Alagebrium Chloride Protects the Heart Against Oxidative Stress in Aging Rats

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To investigate the possible effects of alagebrium chloride (ALT-711) on oxidative stress (OS) process in aging hearts, we examined the role of ALT-711 in cardiac function and OS in the heart of aging rats. Increased mitochondrial DNA (mtDNA) deletion as well as a nearly twofold increase in advanced glycation end products (AGEs) accumulation were observed in aging heart, whereas only about 50% of the superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities were seen. However, after treatment with ALT-711, preserved cardiac diastolic function accompanied with reduced mtDNA deletion and about 30% of AGEs decrease was observed in aging hearts. In addition, ALT-711 can increase SOD and GSH-PX activities in aging hearts as well as in cultured cardiomyocytes. In conclusion, our study suggests that AGEs accumulation and the abnormalities in the OS in aging hearts can be attenuated by ALT-711, and this might be a novel underlying mechanism for ALT-711 in the treatment of cardiovascular diseases that develop with aging.

Key Words: Advanced glycation end products (AGEs)—Alagebrium chloride (ALT-711)—Oxidative stress—Aging heart.

Glucose and other reducing sugars react with proteins via a nonenzymatic modification process, thus forming reversible adducts, which rearrange over time to form a class of products termed advanced glycation end products (AGEs) (1). The formation of AGEs in long-lived connective tissue and matrix components accounts largely for the increase in collagen cross-linking that accompanies the increased arterial wall stiffness and decreased myocardial compliance observed with aging (2–6). Furthermore, AGEs, oxidative stress (OS), and even prooxidant factors linked to chronic diseases such as cardiovascular disease and renal disease (7) have been implicated as causal factors in the aging process (8). OS, which is initiated by mitochondrial dysfunction, can also lead to cellular protein modification and cell damage. Mitochondria act as biosensors of OS, which are the principal source of reactive oxygen species (ROS) in cells and are formed as a result of defects in coupled electron transport. ROS enable cells to undergo changes with aging and other age-related diseases. Moreover, the overproduction of ROS could cause a wide spectrum of oxidative damage to various cellular components, which would lead to cell death or elicit apoptosis by inducing changes in mitochondrial membrane permeability. Further, impairments in mitochondrial respiration and oxidative damage cause the large-scale deletion and duplication of mitochondrial DNA (mtDNA), and these changes in the mtDNA have been found to increase with aging in various human tissues (9,10).

A thiazolium derivative, alagebrium chloride (3-phenacyl-4,5-dimethylthiazolium chloride; ALT-711), which breaks the established AGE cross-links, improves the distensibility of the left ventricles (LV) and the arterial compliance in experimental animals (6,11–15). Initial clinical trials with ALT-711 revealed that this compound increased arterial compliance in elderly patients with systolic hypertension (16). These data suggest that drugs that can disrupt the cross-linking between proteins have potential applications in the prevention and treatment of cardiovascular complications. However, the effect of these drugs on the process of OS, aging, and cardiac remodeling remains to be examined. Studies that examine the effects of ALT-711 on diabetic hearts and kidneys revealed that in addition to the effects of ALT-711 on AGE cross-links, it might exert some effects that are independent of this function, namely, effects in OS pathway in diabetic renal injury. Besides AGE accumulation, mitochondrial dysfunction and subsequent oxidative damage have been implicated in the aging of hearts. Therefore, we determined the extent of these aging-associated cardiac changes and investigated the possible effects of ALT-711 on OS process in aging hearts.

Materials and Methods

Animal Studies

Ten 4-month-old female rats were grouped into adult group, and two groups of 20-month-old female Sprague-Dawley (SD) rats (n = 20; Shanghai Animal Breeding Laboratories, Shanghai, China) were fed regular rat chow ad libitum during the study, which was conducted in accordance with the guidelines of the Nanjing Medical University Laboratory Animal Use and Care Committee. All animals were allowed free access to the chow and water, and three to four animals per cage were housed under a
12-hour light/dark cycle in an animal room. Periodic checks of the cages and body weights were performed to ensure that the food was administered properly. The 20-month-old rats were randomized into two groups: old (n = 10) and old + ALT-711 (n = 10) groups. The animals in the old + ALT-711 group were treated with 10 mg/kg body weight of ALT-711 (Alteon, Inc., Ramsey, NJ) per day via oral gavage for 16 weeks.

Echocardiographic Measurement

After 16 weeks of treatment, two groups of old rats together with the adult counterpart were anesthetized with 7.5% chloral hydrate. Transthoracic echocardiography was performed using a Sonos 5500 ultrasonograph with a 12-MHz transducer (Hewlett-Packard, Palo Alto, CA). All the parameters, namely, the left atrium diameter (LAD), left ventricular posterior wall (LVPW), interventricular septum (IVS), fractional shortening, ejection fraction (EF), and the EF slope, were measured over at least three consecutive cardiac cycles. LV mass was calculated using a standard formula: LV mass = 1.04 × ([IVSd + LVPWd + LVDd]3-LVDd3). Transmitral Doppler flows (E and A velocities, and their ratio) were measured in an apical four-chamber orientation with the sample volume placed at the tips of the mitral leaflet. For tissue Doppler imaging, the smallest sample volume was placed at the septal side of the mitral annulus. Measurements performed include early diastolic (Ea) and late diastolic (Aa) maximal velocities. Gains were adjusted to eliminate background noise and allow for clear tissue signal, and 5–10 cycles were recorded.

After the echocardiography was completed, the body weight of the rats was measured and blood samples were obtained. The rats were then decapitated; their hearts were removed rapidly; and LVs were dissected, snap frozen, and stored at –80°C for subsequent analysis.

Cardiac Collagen AGEs, Cardiac Malondialdehyde, and Glutathione Peroxidase and Superoxide Dismutase Activity Measurement

Fluorescence emitted by the AGEs in collagen was determined using acid-hydrolyzed cardiac collagen preparations via a flow injection system adapted from Wrobel (17) using a fluorescence spectrophotometer (Waters HPLC, Brockton, MA) set at 370-nm excitation and 440-nm emission (18). The fluorescence was expressed as arbitrary units of fluorescence (AUF) per milligram of hydroxyproline (AUF/mg HYP).

To identify the AGE-modified protein, that is, the collagen content in rat’s heart, excised hearts were rinsed in phosphate-buffered saline and then fixed in 10% formalin. Samples were dehydrated with ethanol, mounted in paraffin, and sectioned at 5-μm thickness. Sections were stained with Masson’s trichrome stain to distinguish the content of collagen in the heart.

The level of OS in the aging heart of the rats was estimated by measuring the glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) activities, and malondialdehyde (MDA) concentration using the commercially available kits for those indicators from Nanjing Jiancheng Biology Company, Nanjing, China.

Cell Culture and Treatment

Cardiac myocytes were prepared from ventricles of 3-day-old SD rats, and bromodeoxyuridine (0.6 mg/mL) was added during the first 72 hours to prevent proliferation of nonmyocytes. Cells were plated in six-well plates and maintained in Dulbecco’s modified eagle’s medium (DMEM) (Life Technologies, Inc., Carlsbad, CA) supplemented with 5% heat-inactivated fetal bovine serum and 1% penicillin or streptomycin. All cells were maintained under standard cell culture conditions at 37°C and 5% CO2 environment. After 72 hours of culture, the cardiomyocytes (70%–80% confluent) were treated with AGE–bovine serum albumin (BSA; 20 μM, prepared from d-glucose-oxidized BSA) or bovine serum albumin (control) alone or with ALT-711 (10 mg/L) for 48 hours in complete growth medium. Following the treatment of cells as described previously, whole cell lysate was prepared, and the MDA level and GSH-PX and SOD activities were measured as mentioned earlier.

Isolation of the Mitochondria

Rat heart mitochondria were obtained from the cardiac tissues. Chopped pieces of the heart were rinsed and homogenized in an isolation buffer (210 mM mannitol, 70 mM sucrose, 5 mM N-2-hydroxyethylpiperazine-N’-2-ethane sulfonic acid, 1 mM ethylenediaminetetraacetic acid [EDTA], pH 7.35, and 1% protease inhibitor cocktail P8340). The nuclei and cell debris were removed by centrifugation at 1,000g for 10 minutes. The supernatant was recentrifuged at 10,000g for 10 minutes, and the resulting supernatant was removed. The pellets were resuspended in isolation buffer (1 mL) without EDTA and centrifuged at 1,000g for 5 minutes. After the centrifugation of the supernatant at 10,000g for 10 minutes, mitochondria were obtained. After each centrifugation step, any overlying layer of fat was removed. All the aforementioned procedures were performed at 4°C. The mitochondrial suspensions that were finally obtained were maintained in an ice bath and used immediately for mtDNA measurement. The mtDNA was extracted using a mixture of phenol, chloroform, and isoamyl alcohol.

mtDNA Deletion Analysis

The damage to the mtDNA was estimated, and we quantified the mtDNA4834 deletion (4mtDNA4834) using polymerase chain reaction (PCR) amplification. The following primers were used for the detection and quantification of
the wild-type mtDNA: L4395 (5′-AGGACTTACCAGA-CGCCAACACGC-3′) and H5164 (5′-CCTTTTTCTGA-TAGGCGGG-3′). To quantify the deletion in the mtDNA, the primers L7687 (5′-GCTTAGAGCGTTAACCTTTTAA-3′) and H5164 (5′-CAGCAGTTATGGATGTGGCG-3′) were used. The PCR reactions were performed separately because the size of the products from the two sets of PCR amplification was similar. The PCR mixture contained 20 pmol of each primer, 200 μg/mL of each deoxyribonucleotide triphosphate, 1× PCR buffer (50 mmol/L KCl, 10 mmol/L Tris–HCl, and 2.5 mmol/L MgCl₂; pH 8.4), and 1.5 U Ampli Taq DNA polymerase. The PCR protocol was as follows: (a) 95°C for 2 minutes; (b) 35 cycles: 94°C for 30 seconds, 60°C for 50 seconds, and 72°C for 2 minutes; and (c) 72°C for 10 minutes. The PCR products were electrophoresed on a 1.25% agarose gel, and the gel was scanned using a gel scan system. The amount of ΔmtDNA4834 is expressed as a ratio of the 440-base pair (bp) fragment (deletion products) and the 770-bp product (wild-type mtDNA).

Electron Microscopic Examination

To observe the ultrastructural changes using electron microscopy, the pieces of the rat hearts were fixed in situ with Karnovsky fixative (2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer; pH 7.4) and postfixed in 1% osmium tetroxide. The pieces were then dehydrated, infiltrated, and embedded in Spurr’s resin. Ultrathin sections (each 70 nm thick) were prepared and stained with uranyl acetate and lead citrate for ultrastructural study and observed under a transmission electron microscope (EM-902; Zeiss, Oberkochen, Germany).

Data Analysis

The original data were tested using SPSS 13.0 software. All results were presented as mean ± standard error. One-way analysis of variance was used for statistical analysis, and all tests were considered to be statistically significant at \( p < .05 \) or \( p < .01 \).

### Table 1. Echocardiographic Parameters Determining Cardiac Morphological Characteristics and Systolic Function

<table>
<thead>
<tr>
<th></th>
<th>Adult (( N = 10 ))</th>
<th>Old (( N = 10 ))</th>
<th>Old + ALT-711 (( N = 10 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>584.7 ± 9.60</td>
<td>685.6 ± 8.50</td>
<td>680.0 ± 17.7</td>
</tr>
<tr>
<td>IVS (mm)</td>
<td>2.31 ± 0.28</td>
<td>2.54 ± 0.18</td>
<td>2.39 ± 0.39</td>
</tr>
<tr>
<td>LVPW (mm)</td>
<td>2.81 ± 0.35</td>
<td>3.43 ± 0.43</td>
<td>3.24 ± 0.42</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>4.04 ± 0.63</td>
<td>5.06 ± 0.48</td>
<td>4.63 ± 0.21</td>
</tr>
<tr>
<td>LV mass (mg)/BW (g)</td>
<td>2.61 ± 0.37</td>
<td>3.67 ± 0.41</td>
<td>3.16 ± 0.56</td>
</tr>
</tbody>
</table>

**Notes:** All values are expressed as mean ± standard error. ALT-711 = alagebrium chloride; BW = body weight; IVS = interventricular septum; LVPW = left ventricular posterior wall.

\( ^* \) \( p < .05 \) in adult vs old group.

\( ^\dagger \) \( p < .05 \) in old vs old + ALT-117 group.

\( ^\ddagger \) \( p < .05 \) in adult vs old group + ALT-711 group.

### Table 2. Echocardiographic Parameters Determining Cardiac Function

<table>
<thead>
<tr>
<th></th>
<th>Adult (( N = 10 ))</th>
<th>Old (( N = 10 ))</th>
<th>Old + ALT-711 (( N = 10 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak E (m/s)</td>
<td>0.67 ± 0.09</td>
<td>0.58 ± 0.06</td>
<td>0.66 ± 0.07†</td>
</tr>
<tr>
<td>Peak A (m/s)</td>
<td>0.50 ± 0.08</td>
<td>0.82 ± 0.10†</td>
<td>0.61 ± 0.09†</td>
</tr>
<tr>
<td>E/A</td>
<td>1.46 ± 0.09</td>
<td>0.73 ± 0.12†</td>
<td>1.05 ± 0.15†</td>
</tr>
<tr>
<td>Ea (m/s; DTI)</td>
<td>0.06 ± 0.01</td>
<td>0.03 ± 0.01†</td>
<td>0.05 ± 0.01†</td>
</tr>
<tr>
<td>Aa (m/s; DTI)</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.01†</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Ea/Aa (DTI)</td>
<td>0.93 ± 0.12</td>
<td>0.46 ± 0.10†</td>
<td>0.84 ± 0.11†</td>
</tr>
<tr>
<td>E/Ea</td>
<td>10.21 ± 2.67</td>
<td>27.43 ± 3.29†</td>
<td>13.71 ± 2.48‡</td>
</tr>
<tr>
<td>EF%</td>
<td>74.32 ± 10.13</td>
<td>70.10 ± 4.82</td>
<td>80.91 ± 9.44‡</td>
</tr>
</tbody>
</table>

**Notes:** A = transmitral late filling velocity; Aa = late diastolic maximal velocity; ALT-711 = alagebrium chloride; DTI = Doppler tissue image; E = transmitral early filling velocity; Ea = early diastolic maximal velocity; EF = ejection fraction.

\( ^* \) \( p < .05 \) in adult vs old group.

\( ^\dagger \) \( p < .05 \) in old vs old + ALT-117 group.

\( ^\ddagger \) \( p < .05 \) in adult vs old group + ALT-711 group.

### Results

**Morphometric and Cardiac Functions Analysis**

The left ventricular performance of the aging rats and the cardiac effects of ALT-711 are shown in Table 1. LV mass to body weight ratio was 40% greater in the aging rats and the left ventricular posterior wall end-diastolic thickness also increased. Echocardiographic data for cardiac function are shown in Table 2. The results revealed significant abnormalities in diastolic function in aging hearts, which was demonstrated as an inversed pattern of transmitral E/A velocities, as well as evidently decreased early diastolic (Ea) to late diastolic (Aa) ratio (0.93 ± 0.12 vs 0.46 ± 0.10, \( p < .01 \)) in echocardiography. Furthermore, the ratio of E/Ea, which is the best noninvasive load-independent index to evaluate diastolic dysfunction, increased significantly in the aging group (10.21 ± 2.67 vs 27.43 ± 3.29, \( p < .01 \)). Surprisingly, when treated with ALT-711 for 16 weeks, the aging heart demonstrated significantly decreased LV mass to body weight ratio as well as ratio of E/Ea. Meanwhile, the ratio of transmitral E to A velocities increased significantly (0.73 ± 0.12 vs 1.05 ± 0.15 \( p < .05 \); see Tables 1 and 2).

**Analysis of the AGEs Composition in the LV and mtDNA Deletion**

**Measurement of AGEs in the LV.—**The accumulation of AGE in the myocardial collagen was assessed by collagen fluorescence, and it was significantly increased in aging rats compared with the adult rats (33.51 ± 1.29 vs 18.08 ± 1.24 AUF/mg HYP, \( p < .05 \); shown in Figure 1A). However, this age-related AGE accumulation was decreased in the aging rats administered ALT-711 (24.50 ± 2.26 vs 33.5 ± 1.29, \( p < .05 \); refer to Figure 1A).

Furthermore, the content of collagen in the rat’s heart was examined in tissue sections after Masson’s trichrome...
staining, which demonstrated that old rats had significantly larger areas of collagen accumulation compared with adult rats, and this effect could be attenuated by administering ALT-711, as shown in Figure 1B.

Analysis of mtDNA deletion.—The primers L7825 and H12981 that flank the 16-bp direct-repeat sequence can produce an approximately 5.2-kb PCR product from normal mtDNA genomes and a 440-bp product from deleted mtDNA, respectively (Figure 2A, lanes 1 to 3b). A 770-bp fragment was also amplified from the conservative region of the mtDNA from all samples in each of the two groups (Figure 2, lanes 1 to 3a). The ratio of the 440- to 770-bp fragments in the aging group was significantly higher than in the adult rats (0.1805 ± 0.0718 vs 0.0060 ± 0.0081, p < .01). Surprisingly, this age-related mtDNA deletion could be decreased by administering ALT-711 (0.0068 ± 0.0095 vs 0.1805 ± 0.0718, p < .01).

Detection of MDA Concentration and SOD and GSH-PX Activities

As compared with the aging group, the cardiac SOD and GSH-PX activities significantly decreased in the rats treated with ALT-711 (Figure 3). Further, the aging hearts exhibited a higher level of MDA compared with their adult counterparts, and after being administered ALT-711 for 16 weeks, a significant reduction in the levels of MDA was observed in the hearts of the aged rats compared with those of the aging group that had not been treated with ALT-711 (4.24 ± 0.11 vs 6.73 ± 0.56 nmol/mg, p < .05).

To explore the likely nature of the relationship between ALT-711 and reduction in overall the OS in aging hearts, AGE-BSA and ALT-711 were added into the cultured cardiac myocytes prepared from ventricles of 3-day-old SD rats. Our results demonstrated that AGE prepared from d-glucose-oxidized BSA significantly decreased the SOD and GSH-PX activities in cardiomyocytes. Meanwhile, ALT-711 had the same protective effects as we have seen in the animal model (see Table 3).

Electron Microscope

The results presented in Figure 2 indicated that the myocardial ultrastructures showed obvious damage in all the rats of the aging group. In particular, abnormalities in the mitochondrial structure were observed, such as mitochondrial swelling, variation in the size and shape, disarray, a reduction in the number of cristae, and a mottled matrix. Furthermore, significant disruptions of the myofilament and Z-band were also observed in aging hearts. Other ultrastructural changes were the loss of cell membrane integrity, interstitial edema, and the appearance of intracellular vacuoles. These aging-induced changes can partially be inhibited by treatment with ALT-711 (Figure 4).

Figure 1. (a) Comparison of cardiac tissue levels of advanced glycation end products (AUF/mg HYP) in old, old + ALT-711, and adult groups; ‘*’ indicates p < .05 vs old group. (b) Representative myocardial section stained with Masson’s trichrome of adult (A), old (B), and old + ALT-711 (C) rats observed under microscope (×100). Increased collagen accumulation (blue in [B]) was observed in the heart of old rats compared with the adult rats (A). However, after administering ALT-711 for 16 weeks, significantly decreased collagen accumulation was observed in (C). Note. ALT-711 = alagebrum chloride; AUF/mg HYP = arbitrary units of fluorescence per milligram of hydroxyproline.
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Discussion

Long-lived proteins (e.g., vascular and myocardial collagen) undergo continual cross-linking during the process of aging due to the formation of AGEs. Evidence suggests that aging is a major risk factor for cardiac dysfunction (19). Oxidative modification of the cardiac proteins formed by nonenzymatic glycation, such as AGEs, has been implicated as a causal factor in the aging process (20). ALT-711, a stable 4,5-dimethylthiazolium derivative of the prototype agent N-phenacylthiazolium bromide, belongs to a class of compounds that break the glucose-derived cross-links in proteins such as collagen (21). In this study, we determined the beneficial effects of ALT-711 on cardiac function by affecting the process of OS in addition to breaking down the AGE cross-links in the aged hearts.

The IVS, LAD, LVPW, and LV mass of aged and adult rats were measured by echocardiography. Consistent with results obtained from previous studies with diabetic and nondiabetic aged dogs (14, 22), significant increases in the LV mass, LVPW, and LAD were observed in the senescent animals (Table 1), and these were accompanied by increased accumulation of AGEs in the LV of aged rats. Furthermore, impaired relaxation and augmented chamber stiffness, as reflected by significantly decreased transmitral Doppler E/A ratio and ratio of Ea/Aa as well as increased ratio of E/Ea, were observed in the aging rats. Taken together, these data may indicate that the remodeling process and diastolic dysfunction occur in aging hearts. However, after the senescent rats were administered ALT-711 for 16 weeks, their LV mass prominently decreased, and those indexes for diastolic function improved significantly. These data suggested that ALT-711 could preserve cardiac function by decreasing the LV mass, that is, by reducing the degree of left ventricular hypertrophy and ameliorating cardiac diastolic dysfunction in aging rats.

An increase in the formation of AGEs occurs naturally with aging, as observed in numerous other studies (2, 4). AGEs play an important role in collagen cross-linking. Unlike normally, when modified by AGEs (usually glycation), collagen is hard to be degraded and finally it will deposit in the heart. We therefore evaluated the AGE-modified collagen by measuring its content in the hearts. Our Masson’s trichrome staining results revealed that increased collagen

Table 3. SOD and GSH-PX Enzyme Activities and MDA Changes in Cardiomyocytes After Treatment for 48 Hours

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg/protein)</th>
<th>GSH-PX (U/mg/protein)</th>
<th>MDA (nmol/mg/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>147.71 ± 10.84</td>
<td>621.32 ± 35.83</td>
<td>7.23 ± 1.42</td>
</tr>
<tr>
<td>AGE-BSA</td>
<td>92.61 ± 11.79*</td>
<td>247.67 ± 29.87*</td>
<td>13.81 ± 2.96*</td>
</tr>
<tr>
<td>AGE-BSA + ALT-711</td>
<td>133.52 ± 14.53†</td>
<td>586.13 ± 45.79†</td>
<td>9.77 ± 1.85‡</td>
</tr>
</tbody>
</table>

Notes: All values are expressed as means ± standard error. AGE = advanced glycation end product; ALT-711 = alagebrium chloride; BSA = bovine serum albumin; GSH-PX = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase.

* p < .01 in AGE-BSA vs BSA group.
† p < .01 in AGE-BSA vs AGE-BSA + ALT-711 group.
‡ p < .05 in AGE-BSA vs AGE-BSA + ALT-711 group.
was observed in the old rats’ heart than in adult rat’s heart. However, the increased AGEs-modified collagen in the heart could be reduced by treatment with ALT-711 for 16 weeks, which suggested that ALT-711 had a direct effect on AGEs and AGEs-modified proteins. Furthermore, the accumulated AGEs have the ability to stimulate proinflammatory mechanisms, increase the production of superoxide anions, and then increase OS (23–25). According to the hypothesized central role of the mitochondria in the aging process, a heart that exhibits a high rate of oxygen consumption throughout an individual’s lifetime may be especially prone to oxidative damage (26). Moreover, the mitochondria are a major source and target of oxidative damage. In particular, the accumulation of oxidant-induced somatic mutations in mtDNA is believed to be the underlying cause of the decline in physiological function with age (27), which in turn leads to increased ROS production and further damage to the mtDNA (26,28). As compared with the OS levels in the adult rats, those in the aged group increased significantly, and this increase was manifested as a significant decrease in the GSH-PX and SOD activities and an increase in the levels of MDA. In addition, the rate of mtDNA deletion was observed to be much higher in the aged rats as compared with the adult rats. Subsequently, the ultrastructural changes observed with electron microscopy, such as the disruption of myofilaments and Z-band architecture along with mitochondrial swelling, also occurred in the aging rats. However, after administering ALT-711 for 16 weeks, an increase in GSH-PX and SOD activities and a decrease in the MDA level were observed; further, decreased mtDNA deletion rate was also observed in the aged group, which was accompanied by an attenuation of the ultrastructural changes in the myocardial tissues. Our results, therefore, may indicate that apart from decreasing the protein cross-linking, ALT-711 could protect the aging heart via an antioxidative stress effect. Furthermore, to explore the likely nature of the relationship between ALT-711 and OS, we then investigated the protective effect of ALT-711 on AGEs-treated cardiomyocytes. Our results demonstrated that ALT-711 could inhibit the increased OS induced by AGEs. Consequently, these results indicate that the decrease, at least, partially in OS in cardiomyocytes may be a direct effect of ALT-711.

In summary, our results demonstrated that the compound responsible for the breakage of AGE cross-links, that is, ALT-711, could decrease the aging-induced accumulation of AGEs in myocardial tissues, decrease mtDNA deletions, ameliorate the degree of OS, attenuate the damage to the ultrastructure of the LV, and finally preserve cardiac function. This study shows that ALT-711 may be used in the treatment of the cardiovascular diseases that occur with aging and that this drug might exert its effects via a novel mechanism.

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