Cardiovascular and Renal Effects of a Collagen Cross-Link Breaker (ALT 711) in Adult and Aged Spontaneously Hypertensive Rats

Dinko Susic, Jasmina Varagic, Jwari Ahn, and Edward D. Frohlich

Background: Increased formation of advanced glycosylation end-products on body proteins is a consequence of aging and leads to exaggerated collagen cross-linking eventually increasing cardiovascular stiffness. This study reports our initial inquires into the cardiovascular and renal effects of a cross-link breaker (ALT-711) in aged spontaneously hypertensive rats (SHR).

Methods and Results: The first experiment, in 45-week-old SHR, showed that (among four doses) the dose of 1 mg/kg/d of ALT-711 given for 4 months was most effective in reducing left ventricular and aortic mass indexes. ALT-711 also reduced left ventricular hydroxyproline concentration (5.8 ± 0.2 v 5.1 ± 0.3 mg/g in controls, \( P < .05 \)); however, it did not affect systemic or regional hemodynamics. In older SHR, ALT-711 (1 mg/kg/d) reduced \( (P < .05) \) systolic pressure (tail-cuff) (from 203 ± 3 mm Hg at outset to 187 ± 3 mm Hg at 8 weeks). Systolic pressure remained unchanged in placebo-treated rats. In addition, left ventricular index (3.09 ± 0.10 v 3.44 ± 0.05 mg/g) and aortic mass index (1.54 ± 0.04 v 1.74 ± 0.05 mg/mm) were reduced by ALT-711. In the third experiment, 1-year-old SHR were given vehicle or ALT-711 (1 mg/kg/d) or placebo until natural death. After 3 months, ALT-711 markedly reduced urinary protein excretion (74.5 ± 8.6 v 135.4 ± 11.8 mg/24 h). Echocardiographic studies, performed at the outset and after 3 and 6 months, revealed two changed indexes. Left ventricular end-diastolic diameter increased more in control than in ALT rats, whereas E-wave deceleration time decreased more in control than in ALT rats.


Key Words: Heart, cardiovascular stiff, hemodynamics, proteinuria, collagen.

Increased vascular and myocardial stiffness is the underlying mechanism for risk of cardiovascular morbidity and mortality in the elderly.1–3 Moreover, it is considered to be a very reliable predictor of adverse cardiovascular events.1–3 Furthermore, a progressive increase in cardiovascular stiffness is a part of the overall aging process, and various diseases such as hypertension, atherosclerosis, and diabetes further aggravate it.4,5 A variety of structural and functional variables determine cardiovascular stiffness. Collagen fibers provide firmness in cardiovascular tissue, and increased accumulation of collagen (ie, fibrosis) is often associated with increased stiffness.4,6,7 However, it appears that cardiovascular stiffness is related better to the degree of collagen cross-linking than to the absolute amount of collagen.6,7 Excessive collagen cross-linking increases cardiovascular stiffness and usually results from the formation of advanced glycosylation end-products (AGEs). Increased formation of AGEs occurs in the course of aging, and it is aggravated by comorbid events such as diabetes and hypertension.8 The AGEs accumulate slowly on long-lived proteins, such as collagen, and increase collagen fibers cross-linking and, in this way, they increase cardiovascular stiffness. In agreement with this concept, an inhibitor of AGEs formation has been shown to improve cardiovascular stiffness in diabetic rats.9 More recently, breakers of the AGEs-related protein cross-links have been developed10 and the beneficial effects of one of them (ALT-711) have been shown experimentally,11–13 as well as in one clinical study.14

It is the purpose of the present study to examine further the potential of cardiovascular and renal effects of ALT-711, a representative of a class of thiazolium derivatives.


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The study was supported in part by a grant-in-aid from Alteon, Inc., Ramsey, NJ.

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0895-7061/04/$30.00

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that catalytically break AGES-mediated cross-links between proteins. Because the extent to which AGES-mediated cross-links participate in adverse cardiovascular and renal effects of aging is not known, the present studies were designed to point to potential beneficial effects of breaking collagen cross-links, rather than to characterize the underlying pathophysiologic mechanisms. To this end, the effects of ALT-711 on systemic and regional hemodynamics, cardiovascular mass, left ventricular collagen content, and proteinuria were studied in two groups of spontaneously hypertensive rats (SHR), one “adult” (45 weeks) and the other “aged” (>60 weeks). Dose–response cardiovascular effects of ALT-711 were also determined.

**Methods**

**Animals**

Male SHR, purchased from Charles River Breeding Laboratories (Wilmington, MA), were used throughout the experiment. They were housed in a temperature- and humidity-controlled facility with 12 h light/dark cycle. Standard rat chow and tap water were provided ad libitum. Our Institutional Animal Care and Use Committee previously approved the study protocol.

**Experimental Protocol**

Three separate experiments were performed to answer our preliminary questions. In the first experiment, 25-week-old SHR, were divided into five groups, with 8 rats in each group. They were given either vehicle or 0.01, 0.1, 1.0, or 10 mg/kg/d of ALT-711 by gavage for the next 20 weeks. ALT-711 was dissolved in distilled water immediately before its administration. At the end of treatment, indexes of systemic (arterial pressure, cardiac output, total peripheral resistance) and regional (blood flow and resistance to liver, kidneys, and heart) hemodynamics were determined in conscious, instrumented rats, before and after dipryridamole infusion (4 mg/kg/min for 10 min), as described previously. Radiolabeled microspheres were used for the flow studies. Rats were then killed; hearts and aortas removed and weighted. Left ventricular and aortic mass indexes were calculated from their respective weights normalized for body weight and aortic length, respectively. Hydroxyproline concentration (as an estimate of collagen) was also determined.

Thus, having obtained an idea of an optimal dose in adult SHR, in our second study, 60-week-old rats were divided into two groups (7 in each). One group received ALT-711 (1 mg/kg/d), the other vehicle. These rats were treated for 8 weeks and indirect determinations of systolic blood pressure (tail-cuff) were made at baseline and at weekly intervals thereafter and, at the end, systemic hemodynamics and cardiovascular mass indexes were determined. Rats were anesthetized with pentobarbital (50 mg/kg). Catheters (PE-50; Becton Dickinson, Sparks, MD) were placed into femoral artery and jugular vein and a thermistor probe into aorta (through right carotid artery). Cardiac output was measured (thermodilution) and total peripheral resistance index was calculated from mean arterial pressure and cardiac index, assuming that right atrial pressure is zero. Rats were then killed with pentobarbital overdose, and the heart and aorta were removed for determination of right and left ventricular and aortic mass indexes.

In the third experiment, 1-year-old SHR were divided into two groups with 10 rats in each and were given either ALT-711 (1 mg/kg/day) or vehicle until natural death. After 3 months of treatment, rats were placed in metabolic cages and urinary volume and protein excretion were determined on 3 consecutive days. Echocardiographic studies were made after 3 and 6 months of treatment. Transthoracic echocardiographic examination was performed under anesthesia (pentobarbital, 50 mg/kg) in the left lateral decubitus position using standard echocardiographic machine (Sonos 2000; Agilent Technologies, Palo Alto, CA) and a 7.5-MHz transducer. M-mode tracings were used to determine left ventricular diameters and wall thickness, whereas pulsed Doppler was used to measure

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**Table 1.** Cardiovascular mass indexes and systemic hemodynamics in SHR given either placebo or ALT-711 for 20 weeks

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>ALT-711 (0.01 mg/kg)</th>
<th>ALT-711 (0.1 mg/kg)</th>
<th>ALT-711 (1 mg/kg)</th>
<th>ALT-711 (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>400 ± 7</td>
<td>413 ± 5</td>
<td>413 ± 3</td>
<td>393 ± 6</td>
<td>404 ± 8</td>
</tr>
<tr>
<td>LVI (mg/g)</td>
<td>2.90 ± 0.04</td>
<td>2.94 ± 0.06</td>
<td>2.81 ± 0.07</td>
<td>2.70 ± 0.04*</td>
<td>2.76 ± 0.05*</td>
</tr>
<tr>
<td>LVHC (mg/g)</td>
<td>5.81 ± 0.29</td>
<td>5.41 ± 0.43</td>
<td>5.06 ± 0.27*</td>
<td>5.06 ± 0.27*</td>
<td>5.06 ± 0.27*</td>
</tr>
<tr>
<td>RVI (mg/g)</td>
<td>0.52 ± 0.02</td>
<td>0.56 ± 0.03</td>
<td>0.56 ± 0.02</td>
<td>0.56 ± 0.02</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>AWI (mg/mm)</td>
<td>3.38 ± 0.07</td>
<td>3.31 ± 0.05</td>
<td>3.11 ± 0.08</td>
<td>3.03 ± 0.06*</td>
<td>3.11 ± 0.09</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>160 ± 7</td>
<td>168 ± 8</td>
<td>159 ± 8</td>
<td>143 ± 5</td>
<td>162 ± 5</td>
</tr>
<tr>
<td>CI (mL/min/kg)</td>
<td>233 ± 21</td>
<td>210 ± 12</td>
<td>221 ± 22</td>
<td>195 ± 6</td>
<td>200 ± 16</td>
</tr>
<tr>
<td>TPRI (U)</td>
<td>0.73 ± 0.09</td>
<td>0.81 ± 0.4</td>
<td>0.74 ± 0.06</td>
<td>0.73 ± 0.04</td>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>408 ± 16</td>
<td>442 ± 14</td>
<td>415 ± 10</td>
<td>405 ± 12</td>
<td>447 ± 10</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. * P < .05 when compared to controls. Eight rats in each group.

BW = body weight; LVI = left ventricular mass index; LVHC = left ventricular hydroxyproline concentration; RVI = right ventricular mass index; AWI = aortic mass index; MAP = mean arterial pressure; CI = cardiac index; TPRI = total peripheral resistance index; HR = heart rate.
flow velocities and times at the level of mitral valve and aortic annulus, as detailed elsewhere. Xylazine (2 mg, intraperitoneal) was used to decrease heart rate below 250 beats/min when measuring E and A waves.

Statistical Analysis

Values are expressed as the mean ± 1 SEM. One-way analysis of variance and Student-Newman-Keuls post-hoc tests were used for multigroup comparison, and t tests were used for comparison between the two groups.

Results

Study 1

Body weight and cardiovascular mass indexes are given in Table 1. No difference in body weight was observed between groups. Left ventricular mass index was significantly (P < .05) reduced in rats treated with 1 and 10 mg/kg ALT-711 in comparison to control rats. Left ventricular hydroxyproline concentration, an estimate of ventricular collagen concentration, was also less in rats treated with 1 mg/kg of ALT-711 as compared with controls. No difference in right ventricular mass index was observed between groups, but aortic mass index was significantly (P < .05) lower in rats given 1 mg/kg of ALT-711.

No differences in mean arterial pressure, cardiac index, total peripheral resistance, and heart rate were observed between the groups (Table 1). Similarly, blood flow and resistance to the liver, kidneys, and heart did not differ between groups (Table 2). Furthermore, coronary flow reserve (defined as a difference in flow after dipyridamole and basal flow) and minimal coronary resistance (after dipyridamole) were not different between groups (Table 2).

Study 2

During the 8-week course of therapy, systolic pressure was significantly (P < .01) reduced in the 60-week-old SHR treated with ALT-711 (Fig. 1). Left ventricular and aortic mass indexes were also less (P < .05) in ALT-711 treated rats than in controls. No difference in right ventricular mass index was found (Table 3). At the end of the study, in anesthetized rats, no differences in arterial pressure, cardiac index, and total peripheral resistance were found between control and ALT-711 treated SHR (Table 3).

Study 3

Three months of treatment of 1-year-old SHR with ALT-711 (1 mg/kg/d) significantly (P < .05) reduced protein-

Table 2. Regional hemodynamics in 45-week-old SHR treated with either placebo or ALT-711

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>ALT-711 (0.1 mg/kg)</th>
<th>ALT-711 (1 mg/kg)</th>
<th>ALT-711 (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBF (mL/min/g)</td>
<td>0.184 ± 0.050</td>
<td>0.226 ± 0.039</td>
<td>0.171 ± 0.052</td>
<td>0.165 ± 0.030</td>
</tr>
<tr>
<td>LVR (U)</td>
<td>1115 ± 210</td>
<td>809 ± 164</td>
<td>1170 ± 393</td>
<td>1179 ± 201</td>
</tr>
<tr>
<td>RBF (mL/min/g)</td>
<td>7.342 ± 0.790</td>
<td>7.562 ± 0.711</td>
<td>6.188 ± 0.729</td>
<td>5.370 ± 0.649</td>
</tr>
<tr>
<td>RVR (U)</td>
<td>23.61 ± 3.51</td>
<td>21.71 ± 2.18</td>
<td>23.90 ± 2.59</td>
<td>33.85 ± 4.88</td>
</tr>
<tr>
<td>CBF (mL/min/g)</td>
<td>5.939 ± 0.655</td>
<td>5.436 ± 0.467</td>
<td>4.270 ± 0.264</td>
<td>4.540 ± 0.506</td>
</tr>
<tr>
<td>CVR (U)</td>
<td>28.93 ± 3.51</td>
<td>30.15 ± 3.29</td>
<td>33.83 ± 2.60</td>
<td>39.99 ± 5.94</td>
</tr>
<tr>
<td>CFR (mL/min/g)</td>
<td>2.479 ± 1.537</td>
<td>2.974 ± 0.929</td>
<td>2.313 ± 0.695</td>
<td>3.508 ± 0.808</td>
</tr>
<tr>
<td>CVRmin (U)</td>
<td>22.07 ± 4.57</td>
<td>16.08 ± 0.74</td>
<td>19.64 ± 2.60</td>
<td>19.19 ± 1.16</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. Eight rats in each group.
LBF = liver blood flow; LVR = liver vascular resistance; RBF and RVR = renal blood flow and resistance, respectively; CBF and CVR = left ventricular coronary blood flow and resistance at basal conditions; CFR = coronary flow reserve (defined as a difference between flow at maximal vasodilatation after dipyridamole infusion and basal flow); CVRmin = minimal coronary resistance (after dipyridamole).

Table 3. Cardiovascular mass indexes and systemic hemodynamics in 1-year-old SHR given either placebo or ALT-711

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>ALT-711 (1 mg/kg)</th>
<th>ALT-711 (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>184 ± 7</td>
<td>178 ± 5</td>
<td></td>
</tr>
<tr>
<td>CI (mL/min/kg)</td>
<td>204 ± 12</td>
<td>211 ± 11</td>
<td></td>
</tr>
<tr>
<td>TPRI (U)</td>
<td>0.91 ± 0.06</td>
<td>0.85 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>LVI (mg/g)</td>
<td>3.44 ± 0.05</td>
<td>3.09 ± 0.10*</td>
<td></td>
</tr>
<tr>
<td>RVI (mg/g)</td>
<td>0.52 ± 0.04</td>
<td>0.51 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>AWI (mg/mm)</td>
<td>1.74 ± 0.05</td>
<td>1.54 ± 0.04*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. * P < .05. Seven rats in each group. Abbreviations as in Table 1.
uria. Thus, at 15 months of age ALT-711 treated rats had lower urinary protein excretion and urinary volume than their controls (Fig. 2).

Echocardiographic studies performed after 3 and 6 months of therapy (in 15- and 18-month-old SHR, respectively), revealed several differences between ALT-treated and control rats (Fig. 3). Thus, a progressive increase in left ventricular end-diastolic diameter that occurred in SHR with aging was attenuated by ALT-711. Similarly, a progressive decrease in E-wave deceleration time that occurs in SHR with aging was attenuated by ALT-711. There was no difference in E to A wave ratio or wall thickness. Old SHR given ALT-711 lived somewhat longer than control rats (Fig. 4).

**Discussion**

The presented results clearly demonstrated that ALT-711, a collagen cross-link breaker, exerted beneficial cardiovascular actions in the aged SHR. Thus, it consistently reduced left ventricular and aortic mass, as well as myocardial collagen concentration. This decrease in col-

**FIG. 2.** Urinary volume and protein excretion in 15-month-old SHR treated with placebo or ALT-711 for 3 months. Values obtained in 12-month-old SHR are given for comparison. There were 10 rats in each group. Values expressed as mean ± 1 SEM. *P < .05. Abbreviation as in Fig. 1.

**FIG. 3.** Echocardiographic indices of left ventricular end-diastolic diameter (LVEDD), septum thickness, E to A wave ratio (E/A), and deceleration time (DT) of E wave in 18-month-old SHR treated for 6 months with either ALT-711 or placebo. Values obtained in 12-month-old SHR and normotensive Wistar-Kyoto rats (WK) are given for comparison. There were seven rats in each group. Values expressed as mean ± 1 SEM. *P < .05. Other abbreviation as in Fig. 1.
lager concentration suggests improved metalloproteinase efficacy, as increased collagen cross-linking reduces collagen degradation. These findings are in agreement with previous results that demonstrated that ALT-711 reduced vascular and myocardial stiffness, and in this way decreased ventricular load and improved diastolic function.

Furthermore, a recent study in diabetic rats demonstrated that ALT-711 reduced left ventricular mass and collagen concentration associated with improved expression of several cardiac genes, including natriuretic peptide, connective tissue growth factor, and collagen III (among others). Our results also demonstrated that ALT-711 reduced arterial pressure in the aged SHR, which is also in agreement with previous findings that an inhibitor of AGE formation ameliorated hypertension and oxidative stress in SHR.

Echocardiographic study did not reveal many differences between ALT-711 and placebo-treated old SHR. However, we did find that left ventricular end-diastolic diameter increased with aging in SHR, and demonstrated that ALT-711 attenuated this increase. This is also in agreement with previous findings that ALT-711 reduced left ventricular mass in SHR. The E-wave deceleration time also decreased with aging in SHR, and ALT-711 attenuated the rate of this decrease. Because decreased E-wave deceleration time suggests reduced myocardial distensibility, this finding indicates that ALT-711 may have improved myocardial stiffness in old rats.

Interestingly, we did not detect any changes in E to A wave ratio in SHR with aging as might be expected on the basis of results from early clinical studies. Increased left ventricular stiffness is one of the major contributors to diastolic dysfunction that is often found in otherwise healthy elderly people as well as in hypertensive patients. It is characterized by substantially reduced early diastolic filling and increased late, atrial filling. Pulsed Doppler examination of mitral inflow revealed a characteristic finding in these patients, usually referred to as delayed relaxation pattern, which relates to a decreased E wave and an increased A wave with resulting inversion of E/A ratio. We have not observed this in our study; however, it should be noted that due to high heart rate in rats E and E waves are fused, and to visualize both waves, the heart rate must be reduced pharmacologically. Therefore, the resulting hemodynamic changes may modify echocardiographic presentation.

Finally, a novel and potentially very important finding in our study was that ALT-711 reduced proteinuria in old SHRs. With the progression of time, SHR develop renal damage, so that overt proteinuria is present in rats more than 1 year of age. Therapy with ALT-711 significantly ameliorated progressive increase in urinary protein excretion.

**Perspectives**

Increased cardiovascular stiffness is the underlying mechanism of a risk that contributes significantly to the increased cardiovascular morbidity and mortality, particularly in the elderly. Current pharmacologic therapy does not seem to provide an effective mean for attacking this risk. Because excessive collagen cross-linking due to the formation of AGEs contributes to the increased cardiovascular stiffness associated with aging, hypertension, and diabetes, pharmacologic targeting of this mechanism seems a logical approach. To this end, two therapeutic approaches appear feasible: one is to prevent formation of AGEs and the other is to disrupt already formed cross-links. Our early findings, together with already mentioned studies involving ALT-711 (a representative of a novel class of thiazolium derivatives, whose members can break established AGE cross-links), clearly show that a collagen cross-link breaker therapy exerts beneficial cardiovascular and renal effects. In this way, a novel approach for specific therapy of age-related cardiovascular disorders such as systolic hypertension in the elderly, left ventricular diastolic dysfunction, and renal functional impairment with proteinuria, seems to be at hand.

**References**


