Alagebrium and Complications of Diabetes Mellitus

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ABSTRACT

Glycation is the process of linking a sugar and free amino groups of proteins. Cross-linking of glycation products to proteins results in the formation of cross-linked proteins that inhibit the normal functioning of the cell. Advanced glycation end products (AGEs) are risk molecules for the cell aging process. These ends products are increasingly synthesized in diabetes and are essentially responsible for diabetic complications. They accumulate in the extracellular matrix and bind to receptors (receptor of AGE [RAGE]) to generate oxidative stress and inflammation. particularly in the cardiovascular system. Treatment methods targeting the AGE system may be of clinical importance in reducing and preventing the complications induced by AGEs in diabetes and old age. The AGE cross-link breaker alagebrium (a thiazolium derivative) is the most studied anti-AGE compound in the clinical field. Phase III clinical studies with alagebrium have been successfully conducted, and this molecule has positive effects on cardiovascular hypertrophy, diabetes, hypertension, vascular sclerotic pathologies, and similar processes. However, the mechanism is still not fully understood. The primary mechanism is that alagebrium removes newly formed AGEs by chemically separating α-dicarbonyl carbon–carbon bonds formed in cross-linked structures. However, it is also reported that alagebrium is a methylglyoxal effective inhibitor. It is not yet clear whether alagebrium inhibits copper-catalyzed ascorbic acid oxidation through metal chelation or destruction of the AGEs.

It is not known whether alagebrium has a direct association with RAGEs. The safety profile is favorably in humans, and studies have been terminated due to financial insufficiency and inability to license as a drug.

Keywords: Alagebrium, cross-link breaker, diabetes, diabetic complications

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Mechanism of Glycation

The first step of the glycation reaction involves a reducing sugar with the carbonyl group and the side chain ε-amino group in proteins. The Schiff base intermediate product is formed within hours, which is then converted into Amadori products within days.

The Amadori products are then converted to dicarbonyl compounds and finally to advanced glycation end products (AGEs) within weeks. While the part of the formation of Amadori products is recyclable, the later stages are irreversible (Figure 1).

In a healthy state, AGEs are normally produced in a slow manner, but they are rapidly produced and accumulated in hyperglycemia, diabetes, atherosclerosis, hyperlipidemia, inflammation, renal failure, and neurodegenerative diseases such as Alzheimer's disease. AGEs contribute to the aging process because most of their primary effects are seen on long-lasting proteins such as collagen and lens crystals [1, 2].

AGE Pathophysiology and Mechanism of Action

AGE damages the cells and tissues through various mechanisms; the intracellular glycation of proteins leads to impaired cell function. Circulating AGE binds to cellular receptors and leads to the activation of the signal transduction cascade and alteration in gene expression. The consequent accumulation of AGE in the extracellular matrix results in cross-linking of the proteins and decreases the distensibility in arteries [3].

AGE Accumulation in the Extracellular Matrix

The AGE–RAGE activation increases the transforming growth factor-β (TGF-β) level and matrix metalloproteinase-2 (MMP-2) activity; however, RAGE signaling modulates MMP-9 activity, which determines the changes in collagen-4 transformation [4].

The extracellular matrix is highly sensitive to glycation because of its slow conversion rate, and AGE accumulation is responsible for cross-linking. The subsequent mechanical changes include reduced elasticity, increased vessel myocardial stiffness and thickness, decreased vessel lumen, and development of glomerular sclerosis and atherosclerosis [5-9].

Importance of Metal-catalyzed Oxidation in AGE Formation

Trace levels of redox active transition metals, such as copper(Cu^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), and zinc (Zn^{2+}), are mandatory to maintain biological reactions, but they are cytotoxic when present in excess and result in cellular dysfunction via oxidative stress and free radical production (reactive oxygen products). In addition, these metals can catalyze carbonyl formation in proteins. Furthermore, the inhibition of AGE formation reduces arterial stiffness and the ability to chelate metals [10, 11].

Particularly, for connective tissue, the ability of metals to directly oxidize proteins is mediated by a hydroxyl radical, and a metal catalyst oxidation (MCO) reaction results in the deamination of carbonyl, α-aminoadipic acid δ-semialdehyde lysine residue known as alizine. The physiological cross-linking of collagen and elastin by lysis oxidase is also similar. Other mechanisms for alizine formation could be α-dicarbonyl sugar MCO-mediated Strecker degradation of lysine residues [12].

Mechanism of Protein Damage by Glycation on the Arterial Wall

When the total carbonyl stress increases in a carbonyl-rich environment, such as diabetes and end-stage renal disease, glycation and AGE formation are known to show a theory effect by increasing age-related arterial stiffness. AGE formation begins with the classical Maillard reaction pathway, which is a non-enzymatic reaction of lysyl residues of proteins with reducing sugars. Collagen and elastin are the main proteins on the arterial wall targeted by this reaction [13].

Glycation induces AGE formation and cross-linking for collagen, which leads to an increase in the stiffness of the vessel wall. It is still unclear whether AGEs and cross-links occur in all major glycation sites or in preferential regions of the collagen. However, similar to ribonuclease, glucosepane cross-links have been also found in the lysine and arginine domains in the globular model proteins [14].

Compared to collagen, elastin has a low number of glycation sites due to low lysine content. Importantly, elastin glycation has serious consequences for optimal functioning of the cardiovascular system during aging and diabetes. However, data are scarce on the relationship between AGE and elastin cross-linking.

Therefore, AGE formation and cross-linking are very important in systemic hypertension in which elastin synthesis is stimulated, known as tropoelastin and contains a high amount of lysine residues [15].

According to Santa et al., elastin is prone to the Fenton reaction, whereas it has been shown to cause a vicious cycle of CML, including protein aging with glycation, producing CML formation, binding to redox active metals, such as Cu^{+2} and Fe^{+3}, and resulting in the formation of the CML-metal complex, and inducing lipid peroxidation (providing CML formation by glyoxal) [16].

In addition, myeloperoxidase mediates the formation of CML through oxidation of serine and production of the CML precursor glycolaldehyde at the inflammation sites. The reactive oxygen species (ROS) produced during this cycle induces elastolysis in the arterial wall. Moreover, glycated elastin and collagen show greater affinity for ions of metals such as copper and iron [17].

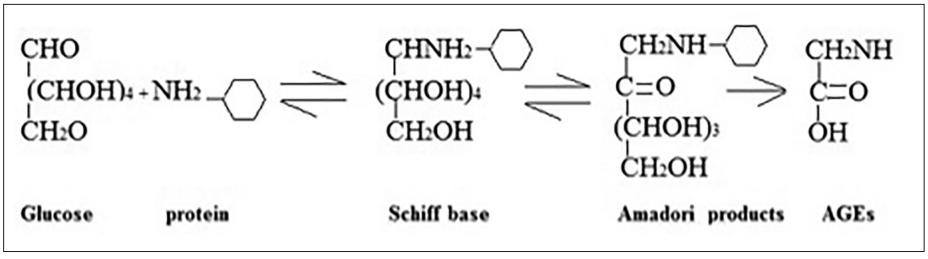

Figure 1. AGE formation.

Chemical Properties of Alagebrium

Since the discovery of N-Phenacylthiazolium bromide, one of the almost completely studied analogs is 4,5-dimethyl-3-phenacylthiazolium chloride (ALT-711®, Alagebrium chloride). Alagebrium (Synvista Therapeutics) is a new generation of cross-link breaker and a thiazolium compound (Figure 2) [18].

This compound has enzymatic properties that breakdown covalent bonds formed in cross-linked proteins, which allows the release of the protein and thereby maintain its normal function. It has been suggested that it non-enzymatically breaks down the cross-linked AGEs formed on long-lived proteins such as collagen and elastin.

Its molecular weight (MA) is 232.32, and the chemical formula is $C_{13}H_{14}NOS$.

The AGE cross-link breaker and methyl glyoxal inhibitor alagebrium (ALT-711) was developed by Alteon Corporation.

Alagebrium is a molecule in the Phase III clinical trials, which showed positive effects in the treatment of diabetes, hypertension, vascular sclerotic pathologies, cardiovascular hypertrophy, and similar diseases. In early studies, after 3 weeks of treatment, diabetic rats showed improved vascular function, decreased IgG binding to red blood cells, and decreased tail-induced collagen cross-linking and ischemia [19].

In humans, this compound exhibits a good safety profile; however, the studies have been discontinued by Alteon Corporation at the beginning of 2009 due to financial difficulties, and it could not be licensed as a drug [20, 21].

The clinical trial results indicate that this compound might be the first-line specific treatment for cardiovascular disorders caused by AGEs [20, 21].

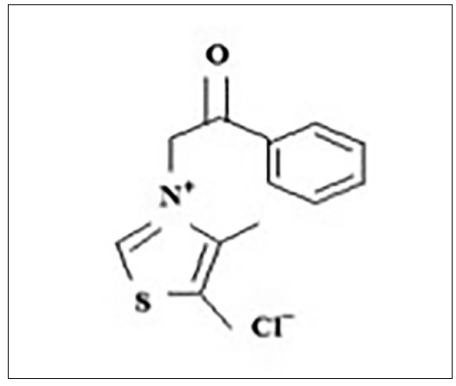

Figure 2. Alagebrium chemical structure.

Mechanism of Action of Alagebrium

The mechanism of action of alagebrium remains controversial. Similar to PTB, initially, alagebrium primarily results in the chemical cleavage of α-dicarbonyl carbon–carbon bonds in cross-linked structures, thereby removing newly formed AGEs. A recent study has supported that alagebrium is an effective inhibitor of MG, reducing α-dicarbonyl compounds [22, 23].

Zinc, iron, and copper are required for normal function integrity but are abundantly present in diabetes and the related complications. Alagebrium is a potent inhibitor of copper-catalyzed ascorbic acid oxidation. Although alagebrium exerts an antioxidant capacity in preclinical animal models and the in vitro hydrolyzed product shows a strong chelating activity, it is unclear whether it is a direct or indirect effect. Therefore, it is difficult to distinguish whether the effect of alagebrium is through metal chelation or AGEs destruction. [24-29].

Also, it is unknown whether alagebrium has a direct link to RAGE. The correlation of the reduction in RAGE protein expression with alagebrium was found in preclinical studies. In transgenic mice without cell surface RAGE, alagebrium treatment has been shown to improve renal function and pathology and cardiovascular disease. Therefore, it is unknown whether the deterioration of AGE pathway is related to RAGE protein translation/transcription through direct feedback or the reduction of metal ions and oxidative stress [30, 31].

Alagebrium Clinical Trials

Preclinical Studies

Many animal model studies of ALT-711 have shown the potential benefit in reversing cardiovascular complications in aging and diabetes.

In a study by the Alton Ochsner Clinic (Hypertension Research Laboratory, Alton Ochsner Medical Foundation, New Orleans, LA, USA) on non-diabetic, spontaneous hypertensive rats, ALT-711 treatment reversed aortic stiffness by regulating left ventricular elasticity. The oral administration of ALT-711 improved late diastolic and stroke volumetric index, decreased left ventricular stiffness, and improved cardiac function in aged dogs [32].

In another study on aged monkeys, the pulse wave velocity and augmentation index decreased significantly and aortic stiffness decreased continuously [33].

Clinical Studies

In several clinical studies sponsored by Synvista Therapeutics, between 2002 and 2010 (DIAMOND [NCT00043836], SAPPHIRE [NCT00045981], SILVER [NCT00045994], SPECTRA [NCT00089713], BREAK-DHF-I [NCT00661611], and BENEFICIAL [NCT00516646]), alagebrium has been extensively studied.

Data have been published, and other studies were terminated early due to the financial insufficiency. In 2001, the data of 93 hypertensive patients randomized to receive placebo or alagebrium were recorded in a pilot study. After 2 weeks of treatment, the patients in the alagebrium group had decreased arterial pulse rate with increased vessel compliance [8].

A 2-year toxicity study of alagebrium on rats showed liver alterations. Since the data were published in 2004, Synvista Therapeutics Inc., which synthesized alagebrium, did not include new patients in clinical trials until the preclinical work for alagebrium was completed [34].

In the subsequent Phase I study, high-dose alagebrium was used to evaluate its effect on triglyceride, total cholesterol, high-density lipoprotein *cholesterol* (HDL-C), and low-density lipoprotein *cholesterol* (LDL-C), and no harmful effects on liver function were detected [34].

In the Phase I studies on healthy subjects, no side effects or deaths were observed after alagebrium treatment. However, it is unknown exactly at which time period these Phase I experiments were made. Between 2001 and 2003, Phase IIb clinical trials were conducted in many regions of North America. Individuals were divided into two cohorts based on left ventricular hypertrophy (LVH). The Systolic Hypertension Interaction with Left Ventricular Remodeling (SILVER) study was based on LVH hypertrophy, and there was no study except for Systolic and Pulse Pressure Hemodynamic Improvement by Restriction Elasticity (SAPPHIRE) [34].

Overall, in these studies, there was no significant change in systolic and pulse pressures when alagebrium therapy was compared with placebo. When individual basal measurements were compared with 3- and 6-month measurements, some changes were observed in the systolic blood pressure [34].

In the Phase II Distensibility Improvement with ALT-711 Remodeling in Diastolic Heart Failure (DIAMOND) study, the effect of alagebrium on many parameters, including exercise oxygen consumption (VO_2), was measured. Compared with baseline, alagebrium reduced left ventricular mass and corrected diastolic function but did not alter blood pressure, VO_2, or aortic distensibility [35].

The effect of 8-week alagebrium treatment on vascular inflammation, inflammation, and collagen turnover was assessed in a double-blind study on individuals with systolic hypertension. Alagebrium improved vascular function and inversely correlated with collagen turnover and serum markers of inflammation [36].

In the BENEFICIAL study, diabetic and non-diabetic heart failure patients were treated with placebo or alagebrium to assess their aerobic capacity and diastolic/systolic function after 36 weeks. The primary (aerobic capacity) and secondary (diastolic/systolic function) results were unchanged. The analysis of diabetes subgroups also revealed no significant interaction [37].

In 4 clinical studies (ClinicalTrials.gov Identifier: NCT00739687, NCT00557518, NCT00662116, NTC00089713), the effects of alagebrium on chronic heart disease and renal dysfunction in individuals with and without diabetes were evaluated by Synvista Therapeutics Inc. between 2004 and 2009. Unfortunately, these studies were terminated in the early period due to the bankruptcy of Synvista Therapeutics Inc. and global financial crisis. In 2013, a randomized, double-blind, placebo-controlled cardiovascular clinic trial aimed to evaluate the effect of multi-dose alagebrium with individual exercise on diastolic heart failure (ClinicalTrials.gov Identifier: NCT01913301). This study was also discontinued because of financial causes. In the last two randomized, double-blind, placebo-controlled studies, exercise was combined with 200 mg/day alagebrium for 1 year in the elderly population. Alagebrium was mostly well-tolerated except for the gastrointestinal symptoms observed in two individuals [38, 39].

In one study, when compared to controls, no independent effect of alagebrium was observed on VO_2 on endothelial function and arterial stiffness. This effect simultaneously disappeared with exercise intervention.

Similar to other pharmaceutical agents reported to date, it is impossible to note the beneficial effects of alagebrium observed in diabetic animal models at the same time in humans due to incomplete clinical trials. To date, clinical trials with alagebrium have not been continued.

Diabetes and Alagebrium

Cardiovascular complications associated with diabetes mellitus are one of the major causes of

morbidity and mortality worldwide. Diabetes is a common disease affecting 346 million people worldwide.

It is expected that this number will increase by 552 million in 2030. In diabetes, micro- and macrovascular diseases cause increased mortality and morbidity. More specifically, the risk of cardiovascular diseases and cardiovascular deaths in diabetic individuals is 2-fold higher than in those without diabetes [40-43].

Diabetes affects the cardiac tissue in many ways and one of them is the direct exposure of endothelial cells to high blood sugar. Toxic products of non-enzymatic glycolysis, particularly MG, induce endothelial cell damage. MG is the reactive ketoaldehyde occurring from glucose metabolism and is also the precursor for AGE and triggers many pathological events, such as type 2 diabetes, pancreatic cell dysfunction, endothelial dysfunction, and hypertension [44].

The administration of ALT-711 for 4 weeks in rats with MG-induced diabetes improved beta-cell dysfunction. Therefore, selective and specific MG inhibitors may be effective against high glucose, high blood pressure, and AGEs. However, currently, there are no selective MG inhibitors; most compounds, such as aminoguanidine and metformin, act non-specifically [44].

In a study, 4-week MG treatment significantly increased blood pressure, total cholesterol, and triglyceride levels and decreased HDL level. The effect of the novel ALT-711 analog (compound Nos. 1-15) on MG was investigated; the inhibitory effect of compound No. 13 on MG was found. It has been observed that compound No. 13 reduces MG-induced metabolic parameters significantly. Alagebrium had a protective effect against the harmful effects of high glucose and MG. ALT-711, which is protective against dicarbonyl-induced AGEs and safe and specific for MG, is therapeutically attractive [45,46].

In the majority of diabetic patients, hypertension develops with increased renin–angiotensin system activity. Methylglyoxal is reactive glucose metabolite and is increased in diabetic patients. In a previous study, the effect of methylglyoxal on the renin–angiotensin system and blood pressure was investigated.

There was a significant increase in the blood pressure and plasma levels of aldosterone, renin, angiotensin, and catecholamine in methylglyoxal-administered rats. The levels of methylglyoxal and angiotensin, angiotensin receptor 1, adrenergic α1D receptor, and renin mRNA were significantly increased in the aorta and kidney of the methylglyoxal-administered rats [47].

It has been shown that long-term alagebrium treatment is effective in many chronic diseases, such as atherosclerosis, erectile dysfunction, cardiovascular disease, hypertension and kidney damage in diabetic animal models [48-51].

The mechanism of restenosis in type 2 diabetes mellitus (T2DM) is not fully understood; however, AGEs most likely contribute to vascular remodeling. AGE-induced collagen cross-linking (ARCC) increases intravenous resistance and induces neointimal hyperplasia (NH) in T2DM by altering intra-stent hemodynamics. Because Alagebrium reduces ARCC, these responses will also decrease. Abdominal aortic stents were implanted to Zucker lean (ZL), Obese (ZO), and diabetic (ZD) rats. Blood pressure, vessel diameter, and vessel wall stress were calculated after 21 days, and the amount of NH was determined [52].

ARCC and TGF-β and RAGE expressions were determined in the arterial segments (the aorta, carotid, iliac, femoral, and arterioles). In the ZD and ZL rats, the flow-direction resistance increased by 60%, but flow and wall shear stress (WSS) decreased significantly (44% and 56%, respectively) [52].

There was no significant difference in RAGE and TGF-β expressions between arterial segments. In decellular matrix cells, ALT-711 modified the lectin-type oxidized LDL receptor 1 but not RAGE expression. In conclusion, ALT-711 reduced ARCC in ZD and ZO rats via a RAGE-independent pathway, increased intra-stent flow rate, and reduced NH. This study aimed to emphasize the important role of NH in AGE-induced remodeling in T2DM [52].

Several previous findings have suggested that there are many complex relationships between biomechanics and biological processes regulating NH development in diabetes and obesity. In ZD rats, AGEs are localized in all vascular locations and their high presence in T2DM has been confirmed. In ZD rats, increased ARCC-induced stiffness in the arterioles was alleviated with ALT-711 treatment. The mean blood pressure decreased in ZD rats and at the same time arteriolar resistance increased. Compared with ZL rats receiving ALT-711 treatment, the stent area WSS was lower in untreated ZD rats. NH was increased in the stent area in ZO rats but not in ZD rats; however, ALT-711 treatment reduced NH in all groups. LOX-1 expression was increased with ALT-711, but there was no effect on TGF-β or RAGE expression; different AGE-mediated pathways were identified as mediating the local NH response [52].

AGEs have recently shown to be associated with vascular calcification through a RAGE-mediated process. Despite the correlation between AGE levels and vascular calcification, there is no evidence that reducing in vivo AGEs or inhibiting the AGE–RAGE signaling pathway reduces medial calcification. In this study, the effect of the inhibition of AGE formation by pyridoxamine and elimination of AGEs by alagebrium on diabetic medial calcification was assessed. When the AGE–RAGE signaling pathway was inhibited, calcification was observed to be prevented. Previous studies have shown that AGE inhibitors prevent time-dependent AGE accumulation in the femoral arteries of diabetic rats. This effect is accompanied by a decrease in accelerated calcification in diabetes. In ex-vivo experiments, it was observed that the RAGE agonist N-methylpyridinium induced diabetic femoral artery calcification, and the inhibition of antioxidants and inhibitors of different signaling pathways were associated with RAGE activation. The physiological significance of oxidative stress has been demonstrated by the reduction of femoral artery calcification in diabetic rats treated with the inhibitor of ROS apocynin. AGE inhibitors have limited or prevented medial calcification. Therefore, the inhibition of AGE–RAGE signaling probably reduces medial calcification [53].

Diabetic Cardiomyopathy

Diabetic heart disease is a clinical condition that can progress to heart failure and sudden death. However, the mechanisms responsible for the changes in excitation–contraction coupling leading to cardiac dysfunction during diabetes are unknown. Hyperglycemia causes the formation of AGEs in longlived proteins, including sarcoplasmic reticulum (SR) Ca^{2+} regulatory proteins. However, their pathogenic role in the use of SR Ca^{2+} in cardiac myocytes is unknown. Thus, it was investigated whether an AGE cross-link breaker could prevent the changes in SR Ca^{2+} cycle leading to in vivo cardiac dysfunction during diabetes. Streptozotocin (STZ)-induced diabetic rats were treated with ALT-711 for 8 weeks and compared with age-matched placebo-treated diabetic rats and healthy rats. Cardiac function was assessed using echocardiographic examination. Ventricular myocytes were isolated to assess the SR Ca^{2+} cycle using confocal imaging and quantitative western blot. Diabetes resulted in in vivo cardiac dysfunction, and ALT-711 treatment partially alleviated diastolic dysfunction (27% vs. 41%, $P < 0.05$ versus untreated diabetic rats, respectively) by reducing the isovolumetric

relaxation time and myocardial performance index. Collectively, that study showed that AGE accumulation in the type 1 diabetes model substantially impaired SR Ca^{2+} re-uptake in cardiac myocytes and that long-term treatment with an AGE cross-link breaker partially improved SR Ca^{2+} utilization and diabetic cardiomyopathy [54].

Diabetic Nephropathy

Diabetic nephropathy (DN) is a global issue that increases in number along with the increasing number of people with diabetes every year, leading to an increase in the incidence of end-stage renal disease [55].

AGEs are important mediators of DN, and they trigger renal inflammation and glomerulosclerosis via RAGE and several other mechanisms. The contribution of RAGE-dependent and independent signaling pathways was investigated in vivo. STZ-induced diabetic RAGE apoE double knockout (KO) mice were treated with ALT-711 (1 mg/kg/day) or the angiotensin-converting enzyme inhibitor (ACEI) quinapril (30 mg/kg/day) for 20 weeks, and the renal parameters were evaluated. RAGE deletion reduced mesangial expansion, glomerular matrix accumulation, and renal oxidative stress associated with diabetes. In contrast, it has not prevented the inflammation and AGE accumulation associated with diabetes. However, the treatment with alagebrium in diabetic RAGE apoE-KO mice reduced renal AGE levels and glomerular matrix accumulation. Even in the absence of RAGE expression, alagebrium reduced cortical inflammation, as indicated by the reduced expression of monocyte chemoattractant protein-1, intracellular adhesion molecule-1, and macrophage marker cluster of differentiation molecule-11b. These novel findings confirm the presence of RAGE-independent signaling pathways as well as RAGE-dependent signaling pathways that may be active in the kidneys through AGEs [56].

Despite the beneficial effects of ALT-711, the renoprotective mechanisms in DN are not fully understood. Since oxidative stress exacerbates diabetic renal damage through the interaction with AGE, a previous study examined the antioxidant properties of ALA in diabetic db/db mice (C57BL/KsJ Jc1 *db/db*) mesangial cells cultured with high glucose or hydrogen peroxide (H_2O_2). ALA not only significantly reduced urinary albumin excretion and renal pathologic changes in db/db mice, but also reduced pentosidine and nitrotyrosine accumulation and expression of nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) oxidase subunits independent of the treatment protocol. In mesangial cells, in collaboration with cellular ROS, ALA effectively prevented the NADPH oxidase subunit (p47 phox, p67 phox, and rac1), protein kinase C (PKC) isoform (α, βI, and βII), and Nox4 messenger RNA expression induced by not only high glucose but also H_2O_2. The direct and indirect antioxidant effect plays an important role in the renoprotective effect of ALA in diabetic kidneys [57].

AGEs play a role in the development of both nephropathy and major arterial diseases. However, it is still unclear which AGE subtypes play a pathogenic role and which RAGEs are associated with the effects of AGE on the cell [58].

In experimental diabetes, similar findings were observed with the use of aminoguanidine and ramipril (ACEI) in fluorescence and immunostable AGE levels, renal PKC activity, nitrotyrosine expression, lysosomal function, and protein utilization. These findings suggest that the inhibition of the renin–angiotensin system blocks the pathways leading to tissue injury. Chemical pathways leading to the formation of AGEs and the renin–angiotensin systems may be induced by glucose and angiotensin II to interact with the formation of free radicals. AGE-dependent pathways play an important role in tubulointerstitial fibrosis in the diabetic kidney. This effect is dependent on RAGE, TGF-β, and connective TGF (CTGF) [58].

STZ-induced diabetic rats were treated with ALT-711 and divided into groups according to treatment periods of 16-32 weeks (early) and 24-32 weeks (late). Treatment with ALT-711 significantly reduced diabetes-induced serum and AGE peptide fluorescence, resulting in reduced renal CML and RAGE. The decrease in tail tendon collagen cross-linking was observed only in the group treated for 16 weeks. ALT-711 delayed albumin excretion rate and reduced blood pressure and renal hypertrophy regardless of the treatment duration. It also reduced diabetes-induced TGF-β1, CTGF, and collagen IV. However, glomerulosclerotic index, tubulointerstitial area, total renal collagen, nitrotyrosine, collagen protein expression, and TGF-β1 only improved in the early treatment group. This study also explains the effect of cross-link breaker treatment on DN not only with renal AGEs, but also with presklorotic cytokine and oxidative stress [59].

A previous study was designed to elucidate the role of AGEs in the development and progression of renal disease and the renal damage in diabetic apolipoprotein E-KO (apoE-KO) mice. Diabetes was induced with STZ in 6-week-old apoE-KO mice. Diabetic animals were left untreated or treated with AGE formation inhibitor aminoguanidine (1 g/kg per d) or cross-link breaker ALT-711. Non-diabetic apo-E mice are characterized by accelerated renal injury, albuminuria, and glomerular tubulointerstitial damage compared to diabetes-induced apo-E mice. These abnormalities include increased type I and type III collagen, TGF-β expression, alpha-smooth muscle actin immunohistochemistry, and macrophage infiltration and serum and renal AGE increase. Both treatments reduced renal AGE accumulation, i.e., resulted in less albuminuria, structural damage, and macrophage infiltration and TGF-β1 and collagen expression [60].

AGE accumulation was suppressed with alagebrium and ramipril to assess the effect of mediators having a role in renal function and diabetic renal injury on renin–angiotensin, AGE, mitochondrial and cytosolic oxidative stress, and intracellular signaling molecules. The treatment groups received ramipril (1 mg/kg/d, wk 0-32), alagebrium (10 mg/kg/d, wk 16-32), or combined treatment. A significant effect on albuminuria was observed in the monotherapy groups, but this effect was not observed in the combined treatment group.

A change in the urinary vascular endothelial growth factor (VEGF) excretion was observed in albuminuria. In diabetes, circulatory angiotensin II suppression is associated with increased levels of AGE and CML in circulation and the kidneys. All treatments reduced circulatory CML but not the renal CML. RAGE I renal gene expression and soluble receptor AGE have been significantly reduced in diabetes, which returned to the normal level with alagebrium. Diabetes-induced renal mitochondrial oxidative stress reduced with alagebrium. In cytosol, both treatments have been shown to be equally effective in reducing ROS. Membrane PKC activity decreased in all treatment groups, but diabetes-associated nuclear factor kappa B p65 translocation remained unchanged [61].

Another study investigated the effect of AGE on PKC isoform expression in DN. STZ-induced diabetic rats were divided into groups that received no treatment, ALT-711, or aminoguanidine. Diabetes also induced beta-I, beta-II, and epsilon isoforms as well as PKC-alpha.

ALT-711 and aminoguanidine treatment reduced renal AGE accumulation and abolished the increase in PKC expression. However, the translocation of phosphorylated PKC-alpha from the cytoplasm to the membrane decreased only with ALT-711. ALT-711 treatment

attenuated the expression of VEGF, extracellular matrix proteins, fibronectin, and laminin with decreased albuminuria. Despite no effect on VEGF expression, aminoguanidine treatment resulted in a slight decrease in fibronectin and laminin. These findings suggest that AGEs are a major stimulus for PKC activation in the diabetic kidney and that PKC-alpha can be directly inhibited by ALT-711 [62].

To evaluate the effect of ALT-711 on DN, ALT-711 was used for 3 weeks in 9 weeks old female db/db mice (A1; 1 mg/kg daily intraperitoneally) and for 12 weeks in 3 months old (A2), 7 months old (A3), and 12 weeks old (A4) female db/db mice. At week 3 of treatment, the serum CML level decreased by 41%, and the CML concentration in the urine increased by 138% from baseline. After 3 months of treatment, serum, skin, and kidney CML levels and urine albumin/creatinine ratios were lower and urinary CML levels were higher in the treatment groups A2, A3, and A4. The authors have suggested that alagebrium can inhibit, delay, and/or reverse the established DN in db/db mice by reducing systemic AGE pools and facilitating urinary excretion of AGEs [63].

Diabetic Neuropathy

Pressure-induced vasodilatation, a neurovascular mechanism based on the interaction between mechanically sensitive C-fibers and vessels, local nociceptive applied pressure response can increase blood flow to the skin and protect against pressure ulcers on return.

In diabetic rats, severe neuropathy can significantly affect vasodilatation induced by pressure. An animal study aimed at determining if there is a pressure-induced vasodilatation change in diabetic mice at 8 weeks. Control and diabetic mice were left untreated or treated with sorbinil (aldose reductase inhibitor) or alagebrium in the last 2 weeks.

Nerve functions were evaluated using the motor neuron speed (MNCV) as well as the C-fiber-mediated nociception threshold. Pressure-induced vasodilatation, endothelial response, C-fiber threshold, and MNCV were completely altered in diabetic animals. None of the treatments had a significant effect on MNCV. Although alagebrium and sorbinil improved Acethycholine-dependent vasodilatation, Sorbinil was the only treatment that improved pressure-induced vasodilatation as well as C-fiber threshold. Thus, the restoration of pressure-induced vasodilatation by aldose reductase-mediated sorbinil inhibition may improve vascular and C-fiber functions that may limit neuropathic diabetic cutaneous pressure ulcers [64].

Diabetic Retinopathy

AGE accumulation is associated with many complications of diabetes mellitus, including diabetic retinopathy. AGE-breakers, such as N-phenylacylthiazolium and alagebrium, have been proposed as therapeutic agents for reversing the increase in protein cross-linking. Epicatechin is the main diet flavonoid with a wide range of biological activities that improve health.

In the study, the potential impact of (-)-epicatechin on reducing the burden of AGEs in vitro and in vivo was evaluated. To examine the effect of (-)-epicatechin on retinal vascular function, rats injected exogenously with AGE (-)-epicatechin (50 and 100 mg/kg i.p.) were treated for 2 weeks.

It was observed that pre-formed glycated bovine serum albumin–collagen cross-linkages were incubated with epicatechin or alagebrium at a range of concentrations. Epicatechin was more effective in vitro than alagebrium. The epicatechin crushing effect was stronger than alagebrium. After overnight incubation with epicatechin or alagebrium, human serum albumin with antigenic glucose decreased in a dose-dependent manner. It was also observed in this case that (-)-epicatechin was effective at lower concentrations than alagebrium [65].

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