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Oxidative Stress and Diabetes

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Abbreviations

ROS: reactive oxygen species
GSH: glutathione
NOD mice: non-obese diabetic mice

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Abstract

Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, protein glycation, and the subsequent degradation of glycated proteins. High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes, and lipid peroxidation. Accumulated evidence also indicates that oxidative stress-activated signaling pathways mediate insulin resistance and β-cell dysfunction. These consequences of oxidative stress can promote the development of diabetes complications. Therefore, oxidative stress, antioxidant defense, cellular redox status should be regarded as the central players in diabetes and its complications.

Introduction

Diabetes mellitus is a metabolic disorder with characteristics of hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of diabetes is not well defined, genetic components, viral infection, autoimmune disease, and environmental factors have been implicated in the disease [1, 2]. Evidence showed that the chronic elevation of plasma glucose causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, and macrovascular and microvascular damage [3, 4].

Experimental diabetes can be induced in rodents by feeding alloxan or strptozotocin. It is well established that alloxan works by generating reactive oxygen species and the β-cells of pancreatic islets are specifically destroyed [7]. It is assumed that free radicals that alloxan generated kill the islet cells. Increased oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, is a widely accepted participant in the development and progression of diabetes and its complications [5, 6]. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses. Glucose oxidation is the main source of free radicals. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcriptional factors, advanced glycated end products (AGEs), and protein kinase C. This review mainly focuses on the correlation between oxidative stress and diabetes as well as the pathogenesis of oxidative stress in the diabetes complications.

Oxidative stress and the onset of diabetes

There are two types of diabetes, type 1 and type 2. Type 1 diabetes is characterized to have insufficient insulin and hyperglycaemia and insulin treatment is very effective in reducing

the plasma glucose level in type 1 diabetes patients, while type 2 diabetes mellitus is characterized as having insulin resistance and hyperglycaemia and treatment of insulin is not very effective in reducing the plasma glucose levels in type 2 diabetes patients. Experimental diabetes can be induced in rodents by feeding alloxan or streptozotocin. Evidence showed that alloxan and strptoxotocin caused diabetes by generating reactive oxygen species [7,8]. This raised the question whether oxidative stress can cause diabetes mellitus.

Oxidative stress and type 1 diabetes mellitus

It is widely accepted that type 1 diabetes mellitus is an autoimmune disease involving T-cell-mediated destruction of pancreatic β-cells. A large genetic component has been implicated in the causation of this disease in that the concordance rate in monozygotic twins is about 13-15% [9]. The MHC DQ region has been identified as that in which the predisposing or protecting determinant lies. However, the diabetes-prone MHC genotypes in human only account for small proportion of this disease. Some other genes might also involve in the etiology. Furthermore, the moderate concordance rate in monozygotic twins suggested that non-genetic components (environmental factors) must be involved.

It is known that alloxan diabetes in rodent is the result of destruction of pancreatic β-cells by radicals, the combined result of active redox enzymes and inadequate antioxidant defences in those cells [7]. And it is very difficult to induce alloxan and streptozotocin diabetes in human pancreatic cells because these cells have very higher levels of SOD and catalase compared with rodent pancreatic cells [10]. Nevertheless, certain xenobiotics can induce diabetes in human, which can be due to the generation of free radicals. Vacor, which could block complex I of the mitochondrial respiratory chain, can lead to generation of $O_2^{\cdot -}$ by causing leakage of reducing

electrons onto O_2, possibly via ubisemiquinone. It is worthwhile taking a look at maternally inherited diabetes. This type of disease is trigged by defects in mitochondrially coded gene (commonly in $tRNA^{Leu(UUR)}$) [11]. This defection seems to produce defective or insufficient complex I. High levels of heteroplasmy in β-cells would produce defective mitochondria, which presumably would generate radicals. It is pretty interesting to look closely the underlying mechanisms of streptozotocin-induced diabetes. There are two ways in which streptozotocin can be used to induce diabetes in rodent. The traditional procedure is to use a single toxic dose which causes β-cell death in 2-4 days. The alternative is to apply multiple low doses, which leads to a more immunologically based disease with insulitis and the activation of C-type retroviruses, perhaps resembling more closely type 1 diabetes in human. It was showed that streptozotocin leads directly to generation of H_2O_2 in cells, more actively indeed than that of alloxan [8]. It is reported that SOD prevents the impairment of islet microcirculation that is an early consequence of streptozotocin in rats [12]. It is apparent that streptozotocin works by a free radical mechanism like alloxan.

Oxidative stress and type 2 diabetes mellitus

A large genetic component also exists in type 2 diabetes mellitus, and the concordance rate in identical twins is around 90%. This suggests that genes essentially determine the disease in the appropriate environment. Insulin resistance is one of the major characteristics of type 2 diabetes mellitus. If the insulin resistance can result from oxidative damage, then a prediction would be that chronic oxidative stress would lead to hyperinsulinaemia if plasma glucose is clamped at normal level by infusing the required insulin. Following experiment supports this hypothesis. Fat-fed mice, infused with insulin and glucose, showed impaired glucose clearance.

However, glucose clearance was slowed 2-3 fold further by prior feeding with low-dose of streptozotocin, which had little hyperglycaemic effect by itself and led to chronic oxidative stress [13]. The streptozotocin effect was presumably not on the β-cell with continuous supplement with external insulin, but seemed to have caused insulin resistance directly. Evidence showed that membrane proteins are early targets of oxidative stress. An early event in the induction of the multiple low dose of streptozotocin diabetes is the gradual loss of GLUT2 glucose transporter from islet cell membranes, which could cause insulin resistance [14].

Protein Glycation

Glycation reaction, also known as nonenzymatic glycosylation or Maillard reaction, is the reaction that free amino groups of protein react slowly with the carbonyl groups of reducing sugars to yield Schiff base intermediates, which undergo Amadori rearrangement to stable ketoamine derivatives. These Schiff bases and Amadori products subsequently degrade into α-dicarbonyl compounds, such as deocyglucosone, nethylglyoxal, and glyoxal, which are more reactive than the parent sugars with respect to their ability to react with amino groups of proteins to form inter- and intra-molecular cross-links of proteins called advanced Maillard products or advanced glycation end products (AGEs). A general scheme of the glycation reaction is shown in Figure 1. The formations of AGEs not only cause damage directly but also the production of free radicals in the glycation reaction or by glycated proteins cause damage. For example, there are three types of free radicals generated in the reaction between methylglyoxal and L-lysine [15]. These three radicals are (1) the cross-linked dialkylimine radical cation; (2) the methylglyoxal radical anion; (3) the superoxide radical anion generated only in presence of oxygen. And the generation of cross-linked radical cations and methylglyoxal radical anions does not require

transition metal ions or oxygen. The cross-linked proteins will be more persistent and could be a reactive site for putative reducing and oxidizing molecules, which produce free radicals for a long duration [15]. These free radicals generated by glycation reaction or by the glycated proteins will initiate cell damage, such as lipid peroxidation and also the diabetes complications in diabetes patients.

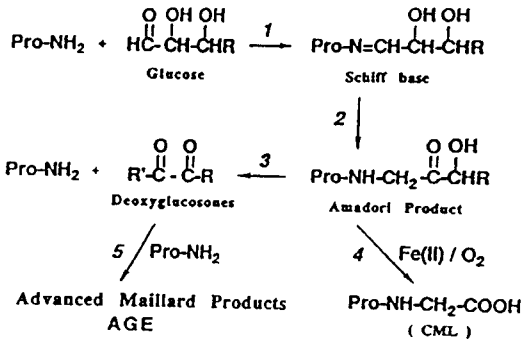

Figure 1. A general schema of the glycation of Maillard reaction. Adapted from [15].

Hyperglycaemia and oxidative stress

Oxidative stress is the imbalance between production and removal of ROS. Increased oxidative stress, which contributes the pathogenesis of diabetes complications, is the consequence of either enhanced ROS production or attenuates ROS scavenging capacity. Several mechanisms, including autooxidative glycosylation, formation of AGEs, and increased polyol pathway activity contribute to increased oxidative stress (Figure 2).

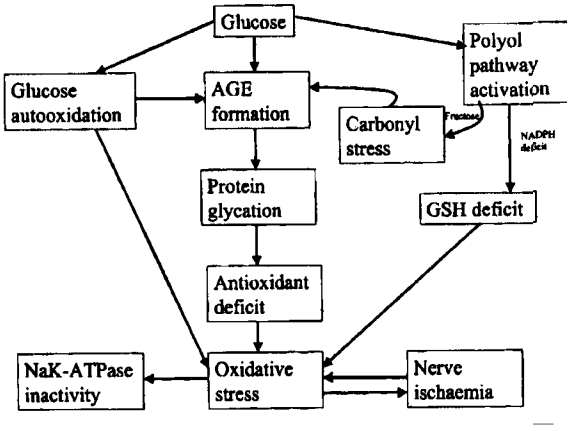

Figure 2. Different pathways leading to hyperglycaemia-associated increased oxidative stress. AGE, advanced glycation end products; GSH, reduced glutathione. Adapted from [18].

Free radicals can be generated in glucose oxidation, which is believed to be the main source of free radicals. In its enediol from, glucose is oxidatized in a transition-metal-dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide aion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transitional metals, can lead to production of extremely reactive hydroxyl radicals [18]. Superoxide anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals. Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway to generate free radicals [19].

Another important source of free radicals in diabetes is interaction of glucose with proteins to lead to protein glycation. Glycation involves the condensation of glucose with the ε-amino group of lysine, the α-amino group of an N-terminal amino acid or the amines of nucleic acids, which will result in the formation of AGEs as discussed early. The increased availability

of glucose in diabetes mellitus induces enhanced production of AGEs. This process has been described as glucosylation, and is probably the major source of increased generation of ROS in diabetes patients [16]. AGEs are believed to be involved in the genesis of many of the irreversible complications of diabetes, including expanded extracellular matrix, cellular hypertrophy, hyperplasia, and vascular complication [17]. The formation of glycoxidation products is not only the result of glucose-induced oxidative stress. Fructose, which is increased as a consequence of activation of the polyol pathway, leads to the formation of AGE precursors methylglyoxal and 3-deoxyglucosone [20]. These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions, promote free radicals formation, and quench and block antiproliferative effects of nitric oxide. By increasing intracellular oxidative stress, AGEs activate the transcription factor NF-κB, thus promoting up-regulation of various NF-κB controlled target genes [21]. NF-κB enhances production of nitric oxide, which is believed to be a mediator of islet β-cell damage. In addition, hyperglycaemia leads to glycation of antioxidant enzymes, which could alter the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals, exacerbating oxidative stress in diabetes. Therefore, the process of glucose oxidation might be responsible not only for increased ROS products but also for decrease availability of antioxidant enzymes.

Oxidative stress and diabetes complications

Chronic hyperglycaemia is a primary cause of most long-term complications in diabetes. Hyperglycaemia leads to protein glycation, which is main source of free radicals. Evidence showed that free radicals make a significant contribution to the progression of diabetes and its complications. These complications include the changes in kidney, nerve, vascular tissue, foot

ulceration, and metabolism. We will mainly discuss diabetic neuropathy and vascular changes in diabetes.

Diabetic neuropathy

Diabetic neuropathy is one of the leading complications of diabetes mellitus. Up to 50% of diabetic patients develop diabetic neuropathy. Hyperglycaemia *per se* is shown to be the pathogenic factor for the onset and progression of diabetic neuropathy. Patients with intensified insulin treatment for 4 years showed a clinically relevant and significant effect on nerve function when compared to the conventional treatment group. Several experiments suggest a multifactorial pathogenesis of diabetic neuropathy with a complex interaction between metabolic and vascular abnormalities. The sorbitol pathway by which glucose is converted to sorbitol and fructose under hyperglycaemia conditions has been shown to lead to reduction of myoinositol, which in turn may reduce the activity of Na^+, K^+ -ATPase, an enzyme known to be important for nerve conduction velocity [22]. Alternatively, or in addition, free radicals may impair the endothelium-dependent vasodilatation either by changes in the generation and bioactivity of nitric oxide or by a reduced synthesis of vasodilating prostaglandins [23]. The diminished generation of vasodilating mediators may lead to reduce endoneurial blood flow, which in turn causes endoneurial hypoxia and/or ischemia. Which are responsible for the destruction of neuronal and Schwann cells and finally nerve degeneration. In animal models of diabetic neuropathy, which show similar neurophysiological and morphometric alterations to those observed in humans, free radical scavengers such as RRR-α-tocopherol and probucol can protect against neurovascular dysfunction [23].

Another important aspect in the progression of diabetic neuropathy is the impaired ability of damaged nerve fibers to regenerate. Some neurotrophic factors, including nerve growth factor, insulin growth factor, and brain-derived neurotrophic factor which are assumed to be essential in nerve regeneration, are reduced in diabetic neuropathy. Although the mechanisms and the biological effects of these neurotrophic factors are not fully understood, it is interesting to note that oxidative stress and impaired microcirculation contribute to the diminution of neurotrophins in the process of neurodegeneration in diabetic neuropathy. In cultured cells, the effects of glucose on nerve tissue are also investigated. It was demonstrated that neuroblastoma cells show decreased Na^+, K^+-ATPase activity when exposed to high glucose for 2 weeks [24]. *In vitro* studies have also demonstrate that ROS and lipid peroxidation can reduce Na^+, K^+-ATPase activities. A model has been proposed based on these *in vitro* experiments (Figure 3). In this model, hyperglycaemia is the cause of peripheral nerve damage via ROS and sorbitol pathway.

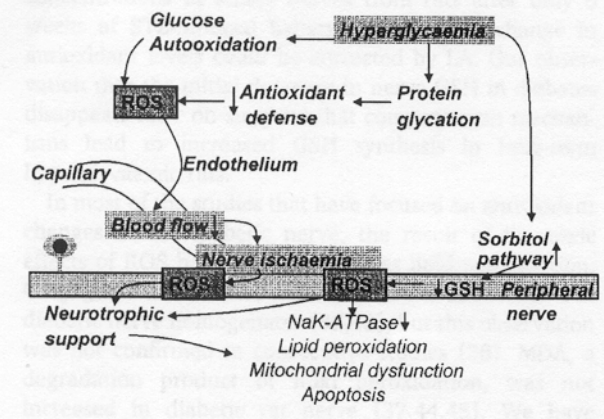

Figure 3. Schema model for the contribution of oxidative stress to diabetic neuropathy. ROS, reactive oxygen species; GSH, reduced glutathione. Adapted from [24].

Diabetic vascular complications

Microvascular complications are one of the most serious aspects of diabetic patients. Microvascular complication can not only cause diabetic retinopathy and nephropathy but also affect the heart and contribute to the development of diabetic neuropathy by limiting the endoneuronal blood flow. The following discuss will focus on the contribution of ROS to the development of vascular complications. Evidence suggests that AGEs and ROS are able to activate PKC and that activation of PKC might be a common downstream mechanism to which multiple cellular and functional abnormalities in the diabetic vascular tissue can be attributed, including changes in vascular blood flow, vascular permeability, extracellular matrix components and cell growth.

AGEs are formed in protein glycation and interaction AGEs with their receptors leads to an impaired of endothelium-dependent vasodilatation, an increased pro-coagulant activity. This results in the functional change of vascular wall. Furthermore, free radicals are generated and some redox-sensitive transcription factors such as NF-κB are activated in vascular wall as a consequent of AGEs interaction with their receptors. The concept of an AGE-induced oxidative stress which activates the transcription factor can explain the concomitant occurrence of oxidative stress and changes in the dynamic endothelial balance form an anticoagulant to a pro-coagulant state, from vasodilatation to vasoconstriction and impaired microcirculation (Figure 4). It is interesting to note that in experiments using vascular cells the generation of ROS and the activation of NF-κB is not only caused by AGE, but also by high concentration of glucose, which indicate a link between elevated postprandial glucose levels and the development of vascular complication. Taken together, evidence suggests that activation of the oxidative stress-sensitive transcription factor such as NF-κB and AP-1 represents an important pathogenic link between hyperglycaemia and the transformation of the vessel wall in diabetes.

Oxidative stress and metabolism

Type 1 diabetes is characterized by insufficiency of insulin production, while type 2 diabetes is characterized by the loss of the ability of insulin-sensitive tissue to respond to insulin, which results in high plasma insulin and high glucose level. Evidence showed that antioxidants influence insulin signaling and glucose uptake. There is a direct link between the imbalance of oxidative stress and antioxidants leading to impaired glucose uptake. In 3T3-L1 adipocytes glucose uptake is rapidly decreased when they are incubated with glucose oxidase which results in steady production of H_2O_2 [25]. The reduction of 2-deoxyglucose uptake is companied by decreased PI-3 kinase activity and GLUT4 translocation. This suggests that ROS and antioxidant depletion could impair the insulin-mediated PI-3 kinase, which results in impaired GLUT4 translocation and defective insulin-mediated glucose uptake. Thus, further evidence supports that the imbalance between ROS and antioxidant capacity may play an important role in the development and progression of insulin resistance in type 2 diabetes. As a matter of fact, treatment with the antioxidant α-lipoic acid increases glucose uptake and glucose oxidation in type 2 diabetes [26]. There is an inverse association between insulin sensitivity and ROS level. Therefore, antioxidant treatment could be an attractive therapeutic strategy for treating type 2 diabetes patients.

In addition, recent studies suggest that ROS may also participate in the development of type 1 diabetes. Antioxidants have been shown to have protective effects against diabetes in non-obese diabetic (NOD) mice, which are known to develop autoimmune diabetes following the infiltration of inflammatory cells into pancreatic islets. However, if NOD mice are overexpressed

thioredoxin, a small protein with antioxidant function exclusively in pancreatic β-cells, then spontaneous diabetes was prevented or delayed in the NOD transgenic mice.

Hyperglycaemia and oxidative stress signaling

As discussed above, diabetic complications come from chronic elevated glucose levels in both type 1 and 2 diabetes. The pathogenic effects of high glucose are mediated via ROS generated by glucose oxidation and protein glycation. In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage, which ultimately lead to late complications of diabetes. Furthermore, these same pathways are also showed to link to insulin resistance and β-cell dysfunction (Figure4).

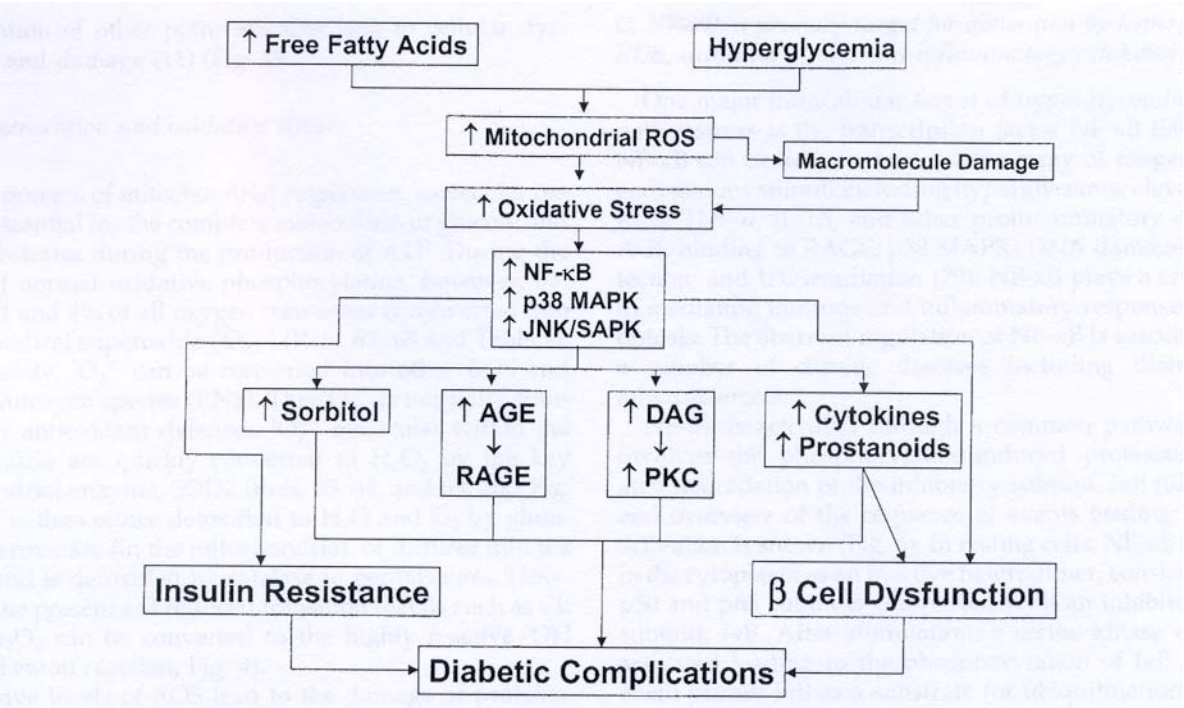

Figure 4. Proposed model of how elevated glucose and possibly FFA levels contribute to the pathophysiology of diabetes via the generation of ROS and the consequent activation of numerous stress-sensitive pathways. Adpated from [28].

We see that NF-κB, JNK/SAPK, p38 MAPK, and hexosamine pathways are stress-sensitive signaling pathways which can be activated by hyperglycaemia and ROS. These pathways are involved in the pathogenesis of diabetes.

Oxidative stress and insulin resistance

ROS and oxidative stress can lead to the activation of multiple serine kinase cascades in vitro. The insulin signaling pathway provides a number of potential targets of these activated kinases, including the insulin receptor (IR) and the family of IR substrate (IRS) proteins. For IRS-1 and –2, an increase in serine phosphorylation decreases the extent of tyrosine phosphorylation and is consistent with the attenuation of insulin action (Figure 5)

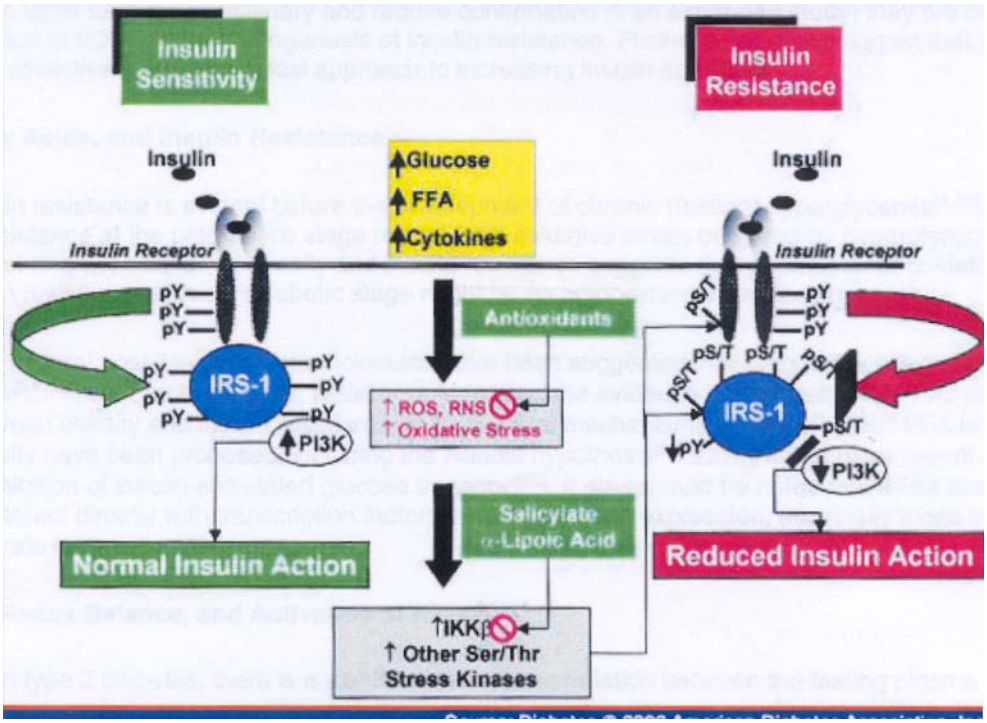

Figure 5. The role of serine kianse activation in oxidative stress-induced insulin resistance. A variety of stimuli increase ROS production and oxidative stress. This results in the activation of multiple stress-sensitive serine/threonine kinase signaling cascades. Once activated, these kinases are able to phosphorylate multiple targets, such as the IR and IRS proteins. Increased phosphorlation of IR or IRS proteins on discrete serine or threonine sites decreases the extent of insulin-stimulated tyrosine phosphorylation. Consequently, the association and/or activities of downstream signaling molecules (e.g. PI-3 kinase) are decreased, resulting in reduced insulin action. Adapted from [28].

Oxidative stress and β-cell dysfunction

β-cells are responsible for sensing and secreting insulin in response to glucose stimulation. β-cells are sensitive to ROS because they are low in antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase [27]. Overexpression of the

antioxidant enzymes in islets or transgenic mice prevents many of the deleterious effects discussed above. Oxygen stress generated by short exposure of β-cells preparations to H2O2 increases production of p21, an inhibitor of cyclin-dependent kinase, decreases insulin mRNA, cytosolic ATP, and calcium flux in cytosol and mitochondria, and cause apoptosis. Insulin secretion stimulated by glucose or methyl succinate can be inhibited shortly, whereas the response to K^+ remains normal. These results suggest that the mitochondrial processes involved in glucose-mediated insulin secretion are particular affected by oxidative stress.

Summary

Emerging evidence indicate that generated ROS is one major factor in the onset and the development of diabetes and its complications. It is demonstrated that ROS is a link between hyperglycaemia and the typical observed pathophysiological features of diabetes. Furthermore, it is showed that ROS can inactivate the signal pathway between the insulin receptor and the glucose transporter system. Recent evidence show that ROS generation is acting as a mediator of insulin resistance and β-cell dysfunction. Therefore, antioxidants might be helpful for treating diabetic patients and their complications.

Future directions and experiments

As discussed above, evidence supports that ROS plays a key role in pathogenesis of diabetes and its complications. NOD mice provide a good model in our understanding diabetes. Pancreatic β-cells have lower expression of antioxidant enzymes. One of the future studies will be to overexpress the antioxidant enzymes such as SOD, GR, or catalase in pancreatic β-cells in NOD mice. Then we will observe the development of diabetes and the associated late

complications in antioxidant enzyme overexpressed mice. We will expect that the development of diabetes as well as the complications in antioxidant enzyme overexpressed mice will be delayed or less deleterious compared with control mice.

Second, we see in Figure 5 that it is proposed that reduction of PI-3 kinase activity induced by oxidative stress is responsible for insulin resistance. If this is true, then expression of constitutive active PI-3 kinase will increase insulin sensitivity. For example, we can overexpress constitutive p85, which is the kinase component of PI-3 kinase in 3T3-L1 adipocytes, then the cells can be incubated with glucose oxidase, which will generate H_2O_2 that achieve a steady state, as reported [25]. We would expect that overexpression of constitutive active PI-3 kinase will increase insulin sensitivity in adipocytes, and restore completely or partial the prolonged oxidative stress impairs insulin-induced GLUT4 translocation.

Third, it is showed that hyperglycaemia causes increase of AGEs, which in turn produce oxidative stress. We could apply AGE receptor antibody or AGE receptor antisense RNAs to interrupt AGE signaling pathways in isolated culture cells which is incubated with certain concentration of glucose. For example, we can inject AGE receptor antibodies or AGE receptor antisense RNAs into neuroblastoma cells. Then the cells are exposed to high glucose concentrations for 2 weeks. After that, we will measure cellular free radical concentration as well as the activity of Na^+, K^+-ATPase [24]. We would expect that low free radicals are generate in cells and Na^+, K^+-ATPase activities are less reduced compared with neuroblastoma cells injected with control antibodies (IgG) or control antisense RNAs.

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