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Molecular Regulators of Diabetic Endothelial Dysfunction

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Abstract

Diabetes is a highly prevalent disease worldwide. It is associated with perturbation in glucose metabolism. Diabetic environment has immense impact on endothelial cells (ECs) which line the endothelium and regulate many vascular functions. Hyperglycemia (HG) is a hallmark of diabetes and it promotes formation of Advanced Glycation End Products (AGEs), oxidative stress, mitochondrial abnormalities, inflammation, polyol pathway, hexosamine pathway, etc. These cellular processes hamper EC function resulting in endothelial cell dysfunction (ED). Several diabetic vascular complications are initiated by ED. The persistence of diabetes related endothelial cell damage even after normalization of glucose levels is known as metabolic memory, which further accelerates diabetic complications. Therefore, diabetes makes patients more susceptible to vascular complications. Herein, some important diabetes related cellular phenomena that perturb EC function are described briefly. Understanding these deleterious phenomena is essential for developing treatment strategies to ameliorate diabetic ED.

Keywords

Diabetes, Endothelial Dysfunction, Hyperglycemia, Oxidative Stress, Inflammation

1. Introduction

Diabetes is a serious global disease [1]. Diabetes immensely increases risk of developing diabetic complications including macrovascular complications such as cardiovascular diseases, atherosclerosis, coronary artery disease, stroke, etc., and microvascular complications such as diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy [2]-[4]. ECs produce vasodilator nitric oxide (NO) and regulate many functions including vascular tone, inflammation, etc. [5] [6]. Many

studies have shown that expression of various genes related to EC pathways including vasoconstriction, vasodilation, inflammation, apoptosis, cell adhesion, extracellular matrix (ECM), etc., is altered under diabetic conditions suggesting prevalence of ED, which is known to act as a stimulus for diabetic vascular disease [7]-[9]. Several studies have analyzed the impact of antidiabetic therapy on endothelial function; no therapy has yet proved to be fully successful in ameliorating ED related to diabetes [10] [11]. Hence, it is imperative to find more potent therapies targeting diabetic ED to ameliorate complications. In this context, it is important to understand the mechanisms of diabetic ED, which is promoted by diverse molecular mediators. Herein, such critical mediators are briefly described. The literature described here provides brief overview of the effect of diabetic conditions mostly on EC function in different types of ECs and is selected from some articles available mainly in PubMed.

2. Advanced Glycation End Products (AGEs)

Nonenzymatic glycation and oxidation of proteins or lipids produce AGEs under normal physiological environment but their production increases in many diseases including diabetes, cardiovascular disease, cancer, etc. [12]. The harmful effects of AGEs in many diseases including diabetic cardiovascular complications arise due to either crosslinking of proteins or by interaction with AGE receptors such as Receptor for AGE (RAGE) [13] [14]. RAGE-expressing cells are usually present in vicinity of sites of high AGE accumulation in regions such as diabetic vasculature. High concentrations of AGEs in serum of type 2 diabetes patients are related to ED [15]. AGE levels are known to be significantly higher in diabetics having vascular complications as compared to those without complications [16].

AGEs act as driver of diabetic ED. HG-induced AGE production involves reactive oxygen species (ROS) and can be prevented by antioxidants in ECs [17]. Albumin-derived AGEs decrease expression of NO-producing enzyme eNOS by decreasing half-life of its mRNA [18]. AGE-modified albumin decreases eNOS activity quickly by causing decrease in its serine phosphorylation and slowly by decreasing its expression [19]. AGE-RAGE binding induces oxidant stress and activation of inflammation related transcription factor NF-κB in ECs, which induces expression of atherosclerosis related adhesion molecule VCAM-1 (Vascular Cell Adhesion Molecule 1), that potentiates their ability to bind with circulating monocytes and contributes to diabetic vascular disease [20]. The oxidant stress is harmful as it can potentially favor formation of vascular lesions [21]. AGE-BSA induces aberration in coagulant and barrier functions of ECs [22]. AGE product of low density lipoprotein induces inflammation related cytokine production [23].

Because of low mitochondrial content, ECs mainly depend on glycolysis for ATP production, rather than on mitochondrial oxidative phosphorylation [24]. HG increases glycolytic flux, resulting in increased concentration of glycolysis by-product methylglyoxal (MGO), which is a dicarbonyl metabolite and AGE precursor and augments ED by multiple mechanisms including apoptosis, oxidative

stress, inflammation, etc. [25]. It alters brain EC permeability by decreasing gene expression, promoting mislocalization, or glycation of tight junction proteins [26]. High plasma MGO levels are linked with cardiovascular disease in diabetes patients [27] [28]. Methylglyoxal-derived hemoglobin-AGEs are involved in inducing ROS formation, apoptosis and cause ED [29]. ED induced by HG is suggested to be mediated by MGO [30]. HG-induced MGO formation also disrupts attachment of EC to ECM by glycation of human type IV collagen, which hinders EC survival and angiogenesis, and is considered to be linked with diabetic vascular dysfunction [31]. MGO is detoxified by glyoxalase system which has two cooperating enzymes Glyoxalase 1 (Glo1) and Glyoxalase 2 (Glo2) [32]. HG mediated AGE formation is abrogated by overexpression of glyoxalase-I in ECs [33]. GLO1-knockdown in Human Aortic Endothelial Cells (HAECs) exposed to HG causes MGO accumulation and resultant inflammation, apoptosis and ED [34]. Overall, AGEs are critically involved in promoting conditions that favour diabetic complications.

3. Mitochondrial Abnormalities

Altered mitochondrial dynamics is observed in diabetic conditions. HG induces EC mitochondrial abnormalities such as mitochondria fragmentation, increased mitochondrial (mt) ROS production, alterations in mitochondrial morphology, membrane potential, fission, fusion, etc., and mitochondrial alterations are associated with many harmful effects such as apoptosis, reduced nitric oxide bioavailability, etc. [35]-[37]. HG induced mtROS production triggers leakage of mtDNA into the cytosol, which is sensed by DNA sensing cyclic GMP-AMP synthase (cGAS). This activates STING (stimulator of interferon genes) signaling, which stimulates inflammation and apoptosis, thus augmenting diabetes related aortic endothelial cell injury [38].

Hyperglycemia causes endothelial damage by activation of Protein Kinase C (PKC), AGE production, sorbitol accumulation, and the hexosamine pathway via increase in mitochondrial ROS production in ECs [39] [40]. Several studies show that HG induces alterations in levels of proteins related to mitochondrial dynamics. HG induces increase in EC mitochondrial fission and increases protein levels of fission proteins Fis-1 (Fission protein 1), Drp1 (dynamin-related protein 1) whereas that of fusion proteins OPA1 (optic atrophy 1) or Mfn2 (Mitofusin 2) is not significantly altered and ECs from diabetes mellitus (DM) patients with ED have higher Fis1 protein levels, modified mitochondria morphology and network compared to healthy volunteers [36]. Drp1 expression is increased while Fis1, MFN2, and OPA1 expression is unaltered by HG treatment of cultured human umbilical vein endothelial cells (HUVEC) in a different study [41]. Another study shows that HG induces decrease in fusion proteins Mfn1, Mfn2, and Opa1 while fission protein Fis1 first increases and then decreases in ECs and suggests that HG mediated mitochondrial fragmentation is regulated mainly by fusion inhibition rather than by fission activation [42]. HG has also been shown to induce altered

mitochondria morphology and enhance Drp1 and reduce OPA1 in ECs [43]. The differences in results of different studies arise because mitochondrial fission and fusion continuously go through dynamic changes and the studies have not fully examined the dynamic changes [42]. Increased mitochondria fragmentation, reduced OPA1 protein levels, higher DRP1, and higher superoxide levels are seen in coronary ECs isolated from diabetic mice compared to ECs from control mice [35]. Overall imbalance of mitochondrial fission and fusion is induced by HG. ED in diabetic conditions is related to mitochondrial abnormalities.

4. PKC Activation

PKC is a family of serine/threonine kinases involved in diverse cell signaling pathways and several studies suggest key role of PKC in impairing endothelial function in diabetes [44]. HG induces EC PKC activation, which triggers ED by several mechanisms such as decrease in NO production or bioavailability, reduction in endothelium-dependent relaxation, increase in EC permeability, oxidative stress, apoptosis, etc. [45]-[48]. Intermittent high glucose is more potent than stable high glucose in stimulating PKC activation related expression of adhesion molecules in ECs via overproduction of mitochondrial free radicals [49]. Overall, PKC plays key role in HG induced ED.

5. Inflammation

Inflammation and ED are induced by postprandial HG, which may augment atherosclerosis and cardiovascular complications [50]. Fluctuating glucose is a critical inducer of inflammation [51]. Fluctuating glucose exposure of ECs triggers increase in several proinflammatory molecules [51]. Monocytes treated with HG show increased adhesion to ECs and increased expression of inflammatory molecules that are important in the pathogenesis of diabetes complications [52]. Monocytes from hyperglycemic diabetes patients without hyperlipidemia also show higher EC binding compared to controls [53]. ICAM-1 (Intercellular Adhesion Molecule-1) and VCAM-1 protein expression in HUVECs isolated from pregnant women with Gestational Diabetes Mellitus (GDM) is elevated compared to HUVECs without GDM. Monocyte adhesion is higher in GDM-HUVECs than in normal HUVECs [54].

PKC dependent endothelial-neutrophil cell adhesion and expression of endothelial adhesion molecules such as (ICAM-1), P-selectin, and E-selectin are induced by HG treatment of HUVECs and EC-neutrophil adhesion is known to augment vascular inflammation leading to vascular diseases [55]. Periodic high glucose is more potent than constant high glucose treatment in inducing secretion and expression of inflammation causing molecules such as IL-6, TNF-α (tumour necrosis factor-alpha) and ICAM-1 in human coronary artery endothelial cells (HCAECs) [56].

HG induced elevation of Microtubule affinity regulating kinase 4 (MARK4) expression mediates activation of NOD-like receptor pyrin domain 3 (NLRP3) in-

flammasome and production of cytokines interleukin (IL)-1β and IL-18 in ECs [57]. Increase in inflammation related transcription factor ELF3 (E74-like ETS transcription factor 3) and decrease in SET8 protein (SET domain-containing protein 8) by HG upregulate MARK4 expression. Diabetic patients and rats show SET8 downregulation and ELF3 upregulation. Inhibition of NLRP3 inflammasome attenuates HG induced ED [58]. Hence, a diabetic environment activates EC inflammation.

6. Polyol Pathway

Under physiological conditions, the glucose metabolism pathway called polyol pathway is insignificant as its first enzyme Aldose Reductase (AR), has low affinity for glucose. However, in diabetic conditions, about 30% of blood glucose is fluxed via polyol pathway and generates oxidative stress in many cell types of the body including lens and nerve [59] [60]. This pathway involves reduction of glucose to sorbitol by AR, which consumes reduced nicotinamide adenine dinucleotide phosphate (NADPH). This creates deficiency of reduced NADPH for enzymes such as antioxidant reduced Glutathione (GSH) regenerating antioxidant enzyme glutathione reductase. This leads to reduction in cellular antioxidant capacity. The second enzyme of this pathway sorbitol dehydrogenase utilizes NAD+ as cofactor and oxidizes sorbitol to fructose, which reduces NAD+ to NADH ratio, and the increase in NADH levels promotes ROS generation by NADH oxidase. The increase in polyol pathway by HG contributes to cellular stress [61]. Accumulation of triose phosphates due to increase in cytosolic NADH/NAD+ ratio provokes AGE precursor (MGO) formation and PKC activation via diacylglycerol formation [62]. Sorbitol is hydrophyllic and cannot easily cross cell membrane; its intracellular accumulation disturbs osmotic balance [63]. HG treatment of HUVECs increases sorbitol levels, fragmented DNA, caspase-3 activity, and oxidative stress, all of which are reduced by aldose reductase inhibitor SNK-860, suggesting involvement of polyol pathway in HG mediated EC damage [64]. Hence polyol pathway is harmful in a diabetic environment.

7. Hexosamine Biosynthesis Pathway (HBP)

In diabetes, increased glucose flux via hexosamine biosynthesis results in increased levels of its end product, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is a substrate of the enzyme O-GlcNAc transferase (OGT), that catalyzes protein O-GlcNAcylation (which is defined as addition of N-acetylglucosamine to serine or threonine amino acids of proteins) [65] [66]. HG induces excessive mitochondria derived superoxide production which triggers activation of hexosamine pathway in ECs [40]. HG induces eNOS O-GlcNAcylation, which is reversed by inhibiting the first and rate-limiting enzyme of the hexosamine biosynthesis using glutamine: fructose-6-phosphate amidotransferase (GFAT) inhibitor azaserine in HCAECs [67]. The presence of eNOS O-GlcNAcylation sites around the Ser1177 residue disables Akt binding to phosphorylate and activate

eNOS. HG induced eNOS O-GlcNAcylation and decrease in serine1177 phosphorylation is reversed by GFAT antisense oligonucleotides or by inhibitors of mitochondrial superoxide production in cultured bovine aortic endothelial cells (BAECs) [68]. O-GlcNAcylation promotes atherosclerosis-related complications in diabetes. Higher EC O-GlcNAcylation was observed in carotid plaques from diabetic patients compared to nondiabetics [67]. Aorta of diabetic rats also exhibits increased GlcNAc modification of eNOS and reduced eNOS activity compared to age-matched nondiabetic rats [68]. Coronary ECs isolated from diabetic mice have reduced levels of O-GlcNAc modification removing enzyme called O-GlcNAcase (OGA), and also have elevated OGT protein levels and higher protein O-GlcNAc modification compared to ECs from control mice. Protein O-GlcNAcylation levels decrease and coronary EC function becomes better in diabetic mice by overexpressing OGA in ECs [69]. HG induces O-GlcNAc modification of transcription factor Specificity protein 1 (Sp1), which upregulates EC expression of inflammatory molecule ICAM-1, which is known to cause damage to the retina in diabetic retinopathy [70]. Enhancement of O-GlcNAcylation using OGA inhibitor PUGNAc decreases glyoxal (AGE precursor) induced ROS generation and apoptosis in Human Retinal Microvascular Endothelial Cells (HRECs) suggesting that it plays protective role in diabetic retinopathy [71]. These studies indicate that O-GlcNAcylation has damaging effect or protective effect on ECs depending on the target protein or the stress type.

8. Metabolic Memory

Several studies show persistence of harmful effects of diabetic conditions even after glucose levels normalization, which is known as metabolic memory. Under diabetic conditions, epigenetic alterations involving DNA methylation, histone modifications, and non-coding RNAs contribute to diabetic complications [72]. Transient HG is shown to trigger persistent NFκB-p65 gene transcription by different mechanisms such as recruitment of SET7 (SET domain containing lysine methyltransferase 7), H3K4 mono-methylation, decrease in H3K9 methylation and enhanced recruitment of Lysine-Specific Demethylase 1 (LSD1) on the NFκB-p65 promoter [73]. Metabolic memory of HG causes persistent increase in pro-inflammatory molecules and elevated atherosclerosis [73].

The mitochondrial adaptor p66Shc acts as a key mediator of vascular hyperglycemic memory in diabetes [74]. HG induces PKC mediated activation of p66Shc via Ser-36 phosphorylation, which persists even after achievement of normoglycemia in ECs. This causes persistent p66Shc dependent effects such as oxidative stress, mitochondrial damage, apoptosis, increase of MGO, ROS mediated persistent PKC activation and PKC-mediated eNOS inhibitory Thr-495 phosphorylation. HG induces p66Shc upregulation via epigenetic modifications such as promoter hypomethylation and increased histone 3 acetylation, which maintain p66Shc upregulation despite glucose normalization. Overexpression of SIRT1 (Sirtuin 1), a class III histone deacetylase, represses HG induced p66Shc expres-

sion, and it has been shown that SIRT1 binds to p66Shc promoter and when overexpressed, it results in reduced acetylated histone H3 binding to p66Shc promoter [75]. HG induced epigenetic modifications of *Sod2* gene, which encodes mitochondrial superoxide scavenging enzyme Manganese Superoxide Dismutase (MnSOD), decrease its expression and are suggested to be involved in metabolic memory [76] [77]. It is important to understand that results of different studies vary depending on several factors such as species of ECs, *in vitro* or in *vivo* conditions, cell type, etc.

HG reduces expression of SET8 and its expression remains low even after glucose normalization in HUVECs suggesting that reduced SET8 is related to cellular hyperglycemic metabolic memory [78] [79]. SET8 overexpression protects EC from the damaging consequences of HG and hyperglycemic memory such as oxidative stress, inflammation, etc., by reducing these damaging conditions. Oscillating glucose is more potent in damaging ECs and in inducing higher metabolic memory effect compared to constant high glucose [80]. Therefore, metabolic memory is dangerous phenomenon as it causes persistent damaging conditions.

9. Conclusions

In summary, diabetic conditions such as HG augment ED by stimulating diverse harmful processes such as excessive AGE formation, PKC activation, polyol pathway, etc. in ECs. Most of these deleterious events converge on molecular mechanisms such as oxidative stress, inflammation, apoptosis, reduction in NO bioavailability, etc. The persistence of glucose induced damaging effects even after glucose normalization is referred to as hyperglycemic memory, which increases risk of diabetes related vascular complications.

The limitation herein is that most of findings described are mainly from EC models used in some studies relevant to diabetic ED. Describing all the findings and mechanisms from all types of diabetic ED models, patients is beyond the scope here.

The conclusion is that diabetes promotes EC damage, which acts as trigger for diabetic vascular complications. Effectively controlling the diabetes induced molecular mediators of EC damage is critical for alleviating diabetic vascular complications.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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