Role of advanced glycation end products (AGEs) and its receptor (RAGE)-mediated diabetic vascular complications

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Abstract
Diabetes Mellitus (DM) is one of the major health problems of the current century. It is associated with accelerating advanced glycation end products (AGEs) formation and accumulation in the circulating blood and various tissues. AGEs, also accelerate the expression of its receptor i.e. receptor for AGEs (RAGE) and plays a pivotal role in the development and progression of diabetic vascular complications through various mechanisms. Hyperglycemia mediated reactive oxygen species generation can induce oxidative stress through four major mechanisms including the polyol pathway, AGEs formation, activation of protein kinase c isomers and the hexosamine pathway. Therapeutic interventions may improve the clinical course of patients having diabetes and its associated vascular complications by reducing the AGEs levels. This review summarizes the recent update on the role of AGE-RAGE mediated mechanisms in the development of diabetic vascular complications.

Introduction
Diabetes mellitus (DM) is one of the major epidemic disorders of the current century [1,2]. It is a group of metabolic disorders leading to defects in insulin secretion and action of insulin or both. Diabetes is influenced by a combination of both hereditary and environmental factors [3]. In the human body, blood glucose levels are controlled by a complex interaction of multiple chemicals and hormones, including insulin and glucagon. Insulin is one of the important peptide hormones produced from the beta cells of the pancreas that allows blood glucose to enter various cells of the body where it is oxidized to yield energy needed by the muscles and tissues to function [4]. Glucagon is also a peptide hormone, secreted from the alpha cells of the pancreas, which causes a rise in the blood glucose concentration. The effect of glucagon is opposite to that of insulin, which lowers the blood glucose concentration.

The global prevalence of DM in adults is increasing at an alarming rate. According to the recent update by the 8th edition of the diabetes mellitus Atlas, it was reported that there are 425 million adults with DM in the world and it is estimated that there will be 693 million people with DM by the year 2045 [5]. This equates to approximately three new cases in every 10 seconds or almost 10 million per year. Diabetes caused 5.1 million deaths in 2013 and every six seconds a person dies from diabetes. Diabetes is rampant in Indian subcontinent. India is the 2nd topmost country having the highest number of people with diabetes. The lack of adequate control in the consistently high level of glucose leads to the appearance of serious vascular complications.

Vascular complications of diabetes
Macrovascular and microvascular complications are the chronic vascular complications of diabetes, which are the major causes of morbidity and mortality (Figure 1). Diabetes, due to its increased prevalence has become the principal cause of blindness and end stage renal disease. About 30-45% of all diabetic subjects suffer from microvascular complications. Among microvascular complications, neuropathy, retinopathy and nephropathy were observed. The characteristic macrovascular complications include cardiovascular diseases. Patients with diabetes are at two to four times increased risk of coronary heart disease, cardiovascular disease and related deaths than those in the general population. Patients with diabetes are at four times

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higher risk of developing peripheral vascular disease (PVD) [6]. Each of these organ specific vascular complications has its own unique, clinical and histologic features, but all are common with increasing duration of hyperglycemia and are driven by its downstream cellular signaling pathways [7].

The Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study Trial (UKPDS) have clearly demonstrated the vital importance of intensive glycemic control in preventing the progression of diabetic complications [8,9]. Hyperglycemia inflicts cumulative long-term structural and functional changes in important macromolecules through advanced glycation end products (AGEs). Hyperglycemia induces a variety of metabolic changes, which includes activation of polyol pathway, activation of the diacylglycerol-protein kinase c, and increased oxidative stress. In this review, we summarize the recent updates on AGEs and the role of AGE-RAGE interaction-mediated various pathways which lead to diabetic vascular complications.

**Hyperglycemia mediated ROS generation**

Oxidative stress (OS) is defined as an imbalance between the reactive oxygen species (ROS) generation and the body’s antioxidant defense system [10-12]. Various studies have evidenced that OS plays an important role in the pathogenesis of a wide range of human disorders such as diabetes, cancer, cardiovascular disorders, kidney diseases and neurodegenerative diseases [13-16]. Also the most important pathogenic role of OS in the initiation and development of diabetes associated complications has been determined. Free radical generation in hyperglycemic conditions may lead to OS in β-cells of the pancreas, which causes β-cell dysfunction and other long term complications of diabetes because of insulin secretion and/or its function impairment [17-20].

Under the normal physiological conditions, ROS generation may help in cell defense, hormone synthesis, signal transduction, transcription factor regulation and gene expression. While under pathological conditions; inflammation, tissue damage, fibrosis and β-cell death may occur [21,22]. There are four major mechanisms involved in the increased intracellular ROS as a result of hyperglycemia, which includes the polyol pathway, advanced glycation end product formation, protein kinase c-diacyl glycerol pathway and hexosamine pathway (Figure 2). It has been reported that all of these pathways are activated by mitochondrial ROS overproduction. The effects of ROS generation can be modified by enzymatic or non-enzymatic antioxidants. Enzymatic antioxidant includes catalase, superoxide dismutase, nitric oxide synthase, glutathione peroxidase, glutathione-s-transferase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Non-enzymatic antioxidant includes vitamins, minerals, polyphenols, carotenoids and some other molecules [23,24].

The polyol pathway mainly focuses on the enzyme aldose reductase. Normally aldose reductase reduces toxic aldehydes in the cells to inactive alcohols, but under hyperglycemic condition, it utilizes NADPH and converts excessive intracellular glucose into the forms of sugar alcohols [15]. In healthier individuals, this pathway utilizes a very small fraction of the total glucose while in diabetic patients aldose reductase activated and induces increased conversion of glucose to sorbitol. After then, sorbitol is oxidized to fructose by enzyme sorbitol dehydrogenase with NAD+ as a cofactor [18]. Consumption of NADPH reduces glutathione reductase (GSH) activity as GSH is well known important scavenger of ROS [18,20,21]. Finally the process induces ROS generation and exacerbates intracellular OS.

![Figure 2. Hyperglycemia-mediated pathways](Image)

Hyperglycemia also induces overproduction of both the intracellular and extracellular AGEs [1]. Advanced glycation end product formation occurs as the result of glyoxal oxidation, 3-deoxyglucosone formation and fragmentation of glycolaldehyde-3-phosphate into methyl glyoxal [15]. Cellular damage may occur due to intracellular production of AGE precursors through mainly three general mechanisms. Firstly, the functional intracellular proteins modification by AGEs may occur. After then abnormal interaction of extracellular matrix compounds which were modified by AGEs precursors with other matrix components and receptors such as integrin is observed. At last, finally the plasma proteins are modified by AGEs precursors binding to cell surface receptors such as receptor for AGEs (RAGE) or macrophage scavenger receptor [18,21]. This AGE-RAGE interaction may contribute to OS via induction of mitochondrial superoxide and cytosolic NADPH oxidase dependent mechanism, which activates multiple signals such as p21RAS, NF-kB, MAP kinase, TGF-β, vascular adhesion molecules, etc. This transcription the number of pro-inflammatory genes and subsequently elicits vascular inflammation, over expression of endothelial growth factor, impaired fibrolytic affinity, platelet aggregation, angiogenesis and thrombosis, thereby playing a central role in the pathogenesis of vascular complications in diabetes by enhancing the OS development [25-28]. These observations suggest that in diabetes, the increased AGES production might alter glucose metabolism through direct attack on pancreatic insulin producing cells.

Protein kinase C (PKC) consists of at least eleven isoforms in mammalian tissues. Increased activation of PKC isoforms is the third most important pathway which induces tissue injury through hyperglycemia mediated ROS. Increased ROS generation inhibits activity of glycolytic enzyme glyceraldehydes-3-phosphate dehydrogenase, which leads to increase the level of diacyl glycerol (DAG) precursors [15]. Tissue phosphate also enhances the de novo synthesis of DAG from glucose. AGE-RAGE interaction also increases the activity of PKC isoforms. Hyperglycemia induced PKC activation may lead to over expression of plasminogen activator inhibitor-1 (PAI-1) and activation of NF-kB [22]. This may lead to vascular damage via inflammation; increase the permeability of basement membrane thickening, angiogenesis and thrombotic vascular occlusion.

Under hyperglycemic condition, when the glucose level is higher inside the cell, most of the glucose is metabolized through glycolysis to glucose-6-phosphate, then to fructose-6-phosphate. Increasing the flux of fructose-6-phosphate into the hexosamine pathway may also
contribute to pathogenesis of diabetic vascular complications [15]. Fructose-6-phosphate is diverted from glycolysis to provide glutamine fructose-6-phosphate aminotransferase (GFAT). After the conversion of fructose-6-phosphate to glucosamine-6-phosphate by GFAT, it is converted into UDP-N-acetyl glucosamine. It has been shown that hyperglycaemia causes four folds increase in UDP-NAG, which induces hyperglycaemia mediated activation of the PAI-1 and TGF-β1 [19,21]. Under normal conditions, very small amount of glucose is metabolized through this pathway.

Therefore, it was believed that under hyperglycemic condition, mitochondria derived, OS leads to AGEs formation, DAG synthesis accelerated, PKC activation, sorbitol or fructose accumulation in the cells as a result of polyol pathway activation. These hypotheses suggest that hyperglycemia mediated OS play an important role in the pathogenesis of vascular disorders.

Biochemistry of advanced glycation end products

Hyperglycemia accelerates non-enzymatic reaction between the free amino groups of proteins and carbonyl groups of reducing sugars or other carbonyl compounds leading to enhanced formation of AGEs, also known as the Maillard reaction [25,26]. Advanced glycation end product formation is a complicated molecular process involving multistep reaction. A reducing sugar, such as glucose reacts non-enzymatically with the free amino group of protein to form an unstable compound, the Schiff base which undergoes a rearrangement reaction to form a more stable product known as Amadori product [27,28]. The Amadori adducts then very slowly undergo irreversible dehydration and condensation reactions leads to the formation of AGEs, which is yellowish brown material with the particular fluorescence. Advanced glycation end products are not produced only from glucose, but also from dicarbonyl compounds produced from auto-oxidation and the degradation products of glucose such as glyoxal, methylglyoxal and 3-deoxyglucosone or α-hydroxy aldehydes such as glyceraldehyde and glycoaldehyde. In addition, AGEs can also act as cross-linkers between proteins, resulting in the production of proteins-resistant aggregates [29].

Under chronic hyperglycemic condition, AGEs are actively produced and accumulate in the circulating blood and various tissues, resulting in vascular complications in diabetes. Furthermore, humans are also exposed to exogenous AGEs including tobacco, smoke, and diet. Food processing methods, such as prolonged heating and microwave cooking, can also accelerate the AGEs formation. As discussed earlier, the AGEs formation reaction also referred as browning reaction, this brown color change of food can be a measure of their AGE content. Over the AGEs formation reaction also referred as browning reaction, this brown color change of food can be a measure of their AGE content. Over a dozen AGEs have been detected in tissues and can be divided into three categories: 1. Fluorescent cross-linking AGEs such as pentosidine and crossline. 2. Non-fluorescent cross-linking AGEs such as imidazolium dilsylene cross-links, alkyl formyl glycosyl pyrrole (AFGP) cross-links and arginine-lysine imidazole (ALI) cross-links. 3. Non-cross-linking AGEs such as pyrraline and N-carboxymethyllysine (CML) [30]. The serum AGEs level was determined spectrofluorometrically at emission maximum (440 nm) upon excitation at 350 nm [26]. Briefly, serum was diluted 1:50 with phosphate buffer saline (PBS) (pH=7.4) and fluorescence intensity was expressed in arbitrary units (AU). Total serum AGEs were also determined by ELISA using commercial kits. Previously, we have reported that higher levels of circulating AGEs were observed in diabetic patients having vascular complications indicating that higher the serum AGEs level, higher the likelihood of development of vascular complication of diabetes [31-33]. Earlier gradual increase in serum AGES-level have been reported with the severity of atherosclerosis in diabetic patients [34,35]. Kalusova et al determined AGES spec trofluorometrically and found AGES were about 23% higher in diabetic patients compared to healthy individuals [36]. In recent studies, AGES level has been suggested to act as a predictor of CVD mortality and diabetic nephropathy [37-41].

Recent studies have evidenced that AGEs may be a key factor in the development of metabolic memory in diabetic vascular complications, because AGEs are produced and accumulated irreversibly in the body, depending on the degree of blood sugar regulation and duration [42,43]. AGEs interact with two main types of cell surface receptors viz, scavenger receptors, which remove and degrade AGEs and the one is receptor for AGEs (RAGE), which triggers specific cellular signaling responses on AGE binding.

AGE-RAGE interaction-mediated pathways

RAGE is one of the best characterized receptor which is responsible for AGEs related diabetic vascular complications, leads to activating the stress response leading to inflammation and cellular dysfunction [44-46]. RAGE is a 45kD transmembrane receptor of immunoglobin superfamily composed of 404 amino acid. It binds to many ligands apart from AGEs, such as high mobility group proteins B1, S100 calcium binding proteins including calgranulin, amyloid β protein and amphotericin [47-51]. Apart from the full length, RAGE also available as soluble circulating isoform including sRAGE1/2/3, esRAGE (endogenous soluble RAGE) and hRAGEsec (human RAGE secreted). A number of mechanisms have been reported that lead to the production of soluble proteins, alternative splicing of the mRNA to remove the transmembrane domain and the proteolytical cleavage from the cell surface. Various studies of RAGE have shown that sRAGE can be formed by both alternative splicing and proteolytic cleavage [52-54]. AGE-RAGE interaction activates signals through TGF-β, NF-κB, MAP kinase and NADPH oxidases, which induces the expression of E-selectin, vascular adhesion molecule-1, VEGF and various pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α (Figure 3). Under hyperglycemic conditions, activation of these signaling pathways is increased in vascular smooth muscle cells, leads to vascular fibrosis, calcification inflammation, prothrombotic effects and vascular damage processes like diabetic nephropathy, neuropathy, retinopathy and cardiovascular diseases. AGE-RAGE interaction mediated OS not only responsible for vascular disorders by activating renin angiotensin system (RAS) but also aggravate organ dysfunction, because RAS activation causes NADPH oxidase-mediated OS that may enhance RAGE expression and AGEs formation. In endothelial cells, AGE-RAGE interaction exacerbates the expression of p22phox and gp91phox, which are the main components of NADPH oxidase, which promotes the production of ROS by activating the cell membrane transport of Rac family small GTPase (Rac1) to cause endothelial cell dysfunction [55-57]. Therefore, targeting the AGE-RAGE interaction has been considered as a potential therapeutic strategy to prevent or reduce vascular complications in diabetes.

Therapeutic intervention of AGEs

Inhibition of AGEs formation and attenuating the AGE-mediated effects may be considered as ideal candidates for pharmaceutical intervention in the amelioration of diabetic vascular complications. Therapies against the AGEs mediated effect can through diverse pathways, like inhibiting the production of Amadori products, decreasing AGE-RAGE interaction, detoxifying dicarbonyl intermediates and interrupting biochemical pathways that impact on
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Table 1. Drugs/inhibitors which modulate AGEs formation

<table>
<thead>
<tr>
<th>Agents</th>
<th>Therapeutic Effects</th>
<th>References</th>
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<tr>
<td>Aminoguanidine</td>
<td>Rapidly reacts with α, β dicarbonyl compounds such as methyl glyoxal, glyoxal and 3-deoxyglucosone to prevent AGEs formation.</td>
<td>[58-60]</td>
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<tr>
<td>Ramipril</td>
<td>An angiotensin converting enzyme inhibitor, inhibit the expression of inflammatory markers by inhibiting signal transduction by AGEs.</td>
<td>[61]</td>
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<td>N-acetyl cystamine</td>
<td>AGE-RAGE mediated ROS generation which induces mesangial cell hypertrophy and fibronectin synthesis has been inhibited</td>
<td>[62-66]</td>
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<tr>
<td>Telmisartan</td>
<td>An angiotensin receptor blocker, inhibit the expression of oxidative stress markers by inhibiting signal transduction by AGEs.</td>
<td>[67,68]</td>
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<tr>
<td>Pravastatin</td>
<td>Inhibit tubular damage in diabetic nephropathy in tubular cells and attenuate AGEs-induced apoptosis.</td>
<td>[69]</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Inhibit AGEs formation</td>
<td>[70]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Inhibits AGE-mediated NF-κB and AP-1 activity.</td>
<td>[71-73]</td>
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<tr>
<td>Linagliptin</td>
<td>Inhibit AGE-RAGE mediated ROS generation.</td>
<td>[74]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Reduces the risk of cardiovascular disease in diabetes by regulating the expression of growth factors and cytokines.</td>
<td>[75,76]</td>
</tr>
<tr>
<td>ALF-711</td>
<td>AGE crosslink breaker</td>
<td>[77]</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>Reduces the expression of RAGE on the myocardium and attenuate cardiac fibrosis and ventricular diastolic function</td>
<td>[78]</td>
</tr>
<tr>
<td>Exendin-4</td>
<td>Inhibit AGE-RAGE interaction mediated damage in tubular cells to attenuate the development and progression of diabetic nephropathy.</td>
<td>[79]</td>
</tr>
<tr>
<td>Alagebrum</td>
<td>AGE crosslink breaker</td>
<td>[61]</td>
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AGEs level. Several drugs are known to modulate AGEs; few of them with their therapeutic effects were shown in Table 1.

Conclusion

There is an increase in the level of AGEs formation under hyperglycemic conditions. These AGEs formation and accumulation may be one of the contributing factors in the development of diabetic vascular complications. The possibility of reducing glycation of protein or circulating AGEs or targeting AGE-RAGE mediated mechanisms may be an approachable target of delaying or preventing the onset of diabetic complications. Various compounds are under investigation for their possible therapeutic intervention. Finally, the use of AGEs as biomarkers/predictors of diabetic complications may be helpful to reduce health problems in diabetic patients.

References


