therefore, we are unable to ascertain whether hypertension in this model is volume dependent. The volume expansion concept in 1K-R rats is supported by the inability of Saralasin to lower blood pressure.

In 1K-R rats, the increment in urinary volume and water intake along with lesser increments in

most severe tubular, interstitial, and vascular changes (p<0.05) and higher water intake, urinary output, and sodium excretion rate (p<0.05).

Tubular and interstitial scores in each rat in all groups were averaged. These values correlated with water intake (r=0.79, p<0.00001) and with urinary output (r=0.77, p<0.0005) but not with sodium excretion rate (r=0.41, p>0.05).

### TABLE 2. Effects of Saralasin on Mean Arterial Pressure in One- and Two-Kidney Models of Renal Radiation*

<table>
<thead>
<tr>
<th>Variable</th>
<th>2K-S (n=6)</th>
<th>2K-1R (n=7)</th>
<th>1K-S (n=4)</th>
<th>1K-R (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infusion</td>
<td>74.5±2.1</td>
<td>74.5±2.1</td>
<td>74.5±2.1</td>
<td>74.5±2.1</td>
</tr>
<tr>
<td>5 ng/kg/min</td>
<td>72.3±2.1</td>
<td>72.3±2.1</td>
<td>72.3±2.1</td>
<td>72.3±2.1</td>
</tr>
<tr>
<td>10 ng/kg/min</td>
<td>70.1±2.1</td>
<td>70.1±2.1</td>
<td>70.1±2.1</td>
<td>70.1±2.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>68.0±2.1</td>
<td>68.0±2.1</td>
<td>68.0±2.1</td>
<td>68.0±2.1</td>
</tr>
</tbody>
</table>

Values are given as the mean±SEM. 2K-S, two-kidney, sham operated; 2K-1R, two-kidney, one radiated; RK, radiated kidney; NRK, nonradiated kidney; 1K-S, one-kidney, sham operated; 1K-R, one-kidney, radiated. *Values significantly different from preceding values (p<0.05).
sodium excretion rate are consistent with the severe tubulointerstitial changes observed. The average of tubular and interstitial scores, however, correlated with intake and output but not with sodium excretion. Thus, although the latter did increase after radiation in 1K-R rats, its theoretical negative role in the development of hypertension is yet to be defined.

In 1K-R rats, hypertension is undoubtedly associated with normal PRA but diminished renal function. The latter could result from severe glomerular sclerosis and also from extensive tubular and interstitial changes.\(^\text{11}\)

Finally, the increased protein excretion rates evidence tissue injury. Despite similar rise in blood pressure and similar radiation dose, however, proteinuria was worse in the uninephrectomized group. Diminished renal mass could have played a role in such a difference.\(^\text{12}\)

In summary, in the two-kidney model, unilateral renal radiation causes hypertension, high PRA, and normal renal function; histological changes are mild-to-moderate in the RK and mild in the contralateral NRK. In the uninephrectomized rats, renal radiation is accompanied by hypertension, unchanged PRA, and renal failure. Here, the histological changes are most severe and accompanied by polyuria, polidipsia, and markedly increased protein output. Sodium excretion rate increases less and does not correlate with tubulointerstitial changes. Further studies are needed to better define the roles of renin and volume in both models.

References


Key Words: renin, renal function, glomerulosclerosis, nephritis
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