**ROLE OF ADVANCED GLYCACTION END PRODUCTS IN THE ONSET OF DIABETIC KIDNEY DISEASE COMPLICATIONS**

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**ABSTRACT**

Advanced glycation end products are known to play an important role in diabetes complications, such as diabetic nephropathy. Most known pathways of diabetic complications involve oxidative stress, that have pivotal role in cell dysfunction onset and progression of angiopathies. This review will explore how AGEs cause endothelial dysfunction in diabetes and what current biochemical mechanisms have been proposed as an explanation for the development of diabetic nephropathy.

**Keywords:** Type 2 diabetes mellitus; advanced glycation end products; endothelial dysfunction; hyperglycemia; oxidative stress; vascular complication; diabetic nephropathy

The prevalence of diabetes mellitus (DM) has risen to epidemic proportions worldwide. The number of cases of diabetes is projected to reach 360 million people in 2030¹,². Diabetes is a chronic disease characterized by elevated blood sugar levels due to lack of insulin production (type 1 diabetes-T1DM), insulin resistance (type 2 diabetes-T2DM) or both, among other mechanisms. T2DM represents 90% of the cases, implicated in insulin resistance and progressive β-cell dysfunction and loss of insulin release, leading to increasing blood glucose concentrations³,⁴. The consequences of uncontrolled glycemic levels are often related to diabetic chronic complications which include microvascular (nephropathy, retinopathy and neuropathy) and macrovascular disease (stroke and atherosclerosis). Thus, cardiovascular complications have the worst prognostic as they are associated with high morbidity and mortality, being the main cause of death⁵,⁶. Moreover, diabetic nephropathy (DN) is a representative disorder of chronic kidney disease (CKD) and the leading cause of end-stage kidney disease (ESKD)⁷-¹⁰. Thus, prevention of the development and progression of this disorder is of clinical importance to improve prognosis in diabetic patients.

The diagnosis of DM has been based on fasting glycemia ≥ 126 mg/dl; 2 hours glycemia, after 75 g oral glucose ≥ 200 mg/dl; hemoglobin A1c (HbA1c) ≥ 6.5% on at least two occasions or plasma glucose levels ≥ 200 mg/dl in patients with classic symptoms of hyperglycemia or hyperglycemic crises¹¹. The diagnosis of diabetic nephropathy is based on the presence of proteinuria, divided into two stages: micro- and macroalbuminuria with or without loss of glomerular filtration rate¹²,¹³. Microalbuminuria is an early indicator of renal impairment. Albumin is present in large amounts in the blood, but almost none is excreted in the urine when renal function is normal. However, when kidney disease ends, urinary albumin levels rise (30-300 mg/24h) and higher amounts of protein in urine reflect higher degrees of renal insufficiency¹²,¹⁴. Being a small molecule, albumin is one of the first proteins detected in the urine when there is kidney damage. Patients with microalbuminuria have higher risk of developing progressive renal failure in the future¹⁵-¹⁶. Macroalbuminuria is defined for urinary excretion of albumin ≥300 mg/24 hours¹⁷. Albuminuria can be estimated by 24 hours urine collection or at a random time after at least
4 hours without urine excretion. As the concentration (or dilution) of urine varies during the day, urine creatinine levels that are stably excreted can be used as a correction factor for random urine samples. It is considered the test of choice for screening for microalbuminuria using the albumin/creatinine urinary ratio.

Although the consequences of the uncontrolled glycemic levels are well known, the relationship between hyperglycemia and the onset of diabetes complications is not totally clarified. Evidence suggests that it is associated with advanced glycation end-products (AGEs) formation, leading to cellular dysfunction. In this review, we will consider the consequences of AGEs and the mechanisms involved in the pathophysiology of diabetic nephropathy.

**AGEs**

AGEs are mainly formed non-enzymatically by the interaction of reducing sugars (glucose, glucose-6-phosphate, and fructose) with free amino groups of proteins (more often lysine and arginine), lipids, and nucleic acids, through complex and sequential reactions known as the Maillard reaction. Under hyperglycemic and/or oxidative stress conditions, this process begins with the conversion of reversible Schiff base adducts to more stable, covalently bound Amadori rearrangement products. Over the course of days to weeks, these Amadori products undergo further rearrangement reactions to form AGEs, irreversibly. As glycation depends only on glucose concentration, this explains AGEs enhancement in DM. Decreased serum AGEs clearance may further increase tissue AGEs accumulation. Other factors that influence AGEs accumulation are: hyperglycemia duration, oxidative stress, inflammation, reduced kidney function and diet.

Alternatively, AGEs can derive from highly reactive dicarbonyl such as glyoxal, methylglyoxal and 3-deoxiglucosone. Methylglyoxal - the main AGE precursor - is formed from the spontaneous dephosphorylation of triose phosphates during anaerobic glycolysis, spontaneously fragmentation of a Schiff base during the Maillard reaction and from ketone and threonine metabolism. These compounds may be known as products of advanced lipoxidation. In metabolic syndrome, the combination of glucose intolerance, dyslipidemia, hypertension, and insulin resistance may generate more AGEs for synergic effect through both glycation and lipoxidation pathways.

In general, the damage associated to AGEs accumulation involves three basic mechanisms: 1) modification in protein structures involved in gene transcription; 2) interaction with extracellular matrix proteins which alters the signaling between the cell and the matrix; 3) changes in serum lipids and proteins that can bind to specific receptors, stimulating production of inflammatory cytokines and intracellular growth factors. In mesangial cells of the renal glomerulus, the cross-linking between collagen, AGEs and lysine and/or arginine residues of protein can contribute to rigidity, loss of elasticity, increased resistance to proteolysis, inhibition of tissue remodeling and thickening of the glomerular capillary membrane leading to changes such as glomerular sclerosis.

Vascular cell dysfunction related to AGEs accumulation can be due to its interaction with proteins, forming stable and irreversible bonds. Moreover, AGEs bioavailability depends on the half-life of the proteins. This explains why the main sites of DM chronic complications are tissues where proteins have longer half-lives, such as proteins of the glomerular basement membrane and lens, featuring nephropathy and retinopathy, respectively. Importantly, chronic kidney disease occurs where long-lived proteins are present, such as glomerular basement membrane. On the other hand, bonds to other types of proteins with shorter half-lives are unstable, leading to rapid AGEs degradation followed by renal excretion.

Increased intracellular glucose is related to pathophysiological mechanisms that trigger DM chronic complications. These mechanisms include: 1) Stimulation of the polyol pathway: in the persistent hyperglycemia, part of the glucose is converted to sorbitol, which increases ROS production, leading to cell dysfunction. 2) Activation of protein kinase C (PKC) isoforms, which induce the synthesis of diacylglycerol, fibronectin, collagen type IV, contractile proteins, and extracellular matrix protein genes in endothelial cells and neurons. 3) Activation of hexosamine pathway, which may interfere with the glycosylation of proteins, changing their final structure and also altering gene expression. 4) Increase of intracellular AGEs levels leads to increase glycation of plasmatic and extracellular matrix proteins. The effects of AGE accumulation in the body can be attributed to the crossinglinking of extracellular matrix proteins, which leads to alteration of the cell structure.

The four pathways described are the result of persistent hyperglycemia and converge in the formation of AGEs, with formation of ROS, mainly superoxide anion, contributing to altering intracellular redox status, leading to oxidative stress. Therefore, the increased ROS in DM, together with the reduction of antioxidant defenses are related to the onset of diabetic complication. ROS increase leads to DNA breaking, which activates poly ADP-ribose polymerase (PARP), reducing the
activity of glyceraldehyde-3-phosphate (GAPDH), a key enzyme in the glycolytic pathway, which, in turn, activates the polyol pathway, increases the formation of AGEs, PKC activation, and increases the flow of hexosamines.

Thus, there seems to be a cyclic activation of AGEs through increased ROS, suggesting the induction of a ROS-AGE-RAGE pathway. In this context, the increased O$_2^-$ production by the electron transport chain appears to be the link between oxidative stress, AGE, and diabetes complications. However, the mechanism by which the increased O$_2^-$ production resulting from hyperglycemia is related to tissue damage that results in diabetic complications still needs to be elucidated.

The mechanism of action of AGEs in target cells involves the interaction with its receptor (RAGE). RAGE is a member of the immunoglobulin superfamily of cell transmembrane surface molecules, playing key roles in the pathogenesis of diabetic complications. The binding of AGE to RAGE induces apoptosis in various cell types and causes endothelial dysfunction by increasing oxidative stress and stimulates the release of cytokines and growth factors by macrophages, renal mesangial, and vascular endothelium, for example. Growth factors can be involved in the complications of diabetes, as they are increased in hyperglycemia. Transforming Growth Factor Beta (TGF-β) is increased in diabetic nephropathy, stimulating production of collagen in the glomerular capillary membrane and fibronectin by mesangial cells. Other factors, such as platelet activating factor (PAF), epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), growth hormone (GH) and insulin may play a role in DM complications. This factors can in turn induce multiple signaling pathways that generate reactive oxygen species (ROS).

There is evidence that the AGE-RAGE interaction induces inflammatory responses and oxidative stress, leading to more AGE formation and accumulation, and hence to RAGE overexpression in diabetic patients. It is well known that the AGE-RAGE interaction leads to stimulation of intracellular signaling pathways whose target is the nuclear factor Kβ (NF-Kβ). NF-Kβ induces the transcription of proinflammatory genes and stimulates transcription of RAGE itself. This positive feedback loop (AGE-RAGE) creates a vicious circle that may explain the phenomenon called metabolic memory in diabetic vascular complications, in which even after a period of glycemic control, vascular complications do not recede. Apart its transmembrane localization, RAGE structure consist of a soluble isoform (sRAGE), formed by two processes: alternative splicing (endoogenous secretory receptor for AGE (esRAGE) or proteolytic cleavage (cRAGE) mediated by disintegrins and metalloproteinases. Studies suggest that sRAGE are risk predictors for vascular complications and therapeutic targets for the prevention of DM complications.

Quantification of glycation products is routinely analyzed through the measurement of glycated hemoglobin (HbA1c), which is used to assess metabolic control in diabetic patients. HbA1c is caused by an irreversible reaction between glucose and the N-terminal valine amino acid unit of the beta chain of hemoglobin. In a non-diabetic individual, approximately 4% to 6% of total HbA1c is glycated, whereas in patients with marked uncontrolled diabetes, this percentage can reach levels two to three times higher than normal. The rate of formation of this compound is directly proportional to the plasma glucose concentration. HbA1c levels above 7% are associated with a progressively increased risk of chronic complications, such as diabetes kidney disease. In this sense, HbA1c dosage plays a key role in the monitoring of glycemic control in diabetic patients, since it provides information about the retrospective index of plasma glucose.

**DIABETIC KIDNEY DISEASE**

Among individuals with T2DM, about 20-40% will be affected by diabetic nephropathy, which is the leading cause of end-stage renal disease in these individuals. The risk factors for onset and progression of diabetic nephropathy include familiar history, diabetes duration, hypertension, presence of retinopathy, smoking, and hypercholesterolemia. Diabetic nephropathy is the leading cause of chronic kidney disease and is associated with increased risk of death from cardiovascular disease (CVD). Hemodynamic changes in renal microcirculation plays an important role in the onset and progression of nephropathy. These changes include glomerular hyperfiltration or hyperperfusion and increased glomerular pressure, where the glomerulus is the main focus of the lesion. ACE-inhibitor drugs such as angiotensin II receptor blockers have shown beneficial effect in preventing the progression of diabetic nephropathy by reducing intraglomerular pressure, dilating the efferent arterioles.

Microvascular complications of T1DM and T2DM appear to be related to genetic susceptibility, as there are individuals who have the disease for a long time and never developed nephropathy or retinopathy and individuals with the same glycemic control who develop microvascular complications. Also, the risk of diabetic nephropathy development increase in individuals who have relatives with T1DM with diabetic
nephropathy. Thus, genetic susceptibility may affect the development of complications.

Among the metabolic factors, hyperglycemia is the major determinant in the onset and progression of diabetic nephropathy. Renal mesangial cells activated by AGEs induce excess extracellular matrix, which would explain the structural changes in the glomerulus, such as, mesangial expansion that occurs in diabetes and other renal diseases. In vitro studies with mesangial cells cultured in hyperglycemic condition demonstrated increased expression of collagen type IV, and its accumulation is proportional to the degree of glomerular sclerosis. In sclerotic glomerulus there is also accumulation of collagen types I and III, which are not usually seen in healthy glomeruli. Mesangial cells cultured in hyperglycemic conditions showed increased expression of GLUT1, which is the main glucose transporter in mesangial cells. In addition, these cells showed higher PKC activity and the subsequent stimulation of extracellular matrix proteins expression. The increased expression of GLUT1 in vitro upregulated GLUT1 expression, through the activation of PKC and promoted the stimulation of extracellular matrix synthesis in mesangial cells. The expression of GLUT1 seems to be the limiting step for the use of glucose by mesangial cells.

Angiotensin II (AII) also increases the expression of messenger RNA (mRNA) of GLUT1 in mesangial cells and GLUT2 (responsible for tubular reabsorption of glucose) also favored increased interstitial glucose, which is transported intracellularly by GLUT1. The production of AII mesangial cells under glucose stimulation leads to an intracrine action within the cells, mediating inflammatory and proliferative effects, with consequent cell proliferation, matrix expansion, and inflammation, contributing directly to the glomerular sclerosis observed in diabetic nephropathy. Thus, glucose and angiotensin II seem to have similar effects on cultured mesangial cells, promoting cell growth, proliferation, extracellular matrix and inhibiting their degradation. Possibly by using similar signal transduction pathways, glucose and AII can ultimately lead to an increase in cellular production of TGF-β.

Oxidative stress, especially nitric oxide (NO) accumulation, is related to the onset of diabetic nephropathy, although the mechanism by which its accumulation is related to kidney damage is still poorly understood. However, nitric oxide acts as a physiological regulator of arterial blood pressure and of mesangial cells proliferation. Moreover, its accumulation is involved in early and in late-stage renal disease. Nitric Oxide has a very short half-life and is formed from L-arginine, in an oxidation reaction catalyzed by three nitric oxide synthases isoforms (NOS). Two NOS are calcium dependent, one expressed in endothelial cells (eNOS) and the other in neuronal cells (nNOS). The third isoenzyme is independent of calcium, but induced by inflammatory or immune response, and is widely expressed (iNOS). The NO's mechanism of action consists of its diffusion through the cell membrane and activation of a second messenger, guanylate cyclase, which acts on various target cell proteins by activating protein kinase cascades, including the mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinases (JNK) pathways. Intracellular responses unleash important physiological effects, such as being a potent vasodilator with effects on both blood pressure regulation and renal function.

Recent studies have shown that AGEs induce iNOS expression in mesangial cells by activation of cytokines 2 - NF-kB and p38 MAPK. Mesangial cells cultured in high glucose medium showed an increase in iNOS expression related to expansion of extracellular fibronectin and glomerular endothelial cells contributes significantly to the initiation of diabetic nephropathy. In mesangial cells cultured in high glucose medium, it was shown an increase in the expression of both iNOS mRNA and protein via activation of PKC, promoting accumulation of extracellular matrix, corroborating in vivo assessments.

The increased expression of iNOS is generally associated to the inflammatory process that accompanies diabetic nephropathy, having no participation in normal renal hemodynamics. Moreover, the eNOS found in glomerular endothelial cells contributes significantly with nitric oxide to normal kidney function and can be involved in the early stages of nephropathy.

The importance of NFκB as a common mediator in the functional and structural changes observed in the pathophysiology of diabetes kidney disease is increasingly well established. NFκB can be activated by hyperglycemia among other mediators such as cytokines, oxidant molecules, AGEs and angiotensin II, which are increased in diabetes. Experimental and human studies have shown increased NFκB activation and increased expression of proinflammatory genes in endothelial cells, vascular smooth muscle cells, and proximal tubule cells, correlated with renal cell infiltration and magnitude of proteinuria in patients with diabetes. After activation, NFκB induces the...
transcription of a number of genes, including adhesion molecules, cytokines, and nitric oxide synthase, among other inflammatory proteins involved in the pathogenesis of the renal complications of diabetes \(^{59-61}\).

In patients with insulin-dependent diabetes, intensive insulin therapy (insulin pump or three or more daily injections) for the control of blood glucose levels can reduce the frequency and severity of nephropathy. Intensive therapy reduces the occurrence of microalbuminuria up to 30% and albuminuria in 54% of cases, compared to conventional therapy. The main adverse effect associated with intensive therapy is the increased occurrence of severe hypoglycemia \(^{8,16}\).

Some evidence demonstrates that AGEs play a pivotal role in the development and progression of diabetic vascular damage. The attenuation of AGE formation and/or inhibition of RAGE activation manifests in improved renal function. All compounds have been evaluated for their anti-AGE effects and are classified as 1) anti-AGEs that prevent AGE formation, 2) AGE breakers that break preformed AGEs and Amadori products, 3) RAGE inhibitors, 4) redox regulators, and 5) nutraceuticals that serve as anti-glycating agents \(^{62-64}\).

Pimagedine (also known as aminoguanidine) inhibited the formation of AGEs and slowed the progression of diabetic complications in animal models. Pyridoxamine, the natural form of pyridoxine (vitamin B6), also prevents AGE formation. AGE breakers include AGE-detoxifying enzymes and small chemical molecules that are able to recognize and break the Amadori products or AGE crosslinks. An example is ALT – 771. Studies with this molecule suggested the pharmacological potential of these molecules in preventing AGE-mediated diabetic complications. The use of RAGE inhibitors such as pravastatin, curcumin could be a potential strategy to combat AGE-mediated adverse cellular manifestations. The rate of conversion of reversible Schiff’s base and partially reversible Amadori products to AGEs is accelerated in oxidative stress conditions. The use of antioxidants such as lipoic acid, vitamin E, and ubiquinol or redox regulators may to prevent AGE-mediated cellular toxicity \(^{62,63}\). However, new agents in treatment of diabetic complications required more studies to be validated.

CONCLUSIONS

The prevalence of DM has increased considerably worldwide and recent estimates suggest an even greater progression, associated with the epidemic of overweight and obesity. As a model of chronic disease, its complications significantly affect the quality of life of diabetic patients, configuring a public health problem \(^{1,7}\).

Diabetic nephropathy has been widely recognized as one of the major complications of type 2 diabetes mellitus, being a more common cause of chronic end-stage renal disease \(^{11}\). It is functionally characterized by proteinuria and albuminuria and pathologically by glomerular hypertrophy, mesangial expansion, and tubulointerstitial fibrosis.

The main mechanism involved in diabetic nephropathy occurs due to the excessive formation of AGEs, which are physiopathologically based on hyperglycemia. The AGE-RAGE interaction increases the formation of ROS and RNS, altering the redox state and causing oxidative stress, which contributes to the development and progression of diabetes and related complications. Another consequence of this interaction is increased expression of inflammatory mediators and, consequently, cell injury \(^{30,35,37}\).

Avoiding the installation as well as the progression of this complication is of paramount importance to improve the prognosis in diabetic patients. Diabetic kidney disease may be present at diagnosis of T2DM or after 5 years of T1DM, and renal injury screening under these circumstances is recommended by albuminuria using the albumin/creatinine ratio in a random urine sample. Studies have shown that intensive blood glucose control significantly reduces the risk of complications, including nephropathy. In addition, other effective strategies are the remission of proteinuria in these patients, which may be obtained by the use of drugs with potentially nephroprotective properties such as angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (BRA II), as well as by the control of cardiovascular risk factors, such as dyslipidemia, hypertension, and obesity \(^{65}\). Additional potential therapies for diabetic kidney disease have been or are currently being evaluated \(^{62,63}\).

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