Effects of Angiotensin II Inhibition on the Aging Process of the Cardiovascular System.

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Introduction
During the natural process of aging there is a progressive modification, and ultimately a loss, of organ function. These changes are common to all species. In general, there is a correlation between the structural and functional alterations associated with aging. In mammals, degenerative processes such as arteriosclerosis, the development of senile plaques in the brain and the replacement of functional parenchyma by fibroconnective tissue in a variety of organs, are considered manifestations of aging [1].

Other authors [2] found that mice chronically treated with enalapril, a non-sulphhydryl-containing angiotensin converting enzyme inhibitor (ACEI), showed a reduction of age-associated myocardial and glomerular sclerosis and an increase in the number of mitochondria in myocardocytes and hepatocytes. Both parameters were associated with an increase in mice survival when compared with untreated controls. It has been widely postulated that reactive oxygen species (ROS) are causally involved in the aging process. In this context, it has been shown that enalapril can alter the oxidant/antioxidant balance in favor of the latter, probably protecting cells from oxidative damage [3].

Kidney
The progressive development of glomerulosclerosis is a well-known phenomenon that occurs in the aging kidney and in a variety of experimental models of renal injury. In the remnant kidney model, Brenner and colleagues [4] demonstrated that ACEI and angiotensin II (AngII) antagonists can attenuate glomerulosclerosis. They also showed that both types of compounds can lower intraglomerular pressure by altering the resistance of the efferent arteriole. Based on these findings they proposed that glomerular hyperfiltration is an underlying factor in the development of glomerulosclerosis.

Previous reports indicated that enalapril, administered to mice during 24 months, significantly decreases both mesangial expansion and glomerulosclerosis and it also attenuates the loss of glomeruli normally associated with aging [5]. The mechanisms involved in these protective effects of enalapril are not well established. In other models, the decrease of intraglomerular pressure by ACEI, as well as AngII type 1 receptor antagonists (AT1A), accounts for the attenuation of glomerular injury [6].

In addition to its protective actions on glomerular structure, chronic enalapril administration has been shown to decrease both peritubular and medullar interstitial sclerosis in aging mice [7]. Again, the underlying mechanisms are not entirely clear.

In an attempt to further define the role of the renin-angiotensin system (RAS) in aging, we administered the converting enzyme inhibitor, enalapril (E) (10 mg/kg/day) or the AT1A losartan (L) (30 mg/Kg/day) to Wistar rats since weaning.

After 7 months of treatment, both agents significantly decreased tubular atrophy and glomerular and interstitial fibrosis (Table 1). Moreover, when treatment was maintained during 18 months (old animals), protection was even more evident (Table 2). At the same time, proteinuria was significantly diminished in treated animals: C: 244 ± 54; E: 110 ± 43*; L: 57 ± 17* mg/24 h (*<0.05 with respect to C). All data presented in the present article were statistically evaluated with one way analysis of variance (ANOVA). These data clearly indicate that the renin-angiotensin system plays a role in the natural aging of the kidney.

Heart and vessels
Aging of the cardiovascular system is mainly characterized by the occurrence of myocardiosclerosis and a variety of changes in the vasculature [8]. These age-associated alterations are accelerated by high arterial blood pressure [9] and, in fact, the aging process itself induces changes which are similar to those produced by hypertension [10]. For example, age-related myocardiosclerosis is frequently accompanied by the
development of left ventricular hypertrophy (LVH) [11].

AngII has been shown to have structural effects on the cardiovascular system including the modification of vascular structure [12] probably via its hemodynamic effects, but also through its growth-promoting properties [13]. In contrast, ACEI and AT1A have both been shown to prevent vascular hypertrophy in a variety of experimental models [13-15], and to protect against the structural changes induced in the vasculature by hypertension [16]. These protective actions seem to be independent of arterial pressure, since they are similar in normotensive and hypertensive animals, and arterial pressure and structural protection are poorly correlated [13].

Table 1: Kidney Alterations. Effects of Losartan and Enalapril in 7 Months Old Animals.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Enalapril</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular Fibrosis</td>
<td>1.43±0.15</td>
<td>0.42±0.07*</td>
<td>0.65±0.03*</td>
</tr>
<tr>
<td>Interstitial Fibrosis</td>
<td>1.39±0.13</td>
<td>0.26±0.8*</td>
<td>0.39±0.07*</td>
</tr>
<tr>
<td>Tubular Atrophy</td>
<td>1.22±0.12</td>
<td>0.05±0.05*</td>
<td>0.16±0.03*</td>
</tr>
<tr>
<td>Collagen-III</td>
<td>1.17±0.12</td>
<td>0.44±0.10*</td>
<td>0.80±0.12*</td>
</tr>
<tr>
<td>α-SM-actin</td>
<td>0.09±0.14</td>
<td>0.11±0.07*</td>
<td>0.39±0.14*</td>
</tr>
</tbody>
</table>

These results were evaluated by a semiquantitative score 0-4. *p<0.05 vs. Control.

Table 2: Kidney Alterations. Effects of Losartan and Enalapril in 13 Months Old Animals.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Enalapril</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular Fibrosis</td>
<td>2.50±0.31</td>
<td>0.50±0.17*</td>
<td>1.25±0.18*</td>
</tr>
<tr>
<td>Interstitial Fibrosis</td>
<td>2.86±0.13</td>
<td>1.30±0.21*</td>
<td>1.72±0.12*</td>
</tr>
<tr>
<td>Tubular Atrophy</td>
<td>2.50±0.27</td>
<td>1.44±0.18*</td>
<td>1.64±0.20*</td>
</tr>
<tr>
<td>Collagen-III</td>
<td>1.75±0.17</td>
<td>0.80±0.20*</td>
<td>1.14±0.12*</td>
</tr>
<tr>
<td>α-SM-actin</td>
<td>1.29±0.34</td>
<td>0.30±0.17*</td>
<td>0.32±0.17*</td>
</tr>
</tbody>
</table>

* p<0.05 vs Control.

ACEI are able to prevent LVH and myocardiosclerosis in animals as well as in humans [11,17,18]. In agreement with those reports, data from our laboratory show that, in Wistar rats, chronic (18 months) enalapril (10 mg/Kg/day) or losartan (10 mg/Kg/day) treatment can attenuate several age-related cardiovascular changes. The protective effects of these compounds include smaller increases in heart weight and myocardiosclerosis compared with untreated controls in aged rats (Table 3). However, at the doses used, losartan was significantly less effective than enalapril. In a separate study, cardiovascular protection by losartan (30 mg/kg/day) was comparable to that of enalapril (10 mg/kg/day) even when treatment was begun in adult 12 months old rats (Table 4).

Michel et al. observed that the ACEI, perindopril, did not prevent the age-associated enlargement of the aortic lumen observed in normotensive male rats, but did postpone the increase in medial and intimal thickening [19]. They also found that in normotensive and hypertensive rats treated with perindopril for one year, left ventricular mass and collagen accumulation were significantly reduced compared to untreated animals [20]. The authors suggested that these effects may be related to hemodynamic factors since perindopril treatment significantly decreased blood pressure.

Atkinson et al. reported that chronic perindopril administration to rats delayed the age-associated decrease in endothelial function within the mesenteric arterial bed [21]. It is unclear whether this effect was dependent on the reduction of arterial pressure by perindopril, and/or on hormonal changes, such as a decline in AngII concentration or a rise in kinin or prostaglandin levels.
In this sense, we have analyzed the activity of nitric oxide synthase - NADPH-diaphorase dependent by histochemistry as optical density (OD) in the aorta of adult rats. Results demonstrated that AngII inhibition by either enalapril or losartan increased the enzymatic activity suggesting that an enhanced release of NO at the aortic wall might be involved in vascular protection (Table 3). The effect seems to be mediated by AT1 receptors. Moreover, the enzymatic activity was lower in the aorta of aged normal animals supporting the existence of a relation between the NO system and vascular structural and functional changes due to aging [22].

Aging is accompanied by decreased aortic compliance that is a major promoter of LVH. Decreased aortic compliance in our older untreated animals has been corroborated by their higher vascular mass. Aged normal rats increased their cardiac and left ventricular mass due to increased vascular resistance, a primary mechanical contributor to higher afterload. Nonetheless, a series of humoral factors have stimulatory effects on protein synthesis, interstitial matrix formation and myofibrillar size. The most potent trophic factor is AngII that promotes myocyte hypertrophy and matrix deposition independently of its effect on blood pressure. Thus, agents that block the RAS may allow regression of LVH to a greater degree. Our results have confirmed that both AngII inhibitors were equally active in the prevention of LVH and increased aortic growth. These effects indicate that even in the normal aging rat these agents exert a protective cardiovascular action.

Experimental data suggest that ACEI improve endothelial function in hypertensive rats. Since the pharmacological effects of the ACEI are due to more than one mechanism, mainly inhibition of AngII formation and blockade of bradykinin degradation, it was necessary to confirm that this action was mostly related to blockade of AngII formation either in the plasma or in the involved tissues. Comparison of the effect of enalapril with an AT1A which specifically impedes the binding of the peptide to its AT1 receptor has helped to clarify the matter. Present information confirms that NOS activity in the aorta and NO production diminishes with age, as previously reported in WKY rats [23]. This finding is consistent with the fact that, in the rat aorta, endothelium dependent relaxation to acetylcholine (Ach) is decreased with aging and hypertension involving an impaired formation of NO [24]. According with present results, chronic long-term administration of RAS inhibitors maintains, in old rats, endothelial NOS activity at the same level detected in young adult rats, suggesting a protective effect on vascular function and confirming the observation that prolonged administration of ACEI can improve endothelial relaxation to Ach in SHR [25]. In general, long-term blockade of AngII seems to delay the development of age-related changes in the cardiovascular system.

In addition to their hemodynamic effects, ACE inhibitors may function as free radical scavengers. Captopril and other ACE inhibitors containing a sulfhydryl group have a nonspecific anti-oxidant action under certain circumstances [26].

Although most studies have been unable to support a free-radical scavenger action of enalapril, the possibility that the beneficial changes observed in the heart of mice receiving enalapril were related to other antioxidant properties of this compound, can still be considered. Conceivably, enalapril may stimulate mechanisms that

| Table 3: The Effect of Enalapril and Losartan in Heart and Vessels. |
|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|
|                 | Control 6 months | Enalapril 6 months | Losartan 6 months | Control 18 months | Enalapril 18 months | Losartan 18 months |
| Heart weight (g) | 1.5±0.05        | 1.25±0.07*       | 1.50±0.06        | 1.82±0.07       | 1.54±0.05*       | 1.60±0.05*       |
| Aortic weight (mg) | 172±8          | 163±8           | 156±10          | 286±16          | 171±7*          | 215±30*         |
| Heart fibrosis (%) | 0.81±0.09      | 0.50±0.10*      | 0.79±0.08       | 1.76±0.23       | 0.50±0.19*      | 1.89±0.21       |
| Aortic thickness (μ) | 35.2±1.7       | 34.1±1.9        | 34.5±1.2        | 60.5±7.0        | 47.9±2.4*       | 54.4±3.9        |
| Aortic Endot. (ODx10) | 0.83±0.02      | 1.30±0.05*      | 1.50±0.07*      | 0.42±0.09#      | 0.96±0.07#      | 0.81±0.07#      |

*p<0.05 vs. Control. #p<0.05 vs 6 months.

Table 4: The Effect of Enalapril and Losartan in Heart And Vessels in Animals Treated from 6 to 18 Months of Age.

<table>
<thead>
<tr>
<th>Group</th>
<th>HWg</th>
<th>LWVg</th>
<th>AoWmg</th>
<th>CF score</th>
<th>α-act score</th>
<th>C III Anal. %</th>
<th>SPF Anal. %</th>
<th>SEF Anal. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.67±0.07</td>
<td>1.19±0.02</td>
<td>200±16</td>
<td>2.00±0.29</td>
<td>1.71±0.29</td>
<td>4.61±2.6</td>
<td>4.36±0.92</td>
<td>1311±145</td>
</tr>
<tr>
<td>L</td>
<td>1.34±0.06</td>
<td>0.50±0.02</td>
<td>157±16</td>
<td>0.92±0.04</td>
<td>0.66±0.16</td>
<td>0.77±0.40</td>
<td>1.13±0.64</td>
<td>2.49±0.53</td>
</tr>
<tr>
<td>E</td>
<td>1.35±0.10</td>
<td>0.86±0.08</td>
<td>177±12</td>
<td>0.33±0.21</td>
<td>0.25±0.17</td>
<td>1.09±0.50</td>
<td>3.42±0.00</td>
<td>3.45±0.06</td>
</tr>
</tbody>
</table>

HW: Heart weight; LW: Left Ventricular Weight; AoW: Aortic Weight; CF: Cardiac Fibrosis; α-act: α-actina; C.III: Collagen III; SPF: Subpericardial Fibrosis; SEF: Subendocardial Fibrosis.

In this sense, we have analyzed the activity of nitric oxide synthase - NADPH-diaphorase dependent by histochemistry as optical density (OD) in the aorta of adult rats. Results demonstrated that AngII inhibition by either enalapril or losartan increased the enzymatic activity suggesting that an enhanced release of NO at the aortic wall might be involved in vascular protection (Table 3). The effect seems to be mediated by AT1 receptors. Moreover, the enzymatic activity was lower in the aorta of aged normal animals supporting the existence of a relation between the NO system and vascular structural and functional changes due to aging [22].

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offset the action of free radicals. In this context, an increase of cytosolic and mitochondrial superoxide dismutase activities in the liver of mice treated with enalapril or captopril for three months has been described [3].

Moreover, experimental data from our laboratory show that 7 months losartan treatment can increase superoxide dismutase activity and total glutathione content in several rat tissues (unpublished data). This enhancement of antioxidant defenses is associated to a decrease in lipid oxidation relative to untreated controls.

The protective role of enhanced antioxidant defenses has been reported in several experimental circumstances. Superoxide dismutase and catalase, exogenously administered, reduce the cellular lesions caused by myocardial ischemia-reperfusion in dogs [27]. Moreover, the simultaneous overexpression of superoxide dismutase and catalase diminished oxidative stress and increased maximum life-span in Drosophila melanogaster [28]. We would anticipate similar effects for the ACEI and AT1A if they increase the activity of antioxidant enzymes.

The mechanism(s) by which these compounds could increase the antioxidant enzyme activities might include a direct effect of these drugs on antioxidant enzyme synthesis or activity, or a secondary effect resulting, for example, from inhibition of AngII synthesis or reduced aldosterone levels, altered cellular sensitivity to catecholamines, or increased tissue bradykinin levels. In this context, AngII apparently increases the expression of a membrane-associated NADP-NADPH-dependent oxidase that is a major source of reactive oxygen species.

The fact that animals treated with an ACE inhibitor or an angiotensin II antagonist showed less myocardiosclerosis and glomerulosclerosis in older animals, suggests that these compounds might have modified the natural aging mechanisms, and that the renin-angiotensin system might play a role in organ damage due to aging.

Overview

The extrapolation of results from experiments conducted with animals to the situation in humans must be made with due caution, and no definitive data exist on the issue of whether ACE inhibitors or angiotensin II antagonists can retard the aging process in normal, healthy individuals. Whether rodents and humans behave similarly in this regard, remains to be established.

References

24. König CF, Lüscher TF. Different mechanisms of endothelial dysfunction with aging and hypertension in rat aorta. Hypertension. 25: 194-200, 1995

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