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Minireview

Therapeutic potential of breakers of advanced glycation end product–protein crosslinks

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Abstract

Long-lived structural proteins, collagen and elastin, undergo continual non-enzymatic crosslinking during aging and in diabetic individuals. This abnormal protein crosslinking is mediated by advanced glycation end products (AGEs) generated by non-enzymatic glycosylation of proteins by glucose. The AGE-derived protein crosslinking of structural proteins contributes to the complications of long-term diabetes such as nephropathy, retinopathy, and neuropathy. AGE-crosslinks have also been implicated in age-related cardiovascular diseases. Potential treatment strategies for these AGE-derived complications include prevention of AGE-formation and breaking of the existing AGE-crosslinks. The therapeutic potential of the AGE-inhibitor, pimagedine (aminoguanidine), has been extensively investigated in animal models and in Phase 3 clinical trials. This review presents the pre-clinical and clinical studies using ALT-711, a highly potent AGE-crosslink breaker that has the ability to reverse already-formed AGE-crosslinks. Oral administration of ALT-711 has resulted in a rapid improvement in the elasticity of stiffened myocardium in experimental animals. Topical administration of ALT-711 was effective in improving the skin hydration of aged rats. The therapeutic potential of crosslink breakers for cardiovascular complications and dermatological alterations associated with aging and diabetes is discussed.

Keywords: Advanced glycation end products; Crosslink breakers; ALT-711; Arterial stiffness; Left ventricular hypertrophy; Diastolic dysfunction

Non-enzymatic, glucose-derived, crosslinking of proteins is a pathophysiological event that has been recognized as a causative factor in diabetic complications and age-related diseases [1–5]. The glucose-modification of proteins is initiated by non-enzymatic interaction of glucose-carbonyl groups with protein amino groups, to form an unstable Schiff's base, which rearranges rapidly to more stable Amadori products. The Amadori structure undergoes a series of slow reactions involving rearrangement, oxidation, and dehydration to form stable, heterogeneous adducts known as advanced glycosylation end products (AGEs)[1] that remain tightly bound to the protein. The post-Amadori products include highly reactive α-dicarbonyls such as Amadori dione, 3-deoxyglucosone, glyoxal, and methyl glyoxal [1–7]. Glyoxal is also generated by oxidative cleavage of glucose and methyl glyoxal from triose-phosphate intermediate of anaerobic glycoslysis [8]. These reactive intermediates cause crosslinking of the glycated proteins with –SH and $-NH_2$ groups on adjacent proteins or within domains of the same protein to form irreversible AGE-crosslinks [4].

The presence of AGEs has been demonstrated in reaction mixtures of proteins with glucose or other reducing sugars as well as in tissues from animal models of diabetes and diabetic patients by several methods. These include characteristic fluorescence of glycated proteins [9,10,17] by immunoreactivity to AGE-specific antibodies [11–16] and by resistance of AGE–protein crosslinks to proteolytic and chemical digestion [17–21].

AGE-formation from Amadori product occurs slowly over a period of months and years [1,2]. Thus, only

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[1] *Abbreviations used:* AGEs, advanced glycation end products; PTB, phenyl thiazolium bromide; RBC, red blood cells; TTC, tail tendon collagen; SHR, spontaneously hypertensive rats; DHF, diastolic heart failure; RAAS, renin–angiotensin–aldosterone system.

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Table 1
Structures of AGE-crosslinks

Crosslink	Nomenclature	Proposed pathways
Lysyl-arginine	Pentosidine	Oxidative rearrangement of Amadori products, 3-deoxyglucosone glycolaldehyde, and pentoses [22,23]
	GODIC, MODIC, DOGDIC, Glucosepane	Enzymatic hydrolysis of reaction mixtures of serum albumin and glyoxal, methyl glyoxal, deoxyglucosone and glucose [23–25]
Lysyl-lysine	GOLD,MOLD DOLD	Interaction of two lysines with two molecules of glyoxal and methyl glyoxal and deoxyglucosone [25–29]
	Crosslines	Crosslinks between two lysyl $-NH_2$ groups of two different stereoisomeric forms. Prepared by incubation of D-glucose and *N*-acetyl lysine [30,31]
	Vesperlysine	Interaction of lysine and oxidative degradation products of glucose [32,33]
bis-Lysinamides	GOLA	Isolated from the reaction mixtures of BSA and glyoxal [34]

proteins with long half-lives such as collagen will accumulate substantial amounts of AGEs in vivo. AGEs remain tightly bound to proteins and form intra- and intermolecular crosslinks with adjacent proteins. A spectrum of AGE-crosslink structures has been isolated by in vitro glycation (Table 1) and many of these structures have been identified in vivo.

AGE-crosslinking causes proteins that are normally flexible to become rigid. The cells, tissues, and blood vessels become stiff and increasingly dysfunctional. In healthy individuals, this process occurs slowly as the body ages. In diabetic patients, the rate of AGE accumulation and the extent of protein crosslinking are accelerated due to exposure to highly elevated concentrations of glucose. AGE- crosslinking of long-lived structural proteins has been correlated with the severity of diabetic complications as well as in certain pathophysiological states commonly seen in aging [2]. Numerous studies have established the role of AGEs in the development of renal, ophthalmologic, neurological, and cardiovascular complications in diabetes and aging [3,35–49].

Potential therapeutic approaches for the AGE-derived pathologies include prevention of AGE-formation and breaking the already existing AGE–protein crosslinks. AGE-inhibitors of various structural classes have been identified [50–56]. Pimagedine (aminoguanidine) has been extensively tested in animal models [57–61] and in Phase 3 clinical trials [62–64]. The therapeutic potential of pimagedine has been reviewed recently [64,65].

This review is focused on AGE-crosslink breakers, a novel class of agents capable of breaking the glucose-derived crosslinks and have the potential to reverse cardiovascular and dermatological stiffness related to aging and diabetes.

Ages and cardiovascular complications

One of the consequences of aging and diabetes is the increased stiffening of the cardiovascular system [66–69]. The large arteries lose their elasticity and the aorta becomes stiff and less compliant, leading to systolic hypertension and eventual heart failure. These changes are associated with quantitative and structural changes in the myocardial matrix proteins [68–74], which consist primarily of Type I and Type III collagens, small amounts of Type IV, Type V, Type VI collagens, and elastin [70–72]. Nearly 80% of cardiac collagen is Type I, that exists in the form of thick fibers. Fine fibers of Type III collagen make up 10% and all other collagen types together form 10% of total cardiac collagen [72]. This fibrillar collagen network connects the individual myocytes, forming the interstitial and perivascular framework. The integrity of this framework is essential to preserve the architecture of the heart, the chamber geometry, and mechanical properties. The tensile strength and flexibility of collagen maintain the level of stiffness and elasticity necessary for normal functioning of the arteries. Stability of the fibrillar matrix is derived from the post-translational covalent crosslinks mediated by lysyl oxidase [72]. This enzymatic crosslinking is a normal physiological process necessary to maintain the stability of fibrillar collagen and the normal dynamic range of elasticity of the myocardium.

The non-enzymatic, glucose-derived, crosslinking of collagen matrix occurs gradually as one ages and also as a result of diabetes. One of the consequences of AGE-crosslinking of collagen is decreased susceptibility to proteolytic and chemical degradation. The decreased proteolytic turnover results in an increased accumulation and continued AGE-derived crosslinking of collagen in the myocardial interstitium leading to the loss of flexibility. Results from numerous studies support the view that myocardial stiffening is associated with an increase in the concentration and glycation of collagen matrix. Collagen from aorta of streptozotocin (STZ)-diabetic rats has been shown to be more crosslinked compared to normal rats [75]. An increase in collagen content and fluorescence of left ventricular myocardium of alloxan-diabetic dogs has been reported by Avendeno et al. [76]. Mitzutani et al. [77,78] have shown that aorta from genetically hypertensive (SHR) rats contained increased collagen AGE-content with associated decrease

in pepsin solubility and aortic distensibility compared to normotensive rats. Studies with aorta from diabetic patients showed a correlation between an increase in fluorescent and non-fluorescent glycation induced crosslinks and stiffening of aorta [79]. Treatment with pimagedine prevented further cardiac damage in the above animal models, indicating a role of AGEs in cardiac remodeling. Herrmann et al. [80] have shown that AGE-crosslinks play a role in increased myocardial stiffening in volume overload hypertrophy in rats and treatment with pimagedine improved myocardial compliance.

The ability of crosslink breakers to disrupt the AGE-derived crosslinks in proteins offers a valuable therapeutic approach to reverse the cardiovascular stiffening. In 1996, we reported the first of AGE-crosslink breakers, phenyl thiazolium bromide (PTB) [81]. Cooper et al. [82] investigated the effect of PTB on vascular hypertrophy in streptozotocin-diabetic rats. Intra-peritoneal administration of PTB resulted in reduction of AGE accumulation on blood vessels and attenuated the diabetes induced mesenteric vascular hypertrophy. Because of the unstable nature of PTB in physiological buffers [83] analogs of PTB were tested. ALT-711 (Fig. 1), a highly potent crosslink breaker and more stable than PTB, was identified. The ability of ALT-711 to break AGE-derived crosslinks in vivo was tested by oral administration to STZ-diabetic rats after 7 weeks of diabetes. During this period of diabetes, the red blood cells (RBC) of diabetic rats showed four times more IgG crosslinks (RBC-IgG) compared to age-matched non-diabetic rats. Single, daily oral administration of ALT-711 (Fig. 2) resulted in 95% reduction in RBC-IgG within a week. A dose–response study showed an EC_{50} of 0.06 mg/kg (Fig. 3) after 1 week of dosing, indicating the potent nature of this compound.

Crosslink breaking by ALT-711 was confirmed using highly crosslinked tail tendon collagen from 32-week diabetic rats (DTTC). Tail tendon collagen (TTC) from diabetic rats and age-matched non-diabetic rats was incubated with and without ALT-711, digested with cyanogen bromide (CNBr), and then analyzed for peptides by SDS–PAGE (Fig. 4). No peptide band was detected with CNBr digest of TTC from diabetic rats incubated with PBS alone (lane 4), suggesting that the

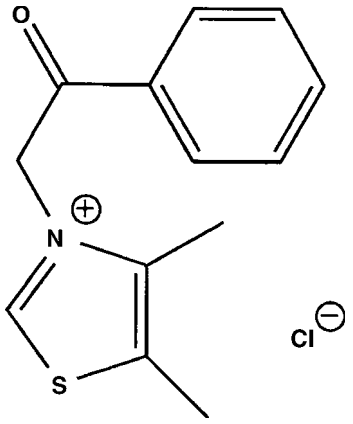

Fig. 1. Structure of crosslink breaker ALT-711.

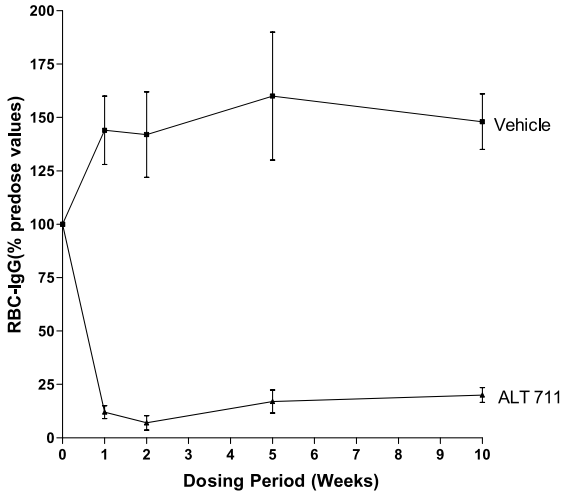

Fig. 2. In Vivo efficacy of orally administered ALT-711 in diabetic rats: Rapid breaking of RBC-IgG crosslinks. Charles River Lewis rats were made diabetic by administration of STZ (65 mg/kg body wt. in citrate buffer, pH 4.5, i.v.) and blood glucose levels were monitored after one week and throughout the course of the study. Rats with blood glucose levels of >250 mg/dl were maintained for 8 weeks in persistent hyperglycemic state. Animals were dosed once a day with ALT-711 (10 MPK) or vehicle (water) by oral gavage. Blood samples were drawn prior to starting the treatment and at the time points indicated during treatment period. The red blood cells were collected and tested for the presence of immunoreactive IgG using a filter trap ELISA format [81].

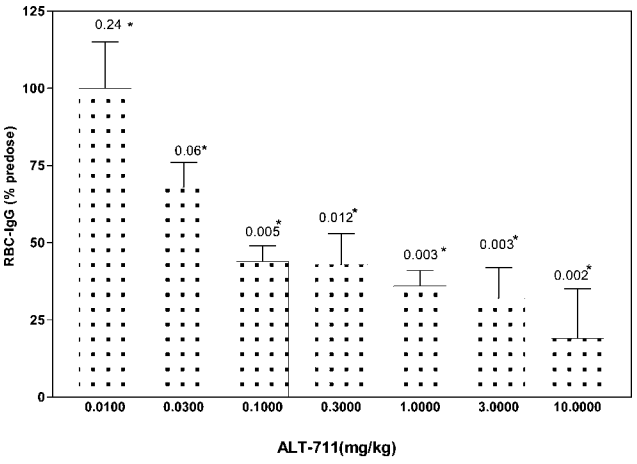

Fig. 3. In vivo efficacy of ALT-711 in diabetic rats: RBC-IgG levels after 8 days of oral dosing indicate $EC_{50} = 0.06$ mg/kg. Charles River Lewis rats were made diabetic by administration of STZ and maintained for 8 weeks in a persistent hyperglycemic state. The indicated dosages of ALT-711 were administered once a day by oral gavage. Blood samples were drawn prior to and after one week of treatment. Red blood cells were collected and tested for IgG levels [81].

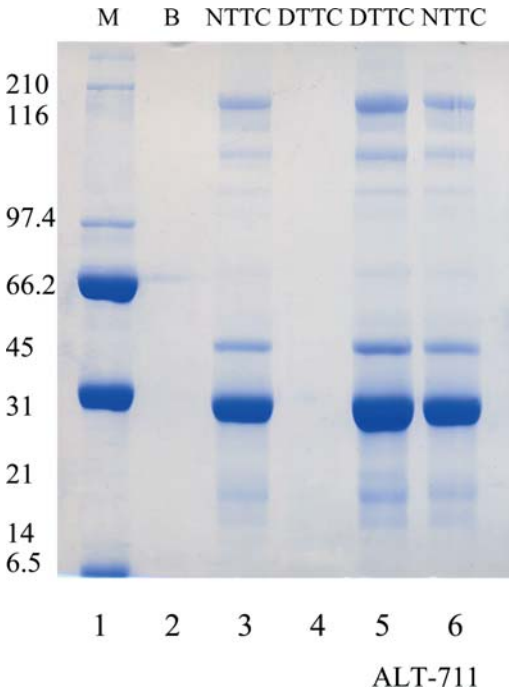

Fig. 4. ALT-711 breaks AGE-crosslinks in tail tendon collagen from diabetic rats. Tail tendon collagen was isolated from 32-week diabetic rats (DTTC) and age-matched non-diabetic rats (NTTC). The TTC samples were incubated with ALT-711 and PBS. The collagen pellets were washed with PBS, then digested with CNBr, and analyzed by SDS–PAGE under reducing conditions and the collagen peptides were visualized by Coomassie blue stain [81]. Lane 1, broad range molecular weight standards (Bio-Rad, No. 161-0147); lane 2, blank; lane 3, NTTC + PBS, CNBr digestion produced a spectrum of collagen peptides; lane 4, DTTC + PBS, CNBr peptides were too large to penetrate the gel and no peptide band was visualized on the gel; lane 5, DTTC + ALT711, Peptides similar to those from NTTC were produced by CNBr digestion of DTTC pre-incubated with ALT-711 to break AGE-crosslinks; and lane 6, NTTC + ALT-711, pre-incubation with ALT-711 did not change the CNBr peptide map of NTTC.

TTC existed as a highly crosslinked structure, too large to penetrate the gel, even after fragmentation with CNBr. Disruption of the AGE-crosslinks by pre-incubation with ALT-711 prior to CNBr digestion resulted in a spectrum of collagen peptides (lane 5). TTC from age-matched non-diabetic rats (NTTC), pre-incubated only with PBS, showed a spectrum of peptides similar to that seen from DTTC pre-incubated with ALT-711 prior to CNBr digestion (lane 3). There was no apparent difference in the peptide maps of NTTC pre-incubated with either PBS or ALT-711 (lane 6). The results indicated that ALT-711 did not affect the physiological crosslinks in TTC while breaking the AGE-crosslinks in DTTC.

Preclinical studies

Studies in several animal models have indicated the potential utility of ALT-711 in reversing the cardiovascular complications of aging and diabetes. A study conducted at Alton Ochsner Clinic (Hypertension Research Laboratory, Alton Ochsner Medical Foundation, New Orleans, LA) using non-diabetic, spontaneously hypertensive rats (SHR) showed a reversal of aortic stiffening along with a restoration of left ventricular elasticity [84] after treatment with ALT-711. Oral administration of ALT-711 to aged dogs resulted in a significant improvement in end-diastolic and stroke volume index, decrease in left ventricular stiffness, and improvement in cardiac functions [85]. In a study conducted with ALT-711 in aged monkeys [86], a significant decrease in pulse wave velocity, augmentation index, and sustained decrease in aortic stiffness, was found compared to the predose values. The effect of ALT-711 on cardiovascular distensibility was assessed in STZ-diabetic Wistar rats [87] that were diabetic for 8 weeks prior to treatment with ALT-711. A statistically significant improvement in the ability of the carotid artery to expand upon systole was observed in ALT-711 treated rats. Oral administration of ALT-711 to 16 week STZ-diabetic Sprague–Dawley rats has been shown to result in significant reduction in left ventricular mass, decrease in cardiac AGE-levels, and an increase in LV collagen solubility [88]. These effects of ALT-711 are taken as evidence that compounds with ability to break AGE-crosslinking of matrix proteins may also have the potential to reduce vascular complications of diabetes and aging.

Clinical trials

Phase 2 clinical trials of ALT-711 were initiated in 1998 and four trials have been completed in healthy volunteers of both sexes and various age groups. In these trials, ALT-711 was well tolerated. In a recently completed Phase 2a clinical trial, investigators at nine US clinical sites tested the effects of ALT-711 on blood pressure and vascular elasticity in 93 individuals over the age of 50 with stiffened cardiovasculature. Study results [89] have shown that patients ($n = 63$) who received ALT-711 (210 mg/day, 8 weeks) experienced statistically significant reduction in arterial pulse pressure and an increase in large artery compliance compared to patients who received placebo ($n = 31$). ALT-711 was well-tolerated by the study subjects with similar proportions of patients reporting adverse events in ALT-711 (54.8%) and placebo (61.3%) groups.

The ability of ALT-711 to decrease the pulse pressure and increase the large artery compliance offers a treatment option for patients with age-related stiffening of large arteries and isolated systolic hypertension. The data from animal models and the preliminary data from human studies indicate that ALT-711 has potential as a therapeutic agent in cardiovascular diseases in the older population.

Two Phase 2b trials, initiated in July 2001 and October 2001, are currently ongoing. (1) SAPPHIRE (systolic and pulse pressure hemodynamic improvement by restoring elasticity) and (2) SILVER (systolic hypertension interaction with left ventricular remodeling). These trials cover a larger population with isolated systolic hypertension with and without enlargement of the left ventricle. The SAPPHIRE trial evaluates the effectiveness of ALT-711 in 750 patients over the age of 50 having elevated systolic blood pressure (systolic hypertension) without left ventricular hypertrophy (LVH). The patients are randomized into one of the five treatment arms (four different oral tablet doses of ALT-711 or placebo) and dosed once a day for six months. This trial extends the range of doses and dosing period compared to completed Phase 2a trials in which patients were treated for eight weeks with a single dose of 210 mg per day.

In the SILVER trial, patients enrolled are also older than 50 years of age and have a systolic blood pressure greater than 150 mmHg, diastolic blood pressure less than 90 mmHg, and thickening of the left ventricle of the heart as measured by echocardiography. The patients are randomized to two treatment arms (ALT-711 or placebo) and dosed once a day for six months. The trial includes male and female, non-diabetic and diabetic patients. The primary endpoints of both Phase 2b studies are a change in systolic blood pressure and a change in pulse pressure (the difference between the systolic and diastolic blood pressures). In addition, secondary endpoints include additional blood pressure measurements and changes in certain urological characteristics. Results of these studies are expected in 2003.

Another Phase 2a clinical trial, DIAMOND (distensibility improvement and remodeling in diastolic heart failure), was initiated in July 2002 to examine the effectiveness of ALT-711 in diastolic dysfunction. Diastolic dysfunction is the inability of heart to fill with blood due to stiffening of the heart and impaired relaxation of the left ventricle. The reduced cardiac output that occurs increases pressure on the left ventricle to maintain the cardiac output, leading to diastolic heart failure (DHF), a leading cause of congestive heart failure in people over the age of 65.

There is currently no specific therapy to improve left ventricular diastolic function directly. Treatment strategies focus on reducing the ventricular filling pressure, control systemic arterial pressure, and LVH regression and improve LV and aortic distensibility. These include the use of nitrates and diuretics, calcium channel blockers, β-blockers, and ACE-inhibitors [90–92]. The DIAMOND study is designed to determine if ALT-711 can have a direct remodeling impact on the stiff heart, thus offering promise as a novel therapy for a medical condition that is currently poorly treated. The trial is being conducted at Wake Forest University Baptist Medical Center and the Medical University of South Carolina in approximately 20 patients at least 60 years of age with isolated diastolic heart failure. Primary endpoints will include aortic distensibility, as measured by state-of-the-art magnetic resonance imaging, and exercise tolerance. Effects on LVH, diastolic filling and quality of life will also be assessed. Patients are receiving 210 mg ALT-711 twice daily on an open-label, outpatient basis for at least 12 weeks. A historical control group of DHF patients who did not receive ALT-711 will be used for comparison. Data from this study, reported recently in The American Journal of Geriatric Cardiology [93], have shown statistically significant reduction in LV mass, left ventricular diastolic filling, and a positive effect on three key quality-of-life measurements.

Potential dermatological application of ALT-711

One of the consequences of aging is the progressive reduction in the thickness of the dermis causing dehydration of skin and loss of elasticity. Non-enzymatic crosslinking of skin collagen via AGEs is considered to contribute to age-related skin stiffening [94,95]. The effect of topical application of ALT-711 on skin elasticity and hydration of aged Fisher 344 male rats was studied. ALT-711 was mixed at 5% concentration with a moisturizing lotion and applied to the skin of 24-month-old rats for 3 days. The treatment was stopped and skin elasticity and hydration were measured at 3, 12, and 36 days. Results (Figs. 5 and 6) indicated that ALT-711-

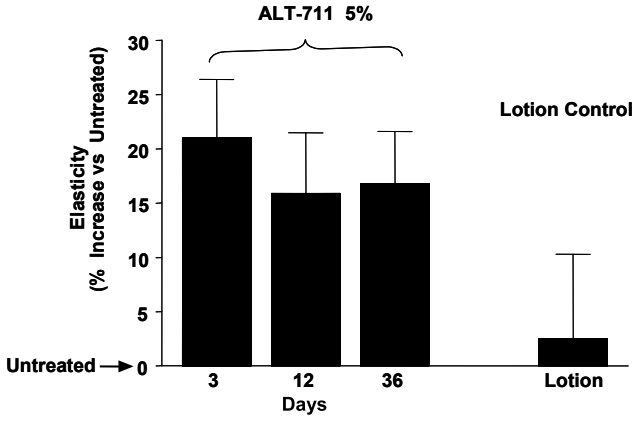

Fig. 5. Topical ALT-711 application increases skin elasticity of aged rats. Twenty-four-month-old Fischer 344 rats were used to determine the effect of topical application of ALT-711 on skin elasticity. A 5% solution of ALT-711 (w/w) in a generic skin crème or the crème alone was applied for 3 days on a shaved area on the backside of the animals. Skin strips were analyzed for tensile strength using an Instron automated materials testing system. Elasticity was determined by calculating Young's modulus from the linear portion of the stress-strain curve. Two groups of animals were similarly treated for 3 days and then left untreated. The elasticity measurements on these animals were repeated on the 9th and 33rd days after stopping the treatment.

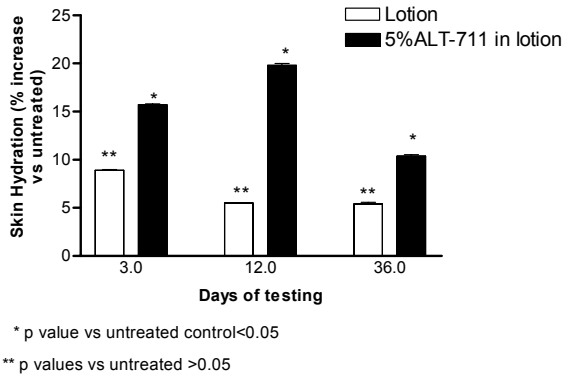

Fig. 6. Effect of ALT-711 on skin hydration is rapid and persistent. Twenty-four-month-old Fischer-344 rats were treated topically for 3 days with a generic skin cream alone or cream containing 5% (w/w) ALT-711. Age-matched untreated rats were used as controls. The water content was determined by weighing skin samples initially and after dehydration at 160 °C, under vacuum. Skin hydration was expressed as the ratio of water weight/dry weight. Two additional groups of animals were similarly treated for 3 days and then left without further treatment. These rats were tested for skin hydration on the 9th and 33rd days after terminating the treatment.

treatment improved the water content and elasticity of the aged rat skin, compared to the control rats treated only with the lotion. Whether this action by ALT-711 in animals can be extended to human remains to be studied.

Summary and perspectives

The results of pre-clinical and clinical studies using ALT-711 show that the crosslink breakers offer a breakthrough in the treatment of AGE-derived complications of cardiovascular system. Administration of this compound to various animal models of diabetes, aging, and spontaneous hypertension has resulted in an improvement in large vessel elasticity and decrease in stiffness and peripheral resistance. ALT-711 may also have potential application in reversing the effect of age on skin.

The exact mechanism of crosslink breaking is not clearly understood. Based on the structural characteristics of the breaker compounds and the predicted dicarbonyl structural motif of the crosslinks, a mechanism for breaking AGE-crosslinks has been proposed [80]. This mechanism precludes the action of crosslink breakers on other AGE–protein crosslink structures such as MOLD, GOLD, glucosepane, DOGDIC, MODIC, and GODIC (Table 1). Whether these crosslink motifs are amenable to breaking by crosslink breakers is not clear, at present. It is possible that these compounds may effect the crosslink breaking by more than one pathway. Regardless of the mechanism of action, the results of pre-clinical and clinical studies discussed here show that, ALT-711 offers a potential therapy for the pathological conditions caused by AGE–protein crosslinks.

Some investigators attribute the pharmacological effects of ALT-711 to inhibition of AGE-formation rather than to breaking of AGE-crosslinks. The rationale behind this theory includes the study by Price et al. [96] that showed ALT-711 to be an inhibitor of copper-catalyzed oxidation of ascorbic acid and certain in vitro studies in which the effect of ALT-711 was not apparent [97,98]. The latter studies include that of by Yang et al. [97] who reported that ALT-711 did not improve the solubility of tail tendon and skin collagens isolated from 7 month diabetic rats and incubated with ALT-711. Also, Mentink et al. [98] did not detect the breaking of AGE-crosslinks when in vitro glycated tail tendon collagen was incubated with ALT-711 and analyzed using differential scanning calorimetry technique. The latter authors however were able to show a change in solubility of tail tendon collagen when diabetic rats were dosed with the ALT-711. The failure to detect the effect of ALT-711 in the above in vitro experiments has led to the hypothesis that ALT-711 acts in vivo to prevent metal-catalyzed glycoxidation and hence AGE-formation.

The reason why ALT-711 failed to show any effect in the above studies is not clear. However, our studies on CNBr digestibility of DTTC before and after incubation with ALT-711 (Fig. 4) clearly substantiate the crosslink breaking effect of ALT-711.

Other in vivo and in vitro studies also corroborate the crosslink breaking action by ALT-711. Candido et al. [88] have shown that ALT-711 treatment of 16-week diabetic rats normalized the pepsin solubility of aortic collagen. Since ALT-711 was administered after the rats have been diabetic for 16 weeks, the normalization of collagen solubility would entail breaking of pre-formed AGE-crosslinks.

Studies by Hollenbach et al. [99] have shown that incubation of high molecular weight aggregates isolated from pre-glycated α-A-crystallin as well as α-A-crystallin isolated from diabetic human lenses with ALT-711 resulted in the that the monomeric form of the protein that could be isolated by using HPLC.

This result provides further evidence of AGE-crosslink breaking by ALT-711. Whether ALT-711 also has inhibitory activity for AGE-crosslink formation is not clearly established, even though our in vitro studies have shown no significant inhibition of AGE-crosslink formation by ALT-711 (unpublished results).

Finally, it should be emphasized that the mechanism underlying the pathogenesis of cardiac stiffening is multifactorial. Alterations in structural matrix can also be brought about by mechanisms other than AGE–protein crosslinks. Binding of AGEs to AGE-specific macrophage receptors (RAGE) [100,101] can stimulate the production of growth factors. Up-regulation of

growth factors results in the proliferation of arterial smooth muscle cells with gradual accumulation of additional myocardial interstitial collagen. Glycated collagen may inhibit collagen degradation by interfering with the activation of pro-metalloproteinases (MMP), the enzymes responsible for the degradation of collagen [102]. This may be an alternate mechanism for diminished proteolysis and increased collagen accumulation in myocardium.

Other pathways, besides non-enzymatic crosslinking, may also lead to changes in collagen matrix. Cardiac collagen metabolism is regulated by fibroblasts, which express mRNAse for Types I and III collagens and for metalloproteinase 1 (MMP1). Various studies with animal models of hypertension have indicated the role renin–angiotensin–aldosterone system (RAAS) plays in the development of fibrosis. In human fibroblast cultures, collagen 1 synthesis was increased by angiotensin-II while inhibiting the MMP-1 activation, thus showing a role of the RAAS system in the collagen modification [103].

The present review has addressed an approach to treat the cardiac complications related to aging and diabetes, using a new class of drugs, the crosslink breakers, that act by reducing the AGE-crosslink burden on the structural matrix.

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