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Hyperglycemia / hypoglycemia-induced mitochondrial dysfunction and cerebral ischemic damage in diabetics

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Abstract

Enhancement of ischemic brain damage is one of the most serious complications of diabetes. Studies from various *in vivo* and *in vitro* models of cerebral ischemia have led to an understanding of the role of mitochondria and complex interrelated mitochondrial biochemical pathways leading to the aggravation of ischemic neuronal damage. Advancements in the elucidation of the mechanisms of ischemic brain damage in diabetic subjects have revealed a number of key mitochondrial targets that have been hypothesized to participate in enhancement of brain damage. The present review initially discusses the neurobiology of ischemic neuronal injury, with special emphasis on the central role of mitochondria in mediating its pathogenesis and therapeutic targets. Later it further details the potential role of various biochemical mediators and second messengers causing widespread ischemic brain damage among diabetics via mitochondrial pathways. The present review discusses preclinical data which validates the significance of mitochondrial mechanisms in mediating the aggravation of ischemic cerebral injury in diabetes. Exploitation of these targets may provide effective therapeutic agents for the management of diabetes-related aggravation of ischemic neuronal damage.

Keywords

Ischemic brain injury; Diabetes; Mitochondria; pharmacological interventions; stroke; ischemia

Introduction

Diabetes mellitus is a chronic metabolic disease involving high blood glucose levels which result from dysfunction in insulin secretion and/or action (American Diabetes Association 2012). World Health Organization data shows that 347 million people worldwide are presently suffering from diabetes. Further, diabetes-related deaths have been projected to double between 2005 and 2030 (World Health Organization 2011). Worldwide, healthcare

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expenditure for diabetes was 471 billion USD in 2012 (International Diabetes Federation 2012). Diabetes is noted to result in high healthcare costs, loss of labour productivity and decreased rates of economic growth.

High blood glucose levels associated with diabetes are known to alter the normal physiology of various organs such as eyes, kidneys, heart, blood vessels and brain (American Diabetes Association 2012). Diabetic retinopathy is an important cause of blindness that occurs as a result of long-term damage to small blood vessels in the retina. One percent of cases of blindness worldwide have been attributed to diabetes (World Health Organization 2012). Diabetes is one of the important causes of kidney failure (World Health Organization 2011). Diabetes also enhances the risk of cardiovascular diseases like stroke. Presently, eighty four percent of people suffering from diabetes die of cardiovascular disease (primarily heart disease: 68% and stroke: 16%) (Morrish et al. 2001; American Diabetes Association, 2011). Many studies have identified diabetes mellitus as an independent and significant risk factor for stroke as well as stroke-related mortality (Abbott et al. 1987; Barrett-Connor and Khaw 1988; Chukwuma and Tuomilehto 1993). Diabetic subjects compose roughly 8.3 % of the U.S. population but account for 15–27 % of all incident strokes (Centers for Disease Control and Prevention 2011; Grau et al. 2001; Kissela et al. 2005). Moreover, diabetic patients are 2.9 times more likely to suffer from stroke than their non-diabetic counterparts (Almdal et al. 2004; Grau et al. 2001; Kissela et al. 2005; Ottenbacher et al. 2004; Benjamin et al. 2003; Wolf et al. 1991). This increase in incidence of diabetes-related stroke episodes is specifically ascribed to an increase in the rate of ischemic stroke rather than hemorrhagic stroke (Abbott et al. 1987; Kissela et al. 2005). Moreover, diabetic patients are more likely to experience the development of cerebral infarction, indicating that ischemia in diabetic patients is less likely to be reversible (Fritz et al. 1987; Kissela et al. 2005; Lithner et al. 1988; Weinberger et al. 1983).

Recent studies demonstrate that besides hyperglycemia, recurrent hypoglycemia also affects brain functions in terms of causing cognitive dysfunction (Schultes et al. 2005), anxiety and depression (Strachan et al. 2000). In an earlier study we demonstrated that exposure to recurrent hypoglycemia before cerebral ischemia can increase cerebral ischemic damage in a rat model of Type 1 diabetes (Dave et al. 2011).

Overall, diabetes increases the risk of cerebral ischemia as well as the severity of cerebral ischemic damage. The high number of diabetics throughout the world ensures that healthcare expenditures on preventing cerebral ischemia and lowering the severity of cerebral ischemic damage in the diabetic population will need to be substantial. In this review article we provide an overview of various available pharmacotherapeutic approaches for the prevention of cerebral vascular disease among diabetics, limitations of the available clinical treatment options, pathophysiology of ischemic brain injury in diabetes and role of mitochondrial mechanisms in mediating aggravation of ischemic stroke among diabetic subjects, followed by potential future directions.

Pharmacological management of cardiovascular disease in diabetic subjects

The International Diabetes Federation (2005) has defined the requirement of multiple strategies for lowering cardiovascular risk among diabetics. These strategies include measures such as control of blood glucose and blood pressure, lifestyle interventions, cardiovascular risk assessment, lipid-modifying therapy and anti-platelet therapy. Blood glucose control can be achieved by lifestyle management, educating patients about the importance of blood glucose level monitoring and its methodology, and pharmacotherapy-based modulation of blood glucose levels [using metformin, sulfonylureas, PPAR- γ agonists (thiazolidinedione), α -glucosidase inhibitors and insulin]. Blood pressure control methods known to mitigate cerebrovascular complications among diabetics involve continuous blood pressure monitoring and related lifestyle modifications, such as reducing intake of calories, salt, alcohol, and inactivity. Pharmacotherapeutic approaches used to treat cardiovascular complications associated with diabetes involve the usage of antihypertensive drugs (ACE-inhibitors and angiotensin-2 receptor blockers), standard hypolipidemic drugs, and anti-platelet agents (International Diabetes Federation 2005). These pharmacological therapies do not target the mechanism involved in the pathophysiology of the disease but only ameliorate secondary damaging effects of chronic diabetic conditions.

These cerebrovascular risk protection strategies for diabetics are found to be very useful in terms of affecting clinical outcome of the disease condition involving ischemic brain damage. Moreover, because prevalence of stroke is much higher in people with diabetes, and because the processes of arterial damage in people with diabetes are similar pathologically to those occurring in the general population (though usually present to a more abnormal degree), these relatively general prevention strategies are substantially beneficial to diabetic patients predisposed to cardiovascular accidents; viz., ischemic stroke (International Diabetes Federation 2005) (Table: 1).

Limitations of existing treatment strategies

Diabetic patients have an increased propensity for cerebrovascular accidents, in part attributed to: a high level of platelet dysfunction, progressive atherosclerosis arising from an altered lipid profile, insulin resistance, hypertension, microalbuminuria, obesity, carotid intima-media thickness, and endothelial dysfunction (Air and Kissela 2007). The benefit of existing therapeutic strategies in lowering incidence of stroke is quite evident. Anti-hypertensive drugs such as angiotensin-converting enzyme (ACE) inhibitors, hypolipidemic drugs (statins) and anti-platelet drugs (aspirin and clopidogrel) are known to be therapeutically beneficial in the treatment of cardiovascular disease in diabetics (International Diabetes Federation 2005). A meta-regression analysis of controlled trials of stroke has shown that aspirin-based anti-platelet therapy evinces a significant efficacy in stroke patients (Johnson et al. 1999). Moreover, such efficacy has been recapitulated in ischemic stroke among diabetic subjects (Phipps et al. 2012). Systematic assessment of data from clinical studies have shown that anti-hypertensive drugs (diuretics or angiotensin-converting enzyme (ACE) inhibitors) and hypolipidemic drugs (simvastatin) reduces the recurrence of stroke (Rashid et al. 2003). These secondary prevention strategies have also

been noted to be useful in treating ischemic stroke patients suffering from diabetics (Phipps et al. 2012). These pharmacotherapeutic strategies display a favourable effect with regard to primary outcome parameters in stroke patients (Al-Qudah et al. 2011; CAPRIE Steering Committee 1996; ONTARGET Investigator et al. 2008). Although these currently approved treatment strategies have proven useful in improving outcomes (Antoniou et al. 2013), diabetic patients continue to have a higher risk of adverse cerebrovascular events compared with that in non-diabetic patients (Mackenzie et al. 2013; Wang and Reusch 2012).

Cerebral ischemia-induced infarction is more widespread and frequent in diabetic subjects and leads to delayed recovery and poor survival rates compared to non-diabetic subjects (Jorgensen et al. 1994). Despite tremendous progress in our understanding of the pathophysiology of stroke, drugs that have been effective in preclinical trials (e.g., excitatory receptor antagonists) have not been effective in stroke patients (Stroke trials registry, 2008; Vosler et al. 2009). First, there is a need to establish an effective therapy to reduce cerebral ischemic damage in non-diabetic patients. Second, in view of secondary complications, the development of a therapeutic strategy for diabetic stroke patients may require creation of a therapeutic approach tailored for diabetics instead. In order to effectively lower cerebral ischemic damage among diabetics it is important to understand cell death pathways involved in cerebral ischemia, and the additional mechanisms which lead to upregulation of the cell death pathways during diabetes. The present review aims at giving an overview of these pathways (with special emphasis on mitochondrial pathways) and discusses how these pathways are different in diabetics.

Pathophysiology of ischemic neuronal injury

Lack of perfusion during cerebral ischemia causes an abrupt depletion of oxygen and glucose in brain (Lipton 1999), which results in various detrimental changes in brain cells. Mitochondria related pathways that participate in cerebral ischemic damage are summarized below.

ATP Depletion

Persistent ischemia has been shown to cause a marked decrease in the mitochondrial production of adenosine triphosphate (ATP), which inhibits active sub-cellular processes leading to generalized neuronal depolarization in brain (Kastura et al. 1993; Nedergaard and Hansen 1993). This widespread excitation of brain cells causes a profound increase in the release of the excitatory neurotransmitter glutamate, a biochemical event which is further facilitated by a concomitant inhibition of the energy-dependent glutamate reuptake process (Rader and Lanthorn 1989). The resultant increase in glutamate release then causes an unchecked activation of NMDA receptors which ultimately leads to massive calcium influx (Strijbos et al. 1996), a phenomenon central to the pathogenesis of ischemic neuronal injury. Hyperglycemia is known to enhance cerebral ischemia-induced release of glutamate suggesting increased excitotoxicity (Wei and Quast 1998).

Calcium influx

Increase in calcium influx associated with NMDA receptor activation-based neuronal excitation during cerebral ischemia causes the activation of a number of calcium-dependent enzymes responsible for the digestion of proteins, lipids and nucleic acids (Lipton 1999; Wang et al. 1996). Moreover, the increase in the influx of calcium is documented to cause further disruption of mitochondrial physiology (Nowicky and Duchon 1998) leading to heightened energy exhaustion and mitochondrial swelling, which ultimately triggers cell death (Lipton 1999; Strijbos et al. 1996). Diabetes leads to impaired mitochondrial calcium buffering capacity leading to the aggravation of ischemic damage (Moreira et al. 2006).

Free radical generation

The ischemia-related biochemical events explained above are known to divert the biochemical machinery of the mitochondrial electron transport chain more from generation of ATP to the production of reactive oxygen species (Chen et al. 2008; Newsholme et al. 2007). Generation of free radicals is further heightened during diabetes because of the associated activation of polyol pathway, related synthesis of advanced glycation end products (AGEs), and inhibition of intrinsic antioxidant enzyme systems (Cameron et al. 1996; Kaneto et al. 1994; Maritim et al. 2003; Monnier 2003; Yorek 2003). These free radicals are known to elicit a direct detrimental influence on various biochemical components of neuronal cells; viz., lipids, proteins and nucleic acids ultimately contributing to ischemic damage (Yorek 2003).

Apoptosis/Necrosis

ATP depletion, calcium influx, and the associated generation of free radicals are known to elicit neuronal cell death during ischemia (Lipton 1999). The precise nature of ischemic cell death in brain depends on the intensity of these biochemical changes that disrupt vital cell functions. While a lower rate of such alterations causes apoptotic cell death, a higher magnitude of such changes is documented to facilitate necrotic cell death (Lipton 1999; Nicotera and Lipton 1999; Hengartner 2000; Graham and Chen 2001). Necrosis, the principal form of cell death during ischemia, involves disruption of plasma membranes and the release of cellular contents. Apoptosis refers to a specialized mode of programmed cell death plays a major role in the progression of ischemic brain damage (Nicotera and Lipton 1999; Hengartner 2000; Graham and Chen 2001). Appearance of pan necrosis is common feature of ischemic damage in diabetic brains (Duverger and MacKenzie 1988; Lascola and Kraig 1997; Li et al. 1998; Wang et al. 2001). Rizk and colleagues reported that focal ischemia / reperfusion in diabetic rats increases neuronal apoptosis in the CA1-hippocampus, while neuronal necrosis in the cerebral cortex (Rizk et al. 2005). Also, hyperglycemia-induced selective vulnerability of certain areas of brain otherwise resistant to ischemic damage have been noted to mediate aggravation of ischemic outcome in hyperglycemia (Duverger and MacKenzie 1988; Ichikawa et al. 2012; Li et al. 1998; Miyamoto et al. 2010; Toni et al. 1994). These studies suggest that both pan-necrosis and apoptosis play a role in brain damage following ischemia in diabetics. Diabetic hyperglycemia is shown to enhance apoptosis by facilitating mitochondrial fission (Leininger et al. 2006; Otera et al. 2013).

Mitochondrial dysfunction

Mitochondria plays an important part in the production of ATP, buffering of excessive calcium influx, generation of free radicals and release of pro-apoptotic factors in ischemic brain (Calo et al. 2013; Gouriou et al. 2011; Niizuma et al. 2010; Robertson et al. 2009). Therefore, mitochondrial dysfunction during cerebral ischemia is observed to cause an increased intracellular calcium level, production of oxidative stress and related synthesis of reactive peroxynitrite species. Moreover, concomitant mitochondrial permeability transition pore activation facilitates the release of cytochrome *c* and thus causes the stimulation of apoptotic cell death pathways leading to the stimulation of terminal executioner caspases and apoptotic cell death, one of the important modes of cell death in ischemic stroke (Liu et al. 2012; Sanderson et al. 2013). Mitochondrial dysfunction therefore assumes a considerable role in the mediation of ischemic brain damage. It should be noted that mitochondrial functions are drastically impaired during diabetes (Katyare and Patel 2006).

The role of mitochondria in ischemia-induced brain damage in diabetics

Given the high rate of metabolism of brain tissue and its essential dependence on aerobic respiration as the principal source of energy, mitochondria play a central role in maintaining neuronal physiology during normal as well as pathological states; viz. ischemic stroke in diabetes. Developments in the last few decades have exposed many more important roles of mitochondria such as generation and regulation of cellular free radicals, involvement in cell death pathways, and calcium buffering, among others (Calo et al. 2013; Liu et al. 2012; Sanderson et al. 2013). In this section we review the literature on how diabetes affects mitochondrial function and how these, when altered, participate in exacerbation of cerebral ischemic damage in diabetics (Figure 1).

Bioenergetics

Mitochondria are the major source of energy in the cell, and in neurons in particular, which are highly dependent on ATP to maintain plasma membrane potential. Minor alterations in ATP production by mitochondria may not affect neuronal function at baseline, but may have drastic effects when cells are stressed by an event like cerebral ischemia (Villa et al. 2013). Electron donors (NADH and FADH₂) generated in the tri-carboxylic acid cycle upon oxidation of pyruvate feed electrons to the mitochondrial electron transport chain (Nelson and Cox 2004). These electrons flow through mitochondrial respiratory chain complexes generating a trans-membrane proton gradient, which is then utilized by transport chain complex V to generate ATP (Nelson and Cox 2004; Schultz and Chan 2001).

Several studies have examined the effect of diabetes on brain mitochondria. For example, Katyare and Patel (2006) observed that the rate of respiration / oxygen consumption is lower in brain mitochondria isolated from male streptozotocin (Stz)-diabetic rats when measured in presence of pyruvate + malate (related to the efficiency of complex I – III – IV), succinate (related to the efficiency of complex II – III – IV), or ascorbate + TMPD (related to the efficiency of complex IV). Mastrocola et al. (2005) observed impaired electron transport chain complex III, IV and V activities in mitochondria harvested from brains of Stz-diabetic rats. They also observed a significant reduction in cellular ATP content. Another study also

demonstrated that Stz-diabetes leads to lower ATP levels in the brain (Moreira et al. 2006). These results were confirmed in a rat model of Type 2 diabetes Goto-Kakizaki (GK) rats (Moreira et al. 2003). Stz-diabetes leads to lowered respiratory control ratio (RCR: an index of mitochondria electron transport chain leakage), State 3 and State 4 respiration, and ADP/O ratio (~ efficiency of mitochondrial ADP phosphorylation coupled with oxygen consumption) in hippocampal mitochondria, but not in cortical mitochondria, indicating that the effect of diabetes on brain mitochondria is region-specific (Cardoso et al. 2012). Moreira et al. (2003) observed that long-term diabetes facilitated lowered RCR, ADP/O ratio, and ATP/ADP ratio (an indicator of cellular ATP content in brain mitochondria). In view of the link between Alzheimer's disease (AD) and diabetes, they also determined the effect of *ex vivo* exposure of mitochondria isolated from GK-diabetic rat brains to amyloid β -peptides (A β). In the presence of A β (an additional stress) the above-mentioned mitochondrial deficits were potentiated and the effect was larger in GK-diabetic rats compared to non-diabetic control rats (Moreira et al. 2003). Overall, the literature demonstrates impaired electron transport chain efficiency in diabetic brain mitochondria. Confirming these results in animal model of treated diabetic may have more clinical values. It also supports the idea that functional impairment of diabetic mitochondria may be amplified in the presence of additional stress such as cerebral ischemia. These results also surmise that a lower availability of ATP during ischemia / reperfusion owing to pre-existing mitochondrial dysfunction in diabetic brains may contribute to exacerbated cerebral ischemic damage in diabetics.

Generation of free radicals

Enhanced generation of reactive oxygen species (ROS) and a compromised endogenous antioxidant system is documented to play a vital role in secondary complications of diabetes including cardiovascular diseases (Ceriello 2003; Selvaraju et al. 2012). Further, diabetes is documented to increase glucose auto-oxidation that causes non-enzymatic glycation of proteins, which leads to further elaboration of reactive oxygen species (Karasu 2010) that play a key role in ischemic brain injury. Many specialized biochemical mechanisms involved in the pathogenesis of diabetes, such as the auto-oxidation of glucose, stimulation of the polyol pathway, related synthesis of advanced glycation end products (AGEs), and inhibition of endogenous antioxidant enzyme systems are documented to enhance oxidative stress in the central nervous system of diabetic subjects (Cameron et al. 1996; Kaneto et al. 1994; Maritim et al. 2003; Monnier 2003; Yorek 2003). Moreover, chronic imbalance between free radical generation and their quenching by endogenous antioxidant enzymes during diabetes was noted to cause damage to important biochemical constituents of neurons such as proteins, lipids and nucleic acids (Valko et al. 2007).

Hyperglycemia augments the tri-carboxylic acid cycle-based flux of electron donors into the mitochondrial electron transport chain, which blocks electron transfer to complex III owing to a high mitochondrial membrane potential (Giacco and Brownlee 2010; Trumper 1990). The electrons are then accumulated at coenzyme Q instead, resulting in their donation to molecular oxygen, which denotes the conversion of oxygen molecules into superoxide free radicals (Giacco and Brownlee 2010). The role of coenzyme Q in diabetes is further highlighted by a study of Moreira et al. (2005) who showed that coenzyme Q-9 levels are

decreased in mitochondria isolated from brains of Stz-diabetic rats. Further, a major fraction of ROS generated in mitochondria has been attributed to the activation of complexes I and III (Boveris et al. 1976; Turrens and Boveris 1980).

Stz-induced diabetes in laboratory animals leads to an increased production/activation of nitric oxide, mitochondrial nitric oxide synthase, oxidized / reduced glutathione ratio (GSSG/GSH) and hydrogen peroxide levels (H_2O_2), while the levels of mitochondrial superoxide dismutase (manganese superoxide dismutase: MnSOD) are decreased (Mastrocola et al. 2005; Cardoso et al. 2010). Similar results are also reported by Singh et al. (2004) who observed that significant decrease in mitochondrial catalase activity, Mn-SOD activity and decrease in GSH levels as well as increases in lipid peroxidation and glutathione peroxidase activity in the ischemic diabetic brain. These studies suggest an imbalance between the production and scavenging of mitochondrial reactive oxygen species.

In order to determine if brain mitochondria from diabetic rats (GK-diabetic) are more susceptible to oxidative stress, Santos et al. (2001) exposed brain mitochondria isolated from brains of diabetic and control rats *in vitro* to the oxidant pair ADP/ Fe^{2+} . They observed that brain mitochondria isolated from diabetic rats were more susceptible to oxidative damage compared to control non-diabetic rats, and this increased susceptibility was inversely correlated with their antioxidant levels (α -tocopherol and coenzyme Q). Similarly, alloxan-induced diabetes has also been noted to cause an imbalance between pro-oxidant and anti-oxidant systems. This effect varied among different brain regions (Ceretta et al. 2012). In summary, these studies indicate that diabetes alters the balance between pro-oxidant and anti-oxidant systems in the brain, leading to oxidative stress. This increased baseline of oxidative stress along with post-ischemic oxidative stress may lead to the activation of multiple mechanisms resulting in cell death, thus amplifying cerebral ischemic damage in diabetes. Determining the efficacy of anti-oxidant therapy in reducing oxidative damage in animal models of diabetes (treated with and without glucose lowering drugs) may help develop new strategies to lower oxidative damage in diabetic patients.

Involvement in programmed cell death

Mitochondria possess a vital role in the pathogenesis of cerebral ischemia, by means of mediating multiple cell death pathways. The continued stimulation of these death pathways and mitochondrial destabilization causes mitochondrial damage, resulting in the opening of mitochondrial membrane permeability transition pores (Hajnóczky et al. 2006; Hengartner 2000; Horbinski and Chu 2005; Lumini-Oliveira et al. 2011; Naderi et al. 2006; Oliveira 2005). Further, mitochondrial membrane destabilization results in the release of mitochondrial components such as cytochrome *c* and apoptosis-inducing factor (AIF), which in turn regulate intrinsic programmed cell death pathways. In the caspase cascade-based process of cell death, the released cytochrome *c* activates caspases which in turn cause the breakdown of many cellular proteins and DNA (Taylor et al. 2008). Other ischemic cell death pathways independent of caspase activation involve calpain- and poly (ADP-ribose) polymerase-1 (PARP1)-mediated AIF release from the mitochondria, with subsequent translocation to the nucleus (Galluzzi et al. 2009; Liu et al. 2011). Moreover, Apaf-1 interacting protein (AIP), an inhibitor of Apaf-1 and Bcl-xL, mediates mitochondrial

pathways leading to ischemic cell death (Galluzzi et al. 2009). End effectors of mitochondrial membrane disruption that lead to ischemic neuronal death via pore formation in the outer mitochondrial membrane include Bcl-2 family proteins; viz., Bax, Bak, Bid, Bad, Bim, and PUMA (Youle and Strasser 2008). Biochemical pathways upstream of mitochondrion that is noted to induce neuronal death involves the activation of the Mitogen Activated Protein (MAP) kinase c-Jun N-Terminal Kinase (JNK) signalling by modulation of various proteins such as Bcl-2, Bax, Bad, Bim, Bcl-xL, Bcl-2 and c-Jun (Johnson and Nakamura 2007). During an ischemic event, activation of these pathways is noted to cause calcium accumulation-based swelling and lysis of mitochondria. Such mitochondrial disruption results in massive release of biochemical stressors which are known to activate certain critical pathways of cell death (Vosler et al. 2009). Activation of such pathways has been proposed to be particularly significant during diabetes. Myranyi et al (2003) undertook to study if diabetes activates cell death pathways following cerebral ischemia. They observed that release of cytochrome *c* into the cytosol was markedly enhanced in diabetic rat brains compared to non-diabetic rats. They also observed increased activation of cell death pathways (activated caspase-3 and cleaved poly-ADP ribose polymerase) following cerebral ischemia in diabetic rat brains compared to non-diabetic rat brains. Their results suggest that enhanced activation of cell death pathways in diabetic rats may play a key role in increased cerebral ischemic damage (Muranyi et al. 2003). Further, diabetes is shown to increase caspase-9 activity in hippocampus (Cardoso et al. 2012). Long-term diabetes causes morphological and immunohistochemical abnormalities in mitochondria of dorsal root ganglion neurons in Stz-diabetic rats (Correia et al. 2012; Schmeichel et al. 2003). Hyperglycemia in cultured dorsal root ganglion leads to increased mitochondrial fission / fragmentation which in later stages cause apoptosis (Leininger et al. 2006). Mitochondrial fission also plays an important role in apoptosis signalling (Otera et al. 2013). It is plausible that excessive mitochondrial fission in diabetes may exacerbate post-ischemic mitochondrial fission leading to increased cerebral ischemic damage. Therefore, diabetes-related aggravation of ischemic neuronal cell death might be primarily ascribed to the excessive elaboration of mitochondria-driven cell death pathways. The literature further demonstrates that diabetic hyperglycemia-based upregulation of principal mediators of cell death; viz. cytochrome *c*, poly-ADP ribose polymerase and caspases, form the mechanistic basis of enhanced ischemic cell death in diabetic subjects.

Calcium homeostasis

Besides being the major contributor to energy in the cell, mitochondria also play an important role in cellular Ca^{2+} buffering (Hajnóczky et al. 2006; MacAskill et al. 2010; Rizzuto et al. 2012; Walsh et al. 2009). Mitochondria have Ca^{2+} transport machinery that accumulates and releases Ca^{2+} in a controlled manner (Rizzuto et al. 2012). Mitochondrial Ca^{2+} buffering participates in the regulation of metabolism, cell survival, and synaptic function in neurons (Hajnóczky et al. 2006; MacAskill et al. 2010; Rizzuto et al. 2012; Walsh et al. 2009). As mentioned earlier, post-ischemic activation of NMDA receptors, owing to excitotoxicity, leads to cellular Ca^{2+} influx (Lipton 1999). Once this influx surpasses mitochondrial Ca^{2+} buffering capacity, it leads to mitochondrial swelling, which results in necrotic cell death, and release of pro-apoptotic molecules from mitochondria into the cytosol, which ultimately causes the activation of apoptotic cell death pathways as well

(Hajnóczky et al. 2006; MacAskill et al. 2010; Rizzuto et al. 2012; Walsh et al. 2009). These studies indicate that mitochondrial pathways are involved in both necrotic and apoptotic cell death. Diabetes is shown to lower brain mitochondrial Ca^{2+} accumulation capacity (Moreira et al. 2006). Impaired mitochondrial Ca^{2+} buffering can result in lower threshold for the release of pro-apoptotic pathways leading to increased cell death following cerebral ischemia in diabetics.

Acidosis

Hyperglycemia at the time of cerebral ischemia leads to enhanced lactate and H^+ production, leading to intra- and extracellular acidosis (Siesjo et al. 1996) concomitant with increased cerebral ischemic damage beyond the “lactate threshold” (Combs et al. 1990). Acidosis contributes to cerebral ischemia-induced brain damage by affecting multiple mechanisms including mitochondrial cell death pathways. Acidic pH is associated with loss of activity of mitochondrial electron transport chain complexes (Rouslin 1983; 1991). The degree of enzyme activity loss was correlated with the severity of acidosis, and different electron transport chain complexes had different labilities to acidic pH (Rouslin 1983). This study suggests decreased energy production during acidosis. On the other hand, acidosis is suggested to decrease the rate of ATP hydrolysis, which may be beneficial during ischemia / reperfusion when energy demand is high (Rouslin et al. 1986). Acidosis is shown to lower mitochondrial calcium buffering capacity (Fedorovich et al. 1996; Robertson et al. 2004). Lowered extracellular pH results in depolarization of intrasynaptosomal mitochondria and increased oxidative stress in isolated rat brain synaptosomes (Pekun et al. 2013). The acid-sensing ion channel 1a (ASIC1a) mediates acidosis-induced neuronal injury in several pathophysiological conditions is present on the plasma membrane as well as in mitochondria (Wang et al. 2013). ASIC1a is suggested to play a role in regulation of mitochondrial permeability transition pores (Wang et al. 2013). The literature suggests that the deleterious effects of acidosis on mitochondrial function are much outweighed by its beneficial effects, further supporting the participation of acidosis in brain damage following cerebral ischemia in hyperglycemic conditions. Testing efficacy of pharmacological agent that buffer intraischemic pH in animal models of diabetes may help design novel therapeutic approach to lower cerebral ischemic damage in diabetics.

Hypoglycemia

Hypoglycemia is an unwanted side effect of intensive glucose-lowering therapies in diabetes (Cryer 2010; Dagogo-Jack 2004; Oyer 2013). Patients exposed to repeated hypoglycemia develop “hypoglycemia unawareness” leading to a vicious cycle of recurrent hypoglycemia (RH) (Bakatselos 2011; Cryer 2010; Dagogo-Jack 2004; Oyer 2013). RH is widely reported in both Type 1 and Type 2 (more commonly in advanced stages) diabetics (Cryer 2010; Dagogo-Jack 2004; Oyer 2013). Earlier we observed that prior exposure of insulin-treated diabetic rats to RH exacerbates cerebral ischemic damage (Dave et al. 2011). Hypoglycemia results in substrate limitation and thus affects mitochondrial functions. Earlier studies demonstrated that mitochondrial substrate limitation following hypoglycemia increases mitochondrial free radical production (McGowan et al. 2006). Acute insulin-induced hypoglycemia in Stz-diabetic rats resulted in decreased ADP/O index, increases the

repolarization lag phase (the time necessary for ADP phosphorylation) and decreases the GSH/GSSG ratio (decreased reduced glutathione levels) in cortical mitochondria while increased H₂O₂ production in hippocampal mitochondria (Cardoso et al. 2010). Chronic (1 week) moderate hypoglycemia in non-diabetic rats resulted in lower state 3 respiration and RCR (Pelligrino et al. 1989). This decrease in state 3 respiration and RCR was more pronounced when Stz-diabetic rats were exposed to chronic moderate hypoglycemia (Pelligrino et al. 1989). Sub-acute (5 days) recurrent hypoglycemia in Stz-diabetic rats does not alter mitochondrial respiration parameters (Dave et al. 2011). However, chronic (14 day) exposure to recurrent hypoglycemia results in decreased state 3 respiration, RCR, ADP/O ratio and higher state 4 respiration (Cardoso et al. 2012). The precise mechanism by which recurrent hypoglycemia mediates mitochondrial dysfunction is unknown. It has been shown that, during hypoglycemia, glutamate-induced excitotoxicity elicits activation of a phosphatidylinositol 4,5-bisphosphate and μ -calpain-dependent increase in the intracellular release of Ca²⁺ (Li and Gong 2007; Tang et al. 2002; Yamashima et al. 1996). This increased level of calcium leads to mitochondrial dysfunction, possibly owing to free radical production associated with the activation of calcium binding proteins (Monje et al. 2000; Adam-Vizi and Starkov 2010). However, detailed dissection of the complete transduction system involved in mediating hypoglycemia-based mitochondrial dysfunction and associated aggravation of ischemic neuronal injury, and the way by which these mechanisms may be likened to the above-mentioned mechanisms of hyperglycemic mitochondrial dysfunction based on exacerbation of ischemic brain damage, still requires an exhaustive experimental evaluation. Overall, the literature demonstrates that exposure to acute, chronic, or RH leads to cerebral mitochondrial dysfunction. This likely renders the brain more susceptible to the exacerbation of damage during cerebral ischemia

Recent advances and future directions

Although some of the medications being tested have shown promise in preclinical settings, the realization of an all-encompassing and foolproof clinical approach to treat ischemic stroke in non-diabetic and diabetics is yet to be realized. The potential of mitochondrial therapeutic targets as the basis of neuroprotective strategies in ischemic stroke has been affirmed by the preclinical data, which seem to hold promise and therefore should be used as an adjunct therapy to provide maximal brain tissue salvation. However, clinical trials need to be carried out, not only with a view of individually validating the clinical efficacy of modulating the mitochondrial targets *per se* but also to assess the therapeutic potential of such interventions in the clinical setting, particularly for long-term neuroprotection, even with delayed administration. Further, among the wide arrays of mitochondrial therapeutic targets proposed in the literature and discussed above, it is also important to define firstly, the bigger picture of interrelationship between the same; secondly, the comparisons between the relative quantitative significance of various mitochondrial enzyme/receptor systems in mediating pathogenesis of diabetes-related ischemic brain damage; and thirdly, identification of the principal rate-limiting steps that may be effectively regulated by pharmacological methods. Further, it is also important to study correlations among the pharmacological data, in which is evaluated the actual effect of selective modulation of

different therapeutic targets proposed to play a key role in mitochondria-mediated ischemic neuronal damage in diabetics.

Conclusion

Scientific advances in the pathobiology of ischemic brain disease in diabetes have revealed a multitude of potential mitochondrial targets in the management of ischemic neuronal injury in diabetics, against which drug discovery efforts have and can be directed. However, the clinical usefulness of most of these targets is still to be explored. Given that many of these therapeutic targets appear to be interrelated, targeting them individually may not yield sufficient clinical impact. Research integrating these individual potential targets may give a clearer picture of composite mechanisms worthy of future attention. Such work will ultimately help address some of the severe medical challenges in managing ischemic stroke associated with diabetes.

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Figure 1. Schematic representation of mitochondrial mechanisms involved in mediating the pathophysiology of ischemic cell death in diabetes.

Table 1

Available clinical approaches to treat cardiovascular disease associated with diabetes (International Diabetes Federation 2005).

Serial Number	Clinical Approach	Agents/ Interventions Employed
1	Control of blood glucose	Metformin, sulfonylureas, PPAR- γ agonists (thiazolidinedione), α -glucosidase inhibitors and insulin
2	Control of blood pressure	ACE-inhibitors and angiotensin-2 receptor blockers
3	Lifestyle interventions	Reducing intake of calories, salt, alcohol, and inactivity
4	Cardiovascular risk assessment	Smoking and serum lipids, and family history of premature cardiovascular disease
5	Lipid-modifying therapy	Statins viz., simvastatin
6	Anti-platelet therapy	Aspirin and clopidogrel