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Hyperglycemia, Acute Ischemic Stroke and Thrombolytic Therapy

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Abstract

Ischemic stroke is a leading cause of disability and is considered now the 4th leading cause of death. Many clinical trials have shown that stroke patients with acute elevation in blood glucose at onset of stroke suffer worse functional outcomes, longer in-hospital stay and higher mortality rates. The only therapeutic hope for these patients is the rapid restoration of blood flow to the ischemic tissue through intravenous administration of the only currently proven effective therapy, tissue plasminogen activator (tPA). However, even this option is associated with the increased risk of intracerebral hemorrhage. Nonetheless, the underlying mechanisms through which hyperglycemia (HG) and tPA worsen the neurovascular injury after stroke are not fully understood. Accordingly, this review summarizes the latest updates and recommendations about the management of HG and co-administration of tPA in a clinical setting while focusing more on the various experimental models studying: 1. the effect of HG on stroke outcomes; 2. the potential mechanisms involved in worsening the neurovasular injury; 3. the different therapeutic strategies employed to ameliorate the injury, and finally; 4. the interaction between HG and tPA. Developing therapeutic strategies to reduce the hemorrhage risk with tPA in hyperglycemic setting is of great clinical importance. This can best be achieved by conducting robust preclinical studies evaluating the interaction between tPA and other therapeutics in order to develop potential therapeutic strategies with high translational impact.

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DISCLOSURE/CONFLICT OF INTEREST

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INTRODUCTION

Thrombolytic therapy with tissue plasminogen activator (tPA) to reopen occluded cerebral blood vessels is currently the best chance acute ischemic stroke patients have of recovering normal function. Elevated blood glucose at the time of acute stroke increases the risk of hemorrhagic transformation with tPA treatment and it is associated with poor clinical outcomes, longer in-hospital stay, increased cost, and mortality. As almost 40% of stroke patients present with hyperglycemia, this is an important clinical problem. The optimal approach to manage these patients, especially with respect to glucose control and tPA treatment, is not clear and the various professional guidelines differ in their recommendations. The mechanisms contributing to exacerbated neurovascular injury and poor outcomes are not fully understood. The purpose of this review is to briefly summarize the clinical evidence on hyperglycemia and tPA interactions in acute ischemic stroke, and discuss how preclinical studies approach this problem with an emphasis on the experimental models of hyperglycemia and methods of reperfusion used in these studies.

I. CLINICAL EVIDENCE

Acute stroke patients who have hyperglycemia on admission or persistent hyperglycemia during the first three days of hospitalization have worse functional outcomes than patients without hyperglycemia [1-3]. This finding has been confirmed by many, although not all [4] observational stroke studies. A large proportion of acute stroke patients with hyperglycemia have diabetes mellitus. The complications associated with chronic diabetes mellitus may be contributing to a worse functional outcome in stroke patients compared to those without diabetes. However, many studies show worse clinical outcomes in acute stroke patients with hyperglycemia without a history of diabetes [5, 6]. The interpretation of such findings is complicated by the fact that some acute stroke patients with hyperglycemia have undiagnosed (unknown) diabetes mellitus.

The exact mechanisms by which hyperglycemia (during acute ischemic stroke) leads to worse functional outcome have not been established. It could be that the hyperglycemia during ischemia somehow results in greater brain injury compared to normoglycemia. Hyperglycemia during acute brain ischemia may impair thrombolysis and reperfusion [7, 8]. For example, HG increases coagulation by increasing thrombin production and stimulating the tissue factor pathway [9, 10]. HG also reduces the fibrinolytic activity of tPA by increasing the production of plasminogen activator inhibitor (PAI)-1 [11]. Additionally, hyperglycemia during acute brain ischemia may exacerbate or accelerate some of the pathologic processes involved in ischemic brain injury [12]. In addition, hyperglycemia increases the risk of cerebral hemorrhage in acute stroke patients treated with intravenous tPA [5, 13-16]. A list of major clinical and preclinical studies that reported increased bleeding with tPA and HG are summarized in Table 1.

It is not clear whether there is a blood glucose threshold that increases the risk of unfavorable functional outcomes in acute stroke. Some acute stroke studies report a hyperglycemia threshold for worse functional outcomes [17], but other report a linear relation between blood glucose and poor functional outcomes [5]. If hyperglycemia during

acute stroke is detrimental, it might be beneficial to lower the blood glucose level swiftly during the initial few hours or days after stroke onset. However, this remains controversial. In traumatic brain injury, intensive insulin therapy was associated with increased risk of hypoglycemia and no improvement in the neurologic outcomes [18, 19]. In ischemic stroke, one limited clinical efficacy trial did not show a benefit of intravenous insulin treatment for mild hyperglycemia [20]. Yet, another small study showed that insulin treatment was associated with a high incidence of hypoglycemia and greater infarct growth in patients with persistent arterial occlusion compared with controls [21]. Another trial of intravenous insulin treatment for patients with greater hyperglycemia and predominantly with diabetes mellitus in acute stroke is ongoing [22].

As might be expected from the paucity of scientific evidence for efficacy, there are limited guideline recommendations for the blood glucose goals during acute stroke. The American Heart Association/American Stroke Association current guidelines [23] recommend maintaining the blood glucose level in the range 140-180 mg/dL during the acute stroke hospitalization. The European Stroke Organization guideline [24] recommends lowering the blood glucose with insulin to below 180 mg/dL. No specific mention is made in the USA or the European guidelines regarding lowering the blood glucose during tPA therapy [23, 24]. Since hyperglycemia increases the risk of cerebral hemorrhage when tPA is used in acute stroke, it might be beneficial to correct the hyperglycemia as soon as possible. Intravenous insulin has an onset of action of approximately 1-2 minutes and could be administered as soon as tPA therapy is contemplated. As each patient's insulin needs and reaction to stress are individualized, a general insulin dose recommendation cannot be made. Hypoglycemia (<60 mg/dL) can be detrimental and should be avoided. Nonetheless, if the blood glucose is for example, 400 mg/dL, an intravenous dose of 10 units regular insulin seems reasonable, as it should lower the blood glucose by a clinically significant amount with little risk for hypoglycemia. Additional doses will likely be needed to maintain the blood glucose <180 mg/dL.

Thrombolytic therapy for acute stroke has been approved in many countries. It should be noted that in Europe, prior stroke with concomitant diabetes is an exclusion criteria from tPA treatment [25, 26, 1-3]. For this reason, the European Cooperative Acute Stroke Study (ECASS) III, which provided the clinical evidence to extend the therapeutic window up to 4.5 hours for the administration of tPA, did not include patients with prior stroke and concomitant diabetes or patients with blood glucose over 400 mg/dL [26]. However, whether hyperglycemia shortens the therapeutic window for tPA is not yet determined. Developing strategies to reduce the hemorrhage risk with tPA thrombolysis in hyperglycemic settings is therefore of great importance. Equally important, determining the therapeutic potential and known side effects of tPA under hyperglycemic conditions along with other commonly found comorbidities/risk factors in stroke patients is critical. In this regard, the potential interaction of tPA with experimental therapeutics needs to be carefully evaluated, and experimental studies are critical to provide the much needed preclinical data to move the field forward.

II. PRECLINICAL EVIDENCE

In this section, we will first summarize different experimental models of hyperglycemia and the effect of hyperglycemia on short-term stroke outcomes. We will then discuss potential mechanisms contributing to exacerbated reperfusion injury and finally, review the different therapeutic strategies employed to reduce the injury (pretreatment and acute post-stroke treatment) and promote recovery (chronic post-stroke treatment).

A. Models of Hyperglycemia and Impact on Short-term Outcomes

Acute hyperglycemia may result from a stress response or due to preexisting diabetes. Early experimental studies in this field used mainly acute changes in blood glucose in which hyperglycemia was induced by glucose injection or by depleting insulin producing islet cells with streptozotocin (STZ) injection a few days prior to stroke surgery and reported short-term outcomes. As recently reviewed, most, if not all of these studies, used the suture occlusion model of stroke and showed that acute hyperglycemia increases neuronal and vascular injury after ischemic stroke leading to poor outcomes as reported in clinical studies [27, 28].

Studies using different diabetic animal models highlighted the importance of preexisting disease on stroke injury and functional outcomes [29]. Nedergaard et al reported that compared with normoglycemia, the infarct volume was decreased in hypoglycemic rats, unaltered in acute diabetes induced by single STZ injection 2 days before middle cerebral artery occlusion (MCAO) and increased in chronic diabetes induced by STZ injection 4 months before MCAO [30]. When Zucker Diabetic Fatty Rats (ZDF) (a type 2 diabetic rat model that mimics the chronic metabolic and inflammatory abnormalities observed in humans) with blood glucose 350-450 mg/dl were subjected to 2 h suture occlusion and 4 h reperfusion, there was a significant increase in neutrophil adhesion and aggregation after reperfusion. This was associated with an increase in the cerebral expression of the inflammatory mediators sICAM, IL-1β and E-selectin, infarct size, and worse neurological outcomes [31]. Several studies also reported greater gray and white matter injury, higher mortality, increased cerebral edema and worsened neurological outcomes in type 2 diabetic (db/db) mice after brain ischemia which was associated with upregulation of matrix metalloprotease-9 (MMP-9) [32-36]. Using the lean Goto-Kakizaki (GK) model of type 2 diabetes, we showed that diabetes mediates pathological remodeling and neovascularization of the brain, and ischemia/reperfusion injury superimposed on this preexisting vascular disease causes extensive vascular injury and bleeding into the brain [37-41]. Despite the fact that GK rats developed relatively smaller infarcts compared to control animals at 24 h after stroke, the functional outcome was worse most likely due to increased bleeding and edema.

While discrepancies in infarct size in these studies may result from differences in the duration of ischemia, an important observation that stems out from the review of these preclinical studies is the vulnerability of the vasculature to relatively small changes in blood glucose and how this in turn worsens the functional outcomes. There is no doubt that neuroprotection is very important but poor outcomes of stroke cannot be solely explained by greater infarcts in hyperglycemic stroke. For example, Xing et al reported that hyperglycemia increases blood brain barrier (BBB) permeability and hemorrhagic

transformation (HT) and worsens outcome but does not affect the infarct volume [42]. We also showed that a mild elevation in blood glucose (140-200 mg/dl) achieved by intraperitoneal injection of 40% glucose solution did not increase infarct size yet worsened vascular injury and neurological outcomes [43]. As discussed above, diabetic GK rats develop greater HT and edema compared to their nondiabetic counterparts leading to unfavorable outcomes without a significant increase in infarct size. Therefore, as we move forward in stroke research, the roles of the vasculature and HT on functional outcomes and recovery need to be carefully delineated.

B. Mechanisms of Hyperglycemic Reperfusion Injury

Although restoration of blood flow to the ischemic tissue is essential to rescuing the penumbral tissue, reperfusion can increase the ischemic injury [44] and hyperglycemia further exacerbates the process. Mechanisms contributing to exacerbated neurovascular injury, HT and poor outcomes in hyperglycemic stroke are likely to be multifactorial and different mechanisms may be at play at the neuronal, glial and/or vascular levels as recently reviewed [27, 29, 28]. Augmented oxidative and nitrative stress under hyperglycemic conditions can modify tight junction proteins and structure compromising BBB integrity. For example, occludin levels are decreased to a greater extent under hyperglycemic hypoxic conditions [45]. HG also heightens endothelial mitochondrial damage and decreases BBB transport which can lead to loss of BBB integrity and increased hemorrhage [46, 47]. Since the goal of reperfusion therapies after stroke is to open the occluded artery and reestablish the cerebral blood flow (CBF), in this review we will focus on cerebrovascular mechanisms and review how hyperglycemic reperfusion affects cerebrovascular function and ultimately CBF. We will also summarize the limited number of studies investigating the effect of tPA on cerebrovascular tone.

1. Hyperglycemia and Cerebral Blood Flow After Stroke—To study the influence of acute hyperglycemia on CBF during ischemia/reperfusion injury, Kawai et al occluded the middle cerebral arteries (MCA) for 2 or 4 h followed by 2 h of reperfusion in rats made acutely hyperglycemic by intraperitoneal administration of glucose 20 minutes before MCA occlusion. They showed that CBF was reduced in the ischemic hemisphere of hyperglycemic rats compared to normoglycemic controls. They also found that poor restoration of CBF in hyperglycemic rats was associated with increased infarct size [48]. These results are in agreement with other studies in rats [49-51] and cats [52-54] which reported reduced CBF in hyperglycemic compared to normoglycemic animals that could limit compensatory blood flow mechanisms and accentuate brain dysfunction after ischemic injury. In contrast, Gisselsson et al found that acute hyperglycemia had no effect on reperfusion blood flow after 30 min ischemia and 1 h of reperfusion, refuting the hypothesis that exacerbated injury after acute hyperglycemic stroke is caused by disturbances in CBF [55]. This discrepancy between studies could result from differences in hyperglycemia severity and its duration.

2. Hyperglycemia and Cerebral Autoregulation—Cerebral autoregulation is critical for maintaining constant blood flow despite changes in perfusion pressure [56-58]. Autoregulation is highly pronounced in the brain and large cerebral arteries contribute

significantly to cerebral autoregulation to protect downstream microvessels [59, 60]. Although the mechanisms of autoregulation in the brain are not fully understood, several studies have shown that the myogenic behavior of the cerebral smooth muscles play a crucial role in the development of autoregulation. Myogenic response describes the intrinsic ability of the smooth muscle cells to constrict in response to increased pressure and dilate in response to decreased pressure to achieve constant blood flow [57, 61, 62]. To investigate the influence of acute exposure to high glucose levels on cerebral myogenic behavior, rat posterior cerebral arteries were exposed to 44 versus 5.5 mmol/L l-glucose and the amount of basal tone and the response to transmural pressure were determined. High glucose levels induced vasodilation and loss of basal tone which made the vessels incapable of responding to changes in transmural pressure [63]. These results are consistent with the findings of Sieber et al which demonstrated that acute hyperglycemia leads to an elevation in CBF and a reduction in cerebrovascular resistance in nondiabetic dogs [64]. Helpern et al also showed that rats infused with 25% D glucose experienced impaired cerebral autoregulation and increased CBF which persisted for 15 min even after normalization of plasma glucose levels [65].

Chronic hyperglycemia has a profound effect on vascular and endothelial function. However, very few studies have focused on the effect of diabetes on the myogenic reactivity of cerebral vessels. Similar to acute hyperglycemia, diabetes can modify cerebrovascular resistance and CBF. Enhanced myogenic tone was reported in cerebral arteries isolated from STZ-induced diabetic rats compared to control [66]. Similar findings were observed in cerebral arteries isolated from type 2 diabetic rats [67-69]. While these findings contradict with a previous report showing a decrease in myogenic tone in cerebral vessels isolated from diabetic rats, differences in the degree of HG may account for this disparity [70]. Taken together, these findings indicate that acute and chronic exposure to high glucose has a deleterious effect on vascular reactivity which may ultimately affect CBF.

3. Hyperglycemia and Cerebral Myogenic Behavior After Stroke—Little attention has been given to the impact of acute and chronic hyperglycemia on myogenic behavior of cerebral vessels during ischemia/reperfusion injury. Cipolla et al investigated the effect of acute hyperglycemia on CBF and the reactivity of penetrating arterioles before and after MCAO. The penetrating arterioles are the major vessels involved in lacunar stroke, a type of stroke characterized with favorable outcomes after moderate hyperglycemia [4]. They showed that reperfusion blood flow was not influenced by acute hyperglycemia prior and after stroke. Basal tone was reduced in both normoglycemic and hyperglycemic animals to the same degree suggesting that it was due to ischemia/reperfusion injury and independent of glucose. They also demonstrated that acute hyperglycemia had no effect on endothelium dependent vasodilator production in penetrating arterioles which may explain why lacunar strokes are not worsened by hyperglycemia [71]. However, studies done on cortical stroke revealed a deleterious effect of acute hyperglycemia on vascular reactivity. When naïve MCAs were perfused intraluminally with plasma of acutely hyperglycemic rats that underwent 2h MCAO/2h reperfusion, they reported increased myogenic tone and endothelial dysfunction, suggesting that circulating factors in plasma due to acute hyperglycemic stroke are vasoactive in nonischemic cerebral vessels [72].

We recently showed that ischemia/reperfusion injury has the global effect of decreasing the myogenic tone of MCAs in both ischemic and nonischemic hemispheres. We also demonstrated that ischemia/reperfusion injury superimposed with acute elevation of blood glucose caused exacerbation of myogenic dysfunction in the nonischemic hemisphere, which was associated with worse stroke outcomes [73]. Similar results were reported by our group showing that myogenic tone of MCAs isolated from control and type 2 diabetic rats was reduced after exposure to 20 min of oxygen glucose deprivation to mimic ischemic injury, which was greater in the diabetic group [68]. Taken together, these findings suggest that acute or chronic HG may exacerbate myogenic dysfunction and impair effective reperfusion, which ultimately can contribute to the detrimental effect of hyperglycemia on stroke outcomes.

4. tPA and Cerebrovascular Function—As discussed above, the only effective and FDA approved treatment for ischemic stroke is to reestablish CBF using recombinant tPA. The most critical adverse effect of tPA administration, hampering its use in stroke patients, is symptomatic intracerebral hemorrhage [74]. To determine whether the hemorrhagic complications associated with tPA are due to a direct deleterious effect on vascular reactivity, MCAs were perfused with tPA and myogenic tone was determined. Intraluminal perfusion of tPA significantly impaired myogenic reactivity in isolated MCAs (66). This myogenic dysfunction was augmented in arteries exposed to ischemia/reperfusion injury and perfused with tPA. In addition, treatment with tPA caused endothelial dysfunction and diminished vasodilation to acetylcholine and 5HT reactivity, and again these effects were exacerbated if vessels were exposed to ischemia/reperfusion [75]. In control rats, tPA diminished myogenic tone of MCAs and caused concentration dependent vasodilation which was prevented by the stabilization of actin cytoskeleton of smooth muscle [76]. These findings suggest that tPA administration has a direct detrimental effect on myogenic reactivity which could contribute to vascular injury after stroke.

Vascular tone is highly regulated by the activity of potassium channels which is a major determinant of membrane potential [60]. A prior study reported that potassium channel activity was impaired after cerebral hypoxia/ischemia, which was aggravated by tPA treatment leading to autoregulation impairment via upregulation of extracellular signal-regulated kinases/mitogen-activated protein kinases [77]. These findings are in agreement with the data which showed that exogenous plasminogen activator administration augmented the hypercapnic and hypotensive cerebrovasodilation impairment in the newborn pig [78, 79]. Taken together, all these studies provide evidence that tPA treatment may adversely affect the cerebrovascular reactivity, especially if superimposed on ischemic injury. However, the impact of tPA administration on vascular function in the presence of hyperglycemia is yet to be determined. Better understanding of how tPA therapy affects vascular reactivity during acute hyperglycemic stroke might be crucial for avoiding its deleterious effects that profoundly constrain its clinical utility.

C. Treatment Strategies in Experimental Hyperglycemic Acute Ischemic Stroke

This section will summarize different therapeutic strategies employed to reduce ischemic injury (pretreatment and acute post-stroke treatment) and promote recovery (chronic post-stroke treatment).

1. Preventive Treatment Strategies—Recent clinical and experimental studies suggest that statins play a pivotal role in reducing the incidence of stroke and myocardial infarction in patients with vascular diseases. This was suggested to be due to statins' pleiotropic rather than lipid lowering effects [80, 81]. After 4 weeks of diabetes induced by STZ injection, animals were treated with vehicle or simvastatin (1 mg/kg/day) for 14 days. Subsequently, mice were subjected to 90 min MCAO and 24 h reperfusion. Diabetes aggravated the stroke outcome and increased the infarct size compared to non-diabetic mice. Pretreatment with simvastatin for 14 days prior to stroke significantly reduced the infarct volume and improved the neurological outcomes in both diabetic and non-diabetic mice [82].

As discussed above, diabetic GK rats develop greater vascular injury and have poor functional outcomes. These rats also exhibit extensive cerebrovascular remodeling and neovascularization, which was prevented by early glucose control with metformin or inhibition of matrix metalloproteases with minocycline for 5 weeks starting at the onset of diabetes [83]. Prevention of vascular remodeling significantly reduced the vascular injury and improved the functional outcome when compared to non-treated GK rats [39]. This suggests that glycemic control and vasculoprotective treatments may be effective preventive strategies in reducing stroke injury.

Thiazolidinediones (TZD) are peroxisome proliferator activated receptor agonists that are commonly used to lower blood glucose. One study evaluated the neuroprotective effects of rosiglitazone in type II diabetic db/db mice and their non-diabetic db/+ littermates. All animals were subjected to 45 min focal cerebral ischemia (suture occlusion) followed by 3 days of reperfusion. Rosiglitazone 4 mg/kg (i.p.) treatment 4 h prior to stroke or 2 mg/kg (i.p.) at 2 h reperfusion significantly decreased the infarct volume and induced neuroprotection without affecting blood glucose. This suggests that TZDs have direct neuroprotective effects independent of blood glucose lowering. However, feeding mice with a chow fortified with rosiglitazone for 3 weeks before inducing MCAO significantly decreased blood glucose in db/db mice without affecting the db/+ (normoglycemic genetic control of db/db) blood glucose levels. The long term oral pretreatment with rosiglitazone induced significantly better neuroprotection and reduction in the infarct size compared to the bolus injection [84]. In an interesting study, Kumari and his group showed that cerebral inflammatory response is needed for recovery after stroke and this response was delayed and diminished in the brains of diabetic stroked mice [85]. In a recent study, they also showed that the administration of Draglitazone for 7 days before induction of hypoxia/reperfusion significantly reduced the infarct size in diabetic mice. Draglitazone also restored the compromised inflammatory response through increasing the expression of TNF-α, IL-1β and IL 6 at the early phase of recovery [34].

2. Acute-Subacute Treatment Strategies—The key question now is whether the detrimental effects of hyperglycemia during acute brain ischemia can be reversed by rapidly correcting the hyperglycemia, consequently improving outcomes. In animals, rapid correction of hyperglycemia during focal brain ischemia resulted in less brain injury than persistent hyperglycemia [86-93] unless there was hypoglycemia [94, 93]. Insulin is the most commonly used agent to regulate blood glucose and has been shown to reduce ischemic brain damage when given immediately before [87, 92, 95, 96] or within minutes after experimental brain ischemia [97-100]. When type 1 diabetic rats were subjected to 2 h MCAO and 24 h reperfusion by the suture occlusion model, acute or chronic administration of low dose of insulin (2U/kg) did not alter the lesion size or the number of apoptotic cells in the brain. However, the chronic treatment with high dose of insulin (12u/kg) for 7 days significantly reduced the lesion volume and the apoptotic levels [101].

These observations suggest that the deleterious effects of hyperglycemia during acute brain ischemia may be at least partly reversible by rapid correction of hyperglycemia as recently reviewed [1]. Although underlying mechanisms are not fully understood, it has been suggested that insulin might be neuroprotective independent of its blood glucose lowering effects. However, there are several key studies that provided strong evidence that regulation of blood glucose is the main factor for reducing ischemic damage with insulin therapy. One study investigated 3 groups of rats with transient focal brain ischemia: 1. Control vehicle treatment, 2. Insulin pretreatment (2-3 IU/kg) 60 min prior to ischemia, and 3. Insulin pretreatment plus glucose to maintain normal blood glucose the same as in the control group. The resulting mean blood glucose in groups 1, 2, and 3 was 151, 61, and 182 mg/dL, respectively [90]. There was no difference in infarct size between groups 1 and 3 whereas group 2 with lower blood glucose had smaller infarcts. This suggests that the reduction in the infarct size might be due to the blood glucose lowering but not due to direct insulin's neuroprotective effect. Earlier studies reported a linear relationship between blood glucose and pathological stroke outcomes [88]. When hyperglycemic cats were given insulin after MCAO, blood glucose decreased to hypoglycemic levels and this caused increased infarct size and early death when compared to cats receiving saline, again suggesting that when glucose levels are not optimal, presence of insulin does not confer neuroprotection [88]. Interestingly, one study showed that the administration of insulin like growth factor-1 (IGF-1) 30 min before MCAO significantly decreased the lesion volume (Infarct size) and decreased the number of apoptotic cells in the central nervous system as indicated by TUNEL staining and caspase 3 immunoreactivity [102].

In addition to glycemic control, other successful tactics have been reported. For example, deferoxamine (DFX) administration, which is an iron chelator, immediately after MCAO attenuated the mortality rate, HT, infarct volume and brain edema in diabetic rats. These findings suggest that DFX may provide potential means to reduce HT in acute ischemic stroke patients [103]. Another study reported that the inhalation of hydrogen gas during reperfusion reduces hyperglycemia-induced HT and brain infarction resulting in improved neurological function [104]. A recently published study demonstrated the neuroprotective effect of Ginkgo biloba extracts in hyperglycemic rats. Diabetes was induced by single intravenous injection of STZ (50 mg/kg) 4-6 weeks before MCAO. They showed that the

Ginkgolide B was able to reduce the levels of malondialdehyde (lipid peroxidation product), reactive oxygen species and the infarct size leading to better outcome [105], suggesting that oxidative stress might be contributing to hyperglycemic stroke injury.

We showed that therapeutic targets after stroke, especially vasculoprotective approaches, may differ in diabetic models. Atorvastatin (15mg/kg) administration, one dose directly after reperfusion and the second dose 12 hours after a 3 hour MCAO, reduced the bleeding rates, hemoglobin content and infarct volumes in both control and type 2 diabetic GK rats [40]. These effects, however, were independent of changes in plasma and brain tissue lipid peroxides and nitrotyrosine levels. A follow-up study evaluated the effects of acute manipulation of potential targets for vascular protection (i.e., NFkB, peroxynitrite, and matrix metalloproteinases) on vascular injury and functional outcome in GK rats. Animals received a single dose of either FeTPPS (peroxynitrite decomposition catalyst), curcumin (NFκB inhibitor) or minocycline (broad spectrum MMP inhibitor) at reperfusion. All treatments reduced hemorrhagic transformation in diabetic animals and this was associated with a reduction in the MMP9 activity. The different treatments improved the neurological outcomes in varying degrees. In control animals, all treatments reduced MMP9 activity yet bleeding was not reduced suggesting that therapeutic targets for neurovascular protection and dosing of potential treatments may differ in control versus diabetic states [37].

3. Chronic Treatment Strategies for Recovery—Type 1 diabetic rats, subjected to temporary MCAO by the suture model two weeks after induction of diabetes, did not show increased lesion volume. However, they exhibited significantly increased brain hemorrhage, BBB disruption and worsened functional outcomes after 14 days of MCAO. In this model, mortality occurred within the first 3 days after surgery and all animals that died exhibited hemorrhage. Niaspan (40 mg/kg), a prolonged release formulation of Niacin which is in current clinical use for increasing HDL cholesterol and also known to improve endothelial function. Niaspan administration did not alter the lesion volume or the mortality rate in diabetic rats when started at 24 hours after MCAO for 14 days. However, this approach significantly reduced the hemorrhage volume, the BBB leakage and improved the functional outcomes. Niaspan also promoted cerebrovascular remodeling which was accompanied by reduced expression of Angiopoietin 2 (Ang 2) and increased the expression of Ang 1 in the ischemic brain [106]. The same group recently investigated the long term therapeutic effects of Niaspan on axonal remodeling after stroke in type 1 diabetic rats. Interestingly, Niaspan (40 mg/kg) treatment for 28 days, starting 24 h after stroke in diabetic rats, significantly increased the axonal density in the ipsilateral motor cortex compared to saline treated diabetic animals [107]. Previously, the same group also showed that long term Niaspan (40 or 80 mg/kg) treatment for 14 days after MCAO improved the functional outcomes and promoted angiogenesis in non-diabetic male Wistar rats [108].

We have previously shown that GK rats exhibit dysfunctional cerebral neovascularization [83] and when these rats are subjected to ischemia reperfusion injury, they develop HT and worse neurological outcomes [39]. A follow-up study was conducted to investigate the effect of diabetes and glycemic control on reparative neovascularization and functional recovery in diabetes. While control animals showed angiogenesis in the peri-infarct area and even in the contralateral hemisphere, diabetic animals did not only show impaired

angiogenesis but also there was regression of existing vessels and exacerbated astrogliosis. Diabetic animals also exhibited worse neurological outcomes, anxiety-like behavior and cognitive deficits after stroke when compared to the normoglycemic stroked rats. Glycemic control with metformin (300 mg/kg/day) for 14 days after stroke significantly improved the cerebrovascular repair and the functional outcomes in these animals suggesting that glucose control in the recovery phase may be very important for neurovascular repair [109].

Therapeutic angiogenesis is being pursued as a potential treatment for stroke recovery. The use of bone marrow stromal cells (BMSC) therapy was shown to be promising in promoting and improving functional recovery in non-diabetic rats after stroke [110]. However, this was not the case with diabetic rats. Chen et al showed that treating type 1 diabetic rats with BMSCs 24 hours after stroke worsened the long-term outcomes at 14 days. BMSCs also increased the mortality, BBB leakage and brain hemorrhage when compared to diabetic untreated animals [111]. Along with our findings in GK rats, these results suggest that promoting new vessel formation after stroke when there is preexisting diabetic vascular disease may not be useful. Stabilization and maintaining the integrity of existing blood vessels may prove more beneficial. Along with our previous study in which we showed vasculoprotective treatment approaches exert differential effects in control vs diabetic rats [37], this study strongly suggests that stroke treatment strategies should be compared in control and disease models.

D. Hyperglycemia, Stroke and tPA

In spite of the importance of tPA in the clinical setting, only a few experimental studies were conducted to evaluate the role that tPA plays in either improving or worsening the outcomes in hyperglycemic stroke. In an acute hyperglycemia model, ischemia was induced by occluding both common carotid arteries and the left proximal MCA with microaneurysm clips for 90 min. tPA was infused 10 minutes before reperfusion and outcome was assessed at Day 3. Hyperglycemia exacerbated the brain damage in tPA-treated animals through increasing the infarct size, brain hemorrhage and edema [112]. It is also worth mentioning that the tPA-induced brain hemorrhage increased with elevated levels of hyperglycemia. In the same study, the administration of the NADPH oxidase inhibitor, apocynin, significantly reduced the tPA-induced brain hemorrhage [112].

In another study, Ning et al. showed that the administration of tPA 2 h after embolic stroke in type 1 diabetic rats significantly increased HT and brain swelling. tPA failed to reduce the brain infarct and improve functional outcomes [113]. However, a similar study conducted by Fan et al reported decreased infarction with the use of tPA in diabetic rats as compared to untreated diabetic rats. Diabetes increased the infarct volume, edema and brain hemorrhage when compared to control rats. The administration of tPA slightly but significantly decreased the infarct size in diabetic rats but it failed to ameliorate the brain swelling. However, tPA significantly increased intracerebral hemorrhage in diabetic rats compared to control animals [114]. In a recent study by the same group using the same diabetic embolic stroke model, they showed that co-administration of minocycline with tPA after stroke significantly reduced the brain infarction, brain swelling and tPA-induced brain HT, providing evidence that combination therapy with minocycline plus tPA may be beneficial

in improving stroke outcomes in diabetes [115]. The same group used again the embolic stroke model to evaluate the effects of early glycemic control by insulin in combination with tPA on stroke outcomes. Insulin was administered at 1 h after stroke and tPA (10 mg/kg) was given at 1.5 h after stroke. The use of either insulin alone or tPA alone had no effect on the ischemic infarction. However, the early glycemic control with insulin in combination with tPA significantly reduced the brain infarction, brain swelling and the tPA induced hemorrhage [116].

Moving forward: Lessons Learned from Clinical and Experimental Studies— Based on the literature discussed above, there are several important points that should be emphasized in order to advance the field.

1. The severity of hyperglycemia seems to be important for the neuronal injury as mild elevations do not increase the infarct size. However, the vasculature is more susceptible to even small elevations in blood glucose which mediate greater edema and HT leading to poor outcomes in hyperglycemic ischemic brain injury. Preventive and therapeutic strategies that offer vasculoprotection seem to promote neuronal protection and repair.
2. The stroke model with respect to method of occlusion and reperfusion and the duration of ischemia prior to reperfusion may impact the injury and recovery.
3. With no exception, all the above studies were conducted with male and relatively young animals. One study reported that male diabetic mice showed higher mortality rates and larger infarct size than females [35, 117]. Thus, there is a great need for studies involving female and older animals in hyperglycemic stroke research.
4. Therapeutic strategies tested focus on reductions in infarct size and improvement of neurological function. However, there is a need to better understand how these treatment approaches would affect vascular function and CBF.
5. Therapeutic interventions in hyperglycemic stroke should evaluate tPA interactions. This is important as despite it was previously demonstrated that single use of erythropoietin (EPO) in mechanical occlusion [118] or embolic [119] models of stroke was protective, EPO failed to improve clinical outcomes and increased the mortality in patients receiving tPA [120]. This was further investigated and Jia et al later showed that the late administration of tPA in combination with EPO worsened the outcome in embolic model of stroke [121]. As reviewed above, there is only one study that evaluated the interaction of tPA with another therapeutic agent in experimental hyperglycemic stroke setting, highlighting the need for additional studies of this kind.

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Table 1

summarizes the outcomes from available clinical and experimental studies.

I- Clinical Studies		
Study [ref #]	**Purpose**	**Outcomes**
Putaala et al, 2011 [14].	Investigate the impact of admission and persistent HG on Stroke outcomes after thrombolysis.	HG at admission and persisting for 48 h after tPA thrombolysis was associated with unfavorable clinical outcome, sICH and death.
Alvarez-Sabin et al, 2003 [15].	Investigate the effect of admission HG on stroke outcomes in tPA treated patients.	Admission HG is an independent predictor of poor clinical outcomes despite of tPA induced recanalization.
Poppe AY et al, 2009 [16].	Investigate the effect of admission HG on long term stroke outcomes in tPA treated patients.	Admission HG was independently associated with increased risk of death, sICH and poor functional outcomes at 90 days.
Bruno et al, 2002 [5].	Analyze the relationship between admission glucose level and clinical outcomes from acute ischemic stroke.	High admission blood glucose levels were associated with undesirable clinical outcomes and significant increase in sICH.

II- Experimental Studies			
Study [ref #]	**Animal Model**	**Treatment**	**Outcomes**
Fan et al, 2012 [114].	Type1 DM rats, embolic	tPA (10 mg/kg) at 1.5 h after stroke.	tPA slightly but significantly reduced the infarct size, but increased the cerebral hemorrhage.
Fan et al, 2013 [115].	Type1 DM rats, embolic	- Minocycline (10 mg/kg IV) at 1 h. - tPA (10 mg/kg IV) at 1.5 h. - Minocycline (45 mg/kg IP) at 12 h	Combination of Minocycline with tPA significantly reduced brain infarction, brain swelling and tPA induced HT.
Fan et al, 2013 [116].	Type1 DM rats, embolic	- Insulin (2 U IV combined with 4 U SQ) at 1 h. - tPA (10 mg/kg IV) at 1.5 h.	Early use of insulin in combination with tPA significantly reduced brain infarction, brain swelling and tPA induced HT.
Ning et al, 2012 [113].	Type1 DM rats, embolic	- tPA (10 mg/kg IV) at 2 h.	tPA significantly increased HT and brain swelling and failed to reduce the brain infarct.
Won et al, 2011[112].	SD rats, acute HG. Mechanical occlusion with micro-aneurysm clips for 90 minutes.	- tPA (10mg/kg IV) was infused 10 minutes before reperfusion.	tPA exacerbated brain infarct, edema and hemorrhage in hyperglycemic rats.