Molecular Targets of Diabetic Vascular Complications and Potential New Drugs

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Abstract: In diabetes, oxidative stress plays a key role in the pathogenesis of vascular complications, and an early step of such damage is considered to be the development of an endothelial dysfunction. Hyperglycemia directly promotes an endothelial dysfunction inducing process of overproduction of superoxide and consequently peroxynitrite, that damages DNA and activates the nuclear enzyme poly(ADP-ribose) polymerase. This process, depleting NAD+, slowing glycolysis, ATP formation and electron transport, results in acute endothelial dysfunction in diabetic blood vessels and contributes to the development of diabetic complications.

These new findings may explain why classical antioxidants, like vitamin E, that work scavenging already formed toxic oxidation products, have failed to show beneficial effects on diabetic complications, and suggest new and attractive “causal” antioxidant therapy. New, low molecular mass compounds that act as SOD or catalase mimetics or L-propionylcarnitine and lipoic acid, that work as intracellular superoxide scavengers, improving mitochondrial function and reducing DNA damage, may be good candidates for such strategy, and preliminary studies support this hypothesis. This “causal” therapy would also be associated with other promising tools such as LY 333531, PJ34 and FP15, which block protein kinase B isoform, poly(ADP-ribose) polymerase and peroxynitrite, respectively. It is now evident that, statins, ACE inhibitors, AT-1 blockers, calcium channel blockers and thiazolidinediones have a strong intracellular antioxidant activity, and it has been suggested that many of their beneficial ancillary effects are due to this property. This preventive activity against oxidative stress generation can justify a large utilization and association of this compounds for preventing complications in diabetic patients where antioxidant defences have been shown to be defective.

Key Words: Diabetic complications, oxidative stress, statins, ACE inhibitors, AT1 receptor inhibitors, calcium channel blockers, thiazolidinediones.

INTRODUCTION

The relationship between diabetes and premature vascular disease is well established [1]. Recent prospective studies indicate that long-term glycemic control is an important predictor not only of microvascular disease [2, 3], but also of macrovascular complications [4]. Vascular endothelial cells are an important target of hyperglycemic damage, but the mechanisms underlying this damage are not fully understood.

In diabetes, oxidative stress plays a key role in the pathogenesis of vascular complications, and an early step of such damage is considered to be the development of an endothelial dysfunction [5, 6]. In vivo and in vitro studies have demonstrated that hyperglycemia directly induces, both in diabetic and normal subjects, an endothelial dysfunction and attenuates endothelium-dependent relaxation [7-9]. Studies in vitro and in vivo showed that this effect, mediated by free radicals production, is contrasted by antioxidants [10-12]. Recent basic and clinical studies have uncovered new insights on the role of oxidative stress in diabetic complications, suggesting a different and innovative approach to a possible “causal antioxidant therapy.

Hyperglycemia, Diabetes and Oxidative Stress

Recently, Brownlee has pointed out the key role of superoxide production in endothelial cells during hyperglycemia, in the pathogenesis of diabetic complications [13]. This is consistent with the four pathways involved in the development of diabetic complications: increased polyol pathway flux, increased advanced glycosylation end products (AGEs) formation, activation of protein kinase C (PKC) and increased hexosamine pathway flux. However, superoxide generation in hyperglycemia represents only a first step in the production of the endothelial dysfunction in diabetes. Nitric oxide (NO) production plays a central role in modulating endothelial function [14]. NO is generated from the metabolism of l-arginine by the enzyme NO synthase (NOS), of which there are three isoforms: the constitutive types bNOS and eNOS, and the inducible type iNOS [15]; the latter is induced de novo by various stimuli, including hyperglycemia, and leads to the production of large amounts of NO [16]. The superoxide anion may quench NO, thereby reducing the efficacy of a potent endothelium-derived vasodilator system that participates in the general homeostasis of the vasculature [17], and evidence suggests that during hyperglycemia, reduced NO availability exists [8]. Consistently, in hyperglycemic conditions, an overproduction of both superoxide and NO has been reported, with threefold increase in superoxide generation [18]. The simultaneous over-genera-
tion of NO and superoxide favours the production of a toxic reaction product, the peroxynitrite anion [19]. The peroxynitrite anion is cytotoxic, because it oxidizes sulphhydryl groups in proteins, initiates lipid peroxidation and nitrates amino acids such as tyrosine, which affects many signal transduction pathways [19]. Peroxynitrite is a potent initiator of DNA single-strand breakage, which is an obligatory stimulus for the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) [20]. These reactive species trigger DNA single-strand breakage, which induces a rapid activation of PARP [21]. PARP activation in turn depletes the intracellular concentration of its substrate NAD+, slowing the rate of glycolysis, electron transport, and ATP formation, and produces the ADP-ribosylation of GADPH. A recent study shows the pivotal role of ADP-ribosylation of GADPH on activation of the three major pathways of hyperglycemic damage (AGEs formation, activation of PKC, and hexosamine pathway flux) [20]. This process results in acute endothelial dysfunction in diabetic blood vessels [21]. In addition to the direct cytotoxic pathway regulated by DNA injury and PARP activation, PARP also appears to modulate the activation of nuclear factor-kB, and the expression of genes, including gene for intercellular adhesion molecule-1, iNOS and NADPH oxidase [22].

Therapeutic Approach: the “Causal” Antioxidant Therapy

As previously stated, convincing evidence is now available about the possible role of oxidative stress in the development of diabetic complications [5]. However, clinical trials with antioxidants, in particular with vitamin E, have failed to demonstrate any beneficial effect [23].

On this matter, it has recently been suggested that antioxidant therapy with vitamin E or other antioxidants is limited to scavenging already formed oxidants and may, therefore, be considered a more “symptomatic” rather than a causal treatment for vascular oxidative stress [24]. Fig. (1) contributes to explain this concept.

According to the evidence discussed in this article, it is suggested that interrupting the overproduction of superoxide by the mitochondrial electron-transport chain would normalise the pathways involved in the development of diabetic complications [25]. It might however be difficult to accomplish this using conventional antioxidants, as these scavenging reactive oxygen species in a stoichiometric manner. Now, low molecular mass compounds that act as SOD or catalase mimetics have the theoretical advantage of scavenging reactive oxygen species continuously by acting as catalysts with efficiencies approaching those of native enzymes [26]. Such compounds normalise endothelial dysfunction in streptozotocin-induced diabetic rats [27] and improve diabetes-induced decreases in endoneurial blood flow and motor nerve conduction velocity [28]. Another interesting compound is L-propionyl-carnitine. This substance has been shown to act as an intracellular superoxide scavenger, improving mitochondrial function and reducing DNA damage [29-32]. These properties have been shown to have beneficial effects on diabetic heart function, peripheral nerve function and vascular blood flow in experimental diabetes [30, 32, 33].

In the last years, another substance has received much attention: the lipoic acid [34]. It may have a unique self-regenerating capacity as a mitochondrial antioxidant [34], and it has reported the possibility that it restores endothelial dysfunction in both animal models of diabetes and in diabetic patients [35, 36].

Other promising tools are LY 333531, PJ34, and FP15, which block protein kinase β isoform, poly(ADP-ribose) polymerase and peroxynitrite, respectively. Not surprisingly, they have been shown to ameliorate the endothelial dysfunction induced by hyperglycemia [24, 25, 37, 38]. LY 333531 has been demonstrated to reduce oxidative stress generation in the retina: this is consistent with the evidence that PKC activation may increase superoxide generation through NAD(P)H [39]. PJ34 is not an antioxidant and does not directly interfere with the reactivity of peroxynitrite [40], however, poly(ADP-ribose) polymerase inhibition can suppress the expression of the inducible form of nitric oxide synthase (iNOS) [41, 42] and that poly(ADP-ribose) polymerase can regulate the function of mitochondria in oxidatively challenged cells: poly(ADP-ribose) polymerase inhibitors or poly(ADP-ribose) polymerase deficiency can suppress mitochondrial permeability transition and mitochondrial oxidant generation [43]. Since mitochondria represent a principal source of reactive oxidants in endothelial cells placed in high glucose [22], may be that poly(ADP-ribose) polymerase inhibition suppresses nitrotyrosine generation via preserving mitochondrial integrity.

FP15 is a potent peroxynitrite decomposition catalyst [38]. It inhibits tyrosine nitration and reduces the toxicity of peroxynitrite for the β-cells and vascular endothelium during the development of diabetes in rats [38].

Therefore, in the near future, a causal antioxidant therapy may include SOD and catalase mimetics, L-propionyl-carnitine, lipoic acid, PKC β and poly(ADP-ribose) polymerase inhibitors, peroxynitrite catalysts. This combination would aim to block the noxious cascade activated by hyperglycemia through the overproduction of superoxide and NO.

However, while waiting for these focused tools, we may already have other possibilities. It is now evident that statins, ACE inhibitors, ATI inhibitors and thiazolidinediones have a strong intracellular antioxidant activity, and it has been suggested that many of their beneficial ancillary effects are due to this property.

3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE INHIBITORS

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins are potent inhibitors of cholesterol biosynthesis, they decrease LDL levels, increase HDL cholesterol and lower triglycerides. Important clinical trials have demonstrated that statins decrease the incidence of coronary heart disease and stroke in patients with atherosclerosis [44-47]. This effect was primarily attributed to its LDL-lowering effects. Recent works have challenged this notion and suggest that lowering of cholesterol may not be
the lone benefit of statin therapy [48,49]. The lower risk of myocardial infarction in patients treated with statins than in patients treated with other cholesterol-lowering drugs, have suggested that the protective effects of statins may extend to mechanisms beyond cholesterol reduction [50, 51]. Moreover, recent trials founded lower coronary risk, despite comparable cholesterol levels, in patients treated with statins compared to the placebo group [46, 52-54]. Most of statins protective effects are mediated by inhibition of isoprenoid compounds and restoration of endothelial function increasing NO bioavailability [55].

Blocking the HMG-CoA Reductase, statins inhibit synthesis of mevalonic acid, a precursor of many non-steroidal isoprenoid compounds like farnesylpyrophosphate and geranylgeranyl-pyrophosphate [56]. These intermediate compounds are important for the subcellular localisation and intracellular trafficking of several membranes bound proteins and...
(Rho, Ras, Rac, Rab, Ral and Rap). In endothelial cells, farnesylation and geranylgeranylation are responsible of translocation of Ras and Rho from cytoplasm to the membrane where these proteins act [57]. During statins treatment, this translocation is blocked and inactive intermediates such as Rho and Ras GTPase family, are accumulated in cytoplasm with reduction of adhesion complexes formation and alteration of sensitivity to calcium of vascular smooth cells [57,58]. Important consequence of inhibition of Rho is the modulation of eNOS expression. In fact, Rho can negatively regulate eNOS expression, in particular, reducing gene expression and mRNA stability [59, 60]. Indeed statins upregulate eNOS expression by prolonging eNOS mRNA half-life in cultured human endothelial cell, increasing the bioavailability of endothelial dependent NO [61, 62]. Moreover, statins can improve eNOS expression by reduction of cholesterol LDL levels that are correlated with downregulation of eNOS mRNA expression [63]. LDL and oxidised LDL appear to regulate eNOS expression trough their action on caveolin-1, a protein involved in trafficking and assembly of cholesterol [64,65]. Increased levels of caveolin-1, like in presence of hypercholesterolemia, leads to inhibition of eNOS activity [66]. Caveolin bind eNOS in plasmalemmal caveola interfering with the calmodulin binding that usually enhances eNOS activity.

Another mechanism by which statins may improve endothelial function is the action on NADPH oxidase activity. Statins attenuate angiotensin II-induced free radical production in vascular smooth muscle cells by inhibiting Rac1-mediated NADPH oxidase activity and downregulating angiotensin type 1 receptor expression [67]. Activation and translocation of Rac-1 GTPase from the cytosolic compartment to the cell membrane is a prerequisite of NADPH oxidase activation [68]; further withdrawal of statins inducing a translocation of Rac-1 from the cytosol to the membrane, transiently increased NADPH oxidase. Consistent with these results, Waasman et al. founded that angiotensin II-induced reactive oxygen species (ROS) were significantly reduced in rats treated with atorvastatin [69]. Furthermore, statins were described to influence expression of endothelin-1 (ET-1), a mitogenic agent secreted by activated endothelial cells [70]. ET-1 has chemotactic properties on monocytes, it is a potent inducer of cell adhesion molecules in endothelial cells and is thought to regulate vascular tone and endothelial functions [71,72]. Therefore, inhibition of ET-1 production might influence the pathogenesis of atherosclerosis, blocking the events that are critical for the re-entry of quiescent vascular smooth muscle cells into the cell cycle.

In vivo studies may support that the most important “ancillary” effect of statin is their preventive antioxidant activity: statin modulates nitrotyrosine generation, both in diabetic and non-diabetic subjects [73, 74]. Interestingly, NT has recently been shown as an independent risk factor for cardiovascular disease [75].

ACE-Inhibitors and AT1 receptor Antagonists

Angiotensin II (AG II) is a multifunctional peptide that modulates blood pressure, water and sodium homeostasis, neural function and other neurohumoral systems [76, 77]. The effects of AG II are mediated by plasma membrane receptors: AT1 and AT2. AT1 receptor mediates the most known effects of angiotensin II, and they are present in vascular smooth muscle cells, heart, lung, brain, liver, kidney and adrenal glands [77]. AT1 receptor are coupled to a variety of intracellular signalling molecules; stimulation of these signal transduction pathways leads to cellular contraction, hypertrophy, proliferation and apoptosis [76-78]. Activation of AT1 receptor by AG II modifies cell activity, vascular inflammation and oxidative stress.

It is now recognised that angiotensin II and activated AT1 receptors produces intracellular oxidative stress [78], and evidence shows that hyperglycemia is able to directly modulate cellular angiotensin production [79]. Mechanism by which angiotensin II produces oxidative stress seems the activation of NADPH and NADH oxidase that results in increasing intracellular superoxide generation [80]. This activation is mediated by intracellular signalling pathways such as arachidonic acid metabolites [81]. Moreover, AG II enhances gene expression of GTPase rac1 and induces translocation of rac1 to the cellular membrane that is essential for NADPH activation [82]. Activation of this system has important value in increasing oxidative stress with production of peroxynitrite, impaired bioactivity of NO and promotes vascular oxidative stress. In fact NADPH oxidase and eNOS are the major source of reactive oxygen species in endothelial cells and vascular smooth muscle cells [83, 84]. Furthermore treatment with ACE inhibitors and AT1 receptor antagonists was founded to increase EC-SOD activity, the major antioxidant system of the human arterial wall [85].

Therefore, if angiotensin II and activated AT-1 receptors are involved in intracellular oxidative stress, is not surprising that ACE inhibitors and AT1 receptor antagonist, can prevent hyperglycemia derived-oxidative stress, endothelial dysfunction and reduce the progression of atherosclerosis [86]. Moreover we have to consider that these drugs act directly on the first step of superoxide formation, resulting in a causal and not symptomatic treatment. Their action is not limited to scavenging already formed oxidants, but they prevent the formation of oxidants [86]. The interruption of superoxide overproduction would normalise the pathways involved in the development of diabetic complication [87]. This possibility is confirmed by important trials that have already shown the efficacy of these compounds in preventing diabetic complications, such as retinopathy [88], nephropathy [89-91] and also cardiovascular disease [89]. Several experimental studies confirmed the influence of ACEI and AT1 antagonist treatment on oxidative stress in vitro and in vivo. In streptozotocin-induced diabetic rats, enalapril treatment improved antioxidants defences, reduced protein and lipid oxidation, and protected against heart and kidney diabetes-related lesions [92,93]. In human endothelial cells, AG II regulate superoxide anion formation in a dose-dependent manner via AT1, therapy with AT1 antagonist down regulates NADPH oxidase expression and consequently reduces oxidative stress [94]. In hypertensive and hypercholesterolemic patients, AT1 blockade improved endothelial dysfunction and reduced oxidative stress and inflammation, evaluated by endothelium-dependent relaxation [95, 96] and by the levels of 8-isoprostane, MCP-1 and ICAM-1 [96],

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Calcium Channel Blockers

Beyond causing vasodilation through inhibition of calcium channels, long-acting CCBs have been demonstrated to produce clinical benefits in patients with coronary artery disease that might be independent of blood pressure change [97,98]. A pleiotropic effect reported for CCBs that might affect the development of atherosclerosis is the ability of these agents to reduce oxidative stress [99]. Both in vitro and in vivo studies have shown that lipophilic CCBs inhibit oxidative damage to lipids associated with cellular membranes and lipoproteins particles [100,101].

Thiazolidinediones

Thiazolidinediones are a new class of insulin-sensitizer agents that bind and activate the nuclear peroxisome proliferator-activated receptor gamma (PPARγ) [102]. This receptor is a member of the nuclear receptor superfamily of ligand-dependent transcription factors that regulates fat-cell development and glucose homeostasis [103]. PPARγ is expressed predominantly in adipose tissue where it induces differentiation and triglyceride synthesis, but PPARγ are also known to be expressed in adrenal gland, spleen, arterial wall cells, vascular smooth muscle cells and macrophage foam cells [104-106]. However, while these drugs are actually utilised principally for lowering glycaemia, important experimental trials show important effects on reduction of arterial inflammation [107-109] and oxidative stress [110]. The studies of Ricote et al. [107] and Jiang et al. [108] showed that activation of PPARγ could down regulate inflammatory responses in monocyte/macrophages. They found that PPARγ is markedly up regulated in activated macrophages where it promotes the expression of the inducible nitric oxide synthase [107,108]. PPARγ ligands, blocking the PPARγ receptor, were found to inhibit the expression of the inducible nitric oxide synthase interfering negatively with the nuclear factor-κB (NF-κB), the signal transducer and activator of transcription (STAT) and the activating protein-1 (AP-1) signalling pathways [107,108]. The molecular mechanism of this inhibition is not fully known; it is considered a trans-repression because it does not appear to involve direct binding to the iNOS promoter [111]. PPARγ-ligands efficacy on inhibiting iNOS expression was found also in mesangial cell [112,113], cerebellar granule cells [114] and peritoneal macrophages [108]. Reduction of iNOS has several important consequences like the reduction of oxidative products and the prevention of arterial inflammation. These documented properties can explain the effectiveness as “causal and preventive” antioxidants.

CONCLUSION

Many evidences suggest a possible role of oxidative stress in the pathogenesis of diabetic complications. Antioxidant therapy may be of great interest in these patients, even because it has recently been suggested that diabetic subjects with complications may have defective cellular antioxidant response against the oxidative stress generated by hyperglycaemia [124, 125]. New insights on the mechanisms leading to the generation of oxidative stress in diabetes are now available. Presumably these findings lead to the discovery and to the evaluation of new antioxidant molecules, such as SOD and catalase mimetics, that hopefully may inhibit at an early stage, the mechanism leading to diabetic complications. While waiting for these new and specific compounds, it is reasonable to suggest that substances already available, such as statins, ACE inhibitors, ATI blockers, CCBs and TDZs should also be used for their effectiveness as “causal and preventive” antioxidants.

ABBREVIATIONS

AGEs = Advanced glycosylation end products
PKC = Protein kinase C
NO = Nitric oxide
NOS = NO synthase
PARP = Poly( ADP-ribose) polymerase
ET-1 = Endothelin-1
AG II = Angiotensin II
CCBs = Calcium channel blockers
PPARγ = Peroxisome proliferator-activated receptor gamma
TDZs = Thiazolidinediones

REFERENCES


