Verapamil, a possible repurposed therapeutic candidate for stroke under hyperglycemia

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Admission hyperglycemia is an independent predictor, that contributes to hemorrhagic transformation (HT), and worsened functional outcome following reperfusion therapy with tissue plasminogen activator (tPA) in ischemic stroke. Clinical studies have revealed a strong association between hyperglycemia and incidence of HT independent of prior diabetes diagnosis (Alvarez-Sabín et al., 2003). Experimental studies showed that hyperglycemia reduces the efficacy of reperfusion and cerebral blood flow following ischemic stroke in rats (Kawai et al., 1997). Hyperglycemia accelerates the production of super oxides and advanced glycation end products, which contribute to blood brain barrier disruption (Won et al., 2011). Additionally, hyperglycemic reperfusion induces glucose overload to the ischemic brain, which accelerates the synthesis reactive oxygen free radicals and worsens neurovascular damage. The increased incidence of admission hyperglycemia in stroke patients along with HT, necessitates the development of novel adjunctive therapies for the management of ischemic stroke.

Recently, we found that reperfusion therapy with tPA exacerbated ischemic reperfusion injury in diabetic mice (Saifudeen et al., 2020). tPA induced hemorrhagic transformation, worsened neurological outcome, and increased expression of thioredoxin interacting protein (TXNIP) in acute hyperglycemia. TXNIP is an endogenous negative regulator antioxidant, thioredoxin, and known to be activated in ischemic stroke. Genetic and pharmacological inhibition of TXNIP improved neurological outcome, infarct size and inflammation in mice model of embolic middle cerebral artery occlusion. We found that activated TXNIP exacerbated ischemic injury by activation of nucleotide binding oligomerization domain like receptor protein (NLRP)-3, aggregation with apoptosis-associated speck-like protein, and cleaved caspase-1. This resulted in the subsequent release of mature interleukin-1β. Hence, the identification of molecules that can target TXNIP/NLRP3 inflammasome activation is of clinical importance to counteract the detrimental effect of tPA in the hyperglycemic condition. Several pharmacological agents such as umbelliferone, resveratrol and ruscogenin have shown their protective effect on ischemic stroke by inhibiting TXNIP/NLRP3 inflammasome activation. Similarly, Guo et al demonstrated that treatment with hyperbaric oxygen prevents hemorrhagic transformation through inhibition of ROS/TXNIP/NLRP3 inflammasome activation in diabetic stroke rats (Guo et al., 2016). However, none of these agents have passed clinical trials with observable effects seen in animal models. Lack of a specific TXNIP inhibitor limit its use as the direct therapeutic target for ischemic brain damage. Verapamil is a phenylalkylamine L-type calcium channel blocker, being used for the treatment of angina and arrhythmia. It has been shown to inhibit TXNIP activation and subsequent inflammation (Ahmed et al., 2021). Recently, Jangholi et al. (2020) demonstrated that verapamil ameliorated mitochondrial oxidative stress, apoptosis, and neuro inflammation after cerebral ischemic reperfusion injury. In addition, intravenous administration of verapamil has been considered as an effective therapy after thrombectomy in preclinical animal models of ischemic stroke without affecting heart rate and blood pressure (Maniskas et al., 2016). We have previously reported that verapamil could ameliorate age associated neuro inflammation and senile dementia by inhibiting TXNIP/NLRP3 inflammasome activation (Ismael et al., 2021). Similarly, verapamil prevented the development of cognitive impairment in mouse model of sporadic Alzheimer's disease (Ahmed et al., 2021). Further, verapamil could improve diabetic neuropathy in high fat diet-fed animals by inhibiting TXNIP/NLRP3 activation (Xu et al., 2019). Hence, verapamil can be considered as the repurposed therapeutic candidate for attenuation of neuroinflammation and neurodegeneration. Drug repurposing is an attractive strategy for identifying novel application of approved drugs to new therapeutic indications as it reduces time, economic cost, and risk of failure during drug development.

Verapamil is known to reduce TXNIP expression at the transcriptional level by preventing the binding of carbohydrate response element binding protein to the promotor region of the gene (Xu et al., 2012). However, its potential benefit in mediating neurovascular damage after hyperglycemic stroke is not yet validated. Recently, we demonstrated that intravenous administration of low dose of verapamil along with tPA attenuated cerebrovascular damage following stroke in hyperglycemic animals (Xu et al., 2012). We found that tPA worsened neurovascular outcome even in intraluminal filament model of ischemic stroke demonstrating the toxicity of tPA independent of thrombolytic effect in hyperglycemic mice. Based on previous reports, tPA and hyperglycemia worsened neurovascular damage independent of reperfusion method adopted (Hafez et al., 2015). tPA moderately aggravated neurological functional deficit after ischemic stroke, which is significantly modulated by verapamil. Verapamil significantly attenuated TXNIP expression in the penumbra along with attenuation of brain edema and infarct volume.

Hyperglycemic reperfusion with tPA induced blood-brain barrier damage and HT even in the golden therapeutic time window. tPA elevated blood-brain barrier damage as evidenced by increased ipsilateral hemorrhage in coronal sections and extravasation immunoglobulins. tPA doubled the blood brain barrier damage when administrated at hyperglycemic reperfusion as evidenced by increased matrix metalloprotease-9 activation and down regulation of junctional proteins (occludens-1 and Caudin 5). Inhibition of TXNIP with verapamil significantly attenuated elevated ipsilateral hemorrhage, parenchymal blood cell infiltration, and immunoglobulin extravasation confirming the attenuation of hemorrhagic transformation. Further, verapamil attenuated down regulation of junctional proteins, such as claudin 5 and zonula occludens-1, despite having no effect on activated matrix metalloprotease-9.

tPA increased TXNIP associated NLRP3 inflammasome activation at hyperglycemic reperfusion. This phenomenon was illustrated by the increased expression of apoptosis-associated speck-like protein and cleaved caspase-1 and interleukine-1β. Elevated expression of NLRP3 inflammasome components such as apoptosis-associated speck-like protein, Cleaved caspase-1 and interleukine-1β were completely blocked with verapamil infusion despite no change in

NLRP3 expression. We have previously demonstrated that pharmacological inhibition NLRP3 activation mitigates neurovascular damage after ischemic stroke. This is in parallel with reduced expression of high mobility group box-1/ nuclear factor kappa-light-chain-enhancer of activated B cells, suggesting the reduced level of priming of inflammasome components. In addition, modulation NFkB p65 expression significantly inhibited the tumor necrosis factor α expression in hyperglycemic penumbra. All together these findings confirm the anti-inflammatory benefit of TXNIP inhibition in hyperglycemic reperfusion.

Collectively, this is the first report demonstrating verapamil as the adjuvant therapy to mitigate the detrimental effects of hyperglycemic reperfusion injury. The beneficial effect is mainly mediated through inhibition of TXNIP/NLRP3 inflammasome activation, independent of vasodilatory effect (Fraser et al., 2017). Consistently, Jangholi et al. (2020) demonstrated that verapamil inhibited post stroke oxidative stress, mitochondrial dysfunction, and apoptosis in rats. Many clinical studies have addressed the potential benefit of verapamil in various neurological diseases. The SAVER-1 (phase1 clinical trial) demonstrated that intra atrial administration of verapamil is safe and had no evidence of intracranial hemorrhage following thrombectomy in human subjects (Fraser et al., 2017). Intraarterial administration verapamil along with tPA improved neurological outcome in patients with anterior spinal artery stroke (Haynes et al., 2021). In addition, retrospective analysis showed that intraarterial administration verapamil improved functional outcome in patients with high-risk aneurysmal subarachnoid hemorrhage (Mao et al., 2021). Verapamil did not affect the blood glucose levels in our treated animals; however, recent clinical studies revealed that verapamil can be an effective therapeutic candidate to delay the pathogenesis of diabetes (Ovalle et al., 2018). Our study concludes that verapamil can be used as an adjunctive therapy to mitigate the damaging effect of tPA in hyperglycemic stroke. The current study only addressed the benefit of verapamil in intraluminal filament model of middle cerebral artery occlusion to study the reperfusion independent toxicity of tPA. Further investigations are needed to confirm the beneficial effect of TXNIP inhibition on long term functional outcome in clinically relevant embolic model of middle cerebral artery occlusion with tPA recanalization.

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