Advanced Glycation End-Product Cross-Link Breakers

A Novel Approach to Cardiovascular Pathologies Related to the Aging Process


Advanced glycation end product (AGE) formation that occurs with aging and diabetes leads to the cross-linking of proteins and subsequent changes in the physicochemical properties of tissues. Cellular responses to AGE that lead to either pathological conditions or removal of AGE are mediated by a number of receptors that have been identified on various cell types such as macrophages, endothelial cells, and smooth-muscle cells.

Mechanisms by which AGE affect the cardiovascular system include AGE cross-linking of long-lived proteins such as collagen and elastin and altered cellular responses. Alagebrium (3-phenacyl-4,5-dimethylthiazolium chloride, ALT-711) is the first drug in a new class of thiazolium therapeutic agents that break established AGE cross-links between proteins. In animal studies, alagebrium was effective in reducing large artery stiffness, slowing pulse-wave velocity, enhancing cardiac output, and improving left ventricular diastolic distensibility. In human studies to determine safety and efficacy, alagebrium was safe and well tolerated. In the first phase 2 clinical study, alagebrium improved arterial compliance in elderly patients with vascular stiffening. In two subsequent phase 2 clinical studies, one addressing diastolic heart failure and the other addressing systolic hypertension, alagebrium was effective in improving cardiac function and uncontrolled systolic blood pressure, particularly in more severely affected patients. Additional clinical studies to determine the utility of alagebrium in treating cardiovascular disorders associated with aging are in progress. Am J Hypertens 2004;17:23S–30S © 2004 American Journal of Hypertension, Ltd.

Key Words: Advanced glycation end products, aging, alagebrium, cardiovascular stiffness, collagen, cross-link breaker, diastolic heart failure, systolic hypertension.

Non-enzymatic glycation is a reaction between reducing sugars, such as glucose, and biological amine groups on proteins and other biomolecules in the tissues of the body. Over time, these glycation sites on biomolecules undergo rearrangements that result in irreversible and tightly bound products referred to as advanced glycation end products (AGE). Cross-linking of proteins is a result of AGE formation that leads to changes in the physicochemical properties of tissues such as elasticity. Depending on their location, these AGE cross-linked proteins can accumulate over a long period.

There is growing evidence that AGE elicit multiple cellular responses mediated by a number of receptors that bind them. One such receptor, found on macrophages, epithelial cells, mesangial cells, endothelial cells, smooth-muscle cells, and other cell types, is the receptor for AGE (RAGE). Interaction of AGE with RAGE on endothelial cells mediates initiating events in atherogenesis including induction of oxidative stress and a resulting increase in vascular cell adhesion molecule-1 (VCAM-1), an adhesion factor that enhances the binding of macrophages to the endothelial surface. In the aging heart, AGE cross-linked collagen has been implicated in the signaling of macrophage recruitment in hypertensive myocardial fibrosis that results in deteriorating diastolic function. Cytokines are produced in response to AGE in a range of cell types, most notably on endothelial and smooth muscle cells. Upregulation of connective tissue growth factor, a proscler-
rosis cytokine, occurs in fibroblasts in response to the presence of AGE and leads to the expansion of extracellular matrix. These proinflammatory responses to AGE lead to enhanced atherosclerosis and the vascular complications of diabetes.

Over the last two decades of research, AGE began to be recognized as important mediators of vascular disease that develops due to aging and is accelerated by diabetes. Mechanisms by which AGES affect the cardiovascular system include AGE cross-linking of long-lived proteins (eg, collagen and elastin) and altered cellular responses. It is believed that AGE cross-links play an important role in the arterial and myocardial stiffening that contribute to the increase in cardiac risk with aging and diabetes and against which new pharmacological approaches are being developed.

**Alagebrium and AGE Cross-Link Breaking**

Alagebrium (3-phenacyl-4,5-dimethylthiazolium chloride [ALT-711]) is the first drug in a new class of thiazolium therapeutic agents that break established AGE cross-links between proteins. Animal studies have provided important experimental evidence for the role of AGE cross-linking in the pathogenesis of arterial and myocardial stiffening. AGE accumulation on collagen in experimentally induced diabetic rats significantly reduced large artery compliance compared to nondiabetic rats, providing evidence that tissue changes occur due to AGE cross-link formation. In animal models, alagebrium is effective in reducing large artery stiffness (as measured by systemic arterial compliance, aortic impedance, and carotid artery compliance and distensibility), slowing pulse-wave velocity, enhancing cardiac output, and improving left ventricular diastolic distensibility. Additionally, in experimental diabetes, alagebrium treatment reduced left ventricular mass and cardiac expression of brain natriuretic peptide, and attenuated atherosclerosis.

The large and compelling body of nonclinical evidence of the effectiveness of alagebrium in treating cardiovascular disease, particularly among the elderly, was attributed in part to AGE cross-linking. Alagebrium (210 mg) in older patients with evidence of stiffened vasculature decreased pulse pressure and increased large artery compliance compared to nondiabetic rats, providing evidence that tissue changes occur due to AGE cross-link formation. In animal models, alagebrium is effective in reducing large artery stiffness (as measured by systemic arterial compliance, aortic impedance, and carotid artery compliance and distensibility), slowing pulse-wave velocity, enhancing cardiac output, and improving left ventricular diastolic distensibility. Additionally, in experimental diabetes, alagebrium treatment reduced left ventricular mass and cardiac expression of brain natriuretic peptide, and attenuated atherosclerosis.

**Vascular Stiffness**

Aging is associated with a general increase in tissue stiffening. Arterial wall stiffening is recognized as the major cause of reduced total arterial compliance and increased central pulse-wave velocity, dominant risk factors for cardiovascular disease, particularly among the elderly. Arterial compliance is determined by ambient mean pressure, endothelial function, vessel tone, and structure and composition. While current antihypertensive therapies target the first three factors, interventions targeting structural factors remain largely unexplored.

The effect on vascular stiffness due to changes in structural matrix protein interaction with regard to AGE cross-linking had not been previously investigated, and was the focus of the first clinical efficacy trial of an AGE-breaker (alagebrium) conducted in 2002, described below.

A multicenter, double-blind, placebo-controlled phase 2 study was conducted to evaluate the safety of a high dose of alagebrium (210 mg) and its effects on stiffened vasculature in older male and female patients when administered orally once daily for 8 weeks. Patients were recruited and screened from hypertension and general medicine clinics, and hypertension databases. A total of 93 individuals aged ≥50 years, with evidence of vascular stiffening (pulse pressure ≥60 mm, systolic blood pressure (BP) ≥140 mm Hg, and large artery compliance of ≤1.25 mL/mm Hg) were enrolled. Vascular stiffness was evaluated by echo-Doppler ultrasonography.

Results of the study showed a statistically significant difference from baseline pulse pressure after 8 weeks between treatment groups (P = .024). Mean pulse pressure in the alagebrium group declined 7.4% (5.5 mm Hg) from pretreatment values. A significant reduction in pulse pressure was observed as early as 3 days after randomization in the alagebrium group and persisted throughout the 8-week treatment period. Small decreases in pulse pressure were observed in the placebo group, but appeared to diminish after 6 weeks. No statistically or clinically significant changes were observed in cardiac output or pulse wave velocities. Additionally, there was an increase in overall vascular compliance, defined as the ratio of stroke volume (echo-Doppler determination) to pulse pressure. After 4 weeks, this measure increased by 12.1% (mean) in the alagebrium group, while decreasing slightly in the placebo group, and the treatment difference was statistically significant (P = .009). After 8 weeks, a similar difference between treatment groups did not attain statistical significance (P = .092).

It was concluded that 8 weeks of once daily oral alagebrium (210 mg) in older patients with evidence of stiffened vasculature decreased pulse pressure and increased vascular compliance. The drug was well tolerated with only one patient of 62 on alagebrium discontinued due to adverse events. This study provided the first clinical evidence of beneficial effects of an AGE breaker on vascular stiffness associated with age.

**Diastolic Heart Failure**

Diastolic heart failure accounts for 30% to 50% of heart failure in clinical practice, and hypertension is its major cause in the elderly population. After age 65 years, the incidence of heart failure in the US approaches 10 per 1000 (1%). Diastolic heart failure most frequently occurs in elderly patients with left ventricular and arterial hypertension that is accompanied by increased left ventricular and arterial stiffness, conditions which have been attributed in part to AGE cross-linking.

Considering the improvement in left ventricular disten-
sibility and arterial compliance in animal studies with alagebrium and the beneficial effects demonstrated by alagebrium on arterial compliance in elderly patients, a second phase 2 clinical study was initiated in 2002 to investigate the effects of alagebrium on diastolic heart failure. The Distensibility Improvement and Remodeling in Diastolic Heart Failure (DIAMOND) trial was an open-label exploratory study conducted to determine effects of alagebrium on key variables related to diastolic heart failure.

After 16 weeks of treatment with alagebrium (210 mg twice daily, corresponding to a total daily dose of 420 mg), exercise tolerance, aortic distensibility, left ventricular hypertrophy, diastolic filling, and quality of life were assessed in male and female patients >60 years old with stable heart failure symptoms (New York Heart Association [NYHA] class II or III) and ejection fraction >50% (n = 23). Patients were maintained on their current therapy. Cardiovascular variables were assessed by echo- and tissue-Doppler imaging.

Left ventricular mass was reduced from 124 ± 35 g at baseline to 119 ± 34 g after 16 weeks of treatment (P = .04). Two patients did not complete the trial because of serious adverse events (one myocardial infarction and one cardiac sudden death), and adverse events were as expected for this patient population. Patients with NYHA class III heart failure had a higher mean reduction in mass (9.95 ± 5.67 g, P = .008) than did patients with class II heart failure (0.92 ± 10.21; P = .772). Additionally, 14 of the 21 patients who completed the trial showed an improvement in their NYHA class. The DIAMOND patients also had a marked improvement in left ventricular diastolic filling, as evidenced by a decrease in the ratio of Doppler early diastolic flow velocity (E) to Doppler early diastolic mitral annulus velocity (E') from 10.6 ± 2.7 to 9.4 ± 1.9 cm/sec (P = .07) and an increase in E' from 7.3 ± 1.2 to 8.4 ± 1.7 cm/sec (P = .05). Alagebrium treatment also had a positive effect on three key quality-of-life measurements, as determined by the Minnesota Living with Heart Failure (MLHF) questionnaire: the MLHF total score improved from 41.2 ± 21.3 to 31.8 ± 20.6 (P = .001), the physical score improved from 20.9 ± 9.5 to 15.5 ± 9.8 (P = .006), and the mental score improved from 7.5 ± 6.0 to 6.1 ± 6.1 (P = .046). Blood pressure, exercise tolerance (peak exercise VO_2), and aortic distensibility remained unchanged over the duration of the trial.

The results of this preliminary trial provide the first clinical evidence that alagebrium has statistically and clinically significant beneficial effects in patients with diastolic heart failure who were maintained on their current cardiovascular therapy.

**Systolic Hypertension**

In 2001, the prevalence of hypertension in the total US population was 50 million. With increasing age, systolic BP and pulse pressure become better predictors of coronary heart disease risk. Hypertension, an important clinical manifestation of AGE-related cardiovascular disease, is uncontrolled in the majority of affected patients despite conventional treatment.

There is growing opinion that, in addition to traditional antihypertensive treatments, new therapeutic agents should be added that reduce systolic BP or pulse pressure and arterial stiffness. The strong evidence for the beneficial effects of alagebrium on arterial stiffness and the unmet need for new therapy led to a third phase 2 clinical trial to evaluate the efficacy of alagebrium in the treatment of systolic hypertension.

In a phase 2b, double-blind, placebo-controlled clinical trial conducted at 60 clinical sites from 2001 and 2003 in the United States, various doses of alagebrium were compared to placebo in the treatment of uncontrolled systolic hypertension. The primary efficacy measure was change from baseline in systolic and pulse pressures as assessed by office BP measurements using a mercury sphygmomanometer. Patients were separated into two cohort studies based on left ventricular hypertrophy (LVH) status: the Systolic and Pulse Pressure Hemodynamic Improvement by Restoring Elasticity (SAPPHIRE study) in patients without LVH, and the Systolic Hypertension Interaction by Left Ventricular Remodeling (SILVER study) in patients with LVH. It was postulated that alagebrium might increase the compliance of the left ventricle, allowing it to receive more blood resulting in an increase in cardiac performance (stroke index). This sequence of events could lead to an unexpected increase in systolic BP. Thus, the separate cohort studies were designed to enable distinction between the two possible outcomes.

The SAPPHIRE study had five dosing arms (alagebrium at oral doses of 210, 140, 70, and 35 mg/day v placebo), and the SILVER study had two dosing arms (alagebrium at a dose of 210 mg/day v placebo). There was a single-blind, 2-week run-in period (a common strategy designed to ameliorate a potential placebo effect) followed by the randomized, 6-month double-blind phase of the trial. The primary efficacy variables were systolic blood pressure and pulse pressure (office cuff pressure) determined by procedures conforming to Joint National Committee (JNC VI) recommendations; ie, three blood pressure (BP) measurements were taken after patients were seated comfortably for 10 to 15 mins, and the second and third BP measurements were taken 2 to 3 min after the previous reading. The primary analysis was the 6-month change from baseline cuff pressure with the last observation carried forward in patients who took at least one dose of study medication. The study was powered at 90% using a two-tailed test (0.05 significance level) to detect a 5–mm Hg change in systolic BP. Men and women ≥50 years old with uncontrolled systolic hypertension (cuff systolic BP ≥150 mm Hg or cuff diastolic BP ≤90 mm Hg) maintained on their current background antihypertensive medication were enrolled. Before enrollment, screening mean
Baseline characteristics were similar across dosing arms.

Another post hoc analysis summarized the proportion of responding participants at month 6 in each dose group of the SAPHIRE population (ie, those without LVH). Responder status was defined as meeting a threshold decrease in 24-h ABPM systolic BP of \( \geq 5 \) mm Hg net of placebo at the 35-mg dose and nonsignificant decreases at the other doses.

The large decrease in cuff BP in the placebo group was not observed in the 24-h ABPM data, so a post hoc analysis compared the mean change from baseline in systolic BP by 24-h ABPM at 3 and 6 months in participants with baseline 24-h ABPM systolic BP \( \geq 140 \) mm Hg (Fig. 1). The analysis showed a statistically significant BP reduction compared with placebo at 3 months for the 35-, 70-, and 140-mg doses of approximately the same magnitude (Fig. 1). After 6 months, there were a statistically significant mean reduction in 24-h ABPM systolic BP of \( >5 \) mm Hg net of placebo at the 35-mg dose and nonsignificant decreases at the other doses.
though the other dose groups had similar but not statistically significant responses (Fig. 2).

Participants with more severe hypertension at baseline had statistically and clinically significant reductions in 24-h ABPM systolic BP in response to 35 mg alagebrium compared with placebo after 6 months of treatment (Table 3). The magnitude of the systolic BP response was greater in participants with higher baseline pressures and in participants on at least two antihypertensive medications: values ranged from ~7 mm Hg net of placebo in patients in the lower baseline systolic BP stratification group (≥140 mm Hg) on at least one concomitant antihypertensive medication to ~18 mm Hg in the higher baseline systolic BP stratification group (≥150 mm Hg) on at least two concomitant antihypertensive medications. At the 35-mg dosage, there was a significant decrease in 24-h ABPM BP after 3 months that continued to be evident at 6 months (Fig. 1). Reductions in pulse pressure also occurred at 3 and 6 months, but they were not statistically significant (data not shown).

In conclusion, alagebrium is a novel agent that acts to reverse structural changes that lead to stiffness in the cardiovascular system that occurs with age. In this hypertension study, alagebrium was efficacious in patients with uncontrolled systolic hypertension (≥140 mm Hg), which is consistent with its mechanism of action. An apparent dose–response relationship was observed in doses from 35 mg to 140 mg/day, suggesting that the best dose in this study is 35 mg.

The systolic BP response rate at the 35-mg dose was significantly higher than the placebo rate and was comparable to currently available antihypertensive medications. Clinically important efficacy was observed in patients who did not respond to standard antihypertensive medications, and alagebrium was found to be safe and well tolerated with few side effects at all doses studied.

The results of this study support the concept that alagebrium may offer a new approach to lowering BP. Further studies are needed to confirm the findings in this study and to establish the effective dose range.

**Clinical Safety Experience of Alagebrium**

In safety/pharmacokinetics studies in healthy individuals, alagebrium has been evaluated at single oral doses ranging from 0.7 to 350 mg (men) or 315 mg (women) and 2 weeks at a dosage of 210 mg twice daily; 185 individuals were treated with alagebrium and 14 treated with placebo. Across studies in healthy individuals, euphoria occurring only at single doses ≥280 mg was the only nervous system-related adverse event reported at a higher frequency in alagebrium-treated individuals compared with placebo-treated individuals. Euphoria was usually associated with a diffuse group of mild transient symptoms of sensation and perception (eg, inability to focus, pressure in eyes, dizziness, relaxed feeling, blurry vision, tingling) and had a predictable onset and duration. This pattern was not observed in any studies in patients. In the early phase 1 studies, elevation in triglyceride levels was the most common clinical laboratory observation with alagebrium. This effect was also observed in some of the toxicity studies with the drug. A subsequent phase 1 study (which included special lipid analyses in addition to the standard serum chemistry evaluations) using a higher alagebrium dose to evaluate further these findings, gave no indication of any effect of alagebrium on total cholesterol, triglycerides, HDL-C, or LDL-C. In phase 1 studies in healthy individuals, there were no serious adverse events, deaths, or discontinuations due to adverse events. In the few patients in phase 1 studies, the incidence of nonlethal

### Table 2. Change in cuff systolic blood pressure

<table>
<thead>
<tr>
<th>Dose</th>
<th>Screening (−2 weeks)</th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 24</th>
<th>24-week Change From Baseline*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg (placebo)</td>
<td>164.16</td>
<td>162.61</td>
<td>153.69</td>
<td>154.63</td>
<td></td>
</tr>
<tr>
<td>35 mg</td>
<td>161.77</td>
<td>160.72</td>
<td>151.14</td>
<td>150.16</td>
<td>−2.58</td>
</tr>
<tr>
<td>70 mg</td>
<td>163.80</td>
<td>158.35</td>
<td>152.98</td>
<td>150.09</td>
<td>−0.28</td>
</tr>
<tr>
<td>140 mg</td>
<td>161.88</td>
<td>158.65</td>
<td>149.02</td>
<td>151.81</td>
<td>1.14</td>
</tr>
<tr>
<td>210 mg</td>
<td>165.86</td>
<td>162.33</td>
<td>154.16</td>
<td>155.87</td>
<td>1.52</td>
</tr>
</tbody>
</table>

* Change net of placebo, including all patients with and without LVH; intent to treat, last observation carried forward.

**FIG. 1.** Mean change from baseline of available data points in systolic blood pressure measured by 24-h ambulatory blood pressure monitoring (ABPM) in participants with baseline 24-h ABPM systolic blood pressure ≥140 mm Hg. *P ≤ .05; **P ≤ .01 v placebo (two-sided t test; adjusted for baseline systolic blood pressure and dose).
serious adverse events was low and comparably distributed across treatment groups; the single (placebo-treated) patient died.

In the three phase 2 trials (one including a safety extension) described above, the safety of alagebrium in clinical populations with vascular pathology consistent with formation of AGE cross-links was also assessed. In the phase 2 studies, 608 patients were treated with alagebrium and 276 treated with placebo. Although the patient populations in these studies were at high risk for serious cardiovascular events (eg, stroke, myocardial infarction, congestive heart failure), the occurrence of these events was less than or equivalent to the published incidence in comparable age groups in the general population.35 Seven deaths occurred, one in a placebo-treated patient and six in patients treated with alagebrium—all with multiple cardiovascular risk factors or evidence of target-organ damage. In the large placebo-controlled multicenter study in patients with systolic hypertension, there were no clinically significant differences in adverse events between the treated and placebo arms (Table 4).

**Summary and Conclusions**

A new class of therapeutic agents is being developed to address major unmet needs in elderly patients and those with diabetes in which vascular stiffening associated with AGE cross-linking is the root cause. Alagebrium, a member of a new class of agents developed to reverse AGE cross-linking of proteins, has emerged as the leading therapeutic candidate for reversing AGE-related pathologic conditions and the first AGE cross-link breaker to enter human trials.

In a series of preliminary human trials to determine safety and efficacy, alagebrium was found to be safe and well tolerated. Alagebrium improved arterial compliance in elderly patients with vascular stiffening, and these results provided a platform for exploring further its efficacy and safety in cardiovascular conditions in which AGE have been implicated, such as diastolic heart failure and systolic hypertension. The results of the subsequent phase 2 clinical studies, one addressing diastolic heart failure in patients with preserved ejection fraction and the other

### Table 3. Change in 24-h ambulatory blood pressure monitoring blood pressure (BP, mm Hg) after 6 months of alagebrium (35 mg)

<table>
<thead>
<tr>
<th>Concomitant antihypertensive medications</th>
<th>Baseline BP ≥ 140 mm Hg</th>
<th>Baseline BP ≥ 150 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>35 mg</td>
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<tr>
<td>≥ 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2.9</td>
<td>-10.4*</td>
</tr>
<tr>
<td>≥ 2</td>
<td>-1.5</td>
<td>-14.0*</td>
</tr>
</tbody>
</table>

* P ≤ 0.01 v placebo. † P ≤ 0.05 v placebo (two-sided t test), all evaluable subjects.
addressing systolic hypertension, revealed that alagebrium had beneficial cardiovascular effects, particularly in more severely affected patients.

Two new studies that focus on systolic hypertension and heart failure are currently being conducted to enable further evaluation of dose response, safety, and efficacy. Systolic Pressure, Efficacy and Safety Trial of Alagebrium (SPECTRA) is enrolling patients with systolic hypertension in a double-blind, randomized, multidose (10, 50, and 150 mg and placebo) clinical trial for 12 weeks. Patients stabilized on the same concomitant antihypertensive medication (hydrochlorothiazide) are being evaluated for change in blood pressure using an ABPM device. Patients with Impaired Ejection Fraction and Diastolic Dysfunction: Efficacy and Safety Trial of ALagebrium (PEDESTRAL) is a smaller, 24-week, open-label study using two doses (35 mg once daily or 210 mg twice daily) of alagebrium in patients with impaired ejection fraction. The primary end-points include quantification of left ventricular mass and complete Doppler evaluation of changes in diastolic function in men and women at least 30 years old with or without diabetes having New York Heart Association, class II to IV heart failure symptoms. In addition, a single-blind study is being conducted in patients with systolic hypertension and elevated pulse pressure to determine whether increasing vascular distensibility by decreasing AGE cross-links via alagebrium treatment improves endothelially mediated vasoreactivity at rest and after exercise.

**References**

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