Oxidative Stress Biology and Cell Injury During Type 1 and Type 2 Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) affects approximately 170 million individuals worldwide and is expected to alter the lives of at least 366 million individuals within a future span of 25 years. Of even greater concern is the premise that these projections are underestimated since they assume obesity levels will remain constant. Type 1 insulin-dependent DM accounts for only 5-10 percent of all diabetics but represents a highly significant health concern, since this disorder begins early in life and leads to long-term complications. In contrast, Type 2 DM is recognized as the etiology of over 80 percent of all diabetics and is dramatically increasing in incidence as a result of changes in human behavior and increased body mass index. Yet, the pathological consequences of these disorders that involve the both the neuronal and vascular systems are intimately linked through the pathways that mediate oxidative stress. Here we highlight some of the relevant oxidative pathways that determine insulin resistance through reactive oxygen species, mitochondrial dysfunction, uncoupling proteins, and endoplasmic reticulum stress. These pathways are ultimately linked to protein kinase B (Akt) and the insulin signaling pathways that determine the initial onset of glucose intolerance and the subsequent course to apoptotic cell injury. Through the elucidation of these targets, improvement in current strategies as well as the development of future clinical applications can move forward for both the prevention and treatment of Type 1 and Type 2 DM.

Keywords

Apoptosis; Akt; diabetes; endoplasmic reticulum stress; erythropoietin; exercise; mitochondria; oxidative stress; uncoupling proteins

THE GLOBAL SCOPE OF DIABETES MELLITUS

Diabetes mellitus (DM) is a condition that leads to elevated levels of serum glucose and is believed to affect at least 16 million Americans and approximately 170 million individuals worldwide (Quinn, 2001). When data is extrapolated on the prevalence of diabetes for the year 2030, approximately 366 million individuals may be afflicted by the disease. These projections are considered to be underestimated, since they assume levels of obesity will remain constant (Wild et al., 2004). Type 2 DM is recognized as the etiology of over 80 percent of all diabetics and is dramatically increasing in incidence as a result of changes in human behavior and increased body mass index (Laakso, 2001). However, it is Type 1 insulin-dependent diabetes

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mellitus (IDDM) which accounts for only 5-10 percent of all diabetics that represents a highly significant health concern, since this disorder begins early in life and leads to long-term complications throughout the body involving cardiovascular, renal, and nervous system disease (Daneman, 2006). Interestingly, disease of the nervous system can become the most debilitating and affect sensitive cognitive regions of the brain, such as the hippocampus that modulates memory function, resulting in significant functional impairment and dementia (Awad et al., 2004, Gerozissis, 2003). Furthermore, both focal and generalized neuropathies, especially in conjunction with vascular disease, can result in severe disability (Perkins and Bril, 2002). The incidence of undiagnosed diabetes and impaired glucose tolerance in the young raises further concerns. Individuals with impaired glucose tolerance are at 2.5 times greater risk for the development of diabetic complications than individuals with normal glucose tolerance (Harris and Eastman, 2000). As a result, healthcare costs for diabetic complications are a significant driver for government resource consumption with costs of $214.8 million for outpatient expenditures and $1.45 billion for inpatient expenditures (Maciejewski and Maynard, 2004). If one examines cognitive impairments resulting from diabetes in the general population that can mimic Alzheimer's disease (Chong et al., 2005d), annual costs equal $100 billion (Maiese and Chong, 2004, McCormick et al., 2001, Mendiondo et al., 2001).

Type 1 DM is associated with the presence of alleles of the Human leukocyte antigen (HLA) class II genes within the major histocompatibility complex (MHC). The disorder is an autoimmune disease resulting from inflammatory infiltration of the islets of Langerhans and the selective destruction of β-cells in the pancreas that leads to insulin loss (Bonner-Weir, 2000). Monogenic inheritance does not appear to lead to Type 1 DM with work illustrating that multiple loci with possible epistatic interactions among other loci may be responsible for genetic transmission (Bottino and Trucco, 2005, Davies et al., 1994). Yet, a variety of environmental factors also may play a significant role with Type 1 DM. For example, some studies have suggested that Type 1 DM in monozygotic twins can occur with a cumulative risk from birth to 35 years of age of 70% (Kyvik et al., 1995, Melanitou 2005). In addition, other work suggests a concordance between monozygotic twins to be approximately 50% (Redondo et al., 2001) illustrating that environmental factors form a platform for the predisposition of Type 1 DM.

In Type 1 DM, activation of T-cell clones that are capable of recognizing and destroying β-cells lead eventually to severe insulin deficiency. These T-cell clones are able to escape from control of the thymus during circumstances that yield high affinity for major histocompatibility complex (MHC) molecules with T-cell receptors but incorrect low affinity for self-peptides. Once released into the bloodstream, these T-cell clones can become activated to destroy self-antigens. Upon initial diagnosis, approximately ninety percent of individuals with Type 1 DM have elevated titers of autoantibodies (Type 1A DM). The remaining ten percent of Type 1 DM individuals do not have serum autoantobodies and are described as having maturity-onset diabetes of the young (MODY) that can be a result of β-cell dysfunction with autosomal-dominant inheritance (Type 1B DM) (Permutt et al., 2005). Other variables reported in patients with Type 1 DM include the presence of insulin resistance that is usually characteristic of Type 2 DM and can lead to neurological and vascular disease (Kernan et al., 2002, Orchard et al., 2003). It is important to note that there is an converse overlap with Type 1 and Type 2 DM, since almost ten percent of Type 1 DM patients may have elevated serum autoantibodies (Pietropaolo et al., 2000).

Loss of autoimmunity in Type 1 DM can be precipitated by a number of exogenous events, such as the exposure to infectious agents (Luppi et al., 1995). In most cases but not all, the insulin gene (INS) and the human MHC or HLA complex are believed to contain the loci with *IDDM1* and *IDDM2* to account for the susceptibility to Type 1 DM with defective antigen presentation (Awata et al., 1997, Baisch et al., 1990). Interestingly, a HLA class II molecule

has been linked to Type 1 DM inheritance. Specifically, HLA-DQ that lacks a charged aspartic acid (Asp-57) in the β-chain is believed to lead to the ineffective presentation of autoantigen peptides during thymus selection of T-cells (Todd et al., 1987). Animal models that involve the nonobese diabetic (NOD) mice further support these findings, since these mice spontaneously develop diabetes with the human predisposing HLA-DQ corresponding molecule of H2 I-Ag. Yet, NOD mice without H2 I-Ag do not develop diabetes (Lund et al., 1990).

In contrast to Type 1 DM, Type 2 DM, also called noninsulin-dependent diabetes mellitus (NIDDM), is characterized by insulin resistance with significant metabolic dysfunction that include obesity, impaired insulin function and secretion, and increased endogenous glucose output. It should be noted that although insulin resistance forms the basis for the development of Type 2 DM, elevated serum glucose levels are also a result of the concurrent impairment in insulin secretion. This abnormal insulin secretion may be a result of defective β-cell function, chronic exposure to free fatty acids and hyperglycemia, and the loss of inhibitory feedback through plasma glucagon levels (Del Prato and Marchetti, 2004). Type 2 DM is the most prevalent form of diabetes and generally occurs more often in individuals over 40 years of age. The disorder is characterized by a progressive deterioration of glucose tolerance with early β-cell compensation for insulin resistance (achieved by β-cell hyperplasia) and subsequently followed by progressive decrease in β-cells mass. Another type of diabetes, gestational diabetes mellitus (GDM), is defined as a state of glucose intolerance during some cases of pregnancy, but usually subsides after delivery.

THE PRECIPITANTS AND CONSEQUENCES OF INSULIN RESISTANCE

Insulin resistance or defective insulin action occurs when a normal level of insulin produces a subnormal physiologic response. Skeletal muscle and liver are two of the primary insulin-responsive organs responsible for maintaining normal glucose homeostasis. Insulin normally lowers the level of blood glucose through suppression of hepatic glucose production and stimulation of peripheral glucose uptake, but a dysfunction in any step of this process can result in insulin resistance. Exposure to high concentrations of glucose and insulin results in insulin resistance, a characteristic feature of Type 2 DM.

Hyperglycemia also has been associated with oxidative stress and increased levels of reactive oxygen species have been proposed to lead to insulin resistance. Oxidative stress occurs when oxygen free radicals are generated in excess through the reduction of oxygen. Reactive oxygen species (ROS) consist of oxygen free radicals and associated entities that include superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite (Chong et al., 2005c). Most species are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems that include superoxide dismutase (SOD), glutathione peroxidase, catalase, and small molecule substances such as vitamins C and E. Superoxide radical is the most commonly occurring oxygen free radical that produces hydrogen peroxide by dismutation. Other enzymes capable of producing superoxide are xanthine oxidase, NADPH oxidases and cytochrome P450. Superoxide produces hydrogen peroxide through the Haber-Weiss reaction in the presence of ferrous iron by manganese (Mn)-SOD or copper (Cu)-SOD. In the presence of transition elements, a reaction of hydrogen peroxide with superoxide results in the formation of hydroxyl radical, the most active oxygen free radical. Hydroxyl radical alternatively may be formed through an interaction between superoxide radical and NO (Fubini and Hubbard, 2003). NO interacts with superoxide radical to form peroxynitrite that can further lead to the generation of peroxynitrous acid. Hydroxyl radical is produced from the spontaneous decomposition of peroxynitrous acid. NO itself and peroxynitrite are also recognized as active oxygen free radicals. In addition to directly altering

cellular function, NO may work through peroxynitrite that is potentially considered a more potent radical than NO itself (Pfeiffer et al., 2001).

Oxidative stress represents an important pathway for the destruction of cells (Chong et al., 2005d, Li et al., 2006a). The production of ROS can lead to cell injury through cell membrane lipid destruction and cleavage of DNA (Vincent and Maiese, 1999, Wang et al., 2003). ROS result in the peroxidation of cellular membrane lipids (Siu and To, 2002), peroxidation of docosahexaenoic acid, a precursor of neuroprotective docosanoids (Greco and Minghetti, 2004), the cleavage of DNA during the hydroxylation of guanine and methylation of cytosine (Lee et al., 2002), and the oxidation of proteins that yield protein carbonyl derivatives and nitrotyrosine (Adams et al., 2001). In addition to the detrimental effects to cellular integrity, ROS can inhibit complex enzymes in the electron transport chain of the mitochondria resulting in the blockade of mitochondrial respiration (Yamamoto et al., 2002).

The pathogenic effect of hyperglycemia, possibly in concert with free fatty acid release, is mediated to a significant extent *via* increased production of ROS. In addition to their ability to directly inflict damage on macromolecules, ROS indirectly lead to tissue damage by activating a number of cellular stress-sensitive pathways. Oxidative stress may decrease insulin sensitivity and injure the insulin-producing cells within the pancreas. For example, ROS can penetrate through cell membranes and cause damage to β-cells of pancreas (Chen et al., 2005, Lepore et al., 2004). In addition, free fatty acids which can lead to ROS, have been shown to also contribute to mitochondrial DNA damage and impaired pancreatic β-cell function (Rachek et al., 2006). Oxidative stress also is believed to modify a number of the signaling pathways within a cell that can ultimately lead to insulin resistance. As a result, it is possible that activation of oxidative stress pathways plays a key role in the development of not only the late complications in Type 1 and Type 2 DM, but also insulin resistance.

A number of oxidative stress pathways responsible for insulin resistance can be highlighted. In non-diabetic rats, hyperglycemia was shown to lead to a significant decrease in insulin-stimulated glucose uptake, a significant increase in muscle protein carbonyl content (used as an indicator of oxidative stress), and elevated levels of malondialdehyde and 4-hydroxynonenal as an indictor of lipid peroxidation (Haber et al., 2003). These biological markers of oxidative stress and insulin resistance were normalized during the application of the antioxidant N-acetylcysteine or taurine to suggest that oxidative stress contributes to the pathogenesis of hyperglycemia-induced insulin resistance (Haber et al., 2003). Furthermore, hyperglycemia can lead to increased production of ROS in endothelial cells, liver and pancreatic β-cells (Ceriello et al., 1996, Ihara et al., 1999, Ling et al., 2003, Yano et al., 2004). In a model of nonobese Type 2 DM, higher levels of 8-OHdG and HNE-modified proteins in the pancreatic beta-cells of diabetic rats than in the controls were observed with levels increasing with age and fibrosis of the pancreatic islets (Ihara et al., 1999). Elevated glucose has been shown to increase antioxidant enzyme levels in human endothelial cells, suggesting that elevated glucose levels may produce an oxidative stress in the cells (Ceriello et al., 1996). Even during brief period of hyperglycemia, ROS can be generated to lead to oxidative stress such as in vascular cells (Yano et al., 2004) or in neurons (Fig. 1). Recent clinical correlates support these experimental studies with acute glucose elevation acting as a trigger on oxidative stress than chronic sustained hyperglycemia (Monnier et al., 2006).

Although the precise role of insulin resistance in neuronal injury remains to be established, it has been shown that direct insulin stimulation of neurons may reverse diabetic neuropathy (Brussee et al., 2004). It suggested that defective insulin signaling in peripheral neurons could partly contribute to the development of diabetic neuropathy. Direct intrathecal delivery of doses insufficient to reduce glycemia of insulin or equimolar insulin growth factor-I improved and reversed slowing of motor and sensory conduction velocity in streptozotocin (STZ) -induced

diabetic rats. However, intrathecal saline or subcutaneous insulin did not have this effect. Interestingly, the neurotrophic and neuroprotective actions of insulin appear to enhance mitochondrial inner membrane potential and increase ATP levels (Huang et al., 2005). Huang *et al.* used real-time whole cell fluorescence video microscopy to analyze mitochondrial inner membrane potential ($\Delta\psi_m$) in cultured adult sensory neurons. Compared with control, insulin and other neurotrophic factors induced a 2-fold increase in $\Delta\psi_m$ over a 24 hour period. Insulin was also found to phosphorylate the cAMP response element binding protein (CREB).

Proper cellular function during a number of disorders including diabetes requires the maintenance of mitochondrial membrane potential (Maiese et al., 2005a). Mitochondria are a significant source of superoxide radicals that are associated with oxidative stress (Smeitink et al., 2004). Blockade of the electron transfer chain at the flavin mononucleotide group of complex I (NADPH ubiquinone oxidoreductase) or at the ubiquinone site of complex III (ubiquinone-cytochrome c reductase) results in the active generation of free radicals which can impair mitochondrial electron transport and enhance free radical production (Floyd and Hensley, 2002). Furthermore, mutations in the mitochondrial genome have been associated with the potential development of a host of disorders, such as hypertension, hypercholesterolemia, and hypomagnesemia (Wilson et al., 2004). Loss of mitochondrial membrane potential during a variety of conditions through the opening of the mitochondrial permeability transition pore represents a significant determinant for cell injury and the subsequent induction of the apoptotic cascade (Chong et al., 2003a, Deng et al., 2002, Ieraci and Herrera, 2006, Lin et al., 2000).

As a result, mitochondrial dysfunction plays a role in the development of diabetes and insulin resistance. In patients with Type 2 DM, ADH:O(2) oxidoreductase activity and citrate synthase activity were found to be depressed with skeletal muscle mitochondria being smaller than in control subjects (Kelley et al., 2002). Recently, a decrease in the levels of mitochondrial proteins and mitochondrial DNA in adipocytes was correlated with the development of Type 2 DM (Choo et al., 2006). Furthermore, insulin resistance in the elderly has been associated with elevation in fat accumulation, and reduction in mitochondrial oxidative and phosphorylation activity (Petersen et al., 2003). In addition, an association exists with insulin resistance and the impairment of intramyocellular fatty acid metabolism in young insulin-resistance offspring of parents with Type 2 DM (Petersen et al., 2004).

In addition to the importance of the presence and integrity of mitochondria, uncoupling proteins (UCPs) are carriers expressed in the mitochondrial inner membrane that uncouple oxygen consumption by the respiratory chain from ATP synthesis and can play a significant role during diabetes. These proteins are a family of carrier proteins found in the inner membrane of mitochondria that can control ROS. They catalyze an inducible proton conductance and disperse the proton electrochemical potential gradient across the mitochondrial inner membrane (Douette and Sluse, 2006). This uncoupling of respiration results in the activation of substrate oxidation and dissipation of oxidation energy as heat instead of ATP. In addition, mild uncoupling of respiration may play a significant role in regulating ATP synthesis, and fatty acids and glucose oxidation. Members of UCP family include UCP-1, 2,3,4,5 in mammals (Criscuolo et al., 2006). Members of the UCP family are distinctly distributed among different tissues. UCP-1 and UCP-4 are exclusively expressed in brown adipose tissue and in the brain, respectively. UCP-2 is expressed in most tissues while UCP-5 is present in multiple tissues with an especially high level in the brain and testis. UCP-3 is expressed predominantly in skeletal muscle.

UCP-1 is important for controlling the dissipation of oxidation energy as heat. Overexpression of UCP-1 is potentially beneficial for diabetes by reducing excessive energy in obesity. Indeed, muscle-specific overexpression of UCP for skeletal muscle can increase energy expenditure

and enhance insulin action to protect in animal models from high-fat diet induced insulin resistance (Li et al., 2000). In addition, skeletal muscle respiratory uncoupling was also shown to enhance insulin sensitivity in genetic obesity (Bernal-Mizrachi et al., 2002). Similar beneficial effects were also observed in specific overexpression of UCP-1 in white adipose tissues (Kopecky et al., 1995).

Uncoupling protein 2 (UCP-2) and uncoupling protein 3 (UCP-3) are expressed in tissues important for thermogenesis and/or in substrate oxidation, such as adipose tissue and skeletal muscles. UCP-2 is a member of the multigenic UCP family that is expressed in a wide range of tissues and organs. Possible functions of UCP-2 include control of ATP synthesis, regulation of fatty acid metabolism and control of reactive oxygen species production. UCP-2 expression in tissues involved in lipid and energy metabolism and mapping of the gene to a region linked to obesity and hyperinsulinemia has furthered investigations with UCP-2 and diabetes. In human adipose tissue and skeletal muscle, UCP-2 expression is increased during fasting. The carrier was shown to be under the control of fatty acids and thyroid hormones *in vivo*. There are reports implicating UCP-2 in the pathogenesis of diabetes. Overexpression of UCP-2 in isolated pancreatic islets results in decreased ATP content and blunted glucose-stimulated insulin secretion while UCP-2-deficient mice show an increased ATP level and an enhanced insulin secretion. Lack of UCP-2 dramatically improves insulin secretion and decreases hyperglycemia in leptin-deficient mice (Zhang et al., 2001). Furthermore, overexpression of UCP-2 could enhance resistance of beta-cells hydrogen peroxide toxicity (Li et al., 2001). It is of note that elevated expression of UCP-2 was also reported to exert substantial negative regulation of β-cell insulin secretion and contributes to the impairment of β-cell function (Chan et al., 2001). A role for UCP-3 in carbohydrate metabolism and in Type 2 DM also has been suggested. Mice overexpressing UCP-3 in skeletal muscle showed reduced fasting plasma glucose levels, improved glucose tolerance after an oral glucose load, and reduced fasting plasma insulin levels. It was found that UCP-3 levels were at least twice as low in patients with Type 2 DM compared with controls, suggesting a role for UCP-3 in glucose homeostasis (Schrauwen et al., 2001). In addition, UCP-3 may function to facilitate fatty acid oxidation and minimize ROS production (MacLellan et al., 2005).

Endoplasmic reticulum (ER) stress also is a feature of peripheral insulin resistance. ER stress is characterized by the accumulation of unfolded proteins in the lumen of the ER (Robertson et al., 2006). It is associated with an unfolded protein response (UPR) or by excessive protein traffic that may be triggered by events such as viremia. UPR regulates ER function and functions to coordinate the activity and participation of the processing and degradation pathways for unfolded proteins (Hampton, 2000). Obesity is associated with induction of ER stress predominantly in liver and adipose tissues. Under hyperglycemic conditions, the production of glucosamine by hexosamine pathway may initiate ER stress. This stress can promote a c-Jun N-terminal kinase (JNK)-dependent serine phosphorylation of insulin receptor substrate 1 (IRS-1), which results in suppression of insulin-receptor signaling pathway. In addition, the absence of X-box-binding protein-1 (XBP-1), a transcription factor that modulates the ER stress response, promotes insulin resistance in mice (Ozcan et al., 2004). Furthermore, gene silencing of oxygen-regulated protein 150 (ORP150), a molecular chaperone resident in the ER, has been shown to promote insulin resistance in non-diabetic control mice. In contrast, overexpression of ORP150 significantly decreases insulin resistance and markedly improves glycemic control in the liver of obese diabetic mice. Although not entirely clear, these observations may be associated with phosphorylation state of IRS-1 and protein kinase B (Akt) as well as the expression levels of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (Nakatani et al., 2005).

TARGETED PATHWAYS OF AKT AND ITS EFFECTORS AGAINST OXIDATIVE STRESS AND DIABETES

The protein Akt, also known as protein kinase B (Chong et al., 2004b, Chong et al., 2005b) is ubiquitously expressed in mammals. Three family members of this serine/threonine kinase are now known to exist that were termed Akt after the molecular cloning of the oncogene *v-Akt* and two human homologues (Staal, 1987, Staal et al., 1988). They are PKBα or Akt1, PKBβ or Akt2, and PKBγ or Akt3 (Chong et al., 2005b). Akt is part of the AGC (cAMP-dependent kinase/protein kinase G/protein kinase C) superfamily of protein kinases and consists of three functional domains.

Defective glucose transport and insulin resistance are believed to be closely linked to the activity of Akt. Insulin controls glucose transport through insulin receptor substrate-1 (IRS-1) and in part through Akt as well as atypical protein kinase C. Perturbations in this pathway can substantially alter hepatic glucose output and triglyceride release (Farese et al., 2005). Akt signaling also has a central function to insulin sensitivity in diabetes. In response to insulin, Akt leads to glucose uptake in adipocytes through its stimulation of glucose transporter 4 (GLUT-4) translocation and increased synthesis of GLUT-1 (Kohn et al., 1996). The elevated glucose influx is accompanied by increased lipid synthesis and decreased glycogen synthesis. Conversely, impairment of Akt kinase activity was accompanied by impairment in insulin-stimulated glucose transport in muscle and adipocytes both from obese rats and patients with diabetes (Carvalho et al., 2000, Krook et al., 1998). In addition, mutations in Akt2 are linked to the development of severe insulin resistance and diabetes (George et al., 2004). Reduced activity of Akt1 also leads to impaired glucose tolerance and diabetes (Bernal-Mizrachi et al., 2004).

Akt also is considered a central modulator to prevent apoptotic cell injury during late genomic DNA destruction and early apoptotic signaling with membrane phosphatidylserine (PS) exposure (Chong et al., 2005d, Maiese and Chong, 2004). Overexpression of Akt in cells prevents apoptosis during growth factor withdrawal (Datta et al., 1997) and paradigms with oxidative stress (Chong et al., 2004a, Li et al., 2006b). Further work has demonstrated that Akt is necessary for the survival of cells, since expression of a dominant-negative Akt or inhibition of phosphoinositide 3 kinase (PI 3-K), necessary for Akt activation, precipitates cell death during oxidative stress (Kang et al., 2003a, Kang et al., 2003b). Increased Akt activity can foster cell survival during free radical exposure (Chong et al., 2003b, Matsuzaki et al., 1999), matrix detachment (Rytomaa et al., 2000), neuronal axotomy (Namikawa et al., 2000), DNA damage (Chong et al., 2004a, Chong et al., 2002, Henry et al., 2001, Kang et al., 2003a), anti-Fas antibody administration (Suhara et al., 2001), oxidative stress (Chong et al., 2003b, Kang et al., 2003a, Kang et al., 2003b, Yamaguchi and Wang, 2001), hypoxic preconditioning (Wick et al., 2002), ß-amyloid (Aß) exposure (Martin et al., 2001), metabotropic receptor signaling (Chong et al., 2005a, Chong et al., 2006, Maiese et al., 2005a), and cell metabolic pathways (Chong et al., 2005e, Maiese and Chong, 2003). Activation of Akt also can prevent membrane PS exposure on injured cells and block the activation of microglia during oxidative stress (Chong et al., 2005a, Kang et al., 2003a, Kang et al., 2003b).

In a number of experimental models, modulation of Akt activity can critically affect cell survival during hyperglycemia and the outcome of diabetic complications. During chronic hyperglycemic stress, inhibition of Akt leads to increased endothelial cell injury (Okouchi et al., 2006). In STZ diabetic rats, vascular control and integrity (Shah and Singh, 2006) as well as mitochondrial function (Di Noia et al., 2006) were improved with an agent that leads to activation of Akt. Furthermore, ER stress inducers can lead to dephosphorylation and inactivation of Akt with subsequent cell death (Hyoda et al., 2006). On the converse side,

overexpression of Akt, such as in endothelial cells, can protect cells from injury during elevated glucose concentrations (Varma et al., 2005).

Given the therapeutic potential of targeting Akt to treat both Type 1 and Type 2 DM and the complications of these disorders, recent work brings to light a number of options. Physical exercise is one of the important lifestyle interventions and generally recommended for patient with diabetes. Regular exercise has shown beneficial effects on glycemic control, weight loss and insulin resistance. An exercise results in pronounced improvement in glycemic control usually over relatively short of time (Maiorana et al., 2002). Furthermore, in diet-induced obesity rats, a single session of exercise improved insulin sensitivity by reversing the increased activity of protein tyrosine phosphatase-1B (PTP1B) and enhanced serine phosphorylation of IRS-1 insulin signaling (Ropelle et al., 2006). Other mechanisms that may account for the benefits of physical activity during diabetes involve Akt. In the immediate periods following acute exercise, Akt phosphorylation and activity has been shown to be increased (Howlett et al., 2006).

Combined with the benefits of exercise, erythropoietin (EPO) may another consideration for diabetic therapeutic strategies. EPO is a trophic factor that is approved by the Food and Drug Administration for the treatment of anemia, but a body of recent work has revealed that EPO is not required only for erythropoiesis and that EPO and its receptor exist in other organs and tissues outside of the liver and the kidney, such as the brain and heart. As a result, EPO has been identified as a possible candidate for disorders that involve both cardiac and nervous system diseases (Maiese et al., 2004, Maiese et al., 2005c). Protection by EPO in a number of cellular systems can block apoptotic injury from a number of sources, such as reduced or absent oxygen tension, excitotoxicity, and free radical exposure. Since apoptotic injury involves both genomic DNA destruction and membrane PS externalization that involve stroke, inflammation, Alzheimer's disease, and myocardial infarction (Han and Suk, 2005, Li et al., 2004, Li et al., 2006b), EPO can be considered unusual in its ability to prevent both the exposure of membrane PS residues and inhibit the committed stages of genomic DNA destruction in several experimental models to potentially offer protection against microglial phagocytosis and thrombotic injury (Cai and Semenza, 2004, Chong et al., 2003b, Chong et al., 2002, Chong et al., 2003c, Grimm et al., 2002, Parsa et al., 2003).

Interestingly, EPO leads to the phosphorylation of Akt. Once activated, Akt can confer protection against genomic DNA degradation and membrane PS exposure (Chong et al., 2002, Kang et al., 2003b, Wick et al., 2002). Up-regulation of Akt activity during injury paradigms, such as N-methyl-D-aspartate toxicity (Dzietko et al., 2004), cardiomyocyte ischemia (Parsa et al., 2003), hypoxia (Chong et al., 2002), and oxidative stress (Chong et al., 2003a, Chong et al., 2003b, Chong et al., 2003c), is vital for EPO protection, since prevention of Akt phosphorylation blocks cellular protection and anti-inflammatory mechanisms by EPO (Chong et al., 2003a, Chong et al., 2003b, Chong et al., 2003c). EPO employs the Akt pathway to prevent apoptosis by maintaining mitochondrial membrane potential ($\Delta\psi_m$), preventing the cellular release of cytochrome c, and modulating caspase activity (Chong et al., 2003a, Chong et al., 2003b, Chong et al., 2002).

In clinical studies, plasma EPO is often low in diabetic patients with (Mojiminiyi et al., 2006) or without anemia (Symeonidis et al., 2006). In these diabetic patients, levels of EPO appear to be determined by hemoglobin levels and degree of microalbuminuria (Mojiminiyi et al., 2006). In addition, the failure to produce erythropoietin in response to a declining hemoglobin level represents an impaired EPO response in diabetic patients (Thomas et al., 2005). In light of the ability of EPO to modulate Akt activity and provide cellular protection during oxidative stress, EPO may be efficacious in patients with diabetes. Investigations involving subcutaneous EPO in diabetics and non-diabetics with severe, resistant congestive

heart failure have shown to decrease fatigue, increase left ventricular ejection fraction, and significantly decrease the number of hospitalization days (Silverberg et al., 2003). Yet, one must be cautious with the introduction of even known therapies for new applications. Some studies suggest that elevated plasma levels of EPO independent of hemoglobin concentration can have a poor prognostic value in individuals with congestive heart failure (van der Meer et al., 2004). The use of EPO in patients with uncontrolled hypertension is contraindicated, since both acute and long-term administration of EPO can precipitate hypertensive emergencies. Maintenance treatment with EPO in cancer patients receiving chemotherapy has been associated with nonfatal myocardial infarction, vascular thrombosis, pyrexia, vomiting, shortness of breath, paresthesias, and upper respiratory tract infection (Henry et al., 2004, Maiese et al., 2005b).

DIRECTIONS FOR THE FUTURE

Knowledge gained in understanding the complex cellular and systemic processes of diabetes provide essential insight into the pathogenesis of diabetes and its complications. Oxidative stress is a principal mechanism in the progression of diabetes and its complications and actively leads to cellular injury in both neuronal and vascular cells that can precede the onset of many diabetic complications. Elucidating the mechanisms that determine insulin resistance as well as glucose signaling and cell survival *via* Akt may begin to lay new strategies for both the treatment and prevention of Type 1 and Type 2 DM.

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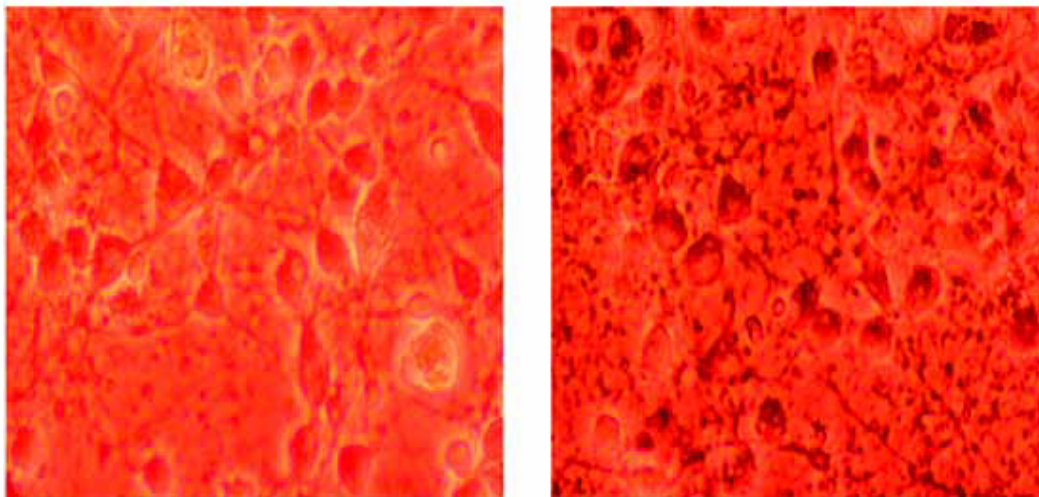
Fig. 1. Acute hyperglycemia leads to neuronal injury
Representative hippocampal neurons obtained from E-19 Sprague-Dawley rat pups were incubated in L-15 growth medium with free serum containing elevated D-glucose of 50 mM for 24 hours at 37°C in a humidified atmosphere of 5% CO_2 and 95% room air. Neuronal cell survival was determined by trypan blue exclusion method and reveals significantly increased dye uptake in injured neurons during hyperglycemia (right panel), but not in untreated control neurons (left panel). Note: Acute neuronal injury with elevated glucose can occur at significantly lower glucose concentrations of 20 mM, but a glucose concentration of 50 mM was chosen for dramatic visualization of cell injury.