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Antioxidant defenses in diabetes mellitus: a clinical and molecular approach

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Abstract

Oxidative and nitrosative stress reactions are implicated in cell and organ damage due to diabetes mellitus. In both type 1 and type 2 DM there is a massive oxidative and nitrosative stresses which have been associated with intensive changes on both antioxidant enzyme systems (SOD, CAT, GPx, GSH) and total antioxidant capacity (TAC) causing peroxidative damage to lipids, proteins, nucleic acids and carbohydrates which can be used as DM biomarkers. Molecular pathophysiology of diabetes mellitus envolves induction of the the NFkB and p38-MAPK cell signaling pathways by Rac, TNF-α, TLR4, decrease of antioxidant defenses, and direct mitochondrial damage. Knowledge of molecular pathways is essential for research of newer preventive and therapeutic approaches in diabetes mellitus complications (atherosclerosis, myocardial damage, endothelial dysfunction, and renal failure).

Introduction

When oxygen, nitrogen or chlorine reactive species are excessively formed in cells and tissues they are implicated in many pathophysiological and disease events, including diabetes mellitus (DM) [1,2]. As a consequence of the oxidative and nitrosative stresses occurs lipid peroxidation (measured by biomarkers like malonaldehyde, 4-hydroxynonenal, and conjugated dienes), protein and aminoacid oxidation (evaluated by protein carbonyls), nucleic acid oxidation (quantifyied by DNA oxidized bases) and carbohydrate oxidation (measured by glycosilation products like glycated hemoglobin [HbA1] and advanced glycation end products [AGEs] commonly found in DM animal models and human patients [3-8].

Oxidative and nitrosative stresses are situations characterized when the production of free radicals surpass the antioxidant defense levels, resulting in many peroxidative and nitrosative damaging reactions which in turn cause since DNA injury and mutations until cell death by necrosis or apoptosis [2].

During at least four decades, an extensive number of research groups have been studied the oxidative and nitrosative stress biomarkers as well as the different antioxidant defense mechanisms by measuring the antioxidant enzymes (superoxide dismutase [SOD], catalase, glutathione reductase [GSH], glutathione peroxidase [GPx], ceruloplasmin, metallothioneins) [1,2,9]. In DM patients its well established that oxidative and nitrosative stress decreases cell antioxidant enzyme defenses, especially the GSH, and increase the SOD activity in order to detoxify the superoxide anion mitochondrial overload [7,8,10,11]. In macrophages of alloxan-induced diabetes, there were higher levels of SOD activity which were further stimulated by melatonin activity, but decreased by insulin action [12].

In the beginning of the 90's, Miller et al. [13], based on techniques from the 1980 decade, had developed a new total antioxidant capacity assay, which was called by "total antioxidant capacity" (TAC). The advantage of this assay is to measure the antioxidant capacity of virtually all elements and compounds of a biological sample (blood, urine, feces, tissue sample), vegetable or fruit extract or even foods or pharmaceuticals [14-16].

This article review and update the clinical, cellular, and molecular knowledge of free radicals and antioxidant defenses in diabetes mellitus.

Ethiopathogenesis of diabetes mellitus I and II

Diabetes mellitus has been conceptualized as a group of metabolic disorders characterized by hyperglicemia as a result of insulin secretion failure and/or lacking of insulin action on target cells. The chronic diabetic hyperglicemic state has been related to long-term organ damage especially to the heart, endothelium and blood vessels, nerves, kidney, and eyes [17-19].

Type I diabetes mellitus

The highly toxic and reactive hydroxyl free radicals are released as a consequence of the pro-inflammatory immunoglobulin-mediated beta cell attack in type I diabetes mellitus (T1DM) patients [20]. In T1DM genetic as well environmental factors induces free radical release by beta cells which in turn activates resident macrophages [21]. Those resident pancreatic macrophages are the primary sources of free radicals in which also participates the recruited and activated macrophages, dendritic cells, and in a later phase the specifically activated T-lymphocytes that decisively contribute to destruction of the insulin secreting β cells [21,22]. Free radical release in T1DM patients is triggered by activation of the NFkB pathway and consequently the

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induction of TNF-α and IL-1β cytokines from antigen-presenting cells (macrophages and dendritic cells) [21,22].

Type II diabetes mellitus

As a consequence of glucotoxicity and lipotoxicity there is a chronic loading of oxygen and nitrogen free radicals in type 2 diabetes mellitus patients [20].

In T2DM, although genes are plausible associated with the disease risk, lacking of exercise, obesity, poor dietary habits, poor sleeping, and other risk factors have been implicated in disease causality [22-26].

Is Diabetes mellitus associated with decrease total antioxidant capacity?

It should be emphasized oxidative stress and consequently the depletion of cell antioxidants is not always present in diabetes, since it correlates with disease progression and severity. In an animal model of diabetes, increased lipid peroxidation was observed with no further changes in antioxidant parameters (SOD, ceruloplasmin, GPx, GSH and TAC) [27]. In other experimental model of diabetes authors observed decreased levels of renal catalase despite of increased levels of heart catalase, kidney and heart SOD, and liver GPx [28].

T2DM patients, with or without proteinuria, a biomarker of renal damage, had very lower TAC levels compared with the control group (DM-free) (1.7 mmol/L and 1.4 mmol/L *versus* 2.7 mmol/L) [3]. In the same manner, it was verifyied a significant impairment of plasma TAC and GSH and an increase of lipid peroxidation in T1DM children patients [5]. Diabetic men had increased values of both lipid peroxidation products and advanced glycoperoxidation end products (AGEs), and reduced TAC in seminal plasma compared to non-diabetic men [29].

Among diabetic patients, obesity has an additional effect on decrement of the TAC, SOD, and vitamin C levels [30].

A study with young adult healthy subjects observed an inverse association between dietary TAC and serum glucose, systolic blood pressure, and free fatty acids [31].

A recent study covering patients with diabetic complications observed a significantly increase on oxidative stress markers as well as the total antioxidant capacity [32].

However, women with gestational diabetes mellitus had lower levels of total antioxidant capacity which was correlated with lower vitamin E and zinc status [33].

An interventional study with pomegranate juice supplementation decreased LDL oxidation and increased TAC in type 2 diabetes mellitus patients [34].

Another study with type 2 diabetic men revealed higher oxidative stress and both lower TAC and zinc values [35] which were corroborated by other studies in different populations [36-40].

In fact, diabetes mellitus has been associated with disruption of cellular zinc turnover with increased loss of zinc and oxidative stress [35,41,42].

Hyperglycemia as the great villain in diabetes mellitus: induction of massive depletion of antioxidant defenses

Hyperglycemia induces impairment on functional activity of the pancreatic islet beta cells, an effect which has been associated to an intense and deleterious oxidative stress [43]. While the functionality of beta cells is preserved, the plasma TAC levels remain higher [44]. Prediabetes pathogenesis characterized by insulin resistance and hyperinsulinemia is followed by the failure on glycemic control and finally the diabetic hyperglycemic status [45]. During the DM pathogenesis plasma TAC levels are being progressively reduced [44-48]. It is important to note that patients with acute pancreatitis due to alcoholism, biliary problems, trauma or idiopathic origin had presented yet a 40% decrease on serum TAC [49]. When the subject becomes diabetic his antioxidant defenses are compromised and this has been correlated with progressive DNA damage [50]. Another study has pointed out that diabetic animals had increased lipid peroxidation than the normal group; and that DNA oxidative damage was higher among those animals with metabolic syndrome features [51]. In this respect, increased oxidative DNA damage is suggested to occur in insulin-resistant prediabetics as well as T2DM patients [52]. Diabetic patients without glycemic control presented reduction on plasma TAC, which was partially recovered among DM patients with adequate glycemic control [46].

An interesting Spanish study evaluated erythrocyte antioxidants from healthy subjects and diabetic patients with or without microvascular complications and found decreased levels of erythrocyte GPX, GSH and increased levels of erythrocyte SOD in both diabetic groups compared to controls [52]. The same study also revealed increased lipid peroxidation and hemoglobin glycation among the diabetic groups, whereas advanced oxidation protein products were higher among those diabetics with microvascular complications. Although TAC can not be affected in well controlled T2DM patients [53], it has been depleted in uncontrolled type 2 DM patients where glycated hemoglobin (HbA_{1c}) was positively associated with both lipid peroxidation products and c-reactive protein (PCR), whereas HBA_{1c} was inversely associated with SOD and TAC levels [54]. This depletion of intracellular GSH reserves was also observed in the liver and kidney [55]. This depletion can be partially explained by the overexpression of the NADPH oxidase in kidneys and subsequent free radical damage in diabetic nephropathy [56], since impaired function of NADPH oxidase has been suggested to have a central role on activation of other oxidative stress enzymes [57]. It has been found that DNA oxidation and nitrosative stress with excessive production of nitric oxide and peroxynitrite were implicated in progression of chronic diabetic nephropathy [58]. It has been suggested that bilirubin and biliverdin can afford protection against diabetic nephropathy by blocking NADPH oxidase [59].

A recent study reported that DM patients had higher levels of lipid peroxidation products, uric acid, total cholesterol, LDL-cholesterol, and tryglycerides, and lower values of enzymatic antioxidants (SOD and GSH) and of HDL-cholesterol [60], confirming previous studies [61].

A previous study confirmed that diabetic polyneuropathy was linked to a significantly lowering on blood TAC [62]. However, in a recent clinical study, TAC was not significantly decreased even in the presence of SOD and GPX depletion [63]. It is possible that this discrepancy could be due to different clinical stages of the patients from the two studies. Further work is necessary to investigate the possible clinical correlations between antioxidant defense and nervous system commitment in diabetic subjects. Diabetic patients with retinopathy had also increased levels of lipid peroxidation and reduced serum concentrations of SOD, GSH, and vitamin C [64]. In aqueous humor of hyperglycemic animals, it was found decreased levels of TAC, ascorbic acid, and GSH [65], which can contribute to diabetic retinal damage.

Molecular pathways of diabetes mellitus pathogenesis

Investigating the molecular shape of diabetes, it has been reported mitochondrial dysfunction and apoptosis of myocardiocytes from diabetic human heart [66]. In this special context, the disruption of the uncoupling mitochondrial protein-2 (UC-2) into the inner membrane has been associated with a phenotype of massive and chronic free radical release [67].

Considering the brain of diabetic rats it has been found impaired thioredoxin response, decreased levels of SOD, and increased levels of GPX [68], confirming previous study in which hyperglycemia had induced inhibition of thioredoxin function by the action of p38-MAPK triggering of thioredoxin-interacting protein (Txnip), causing oxidative stress [69]. In rat experimental model of diabetes, hyperglycemic state had induced Txnip expression which has been implicated with increased inflammation and gliosis [70]. It has been suggested nitric oxide blocks expression of the Txnip improving thioredoxin activity [71].

This mechanism of Txnip inhibition of thioredoxin function associated with increased oxidative stress has also been demonstrated in glucose-exposed endothelial cells [72]. In the same manner, the inflammatory cytokine tumor necrosis factor-α (TNF-α) promotes insulin resistance through p38-MAPK pathway [73]. Beyond higher levels of TNF-α, other important inflammatory mediators (C-reactive protein and interleukins 6 and 8) were also increased among gestational diabetes mellitus patients [74]. TNF-α also causes activation of NFkB which mediates cell signaling through IKK-b inducing insulin resistance and coronary arteriolar dysfunction [75]. NFκB activation triggers potent inflammatory reactions found in acute pancreatitis, DM, metabolic syndrome, brain injury, Alzheimer's disease, and diabetic nephropathy [76]. Into the cortex of the kidneys, NFkB activation was associated with increased expression of plasminogen activator inhibitor-1 (PAI-1) and enhanced expression of the intercellular adhesion molecule-1 (ICAM-1) which were associated with higher degree of renal inflammation in diabetic rats [77]. The activation of p38-MAPK pathway also impairs insulin action into the myocardium increasing free radical-mediated infarction [78]. Diabetic mice with abbrogated gene expression of the p38-MAPK had decreased levels of free radical production as well as lower degree of myocardial damage and apoptosis compared with normal gene expression mice [79]. Cardiomyocytes were protected against oxidative stress-induced hyperglycemic toxicity through inhibition of the p38-MAPK signaling pathway [80].

Another downregulator of the Txnip is represented by activation of the AMP-activated protein kinase (AMPK) which stimulates forkhead transcription factor 3 (FOXO3) inhibiting reactive oxygen species production [81]. This mechanism of AMPK activation has also been suggested to protect kidney tissues from diabetic rats [82].

In the same context, beta cells can adapt against free radical damage by expressing the nuclear-factor E2-related factor (Nrf-2) which triggers the expression of many different antioxidant genes, helping to rescue beta cells from the genotoxic free radical insults [67]. The Nrf-2 induction leads to expression of antioxidant genes and attenuation of the NFkB inflammatory pathway protecting endothelium, the nephron, and other structures against hyperglicemic-load induced toxicity [83-86].

Other molecular regulator of free radical release in diabetes mellitus is represented by Rac proteins (Rac1-ubiquitously expressed, Rac2-restricted to hematopoietic tissues, and Rac3-restricted to the central nervous system). These proteins are small Rho GTPases (Rac1, Rac2 and Rac3) which can provoke endoplasmic reticulum stress and induce mitochondrial ROS production via NFκB and Akt signaling mechanisms [87]. This endoplasmic reticulum stress is mediated by NFκB, JNK, and p38MAPK pathways and operates the endothelial dysfunction in diabetes mellitus patients [88]. Another pathway of endothelial dysfunction in DM is represented by hyperhomocysteinemia, an independent risk factor for both cardiovascular and cerebrovascular diseases [89,90]. Excessive blood homocysteine has been implicated in massive oxidative stress and decreased plasma total antioxidant capacity which could be reversed by both antioxidant and L-arginine supplementation [91,92]. Hyperhomocysteinemia, a common feature in diabetes mellitus, has been associated with increased cardiovascular disease risk and polyneuropathy in DM patients [93-95].

In diabetic cardiomyopathy, hyperglycemia causes activation of Rac1 signaling disturbing endoplasmic reticulum and mitochondria resulting in free radical overload and myocardium cell death [96,97]. Under excessive levels of glucose, Rac1 activation induced expression of a cell surface lipid transporter, the CD36, which triggers mitochondrial dysfunction, oxidative stress and apoptosis of beta cells [98]. In the same study, inhibition of Rac1 abrogated the deleterious effects of high glucose on pancreatic beta cells. In fact, inhibition of Rac1 was associated with abrogation of membrane NADPH oxidase activity and suppression of free radical production [87,99].

Hyperglycemia also triggers activation of the Toll-like receptors-4 (TLR4) in myocardiocytes membrane, a cell surface receptor responsible for pathogen recognition by the immune cells [100]. In this study, the inhibition of TLR4 blocks apoptosis, NADPH oxidase activity and free radical release in cardiomyocytes. The same mechanism is responsible for doxorubicin-induced myocardium toxicity [101]. Another study also demonstrated that blocking TLR4 signaling also rescue neuronal survival in diabetic rats [102]. Chronic triggering of TLR4 pathway induces both insulin resistance and amyloid beta deposition [103]. It has been suggested that TLR4 action is mediated by NFkB signaling pathway [104]. These complex prodiabetic and antidiabetic molecular mechanisms are represented in Figure 1.

Other important proteins involved in oxidative stress and mitochondrial dysfunction in diabetic experimental models include frataxin, duodenal homeobox factor-1 (PDX-1), MafA, and forkhead box protein O1 (FOXO1) [41].

Some important classic laboratory features of diabetes mellitus (hypercholesterolemia, hypertrygliceridemia, glycemia, arterial blood pressure, billirrubin, insulin resistance, creatinine, and C-reactive protein) are inversely associated with TAC, suggesting increased risk of atherothrombosis [105-109]. The unique DM biomarker that is positively associated with total antioxidant capacity is the uric acid [110,111], which has also been positively associated with DM risk and prognosis [111,112]. Increased uric acid levels have been pointed out to be the possible explanation for increased TAC levels in diabetic patients in some studies [32].

Total antioxidant capacity has a potential to be a diabetes diagnostics and therapeutics biomarker since it has been found to be normal or increased in controlled T2DM patients and reduced in uncontrolled and complicated T2DM subjects [44-48,53,54,62,65,113].

However, laboratorial evaluation of antioxidant capacity in diabetic patients should include the total antioxidant capacity test and other biochemical analysis listed in Table 1.

Table 1. Laboratory examination of antioxidant-oxidant balance in diabetic patients.

Test	Use/samples	Comment
Total Antioxidant Capacity (TAC)	Cells, tissues, blood, saliva, and urine	Should be controlled regarding physiological changes
Superoxide dismutase (SOD)	Same. It indicates oxidation	Should be measured at different time intervals
Catalase (CAT)	Same. It indicates antioxidant defense	Should be measured at different time intervals
Lipid peroxides	Same. The first products of lipid peroxidation	Indicates only the inicial phase of lipid peroxidation
Conjugate dienes	Same. The first products of lipid peroxidation	Same as above.
Malonaldehyde	Same. The final product of lipid peroxidation.	Could react with other compounds, yielding false results
Glutathione (GSH) and Glutathione Peroxidase (GPX)	Same. Indicate cell antioxidant defense	
Glycated Hemoglobin (HBA1C)	Blood	Specific marker of oxidation in diabetic patients

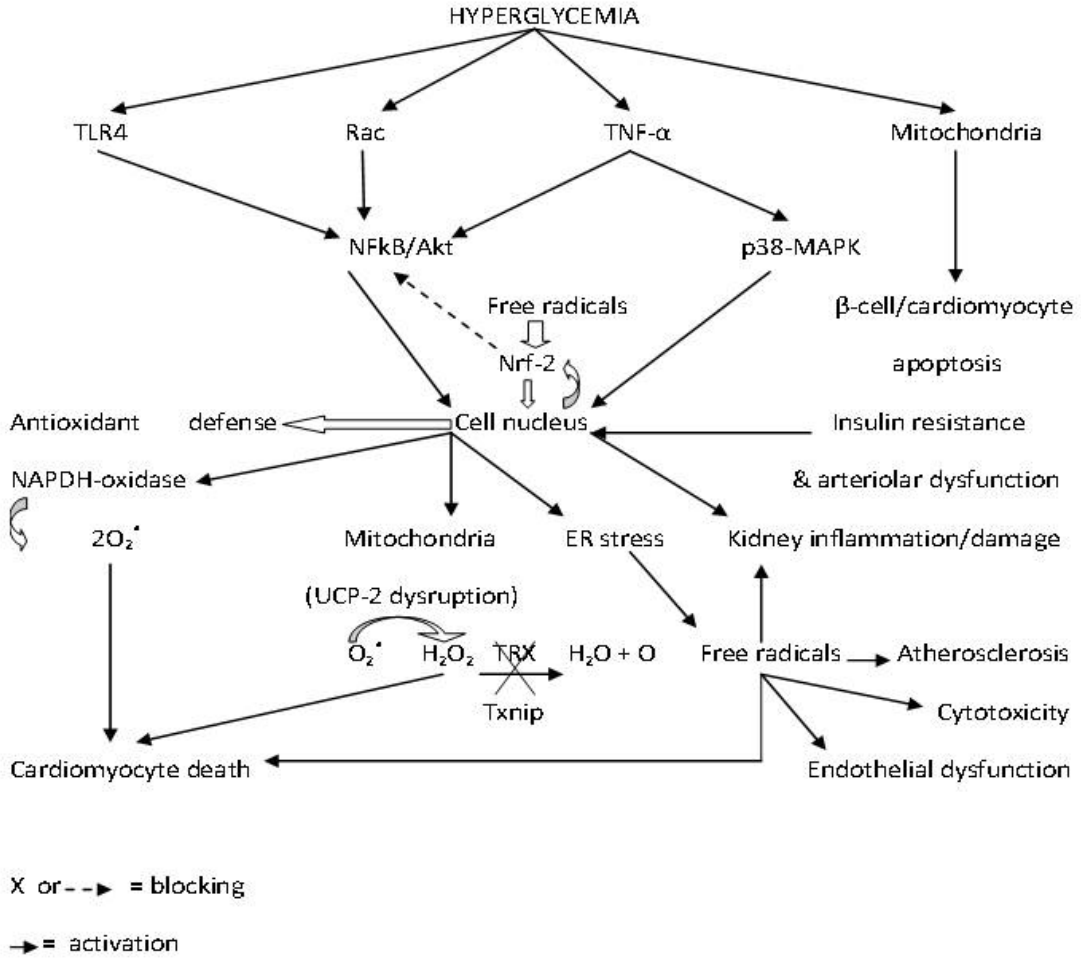

Figure 1. Molecular roles of hyperglycemia in diabetes mellitus-induced pathophysiology.

Drinking of sulphurous water could decrease oxidative stress and improve the glycemic values in diabetes mellitus [114].

Beyond impairment of the antioxidant defenses, DM causes molecular dysfunctions that can be targeted for development of newer therapeutic and preventive strategies like dietary intake of antioxidant-rich foods and exercise training [14,115-124].

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