12. GLYCATION AND AGE IN DIABETES AND COMPLICATIONS

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Epidemiological studies have confirmed that hyperglycaemia is the most important factor in the onset and progress of diabetes complications, both in insulin-dependent and non-insulin-dependent diabetes mellitus. Mechanisms connecting hyperglycaemia with complications of long-term diabetes were investigated. Among others, a large number of useful proofs indicated the involvement of non-enzymatic glycation processes.

Nonenzymatic glycation is a process by which glucose is chemically bound to aminogroups of proteins, but without the help of enzymes. It is a classical covalent reaction in which, by means of N-glycoside bonding, sugar-protein complex is formed through a series of chemical reactions described by a chemist Maillard. Maillard reactions are complex and multi-layer, and can be analyzed in three degrees. First sugar-protein complex is formed (Amadori rearrangement). It is an early product of nonenzymatic glycation, an intermediary which is a precursor of all later compounds. The second degree includes the formation of numerous intermediary products among which some are very reactive and further continue with glycation reactions. The third, final phase, consists of complex product polimerization reaction which occurred in the second stage in the process of which heterogeneous structures called advanced glycation endproducts (AGE) are formed. It was believed that the primary role in Maillard reactions was exclusively played by high glucose concentration. However, recent data show that, in spite of the fact that sugars are the main precursors of AGE compounds, numerous intermediary metabolites, i.e. α-oxoaldehydes also creatively participate in nonenzymatic glycation reactions. Such intermediary products are generated during glycolysis (methylglyoxal) or in the polyolic pathway, and they can also be formed by autooxidation of carbohydrates (glyoxal). Alpha-oxoaldehydes modify AGEs surprisingly fast, in contrast to classical Maillard reactions which are very slow. (Figure 1).
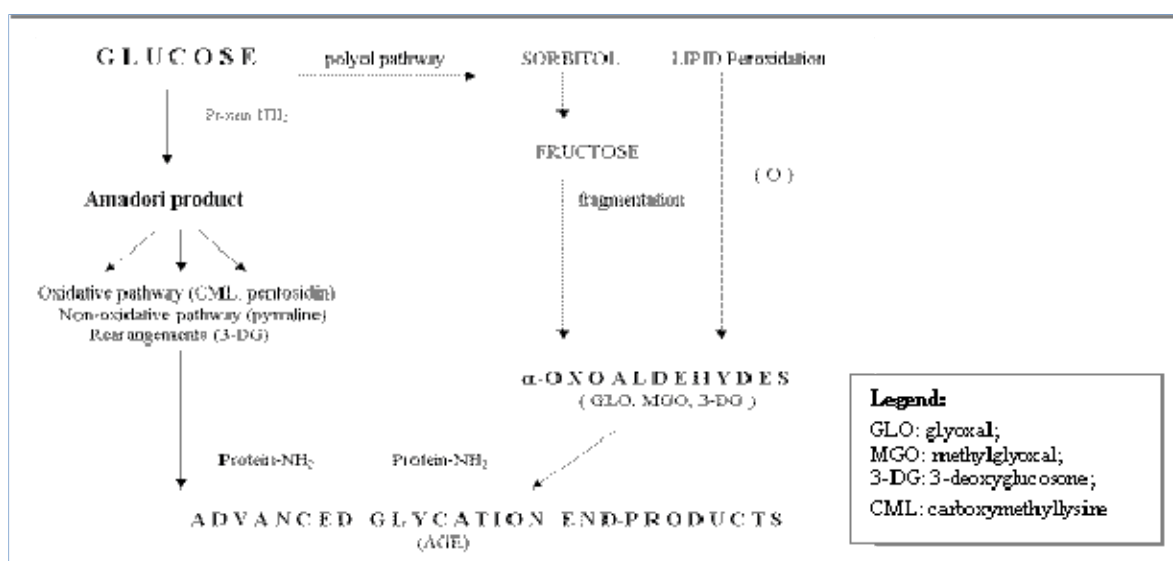

Figure 1. Schematic representation of potential pathway leading to AGE formation: a) AGE

arise from decomposition of Amadori product, b) fragmentation products of polyol pathway, c) as glycoxidative products which all react with amino groups of protein

Glycation has both physiological and pathophysiological significance. In physiological conditions glycation can be detected in the ageing process, and the reactions are significantly faster and more intensive with frequently increased glucose concentrations. In diabetology the importance of these processes is manifest in two essential issues: 1) effect of protein glycation on the change of their structure and function and 2) use of glycated proteins level as a parameter of integrated glycaemia. A classical example of nonenzymatic glycation is the formation of glycated haemoglobin, or, more precisely, HbA1c, its normal value being up to 5%. The degree of nonenzymatic glycation being directly associated with blood glucose level, the percentage of HbA1c in diabetes can be very increased. HbA1c had been the first studied glycated protein, but it was soon discovered that other, various structural and regulatory proteins, are also subject to nonenzymatic glycation forming glycation endproducts.

1.1. Advanced Glycation Endproducts (AGE)

During glycation process first early glycation products are formed, which later rearrange into final AGE structures by a series of very complex chemical reactions. Protein modification with AGE is irreversible, there being no enzymes in the organism able to hydrolyze AGE compounds, which structures consequently accumulate during the life span of a protein on which they had were formed. Examples include all types of collagen, albumin, basic myelin protein, eye lens proteins, lipoproteins and nucleic acid. Today it is well documented that AGE change the function of many proteins, thus contributing to various late complications of diabetes mellitus. The major biological effect of excessive glycation include: inhibition of regulatory molecule binding, crosslinking of glycated proteins, trapping of soluble proteins by glycated extracellular matrix, decreased susceptibility to proteolysis, inactivation of enzymes, abnormalities of nucleic acid function, and increased immunogenicity in relation to immune complexes formation.

It has been well documented that AGEs progressively accumulate on the tissues and organs which develop chronic complications of diabetes mellitus like retinopathy, nephropathy, neuropathy and progressive atherosclerosis. Immunohistochemical methods have proven the presence of different AGE compounds in glomeruli and tubuli cells in both experimental and human diabetic nephropathy. Some papers show that AGE compounds are directly linked with the development of proliferative retinopathy. AGE role in atherosclerosis is also significant. For instance, reticulated and irreversible LDL from the circulation binds to AGE-modified collagen of blood vessel walls. In the majority of blood vessels such reticular binding delays normal outflow of LDL particles which penetrated vessel wall and thus enhances cholesterol depositing in the intima. Such AGE reticulation increases lipoprotein deposition regardless of plasma LDL level. This is followed by an accelerated development of atherosclerosis. The presence of many AGE compounds in atheroma has been proven by immunohistochemical methods and chemical analysis techniques.

1.2. AGE receptors

The level of AGE proteins reflects kinetic balance of two opposite processes: the rate of AGE

compound formation and the rate of their degradation by means of receptors. AGE receptors participate in the elimination and change of aged, reticular and denatured molecules of extracellular matrix as well as all other AGE molecules. However, in diabetes mellitus AGE protein accumulation may exceed the ability of their elimination due to chronic hyperglycaemia and excessive glycation process.

AGE receptors were first detected on macrophage cells. AGE protein binding to macrophage cell receptors causes a cascade of events in the homeostasis of blood vessel walls and their milieu by mediation of cytokines and tissue growth factors. At least four different AGE receptors have been described, among which two belong to the group of receptor scavengers. One of them is very similar, if not identical, to the receptor which internalizes altered LDL particles. Receptors on endothelium cells differ. These are sites on cell membranes which bind AGE-ligands. The abbreviation used to denote them in literature is RAGE, they belong to immunoglobulin receptor family and are prevalent in tissues. Binding of AGE compounds to RAGE leads to cellular stress. Can variations in AGE level explain the differences in susceptibility to develop complications? This is not known, but theoretical reflections indicate that gene diversity in AGE receptors could offer an explanation.

1.3.   Glycotoxins (AGE-peptides)

Tissue macrophages by AGE receptors represent the major pathway of AGE-altered tissue and cell degradation. In this process AGE-peptides are released as degradation products, partly occurring through proteolysis of matrix components, commonly called glycotoxins. Glycotoxins (AGE-peptides) entering blood circulation are very reactive. In case they have not been eliminated through kidneys, recirculating AGE-peptides can generate new AGE-products reacting with other plasma or tissue components. At this stage glycation becomes an autonomic process which significantly accelerates the progress of complication.

The level of serum glycotoxin correlates with the kidney function. In healthy persons renal clearance is about 0.72 ml/min. Diabetic patients with normal renal clearance are capable of eliminating glycotoxins equally fast. However, renal function impairments result in increased level of glycotoxins of even up to 800%, as in diabetic patients with end-stage renal insufficiency. The fact that not even haemodialysis eliminates glycotoxins is particularly disappointing.

1.4.   Pharmacologic inhibition of AGE

It has been attempted with greater or lesser efficacity to pharmacologically influence the process of nonenzymatic glycation and AGE products formation. There are two approaches available:

- Inhibition of the rearrangement from early to advanced glycation endproducts by means of hydrasine: aminoguanidine hydrochloride or analogue
- Breaking of already existing AGE products with substituted thiazolium salts

Pharmacologic activity of aminoguanidine may render impossible or retard some of microvascular complications in animal model. Although the mechanism of aminoguanidine ac-

tion has not been completely understood, it seems it inhibits some stages in a series of chemical reactions leading to glycation end-product formation. In spite of the first encouraging results, clinical trials of aminoguanidine in patients with type 2 diabetes mellitus have been suspended due to adverse effects.

Intensive investigations of new compounds which would break already existing AGE products have recently been started.

1.5. Immunochemical detection of AGEs

Development of high-titer polyclonal and monoclonal anti-AGE antibodies have been applied successfully to enzyme-linked immunosorbent assays (ELISA) and immune-histochemical studies.

Competitive ELISA method is most frequently used in the measurement of AGEs concentration in body fluids. The reaction principle is as follows: the immunoplate wells are over-coated by AGE-antigen, and serum containing an unknown quantity of AGE-antigen is incubated together with anti-AGE antiserum. At the end of the incubation period the wells are treated with secondary antibody enzyme labelled. Then a substrate is added, which gives the absorbance difference to be measured. Competitive immunoreactivity of the samples is read from calibration curve.

Immunofluorescence is a method used for detecting AGE immunoreactivity localization on tissues. It is a simple and sensitive technique based on AGE antibodies binding with a fluorescent matter. Thus marked antibody reacts with antigenic determinants. After illumination by a light of an appropriate wavelength (UV), sites containing antigen can be determined according to the characteristic colour of the light. This method enables identification and precise location of antigens on tissue scars regardless whether they are located in a cell, on cell membrane or in free cells.

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