ADVANCED GLYcation END PRODUCTS IN DIABETES MELLITUS: MECHANISM OF ACTION AND FOCUSED TREATMENT

VALENTIN RADOI¹, DANIELA LIXANDRU¹, MARIA MOHORA¹ and BOGDANA VIRGOLICI¹

¹University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania
Corresponding author: Bogdana VIRGOLICI, E-mail: hvirgolici@yahoo.com

Received December 8, 2011

Data shows that glucose is not only the main energy source for short periods, but also the major source of diabetes mellitus complications, mainly by forming oxidative and proinflammatory advanced glycation end products (AGE). The preclinical and clinical studies of the last decade demonstrated a strong involvement of AGE in all of the micro and macrovascular complications of diabetes mellitus, especially in type 2 diabetes. The treatment handbooks should include not only the quantity and composition of food but also the ways to prepare food, as well as emphasize the role of physical exercise, even moderate, in preventing the formation of AGE compounds. A well controlled glycaemia, combined with a diet which reduces AGE intake and physical exercise, should be considered in the management of diabetes mellitus alongside medication directly targeting AGE. The present review covers the mechanisms of AGE formation and the pathways through which AGE determine diabetic complications, presents proven diet and physical exercise recommendations and explains the pharmacodynamics of current and future drugs that could be used for reducing AGE in diabetic patients.

Key words: AGE, AGER, diabetes mellitus, oxidative stress, diet, treatment, physical exercise.

INTRODUCTION

In the year 2010, 284 million people were recorded as suffering from diabetes mellitus, and it is suggested that this figure will increase to 439 million in the year 2030, thus turning the disease into an epidemic. Knowledge of the involved pathogenic mechanisms is essential for preventing and improving the complications of this illness¹.

OXIDATIVE STRESS MEDIATES AGE EFFECTS

Reactive oxygen species (ROS) are incriminated in diabetes mellitus pathogenesis. These free radicals are formed through non-enzymatic glycation reactions, through mitochondrial electron transport chain dysfunctions or through activation of hexoseamines in the presence of hyperglycemia² (Figure 1).

Oxidative stress and the consequent activation of inflammatory and apoptotic processes are involved in β-pancreatic cell dysfunction in type 1 diabetes patients. An antioxidative treatment could protect insulin producing cells. In the case of type 2 diabetes, ROS are considered as major risk factors for developing micro and macrovascular complications. ROS disrupt transmission pathways between the insulin receptor and the glucose transport system, which leads to an onset in insulin resistance and are involved also in the inactivation of the two critical anti-atherosclerotic enzymes: endothelial nitric oxide and prostacyclin synthase³-⁵.
ROS participate in the formation of advanced glycated end products (AGE), but also mediate AGE effects on target tissues. The connection between clinical complications of diabetes mellitus and oxidative stress arises from the formation of high doses of AGE in this metabolic disorder.

AGE represent a heterogeneous class of compounds formed through non-enzymatic glycation, but also through protein and lipid oxidation (Figure 2). In the glycation reaction free amino group from a proteic structure and reductive monosaccharide (e.g., glucose) or several carbonyl compounds are involved. The glycation reaction is slow, reversible in the first stages and significant for slow turnover proteins (for example proteins comprised in the structure of the lens).

Glycation products (Amadori), formed through the reaction between an aldehyde and a proteic amino group, can be oxidated (for example by reactive oxygen species), thus generating advanced glycated end products (AGE). Examples of such products, identified in diabetes patients, are pentosidine, carboxymethyllysine (CML), methylglyoxal and pyrline. The biochemical process of advanced glycation appears to be increased in diabetes patients, not only because of hyperglycemia and oxidative stress, but also because of high quantities of free fatty acids. AGE induce crosslinkation processes in the structure of long lifespan proteins, such as collagen, modifying blood vessel structure. By binding to their specific receptors (RAGE), they activate intracellular signaling pathways which lead to cytokine production, responsible for the proinflammatory and prosclerotic effects.

During the last decades, a large number of preclinical and clinical studies were conducted, with the purpose of studying the formation, degradation and effects of AGE, but it remains to be established if a protective therapy can be implemented.

Fig.1. Sources of ROS under the influence of hyperglycemia (adapted after Rodrigo).
Advanced glycation end products in diabetes mellitus

**AGE and Diabetes Mellitus Complications**

TAGE (toxic AGE) represent the dominant form of advanced glycation end products, derived from glyceraldehyde and are very aggressive compounds. The interaction between TAGE and their receptors, noted as RAGE, in endothelial and inflammatory cells, leads to intracellular generation of reactive oxygen species (ROS) via the electron transport chain, NADPH oxidase, xanthine oxidase and arachidonic acid metabolism\(^2,5\).

**AGE and diabetic retinopathy**

TAGE have intra and extracellular proinflammatory effects by activating the NF-κB transcription factor via MAP kinases-Ras, which increases the transcription of vascular endothelial growth factor (VEGF) via reactive oxygen species. VEGF has a mitogenic effect on endothelial cells, influencing vascular permeability and being involved in the pathological process of proliferative diabetic retinopathy. It has been demonstrated that TAGE and VEGF have very high levels in diabetic patients, especially in the aqueous humour, and the values were correlated with the severity of diabetic retinopathy neovascularisation\(^6\).

PEDF (Pigment-Epithelium-Derived Factor) is a glycoprotein belonging to the group of serin protease inhibitors, with antioxidant, antiangiogenic, antiinflammatory and neuroprotective effects. It has been recently demonstrated that PEDF can reduce oxidative stress by suppressing reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated generation of reactive oxygen. So, PEDF inhibits the AGE-induced reactive oxygen species generation in a dose-dependent manner. In patients with proliferative retinopathy, PEDF shows decreased concentrations in the humours of the eye, and administration of the compound prevents microvascular complications. Substitution of PEDF could prevent the progression of diabetic retinopathy by attenuating the deleterious effects of AGE\(^7\).

**AGE and diabetic nephropathy**

It has been proven that in physiological conditions, the main source of superoxide in the renal cortex is represented by the NADPH oxidase.

\[
NADPH + 2O_2^{-} \xrightarrow{NADPH oxidase} NADP^+ + \\
+H^+ + 2O_2^- (\text{superoxide})
\]

AGE, hyperglycemia, fatty acids and angiotensin II activate this enzymatic system in vascular muscle cells and mesangial cells\(^8\). So, in diabetes mellitus, there is a hypothesis of excess superoxide anion formation in these cells. However, NADPH oxidase present in endothelial cells is also activated by tumor necrosis factor \(\alpha\) (TNF\(\alpha\)), thrombin and other compounds. In the renal biopsies performed on patients suffering from diabetic nephropathy, glycoxidation compounds, CML and pentosidine, along
with the lipid peroxidation compound called malondialdehyde (MDA), a marker of oxidative stress, have been evidenced through immunohistochemistry techniques. The presence of these AGE compounds has also been proven in glomerular mesangium expansions, thickened glomerular capillaries and renal arteriolar walls. Still, pyraline was not identified in these areas, which led to the hypothesis that only some species of AGE reflect a state of oxidative stress. Nonetheless, all of the precursors of these compounds are extremely reactive and can modify matrix proteins through intermolecular crosslinking.

Angiotensin II induces RAGE expression in pericytes and endothelial cells, making them less vulnerable to TAGE action. TAGE determine a release of VEGF and chemokines, leading to leucocyte recruitment, which, in turn, triggers an inflammatory process with very harmful effects. Administration of renin-angiotensin system inhibitors (ACE) blocks the connection between TAGE and RAGE, thus preventing diabetic nephropathy and retinopathy.

AGE and diabetic neuropathy

AGE determine the modification of the peripheral nerve myelin which becomes susceptible to phagocytosis and determines segmental demyelination, the modification of major axonal cytoskeletal proteins such as tubulin, neurofilament and actin which cause axonal atrophy and degeneration and impaired axonal transport. The glycation of laminin, found in the extracellular matrix also leads to impaired regenerative activity in diabetic neuropathy.

The AGE-RAGE interactions determine the upregulation of nuclear factor kappaB (NFkB), protein kinase C β 2 and various NFkB mediated proinflammatory genes, an increased neurological dysfunction, including altered pain sensation as can be seen in the diabetic foot and an acceleration of the formation of new glycoxidation products such as N-epsilon-(carboxymethyl)lysine and pentosidine. A cumulative rise of AGE products was observed in diabetic peripheral epidermal axons, sural axons, Schwann cells, and sensory neurons within ganglia for streptozocin-induced diabetic mice. Mice lacking RAGE have attenuated features of neuropathy and limited activation of potentially detrimental signaling pathways.

AGE and diabetic cardiomyopathy

AGE determine the acceleration of the crosslinkage process of collagen, leading to the formation of links throughout the entire collagen molecule, as opposed to the more limited terminal positions for normal crosslinking. This modification of the collagen structures leads to myocardial stiffness. Diabetes mellitus can produce a stiff myocardium before the development of myocardial fibrosis. The stiff myocardium in the early stages of the development of the cardiomyopathy of diabetes mellitus is not a consequence of an increase in ventricular resistance afterload and in these circumstances is associated with the formation of collagen AGEs.

AGE also induce the release of profibrotic proteins, such as Transforming Growth Factor-β (TGFβ) and proinflammatory cytokines, such as Interleukin 6 (IL6) and Tumor Necrosis Factorα (TNFα). AGE concentration is correlated with free fatty acids levels which are increased in patients with visceral fat, who are at high risk for cardiovascular diseases.

These mechanisms demonstrate that AGE are involved in early and late diabetic cardiomyopathy through their direct and indirect mechanisms.

AGE and thrombogenesis in diabetes mellitus

In humans, TAGE concentrations in the serum were positively correlated with thrombogenic markers such as PAI 1 (Plasminogen Activator Inhibitor 1) and fibrinogen, whilst hypoglicaeia has potential prothrombotic effects. As such, the limited value of aspirin and other antiplatelet drugs in the treatment of atherothrombotic events in patients with diabetes was to be expected.

AGE cause platelet activation and fibrin stabilization through several mechanisms such the decreased production of Prostaglandin I2 (PGI2), by increasing the levels of mRNA coding for PAI 1 in endothelial cells (EC) which causes an increase in immunoreactive PAI 1 contents and the anti-fibrinolytic activity. The results thus suggest that AGE have the ability to cause platelet aggregation and fibrin stabilization, resulting in a predisposition to thrombogenesis and thereby contributing to the development and progression of diabetic vascular complications.
AGE and other complications of diabetes mellitus

There is increasing evidence that advanced glycation endproducts (AGEs) play a pivotal role in atherosclerosis, in particular in diabetes. AGE accumulation is a measure of cumulative metabolic and oxidative stress, and may so represent the “metabolic memory”. Furthermore, increased AGE accumulation is closely related to the development of cardiovascular complications in diabetes. The receptor for advanced glycation end products (RAGE) is a cell surface receptor whose signaling pathway has been implicated in atherogenesis. RAGE has an endogenous secretory receptor form, called soluble RAGE (sRAGE), that could exert antiatherogenic effects by acting as a decoy. Reduced sRAGE levels in CAD (coronary artery disease) subjects with normal levels of LDL cholesterol could also raise the possibility that the measurement of sRAGE level may improve risk assessment among normolipidemic subjects. sRAGE levels were significantly higher in type 2 diabetic patients than in non-diabetic subjects and positively associated with the presence of CAD. Plasma sRAGE levels are positively associated with endothelial function and predict cardiovascular events in nondiabetic participants with suspected coronary artery disease, suggesting its pivotal role in atherothrombosis.

Low levels of sRAGE have also been associated with stroke, especially in subjects without identifiable conventional risk factors, being even proposed as a potential biomarker. Recently, deranged bone metabolism, including osteoporosis, has been recognized as a complication of diabetes which can cause this effect through multiple pathways, some involving AGE. In type 2 diabetes, fracture risk is increased in spite of a high bone mineral density (BMD) most likely due to deteriorated “bone quality”. However, it is difficult for endocrinologists to clinically assess this type of osteoporosis. Also recently, the relationship between AGE and erectile dysfunction (ED) has been reported.

WAYS OF DECREASING AGE IN THE BODY

DIET – AGE are endogenously formed, but can also arise from exogenous sources. Cigarette smoke, for example, is a well known exogenous source of AGE. During smoking, the combustion of preAGE compounds present in tobacco gives rise to a series of reactive and toxic AGE. AGE or LDL-linked AGE levels in the serum are significantly increased in cigarette smokers. This is the reason why diabetic smokers show higher concentrations of AGE in arteries and in the crystalline lens.

Numerous studies have evidenced that diet is a significant exogenous source of highly reactive AGE. During food processing, food storage and cooking (including microwave usage), the reaction between the amino groups and the carbonyl group of food components occurs randomly. This reaction is called the Maillard (or browning) reaction. In the late stage of this reaction, advanced glycation end products (AGE) are formed. Modern diets are largely heat-processed and as a result, they contain high levels of AGE.

Animal origin products, rich in lipids and proteins, have a high AGE concentration and also have the potential of forming new AGE throughout thermal processing. By contrast, products rich in carbohydrates, such as vegetables, fruit, whole grains and milk contain low AGE concentrations even after being cooked. It has been recently proven that the popular Atkins diet, poor in carbohydrates, increases methylglyoxal and methylglyoxal derived AGE concentrations, having harmful effects. However, it is important to specify that in the case of carbohydrates, fructose is better to be avoided, as it has proatherogenous effects and leads to AGE formation. The Montignac diet has omitted this aspect and therefore it has shown fewer followers.

Food processing, especially heating (even in microwave ovens) has a significant catalyzing effect in generating glyco and lypooxidation compounds. To give products a more intense taste, food producers add synthetic AGE to certain products. Therefore, by increasing the consumption of processed foods, AGE intake in diets has significantly increased in the last 50 years. Animal studies have proven an important relationship between a high AGE intake and the development or progressions of certain diabetic tissular lesions, especially vascular and renal. This is why these lesions can be prevented through a diet poor in AGE.

A large proportion (approximately 10%) of consumed AGE are absorbed together with food. Apparently, there is a correlation between AGE circulatory levels and AGE consumption. Animal studies have proven an important relationship between a high AGE intake and the development or progressions of certain diabetic tissular lesions, especially vascular and renal. This is why these lesions can be prevented through a diet poor in AGE.

A reduction in AGE intake through a strict diet concerning these products has been associated with a significant suppression of plasmatic concentrations
of vascular lesion markers (such as adhesion molecules) and inflammation mediators.

This new evidence suggests that reduction of AGE containing products can become an important component in the management of diabetic patients. Until efficient and secure medicines become available, healthcare practitioners and clinical nutritionists can advise and encourage the consumption of fresh foods, cooked through decreased periods of heating, in the presence of water or humidity, or addition of acid ingredients such as lemon juice or vinegar. High concentrations of salt reduce the formation of Maillard compounds (AGE precursors), and banana flour possesses anti-AGE properties, being used as a diet supplement for diabetic patients. Commercial cookies which contain ammonium bicarbonate and fructose have the highest concentrations of AGE precursors. Consumers should avoid commercial sauces, chips, crispy pretzels, bread crust and beverages rich in AGE (coffee based). Glutamine has been proven to reduce glutamine fructose-6-phosphate transaminase 1, a receptor of advanced glycation end products, and it can easily be found in cabbage, beef, chicken, fish, beans. It is interesting that some of these exogenous AGE can even influence the expression of several proinflammatory genes. Children can be exposed to high AGE concentrations from as early as inside the womb, through the mother’s blood, depending on her diet, and later on through the consumption of sweets, thus presenting a high risk for an early onset of diabetes mellitus. The dry heating of foods increases AGE concentrations 10 to 199 times more than unprocessed ones.

A diet based on decreasing AGE is not tasteless and does not make compromises regarding important nutrients. Such a diet can decrease exogenous AGE intake by more than 50%, which brings AGE concentrations in the blood to decrease by almost 30% in one month, without modifying HbA1C. A short term normal glycaemia or a temporary normal level of HbA1C is not enough to reduce AGE in the serum, the latter being possible after longer periods of time (months or years).

**PHYSICAL EXERCISE**

Physical Exercise, alongside diet, is more and more seen as a necessary part of the lifestyle changes that need to be made by diabetic patients. As such, numerous studies have been done to prove the effectiveness of this approach.

Apart from the obvious benefit of weight loosing seen in type 2 diabetes, physical exercise has several other effects, including the reduction of inflammatory and oxidative markers, as well as AGE. However it was proven that acute (extenuating) exercise only lowers the level of glucose, lipids and insulin whilst aggravating the inflammatory profile and oxidative stress. On the other hand, chronic (habitual) exercise had beneficial effects on all the previously mentioned biomarkers and profiles. Even moderate physical exercise, such as daily power walking has numerous positive effects, such as lowering ischemia-modified albumin (IMA), serum total antioxidant status (TAS), total oxidant status (TOS) and systolic and diastolic pressure. Animal studies have showed that chronic exercise can also attenuate the heart stiffness found in CAD. A note is to be made here about diabetic patients with heart failure who have higher AGE values than non-diabetic persons and at the same left ventricular ejection fraction present a poorer exercise capacity, thus needing a different physical exercise program.

The same moderate exercise was proven to reduce glomerular mesangial and tubulointerstitial fibrobrasis, alongside AGE, N(epsilon)-carboxymethyllysine and advanced oxidation protein products, thus physical exercise being a possible easy and effective nonpharmacological approach to ameliorate early diabetic nephropathy. Physicians have at their disposal several proven types of moderate exercise which can be practiced for a long duration of time for at least several days per week. Amongst this we mention power-walking, especially useful for older patients or patients with a reduced effort capacity and Tai Chi which was accepted and practiced a lot more in the western world in the last few years.

A more complex proven model of exercise can consist of 15 minutes of warm-up (stretching and walking), 60 minutes of aerobics, rhythmic exercises and exercises with dumbbells or balls and a 15 minute cooldown. We would also like to emphasize the importance of a non-sedentary lifestyle as proven by the numerous studies that involved using pedometers as to be able to asses daily walked distance.
**ANTIDIABETIC THERAPIES OF DECREASING ADVANCED GLYcation END PRODUCTS**

Aminoguanidine is one of the first anti-AGE medicines and is believed to act as a nucleophile trap for carbonyl intermediary compounds. Experimental studies on diabetic guinea pigs proved that aminoguanidine prevents the onset of vascular complications, and, in clinical trials, it led to an AGE decrease, independent of decreases in glycated hemoglobin\(^{31}\). Placebo controlled studies were also carried out in type 1 and 2 diabetes patients in order to examine the evolution of their complications\(^{52}\). A decrease in proteinuria was noticed, along with a stagnation in retinopathy evolution, but a statistically significant benefit for the progress of nephropathy could not be obtained.

Following clinical evaluations for this medicine were limited by long-term toxicity, several patients developing glomerulonephritis, anti-mieloperoxidase antibodies and anti neutrophile antibodies\(^{53}\).

**Pyridoxamine** is a vitamin B\(_6\) derived compound that prevents intermediary Amadori protein degradation into AGE proteins. Experiments on guinea pigs have evidenced that pyridoxamine decreases hyperlipidemia, prevents AGE formation by antagonizing the effects of angiotensin II, prevents renal hypertrophy and retinal vascular lesions and decreases salt retention\(^{54}\).

**Benfotiamine**, a lyposoluble thiamine (vitamin B\(_1\)) derived compound, prevents both intracellular AGE formation as well as hexoseamine pathway and DAG-PKC pathway activation by increasing the activity of transketolase, a speed limiting enzyme present on the non-oxidative branch of the pentose phosphate pathway. Animal studies have revealed that therapy with high doses of thiamine and benfotiamine increases transketolase expression in the renal glomeruli, inhibits renal hyperfiltration and microalbuminuria. It has been proven that benfotiamine prevents macro and microvascular modifications, and, by enhancing nerve impulse conduction speed, improves diabetic neuropathy\(^{55}\). Long-term clinical studies which certify the above mentioned benefits have not been carried out yet.

The compound known as LR-90 inhibits AGE formation and was tested only on diabetic guinea pigs for the moment. The first data obtained has indicated a decrease in serum creatinine, circulating AGE compounds and also albuminuria, even in the case of a less rigorous glycaemia control\(^{56}\). At the renal level, prevention of glomerulosclerosis and AGE accumulation has been successful. At present, studies are carried out regarding the effects of this compound on diabetic macrovascular complications. It appears that LR-90 also inhibits the expression of several proinflammatory genes stimulated by the AGER pathway in human monocytes\(^ {57}\), which means that this new substance could possess anti-inflammatory properties, together with a protective effect on diabetic vascular complications. Clinical studies which will be carried out in the near future will continue evaluations of the LR-90 compound.

**Soluble AGER molecules** (sAGER) represent a new therapeutic approach. These substances block AGE, denying them interaction with tissular AGER. It remains to be established if sAGER act as receptor antagonists or they connect with advanced glycation end products, thus denying their contact with AGER or with other scavenger receptors that can bind these highly proinflammatory AGE\(^{58}\). In the following years, sAGER or a non-peptidic AGER antagonist will surely be studied from a clinical point of view, but it could be possible for the research object to be a disease, other than diabetes.

There are also other diseases characterized by a tissular AGE increase, such as atheromatous plaque deposits. At this level, high concentrations of CML, pentosidine and protein or lipid oxidation compounds have been reported. Similar results have been recorded in Alzheimer’s disease and in amyloidosis associated with hemodialysis, an affliction present in patients who have underwent long-term dialysis.

**Angiotensin-converting-enzyme inhibitors** (ACEI) and angiotensin II receptor antagonists decrease AGE formation according to several studies conducted in vitro and on diabetic guinea pigs. Moreover, based on a series of in vitro studies, both preclinical and clinical, ACEI decrease AGER expression, which implies a new inhibition mechanism of AE effects. The possible explanation is that ACEI produce a down regulation of mARN-AGER through a dose-dependent phenomenon\(^{59,60}\).

**SOD/catalase mimetics** reduce superoxide anion synthesis and release, thus preventing macrovascular complications. It is a known fact that AGE levels are correlated with glycaemia levels and that AGE decreases nitric oxide concentration. Experimental studies have proven that hyperglicaemia, via ROS, determine a decrease in endothelial NOS enzymatic activity by 65% and in prostacyclin synthase by 95% in the aorta of diabetic mice.
Fig. 3. Antioxidant therapy which targets advanced glycation end products (adapted after Rodrigo). ACE, angiotensin conversion enzyme; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; AGER, advanced glycation end products receptor; LR-90, methylene bis [4,4′-(2 chlorophenylureido phenoxysobutyric acid].

Being a therapy with antioxidant enzymes, SOD/catalase mimetics prove more efficient than other antioxidants as they act continuously (because the formation of superoxide anion is also continuous) and they are considered as highly promising in the treatment of diabetes. Moreover, in experimental studies and cellular cultures, inhibitors of the poly ADP ribose polymerase have shown significant results in preventing diabetic complications. The PARP (Poly [ADP-ribose] polymerase) inhibitor prevents PKC and NF-kB proinflammatory factor activation, intracellular AGE formation and the hexoseamine pathway.

Bisphosphonates, by containing nitrogen, inhibit the proinflammatory and proliferative effects of TAGE on endothelial cells, by inhibiting the geranylgeranylation of the proteic Rac-1 component present in the structure of endothelial NADPH oxidase. Thus, reactive oxygen species are no longer formed and TAGE effects cannot be intermediated.

Minodronic, by blocking VCAM-1 gene expression, the angiogenic signaling of VEGF and by inhibiting the AGE-RAGE signaling pathways through suppression of ROS generation via inhibition of Rac prenylation might prove the most interesting drug in this class. Seeing the complex nature of diabetic osteoporosis, bisphosphonates would represent an especially useful class of drugs for these patients if they truly have this double effect, both on the bone and on ROS and AGE.

Statins inhibit vascular hyperpermeability and angiogenesis. It seems that protein prenylation is crucial for intracellular TAGE signaling, once bound by RAGE. The antioxidant effects of statins seem to be responsible for inhibiting the formation and release of reactive protein C from TAGE.

A clinical study proved the effect of cerivastatin in reducing AGE-CML, thus determining an improvement in the cardiovascular profile of diabetic patients. Atorvastatin also decreases AGE in patients with type 2 diabetes and influences the expression of sRAGE and esRAGE (a splice variant of sRAGE). Atorvastatin restores the levels of gluthathione and inhibits RAGE upregulation in cultured cells and downregulates the expression of RAGE and Monocyte Chemoattractant Protein-1 (MCP-1) in animal studies, thus demonstrating a potential for reducing the cardiovascular risk in diabetic patients.

In another study multiple stepwise regression analysis revealed that AGE level was a sole independent correlate of proteinuria and atorvastatin could decrease proteinuria in non-diabetic CKD patients with dyslipidemia partly via reduction of serum levels of AGEs. Atorvastatin may have AGE-lowering effects in CKD patients as well that could contribute to renoprotective properties of this agent.

AGE-breakers are medicines which inhibit AGE formation or affect those already formed (they break established AGE cross-links between proteins). So far, experimental studies and studies on humans have been very promising.

Pyridinium, 3-[[2-(methylsulfonyl) hydrazino] carbonyl]-1-[2-oxo-2-2-thienyl] ethyl]-chloride

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
<th>Effect in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEI inhibitors</td>
<td>↓ Angiogenin II Deactivates NADPH</td>
<td>↓ Nephropathy and retinopathy</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>Inhibits AGE formation</td>
<td>↓ Nephropathy and retinopathy</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>Inhibits AGE formation</td>
<td>↓ retinopathy</td>
</tr>
<tr>
<td>LR-90</td>
<td>↓ Oxidative stress</td>
<td>↓ Nephropathy</td>
</tr>
<tr>
<td>Anti-RAGE, sRAGE</td>
<td>Blocks AGER</td>
<td>↓ Nephropathy</td>
</tr>
</tbody>
</table>
Alagebrium (ALT-711) acts by breaking the abnormal crosslinks formed by collagen by being a low-affinity inhibitor of thiamine diphosphokinase (TDPK)\textsuperscript{51}. In a 32 week animal study, ALT-711 restored left ventricular collagen solubility and cardiac brain natriuretic peptide (BNP) in association with reduced AGE levels and abrogated the increase in RAGE, AGE-R3, connective tissue growth factor (CTGF) and collagen III expression\textsuperscript{52}. Alagebrium, in combination with sildenafil showed a decrease in ED in lab animals, mediated by a decrease in AGE, MDA, inducible nitric oxide synthase (iNOS), NFKB, MAP kinase and apoptosis levels, whereas it preserved cyclic GMP (cGMP) contents in diabetic penile tissue\textsuperscript{28}. Other effects of alagebrium, demonstrated in animal studies, are represented by the prevention and reversal of diabetic nephropathy, the treatment of heart failure in diabetic patients, the improvement of systolic pressure and the decrease in myocardial stiffness\textsuperscript{73-76}.

CONCLUSIONS

The preclinical and clinical studies of the last decade demonstrated a strong involvement of AGE in all of the micro and macrovascular complications of diabetes mellitus, especially in type 2 diabetes. As such, inhibition of AGE formation, antagonizing RAGE or suppressing the expression of this receptor have become viable targets in the treatment of this condition. We have presented, at a molecular level, the mechanisms through which AGE cause these complications, as well current and future lifestyle changes or drugs that could be used by clinicians in the treatment of their diabetic patients.

REFERENCES


Advanced glycation end products in diabetes mellitus


