Intraglomerular Expression of α-Smooth Muscle Actin in Aging Mice

Luis A. Romano, Leon Ferder, Felipe Inserra, Liliana Ercole, R. Ariel Gomez

Abstract To determine whether chronic treatment with enalapril initiated early in life prevents glomerular injury secondary to normal aging, CF1 mice received enalapril (20 mg/L, n=10) or nifedipine (40 mg/L, n=10) in their drinking water from the time of weaning to 12 months of life. Control mice (n=10) received tap water ad libitum. Immunocytochemical detection of renin confirmed that angiotensin-converting enzyme inhibition resulted in recruitment of renin-containing cells along the preglomerular vessels. Morphometric analysis of glomeruli included assessment of glomerular diameter and the percentage of mesangial area per glomerulus. Glomerular diameter and mesangial area were higher in control mice (99.7±0.5 μm, 12.7±0.3%) than in enalapril-treated mice (88±0.8 μm, 8.6±0.6%) (P<.05). Glomerular diameter and mesangial area in the nifedipine-treated group (99.1±0.9 μm, 12.4±0.9%) were not different from control mice. These results demonstrate that angiotensin-converting enzyme inhibition prevents the glomerular enlargement and mesangial expansion observed during natural aging. In addition, control glomeruli expressed α-smooth muscle actin in a mesangial distribution. This effect was prevented by enalapril treatment but not by nifedipine. We conclude that long-term treatment with enalapril from early life prevents the early changes associated with glomerular injury and expression of α-smooth muscle actin in the glomerulus. α-Smooth muscle actin may participate in and serve as an early marker of the glomerular injury during the normal aging process. (Hypertension. 1994;23[part 2]:889-893.)

Key Words • renin • glomerular mesangium • enalapril • nifedipine • immunohistochemistry

Actin is a cytoskeletal and contractile protein highly conserved and widely distributed among eukaryotic cells. In addition to their role in muscle contraction, actin filaments are involved in a variety of cellular processes, including cell movement, nerve growth, tubular gland formation, movement of intestinal microvilli, cytoplasmic flows, and gastrulation. In adult mammals, six different actin isoforms have been described. Two of these actin isoforms are specific for striated muscle, including skeletal α-actin and cardiac α-actin. Two other isoforms are specific for smooth muscle and are designated α- and γ-smooth muscle (SM) actin. The remaining two isoforms are known as nonmuscle isoactins. These include β- and γ-nonmuscle actin. These variants predominate in nonmuscle tissues, although they can also be found in undifferentiated skeletal muscle. The terms α, β, and γ designate variants that can be distinguished by their isoelectric points. In mature vascular smooth muscle, α-SM and γ-SM are the predominant isoforms. However, the proportions of each isoform change markedly during embryonic development. Although several studies have examined the development of actin expression in vitro, fewer studies have been performed in vivo. We have recently shown that α-SM actin is a useful marker for the study of the ontogeny of the intrarenal vasculature. The aforementioned studies allowed us to conclude that expression of α-SM actin follows the centrifugal pattern of nephrovascular development and that during early fetal and postnatal life α-SM actin is expressed in glomeruli and peritubular capillaries. As the animal matures, α-SM actin expression disappears from glomeruli and peritubular capillaries and remains localized to the renal vascular tree. It has been shown recently that in experimental models of glomerulonephritis proliferation of mesangial cells is associated with the acquisition of the smooth muscle cell phenotype, as evidenced by the expression of α-SM actin. Angiotensin-converting enzyme inhibitors (captopril, enalapril, and lisinopril, among others) are currently an integral part of our therapeutic armamentarium because of their efficacy in the control of arterial hypertension. In addition, these compounds have shown a beneficial effect in the progression of renal disease in various models of renal disease independently of their effects on systemic blood pressure. Recently, we observed an increase in the life span of CF1 mice chronically treated with enalapril. Furthermore, we showed that the decrease in glomerular and myocardial sclerosis in those animals was related to an increase in the number of hepatic and cardiac mitochondria. These findings suggest a relation between the deletion of the mitochondrial genome and aging. In the present study we provide evidence that natural aging is associated with intraglomerular expression of α-SM actin and that treatment with enalapril from early life prevents the early changes associated with glomerular injury and the expression of α-SM actin in the glomerulus. This study suggests that α-SM actin may serve as an early marker of glomerular injury during the normal aging process.

Methods

Thirty female CF1 mice were obtained from the Centro de Investigaciones Albert Einstein, Buenos Aires, and kept with...
their respective mothers until weaning at 15 days of postnatal age. After weaning, animals were randomly divided into three groups. Ten animals received enalapril maleate in the drinking water (ENL, 20 mg/L). A second group of 10 mice received nifedipine (NIF, 40 mg/L) in their drinking water. Both groups were compared with a control group (CON) of 10 mice receiving water ad libitum. All animals received standard chow and were subjected to a 12-hour light/dark cycle. At 12 months of age animals were killed with intraperitoneal sodium pentobarbital (4 mg/100 mg body wt). In all animals the abdominal aorta was catheterized, and the kidneys were perfused first with saline and after ward with Bouin's solution. Subsequently, the kidneys were removed, sectioned longitudinally, and maintained in Bouin's solution for 3 hours. The kidneys were embedded in Paraplast and stained with the immunoperoxidase technique as we have previously described.13'18'19 Immunodetection of renin was performed with an anti-rat polyclonal renin antibody (gift of Dr T. H. Goldstein, Nashville, Tenn) diluted 1:2500 in phosphate-buffered saline. a-Smooth muscle actin was detected using a monoclonal anti-α-SM actin–specific antibody (clone No. 1A4, lot No. 107F-4806, Sigma Chemical Co) diluted 1:400 in phosphate-buffered saline containing 3% bovine serum albumin. The high specificity and characterization of these two antibodies has been previously described.13'18'20 Sections were washed in tap water and counterstained with hematoxylin. Negative controls included omission of the primary antibody and replacement of the primary antibody with preimmune normal rabbit serum. No staining occurred in any control section.

Morphometric analysis of histological sections was performed using the method of Weibel21,22 as modified by Cockson.23 A total of 20 sections per kidney were examined. In each section, 10 standard microscopic fields (each containing 9.7±12 glomeruli per field) were randomly selected and examined. Glomerular diameters were measured with an ocular micrometer (0.1-mm length). In each glomerulus examined, two glomerular diameters (L1 and L2) perpendicular to each other were obtained. Measurements of glomerular diameters were obtained between points in the visceral portion of Bowman's capsule. Bowman's space was not included in the measurements. The final glomerular diameter was obtained with the formula glomerular diameter= (L1+L2)/2. To determine the percentage of mesangial area per glomerulus, we used a standard Weibel eyepiece graticule (containing 21 lines and 42 points). The total glomerular area was determined first; the total length of tested lines (Lt) was determined with the formula 1—Lt=t/nt, where t is the length of each ocular line, nt is the number of lines in the ocular (45 lines), and m is used the magnification used (×1200). Then the area corresponding to mesangium (Sm) was derived by applying the formula  

$$S_m = 2\pi r^2$$

After total glomerular area and mesangial areas were obtained, mesangial area was expressed as the percentage of the total glomerular area. Quantitative analysis of the immuno histochemical staining was performed with an eyepiece integrator (Zeiss), plate II (containing 100 points in parallel lines), which allows the determination of the structure underneath each point independently of the shape of the object studied. With a magnification of ×200, 300 points per section were counted, representing 12 000 points per kidney. This technique allows a representative population sample to be obtained and eliminates observer bias. The number of structures scoring positive and negative for the respective immunostaining was counted. The percentage of juxtaglomerular apparatuses (%JGA), afferent arterioles (%AA), and arcuate vessels (%AV) stained with renin was determined as previously described in our laboratories.24 The percentage of glomeruli positive for α-SM actin (%SMG) was similarly obtained. Statistical analysis was performed with ANOVA and Scheffé's contrast test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON (n=10)</th>
<th>NIF (n=10)</th>
<th>ENL (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular diameter, μm</td>
<td>99.7±0.5</td>
<td>99.1±0.9</td>
<td>98±0.8†</td>
</tr>
<tr>
<td>Mesangial area, %</td>
<td>12.7±0.3</td>
<td>12.4±0.9</td>
<td>8.6±0.6†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>19.5±1.4</td>
<td>19.9±1.1</td>
<td>27.6±1.2†</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>0.19±0.01</td>
<td>0.19±0.01</td>
<td>0.25±0.02†</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>0.19±0.01</td>
<td>0.18±0.01</td>
<td>0.24±0.02†</td>
</tr>
<tr>
<td><strong>CON indicates control mice; NIF, nifedipine-treated mice; and ENL, enalapril-treated mice. Values are mean±SD; n is number of animals.</strong></td>
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</table>

**Results**

**Morphometric analysis of glomeruli, kidney, and body weights is shown in the Table. Glomerular diameter was smaller in ENL animals than in CON or NIF animals (P<0.05). In addition, the percentage of mesangial area per glomerulus was smaller in ENL than in CON or NIF mice (P<0.05). This effect was not due to a decrease in kidney growth in ENL mice. In fact, somatic and kidney weights were higher in the ENL than CON group (P<0.05, Table).**

Intrarenal distribution of renin is shown in Figs 1 and 2. In CON animals, renin was mainly localized to the classic juxtaglomerular localization (Fig 1). As expected, in ENL animals the expected recruitment of renin-containing cells along the preglomerular vessels was observed (Fig 2). This was certified by the statistical analysis of immunohistochemical ratios: The %JGA containing renin was lower in CON (62.3±1.2%) and NIF (59.1±2.4%) than in ENL (95.3±2.2%) mice (P<0.05). Similarly, the %AA was smaller in CON (21.4±2%) and NIF (23.5±1.8%) than in ENL (48.3±1.3%) mice (P<0.05). Although in CON and NIF animals there was no renin immunostaining in arcuate vessels, the %AV was increased (3.1±1.3%) in ENL animals. Distribution of α-SM immunoreactivity in control and treated animals is shown in Figs 3 and 4. In CON and NIF mice, α-SM actin was detected in intrarenal vessels and glomeruli (Fig 3). Whereas control glomeruli expressed α-SM actin in a mesangial distribution (Fig 3), this effect was prevented by enalapril treatment and was not modified by nifedipine treatment (Figs 3 and 4). The mean percentage of glomeruli positively stained with anti-α-SM actin antibody was significantly higher in CON (93.8±2.28%) and NIF (92.8±3.11%) than in ENL (88±0.8%) mice (P<0.05). Neither enalapril nor nifedipine modified α-SM actin staining of intrarenal vessels.

**Discussion**

The present study demonstrates that the natural process of aging is associated with de novo expression of α-SM isoactin in an intraglomerular location. This effect is prevented by enalapril and not by nifedipine treatment. In addition, enalapril but not nifedipine treatment prevents the early changes of glomerular injury (glomerular enlargement, mesangial expansion) accompanying natural aging. Recent studies have demon-
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FIG 1. Photomicrograph of kidney tissue section from control animal shows renin immunostaining (brown) only at the level of juxtaglomerular apparatus. Counterstaining with hematoxylin (original magnification ×300).

FIG 2. Photomicrograph of kidney tissue section from enalapril-treated animal shows recruitment of renin-containing cells (brown) along the preglomerular vessel. Counterstaining with hematoxylin (original magnification ×300).

strated that in experimental models of glomerulonephritis, mesangial cell proliferation is associated with the acquisition of the smooth muscle cell phenotype as identified by expression of α-SM actin. In addition, we have reported that enalapril treatment markedly decreases glomerulosclerosis in aging mice. In the same study it was also demonstrated that enalapril treatment prevented glomerular enlargement and mesangial expansion. In addition to hyperfiltration, it has recently been shown that glomerular enlargement and mesangial expansion are major factors in the development of glomerulosclerosis. In agreement with those observations, the present study demonstrates that enalapril treatment prevents the early morphologic changes that precede glomerular sclerosis.

We have previously shown that expression of α-SM actin is developmentally regulated. During fetal life, α-SM actin is expressed in an intraglomerular location. Interestingly, a similar response during injury and natural aging is observed in adult animals, suggesting that under the appropriate stimulus adult mesangial cells are capable of reexpressing this protein. As discussed below, these findings are reminiscent of those observed with smooth muscle cells of renal arterioles, capable of reexpressing renin under the appropriate physiological stimulus.

The renin-angiotensin system is one of the first endocrine systems to appear during evolution of the phylogenetic scale. For instance, in bony fish, renin granules can be observed throughout the entire vascular tree. A similar situation is encountered in other lower vertebrates, in which renin distribution is not limited to the juxtaglomerular apparatus extending throughout the renal vasculature. Lower vertebrates have relatively low arterial pres-
It is believed that in these animals, the renin-angiotensin system increases arterial pressure and blood volume, playing an important role in the maintenance of blood flow to the tissues. In mammals, expression of the renin gene is developmentally regulated. In the fetal rat, renin mRNA and its protein are widely distributed throughout the large arterial vessels. In the adult rat, renin mRNA and its protein are circumscribed to a classic juxtaglomerular localization. The mechanisms whereby renal ontogeny recapitulates its phylogeny are currently unknown. As mentioned above, the adult kidney vasculature has the plasticity to elicit a recruitment of renin gene expressing cells under diverse physiological conditions. The present study corroborates those findings and indicates that the ability to elicit renin recruitment is maintained in aged mice.

During mammalian embryologic development, α-SM actin is expressed in an intraglomerular location. As the animal matures and reaches adult life, intraglomerular expression of α-actin disappears, remaining localized to the intrarenal arteries. This study demonstrates that during the natural process of aging α-SM actin is reexpressed in almost 100% of glomeruli, suggesting that the process of recruitment is a phenomenon of general biologic importance not limited to renin-expressing cells. It seems possible that the phenomenon of recruitment is more evident for proteins that have been highly conserved throughout the phylogenetic scale. Supporting this hypothesis, recent unpublished work from our laboratories indicate that α-SM actin is expressed in adult amphibian glomeruli.

The present study also demonstrates that inhibition of the renin-angiotensin system prevents the expression of α-SM actin in glomeruli. These findings suggest an interesting relation between the expression of proteins of the cytoskeleton, aging, and the renin-angiotensin system.
system. It is possible that inhibition of angiotensin II formation with enalapril by preventing hyperfiltration and morphological changes of glomeruli prevents expression of α-SM actin in this structure. In agreement with this hypothesis, the calcium channel blocker nifedipine, which elicits glomerular hemodynamic changes different from enalapril, did not prevent any of the glomerular changes observed. Concomitant with the appearance of early glomerular changes, α-SM actin is expressed in mesangial cells, suggesting that α-actin is an early marker of glomerular injury during the normal process of aging. It remains to be determined whether α-SM actin induces and/or is the result of the aforementioned glomerular changes.

Acknowledgments

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References

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