European Review for Medical and Pharmacological Sciences 2015; 19: 416-422

Combination of thrombolytic therapy and neuroprotective therapy in acute ischemic stroke: is it important?

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Abstract. – Tissue plasminogen activator (tPA) is the only treatment approved by the USA FDA for acute ischemic stroke. There are many obstacles, however, when it is widely used in clinical setting, such as narrow therapeutic window, cytotoxicity and neurotoxicity. In recent years, many neuroprotective agents aiming to different molecular targets in ischemic cascade have been rapidly developed. Although these agents showed remarkable effects in experimental stroke, they failed to be translated to clinical use for many reasons. As the concept of "neurovascular unit" (NVU) is mentioned, combination of thrombolytic agents such as tPA with neuroprotectants gets more and more attention. Evidences supporting the combination therapy have been obtained from a variety of studies in many kinds of animal models, even though clinical evidences are inadequate. Combination of thrombolysis and neuroprotection has been considered as a promising approach for treatment of acute ischemic stroke. There are many advantages for the combination therapy. In this context, we review the sound rationales and researching achievements to support the therapy.

Key Words:
tPA, Thrombolytic therapy, Neuroprotection, Ischemic stroke.

Abbreviations

tPA = tissue plasminogen activator; BBB = blood-brain barrier; PDGF-CC = platelet-derived growth factor-CC; MMP-9 = matrix metalloproteinase-9; MMP-3 = matrix metalloproteinase-3; LRP = low-density lipoprotein receptor-related protein; NMDA = N-methyl-D-aspartic acid; NVU = neurovascular unit; PS-341 = Bortezomib; MCAO = middle cerebral artery occlusion; rhEPO = recombinant human erythropoietin; CEPO = carbamylated rhEPO; G-CSF = granulocyte-colony stimulating factor; VEGF = vascular endothelial growth factor; ERK = extracellular signal-regulated kinase; ER = endoplasmic reticulum; UA = uric acid; TIA= transient ischemic attack; ETA = endothelin type A receptor; ICAM-1 = intercellular adhesion molecule-1; PAR-1 = protease-activated receptor 1; GP IIb/IIIa = glycoprotein IIb/IIIa; MCA = middle cerebral artery; CBF = cerebral flood flow; ATP = adenosine triphosphate; GABA = γ-aminobutyric acid; NO = nitrogen monoxide; O_2 = oxygen.

Introduction

Tissue plasminogen activator (tPA) is the only treatment approved by the USA Food and Drug Administration (FDA) for acute ischemic stroke, which dissolves the obstructive clot so as to restore cerebral blood flow. There are many obstacles, however, when it is widely used in clinical setting. One of the important difficulty is it has a narrow therapeutic window of 3 to 4.5 h[1-2]. Meanwhile, tPA also has a few of untoward effects like cytotoxicity and neurotoxicity, especially when administrated together with L-arginine[3]. It can increase permeability of blood-brain barrier (BBB) to result in the damage of neurovascular unit[4] via activation of latent platelet-derived growth factor-CC (PDGF-CC[5], stromelysin (MMP-3, MMP-9) and receptor–related protein (LRP)/nuclear factor-kB pathway[6]. tPA can also enhance NMDA (N-methyl-D-aspartic acid)-mediated signaling, resulting in neuronal excitotoxicity which make the injury from cerebral ischemia even severer[7-9].

In recent years, many neuroprotective agents aiming to different molecular targets in ischemic cascade (Figure 1) have been rapidly developed. Although these agents showed remarkable effects in experimental stroke, they failed to be translated to clinical use. Potential explanations for the failure are as follows. Firstly, there are apparent differences between human and animals. Secondly, efficacy of some of the neuroprotective agents is very limit when used alone that may not be sufficient to suppress ischemic damages resulted

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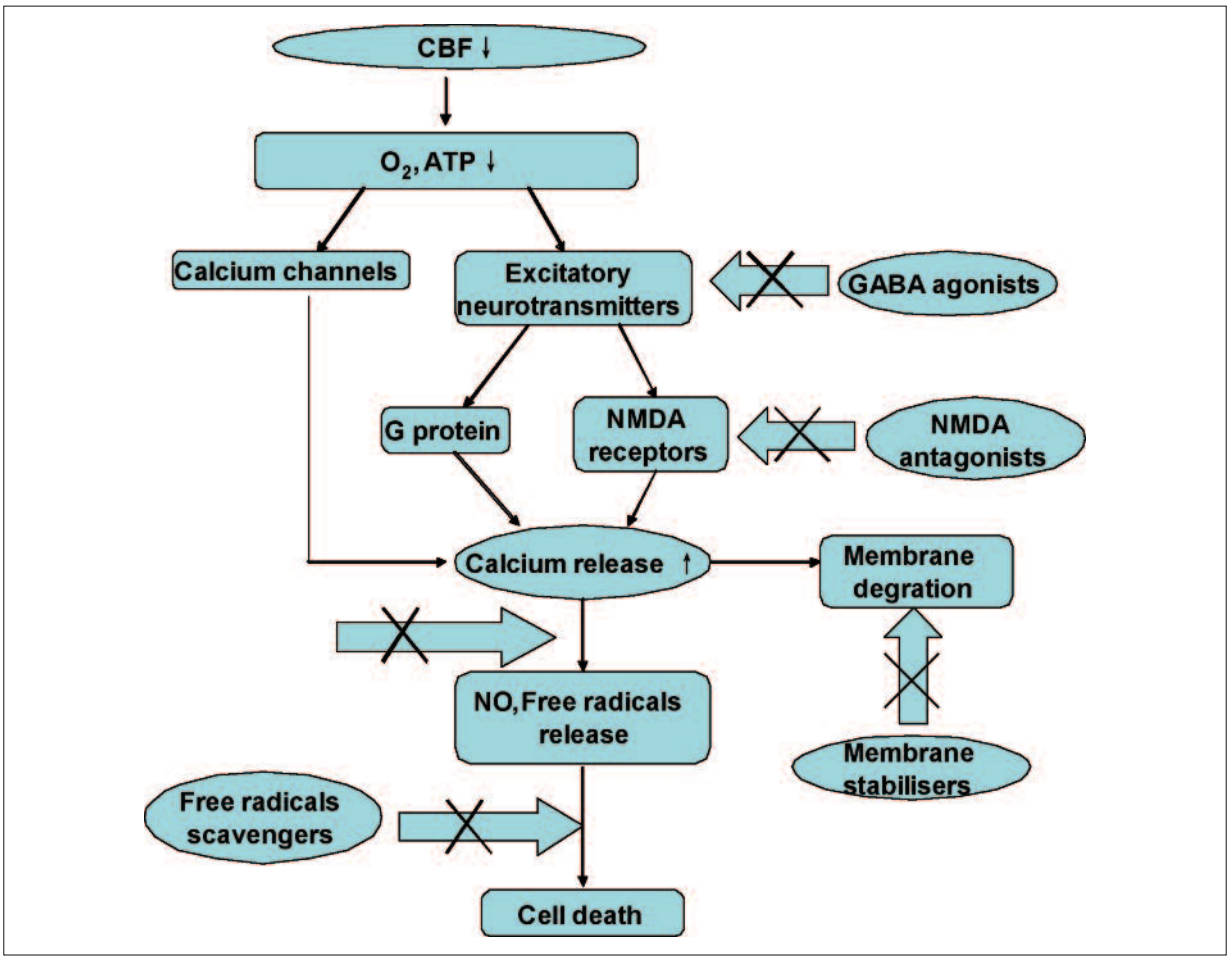

Figure 1. Ischaemic cascade and the targets of different neuroprotective agents.

from different parts of ischemic cascade[10]. Thirdly, ischemic cascade reactions occur in the early stage of stroke, which can not be treated timely because clinical treatment is performed often out of 6h after a stroke attack. Fourthly, doses of drugs are insufficient because deliver of the agents to the target is difficult. Ischemic penumbra is often as a target in experiment stroke but it is different from that of clinical treatment. Fifthly, evaluation of efficacy of neuroprotective agents is mainly dependent on infarct volume in experiment stroke, in contrast it is mostly dependent on behavioral function in clinical setting. Sixthly, there are still a few of shortcomings in methodology[11]. Last but not the least, study for pharmacokinetics of neuroprotective agents is still inadequate[12].

The "neurovascular unit" (NVU) (Figure 2) is a concept framework which consists of microvessels, neuroepithelial cell, neurons and their axons and the extracellular matrix[13]. This concept offers a platform for understanding stroke as a pathological outcome that many related tissues participate in. On account of this concept, interventions in vessels and cells are two fundamental approaches[14] in stroke therapy. Intervention in vessels is to realize reperfusion as soon as possible, while the latter is to interrupt the ischemic cascade, which is known as neuroprotective therapy. Study indicates that some neuroprotectants work basing on their effects to make the canal reperfusion realized[15]. It is obvious that the two therapeutic options are not only independent but also correlative each other. Reperfusion can be spontaneously repaired in human after ischemia, but it is delayed for a long time. In order to obtain maximal benefit from neuroprotective agents, it will be an ideal manipulation to make the reperfusion realized as early as possible[10].

Owing to the pathological feature of ischemic focus, cerebral injuries secondary from reperfusion easily occur if thrombolytic therapy is con-

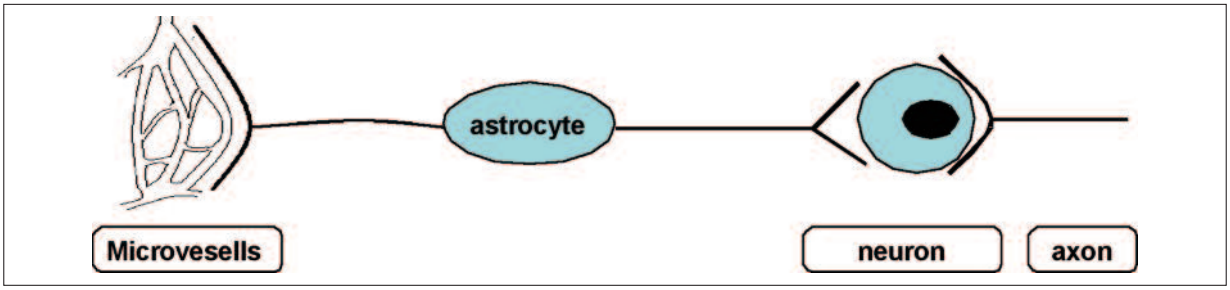

Figure 2. A simplified figure of the "neurovascular unit" (NVU).

ducted beyond therapeutic window including impairment of BBB, activation of cascade reaction mediated by free radicals, cerebral aseptic inflammation and so on[16]. Combination of thrombolytic agents such as tPA with neuroprotectants might, therefore, be needed to protect cerebral tissues from reperfusion injury.

Potential Advantages of Combining Thrombolysis with Neuroprotection

Over the last decade, evidences supporting the combination therapy have been obtained from a variety of studies in many kinds of animal models, even though clinical evidences are inadequate. Combination of thrombolysis and neuroprotection has been considered as a promising approach for treatment of acute ischemic stroke[17]. Sound rationales to support the combination therapy are posted in the present paper as well as others'.

Extension of the Thrombolytic Time Window With Neuroprotective Agents

Neuroprotective agents if given early can prolong the therapeutic window for thrombolyis. For instance, minocycline has been shown a beneficial neuroprotectant for ischemic stroke in many kinds of animal models[18]. In a study[19], rats were subjected to embolic focal ischemia and treated with minocycline at 4h and tPA at 6h. Infarct volumes were obviously reduced and the effect was equal to that when tPA was given 1h after ischemia, suggesting that minocycline could extend the therapeutic window of tPA.

Proteasome inhibitors targeting ubiquitin–proteasome pathways has significantly shown neuroprotective effects by suppressing inflammation, upregulating endothelial nitric oxide synthase and inducing antioxidative enzyme expression. Bortezomib (PS-341) and PS-519 are two different proteasome inhibitors widely investigated in experimental stroke. Combination of tPA and the individual bortezomib[20], PS-519[21] or atorvastatin[22] for therapy in rat embolic ischemic stroke could enhance the neuroprotective effect of these agents, apparently extend the therapeutic window of tPA to 6h, and notably decrease incidence of hemorrhage and other adverse events as well. A meta-analysis [23], extracting data from a number of focal ischemia experiments before 2010, indicated that combinations of tPA and each of other agents could extend the therapeutic window to 4.4-8h. Benefit of combination therapy beyond 6h after ischemia, however, still is needed to be defined by more animal experiments.

Reduction of Neurological Impairment

Neuroprotective agents may reduce reperfusion injuries resulted from thrombolysis. Calcium channel blockers, excitatory amino acid receptor antagonists and free radical scavengers are neuroprotective agents widely investigated. Although most of clinical trial results of the drugs are negative, some could indeed protect brain tissues from ischemic reperfusion injuries. Nerve growth factors are investigated more and more widely because the NVU concept is recently raised. Erythropoietin, a hematopoietic growth factor, given to rats subjected to middle cerebral artery occlusion (MCAO) 2 h after the surgery, could significantly reduce infarct volumes at 24 h, 48 h and 72 h after reperfusion and improve neurological impairment as well[24]. In another study[25], recombinant human erythropoietin (rhEPO) and carbamylated rhEPO (CEPO) were intravenously injected to rats, respectively, subjected to embolic MCAO at 6h after ischemia, infarct volumes were noticeably decreased, behavioral and neurological function were also ameliorated.

Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic growth factor. Under ischemic conditions, it inhibits programmed neuronal cell death and stimulates neural progenitor

cell differentiation[26-27]. When administered 1.5 h after MCAO/reperfusion in rats, G-CSF significantly reduced infarct volumes, improved neurological function and prevented expressions of neuronal and glial pro-inflammatory cytokines[28]. Rats subjected to MCAO were treated with G-CSF 5 h after ischemia, infarct volumes were significantly reduced and neurological function was improved 1w, 2w and 3w[29]. In a recent study[30], G-CSF show a neuroprotective effect through the signaling pathway for the antiapoptotic cascade and antiinflammatory effects. G-CSF is given to mice subjected to MCAO for 1 h and reperfusion, infarct volumes were reduced by 34% ($p < 0.006$) and 25% ($p < 0.04$), neurological deficit was significantly improved by 35% ($p < 0.001$) and 24% ($p < 0.01$), respectively, 24 and 72 h after reperfusion.

Vascular endothelial growth factor (VEGF), a kind of angiogenic peptide, was recently reported to resist ischemic/reperfusion injury via various mechanisms. A study reported that VEGF induced neuroprotective effect by inhibiting extracellular signal-regulated kinase (ERK) signaling pathway[31-32] and endoplasmic reticulum (ER) stress pathway[31]. Rabbits subjected to focal cerebral ischemia/reperfusion induced by 2 h-MCAO are treated with different doses of VEGF. Results indicated[33] that 2.5 ng/ul VEGF significantly reduced infarct volume and ischemic neuronal danger 70 h after reperfusion. In another study[34], VEGF is intracerebroventricularly administered 1 h and 3 h after reperfusion to mice subjected to 1.5 h-MCAO, infarct volumes were decreased by 35% and 46% and neurologic function at 24 h after reperfusion was improved.

Over the last decade, many studies about the neuroprotective effect of nerve growth factor, human chorionic gonadotropin, insulin-like growth factors and acidic fibroblast growth factor in ischemic/reperfusion injury were reported. Deeper investigation about combination therapy of thrombolysis with other kinds of neuroprotectants, like calcium channel blockers, excitatory amino acids receptor antagonists and free radical scavengers are nowadays being conducted and the therapy definitely become a prospective treatment.

Thrombolysis May Increase Delivery of Neuroprotectants to the Target Site

After ischemia, cerebral metabolic rate reduces and tissue constitution is changed, neuroprotective agents to targets may be influenced. Cerebral blood flow after ischemia is reported to reduce clearly and goes down to 5-10% of normal baselines in core ischemic region, and to 30-40% in the penumbra region[35]. When neuroprotective agents are administrated via body circulation, their diffusion to the target may be severely influenced. Besides, effective delivery may be impeded by increased intracranial pressure induced by brain edema and decreased metabolic rates[36]. Thrombolysis can be administered to produce thrombolysis and restore blood flow to the ischaemic brain, therefore, might be beneficial to increase delivery of neuroprotectants to their target sites. However, this remains to be proved by experiments.

Additive or Potentiative Neuroprotection of the Combination

In the experimental stroke the combination therapy shows additive or potentiative neuroprotective effect in variety mechanisms. Neuroprotective agents attenuate the inflammatory response, suppresses molecules that mediate thrombosis and blood brain barrier (BBB) disruption induced by ischemia and rtPA so that extend the benefits of rtPA.

Uric acid (UA) is a natural antioxidant and has noticeable neuroprotective effect in transient ischemic attack (TIA). Rats were given uric acid 20 min after thromboembolic MCAO as well as tPA at 3 h, infarct volumes at 24 h in the group treated with the combination therapy were additively reduced by 17%, compared with the group given tPA alone ($p < 0.05$) and intracranial hemorrhages were also decreased at the point[37]. S-0139, an antagonist of endothelin type A receptor (ETA) was given 2 h after ischemia to rats subjected to embolic MCAO and tPA was given 4 h after ischemia, infarct volumes and parenchymal hemorrhages were additively reduced and behavioral outcome was significantly improved at 7 d[38]. This study also indicated that the synergistic benefit with tPA come from synergistically reduction of ischemia, suppression of intercellular adhesion molecule-1 (ICAM-1) and protease-activated receptor 1 (PAR-1) induced by tPA as well as decrease of platelets accumulation in cerebral microvessels[38].

Moreover, using a factorial design, rats subjected to embolic MCAO were given combination of integrin CD11b/CD18 antagonist (UK-279,276) and rhtPA. They yielded a synergy efficacy for stroke treatment at 7 d after the onset[39]. When glycoprotein IIb/IIIa (GP IIb/IIIa) receptor inhibitor $E(ab')_2$ and rhtPA were administered to

the rats, they also produced an additive effect on improvement of neurological functions, infarct volumes and behavioral outcomes. The additive or synergy effects of combined thrombolysis with neuroprotectant could reduce adverse events and elevate safety as well because doses of the drugs could be decreased when they were combined. On the other hand, the factorial analysis on the additive or synergistic effects of the drugs is very helpful to promote theoretical study of drug interactions.

Neuroprotectants Can Prevent or Decrease Cytotoxicity, Neurotoxicity and Intracranial Hemorrhage of tPA

More and more studies found that combination of thrombolysis and neuroprotective agent could prevent or decrease cytotoxicity, neurotoxicity intracranial hemorrhage and other adverse events resulted from tPA treatment. xenon, an anesthetic gas with good tolerance, was proven to be a very promising neuroprotective agent with few adverse events for treating acute ischemic stroke. Results from a preclinical study[40] showed that, under the ischemic condition, xenon not only appeared significant neuroprotective effects when given after reperfusion induced by tPA but also prevented hemorrhage and BBB permeability.

Estrogen exerted its neuroprotective properties in experimental ischemic stroke by inhibiting neuronal apoptosis through multiple signaling pathways. In ovariectomized female rats subjected to ischemia/reperfusion, combination of 17β-estradiol and tPA could reduce infarct volumes. Results suggested that 17β-estradiol prevented BBB injury induced by tPA by inhibiting matrix metalloproteinase-9 (MMP-9) expression[41]. It also could suppress brain edema, hemorrhage induced by tPA[42]. Moreover, studies indicated that free radical scavenger edaravone could decrease intracalvarium hemorrhage[43-44] and BBB leakage by inhibiting the extravasation of tPA from brain vessels[45,46], clearing free radicals, suppressing peroxidation and MMP-9 expression[44,46].

Melatonin, a natural antioxidant, could also inhibit MMP-9 activity and expression and decrease neurovascular oxidative injury as well as BBB leakage induced by TIA[47-48]. Similar effects were observed in mice subjected to ischemia/reperfusion as well when the mice were treated with both melatonin and tPA at 6 h after the onset of photothrombotic distal middle cerebral artery (MCA) occlusion[49]. Results from an immunosuppressant tacrolimus showed hemorrhage rates induced by tPA were remarkably decreased[50-51], also indicating a promising neuroprotective activity of the combined therapy in animal models of cerebral ischemia.

Conclusions

Improvements have preclinically and clinically been achieved in combination therapy of each of various neuroprotective agents and tPA. It is steadily translating to clinical application. A multicenter, single blind, randomized, open-labeled study[52] revealed that edaravone could recanal vessels if given at early stage of reperfusion induced by tPA. In another randomized double blind clinical trial[53], patients were administrated with uric acid and tPA together, lipid peroxidation was inhibited and early fall of uric acid in serum was successfully prevented. In addition, combination of statins and tPA significantly reduced neurological injury in stroke patients[54].

The combination therapy is a promising approach in treatment of ischemic stroke and is translating to clinical use gradually. Samples in clinical trials which have been put into practice; however, are not enough and there are still a few of defects in designs of some clinical trials. Therefore, larger range of clinical trials designed strictly are urgently needed to definite the effect and safety of the combination therapy. Meanwhile, further studies using animal models are should be deeply conducted to make way for clinical trials.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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