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Effects of gliclazide beyond metabolic control

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Abstract

Evidence implicates hyperglycemia-derived oxygen free radicals as mediators of diabetic complications. Recent studies demonstrate that hyperglycemia-induced overproduction of superoxide seems the first and key event in the activation of all pathways involved in the pathogenesis of diabetic complications. Superoxide overproduction is accompanied by increased nitric oxide generation and, consequently, formation of the strong oxidant peroxynitrite, and by poly(adenosine diphosphate ribose) polymerase activation, which in turn further activates the pathways involved in the pathogenesis of diabetic complications. This process results in acute endothelial dysfunction and activation of inflammation in diabetic blood vessels that, convincingly, contribute to the development of diabetic complications. Gliclazide is an oral hypoglycemic agent that belongs to the class of sulfonylureas: basic and clinical evidences suggest that gliclazide works as an antioxidative drug, independently from its ability to reduce hyperglycemia. The availability of a compound that simultaneously decreases hyperglycemia, restoring insulin secretion, and inhibits oxidative stress produced by high glucose seems to be an interesting therapeutic prospect for the prevention of vascular complications of diabetes.

1. Introduction

Patients with diabetes have an increased risk of cardiovascular disease. The response-to-injury hypothesis of atherosclerosis states that the initial damage affects the arterial endothelium, leading to endothelial dysfunction [1]. Hyperglycemia is a hallmark of both non–insulin-dependent (type 2) and insulin-dependent diabetes mellitus (type 1). In vivo and in vitro studies have demonstrated that hyperglycemia directly induces, both in diabetic and normal subjects, endothelial dysfunction and attenuates endothelium-dependent relaxation [2-4]. There is considerable evidence that elevated glucose levels are associated with increased production of reactive oxygen species by several different mechanisms [5-9]. The hyperglycemia-dependent increase in reactive oxygen species production contributes to the development of impaired endothelium-dependent relaxation [2]. Several experimental and clinical studies suggested that in diabetes oxidative stress plays a key role in the pathogenesis of vascular complications, both microvascular and macrovascular [10], and an early step of such damage is considered to be the development of endothelial dysfunction [10,11].

Recently, Brownlee [12] pointed out the key role of superoxide production in endothelial cells during hyperglycemia in the pathogenesis of diabetic complications. Superoxide production, indeed, can activate the 4 pathways involved in the development of diabetic complications: increased polyol pathway flux, increased advanced glycosylation end (AGE) product 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 [13]. Nitric oxide is generated from the metabolism of L-arginine by the enzyme NO synthase (NOS), of which there are 3 isoforms: the constitutive types bNOS and eNOS, and the inducible type iNOS [14]; the latter is induced de novo by various stimuli, including hyperglycemia, and leads to the production of large amounts of NO [15]. 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 [16], and evidence suggests that during hyperglycemia reduced NO availability exists [3]. Consistently, in hyperglycemic conditions, an overproduction of both superoxide and NO has been reported, with a 3-fold increase in superoxide generation [17]. The

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simultaneous overgeneration of NO and superoxide favors the production of a toxic reaction product, the peroxynitrite anion [18]. The peroxynitrite anion is cytotoxic, because it oxidizes sulfhydryl groups in proteins, initiates lipid peroxidation, and induces nitration of amino acids such as tyrosine, which affects many signal transduction pathways (Fig. 1) [18].

2. Markers of oxidative stress in diabetes

2.1. Lipid peroxidation

Low-density lipoprotein (LDL) in its native state is not atherogenic. Low-density lipoprotein chemical modification, as occurs during oxidative stress-related lipid peroxidation, leads to the formation of oxidized LDL (oxLDL). This modification renders LDL susceptible to macrophage uptake via a number of scavenger receptor pathways, producing foam cells [19]. Moreover, oxLDL induces synthesis of monocyte chemotactic protein 1 [20,21], resulting in the recruitment of inflammatory cells [22], and stimulation of smooth muscle cell proliferation [23]. The presence of oxidized lipids was found in human atherosclerotic lesions [24-28].

2.2. Protein oxidation and nitration

In addition to lipid oxidation, there is also good evidence for protein oxidation in human atherosclerotic lesions [29,30]. Oxidation and nitration products include several reactive species such as hydroperoxides and protein-bound reductants. Some of these products, like nitrotyrosine (NT), have become markers of oxidative stress. Nitrotyrosine formation, derived by nitration of tyrosine, is detected in the artery wall of monkeys during hyperglycemia [31] and is followed by the development of endothelial dysfunction in both healthy subjects [32] and in coronaries of perfused hearts from rats [33], and this effect is not surprising, because it has been shown that NT can also be directly harmful for endothelial cells [34]. The toxic action of NT is supported by evidence showing that increased apoptosis of myocytes, endothelial cells, and fibroblasts in heart biopsies from diabetic patients [35], in hearts from streptozotocin-induced diabetic mice [36], and in working hearts from rats during hyperglycemia [33] is selectively associated with the levels of NT found in those cells. However, limited information is available regarding the relationship between the accumulation of oxidized and nitrated proteins and the severity of oxidative damage.

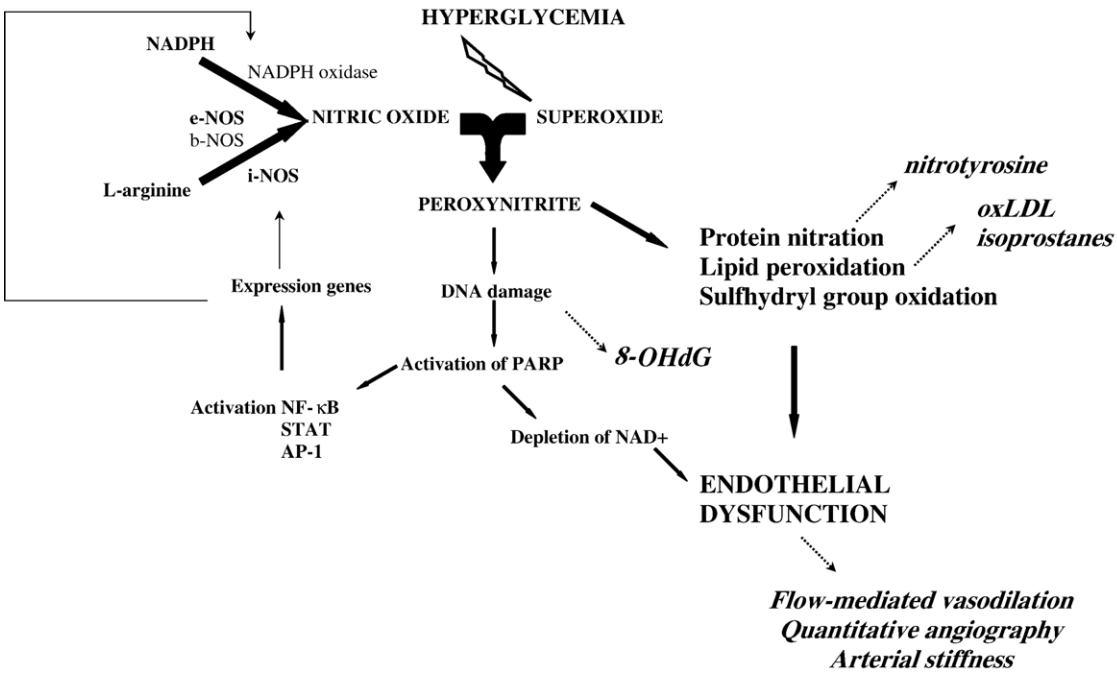

Fig. 1. Hyperglycemia determines an overproduction of superoxide by the mitochondrial electron transport chain and favors increased expression of NADPH and iNOS by activation of NF-κB. Consequently, hyperglycemia results in an overproduction of superoxide and nitric oxide. This condition is associated with overgeneration of a toxic reaction product, the peroxynitrite anion. Peroxynitrite is cytotoxic because it leads to oxidation of sulfhydryl groups in protein, peroxidation of lipids, and nitration of amino acids. Moreover, peroxynitrite is a potent initiator of DNA single-strand breakage, which is a stimulus for activation of PARP, which depletes the intracellular concentration of its substrate, NAD+, slowing the rate of glycolysis, electron transport, ATP formation, and ADP ribosylation of the GAPDH. This process results in acute endothelial dysfunction in diabetic blood vessels, which contribute to the development of diabetic complications. Assessment of endothelial cell function is now possible with a consistent number of biomarkers: DNA damage can be detected with 8-OHdG, nitrotyrosine is a marker of protein nitration, whereas oxLDL and isoprostanes are markers of lipid peroxidation. Moreover, a direct measure of endothelial dysfunction is possible with flow-mediated vasodilation, quantitative angiography, and arterial stiffness. NADPH indicates nicotinamide adenine dinucleotide phosphate (reduced form); AP1, activating protein 1; STAT, signal tranducer and activator of transcription; NAD+, nicotinamide adenine dinucleotide (oxidized form).

2.3. DNA damage

Peroxynitrite is a potent initiator of DNA single-strand breakage, which is an obligatory stimulus for the activation of the nuclear enzyme poly(adenosine diphosphate [ADP] ribose) polymerase (PARP) [37]. These reactive species trigger DNA single-strand breakage, which induces a rapid activation of PARP [37]. Poly(ADP-ribose) polymerase activation in turn depletes the intracellular concentration of its substrate, nicotinamide adenine dinucleotide (oxidized form), slowing the rate of glycolysis, electron transport, and adenosine triphosphate (ATP) formation, and produces the ADP ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A recent study shows the pivotal role of ADP ribosylation of GAPDH on activation of the 3 major pathways of hyperglycemic damage (AGE formation, activation of PKC, and hexosamine pathway flux) [37]. This process results in acute endothelial dysfunction in diabetic blood vessels [38]. In addition to the direct cytotoxic pathway regulated by DNA injury and PARP activation, PARP also appears to modulate the activation of nuclear factor κB (NF-κB) and the expression of genes, including the gene for intercellular adhesion molecule 1, iNOS, and nicotinamide adenine dinucleotide phosphate (reduced form) oxidase [38].

2.4. 8-Hydroxy-2′deoxyguanosine

Oxidative changes to DNA can occur by many routes including oxidative modifications of the nucleotide bases or sugars, or by forming cross-links. In vivo, damaged DNA is repaired by endonuclease- and glycosylase-liberating deoxynucleotides and bases, respectively, that are excreted in urine. DNA damage can be evaluated by measuring 8-hydroxy-2′deoxyguanosine (8-OHdG) and its free base 8-hydroxyguanine in blood cells and/or urine [39]. Studies have shown that hyperglycemia independently increases the levels of 8-OHdG in urine and plasma of patients with type 2 diabetes mellitus and the level of urinary 8-OHdG in diabetes correlated with the severity of diabetic nephropathy and retinopathy [40-42]. Moreover, in human atherosclerotic plaques, there were increased amounts of 8-OHdG [43].

3. Effects of sulfonylureas beyond metabolic control: the antioxidant action of gliclazide

Gliclazide is an oral hypoglycemic agent that belongs to the class of sulfonylureas: it has been demonstrated effective and safe in numerous clinical trials and in clinical practice. Sulfonylureas stimulate insulin secretion from pancreatic beta cells by inhibiting ATP-sensitive potassium channels. Furthermore, various studies have demonstrated, both in vitro and in vivo, that gliclazide shows antioxidative potential, independent from its hyperglycemia-lowering effect.

3.1. In vitro evidence

Gliclazide has been established as a general free radical scavenger in vitro: in comparison with glibenclamide, which lacked any effect up to a 25 μg/mL concentration, gliclazide induced a strong concentration-dependent inhibition of free radical generation at therapeutic concentrations [44].This property convincingly explains the ability of gliclazide to protect, in vitro, LDL from oxidation [45]. However, the antioxidative action of gliclazide may also contribute to explaining why the concomitant incubation of bovine aortic endothelial cells with gliclazide and native LDL is followed by a dose-dependent decrease in monocyte adhesion to the endothelial cells induced by oxidized LDL [46]. In the same conditions, glibenclamide did not have any effect [46]. Consistent with this property is the finding that the pretreatment of endothelial cells with 10 μg/mL gliclazide significantly decreases glycated albumin-stimulated adhesion molecule expression as well as the NF-κB activation [47]. Similarly, human aortic smooth muscle cells with native LDL were exposed to gliclazide (1-10 μg/mL). The incubation of human aortic smooth muscle cells with gliclazide resulted in a marked decrease in oxidatively modified LDL-induced monocyte chemoattractant protein 1 and human heat shock protein 70 expression, both at the gene and protein levels [48]. Finally, it has recently been shown that pretreatment with gliclazide or antioxidants such as vitamin E or *N*-acetyl-L-cysteine resulted in a significant decrease in AGE-induced production of vascular endothelial growth factor and activation of PKC, mitogen-activated protein kinase, and NF-κB signaling pathways in bovine retinal endothelial cells [49].

In 2000, Vallejo et al [50] designed a model of impairment of endothelium-dependent vascular relaxation by exposing both isolated aortic cells and mesenteric vessels taken from normal rats to glycosylated human oxyhemoglobin (GHHb). Preincubation of the vessels with gliclazide (100 nmol-10 μmol) prevented the impairment of the endothelial relaxation. In addition, 10 μmol gliclazide also prevented the reduction caused by GHHb in the relaxation induced by exogenous NO. Determination of superoxide anion release measured by the reduction in ferricytochrome *c* indicated that GHHb produced significant amounts of these free radicals that were inhibited by gliclazide. The impairment of endothelium-mediated responses was also prevented by 100 U/mL superoxide dismutase (SOD) or 10 μmol/L ascorbic acid but not by glibenclamide.

3.2. In vivo evidence

Seventeen type 2 diabetic patients treated with various sulfonylureas were switched to gliclazide [51]. Glycemic control remained unchanged during the whole follow-up. In this setting, on gliclazide treatment, peroxidized lipids significantly dropped [51]. These effects of gliclazide on oxidative stress in clinical conditions have been confirmed by Jennings et al [52], who have documented in gliclazide-

treated type 2 patients with retinopathy a highly significant and sustained decrease in peroxidized lipids and an increase in erythrocyte SOD activity. Interestingly, the glucose control did not differ between therapeutic groups, which emphasizes the hypothesis that the effect results from the molecule gliclazide itself rather than from a general improvement in the metabolic control. A significant decrease in platelet aggregation was also observed with gliclazide treatment, which suggests a causal relationship between the gliclazide free radical scavenging property and its antiplatelet activity.

In 2000, O'Brien et al [53] combined their in vitro assay with a clinical investigational protocol. This study was actually a prospectively planned substudy of a larger multicenter clinical trial to compare the efficacy of the gliclazide 30 mg modified release (MR) formulation with the 80 mg gliclazide formulation in patients with type 2 diabetes with no control arm using a comparator such as a different sulfonylurea or any other oral antihyperglycemic agent. Analysis was performed on 44 patients, 22 receiving gliclazide MR and 22 receiving the 80 mg gliclazide formulation. Both groups were similar with regard to age, duration of diabetes, and rate of complications. Subjects were taken off all antidiabetic medication for 2 weeks before randomization. After this period, baseline blood samples were taken and trial medication was started. Starting daily doses were 1 tablet in each group; doses were titrated upward over the following 4 months aiming for a fasting blood glucose of less than 6.7 mmol/L. The mean daily dose at the end of this titration period was 2 tablets in each group. Blood samples for oxidative parameters were taken at baseline, and after 4 and 10 months of treatment. There was no significant difference in the HbA_{1c} value between groups and from baseline. Treatment with gliclazide improved all of the oxidative parameters measured. Both the gliclazide MR and 80 mg formulation were effective in an identical manner. There was a progressive increase in total plasma antioxidant capacity from baseline to the end of the study, suggesting that gliclazide cumulatively enhanced the radical trapping capacity of the plasma. Thiols and SOD increased as well, both of them having a role in quenching free radicals. Isoprostanes, a marker of general oxidative stress, were reduced during gliclazide treatment. It is possible that the reduction in glucose, induced by gliclazide, contributed to the improvement in oxidation status. However, the change in glucose was modest and the improvement in oxidation status continued after completion of the 4-month dose titration phase, although glycemic control remained stable thereafter.

The antioxidative property of gliclazide convincingly impacts on the vascular system in diabetes. Fava et al [54] studied both the antioxidative potential in vivo and the effect of gliclazide on vascular reactivity. In this experiment, as patients were previously treated, blood glucose control remained unchanged from baseline and similar in both groups, which excludes any glucose-related "bias effect." Thirty type 2 diabetic patients received glibenclamide (n = 15) or gliclazide (n = 15) in a 12-week, randomized, observer-blinded, parallel study. Blood pressure responses to an intravenous bolus of L-arginine were measured pre- and posttreatment. Gliclazide but not glibenclamide significantly reduced systolic and diastolic blood pressure ($P = .0199$ and $P = .00199$, respectively, 2-way repeated measures analysis of variance) in response to intravenous L-arginine. This provides the first demonstration that gliclazide significantly enhances NO-mediated vasodilatation and thus improves vascular reactivity in type 2 diabetic patients.

4. Beta-cell damage and oxidative stress: the possible protective role of gliclazide

Type 2 diabetes mellitus is a heterogeneous disease, resulting from a dynamic interaction between defects in insulin secretion and insulin action. However, some new hypotheses are coming out: because evidence suggests that overnutrition, insulin resistance, impaired glucose tolerance, diabetes, and cardiovascular disease share in common the presence of measurable oxidative stress, oxidative stress itself is proposed as the pivotal common and persistent pathogenic factor mediating the emergence of insulin resistance, while producing, in the meantime, the increased cardiovascular risk condition that is typically associated with prediabetic and diabetic status by facilitating/enhancing macrovascular complications [55]. Furthermore, new evidence is also convincingly demonstrating that the same process of overproduction of superoxide at mitochondrial level is involved in the beta-cell damage induced by both hyperglycemia and free fatty acids, and that this effect leads to the clinical development of beta-cell failure and the appearance of overt diabetes [55]. Interestingly, gliclazide has been proved to have a protective effect on oxidative stress-induced beta-cell damage [56].

5. Conclusion

Basic and clinical evidence suggest that gliclazide works as an antioxidative drug, independently from its ability to reduce hyperglycemia. The availability of a compound that simultaneously decreases hyperglycemia, restoring insulin secretion, and inhibits oxidative stress produced by high glucose seems to be an interesting therapeutic prospect for the prevention of vascular complications of diabetes.

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