Decreased Glomerulosclerosis in Aging by Angiotensin-Converting Enzyme Inhibitors

Leon Ferder, Felipe Inserra, Luis Romano, Liliana Ercole, and Viviana Pszenny

ABSTRACT
To evaluate the effects of angiotensin-converting enzyme inhibition on renal aging, enalapril was administered in the drinking water to three groups of CF1 mice at doses of 20 mg/L (Group A), 10 mg/L (Group B), and 5 mg/L (Group C). These experimental groups were compared with 20 CF1 mice not receiving enalapril (Group D). At 2 yr, total body weight was 48.1 ± 7.5 g in Group A, 47.7 ± 7.1 g in Group B, 47.6 ± 4.6 g in Group C, and 35.1 ± 5.4 g in Group D. The ratio of kidney to total body weight, in percentages, was 18.0 ± 0.3, 17.6 ± 0.3, 17.9 ± 0.2, and 17.5 ± 0.1 in Groups A, B, C, and D, respectively. Morphometric studies of the kidneys revealed the glomerular diameter to be 86.7 ± 18.0 μm, 96.9 ± 6.3 μm, 91.1 ± 11.4 μm, and 106.8 ± 9.3 μm in Groups A, B, C, and D, respectively. The number of glomeruli per square millimeter of renal cortex was 9.6 ± 3.7, 12.3 ± 2.7, 12.4 ± 8.6, and 3.2 ± 1.5 in Groups A, B, C, and D, respectively. The mesangial area per glomerulus, in percentages, was 11.6 ± 4.8, 13.9 ± 2.9, 14.2 ± 3.1, and 20.6 ± 1.9 in Groups A, B, C, and D, respectively. The percentage of glomeruli with sclerosis was 0.1 ± 0.1, 0.3 ± 0.1, 0.6 ± 0.2, and 11.6 ± 1.9 in Groups A, B, C, and D, respectively. For all of the measurements described, the P value was less than 0.01 comparing groups A, B, or C with D. There were no significant differences in blood pressure between treated and control groups. These data suggest that angiotensin-converting enzyme inhibition decreases the renal and glomerular changes that normally accompany the aging in CF1 mice.

Key Words: Kidney, glomerulosclerosis, aging, angiotensin-converting enzyme inhibitors, angiotensin
Morphometric Analysis

Masson's trichrome sagittal sections of kidney were scanned under light microscopy with a serpentine movement from cortex to medulla so as to assure equal sampling of the kidney and to avoid evaluating the same glomerulus twice. All morphometric parameters were performed by the method described by Weibel (11, 12) based on the principle stated by Delesse (13) and improved by Cockson (14).

The number of glomeruli per square millimeter of renal cortex was calculated as follows:

\[ N = \frac{n}{b \times p} \]

n equals the number of glomeruli counted by the area unit; b is a constant depending on body shape rather than body size (bodies are glomeruli in this case, and the value was estimated as 1.38); and p is the area fraction occupied by the bodies.

A graticule eyepiece was used for glomeruli counting, with prior measurement of graticule size. To avoid glomeruli double counting, glomeruli crossed by the upper and left side lines of the graticule were counted in 10 fields of tissue section, 20 sections per kidney. Glomeruli crossed by the lower and right side lines were discarded. The median number of glomeruli per microscopic field was calculated in every section observed (200 fields per kidney). Then, the number per area unit was calculated (15).

To determine the glomerular diameter (GD), a 0.1-mm-long micrometric eyepiece was used. Ten tissue sections per microscopic field were taken at random: two length measurements (L1 and L2) were taken in each glomerulus found. The diameter is represented by the formula:

\[ GD = \frac{L1 + L2}{2} \]

The calculation of the glomerular sclerosis rate was performed with a Zeiss integrator eyepiece on a 100-point parallel-line plate II, to determine on which structure each point was found, regardless of the shape of the object under study. With a 200× magnifier, between 1,000 and 1,200 points were counted per section. After all 10 sections per kidney were counted (i.e., 10,000 to 12,000 points per kidney), points corresponding to sclerosed and nonsclerosed glomeruli are added, determining the rate of each.

For the determination of mesangial area per glomerulus rate, a standard Weibel eyepiece graticule of 21 lines giving 42 points was used. To determine the mesangial area rate, the area of the respective glomerulus was determined first:

\[ V(G) = \frac{\beta}{K} \times (A(G))^{1/2} \]

Glomerulosclerosis was defined as the collapse and/or obliteration of the glomerular capillary tuft accompanied by hyalin material and an increase in the mesangial matrix and/or adhesions of the tuft to Bowman's capsule.

Statistical Method

Results were analyzed by analysis of variance and Scheffe's contrast test.

RESULTS

Potassium levels in serum in Group A were 6 ± 0.1 mEq/L; in Group B, they were 5.9 ± 0.1 mEq/L; in Group C, they were 6.1 ± 0.1 mEq/L; and in the control Group D, they were 4.9 ± 0.2 mEq/L (P < 0.01 for A, B, C, versus D). The mean body weight was higher in all treated animal groups than in control groups (Table 1). Likewise, renal weight was higher in animals receiving EM. Also, the renal weight body weight ratio was higher in the treated groups (Table 1). Mean systolic and diastolic blood pressure showed no significant differences between groups receiving different doses of EM and the control group (Table 2).

Renal Microscopy Assessment

The number of glomeruli per square millimeter of renal cortex was greater in the treated animals than in the control group (Table 3). The glomerular diameter, the percentage of mesangial area in each glomerulus, and the percentage of glomerulosclerosis was significantly greater in control Group D (Table 3).

TABLE 1. Body and renal weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt (g)</th>
<th>Right Kidney (g)</th>
<th>Left Kidney (g)</th>
<th>Kidney Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>48.10 ± 7.50b</td>
<td>0.43 ± 0.04b</td>
<td>0.42 ± 0.04b</td>
<td>1.78 ± 0.25b</td>
</tr>
<tr>
<td>B</td>
<td>47.73 ± 7.09b</td>
<td>0.36 ± 0.08b</td>
<td>0.39 ± 0.05b</td>
<td>1.63 ± 0.30b</td>
</tr>
<tr>
<td>C</td>
<td>47.59 ± 4.60b</td>
<td>0.46 ± 0.03b</td>
<td>0.43 ± 0.03b</td>
<td>1.89 ± 0.20b</td>
</tr>
<tr>
<td>D</td>
<td>35.08 ± 5.40</td>
<td>0.26 ± 0.03</td>
<td>0.26 ± 0.06</td>
<td>1.52 ± 0.09</td>
</tr>
</tbody>
</table>

a Kidney percentage of total body weight.
b P < 0.01, A, B, and C compared with D.
TABLE 2. Blood pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic (mm Hg)</td>
</tr>
<tr>
<td></td>
<td>Diastolic (mm Hg)</td>
</tr>
</tbody>
</table>

<sup>a</sup> P = not significant; A, B, and C compared with D.

TABLE 3. Glomerular morphometry: percentage of glomerulosclerosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Glomeruli per mm&lt;sup&gt;2&lt;/sup&gt; of Renal Cortex</td>
<td>A</td>
</tr>
<tr>
<td>9.6 ± 3.7</td>
<td>12.3 ± 2.7</td>
</tr>
<tr>
<td>Glomerular Diameter (μm)</td>
<td>86.7 ± 18</td>
</tr>
<tr>
<td>Mesangial Area per Glomerulus (%)</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Glomerulosclerosis (%)</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01, A, B, and C compared with D.
<sup>b</sup> P < 0.001, A, B, and C compared with D.

In Group D, the mesangial matrix and the degree of focal or diffuse glomerulosclerosis found in the periphery of the glomerulus associated with capsular adhesion (Figures 2 and 4) were significantly greater than that observed in treated mice. In treated mice, no significant glomerular or mesangial alterations were observed, independent of the dose of EM (Figures 1 and 3).

Autopsy on spontaneously dead animals showed no alterations compatible with hyperkalemia, hypotension shock, or renal anatomic changes due to acute or chronic renal failure. The cause of death was attributed to respiratory infection and tumors, common in this animal species.

DISCUSSION

Previous findings indicated that, by using the same chronically administered doses of EM in CF1 mice, a marked increase in renin-producing cell recruitment and an increase in mRNA encoding the synthesis could be detected both through immunohistochemistry or through in situ hybridization (16). Also, the high potassium levels in our three treated groups are an expression of the inhibition of the renin-angiotensin-aldosterone system and suggest that all three doses were pharmacologically active.

No explanation was found for the higher weight of animals with an inhibited renin-angiotensin system.
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versus animals in the control group. It could be related to the absence of weight reduction occurring during senescence, as described both for animals and humans (17). The higher weight of kidneys in treated groups as compared with control has already been referred to by other authors (18). This finding could have been related to a lower degree of glomerulosclerosis, as well as to the lower interstitial sclerosis described in this model (19).

It is known that in both rats and CF1 mice, the development of glomerulosclerosis is a biologic phenomenon of aging (20), which also occurs in humans (21). The number of sclerosed glomeruli identified by an optical microscope has a 10 to 12% prevalence in humans after the age of 70 (21).

Brenner et al. proposed that glomerular hyperfiltration is responsible for the glomerulosclerosis seen in a variety of experimental models of renal injury (22–24). Fogo et al. have proposed that glomerular hypertrophy may be responsible for the glomerulosclerosis (25). These hypotheses can be used to explain the glomerulosclerosis seen in a variety of human pathologic situations and may also explain the glomerulosclerosis seen in aging, because similar changes have been described in aged animals (26). The total number of microscopically identifiable glomeruli becomes lower with age and is related to the drop in renal weight (27).

The significant decrease in the number of cortical glomeruli in our control animals compared with ACEI-treated animals suggests that ACEI prevents the loss of glomeruli associated with the normal aging process in CF1 mice. Yoshida et al. emphasize the importance of the loss of nephrons, which then activates hypertrophy in the remaining nephrons, provoking sclerosis, independent of changes in glomerular hemodynamics (28). Although the precise mechanism for nephron loss and nephrosclerosis is unknown, we clearly found a high correlation ($r = 0.9971$) between glomerular size and sclerosis ($P < 0.003$), as described in other models (29,30).

The significantly smaller amount of mesangial matrix in the ACEI-treated animals is probably related to changes in the balance of production and degradation as a consequence of the inhibition of the angiotensin II formation (31), without ruling out that other mechanisms or substances altered by the chronic use of ACEI (quinine, prostaglandins) may be involved. In other animal models in which glomerulosclerosis is seen, ACEI have been shown to either prevent or retard its development (7,32–35). The process by which this protective mechanism takes place is not well known, although a decrease in intraglomerular pressure has been demonstrated (32,33).

It is now well known that angiotensin II is a growth factor (36,37). Angiotensin II has many of the characteristics of a "classic" growth factor in that it binds to specific cell surface receptors (38), activates a number of intracellular signaling pathways associated with cell growth (39,40), and induces the proliferation of a variety of cells (41).

Different results have been described regarding the effect of angiotensin II on the proliferation of mesangial cells. Angiotensin II activates DNA synthesis and the expression of the early growth-related genes (42,43). The mitogenic effect on mesangial cells due to angiotensin II has been shown by Unwin et al. (44). That effect can be produced either directly or in a synergistic way with platelet-derived growth factor (45). However, other researchers could not find these effects (46). Important data with regard to the role of angiotensin II in hypertrophy as well as in the increase in the production of the mesangial matrix were reported in studies using mesangial cell cultures (47). Kakinuma et al. suggest that angiotensin II is an important factor in the hypertrophy of the muscular tissue in blood vessels (48). The use of converting enzyme inhibitors in our model has prevented changes in the muscular layer of vessels and myocardioclasclerosis (49,50). A decrease in animal mortality was also found (51).
In those models in which the systemic blood pressure is not increased, ACEI are still protective, suggesting that the effect does not depend on their antihypertensive action. Also, using doses that do not modify systemic arterial pressure still retards the appearance of sclerosis (31,35,48,52). There were no significant differences in our study between blood pressure levels in 24-month-treated and control animals, as shown in Table 2.

Aging-related structural renal changes do not have a known pathogenic mechanism. Anderson et al. found that 24-month rats showed an increase in filtration pressure caused by lower resistance of the afferent arteriole, which did not occur in animals chronically treated with EM (26). Zoja et al. also found that ACEI caused decreased glomerular sclerosis and microalbuminuria in rats treated since their first year of age (53). A number of other renal structural modifications related to aging should be added to these hemodynamic changes, including lower renal weight of about 30% when kidneys are compared at the fourth and eighth decades of life; a lower number of identifiable glomeruli (27); and an increase in sclerosed glomeruli identified with an optical microscope (21). Our findings are in agreement with this information, because aged mice showed lower renal weight, mesangial expansion, increased glomerular diameter, glomerulosclerosis, and a decreased number of identifiable glomeruli. These changes were not found in EM-treated animals.

In summary, these experiments demonstrate that the daily administration of an ACEI in CF1 mice from the time of weaning significantly retards the progressive development of mesangial expansion and focal or diffuse glomerulosclerosis seen in untreated mice as they age. Although the precise mechanism(s) for this protection against the aging process in the kidney are not known, they appear to be similar to the protection offered by ACEI against the progressive glomerulosclerosis seen in models of experimental renal injury.

REFERENCES
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