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Mini-review

OXIDATIVE STRESS AND ITS COMPLICATIONS IN DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus is a widespread disease with a great social impact. The quality of life and the life span of the patients with the disease depend on its complications. Hence, there is an increased interest in dealing with this disorder. Convincing evidences of the role of free radicals and oxidative stress in the pathogenesis and complications of diabetes mellitus have been established over time. It was shown that the patients were put under increasing oxidative stress in conjunction with different biochemical changes that lead to endothelial dysfunction. One of the most important is the inactivation of nitric oxide, which is key to maintaining vascular tonus. These findings underscore their importance as prognostic markers in this disease. Accordingly, a speculation into the possible use of antioxidants as adjuvant to conventional therapy of diabetes mellitus is developed here.

Key Words: Oxidative Stress, Diabetes Mellitus, Endothelial dysfunction

It has been established that oxidative stress lies at the root of a number of pathological processes and diseases such as cancers, atherosclerosis, rheumatic arthritis, haematological and neurodegenerative disorders are not exempt (1, 2), with more making the list among which is diabetes mellitus

Diabetes Mellitus is a widespread disease and affects all nationalities and ages. The number of patients in 2003 has reached an epidemic proportion totalling a whopping 194 million with patients of 20 to 79 years of age affected (5.1 % of the population in this age group). A rise to 50% more is expected in 2010, mainly from new cases in Africa, Asia and South America. A projection of this figure shows that in 2025 diabetes patients will be 333 million or 6.3% of the total population on Earth. To this alarming trend must be added the fact that chronic complications of diabetes – micro- and macroangiopathy, are the causes of 4 times higher mortality in patients with diabetes mellitus in comparison with healthy individuals. Therefore the great social importance of the disease is determined not only by the millions of patients, but also by the high mortality. This explains the intensive studies done on this disease.

Diabetes Mellitus is a heterogeneous disease characterized by broken synthesis and/or secretion of insulin, as well as by resistance of the peripheral tissues to the hormone activity. The pathogenesis of the disease is of multifactorial nature and the functional trouble at the level of β-cells is manifest from its earliest stages of development.

To understand the essence of aetio-pathogenic mechanisms, which are at the root of diabetic complications development is an essential challenge to modern medical science and practice. Nowadays diabetic micro- and macroangiopathy are considered to be poly-aetiological multifactorial diseases where persistent hyperglycaemia plays the leading part (3-11). On the other hand it contributes to the origin of oxidative stress. Along with the others, endogenous and exogenous factors takes a considerable place in diabetes pathogenesis. Hence, the patients are exposed to continuously increasing oxidative stress caused by the prolonged hyperglycaemia and conditioned by different pathophysiological processes (**Figure.1**).

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Hyperglycaemia

From **Figure 1** it is evident that in state of chronic hyperglycaemia non-enzyme glycosylising of proteins sets in. Their formation mechanism is presented on **Figure 2**.

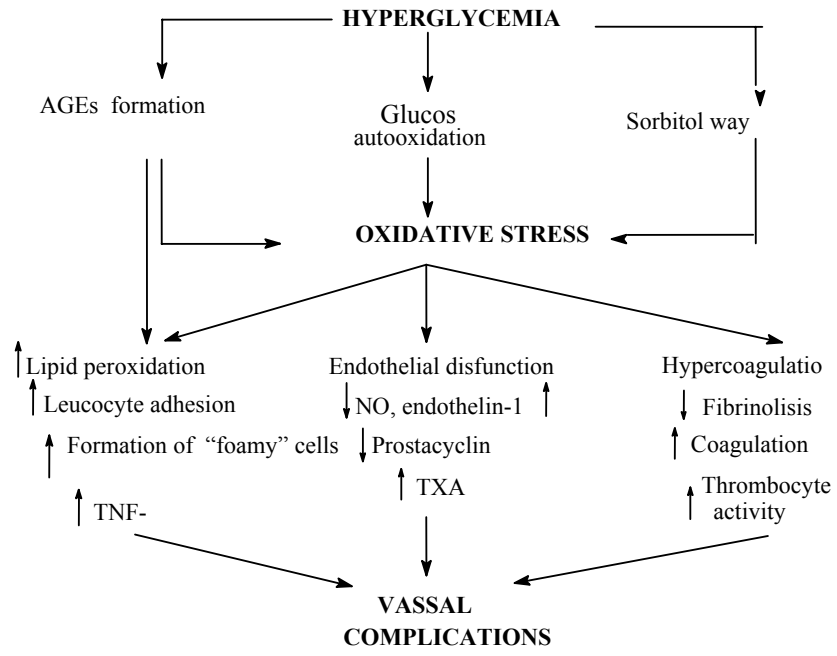

Figure 1. *Schematic representation: hyperglycaemia and biochemical processes lead to oxidative stress and vascular effects*

AGEs – Advanced Glycosylation End products; TXA_2 – Thromboxane A_2; TNF-α - Tumour Necrotising Factor – α; NO – Nitric Oxide

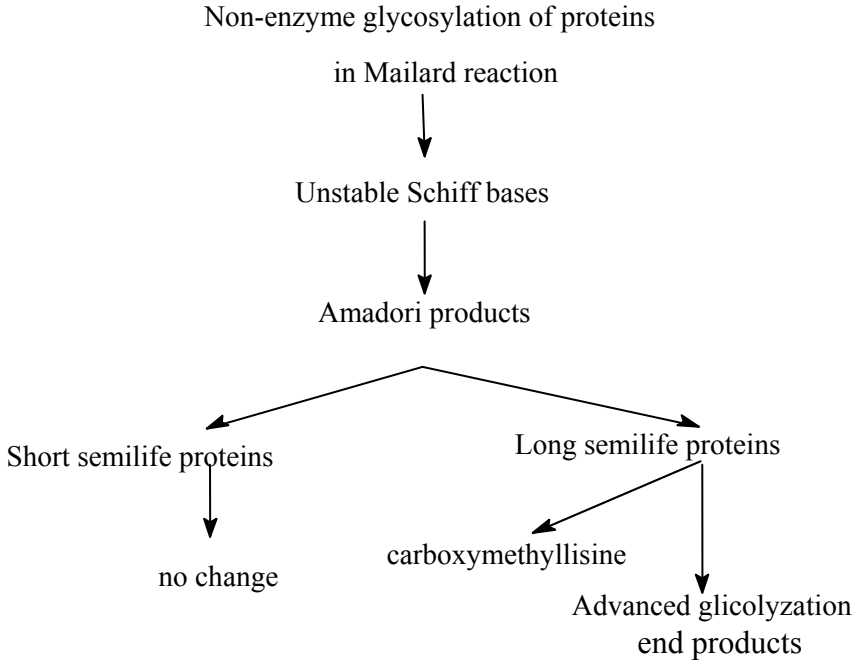

Figure 2. *Influence of hyperglycaemia over plasma proteins*

The combining of glucose with plasma proteins is completed during the Mailard reaction. In glycosylation it reacts by lateral groups (lying under and over the peptide connection plane), to amino acids` remains, as well as by amino acids` remains in C- and N-end of the protein molecule until formation of Schiff bases. They are unstable and a few

days later they are transformed into stable ketoamine called Amadori product.

The brief period of semilife of most cellular and plasma proteins does not provide the possibility for Amadori products to transform further. By contrast, proteins with long semilife, a part of Amadori products, undergo partial oxidative degradation and carboxymethyllysine. The rest are included into series of intermediate and subsequent Mailard reactions until formation of pigmented, fluorescent and containing cross-"links "advanced Mailard products" called also advanced glycosylation end products "(AGEs), for example pentosidine and others. They can be determined as a class of heterogeneous compounds of monosaccharides and proteins, obtained by consecutive reactions of dehydration, condensation, fragmentation, oxidation and cyclisation (5, 12). This produces a combination of glucose with plasma proteins free radicals. Together with the transformed proteins they contribute to the intensification of oxidative stress and vessel injury.

At the same time with the proteins, glycosylising glucose autooxidation occurs. It is catalysed by ions of metals with variable valencies. Reactive metabolites of oxygen and ketoaldehydes are formed. The latter can interact with still unchanged proteins and with Amadori products. In this way the consequences of glucose autooxidation are deepened and widened.

Glucose autooxidation products can attach to specific receptors from the surface of endothelial cells and change their properties. For instance, their combining with nuclear factor kappa-B (NF – kB) stimulates the synthesis of atherogenic circulating adhesive molecules and inflammatory cytokines (e.g., Tumor necrotizing factor α, TNF- α) (5, 13-16). For their part they regulate cellular growth, proliferation and migration and they have a very important role for early formation of atherosclerotic lesions.

Hyperglycaemia in non-insulin dependent cells activate aldosereductases enzyme, which leads to intensive metabolising of glucose into sorbitol and fructose (see **Fig.1**). It reduces the proportion of NADFN/NADF+ and increases the proportion of NADN/NAD+. The trouble in the oxidation of NADN in the respiratory chain is indicated as "hyperglycaemic pseudo hypoxia" and leads to increasing the quantity of reactive oxygen species (ROS) in the cells.

The increased formation of ROS is reinforced also by the real hypoxia related to vessel complications in diabetes mellitus, which has already occurred. Reinforced formation of ROS in the conditions of pseudo- and real hypoxia could be connected with activating of protein kinase C – a key enzyme in transmission of signals (the inclusion of sorbitol way increases de novo diacylglycerols synthesis which is a cause for activating protein kinase C in endothelial cells). Protein kinase C phosphorylates and thus activates phospholipase A. It releases arachidonic acid from membrane phospholipids as at the same time the quantity of superoxide radicals and prostanoides is increased (9).

Clinical troubles accompanying protein kinase C activation are expressed by increasing endothelium permeability, vasodilator troubles, disturbed vascular stream and intensified synthesis of basal membrane proteins (10).

This presentation discusses the three basic consequences of hyperglycaemia – formation of "final products of advanced glycosylation", glucose autooxidation and sorbitol increase. All this contributes to the rise and reinforcement of oxidative stress in diabetes mellitus.

The results of clinical studies published recently (DCCT, UKPDS) confirm plainly the stated hypotheses for "the toxicity" of the excessively high levels of plasma glucose – long-lasting hyperglycaemia or postprandial glucose variations. These two factors, in conjunction with the oxidative stress caused by them, are at the root of the greater part of vascular complications in diabetes mellitus (2). These ones determine the high cardiovascular risk, kidney insufficiency, blindness, amputations and appear to be the principal cause for the high mortality in patients with this disease.

In relation to vascular complications the role of oxidative stress in their pathogenesis is of interest. Before examining this problem, concise data for the basic functions of endothelium I norm will be given.

Basic functions of endothelium

It separates not only the vessel wall from the blood stream, but possesses its own metabolism also. Endothelial cells synthesize factors, which can stimulate them reversibly and maintain them activated for a long time.

One of the most important endothelium functions is providing suitable vessel tone. It is achieved by balance of synthesis and release of vasodilators and constrictors. The basic factor stimulating the elimination of

vasodilators such as nitric oxide, prostacyclin (PGI 2) and hyperpolarizing factor of endothelial origin (EDNF), is the endothelium irritation by the blood stream.

The well-known vasoconstrictor is endothelin - 1. Its actions can be found also among the derivatives of arachidonic acid prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2), obtained by the action of cyclooxygenase. Superoxide anion and angiotensin II constrict blood vessels also. In the endothelium the oxidation of plasma lipids the synthesis of angiotensin II, as well as the breaking up of catecholamines and kinins circulating in the blood, are accomplished.

Endothelium regulates the proliferation of flat-muscular cells of the vessels and the adhesion of leucocytes (granulocytes, monocytes) and thrombocytes. Besides, it modulates vessel permeability and influences inflammatory processes. Endothelium possesses antithrombotic and fibrinolytic properties (17).

Endothelial dysfunction

In some pathological conditions and diseases, for example atherosclerosis and particularly diabetes mellitus, the above mentioned properties of endothelium change (**figure 3**) (6).

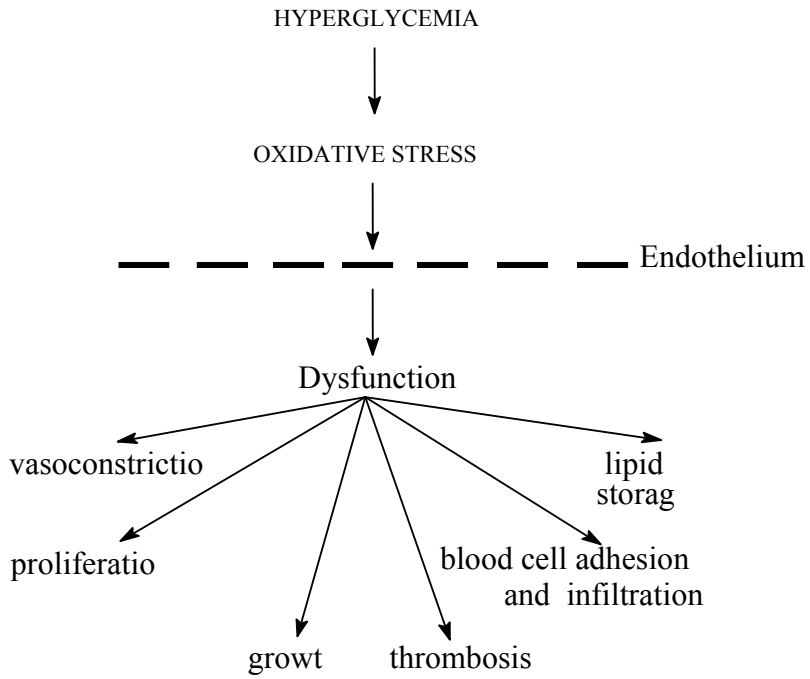

Figure 3. *Endothelial dysfunction due to hyperglycaemia*

By the concept "endothelial dysfunction" is meant the disturbed balance between vasodilators and vasoconstrictors, thrombotic and fibrinolytic mediators, as well as growth retaining and stimulating substances.

The increased vascular tone is responsible for the vessel permeability increase. The loss of antithrombotic and fibrinolytic properties of endothelial cells enables local thrombosis. The upset balance between prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) leads to accelerated aggregation of thrombocytes (17).

The upset functioning of endothelium is manifest by increased expression of adhesion molecules (intracellular adhesive molecules – ICAM-1, VCAM-1, E-selectin) upon cells surface. It intensifies the interaction between them and blood-forming elements- adhesion and extravasal migration of leucocytes occur. The activation of endothelial cells increases the proteins secretion of intercellular matrix. These endothelial disturbances can very depending on the type of lesion and its localization.

Mechanisms by which hyperglycaemia causes endothelial dysfunction were unknown many years. Contemporary clinical observations show that the earliest vessel changes include increased vasodilatation and corresponding increase of blood stream. Consentino et al (6) established that prolonged action of high glucose concentrations intensifies synthesis of nitric oxide and superoxide anions in human aorta. These two free radicals interact and form peroxynitrate. Release of arachidonic acid from membrane phospholipids is stimulated – synthesis of

vasoconstrictors prostaglandins – PGF_{2a} and TXA_2 is reinforced. In the end endothelial dysfunction occurs.

These confirm a large extent previous hypotheses about inactivation of nitric oxide by the present in excess in hyperglycemia superoxide anions (7).

Hence, hyperglycemia leads, on the one hand, to increased inactivation of nitric oxide, and on the other hand – to obtaining of powerful vasoconstrictors. Besides intensified formation of prostaglandin H_2 (PGH_2) TXA_2 stimulates thrombocytes aggregation. Thus, at supraphysiological levels of plasma glucose, endothelium loses its properties of anticoagulant. It was shown that in hyperglycaemia the activity of antithrombin 3, the syntheses of heparin sulphate and release of tissue plasminogenic activator decrease. The followed activation of coagulation cascade increases the quantity of endothelial activators – thrombin and fibrin (18).

Oxidative stress caused by hyperglycaemia

It acts upon endothelium expression of growth factors from its adjacent cells. The transforming growth factor β (TGF- β) secreted by mesangealial cells and growth factor of vessel endothelium (VEGF) stimulate its cells proliferation and suppress their ability for regenerating after damage.

Hyperglycaemia effects are reinforced addition ally by reactive oxygen types formed in the course of non-enzyme glycosylising of proteins and glucose autooxidation occurring simultaneously (5). Obtained "final products of advanced glycosylation" can activate endothelial cells attaching to specific receptors on their surface. In these conditions endothelial permeability increases, accumulation of adhesive molecules deepens and synthesis of interleukins (IL-1, IL-6) and tumour necrotizing factor α (TNF- α) is reinforced (19).

Previously it was considered that osmotic pressure of sorbitol was the cause for vessel damage in diabetes. At present it is known that nitric oxide in norm activates guanylcyclase, which transforms GTF into GMF which for its part acts as a second mediator and activates protein kinase C. It phosphorylates and activates phospholipase A_2. Secretion of arachidonic acid from membrane phospholipids and its metabolism to vasoactive prostanoids is intensified. In conditions of hyperglycaemia however, because of the intensified endothelial transformation of glucose into sorbitol and fructose, a decrease of NADFN quantity is observed and the obtaining of nitric oxide from arginine is compromised. The examined clinical disturbances are expressed by increase of endothelium permeability, vasodilators troubles, damaged vessel stream and reinforced proteins synthesis of basal membrane (9).

In conclusion it must be underlined that the above exposed biochemical troubles can be examined as a connecting link between hyperglycaemia, oxidative stress and diabetic angiopathy.

Microangiopathy

It is a specific complication and is the most clearly expressed and is characteristic for patients with diabetes mellitus type 1 but affects also the other forms of the disease. Important clinical problems are microvascular lesions in retinal, neuronal and kidney vessels leading to retinopathy, neuropathy and nephropathy (20, 21).

Retinopathy

Retina is characterized by high contents of lipids and increased consumption of oxygen. This makes it particularly susceptible to the influence of reactive oxygen types formed in conditions of hyperglycaemia. It is established that markers of oxidative stress (malondialdehyde, sulfhydril protein) in sub retinal liquid in patients with proliferative retinopathy are changed significantly in comparison with healthy controls and patients without retinopathy. In addition to this, activation of protein kinase C intensifies synthesis of vasoactive prostanoids and leads to changes in retinal blood stream.

Nephropathy

It is proved that "final products of advanced glycosylation" take principal position in pathogenesis of diabetic nephropathy. Observed initially kidney hyper perfusion and hyper filtration are connected with activation of phospholipase A_2 by protein kinase C. Advanced diabetes however, leads to kidney vasoconstriction and increased deposition of extracellular matrix, contributing to systemic hypertension and nephrosclerosis.

Neuropathy

The diminished neural perfusion and capillary occlusion due probably to thrombosis, oedema of endothelial cells or proliferation connect neuropathy with micro vascular diseases. It is considered that ROS damage neural fibres.

Macroangiopathy

It is an atherosclerotic process, which because of metabolic troubles in diabetes, sets in earlier age. It occurs more often and shows a faster evolution and heaviness in comparison with the non-diabetic. Macroangiopathy is more characteristic of diabetes mellitus type 2. Atherosclerotic lesions are formed mainly in the big and medium size arteries and can cause ischemic changes in the heart, the brain or the extremities. This can lead to myocardial infarction, brain stroke or necrosis of the foot.

Unlocking factor for atherogenesis is lesion of endothelial cells in persistent hyperglycaemia and oxidative stress. Nowadays the concept that reactive oxygen types and lipid peroxidation products are leading factors in endothelium lesion is being held. Endothelial dysfunction precedes the evolution of macrovascular disease clinically manifested. In studies of patients with diabetes mellitus type 2 in early stage a decrease of endothelium-dependent vasodilation is established.

New essential element of atherogenesis is a disturbance in the relaxation of arterioles, which can be met in anatomically unchanged vessels also. In this case the diminished vasodilator response is expression of occurred endothelial dysfunction. As in endothelial cells nitric oxide (vasodilator) is synthesized, they have great importance for relaxation of arterioles. In conditioned by hyperglycemia endothelial dysfunction nitric oxide is eliminated by increased quantity of superoxide radical. Except this it is accepted that non-enzyme glycosylation products influence the synthesis and inactivation of this vasodilator (7).

In addition to endothelial dysfunction it is shown that *upset lipid metabolism* takes central position in atherosclerosis evolution (19, 22-27). Patients with diabetes type 2 have usually increased levels of triglycerides (especially of VLDL) and diminished quantity of HDL-cholesterol – two risk factors for cardio-vascular diseases. Concentrations of LDL-cholesterol do not differ significantly from these in persons without diabetes, but in contrast to them LDL-particles are smaller, thicker and oxygenated. They are more strongly atherogenetic than normal, bigger and more motile LDL-particles.

Excess of reactive oxygen types contributes to intensive peroxidation of plasma lipids and so plays decisive role in formation of atheromatous plaque. Initially LDL accumulates in extracellular space of small arteries walls and is oxygenated. There are formed the so-called minimum modified LDL (28). This fraction causes migration of monocytes and macrophages and subsequently – peroxidation of lipoproteins. Peroxidated lipoproteins are recognized by scavenger-receptors of macrophages which phagocytise them and are transformed into "foamy" cells (22). In contrast to the case of non-oxidised LDL, phagocytosis of oxidised lipoproteins does not lead to mass inclusion of cholesterol (from oxidised LDL) in macrophages. Except this oxidised LDL influence directly monocytes and cause their attachment to the endothelium (19, 25).

Oxidation of LDL diminishes their ability to fluctuate between the lumen and blood vessels wall. As the oxidised LDL enable extracellular release of lipids and lysosome enzymes and reinforce atherogenesis, they are cytotoxic for endothelial cells (26, 28). That is why oxidative modification of lipoproteins appears to play a key role in formation of "foamy" cells, which is an irreversible stage in the atherogenesis chain (23). The above described relation between lipid oxidation and atherogenesis is an important fact and possibility for favourable therapeutic influence on evolution of ischemic disease of the heart and other manifestations of macroangiopathy.

CONCLUSION

It is obvious from the presented data that a relation exists between hyperglycaemia, oxidative stress, cellular and endothelial dysfunction. Increased formation of superoxide radicals and inactivation of nitric oxide in condition of hyperglycaemia is one of the probable causes for evolution of vascular complications in diabetes mellitus. It gives reason to look for dependence between the oxidative stress degree, evolution of the disease and its chronic complications. This dependence could be used as prognostic marker of course evaluation of diabetes. Not the least is the possibility of reducing oxidative stress by means of different antioxidants as a supplement to the conventional therapy of diabetes mellitus.

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