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Advances in the Mechanisms of Hemorrhagic Transformation and Therapeutic Agents after Intravenous Thrombolysis in Ischemic Stroke

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Abstract

Ischemic stroke is an important disease leading to death and disability for all human beings, and the key to its treatment lies in the early opening of obstructed vessels and restoration of perfusion to the local infarcted area. Intravenous thrombolysis with tissue plasminogen activator (tPA) is one of the effective therapies to achieve revascularization, but it faces strict indications with a narrow therapeutic time window, and significantly increases the incidence of hemorrhagic transformation, HT, after reperfusion of the infarcted foci, which greatly reduces the incidence of patients with ischemic stroke. which significantly increases the incidence of hemorrhagic transformation (HT) after reperfusion of the infarcted focus, greatly reducing patient utilization and clinical benefit. Since the mechanism of HT has not been fully elucidated, and the related molecular mechanisms are complex and interactive, there is no specific and effective therapy to avoid the occurrence of HT. In this article, we focus on the research progress on the mechanism of HT after tPA intravenous thrombolysis in ischemic stroke patients from the aspects of vascular integrity disruption, oxidative stress, and neuroinflammatory response and the corresponding therapeutic strategies, in order to improve the safety and prognosis of tPA intravenous thrombolysis in the clinic.

Keywords

Hemorrhagic Transformation, Tissue-Type Fibrinogen Activator, Acute Ischemic Stroke, Blood-Brain Barrier

1. Introduction

Acute ischemic stroke (AIS) is a group of diseases in which blood flow is im-

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peded due to narrowing or occlusion of cerebral blood vessels, resulting in ischemia and hypoxia in the blood-supplying region of the blood vessels, leading to death of brain cells and corresponding neurological deficits. AIS has a very high morbidity and disability rate globally, which seriously affects people's health and quality of life. Timely restoration of the obstructed blood flow at the early stage of the disease is an effective therapeutic measure to save the ischemic semi-dark band, in which intravenous thrombolysis is more convenient than other endovascular interventions, such as mechanical thrombolysis, and the price is low and the acceptance of the patient is high; therefore, it is the most widely used and mature in the clinic. Currently, tissue plasminogen activator (tPA) is the only effective drug approved by the U.S. FDA for the treatment of AIS, but serious complications such as hemorrhagic transformation (HT) can occur, affecting patient prognosis or even causing early death [1]. Data show that symptomatic intracranial hemorrhage is an independent risk factor for death within 3 months in patients with AIS [2]. Therefore, it is necessary to understand the mechanism of HT and the latest therapeutic agents after tPA intravenous thrombolysis in patients with AIS, which will greatly improve the safety of tPA intravenous thrombolysis in the clinic and greatly reduce the heavy economic burden of the families of the patients while improving their prognosis.

The molecular mechanisms underlying the development of HT by intravenous thrombolysis and reperfusion in ischemic stroke are extremely complex and are still being explored. One of the main pathophysiological mechanisms of HT is the disruption of the integrity of the blood-brain barrier (BBB), which is exacerbated by ischemia-hypoxia-induced BBB disruption in patients with AIS after recanalization of occluded blood vessels with tPA thrombolysis, causing damage to the entire neurovascular unit, including the extracellular matrix, endothelial cells, proteins, astrocyte terminals, pericytes, and basolateral membranes, astrocyte terminals, pericytes, and basement membranes.

2. Physiological Properties of tPA

TPA is a serine protease with multiple structural domains, which can be classified into endogenous tPA and exogenous tPA according to its source. tPA mainly converts inactive fibrinogen into active fibrinolytic enzymes in the vasculature, and then degrades the insoluble fibrinogen in the obstructed vasculature into soluble peptide fragments, thus restoring reperfusion of the infarcted area of the cerebral tissue.

Endogenous tPA is mainly secreted by endothelial cells and neurons, and is involved in synaptic growth and development and the regulation of central nervous system functions. Studies have shown that Exposure of cerebral cortical neurons to ischemic and hypoxic conditions accelerates the release and up-regulates the activity of endogenous tPA, disrupting the integrity of the BBB [3]. However, it has also been shown that tPA increases the survival of injured neuronal cells, is

a modulator of synaptic functional homeostasis in the central nervous system, and has neuroprotective effects [4].

Currently, the most widely used exogenous tPA in clinical application mainly refers to the second-generation thrombolytic agent recombinant human tissue-type plasminogen activator (rt-PA). rt-PA is synthesized through genetic engineering technology, and has a better thrombolytic effect. It has an extremely short half-life of about 4 - 6 minutes, mainly due to the presence of endogenous inhibitors in the circulation that target its rapid action, such as plasminogen activator inhibitor-1 (PAR-1). PAR-1 binds to rt-PA and irreversibly inactivates it, forming an inhibitory complex that is metabolically cleared by the liver. Because of the short duration of the pharmacodynamic effect of rt-PA, larger doses are often used clinically to achieve a therapeutic effect. Traditionally, the time window of cerebral tissue perfusion refers to 0 - 3 h or 4.5 h after the onset of the disease, which is also the window of indication for intravenous thrombolytic therapy with rt-PA in patients with AIS. Moreover, there are strict indications and contraindications for the use of rt-PA in clinical practice, and it has been found that the later the occluded artery is treated with thrombolytic therapy, the greater the risk of HT and the worse the prognosis of patients. The results of a clinical randomized controlled study showed that rt-PA given to patients with mild AIS within 3 - 5 hours of the onset of the disease did not show significant major neurological improvement and increased the incidence of HT after 90 days [5]. Also in the mouse ischemic mechanical model, rt-PA can induce HT in the infarct region in a dose-independent manner, and the presence of the rt-PA-induced in vitro thrombolytic product, TLP, can lead to an increase in infarct size and a deterioration of endothelial function, which can further increase the severity of bleeding [6].

In addition, the risk of developing HT after rt-PA thrombolysis is also related to the underlying conditions of patients with AIS and so on. The arterial walls of patients with long-term hypertension are inherently damaged to varying degrees, and these stroke patients are more likely to develop HT after rt-PA thrombolysis [7]. One study showed that the higher the baseline glucose in patients with AIS, the higher the incidence of HT later in life [8]. Studies have shown that independent risk factors for HT include age, history of comorbid AF, and baseline NIHSS score [9]. Therefore, individualized evaluation of patients with AIS will help us to more standardize the treatment of tPA thrombolysis.

3. Molecular Mechanism of HT

The molecular mechanisms underlying the development of HT by tPA thrombolysis/reperfusion are extremely complex and are still being explored. Currently, one of the main pathophysiologic mechanisms of HT is the disruption of the integrity of the blood-brain barrier (BBB). Here the present study focuses on several aspects of tPA-induced disruption of vascular integrity, oxidative stress, and neuroinflammatory responses in brief.

3.1. Disruption of Vascular Integrity

It is clear that disruption of vascular integrity is the pathologic-structural basis for the development of HT. Studies have shown that thrombolytic therapy with tPA in patients with AIS can lead to hydrolysis of structural proteins such as collagen, laminin, and fibronectin, which increases BBB permeability, leakage and collapse of the intact neurovascular unit, and ultimately leads to blood extravasation, the development of cerebral edema and HT, and further deterioration of the patient's symptoms of neurological deficits [10].

Playing an important role are members of the matrix metalloproteinase (MMP) family, a large class of zinc-dependent endopeptidases that break down proteins that make up the basement membrane and extracellular matrix. In an experimental ischemic stroke model, MMP-2, MMP-3, and MMP-9 are upregulated in ischemic brain tissue and mediate matrix protein degradation to disrupt the BBB [11]. Among them, the activation of MMP-9 has been most extensively studied for HT. Low-density lipoprotein-related receptor protein (LRP) regulates lipoprotein metabolism and is one of the major binding sites for tPA on the surface of neuronal cells. Studies have shown that LRP expression is increased in ischemic brain tissue of AIS patients, and anti-LRP antibody treatment reduces the degree of brain edema. tPA binds to LRP under ischemic conditions, passes through the intact BBB into the brain parenchyma, and activates MMP-9 through the LRP-mediated related signaling pathway, leading to vascular injury causing HT [12]. An experimental study by Clark *et al.* in mice with a middle cerebral artery occlusion (MCAO) model demonstrated that MMP-2 knockout stroke mice exhibited smaller infarct size and lower incidence of hemorrhagic transformation after ischemia/reperfusion compared with wild-type mice [13]. Elevated MMP-2 and MMP-9 in ischemic and reperfused brain tissues can lead to degradation of occludin and claudin-5 and repartitioning in microvascular endothelial cells mediated by caveolin-1, inducing an early opening of the BBB, which corresponds to the time-dependent change in biphasic pattern of BBB infiltration after reperfusion [14]. Serum occludin levels increase proportionally to the degree of BBB injury, and it can be used alone or in combination with NIHSS scores to assess the risk of HT after tPA administration in stroke patients [15] [16]. In addition, high serum MMP-9 levels can be an independent predictor of the occurrence of HT after intravenous thrombolysis [17]. Recent studies have shown that inhibition of tPA activity can attenuate the expression of MMP-12 in brain tissue, thereby reducing the infiltration of immune cells such as macrophages in the infarcted area and inhibiting the expression of microglia and other microglial cells from M2 anti-inflammatory to M1 pro-inflammatory phenotypes, and attenuating reperfusion injury [18].

3.2. Oxidative Stress

Oxidative stress is an important mechanism in the development and progression of many diseases and also plays an important role in tPA reperfusion-mediated

BBB injury induced HT. Free radicals mainly refer to reactive oxygen species (ROS) and reactive nitrogen species (RNS), which act as redox signaling molecules in vivo to participate in cell proliferation, differentiation, and information transfer. The process of ischemia/reperfusion can cause the generation of excess free radicals, the main source of which is the mitochondrial respiratory chain, including NADPH oxidase (NOX) and cytochromes, etc., in addition to macrophages and mitochondrial polyunsaturated membranes, lipid peroxidation can also be generated. Excess free radicals lead to an imbalance in the body's oxidation-reduction regulatory capacity, oxidative stress occurs, destroying neurons, endothelial cells, etc., causing HT to occur [19]. Immunofluorescence analysis of brain sections from ischemic/reperfused rats showed that the mRNA and protein levels of NOX2 and NOX4 of the NOX family were significantly increased in rat neurons, astrocytes and endothelial cells, and peaked at 3 hours after reperfusion. In rats pretreated with the antioxidant drug melatonin, the expression of NOX2 and NOX4 was suppressed, and ROS levels were reduced, which alleviated the oxidative stress damage in neurons [20]. In addition, in another animal study, mice injected with Apocynin (NOX2 inhibitor) and with NOX2 knockout showed less cerebral infarct volume and lower incidence of HT compared with untreated wild-type control stroke mice [21]. 12/15-Lipoxygenase (12/15-LOX) is a key metabolizing enzyme of arachidonic acid. The cytotoxic activity of 12/15-LOX is up-regulated in neurons and endothelial cells under ischemic hypoxic conditions, and it can directly damage membrane-bound organelles, such as mitochondria, causing mitochondrial damage, which in turn causes redox imbalance in the brain microenvironment, and ultimately leads to necrotic apoptosis of neuronal cells [22]. In animal experiments, MCAO mice treated with the 12/15-LOX inhibitor ML351 or knockout showed less HT after tPA thrombolysis, and thus the safety of 12/15-LOX inhibitors can be improved by combining them with tPA [23].

3.3. Neuroinflammatory Response

In recent years, many studies have found that the neuroinflammatory response is closely related to the development of HT after tPA-mediated intravenous thrombolysis. Under ischemic and hypoxic conditions, the body's immune system is strongly activated, and CNS immune cells such as microglia and astrocytes secrete large amounts of pro-inflammatory mediators, which directly damage neurons and the extracellular matrix. In addition, peripheral leukocytes such as neutrophils may migrate and infiltrate toward the lesion in response to chemokines, mediating BBB destruction, neuronal cell death, and HT development through a series of complex cascade reactions.

It has been reported that in patients with AIS who developed HT after receiving arterial thrombolysis, they had significantly higher leukocyte counts than patients who did not develop HT [24]. Neutrophils are the most responsive subpopulation of leukocytes in the inflammatory microenvironment and play an

important role in the early destruction of the BBB. Exogenous rt-PA promotes neutrophil degranulation, which produces and releases large amounts of MMP-9 into the bloodstream, inducing disruption of neurovascular integrity [25]. Studies have reported that serum neutrophil count before intravenous thrombolysis in patients with AIS is an independent predictor of HT after reperfusion [26]. In addition, inflammatory factors such as TNF-α, IL-1, IL-6 and IL-10 also predicted the occurrence of HT after thrombolysis [27]. In addition to this, the dynamic variables neutrophil to lymphocyte ratio, platelet-to-lymphocyte ratio, and lymphocyte-to-monocyte ratio were all associated with neurologic deterioration such as HT after thrombolysis [28]. Hypoxia-inducible factor-1 (HIF-1) is an important transcription factor that regulates cellular oxygen adaptation and inflammatory responses under hypoxia. It has been demonstrated that blockade of β2-adrenergic receptors attenuates reperfusion injury by decreasing HIF-1 levels [29]. Recent studies have shown that tPA administration induces high HIF-1 expression in neurons and endothelial cells at the ischemic site, whereas application of the HIF-1-inhibiting drug YC-1 reduces the occurrence of HT by blocking inflammatory cell infiltration mediated by activation of the HMGB1/TLR4/NF-κB signaling pathway, and by increasing the expression of tight junction proteins in neurovascular units [30]. Platelet derived growth factor C (PDGF-C) has been shown to be a downstream substrate of tPA. Exogenous tPA injected intracerebroventricularly can activate PDGF-α receptors on astrocytes under non-ischemic conditions leading to a significant increase in BBB permeability, and administration of PDGFR-α antagonist imatinib significantly reduced the occurrence of tPA-mediated HT in stroke mice [31]. It should be noted that after stroke, damaged neurons can also secrete endogenous tPA as an intercellular signaling regulator to activate microglia, and activated microglia can continue to express tPA to form a positive feedback mediating the neuroinflammatory response and the development of HT [32].

4. Progress of Research on HT Therapeutic Drugs

Based on the molecular mechanisms and related targets of tPA reperfusion-mediated HT onset, a number of drugs have been developed for the treatment of HT and have been initially validated in relevant animal experimental models and clinical trials.

MMPs can degrade matrix proteins to disrupt BBB integrity, and tPA can induce upregulation of MMPs expression by activating multiple signaling cascade responses. Therefore, inhibition of MMPs activation may be an effective therapeutic strategy for the treatment of HT that occurs after intravenous thrombolysis with tPA in AIS patients. In the endothelial cell oxygen/glucose deprivation and reperfusion (OGD/R) model, pretreatment with the anesthetic agent isoflurane inhibited the activation of the LRP/NF-κB/Cox-2 signaling pathway in endothelial cells, and subsequently down-regulated the expression of MMP-2 and MMP-9 to attenuate tPA-induced ischemia-reperfusion injury [33]. It is note-

worthy that MMP-10 can also be used alone or in combination with tPA to attenuate the neurotoxicity of tPA itself and its mediated excitotoxicity, such as calcium overload, and thereby inhibit neuronal necrosis and apoptosis [34]. In another study, the MMP inhibitor minocycline in combination with tPA widened the time window for thrombolysis from 3 hours to 6 hours in patients with AIS [35]. Although MMPs inhibitors are able to protect BBB and anti-HT at animal level, they are not widely used in clinical practice, which may be due to the fact that MMPs are a class of downstream signaling molecules regulated by many signaling pathways, and only targeting MMPs cannot produce good therapeutic effects. Adenosine A2b receptor (A2bR) is widely known to regulate peripheral vasoconstriction, and recent studies have found that the expression of A2bR on cerebral microvascular endothelial cells is also markedly upregulated after ischemia/reperfusion and can attenuate BBB disruption and secondary cerebral edema, etc., by inhibiting MMP activation; therefore, addition of A2bR agonists can attenuate tPA-induced HT [36]. Vascular endothelial growth factor (VEGF) plays an important role in vascular regeneration and remodeling. During ischemia and reperfusion, VEGF expression increases in neurons and endothelial cells and destroys the BBB by activating MMP-9 [37]. In a rat thromboembolic model, the incidence of HT was significantly lower in stroke mice administered a combination of tPA and an anti-VEGF neutralizing antibody than in those treated with tPA alone, and thus inhibition of the VEGF signaling pathway is also a promising therapeutic strategy to reduce the incidence of HT [38]. Edaravone, a strong free radical scavenger widely used in clinical practice, exerts neuroprotective effects by scavenging overproduced free radicals in ischemic and reperfused brain tissue. In a randomized controlled study, rats treated with Edaravone in combination with r-tPA had a significantly lower incidence of HT after reperfusion compared with control rats treated with r-tPA alone, due to Edaravone's ability to inhibit MMP-9 activity and NF-κB expression in endothelial cells in a dose-dependent manner [39]. The natural active compound baicalin attenuates HT caused by delayed tPA administration in AIS patients by inhibiting ONOO-mediated MMP-9 activation, and baicalin has no effect on the thrombolytic efficiency of tPA [40]. Uric acid is also a natural antioxidant, and co-administration of uric acid with r-tPA significantly reduced the volume of cerebral infarction, attenuated the inflammatory response of ischemic brain tissue, and improved the functional score of neurological deficits in stroke mice by inhibiting ONOO-induced tyrosine nitration in the MCAO rat model [41]. Glycyrrhizin can protect the BBB and ischemically damaged neurons by inhibiting the ONOO-/HMGB1/TLR2 signaling cascade to reduce the production of MMPs, inflammatory factors, and excessive free radicals in ischemic brain tissues [42]. Natural products, with their multi-target properties, safety and efficacy, have become hotspots for innovative drug discovery.

5. Summary

Intravenous thrombolysis with tPA is a very effective and widely used method of

revascularization in the hyperacute phase of AIS, which is more convenient than interventional procedures such as endovascular mechanical thrombolysis. However, HT is a common complication of tPA thrombolysis, which can cause further deterioration of neurological deficit symptoms in stroke patients and even lead to early death. The development of HT is not the result of a single pathogenetic factor, but rather involves the intersection, synergy, and dynamics of multiple molecular mechanisms centered on damage to the entire neurovascular unit, including the extracellular matrix, endothelial cells, tight junction proteins, astrocyte terminals, pericytes, and basement membranes. While exerting thrombolytic effects, TPA also has potential neurotoxicity, which can trigger different signaling pathways in the cerebral ischemic-hypoxic microenvironment and activate a series of oxidative stress and neuroimmune responses mediating reperfusion injury, leading to the occurrence of HT. Continuously exploring the cytological and molecular mechanisms of HT development induced by tPA thrombolysis will help us to develop specific inhibitors and some corresponding physical therapies for the drug targets. In addition, we can explore more biomarkers for early prediction, reduce the incidence of HT before and after tPA thrombolysis.

It is important to note, however, that drug development for currently discovered mechanisms and targets does not address the treatment and prevention of HT. Firstly, the results of animal experiments and clinical trials differ greatly. Some drugs proved to be effective at the animal level, but ineffective or even aggravated HT in the clinic, which is mainly related to the selection of animal models, physiological differences between humans and animals, and pharmacodynamic evaluation criteria. Therefore, it is necessary to choose animal models that are consistent with the pathogenesis of HT in humans and pharmacodynamic evaluation methods that are close to those of humans for the development of HT drugs. Secondly, the efficacy of the same drug in the same animal model varies, which is related to the choice of drug treatment strategy, time of administration, dosage, and observational indexes, etc. Therefore, we should develop a more standardized drug administration strategy, and try to carry out large-scale clinical trials to evaluate the efficacy and safety of drugs. In addition, drug-drug interactions between tPA and other therapeutic molecules are crucial for translating research results into clinical practice. Considering the tPA thrombolytic time window and drug availability, the development of new-generation thrombolytic drugs with long thrombolytic window and low adverse effects and natural therapeutic factors with high biosafety and wide sources are important research directions. Combining nanomaterials for targeted controlled release of drugs is also a hot research topic in HT therapy. In summary, with the continuous exploration of HT mechanisms and therapeutic targets, the development of HT therapeutic drugs will certainly be promoted, and the safety of intravenous thrombolytic therapy with tPA in AIS patients will be increased, so that more patients can benefit from it.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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