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Review article
Pregledni članak

THE EFFECT OF HYPERGLYCEMIA AND OXIDATIVE STRESS ON THE DEVELOPMENT AND PROGRESS OF VASCULAR COMPLICATIONS IN TYPE 2 DIABETES

UTICAJ HIPERGLIKEMIJE I OKSIDATIVNOG STRESA NA NASTANAK I RAZVOJ VASKULARNIH KOMPLIKACIJA U DIJABETESU TIPA 2

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Summary: Oxidative stress is the result of increased production of free radicals, which impair the cell function and cause many pathological conditions and diseases. The development of diabetes, its course and complications are closely associated with an imbalance in pro-antioxidative cell state and change of redox potential. Prolonged exposure to hyperglycemia is currently considered the major factor of the pathogenesis of atherosclerosis in diabetes. Atherosclerosis is the cause of about 80% of mortality in diabetics, and over 75% of all hospitalized diabetic patients have associated cardiovascular complications. Hyperglycemia induces different vascular tissue damage at the cellular level, which potentially accelerates the atherosclerotic processes. The most significant mechanisms responsible for acceleration of atherosclerotic processes in diabetic patients are: a) non-enzymatic protein and lipid glycosylation which interferes with normal function, in the way that it deranges molecular conformation, impairs enzymatic function, reduces the capacity of breakdown and interferes with recognition of protein structures by receptors; b) interaction of glycosylated proteins with their receptors resulting in induction of oxidative stress and proinflammatory reactions; c) polyol pathway; d) hexosamine pathway and e) activation of protein kinase C and impaired growth factor expression.

Keywords: oxidative stress, hyperglycemia, free radicals, diabetes mellitus, vascular complications

Kratak sadržaj: Oksidativni stres nastaje kao posledica prekomerne produkcije slobodnih radikala, koji oštećuju ćelijsku funkciju i dovode do nastanka mnogih patoloških stanja i bolesti. Nastanak dijabetesa, tok i razvoj komplikacija, usko su povezani sa disbalansom pro-antioksidativnog stanja ćelije i promenom redoks potencijala. Prolongirana izloženost hiperglikemiji danas se smatra glavnim faktorom u patogenezi ateroskleroze u dijabetesu. Ateroskleroza je uzrok oko 80% smrtnosti u dijabetičara, a više od 75% hospitalizovanih dijabetičara imaju i prateće kardiovaskularne komplikacije. Hiperglikemija indukuje veliki broj oštećenja vaskularnog tkiva na ćelijskom nivou koji potencijalno ubrzavaju aterosklerotske procese. Istraživanja na ljudima i životinjama rasvetlila su nekoliko mehanizama koja obuhvataju većinu patoloških oštećenja u vaskulaturi: a) neenzimska glikozilacija proteina i lipida koja interferira sa normalnom funkcijom, tako što remeti molekularnu konformaciju, oštećuje enzimsku funkciju, smanjuje kapacitet razgradnje i interferira sa prepoznavanjem proteinskih struktura od strane receptora; b) interakcija glikoziliranih proteina sa njihovim receptorima rezultuje indukcijom oksidativnog stresa i proinflamatornim reakcijama; c) put poliola; d) put heksozamina; i e) aktivacija protein kinaze C i poremećaj ekspresije faktora rasta.

Ključne reči: oksidativni stres, hiperglikemija, slobodni radikali, dijabetes melitus, vaskularne komplikacije

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Introduction

Oxidative stress is the result of increased production of free radicals, which impair the cell function and cause many pathological conditions and diseases. The development of diabetes, its course and complications are closely associated with an imbalance in pro-antioxidative cell state and change of

redox potential. Oxidative stress represents a biochemical mechanism which, due to impaired glucose metabolism and dysregulation of cellular signaling, gives rise to following complications: micro- and macroangiopathy underlying the diabetes nephropathy, neuropathy, retinopathy and cardiovascular diseases. Oxidative stress in diabetes is the consequence of both increased production of free radicals and reduced capacity of antioxidative defense. It has been verified that increased intra- and extracellular glucose concentration leads to oxidative stress (1, 2). Several mechanisms, with the underlying hyperglycemia, are responsible for the development of oxidative stress (3), as follows:

a) autooxidation of glucose, followed by production of free radicals ($O_2^{-\cdot}$, H_2O_2, OH);

b) lower concentration of reduced glutathione because of impaired activity of glutathione reductase due to a deficit of NADPH which is intensively used up in the glucose polyol pathway;

c) low concentration of thiol compounds in cell, especially glutathione, has a positive effect on the activity of transcriptional factor NF-κB responsible for the stimulation of gene expression whose products maintain the pro-oxidative state in the cell;

d) non-enzymatic glycation of proteins (particularly extra- and intracellular SOD) and other antioxidation enzymes, gives rise to reduction of their catalytic activity;

e) hemoglobin glycation brings about the production of HbA1c which releases oxygen less easily, causing hypoxia in peripheral tissues, which triggers both the activation of xanthine oxidase (XO) and other mechanisms initiating the production of free radicals under conditions of deficient oxygen;

f) oxidative modification of structural and functional metalloproteins (transferrin, ceruloplasmin Cu, Zn-SOD) causes their accelerated degradation, and released metals, being involved in Fenton's reaction, further stimulate the production of free radicals (2–4).

In the event of prolonged hyperglycemia, tissues that take over the glucose independently from the existing insulin (retina, lens, nerves and endothelium) sustain extreme damage.

Prolonged exposure to hyperglycemia is currently considered the major factor of pathogenesis of atherosclerosis in diabetes. Atherosclerosis is the cause of about 80% of mortality in diabetics, and over 75% of all hospitalized diabetic patients have associated cardiovascular complications (7–10). Hyperglycemia induces different vascular tissue damage at the cellular level, which potentially accelerates the atherosclerotic processes. Studies on humans and animals have clarified several mechanisms which include the majority of pathological damages occurring in vasculature (3) (*Figure 1*):

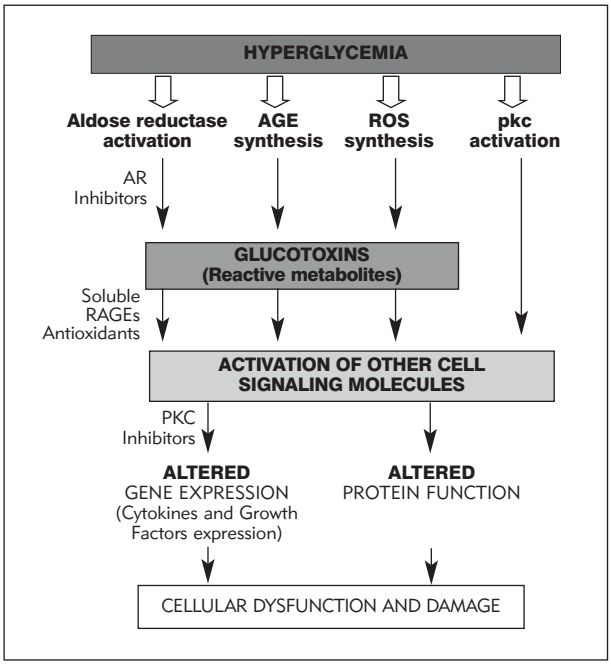

Figure 1 Mechanisms of pathological damages in the vascular tissue produced by hyperglycemia.

a) non-enzymatic protein and lipid glycosylation which interferes with normal function, in the way that it deranges molecular conformation, impairs enzymatic function, reduces the capacity of breakdown and interferes with recognition of protein structures by receptors;

b) interaction of glycosylated proteins with their receptors resulting in induction of oxidative stress and proinflammatory reactions;

c) polyol pathway

d) hexosamine pathway

e) activation of protein kinase C and impaired growth factor expression (11).

Non-enzymatic glycosylation

One of the most significant mechanisms, responsible for the acceleration of atherosclerotic processes in diabetic patients, is the non-enzymatic reaction between glucose, proteins and lipids in the arterial wall, known as »Maillard's« or »Browning« reaction (12). Non-enzymatic glycation of proteins by glucose a complex cascade reaction producing heterogenous substances which are called »Advanced Glycosylated End Products« or AGE products (13). Non-enzymatic protein glycation, first described by Louis Camille Maillard (1912), is included in the pathogenesis of diabetes, renal failure and aging.

In the first phase of glycation, glucose forms chemically reversible »early« products of glycosylation with reactive amino groups of circulating proteins or blood vessel wall proteins named »Schiff's base«,

which, by internal rearrangement, yield more stable glycosylation products of Amadori type. The balance between Schiff's base and Amadori product (the most well-known HbA1c) is achieved in a few hours and lasts several weeks (14). The first stable product of this reaction is fructolysine (FL) whose concentration is directly proportional to ambient glucose concentration and correlates with glycohemoglobin. During a series of chain reactions between proteins and »Maillard's products« (by dehydration and condensation reactions), in reaction with free radicals, keto amine derivative (FL) or its dissociation products such as N-carboxy methyl lysine (CML), pentozidine and pyraline may be transformed into other polymers which are called »Advanced Glycosylated End Products« or AGE products (15). Elevated serum CML level was noted in children and teenagers with DM1. It is interesting that such an increase preceded micro- and macrovascular complications (16). Among these intermediary substances resulting from »cross-linking« bonds between glucose and proteins, methyl-glyoxal is of special importance (17, 18). This compound which originates in the phase of early glycation is the AGE precursor. Short period of hyperglycemia noted in IGT may be sufficient for increased concentration of methyl-glyoxal (19). It reacts with collagen, especially in cellular matrix, in the reaction where arginine residua are lost (20), and it may bind with arginine itself (21). Methyl-glyoxal is physiologically detoxicated via cytosol glutathione-dependent glyoxalase system (22). The changes of glyoxalase expression have been encountered in diabetes (23), while disorder of detoxication via glyoxalase system contributes to cytotoxicity and chronic complications of DM (24). According to Ahmed and Thornalley (25), AGE products may be divided by structure into 4 groups presented in *Table I*.

AGE products are highly oxidized forms, which contain hydroperoxides of amino acids, and they are particularly powerful generators of superoxide anion. Once produced AGE product is stable and irreversible and it continuously accumulates in long-lived proteins of blood vessel walls. This accumulation grows with aging and accelerated progress of diabetes. AGE products accelerate atherosclerotic processes via several mechanisms that are grouped into receptor-non-mediated and receptor-mediated mechanisms (26).

Table I Biologically significant AGE.

1) Monolysyl-products (without cross-link reactions)
- a) N^{ε}-carboxy methyl lysine (CML)
- b) N^{ε}-carboxy ethyl lysine (CEL)
- c) Pyralin

2) Bis-lysyl products (cross-linkage)
- a) Glyoxal-lysine dimer (GOLD)
- b) Methyl-glyoxal lysine dimer (MOLD)
- c) 3-Deoxi-glukozon lysine dimer (DOLD)

3) Hydroimidazolones
- a) Glyoxal-hydroimidazolone (G-H)
- b) Methylglyoxal-hydroimidazole (MG-H)
- c) 3-deoxyglucosone lysine dimer (3DG-H)

4) Other cross-linking bonds
- a) Pentosidine

Action of AGE products through receptor-non-mediated mechanisms

Glycosylation processes are, to a large extent, present in apoprotein B (27) and phospholipid component of LDL particle (28), when they lead to functional changes of LDL clearance and susceptibility of LDL to oxidative modification. Clinical studies have shown that AGE and LDL levels are increased in diabetics in relation to healthy population (29, 30). Glycosylation of apoprotein Apo B, which is a superficial protein of LDL, occurs at positively charged lysine residua at the receptor-binding domain that is important for the »recognition« of LDL particle by LDL receptor (31).

Glycosylation of apo B LDL gives rise to significant reduction of takeover of LDL particles by highly-specific receptors, because LDL molecule is »unrecognizable«; in this way, LDL clearance becomes reduced (32). Takeover of these modified LDLs proceeds now by nonspecific »scavenger« receptors, which are present on the surface of human macrophages, resulting in the formation of foam cells and initiation of atherosclerosis.

Glycosylated LDL becomes very susceptible to oxidative modification. Oxidative modification of LDL during glycosylation may also involve a phospholipid component containing $-NH_2$ group. Glycosylation of the phospholipid LDL component is followed by oxidative modification of unsaturated fat acids.

Diabetics are at 2 to 4 times higher risk of developing cardiovascular disorders. Increased oxidizability of LDL may manifest in severe hyperglycemia or vascular complications (33, 34). The effect of severe hyperglycemia on LDL and VLDL oxidizability has been recently verified in diabetics with hyperketonemia (35). Therefore, the control of glycemia level is extremely important for the prevention of sensitivity of LDL to oxidative modification in DM (36, 37). It has been validated that partially oxidized LDL in plasma correlates well with insulin resistance and metabolic sequelae (38). LDL oxidation produces new epitopes and results in the generation of oxidized LDL particle antibodies. The presence of oxidized LDL antibody has been suggested as a new predictive factor of progression of carotid artery sclerosis. These antibodies have been found in plasma and atherosclerotic plaque in diabetic patients (39). It has been confirmed that serum of type I diabetic patients contains oxidized LDL antibodies as well as oxidized LDL immunocomplexes (antigen-antibody) (40). The pre-

sence of such immunocomplexes may be considered a risk factor for macrovascular diseases in these patients.

Action of AGE products through specific receptors

The action of advanced glycosylated products is mediated by specific cellular receptors (RAGE) found on the surface of many cells, and especially on the surface of monocytes-macrophages, endothelium, smooth muscle cells, etc. (41). In pathological conditions, higher expression of these receptors is mediated by increased synthesis of ligands (to receptors), i.e. increased production of AGE products (42–45). AGE binding to receptors induces both the oxidative stress and nuclear factor (NF-κB) and adhesive molecule VCAM-1 synthesis (46–48), resulting in increased permeability of endothelial cells that may lead to more intensive incursion of lipids in the subendothelium.

Binding of soluble AGE to receptors in monocytes induces the chemotaxis (49), what is followed by infiltration of mononuclears through the intact monocellular layer of endothelium (50). AGE-RAGE interaction in monocytes-macrophages results in increased synthesis of the following mediators: interleukin-1 (Il-1), tumor necrosis factor (TNF-α), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (IGF) (51, 52), which are involved in the pathogenesis of atherosclerosis (53).

Binding of AGE products to receptors in smooth muscle cells causes increased cell proliferation (54). Although the definite mechanism of these reactions is not known, the effects caused by AGE-RAGE binding are the processes where cytokines and growth factors are the promotors. Accordingly, under conditions of increased deposition of AGE products in tissues, the AGE-RAGE interaction in the blood vessel walls allows for migration of inflammatory cells into the very lesion and release of cytokines and growth factors.

Polyol pathway

A number of studies has shown that glucose in abnormally high concentration is, before all, metabolized via the polyol pathway. Glucose is reduced by aldose reductase to sorbitol, which is oxidized to fructose by sorbitol-dehydrogenase. NADPH is required for the activity of aldose reductase. Therefore, intensification of the polyol pathway results in increased consumption of NADPH (54). Antioxidant enzymes such as glutathione reductase which regenerates the reduced glutathione also require NADPH. Accordingly, increased consumption of this cofactor leads to lower activity of glutathione reductase, diminishes the intracellular concentration of reduced glutathione, resulting in redox-imbalance and induction of oxidative stress (55, 56). Intracellular NADPH consumption also gives rise to reduced synthesis of $NO^{\bullet}$, since NADPH is also a cofactor of NO synthesis which synthesizes nitrogen monoxide from L-arginine. Metabolism of $NO^{\bullet}$ may be impaired by excessive production of superoxide anion which is the consequence of higher intracellular glucose concentration. Superoxide anion is involved in the physiological inactivation of $NO^{\bullet}$, where peroxynitrite is generated ($ONNO^{\bullet}$) which is a very strong oxidant, and is disintegrated into NO_2 and $OH^{\bullet}$ (57). It is believed that $NO^{\bullet}$ may be bound in endothelium by proteins (e.g., collagen) containing AGE, and in this way its diffusion to smooth muscle cells becomes restricted (58). Thus, an increase of intracellular glucose concentration results in the abnormalities of $NO^{\bullet}$ metabolism, which may trigger vascular complications in DM.

Hexosamine pathway

In case of higher intracellular glucose concentrations, a major part of glucose is metabolized by glycolysis, primarily to glucose-6-phosphate, and thereupon to fructose-6-phosphate. A part of fructose-6-phosphate is, by fructose-5-phosphate amidotransferase (GFAT), converted to glucosamine-6-phosphate and finally to uridine-diphosphate-N-acetyl-glucosamine (11). N-acetyl-glucosamine may bind to serine and threonine residua of proteins, especially transcriptional factors in the process of phosphorylation, resulting in pathological changes of gene expression (59–61). Thus, for example, modification of transcriptional factor Sp1 results in higher expression of transforming growth factor β (TGF-β) and PAI-1, leading to vascular and coagulation disorders in diabetics (62).

Oxidative stress and modulation of PKC and MAP kinase activities in diabetes

One of the most common ways of signal transduction through the cell is the process of phosphorylation of effector proteins, which is controlled by kinase and phosphatase action. The effects of oxidative stress on the activities of this enzyme are the critical points of signal transduction modulation by oxidative stress in diabetes.

It has been recently suggested that the hyperglycemia causing oxidative stress brings about the diabetic complications via two signal pathways: by activation of protein kinase C and activation of MAP kinases. Since specific PKC isoforms may activate MAP kinases which, by phosphorylation of transcriptional factors leads to alteration of genes expression, included in modification of cell phenotype, proliferation and synthesis of extracellular matrix, it may be considered that activation of MAP kinases is the

critical event in the development of diabetic complications (63, 64).

Metabolic sequelae of hyperglycemia may be manifested in those cells where glucose transport is independent from insulin action. Intracellular hyperglycemia may participate in the pathogenesis of diabetic complications through activation of the protein kinase C (PKC) system (65). High glucose concentration activates PKC by increased concentration of diacylglycerol (DAG), which is the major endogenous cellular cofactor of PKC activation.

PKC belongs to serine-threonine family and it is present in at least 12 isoforms. Several isoforms are found expressed in vascular tissue. PKC system is ubiquitous in the cell, and it is involved in the transcription process of several growth factors as well as in signal transduction as a part of response to growth factor effect (66).

Higher PKC expression, which is associated with development of some forms of type 2 DM, is responsible for »down-regulation« of a number of insulin receptors and reduced activity of protein kinase B (PKB), which results in insulin resistance, hyperinsulinemia and hyperglycemia (67).

In smooth muscle cells of the blood vessel wall, PKC activation modulates the rate of growth, DNA synthesis as well as »turnover« of growth factor receptors. PKC activation increases the expression of transforming growth factor β (TGF-β) which regulates extracellular matrix production by the activation of proteoglycan and collagen synthesis gene, and reduces the synthesis of proteolytic enzymes which disintegrate matrix proteins (68). Higher TGF-β expression causes the thickening of capillary base membrane, one of the structural abnormalities seen in almost all tissues in diabetic patients. PKC is also activated by free radicals, especially hydrogen peroxide.

Vascular endothelium as a valuable endocrine and metabolic organ has a role in the exchange of materials, maintenance of arterial wall integrity and tonus, regulation of hemostasis (coagulation and fibrinolysis), permeability and local platelet activation. Its alteration is considered the initial event in the process of atherogenesis, i.e. in the development of micro- and macroangiopathy in diabetes. A large number of active molecules are synthesized and secreted by endothelial cells.

There is an increasing number of data suggesting that hyperglycemia and PKC activation is associated with premorphological and subsequently morphological changes in the development of endothelial dysfunction. Constant hyperglycemia, through activated PKC, increases the arterial wall tonus leading to vasoconstriction. Besides the increase of a number of angiotensin II receptors, the phosphorylation of myofibrillary proteins and expression of actin-myosin regulatory proteins occur (69). An excessive PKC-bII expression causes the phosphorylation of troponin-I and lower sensitivity of myofilaments to Ca^{2+}, all resulting in reduced contractility of the cardiac muscle. The apoptosis of smooth muscle cells induced by free radicals is also a PKC-dependent process (70).

Increased permeability of blood vessels, that is, loss of endothelial barrier is an early pathophysiological mechanism of diabetic angiopathy etiology. Different stimuli such as higher glucose concentration, and activation of secondary messengers in endothelial cells, before all PKC, give rise to phosphorylation and relaxation of cytoskeletal and adhesive proteins (caldesmon and vimentin) as well as to »up regulation« of nitric oxide synthase (NOS) resulting in increased permeability and dysfunction of the endothelial barrier (71).

Protein kinase C-induced phosphorylation of serine residua on locus 23 of Na^+-K^+-ATPase leads to lower activity of enzymes. In this way, vascular permeability (resulting in proteinuria), deposition and interaction of extracellular matrix as well as Na^+ and H^+ excretion are affected, making the molecular basis of diabetic nephropathy.

Since there is an interaction of multiple receptor proteins in the complex pathway of signal transduction through the cell, whose specific communication and interaction (phosphorylation, dephosphorylation, proteolysis) cause impulse amplification, it is most frequently the question of joint, i.e. cascade pathways of signal transduction. One of these joint pathways, which is the basic molecular substrate of the development of diabetic complications, is the PKC-MAP kinase pathway of transduction of signal to transcription factors which then modify the expression of particular genes whose products initiate, stimulate and maintain the occurrence and development of complications of this disease (4).

Mitogen-activated protein kinases (MAPK) are a group of serine/threonine specific kinases which are activated by the action of extracellular stimuli that initiate phosphorylation of threonine and tyrosine residua in the enzyme. Three subgroups are derived from this group, i.e. ERK kinase, JNK and p38 kinase. Each of these subgroups has its own isoenzymes which are the products of three different genes. Many isoenzymatic forms of these kinases, by further posttranslation modification of enzymes, assume their own specific isoforms which are different from each other in relation to mode of activation and affected substrate. For example, p38 MAPK is activated in diabetes via osmosensors, whose nature has not been specified, but it is known that, in conditions of polyol pathway stimulation, the increase of sorbitol concentration causes the activation of this MAP kinase. Oxidative stress, which develops in diabetes by activation of the polyol pathway, and leads to a decrease of reduced glutathione concentration, activates ERK kinase in

human fibroblasts, but does not activate JNK and p38 kinase (72). On the other hand, hydrogen peroxide activates all three MAP kinases. Activation of ERK is the last link of the MAP-kinase cascade system responsible for the phosphorylation of different cytosol and nuclear proteins, which most commonly represent the transcriptional factors or associated proteins. MAP-kinase cascade, as well as PKC, is subjected to »down regulation« mediated by N-acetyl-L-cysteine and reduced glutathione, while both reduction of the glutathione concentration and present oxidative stress initiate their activation (73, 74).

Reviewing the aforesaid, it may be concluded that oxidative stress occurring in diabetes causes prolonged activation of the various pathways of signal transduction (especially PKC-MAP cascade), providing conditions for the modification of cell phenotype, apoptosis or survival along with an imbalance which results in the complications of particular organs and systems. Therefore, it is not surprising to learn about the attempts to specify new therapeutical options for the treatment of diabetic complications. The application of antioxidants, aldose reductase inhibitors, NOS, poly-ADP-riboso polymerase, ACE inhibitors, polyamines, and especially specific inhibitors of PKC and MAP-kinase isoenzymes, represents a new strategy for diabetes treatment in the future (75–78).

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