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

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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 illness[1].

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 hyperglycemia[2] (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 synthase[3-5].

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[2].

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 (eg. 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)[5].

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 pyraline[6]. 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[7].

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.

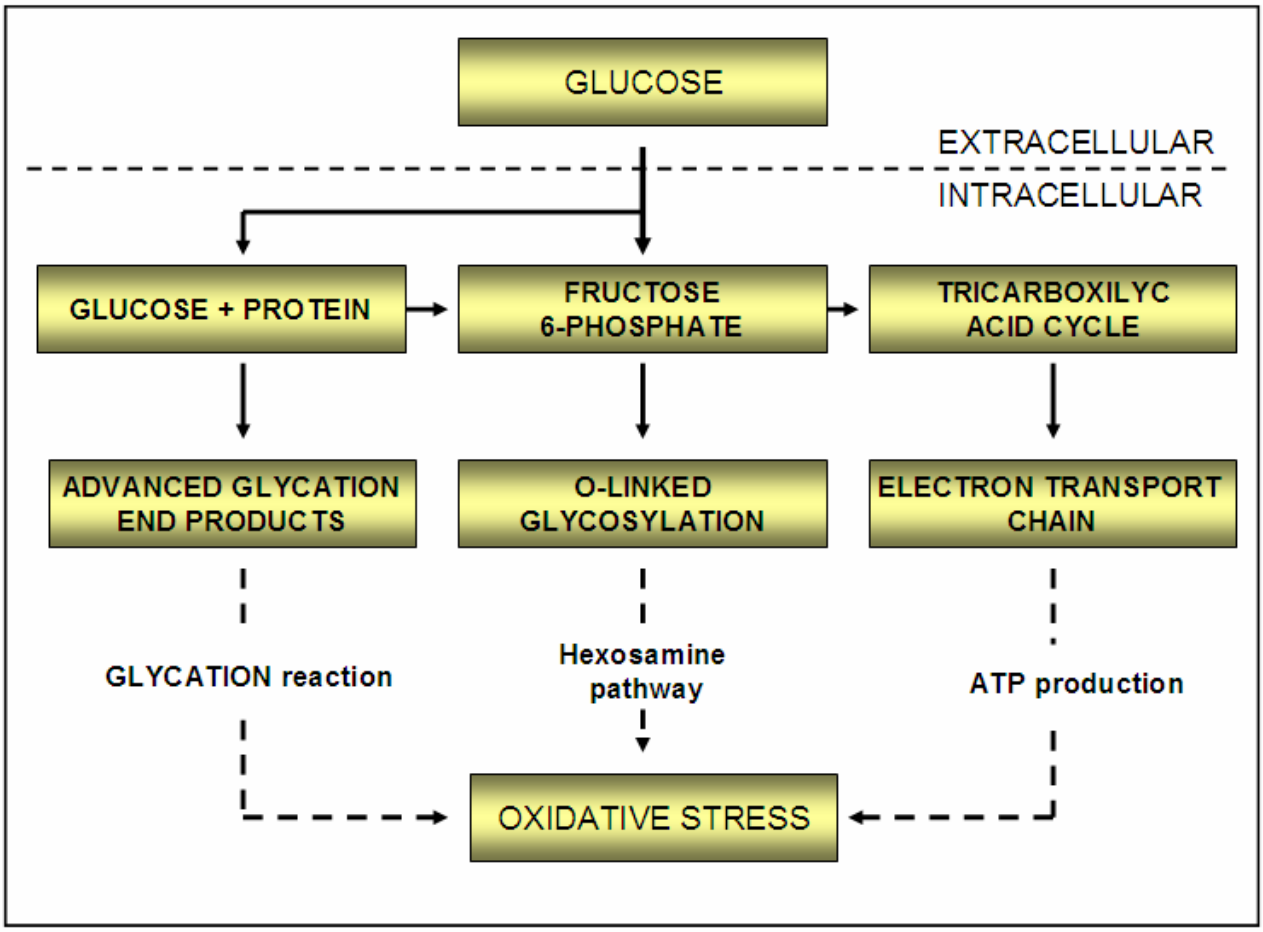

Fig.1. Sources of ROS under the influence of hyperglycemia (adapted after Rodrigo).

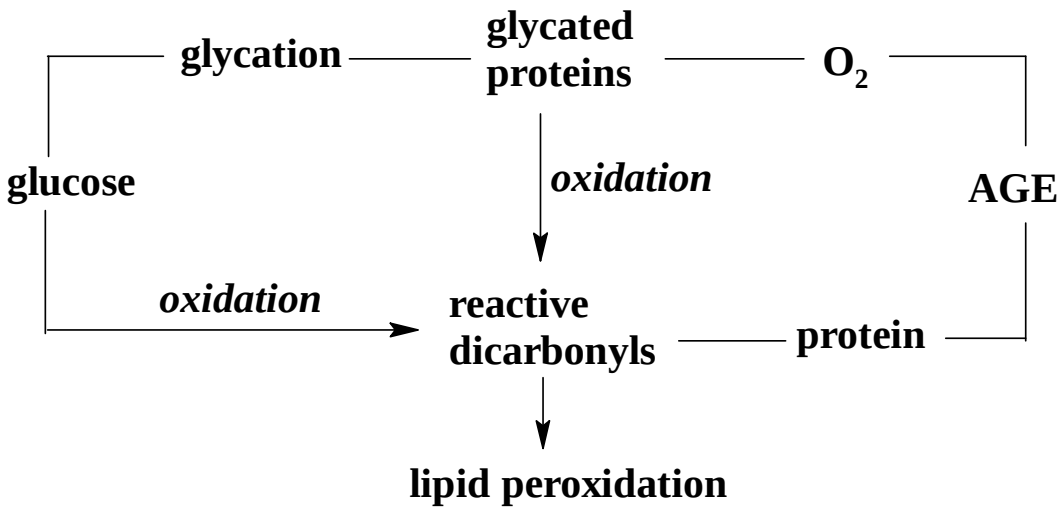

Fig.2. The initial oxidation of glucose with the formation of reactive dicarbonyl compounds which are later condensed with a proteic amino group represents another way of obtaining AGE.

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 micro-vascular 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.

$$NADPH + 2O_2 \xrightarrow{NADPHoxidase} NADP^+ + \\ +H^+ + 2O_2^- \quad (superoxide)$$

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 factorα (TNFα), thrombin and other compounds. In the renal biopsies performed on patients suffering from diabetic nephropathy, glyco-oxidation compounds, CML and pentosidine, along

with the lipid peroxidation compound called malonyldialdehyde (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 glomerullar 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[9].

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[10,11].

AGE and diabetic neuropathy

AGE determine the modification of the peripheral nerve myelin which becomes susceptible to phagocytosis and determines segmental demyelinization, 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 extracelular matrix also leads to impaired regenerative activity in diabetic neuropathy[12].

The AGE-RAGE interactions determine the upregulation of nuclear factor kappaB (NFkB), protein kinase C β 2 and various NFkB mediated proinflamatory 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 glycooxidation products such as N-epsilon-(carboxymethyl)lysine and pentosidine[12,13]. 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[14].

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[15,16]. 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[16].

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)[17]. 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[18].

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[7,19], whilst hypoglicaemia 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[20].

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[21].

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[22]. 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[23]. sRAGE levels were significantly higher in type 2 diabetic patients than in non-diabetic subjects and positively associated with the presence of CAD[24]. 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 [25].

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 [26].

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 asses this type of osteoporosis[27]. Also recently, the relationship between AGE and erectile dysfunction (ED) has been reported[28].

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[29].

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[30,31]. By contrast, products rich in carbohydrates, such as vegetables, fruit, whole grains and milk contain low AGE concentrations even after being cooked[32,33]. 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 proaterogenous effects and leads to AGE formation. The Montignac diet has omitted this aspect and therefore it has shown fewer followers [34].

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.

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[34].

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[35]. Commercial cookies which contain ammonium bicarbonate and fructose have the highest concentrations of AGE precursors[36]. Consumers should avoid commercial sauces, chips, crispy pretzels, bread crust and beverages rich in AGE (coffee based)[37,38]. 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, beats and beans[39]. It is interesting that some of these exogenous AGE can even influence the expression of several proinflammatory genes[40]. 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[41]. 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 HbA_{1C}. A short term normal glycaemia or a temporary normal level of HbA_{1C} 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[42,43]. 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[44]. 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[45]. Animal studies have showed that chronic exercise can also attenuate the heart stiffness found in CAD[46]. 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[47].

The same moderate exercise was proven to reduce glomerular mesangial and tubulointerstitial fibrobrosis, alongside AGE, N(epsilon)-carboxy-methyllysine and advanced oxidation protein products, thus physical exercise being a possible easy and effective nonpharmacological approach to ameliorate early diabetic nephropathy[48].

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 [44,49].

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[50].

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[51]. 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 (s*AGER*) 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.

Compound	Mechanism	Effect in humans
ACEI inhibitors	↓ Angiotensin II Deactivates NADPH	↓ Nephropathy and retinopathy
Aminoguanidine	Inhibits AGE formation	↓ Nephropathy and retinopathy
Pyridoxamine	Inhibits AGE formation ↓ Cholesterol	↓ retinopathy
LR-90	↓ Oxidative stress ↓ Fibrosis of extracellular matrix	↓ Nephropathy
Anti-RAGE sRAGE	Blocks AGER	↓ Nephropathy

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 phenoxyisobutyric 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[61]. 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[2].

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[60].

Minodronate, 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[62,63]. 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[27].

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[60].

A clinical study proved the effect of cerivastatin in reducing AGE-CML, thus determining an improvement in the cardiovascular profile of diabetic patients[64]. Atorvastatin also decreases AGE in patients with type 2 diabetes and influences the expression of sRAGE and esRAGE (a splice variant of sRAGE)[65,66]. 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[67]. 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 (chronic kidney disease) 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[68].

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

(TRC4186) has demonstrated AGE-breaking activities *in vitro* experiments and improvement in the endothelial and myocardial function in animal models of diabetes mellitus with reduction of AGEs accumulation in tissues over time. The safety of TRC4186 has been established *in vitro* and *in vivo* preclinical studies[69]. TRC4186, an AGE-breaker, clearly preserved cardiac function and reduced the severity of renal dysfunction in Ob-ZSF1, an animal model with persistent severe hyperglycemia leading to diabetic heart failure and renal failure[70].

Alagebrium (ALT-711) acts by breaking the abnormal crosslinks formed by collagen by being a low-affinity inhibitor of thiamine diphosphokinase (TDPK)[71]. 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[72]. 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[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[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

1. Farag YM, Gaballa MR, Diabesity: an overview of a rising epidemic. Nephrol Dial Transplant. 2011; 26 (1):28-35.
2. Brownlee M, The pathobiology of diabetic complications. A unifying mechanism. Diabetes, 2005; 54: 1615-1624.
3. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, Perego C, Muscogiuri G, The Role of Oxidative Stress in the Pathogenesis of Type 2 Diabetes Mellitus Micro- and Macrovascular Complications: Avenues for a Mechanistic-Based Therapeutic Approach. Current Diabetes Reviews. 2011; 7(5):313-24.
4. Rodrigo R., Oxidative stress and antioxidants: their role in human disease, Nova Science Publishers, 2009, 1-24.
5. Haliwell B, Gutteridge J, Free radicals in Biology and Medicine, fourth ed., Claredon Press, Oxford, 2007, 642-645.
6. Sheetz MJ, King GL, Molecular understanding of hyperglycemia's adverse effects for diabetic complications. JAMA. 2002, 288, 2579-2588.
7. Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M, Amano S, Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. Diabetologia. 2003;46 (2):284-7.
8. Kappert K, Schmidt G, Doerr G, Wollert-Wulf B, Fleck E, Graf K. Angiotensin II and PDGF-BB stimulate β1-integrin-mediated adhesion and spreading in human VSMCs, Hypertension, 2000, 35:255-261.
9. Wautier M.P., Chappey O., Corda S., Stern D.M., Schmidt A.M., Wautier, J.L., Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via AGER, Am J Physiol Endocrinol Metab, 2001; 280:685-694.
10. Khamaisi M., Schrijvers B.F., De Vriese A.S., Raz I., Flyvbjerg A., The emerging role of VEGF in diabetic kidney disease, Nephrol Dial Transplant, 2003;18:1427-1430.
11. Cha DR, Kang YS, Han SY, Vascular endothelial growth factor is increased during early stage of diabetic nephropathy in type II diabetic rats, J Endocrinol, 2004;183(1):183-94.
12. Sugimoto K, Yasujima M, Yagihashi S, Role of advanced glycation end products in diabetic neuropathy. Curr Pharm Des. 2008; 14(10):953-61.
13. Virgolici B, Mohora M, Radoi V, Lixandru D, Stoian I, Gaman L, Coman A, Greabu M, Manuel-Y-Keenoy B. Correlations between dysglycemia, markers of oxidative stress and inflammation in diabetic foot patients. Farmacia, 2011;59 (2): 216-227.
14. Toth C, Rong LL, Yang C, Martinez J, Song F, Ramji N, Brussee V, Liu W, Durand J, Nguyen MD, Schmidt AM, Zochodne DW. Receptor for advanced glycation end products (RAGEs) and experimental diabetic neuropathy. Diabetes; 2008; 57(4):1002-17.
15. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med. 1988; 318: 1315–1321.
16. Norton GR, Candy G, Woodiwiss AJ. Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. Circulation. 1996; 93: 1905–1912.
17. Simm A, Bartling B, Silber RE, RAGE: a new pleiotropic antagonistic gene? Ann N Y Acad Sci. 2004; 1019:228-31.
18. Lichiardopol R, Florentiu A, Radoi V. Body composition and the metabolic impact of weight excess in patients with type 1 and type 2 diabetes mellitus. Acta Endo; 2010; 6(4): 493-506.
19. Enomoto M, Adachi H, Yamagishi S, Takeuchi M, Furuki K, Hino A, Hiratsuka A, Takajo Y, Imaizumi T. Positive association of serum levels of advanced glycation end products with thrombogenic markers in humans. Metabolism. 2006; 55(7):912-7.

20. Storey R.F. What drives thrombogenesis despite antiplatelet therapy in diabetes mellitus? Diabetes & Vascular Disease Research ; 2010; 7(4) 249–250.
21. Yamagishi S, Fujimori H, Yonekura H, Yamamoto Y, Yamamoto H. Advanced glycation endproducts inhibit prostacyclin production and induce plasminogen activator inhibitor-1 in human microvascular endothelial cells. Diabetologia; 1998; 41(12):1435-41.
22. Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovascular Diabetology 2008, 7:29.
23. Falcone C, Emanuele E, D'Angelo A, Buzzi MP, Belvito C, Cuccia M, Geroldi D. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arterioscler Thromb Vasc Biol. 2005;25(5):1032-7.
24. Nakamura K, Yamagishi S, Adachi H, Kurita-Nakamura Y, Matsui T, Yoshida T, Sato A, Imaizumi T. Elevation of soluble form of receptor for advanced glycation end products (sRAGE) in diabetic subjects with coronary artery disease. Diabetes Metab Res Rev. 2007;23(5):368-71.
25. Chiang KH, Huang PH, Huang SS, Wu TC, Chen JW, Lin SJ. Plasma levels of soluble receptor for advanced glycation end products are associated with endothelial function and predict cardiovascular events in nondiabetic patients.Coron Artery Dis. 2009;20(4):267-73.
26. Park HY, Yun KH, Park DS. Levels of Soluble Receptor for Advanced Glycation End Products in Acute Ischemic Stroke without a Source of Cardioembolism. J Clin Neurol. 2009; 5(3):126-32.
27. Okazaki R, Diabetes Mellitus and bone metabolism. Clin Calcium. 2011; 21(5):669-75.
28. Gurbuz N, Sagdic G, Sanli A, Ciftcioglu A, Bassorgun I, Baykal A, Usta MF. Therapeutic effect of combination of alagebrium (ALT-711) and sildenafil on erectile function in diabetic rats. Int J Impot Res. 2011; doi: 10.1038/ijir.2011.54.
29. Nicholl ID, Bucala RC. Advanced glycation endproducts and cigarette smoking. Cell Mol Biol, 1998;44 (7):1025-33.
30. Visentin S, Medana C, Barge A, Giancotti V, Cravotto G, Microwave-assisted Maillard reactions for the preparation of advanced glycation end products (AGEs). Org Biomol Chem. 2010; 8(10): 2473-2477.
31. Yamaguchi K, Nakawaki R, Noumi Y, Mizude E, Nakajima K, Otsuka Y, Nagatsuka C, Homma T, Aizawa H, Van Chueyn N, Effects of Salt Concentration on the Reaction Rate of Glc with Amino Acids, Peptides, and Proteins. Biosci. Biotechnol. Biochem., 2009; 73 (11), 2379–2383.
32. Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, Yong A, Striker GE, Vlassara H, Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc. 2010;110(6), 911-16.
33. Krajčovičová-Kudláčková M, Sebeková K, Schinzel R, Klvanová, Advanced Glycation End Products and Nutrition. J. Physiol. Res., 2002; 51, 313-316.
34. Turk Z., Glycotoxines, Carbonyl Stress and Relevance to Diabetes and Its Complications, Physiol Res. 2010;59(2):147-56.
35. Bhaskar JJ, Shobha MS, Sambaiah K, Salimath PV, Beneficial effects of banana (Musa sp. var. elakki bale) flower and pseudostem on hyperglycemia and advanced glycation end-products (AGEs) in streptozotocin-induced diabetic rats. J Physiol Biochem. 2011 Sep;67(3):415-25.
36. Arribas-Lorenzo G, Morales FJ, Analysis, distribution, and dietary exposure of glyoxal and methylglyoxal in cookies and their relationship with other heat-induced contaminants. J Agric Food Chem., 2010, 10; 58(5): 2966-72.
37. Sato T, Wu X, Shimogaito N, Takino J, Yamagishi S, Takeuchi M, Effects of high-AGE beverage on RAGE and VEGF expressions in the liver and kidneys. Eur J Nutr., 2009; 48(1):6-11.
38. Förster A, Kühne Y, Henle T, Studies on absorption and elimination of dietary maillard reaction products. Ann N Y Acad Sci. 2005;1043:474–81.
39. Tsai PH, Liu JJ, Yeh CL, Chiu WC, Yeh SL. Effects of glutamine supplementation on oxidative stress-related gene expression and antioxidant properties in rats with streptozotocin-induced type 2 diabetes. Br J Nutr. 2011, 1:1-7.
40. Bartling B., Fuchs C., Somoza V., Niemann B., Silber R.E., Simm A., Lung level of HMBG1 is elevated in response to advanced glycation end product-enriched food in vivo.Mol Nutr Food Res., 2007; 51(4), 479-87.
41. Mericq V, Piccardo C, Cai W, Chen X, Zhu L, Striker GE, Vlassara H, Uribarri J, Maternally transmitted and food-derived glycotoxins: a factor preconditioning the young to diabetes?.Diabetes Care., 2010; 33(10), 2232-7.
42. Boor P, Celec P, Behuliak M, Grancic P, Kebis A, Kukan M, Pronayová N, Liptaj T, Ostendorf T, Sebeková K. Regular moderate exercise reduces advanced glycation and ameliorates early diabetic nephropathy in obese Zucker rats. Metabolism; 2009; 58(11):1669-77.
43. Gano LB, Donato AJ, Pierce GL, Pasha HM, Magerko KA, Roeca C, Seals DR. Increased proinflammatory and oxidant gene expression in circulating mononuclear cells in older adults: amelioration by habitual exercise. Physiol Genomics; 2011; 43(14):895-902.
44. Teixeira de Lemos E, Pinto R, Oliveira J, Garrido P, Sereno J, Mascarenhas-Melo F, Páscoa-Pinheiro J, Teixeira F, Reis F. Differential effects of acute (extenuating) and chronic (training) exercise on inflammation and oxidative stress status in an animal model of type 2 diabetes mellitus. Mediators Inflamm; 2011; 2011:253061.
45. Kurban S, Mehmetoglu I, Yerlikaya HF, Gönen S, Erdem S. Effect of chronic regular exercise on serum ischemia-modified albumin levels and oxidative stress in type 2 diabetes mellitus. Endocr Res; 2011; 36(3):116-23.
46. Woodiwiss AJ, Kalk WJ, Norton GR. Habitual exercise attenuates myocardial stiffness in diabetes mellitus in rats. Am J Physiol; 1996; 271(5 Pt 2):H2126-33.
47. Willemsen S, Hartog JW, Hummel YM, van Ruijven MH, van der Horst IC, van Veldhuisen DJ, Voors AA. Tissue advanced glycation end products are associated with diastolic function and aerobic exercise capacity in diabetic heart failure patients. Eur J Heart Fail; 2010; 13(1):76-82.
48. Boor P, Celec P, Behuliak M, Grancic P, Kebis A, Kukan M, Pronayová N, Liptaj T, Ostendorf T, Sebeková K. Regular moderate exercise reduces advanced glycation and ameliorates early diabetic nephropathy in obese Zucker rats. Metabolism; 2009; 58(11):1669-77.
49. Goon JA, Aini AH, Musalmah M, Anum MY, Nazaimoon WM, Ngah WZ. Effect of Tai Chi exercise on DNA damage, antioxidant enzymes, and oxidative stress in middle-age adults. J Phys Act Health; 2009; 6(1):43-54.

50. Yoshikawa T, Miyazaki A, Fujimoto S. Decrease in serum levels of advanced glycation end-products by short-term lifestyle modification in non-diabetic middle-aged females. Med Sci Monit; 2009; 15(6):PH65-73.
51. Chang KC, Tseng CD, Wu MS, Liang JT, Tsai MS, Cho YL, Tseng YZ, Aminoguanidine prevents arterial stiffening in a new rat model of type 2 diabetes, Eur. J. Clin. Invest. 2006; Aug; 36(8):528-35.
52. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP, Randomized trial of an inhibitor of formation of advanced glycation endproducts in diabetic nephropathy. Am. J. Nephrol., 2004;24:32–40.
53. Whittier F, Spinowitz B, Wuerth JP, Pimagedine safety profile in patients with type 1 diabetes, J. Am. Soc. Nephrol. 1999; 6410:184A.
54. Thomas, M.C., Tikellis, C., Burns, W.M., Bialkowski, K., Cao, Z., Coughlan, M.T., Jandeleit- Dahm, K., Cooper, M.E., Forbes, J.M., Interactions between rennin angiotensin system and advanced glycation in the kidney, J. Am. Soc. Nephrol. 2005; 16: 2976-2984.
55. Haupt, E., Ledermann, H., Köpcke, W., Benfotiamine in the treatment of diabetic polyneuropathy--a three-week randomized, controlled pilot study (BEDIP study), Int J Clin Pharmacol Ther. 2005;43(2):71-7.
56. Figarola, J.L., Scott, S., Loera, S., Tessler, C., Chu, P., Weiss, L., Hardy, J., Rahbar, S., LR-90 a new advanced glycation endproduct inhibitor prevents progression of diabetic nephropathy in streptozotocin-diabetic rats, Diabetologia. 2003; 46(8):1140–1152.
57. Figarola, J.L., Shanmugam, N., Natarajan, R., Rahbar, S., Anti-inflammatory effects of the advanced glycation endproduct inhibitor LR-90 in human monocytes, Diabetes. 2007; 56(3): 647-655.
58. Forbes, J.M., Thorpe, S.R., Thallas-Bonke, V., Pete, J., Thomas, M.C., Deemer, E.R., Bassal, S., El-Osta, A., Long, D.M., Panagiotopoulos, S., Jerums, G., Osicka, T.M., Cooper, M.E., Modulation of soluble receptor for advanced glycation endproducts by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy, J. Am. Soc. Nephrol. 2005; 16: 2363–2372.
59. Yoshida, T., Yamagishi, S., Nakamura, K., Matsui, T., Imaizumi, T., Takeuchi, M., Koga, H., Ueno, T., Sata, M., Telmisartan inhibits AGE-induced C-reactive protein production through downregulation of the receptor for AGE via peroxisome proliferator-activated receptor-gamma activation, Diabetologia. 2006; 49: 3094–3099.
60. Takeuchi M, Takino J, Yamagishi S. Involvement of the Toxic AGEs (TAGE)-RAGE System in the Pathogenesis of Diabetic Vascular Complications: A Novel Therapeutic Strategy. Current Drug Targets, 2010, 11, 1468-1482.
61. Green K, Brand MD, Murphy MP, Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes, Diabetes, 2004; 53:S110-S118.
62. Yamagishi S, Nakamura K, Matsui T, Takeuchi M. Minodronate, a nitrogen-containing bisphosphonate, is a promising remedy for treating patients with diabetic retinopathy. Med Hypotheses; 2006; 66(2):273-5.
63. Yamagishi S, Matsui T, Nakamura K, Takeuchi M. Minodronate, a nitrogen-containing bisphosphonate, inhibits advanced glycation end product-induced vascular cell adhesion molecule-1 expression in endothelial cells by suppressing reactive oxygen species generation. Int J Tissue React; 2005; 27(4):189-95.
64. Scharnagl H, Stojakovic T, Winkler K, Rosinger S, März W, Boehm BO. The HMG-CoA reductase inhibitor cerivastatin lowers advanced glycation end products in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes; 2007; 115(6):372-5.
65. Jinnouchi Y, Yamagishi S, Takeuchi M, Ishida S, Jinnouchi Y, Jinnouchi J, Imaizumi T. Atorvastatin decreases serum levels of advanced glycation end products (AGEs) in patients with type 2 diabetes. Clin Exp Med; 2006; 6(4):191-3.
66. Tam HL, Shiu SW, Wong Y, Chow WS, Betteridge DJ, Tan KC. Effects of atorvastatin on serum soluble receptors for advanced glycation end-products in type 2 diabetes. Atherosclerosis; 2010; 209(1):173.
67. Feng B, Xu L, Wang H, Yan X, Xue J, Liu F, Hu JF. Atorvastatin exerts its anti-atherosclerotic effects by targeting the receptor for advanced glycation end products. Biochim Biophys Acta; 2011; 1812(9):1130-7.
68. Nakamura T, Sato E, Fujiwara N, Kawagoe Y, Takeuchi M, Maeda S, Yamagishi S. Atorvastatin reduces proteinuria in non-diabetic chronic kidney disease patients partly via lowering serum levels of advanced glycation end products (AGEs). Oxid Med Cell Longev; 2010; 3(5):304-7.
69. Chandra KP, Shiwalkar A, Kotecha J, Thakkar P, Srivastava A, Chauthaiwale V, Sharma SK, Cross MR, Dutt C. Phase I clinical studies of the advanced glycation end-product (AGE)-breaker TRC4186: safety, tolerability and pharmacokinetics in healthy subjects. Clin Drug Investig; 2009; 29(9):559-75.
70. Joshi D, Gupta R, Dubey A, Shiwalkar A, Pathak P, Gupta RC, Chauthaiwale V, Dutt C. TRC4186, a novel AGE-breaker, improves diabetic cardiomyopathy and nephropathy in Ob-ZSF1 model of type 2 diabetes. J Cardiovasc Pharmacol; 2009; 54(1):72-81.
71. Krautwald M, Leech D, Horne S, Steele ML, Forbes J, Rahmadi A, Griffith R, Münch G. The advanced glycation end product-lowering agent ALT-711 is a low-affinity inhibitor of thiamine diphosphokinase. Rejuvenation Res; 2011; 14(4):383-91.
72. Candido R, Forbes JM, Thomas MC, Thallas V, Dean RG, Burns WC, Tikellis C, Ritchie RH, Twigg SM, Cooper ME, Burrell LM. A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. Circ Res; 2003; 92(7):785-92.
73. Peppa M, Brem H, Cai W, Zhang JG, Basgen J, Li Z, Vlassara H, Uribarri J. Prevention and reversal of diabetic nephropathy in db/db mice treated with alagebrium (ALT-711). Am J Nephrol; 2006; 26(5):430-6.
74. Little WC, Zile MR, Kitzman DW, Hundley WG, O'Brien TX, Degroof RC. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. J Card Fail; 2005; 11(3):191-5.
75. Susic D, Varagic J, Ahn J, Frohlich ED. Cardiovascular and renal effects of a collagen cross-link breaker (ALT 711) in adult and aged spontaneously hypertensive rats. Am J Hypertens; 2004; 17(4):328-33.
76. Doggrell SA. ALT-711 decreases cardiovascular stiffness and has potential in diabetes, hypertension and heart failure. Expert Opin Investig Drugs; 2011; 10(5):981-3.