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REVIEW ARTICLE

Diabetes Mellitus/Poststroke Hyperglycemia: a Detrimental Factor for tPA Thrombolytic Stroke Therapy

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Abstract

Intravenous administration of tissue-type plasminogen activator (IV tPA) therapy has long been considered a mainstay in ischemic stroke management. However, patients respond to IV tPA therapy unequally with some subsets of patients having worsened outcomes after treatment. In particular, diabetes mellitus (DM) is recognized as a clinically important vascular comorbidity that leads to lower recanalization rates and increased risks of hemorrhagic transformation (HT). In this short-review, we summarize the recent advances in understanding of the underlying mechanisms involved in post-IV tPA worsening of outcome in diabetic stroke. Potential pathologic factors that are related to the suboptimal tPA recanalization in diabetic stroke include higher plasma plasminogen activator inhibitor (PAI)-1 level, diabetic atherogenic vascular damage, glycation of the tPA receptor annexin A2, and alterations in fibrin clot density. While factors contributing to the exacerbation of HT in diabetic stroke include hyperglycemia, vascular oxidative stress, and inflammation, tPA neurovascular toxicity and imbalance in extracellular proteolysis are discussed. Besides, impaired collaterals in DM also compromise the efficacy of IV tPA therapy. Additionally, several tPA combination approaches developed from experimental studies that may help to optimize IV tPA therapy are also briefly summarized. In summary, more research efforts are needed to improve the safety and efficacy of IV tPA therapy in ischemic stroke patients with DM/poststroke hyperglycemia.

Keywords Ischemic stroke · Tissue-type plasminogen activator · Diabetes mellitus · Hyperglycemia · Recanalization rate · Intracerebral hemorrhage

Introduction

Ischemic stroke is a serious cerebrovascular event. Despite enormous research efforts that have been put in finding effective approaches for ischemic stroke management, intravenous administration of recombinant tissue plasminogen activator (IV tPA) remains the only FDA-approved pharmacological intervention for emergent management of ischemic stroke [1, 2]. IV tPA therapy is based on the "recanalization hypothesis," deeming the reopening of occluded vessels by the clot lysis, improvement of regional reperfusion, and subsequent salvage of threatened brain tissues important to improve the clinical outcome in acute ischemic stroke [1]. Nonetheless, the extension and optimization for IV tPA therapy remain a prominent issue in ischemic stroke management. The aim of this mini-review is to summarize recent advances in understanding of the mechanisms mediating IV tPA-associated limitations and complications, particularly in the context of diabetic stroke, and try to provide an insight into how the IV tPA therapy can be possibly improved by combination approaches to enhance its safety and efficacy.

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IV tPA Therapy for Ischemic Stroke and Its Limitations

Recanalization is an important predictor of poststroke outcome in all modalities of thrombolytic therapy [3]. Indeed, the thrombolytic feature of tPA makes it ideal for the management of acute ischemic stroke. However, there are limitations accompanying the IV tPA therapy, including lower recanalization rate, short therapeutic time window, and increased risk of HT [4, 5]. Overall rates of complete or partial recanalization after early IV tPA therapy only reach 33% and, particularly, there is a much lower rate of complete recanalization after IV tPA therapy in large vessel occlusion cases. Even after achieving the successful reperfusion by receiving the current gold standard intervention that combine IV tPA with endovascular thrombectomy (EVT), many patients with large vessel occlusion remain severely disabled [6]. Overcoming these limitations and making tPA work more effective are of high priority in advancing acute ischemic stroke management [7]. Although a flurry of potential thrombolytic agents has been tested, none of them are considered as effective or as a replacement for tPA [8]. Importantly, exogenous tPA may aggravate ischemia-induced blood-brain barrier (BBB) disruption, elevate risks of symptomatic intracranial hemorrhage (sICH), and thus partially contribute to the narrowing of therapeutic time window [2]. Recent clinical investigations have indicated the potential opportunities to improve IV tPA therapy. For instance, European Cooperative Acute Stroke Study (ECASS) III trial showed that IV tPA initiated up to 4.5 h after the onset of symptom still benefits the clinical outcomes [9]. In the Echoplanar Imaging Thrombolytic Evaluation trial, IV tPA started at 4.5–6 h after stroke reduced infarct growth and increased the rate of reperfusion, which was associated with good neurological and functional outcome [10]. Moreover, recent clinical trials have developed potential ways to improve the patient's access to IV tPA by using imaging-based patient selection and emphasized the importance of rapid and complete reperfusion of the penumbra [11], including EVT performed from 6 to 24 h after stroke onset [12].

Importantly, although the perfusion imaging-based approach may help in selection of more ischemic stroke patients to receive IV tPA and therefore prolong the therapeutic time window, sICH remains the most threatening complication for IV tPA therapy [13]. A recent data based on a multicenter, randomized, placebo-controlled trial recruiting 225 stroke patients showed that, combined with automated perfusion imaging to delineate hypoperfused but salvageable brain regions in stroke patients, IV tPA therapy given between 4.5 and 9 h after stroke still improves the neurologic functions. However, the probability of developing sICH is much higher in IV tPA-receiving group [14]. There are a number of risk factors in association with IV tPA therapy-mediated HT, including DM and poststroke hyperglycemia, older age, larger infarct, and high blood pressure [15–17]. In this mini-review, we focus on the potential mechanisms underlying the lower recanalization rate and exacerbation of HT after IV tPA therapy in diabetic stroke patients. However, detailed molecular mechanisms remain to be further elucidated and we believe that a better understanding of these complex pathways may eventually lead us to novel approaches that can counteract the negative impacts of DM in IV tPA therapy for ischemic stroke patients [17–19].

Epidemiology: Prevalence of DM in Ischemic Stroke Patients

DM is a major risk factor for cardiovascular disease including ischemic stroke. Globally, mortality rates of ischemic stroke have fallen, but its incidence and sequelae have significantly increased over the last three decades [20]. DM is a well-recognized independent risk factor for ischemic stroke, and it is associated with higher morbidity and mortality rates in stroke patients [21]. Epidemiological investigations have documented that diabetic patients are 2 to 6 times more susceptible to ischemic stroke. Approximately 30% of ischemic stroke patients are diabetic and more than 90% of them comprise type 2 diabetes mellitus (T2DM) [22]. Additionally, other metabolic risk factors for cardiovascular disease including obesity, dyslipidemia, and hypertension are often present as comorbidities with T2DM and may in concert contribute to the higher incidence of ischemic stroke when compared to patients having similar risk profile without T2DM [21]. Clinically, the presence of DM doubles the mortality rate and worsens neurological outcomes of ischemic stroke patients [23, 24]. Importantly, ischemic stroke patients with T2DM respond less favorably to IV tPA therapy due to the lower recanalization rate [25–27], while higher risk of HT [28, 29].

DM: a Detrimental Factor for IV tPA Therapy in Ischemic Stroke

Mechanisms for Suboptimal Recanalization After IV tPA Therapy in Ischemic Stroke with DM

IV tPA has been proven beneficial for acute ischemic stroke when given within 4.5 h of symptom onset [30]. Although ischemic stroke patients can receive substantial benefit from early IV tPA therapy [31], several large clinical investigations reported that there is an association between DM and/or poststroke hyperglycemia and unfavorable neurological outcomes, such as higher risk of HT, higher mortality, and more severe long-term disability after IV tPA therapy [28, 29]. Other clinical studies also showed that DM/poststroke

hyperglycemia was correlated with lower recanalization rates after IV tPA in ischemic patients [25–27, 32]. A recent report using propensity score-matched data from the Safe Implementation of Treatments in Stroke International Stroke Thrombolysis Register (SITS-ISTR) again consolidated the correlation that DM and/or admission hyperglycemia deteriorate outcomes, including 3-month functional outcome, functional independence, sICH rate, and mortality rate, in IV tPA-treated ischemic stroke patients [33]. These data suggest that there was an impaired fibrinolytic response in the setting of DM and/or hyperglycemia. Although the detailed underlying mechanisms remain to be defined, we speculated that the following pathologic factors might contribute to the thrombolysis resistance or lower recanalization rate after IV tPA in ischemic stroke patients with DM.

The first considerable pathologic factor is the higher level of plasma plasminogen activator inhibitor 1 (PAI-1) in ischemic stroke patients with DM. Hypercoagulative status in DM is mainly attributed to the enhanced platelet activation and higher circulating PAI-1 level, which might be responsible, at least in part, for the lower recanalization rate after IV tPA therapy [34–37]. It has been demonstrated that DM and metabolic syndrome are associated with the increased plasma PAI-1 levels in patients at risk of atherothrombosis [38, 39]. Biologically, PAI-1, as the primary and potent endogenous tPA inhibitor, is supposed to interfere with the exogenously administered tPA and can affect its therapeutic outcomes. Although clinical investigation is largely lacking, a few experimental and clinical reports suggest that admission fibrinolytic profile is associated with sICH and thrombolysis resistance in ischemic stroke patients treated with IV tPA [40–42]. It has been shown that lower baseline levels of plasma PAI-1 and thrombin-activable fibrinolysis inhibitor (TAFI) can predict the occurrence of sICH [41], whereas higher admission plasma PAI-1 levels predict thrombolysis resistance after IV tPA therapy [42]. However, using PAI-1 levels as a predictive indicator has to be carefully interpreted and also needs to be considered with other risk factors of atherothrombosis and pre-existing vascular comorbidities in most patients with ischemic stroke [43]. Thus, the roles of circulating PAI-1 in the variable efficacy of reperfusion and higher risk of HT following IV tPA remain to be further investigated [37].

Another possible pathologic factor is diabetic atherogenic vascular damage. The presence of systemic inflammation and atherogenic pathological process in the cerebral vascular wall of patients with DM results in elevation of vascular inflammation, disruption of BBB integrity, impairment of vascular fibrinolysis, and endothelial NO synthase activity [44]. Once ischemic stroke occurs, there may be more robust platelet activation, fibrin deposition, circulating inflammatory cell accumulation, and new thrombosis formation at the injury site of occluded vessel, which might lead to thrombolysis resistance [45, 46]. Glycation of an endogenous protein called annexin A2 in hyperglycemic state could also be a potential factor since annexin A2 is an important fibrinolytic receptor for normal tPA functions through accelerating tPA-converted plasmin generation [47]. Our previous studies showed that combination of tPA with recombinant annexin A2 enhanced thrombolytic efficacy of tPA, reduced the required dose of tPA for achieving adequate reperfusion, and improved long-term neurological outcomes in rats with embolic focal ischemia [48–50]. Impaired fibrinolysis on the surface of endothelial cells has been identified as one of key pathologic factors mediating the thrombotic vascular complications in patients with DM [51]. An immunochemical and biochemical study with the purpose of uncovering types of glycated endothelial plasma membrane proteins in DM has identified annexin A2 as one of the three major glycated proteins [52]. In cultured human brain microvascular endothelial cells, we found that treating cells with excess glucose, which simulates in vivo hyperglycemic environment, for 7 days significantly reduced the fibrinolytic activity on the cell surface, and also decreased mRNA and protein expression of tPA, plasminogen, and annexin A2, while increased the level of PAI-1. Aberrantly high level of glucose significantly increased AGE-modified forms of total cellular and membrane annexin A2, whereas the reduction in fibrinolytic activity due to the excess glucose was fully restored upon incubation with recombinant annexin A2, but not the AGE-modified annexin A2 or exogenous tPA, supporting the hypothesis that hyperglycemia causes dysfunction of annexin A2 on the endothelial membrane, thereby leading to an overall reduction of fibrinolytic activity [53]. Our experimental findings also support a previous speculation that worse neurological outcome and resistance to tPA thrombolysis in ischemic stroke patients with DM might be partially attributed by impaired fibrinolysis due to the glycation of endothelial annexin A2, which is considered as an acquired annexinopathy [54].

Additionally, higher density of fibrin clot may be another contributor as well. It has been reported that patients with DM exhibit increased the maximal strength as well as the density of fibrin clot [55, 56]. Moreover, prolonged duration of T2DM is associated with prothrombotic phenotype of fibrin clot [57]. These observations suggested that a denser fibrin clot in DM might also play a role in the increased resistance to tPA thrombolysis [27].

Mechanisms for Increased Risk of HT After IV tPA Therapy in Ischemic Stroke with DM

sICH is a devastating complication after receiving IV tPA therapy. The incidence rate of sICH is approximately 3–7% as it varies between studies due to the differences in the criteria used to define sICH [58]. There are a number of risk factors associated with tPA thrombolysis-mediated HT, including poststroke hyperglycemia, older age, larger infarct, and high

blood pressure [15, 16]. Poststroke hyperglycemia is presented in all patients with pre-existing DM, which account for approximately 37% of ischemic stroke patients and 50% of non-diabetic ischemic stroke patients [59]. A history of DM and higher degree of poststroke hyperglycemia are associated with poor clinical outcome after stroke and thrombolysis [60, 61]. For example, in the NINDS rt-PA Stroke Trial, treating patients with IV tPA within 3 h after the onset of stroke, serum glucose level was an independent predictor directly correlating with sICH [62]. This was replicated in the Prolyse in Acute Cerebral Thromboembolism (PROACT) II trial, where sICH occurred in 35% of ischemic stroke patients having serum glucose values greater than 200 mg/dL [63]. In another study using data from the prospective, multicenter clinical trial called Canadian Alteplase for Stroke Effectiveness Study (CASES), admission hyperglycemia was independently associated with increased risk of death, HT, and poor functional status at 90 days in the cohort of IV tPA-treated ischemic stroke patients [61]. Although current evidence supports that an increased risk of HT caused by IV tPA in DM patients with hyperglycemia, the underlying mechanisms of this effect remain to be further investigated [1, 18, 64].

Interactions of multiple factors including original ischemic insult, hyperglycemia-mediated vascular oxidative stress, neuroinflammation-mediated injury, and tPA neurovascular toxicity contribute to the extracellular proteolysis dysfunction-BBB damage-HT pathway [65–68]. Hyperglycemia may cause vascular oxidative stress, which occurs very early after the onset of ischemia/reperfusion injury via the overproduction of reactive oxygen species (ROS). The generation of oxidative stress during ischemic stroke is a critical event leading to BBB disruption with secondary vasogenic edema and HT in infarcted brain tissue, compromising the benefit of IV tPA-induced reperfusion [66, 69]. Evidence showed that ROS can directly oxidize and damage BBB structures [70]. Furthermore, ROS is an upstream intermediate involving in multiple pathophysiological mechanisms during reperfusion injury that link protease activation to vascular leakage [71, 72]. The importance of oxidative stress in ischemic stroke and IV tPA-related vascular disruption has been well-documented in several studies investigating the efficacy of antioxidant plus tPA combination in embolic stroke animal models [73, 74]. Supporting evidence from both clinical and experimental studies showed a direct relationship between increased oxidative stress and enhanced matrix metallopeptidase (MMP)-9 expression, which may subsequently lead to BBB degradation [75].

Poststroke neuroinflammation-mediated BBB leakage is a progressive and interactive process that largely depends on the activation, expression, and secretion of proinflammatory mediators (e.g., cytokines) from both cerebral and peripheral cells [76–78]. Indeed, elevated proinflammatory cytokines as well as altered activation of various immune cells resulting from diabetic state significantly contribute to the aggravated brain damage after ischemic stroke [79]. For instance, experimental data showed that the oxidative stress is a major stimulator of inflammatory cytokine production and protease secretion by leukocytes, microglia, and brain resident cells of the neurovascular unit [67, 80]. Neuroinflammation-mediated dysfunction of extracellular matrix proteolysis is the key pathological mechanism contributing to the BBB disruption after ischemic stroke, mainly by means of early elevated proinflammatory cytokines, release of leukocytes into circulation, adhesion of leukocytes to injured cerebrovascular endothelium, and brain infiltration followed by release and activation of proteases [81]. Leukocyte-microvessel interaction and subsequent infiltration of leukocytes into the ischemic brain play dominant roles in the development of secondary damage resulting in edema formation, microvascular permeabilization, and hemorrhage via secretion of free radicals, cytokines/chemokines, lipid-derived mediators, and proteases [67, 82, 83]. In addition, the neutrophil-platelet interaction has also been targeted for stroke therapy since activated platelets are indispensable for neutrophil extracellular trap (NET) formation via cell-cell contact or soluble mediators and thus contribute to the hypercoagulability in ischemic stroke patients [84]. T2DM-related platelet hyperreactivity may strengthen the neutrophil-platelet interaction and subsequent NET formation, in turn, contribute to the hypercoagulative and prothrombotic state [85] and eventually neutralize the tPA efficacy [86]. It has been clearly recognized that proteases secreted by activated leukocytes is one of the key pathologic factors contributing to BBB leakage and HT in ischemic stroke [67, 68, 80, 87]. Importantly, activated extracellular proteases, such as MMP, act as inflammatory mediators as well, for example, by triggering cytokine expression [87–89].

In the context of extracellular proteolytic dysregulation resulting in IV tPA-related hemorrhagic complication, the primary focus is based on the interaction between tPA and MMP [90–92]. tPA amplifies MMP-9 expression, which may give rise to increased oxidative stress and neuroinflammation [66, 93]. Experimental data suggest that extracellular matrix proteolysis may target multiple cell types at the neurovascular interface and underlie multiple cascades of BBB disruption after IV tPA therapy [80, 93, 94]. Actually, DM-induced upregulation of MMP-9 activity in cerebral vessels increases the likelihood of HT after ischemic stroke even without tPA administration, which could synergistically exacerbate the tPA-related MMP-9 amplification [95].

DM Induces Impairment of Cerebral Collateral Flow Compromising the Benefits of IV tPA Therapy for Ischemic Stroke

Impaired collateral flow compensation is another considerable pathologic factor mediating tPA thrombolysis-related

complications, since more robust collateral grade was associated with better recanalization, reperfusion, and subsequent better clinical outcomes [96, 97] since the cerebral vasoreactivity and collateral circulation are important protective mechanisms against cerebral ischemia [98]. Physiologically, collateral circulation in the brain represents endogenous vessels that maintain residual blood flow to brain areas distal to an arterial occlusion. Usually, the primary collateral network is derived from the circle of Willis that can be rapidly recruited for maintaining the perfusion when there is a large artery occlusion, whereas it fails to do the same compensation during the distal artery occlusion. Fortunately, the presence of secondary collateral flow via leptomeningeal anastomoses, consisting of cortical pial arteries connecting to the major branches of cerebral arteries, enables the continuous blood flow to brain regions distal to the occlusion [99]. In fact, the collateral formation is a subtype of arteriogenesis responsible for cross-connecting the arterial trees in response to tissue ischemia [100]. Clinical observation has linked the degree of collateral circulation in stroke patients to the final infarct size and functional recovery [101, 102]. In addition, collateral flow also positively correlates with the successful recanalization and lower risk of HT after IV tPA in stroke patients [99] while the lower degree of collateral flow is strongly associated with the occurrence of intracerebral hemorrhage after thrombolysis in stroke patients [103]. Indeed, better collateral flow can bring more thrombolytic agent to the major clot as well as help to clear the small clot fragments after thrombolysis, which can migrate to and block distal branches downstream the occluded artery. Furthermore, collateral flow protects against vascular dysfunction and therefore improves postrecanalization reperfusion [99].

Metabolic diseases, including diabetes, have been demonstrated to cause remodeling and structural alternation of cerebrovasculature [104]. Mild hyperglycemia in diabetic rats can induce, within a relatively short period of time, marked vascular medial thickening, and hypertrophic remodeling [105]. Experimentally, metabolic dysregulation also negatively impacts leptomeningeal arteries that are responsible for blood supply to brain surface perfusion by reducing the perfusion, number, and diameter of pial collaterals. In transgenic db/db mice with T2DM, doppler optical coherent tomography showed not only the aggravated early reduction in vessel diameters of the main feeding arteries in the circle of Willis, but also a disabled collateral recruitment, leading to a worse outcome after stroke [106]. Also, a combination of endothelial and smooth muscle cell dysfunction and downregulation of both endothelial and inducible nitric oxide synthase in the diabetic status may compromise the dilation of cerebral arterioles, impairing the adaptation of blood flow to metabolic changes and potentially contributing to the infarct enlargement and deterioration of neurological deficits after stroke [107]. Moreover, neovascularization in diabetic animal might be abnormal and accompanied by increased permeability and MMP-9 activity, rendering the increased tortuosity, narrowed lumen diameter, increased permeability, and subsequently more vulnerable to ischemia/reperfusion injury of newly formed collaterals [108]. Another experimental data has shown an impaired collateral flow compensation during chronic cerebral hypoperfusion and after focal ischemic stroke in mice with DM [109], suggesting that the collateral flow impairment might not directly affect the thrombolysis, but may contribute to the lower reperfusion efficacy of IV tPA therapy and worse neurological outcomes in ischemic stroke patients with DM [27, 96]. Hence, further studies focusing on the role of collateral flow in the pathogenesis of worsened outcomes after tPA thrombolysis in ischemic stroke with DM are needed.

As discussed earlier, there are multiple upstream mediators of abnormal extracellular proteolysis-BBB disruption process, including hyperglycemia, oxidative stress, and neuroinflammation. Exogenous tPA may contribute to this pathological process, which counteracts its beneficial thrombolytic effect and thus limits the wide use of tPA in diabetic stroke patients [18, 69, 90–93]. Fundamentally, the precise molecular mechanisms involved in these pathological interactions remain poorly characterized [60, 65] and, therefore, further studies regarding assessing the more detailed mechanisms underlying the interaction between tPA and these regulators are essentially required for modification and optimization of IV tPA therapy. However, we should also see the challenges that are still remaining, especially the technical difficulty evaluating dynamic changes of multiple factors and their interactions, and dissecting them based on a temporospatial manner in animal models. Meanwhile, finding a more suitable and optimized animal model is still a top priority in tPA research since a mismatch between preclinical studies and real clinical scenarios has always been a barrier hampering the translation [6].

Combination Therapy for IV tPA Thrombolysis

Although the emergence of EVT substantially extend the effective therapeutic time window for large cerebral vessel occlusion [110], IV tPA therapy still has advantages in regard to its availability and feasibility as well as the effectiveness particularly for those ischemic stroke patients with small artery occlusion, which accounts for approximately 50% of ischemic stroke cases [7]. Although, the current guidelines for ischemic stroke management does not specifically exclude stroke patients with DM and/or hyperglycemia from receiving IV tPA therapy [111], both are major risk factors for increased HT after IV tPA therapy and associated with poor posttreatment outcomes [112], rendering these patients much less likely to obtain benefits from IV tPA therapy [22]. Hence, the optimization of IV tPA to benefit diabetic stroke patients has been

considered a clinical high priority. As we discussed earlier, DM causes pre-existing prothrombotic and proinflammatory vascular pathology. We believe that combining conventional tPA with other approaches targeting single or even multiple metabolic dysfunctions and cerebrovascular pathological cascades may ultimately improve both safety and efficacy of IV tPA for diabetic stroke patients [18, 64, 113].

Unfortunately, despite the strong relationship between the DM and higher rates of complications after IV tPA therapy, only a few of studies have investigated the tPA-based combination therapy in diabetic stroke animals [18]. Technically, pharmacological induction of DM/hyperglycemia in rats causes various pathological changes including cerebrovascular inflammation and coagulation dysfunction. These rats are suffered from increased brain infarction and HT after IV tPA, which closely mimic the clinical situation [114]. In addition, the embolic focal stroke model, which closely simulates the ischemic stroke in humans involving the use of a tPA-breakable homologous blood clot to occlude the origin of middle cerebral artery, enables us to test different thrombolytics [115]. In this type of animal model consisting of both diabetes and stroke, significantly increased infarct size, poorer neurological outcomes and the aggravation of HT after IV tPA therapy have been observed, while combination of tPA with minocycline, a versatile compound possessing multiple wide array of abilities including anti-oxidative stress, anti-inflammation, anti-apoptosis and MMP inhibition, can significantly reduce the infarct size, brain swelling, brain MMP-9 level, and HT after IV tPA therapy in diabetic rats [116]. Furthermore, conventional blood-lowering agent insulin, when co-treated with tPA, also ameliorates the HT induced by IV tPA in these diabetic rats, suggesting simultaneously suppression of metabolic dysregulation, such as hyperglycemia as the major detrimental factor in tPA-related HT, might be an effective way to improve IV tPA therapy in diabetic stroke [117]. However, using insulin administration has a major safety concern that hypoglycemic complication may cause severe neurological injury and compromise the efficacy of IV tPA therapy [118]. Indeed, from the translational perspective, more experimental stroke studies using animals with comorbidities, such as T2DM, are urgently needed for better understanding and addressing this issue in clinical practice.

Although, most of preclinical studies testing tPA combination therapy were conducted in non-diabetic animals. We believe that the current identified pathological factors involving in IV tPA-associated complications, including HT, may be shared between diabetic and non-diabetic animal model [46]. Thus, tPA-based combination approaches that have been experimentally tested in non-diabetic animal models are also briefly discussed below.

Pharmacological chemical compounds, combined with tPA therapy, have been extensively investigated during past decades. One of the main purposes of these combinations is for inhibiting HT after IV tPA therapy, especially via combining free radical scavengers and MMP inhibitors with tPA. For example, the combination of the potent ROS extinguisher Edaravone or the antioxidant baicalin with tPA showed significant reduction of HT after IV tPA therapy [119, 120]. Broad-spectrum MMP inhibitor-tPA combination has also showed protective effects in reducing IV tPA-related hemorrhagic complication [121]. Very interestingly, besides MMP inhibitor, combination of MMP-10 plus tPA exhibited effects of BBB protection, neuronal excitotoxicity reduction, and calcium overload mitigation [122]. Molecules targeting coagulation pathway have also been included as a potential adjunct of IV tPA therapy. For instance, Eptifibatide, a glycoprotein IIb/IIIa inhibitor, combined with tPA showed safe and potential clinical benefits in a phase 2 clinical trial [123]. It would be also interesting if this combination could be further tested in diabetic stroke since platelet activation is a contributor to prothrombotic state in T2DM and may compromise the therapeutic effects of tPA [124]. Other agents such as thrombin inhibitor Argatroban and inhibitors of thrombin-activated fibrinolysis inhibitor are also tested in the combination with tPA [123]. Experimentally, targeting multiple platelet functions, including adhesion and secretory activity, together with tPA may provide a safe and effective way for preventing ischemia-reperfusion injury [125]. Another recent animal study reported that Vepoloxamer, a nonionic surfactant showed potent hemorheological and antithrombotic properties, can facilitate fibrinolytic efficacy of tPA via diminishing endothelial permeability and lowering PAI-1 and tissue factor levels. Such combination could also be considered to investigate in diabetic stroke model [126].

Anti-inflammatory agent is another mainstay that might be able to optimize the tPA therapy. A recent report combining inhibition of phosphoinositide 3-kinase gamma, a mediator in a wide range of immune and inflammatory responses, and tPA demonstrated that, through suppression of nuclear transcription factor-kB–dependent MMP-9 and PAI-1 in the ischemic brain endothelium, this combination protects BBB, alleviates delayed tPA therapy-related ICH, and improves microvascular patency [127]. Compound 21, a novel selective angiotensin type 2 receptor agonist with neuroprotective and anti-inflammatory properties, has been shown to extend the tPA therapeutic time window and improve the cognitive function in a randomized, blinded preclinical trial using rat model of thromboembolic stroke [128]. One more example is tPA combination with granulocyte colony–stimulating factor, which is produced by bone marrow stromal cells and fibroblasts that exert neuroprotective effects in animal models of ischemia. The combination markedly prolonged the effective tPA therapeutic time window up to 6 h after stroke, significantly reduced infarct volume, and delayed tPA treatment-associated HT and mortality rate [129].

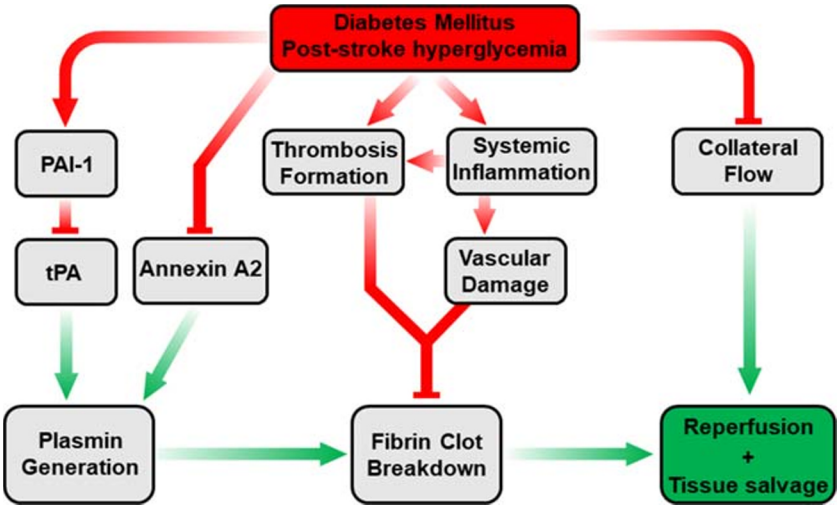

Fig. 1 A schematic showing the association between diabetes mellitus (DM) plus poststroke hyperglycemia and the lower recanalization rate after IV tPA therapy. Detrimental effects of the comorbidity DM and poststroke hyperglycemia on the process of tPA-induced thrombolysis that may responsible for lower recanalization rate include higher plasma PAI-1 level that neutralizes the tPA activity, prothrombotic status, and inflammation-related diabetic atherogenic vascular injury that may contribute to the increased fibrin clot density, and glycation and dysfunction of annexin A2, which acts as a fibrinolytic receptor for normal tPA functions through accelerating tPA-converted plasmin generation. They all involve in the impairment of fibrinolytic activity for the exogenous tPA, which exhibits clinically as a lower recanalization rate after tPA thrombolysis in ischemic stroke patients with DM

We know that due to the existence of multiple and complicated neurovascular pathological factors and cascades, targeting single pathway might not be enough for treating ischemic stroke with T2DM [46]. Emerging research efforts have been shifted to investigate the therapeutic potential of compounds having multiple pharmacological functions. For example, N-acetyl-seryl-aspartyl-lysyl-proline is a molecule that has multiple pharmacological functions such as anti-inflammatory, antifibrotic, and proangiogenic effects. It can not only augment microvascular perfusion but lower thrombosis and BBB leakage and subsequently improve tPA therapeutic effects [130]. Taurine is a pleiotropic, endogenous amino acid mediating in various biological processes. This molecule has also demonstrated to be able to prevent tPA-associated hemorrhage, improve microvascular patency by reducing downstream intravascular deposition of fibrin/fibrinogen with platelets, and strongly suppress CD147-dependent MMP-9 pathway in brain endothelium [131]. One more example, FGF21 acts as a pleiotropic endocrine regulator having metabolic regulatory, anti-oxidative stress, and anti-inflammatory and tissue protective functions [132]. Previous experiments demonstrate that FGF21 regulates metabolic derangements, alleviates BBB disruption, inhibits proinflammation, preserves white matter integrity, and eventually improves neurologic outcomes in diabetic stroke mice [133, 134]. Additionally, compared to insulin, which requires constant monitoring during the administration to prevent adverse effects such as severe hypoglycemia, exogenous FGF21 treatment can effectively lower the blood glucose without causing hypoglycemic events, rendering this molecule ideally for tPA combination. These data strongly indicate that FGF21 might also deserve a thorough investigation for potential tPA combination therapy in diabetic stroke. Lastly, as we discussed in the previous section, hyperglycemia-induced glycation of annexin A2 might be one of the key factors responsible to the low recanalization in diabetic stroke, since annexin A2 acts as an accelerator facilitating tPA-converted plasmin generation. Exogenous administration of non-glycated normal annexin A2, which is also a pleiotropic molecule, in combination with tPA might improve IV tPA efficacy in diabetic stroke [53].

Cellular components have also been proposed as a promising partner for tPA combination therapy. Multiple types of stem cell, such as mesenchymal stem cells (MSCs), have been shown the protection against ischemic stroke [135]. MSCs exert protective and regenerative effects by producing multiple factors targeting different aspects of pathophysiology after stroke [136]. A recent clinical trial showed that intravenous MSC administration significantly improved motor functions in stroke patients [137]. Experimentally, tPA-MSC combination markedly ameliorated delayed tPA therapy–induced escalation of brain damage by BBB protection and suppression of proinflammatory factors and MMP-9 expression [138]. Another experimental report showed that regulatory T cell adoptive transfer plus tPA combination improves both efficacy and safety of thrombolytic therapy through, at least partially, significant reduction of tPA-upregulated MMP9 and chemokine (C-C motif) ligand 2 [139].

Taken together, it is highly significant to develop an effective tPA-based combination to overcome the lower recanalization rate and higher risk of HT, which would ultimately make the IV tPA therapy more beneficial in both efficacy and safety to this subgroup of ischemic stroke patients with

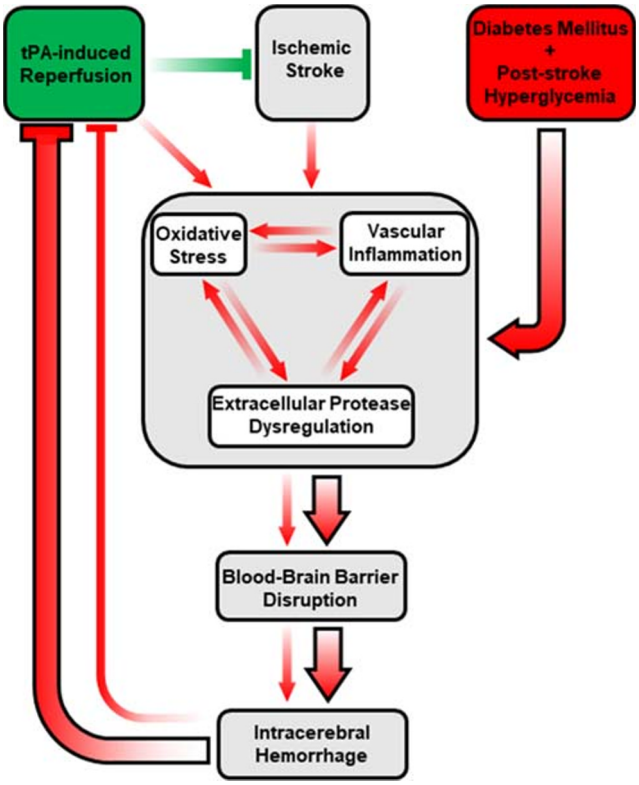

Fig. 2 A schematic presenting multiple interactions between pathologic factors and consequently BBB breakdown and hemorrhagic transformation after tPA thrombolytic therapy in ischemic stroke with DM. Properly administration of exogenous tPA is beneficial to reperfuse ischemic brain and rescue the compromised tissue. Ischemic stroke fuels early elevation of oxidative stress and neuroinflammation and underlies the extracellular matrix proteolytic dysfunction at the neurovascular interface. Exogenous tPA administration may further promote oxidative stress, activate extracellular protease, and neuroinflammation to potentiate extracellular proteolysis dysfunction. Additionally, DM and poststroke hyperglycemia are direct precondition for aggravated oxidative stress and inflammation in cerebral vasculature. All these pathological pathways and interactions worsen BBB breakdown and hemorrhagic transformation after IV tPA therapy in ischemic stroke with DM. Indicators with black-colored outline: DM and/or poststroke hyperglycemia exacerbates stroke- and IV tPA-related brain injuries and thus hampers IV tPA-related beneficial effects

DM. To fulfill this goal, future studies in better understanding the pathological mechanisms underlying the higher risk of complications after IV tPA in diabetic stroke are urgently needed [114].

Summary

DM is considered as a clinically important vascular comorbidity that leads to reduced rates of recanalization while increased risks of HT after IV tPA therapy for ischemic stroke. In this mini-review, we summarized the recent advances in the understanding of underlying mechanisms mediating the exacerbation of detrimental effects of IV tPA therapy in ischemic stroke patients with DM. Potential pathologic factors that relate to suboptimal recanalization include higher plasma PAI-1, diabetic atherogenic vascular damage, glycation of the tPA receptor annexin A2, alterations in fibrin clot density, and impaired collaterals (Fig. 1). Factors that may contribute to increased HT include hyperglycemia, vascular oxidative stress and inflammation, tPA neurovascular toxicity, and dysfunction in extracellular proteolysis balance (Fig. 2). A further and thorough investigation of these complex pathways may eventually lead us to novel ways of counteracting the negative effects of diabetes in stroke. It is clinically highly significant to develop effective tPA-based thrombolytic combination approaches, in order to overcome the lower recanalization rate and higher risk of HT in the subgroup of stroke patients with diabetes and poststroke hyperglycemia.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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