ABNORMAL GLUCOSE METABOLISM IN DIABETES MELLITUS: FORMATION OF ADVANCED GLYCATION END PRODUCTS AND THEIR CONSEQUENCES

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Abstract

Diabetes mellitus is a diverse metabolic disorder characterized by elevated blood glucose resulting from defects in insulin secretion, action, or both. In diabetes glucose is metabolized by alternate pathways leading to sorbitol and hexoseamine formation, non-enzymatic glycation and activation of protein kinase C. However, all these pathways will triggers a common pathway that is the formation of reactive oxygen species causing chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion. The aldehyde group of glucose reacts with amino groups in proteins forming Amadori products over a long period time the amadori product undergoes rearrangement to form irreversible advanced glycation end products (AGEs). Accumulation of AGEs in different cell types alters the structural and functional properties of protein. The recognition and binding of AGEs to receptors of AGE (RAGE) and the subsequent activation of transcription factors followed by downstream events contribute to the microvascular and macrovascular complications. A variety of compounds that inhibit AGEs have been under investigation. Thus all the complications associated with diabetes have a common mechanism resulting in

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diverse end points in different tissues and cells, and all of them result from elevated blood glucose concentration.

Key words: Diabetes, Hyperglycemia, Non enzymatic glycation, AGE, glucose,

Diabetes mellitus is a diverse metabolic disorder characterized by elevated blood glucose level and distinctive complications which results from defects in insulin secretion, insulin action, or both (American Diabetes Association. 2010, Fowler et al. 2008). The DCCT (Diabetes Control and Complications Trial) and the UKPDS (U.K. Prospective Diabetes Study) established that hyperglycemia, is the initiating cause of the diabetic tissue damage. Prolonged hyperglycemia leads to abnormal metabolism of glucose in diabetes which ultimately results in chronic micro and macrovascular complications.

One of the consequences of hyperglycaemia in human diabetes mellitus is increased metabolism of glucose by the sorbitol pathway. Aldose reductase present in human brain, nerves, aorta, muscle, erythrocytes and ocular lens reduces glucose to sorbitol, followed by oxidation of sorbitol to fructose by sorbitol dehydrogenase. Sorbitol is not permeable to cell membranes and tends to accumulate in the cell. Resulting in osmotic damage to microvascular cells (Michael Brownlee 2001).

When glucose is high inside a cell, the glycolytic pathway is diverted into a signaling pathway by an enzyme glutamine: fructose-6 phosphate aminotransferase (GFAT) where an intermediate fructose-6-phosphate gets converted into glucosamine-6-phosphate finally to UDP -N-acetyl glucosamine. The N-acetyl glucosamine gets put onto serine and threonine residues of transcription factors, and often results in pathologic changes in gene expression (Sayeski and Kudlow 1996) and protein function, which together contribute to the pathogenesis of diabetic complications.

Under hyperglycemic condition the glycolytic pathway of glucose takes an alternative pathway, where glyceraldehyde 3-phosphahte gets autoxidized. Autoxidation of these α-hydroxyaldehydes generates forms two potentially toxic substances hydrogen peroxide (H$_2$O$_2$) which generates reactive oxygen species that can cause mutagenic alterations in DNA and α-ketoaldehydes., which

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contribute to glycosylation of protein. Glyceraldehyde when present in excess inhibits insulin secretion (Wolff and Dean 1987).

Hyperglycemia induces non-enzymatic reactions between extracellular proteins and glucose which gradually form advanced glycation end product (AGE). Production of intracellular AGE precursors and AGEs damages target cells by, intracellular proteins modification. AGE precursors interact abnormally with other matrix components and with the receptors for matrix proteins like integrins on cells. Through the receptor for AGE (RAGE) AGE activates the pleiotropic transcription factor NF-kB, causing pathological changes in gene expression (Thornalley 1999).

Due to hyperglycemia not only glucose but also lipids and protein metabolism gets adversely affected. Decreased glycolysis due to Insulin resistance or lack of insulin effects the ATP generation, and hence hampers the biosynthesis of lipids, fatty acids and proteins. Lypolysis occurs in the adipose tissues where triacyl glycerol gets converted into more of free fatty acids increased acetyl Co A by the fatty acid degradation activates the gluconeogenic enzymes pyruvate carboxylase. They finally retard TCA cycle. The Acetly Co A results in the formation of ketone bodies which accumulate in blood and excreted in urine (Ketouria). The excess of fatty acid in the liver also gets converted into very Low Density Lipoproteins rich in tryacyl glycerol which leads to the increased TG in the blood. It also increases blood cholesterol level (Michael A.cawthorne 1987)

Hyperglycemia increases the synthesis of diacyl glycerol a lipid second messenger which is a critical activating cofactor for the isoforms of protein kinase-C, β, δ and α (Koya and King 1998). Activation of PKC has a variety of effects on gene expression. It induces expression of the permeability enhancing factor VEGF in smooth muscle cells (Williams B1997). Activation of PKC contributes to increased microvascular matrix protein accumulation by inducing expression of TGF- β, fibronectin and type IV collagen in cultured mesangial cells (Studer1993) Hyperglycaemia-induced activation of PKC has also been implicated in the overexpression of the fibrinolytic inhibitor PAI-1 (Feeneret al. 1996), the activation of NF-κB a pro inflammatory factor in cultured endothelial cells and vascular smooth muscle cells (Pieper and Riaz-ul-Haq1997,Yerneniet al.

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Abnormal Glucose Metabolism in Diabetes Mellitus: Formation of Shubha M.C. Cletus and J.M.D’Souza* (1999) and in the regulation and activation of various membrane-associated NADPH-dependent oxidases

**Oxidative Stress and its Consequences on Beta Cell Function**

Hyperglycemia induced pathological mechanisms are all linked to a common upstream event. This single unifying process is the overproduction of superoxide by the mitochondrial electron-transport chain1, (Nishikawaet al.2000). Many studies have shown that diabetes and hyperglycaemia increase oxidative stress (Giugliano et al. 1996) All the above damaging pathways are activated by the inhibition of a key glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH). The GAPDH activity gets modified or decreased by the enzyme poly (ADP-ribose) polymerase (PARP). This enzyme is involved in ROS induced DNA damage. Continuous exposure of the beta cell to increased concentrations of glucose causes defective insulin gene expression accompanied by marked decreases in insulin content and abnormal insulin secretion (Robertson et al ,1992). One potential central mechanism for glucose toxicity is the formation of excess ROS levels, which takes place within multiple mitochondrial and non-mitochondrial pathways. The islet is especially vulnerable to ROS because of its low intrinsic level of antioxidant enzymes. Chronically excessive glucose and ROS levels can cause decreased insulin gene expression via loss of the transcription factors PDX-1 and MafA and can also accelerate rates of apoptosis

Hyperglycemia induced unfavoured biochemical pathways in diabetic patients, abolishes structure and functions of important proteins and enzymes in the physiological condition. And these pathways results in generation of free radicals which end up in oxidative stress. All these mechanisms ultimately results in microvascular and macrovascular complications. Hyperglycemia and insulin resistance both seem to have important roles in the pathogenesis of macrovascular complications.

**Non-enzymatic glycation and AGE formation**

Non-enzymatic glycation occurs through the covalent binding of aldehyde or ketone groups of reducing carbohydrates (e.g., glucose, fructose, ribose) or derivatives (ascorbic acid etc) with either terminal or epsilon amino groups in

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lysines or guanidine groups in arginine residues of amino acids, peptides, and proteins to form reversible Schiff’s base intermediate which rearranges to form a relatively stable ketoamine compound called amadoric compound. (Acosta et al. 2000)

Under oxidative condition the Amadori compound further undergoes a series of dehydration and fragmentation reactions generating a variety of carbonyl compounds which are generally more reactive than the original carbohydrates and act as propagators by reaction with free amino groups. Among the most active of the enhancers are α-dicarbonyls such as methyl glyoxal, glyoxal, (Takeuchi 2001)glucosones, deoxyglucosones and dehydroascorbates. Glyoxal and methyl-glyoxal can be also formed by glucose auto-oxidation and by products from glycolipids (Thornalley 1999).

Finally these, initial and intermediate glycation products slowly undergo a complex series of further chemical rearrangements, to yield irreversible advanced glycation end products (AGE). AGEs are fluorescence molecules with wide structural and physiochemical diversity. An extensive variety of AGEs has been discovered like pentosidine, pyrraline, argpyrimidine, tetrahydropyrimidine, carboxymethyllysine (CML), a number of imidazolones and lysine-lysine crosslinks such as glyoxal-lysine dimer (GOLD), methyl-glyoxal-lysine dimer, (MOLD) and imidazolysine (Vlassara 1996).

Glycosylation of proteins and lipoproteins can interfere with their normal function by disrupting molecular conformation, alter enzymatic activity, reduce degradative capacity, and interfere with receptor recognition, thus, changes in the normal physiology of proteins that are relevant to atherogenesis, may promote atherosclerosis in diabetic individuals.

**AGE and its consequences**

AGE promotes the acceleration of atherosclerotic process through non-receptor-mediated and receptor-mediated mechanism. Accumulation of AGEs on proteins in the ECM can cause formation of cross-links, which can “trap” other local macromolecules (Schmidt 1994) AGEs can alter properties of the large matrix proteins collagen, vitronectin, and laminin (Charonis 1990), through AGE-
AGE intermolecular covalent bonds, or crosslinking (Hammes 1996, Howard 1996), AGE cross-linking on type I collagen and elastin causes an increase in the area of ECM, resulting in increased stiffness of the vasculature (Haitoglou 1992, Tanaka 1988). Formation of AGEs on laminin results in reduced binding to type IV collagen, reduced polymer elongation, and reduced binding of heparin sulfate proteoglycan (Charonis 1990). Glycation of laminin and type I and type IV collagens, key molecules in the basement membrane, causes inhibited adhesion to endothelial cells for both matrix glycoproteins (Paul 1999, Haitoglou 1992). These AGE-induced abnormalities in the function of extracellular matrix alter structure and function of intact vessels. Soluble plasma proteins, such as LDL and immunoglobulins IgG, are also entrapped and covalently cross-linked by AGEs on collagen (Vlassara 1996). The glycation process occurs both on the apoprotein B (apoB) and on phospholipid components of LDL, leading to both functional alterations in LDL and increased susceptibility to oxidative modifications (Bucala 1995). Later the uptake of glycated LDL by human monocyte-derived macrophages occurs to a greater extent than for native LDL. Diabetic LDL samples revealed significantly elevated levels of both apoB- and lipid-linked AGEs, which correlated with levels of oxidized LDL (Bucala 1994).

**RAGE**

The cellular interactions of AGEs are mediated through a specific receptor for AGE determinants on cell surfaces (Schmidt 1994). The AGE receptor (RAGE) is a member of the immunoglobulin Superfamily of receptors (Neeper 1992). It has been demonstrated in all cells involved in atherosclerotic process including monocyte-derived macrophages, endothelial cells, and smooth muscle cells (Brett et al. 1993). AGE interaction with RAGE on endothelial cells results in the induction of oxidative stress and consequently of the transcription factor NF-κB (Yan et al. 1994) and VCAM-1 (Schmidt et al. 1995). AGEs with their specific receptors increases permeability of endothelial cell monolayers (Esposito et al. 1989). This in turn increases the lipid entry into the sub endothelium. Monocyte-macrophage interaction with AGEs also results in the production of mediators such as interleukin-1, tumor necrosis factor-α, platelet-derived growth factor, and insulin growth factor-1 (Kirstein et al. 1990, 1992, Vlassara et al. 1988), which have
a pivotal role in the pathogenesis of atherosclerosis (Ross 1999). Receptor-mediated interaction of AGE-proteins with vascular wall cells facilitate the migration of inflammatory cells into the lesion with the subsequent release of growth-promoting cytokines. AGEs that bind to RAGE on the endothelial cell surface lead to a signaling cascade, stimulating NAD(P)H oxidase and increasing ROS, p21 RAS, and MAPKs. AGE may decrease NO availability by the decreased activity of NOS and by quenching NO. AGEs activate monocytes, causing increased expression of macrophage scavenger receptor (MSR) class A receptors and CD36 receptors, leading to increased OxLDL uptake and foam cell formation.

**AGE inhibition**

A variety of different compounds that inhibit AGEs have been under investigations which are protective against the development of the complication of diabetes. Aminoguanidine an hydrazine derivative reduces the formation of AGEs by binding of early glycation and glycoxidation products (Yan 1994). It has reduced the development of neuropathy, retinopathy and nephropathy. Aminoguanidine is also an NOS inhibitor. Metformin is an insulin sensitizer which has been shown to inhibit alpha-dicarbonyls formation (Beisswenger 2003). Pyridoxamine as a natural intermediate of vitamin B6 metabolism reacts with carbonyl intermediates of the Millard reaction, blocking the formation of advanced glycation and lipoxidation end products (Metz et al. 2003). Benfotiamine and thiamine are novel compounds which have the ability to reduce AGE (Babaei-Jadidi et al. 2003) accumulation in diabetes. Betformine is able to block the biochemical pathways of AGE formation .it reduces the triose phosphate accumulation which triggers the diabetic complications.

N-(2-Acetamidoethyl) hydroxinecarboximidamide hydrochloride (ALT-946) has been shown to be an effective inhibitor of AGE-induced cross-links (Forbes 2001). 4, 5-Dimethyl-3-phenacyltiolzolium chloride (ALT-711) is a compound that breaks the cross-links of AGEs. It has a thiazolium structure that is able to sever α-carbonyl compounds by breaking the carbon-carbon bonds between carbonyls (Kass 2003). Inhibitors of HMG CoA reductase are also known to inhibit the signaling pathways triggered by the AGEs by blocking the
synthesis of isoprenoid intermediates (Okamoto 2002) Another approach to inhibit AGE accumulation is through soluble RAGE (sRAGE). sRAGE competes with the cellular associated RAGE for binding AGE thereby reducing the endogenous activation (Wautier 1996).

Conclusion

Glucose is an essential metabolite for all the cells. However if it remains unutilized in cells or in blood by the glycolytic pathway, will be metabolized by alternative pathways and can produce toxic metabolites like glyoxal and methyglyoxal. These metabolites along with glucose can glycate proteins non enzymatically. Non enzymatic glycation and its subsequent action through specific receptors is the underlying mechanism of diabetes complications. Hence reduction of high blood glucose and inhibition of AGE formation are the strategies used for reducing diabetes complications.

References

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