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# Effect of recombinant plasminogen activator timing on thrombolysis in a novel rat embolic stroke model

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ABSTRACT

The treatment of acute ischemic stroke (AIS) using thrombolysis with recombinant tissue-plasminogen activator (rtPA, alteplase) is limited by its narrow time window and the risk of hemorrhage. Recombinant plasminogen activator (rPA, reteplase) has been used clinically on coronary artery thrombosis and acute myocardial infarction. It is necessary to induce strokes experimentally as a means of validating the rPA timing on patients with AIS. However, current embolic models cannot mimic clinical situations well due to the embolus's composition of dried blood clots or artificial materials. In this paper, we used two novel rat thromboembolic models to determine the dosage-effect relationship and therapeutic time window of r-PA. Male rats were administered rPA or rtPA intravenously at 2–12 h postischemia. Cerebral blood flow, behavioral outcomes and infarct volume within the same animal group were determined. Our results demonstrated that rPA (0.2 and 0.4 mg/kg) or rtPA (0.2 mg/kg) restored focal perfusion, reduced cerebral infarction, and improved behavioral outcomes at 2–4 h postischemia. rPA but not rtPA significantly restored focal perfusion at 6 h postischemia. However, delayed rPA-treatment neither decreased infarct volume nor improved the neurological disorder. Cerebral hemorrhage occurred at 6 h postischemia detected by Evan's blue leakage and tissue hemoglobin content. Collectively, Thrombolysis with rPA may be beneficial in revascularization at an acceptable dosage of 0.2–0.4 mg/kg within 6 h after the cerebral infarct onset.



## 1. Introduction

Acute cerebral ischemic stroke (AIS), resulting from the reduction of cerebral perfusion, is a leading cause of disability worldwide in patients over the age of 60 [1]. Many factors participate in AIS, including thrombosis, embolism, stenosis, and intracerebral hemorrhage. Both embolic and thrombotic stroke are primary causes of occlusion of arteries in the brain. Currently, recombinant tissue-plasminogen activator (rtPA) is the only thrombolytic approved by the US Food and Drug Administration for the treatment of AIS. However, thrombolysis with rtPA is limited by its narrow time window and the risk of hemorrhagic complication. Treatment of patients with rtPA more than 4.5 h after the onset of symptoms is not recommended. Thus, fewer than 3% of potential patients currently receive rtPA [2]. Therefore, a thrombolytic with additional safety and efficacy for AIS is urgently required.

rPA has been authorized in treating acute myocardial infarction for years, and its therapeutic effect was notable [3]. As single-chain, nonglycosylated peptide, reteplase is a fibrin-specific recombinant form of tissue (t-PA) plasminogen activator that contains the kringle 2 and protease domains of native t-PA, but lacks the kringle 1, fibronectin finger, and epidermal growth factor domains [4] However, the study of rPA on thrombotic stroke has been limited because current AIS models are reluctant to mimic the clinical situation and not suitable for thrombolytic timing studies. In this

*Abbreviations:* AIS, acute ischemic stroke; rtPA, recombinant tissue-plasminogen activator; rPA, Recombinant plasminogen activator; SD, Sprague–Dawley; EJV, external jugular vein; CCA, common carotid artery; ECA, , external carotid artery; ICA, internal carotid artery; PPA, pterygopalatine artery; CBF, cerebral focal perfusion; PU, perfusion unit; TTC, 2,3,5-triphenyltetrazolium chloride; MCAO, middle cerebral artery occlusion.

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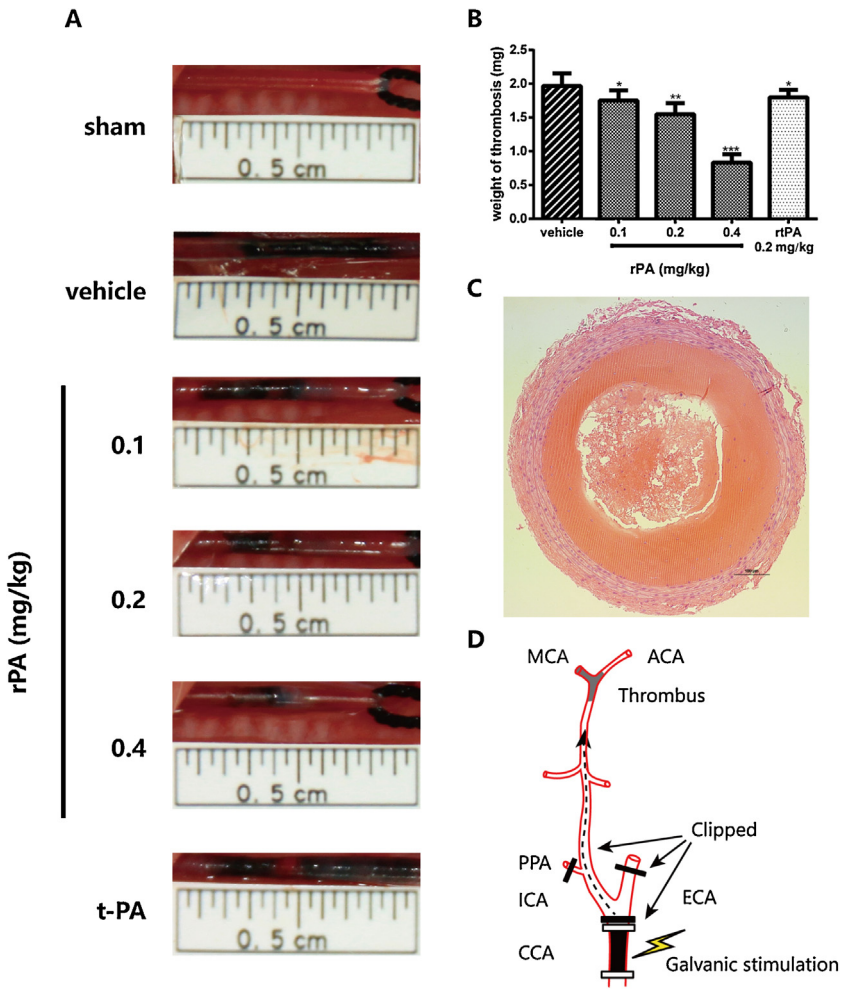


**Fig. 1.** Thrombolytic effects of rPA on CCA occlusion. (A) Representative images of the thrombus in each group. (B) Vehicle, rtPA (0.2 mg/kg) or rPA (0.1 0 2 or 0.4 mg/kg) was administered immediately after the thrombosis. rPA reduced the weight of the thrombus in a dose-dependent manner (n = 10, *P < 0.05, **P < 0.01, ***P < 0.001 *vs.* vehicle, ANOVA). (C) HE staining of a 10 μm section of embolic CCA. Most of the clot is composed by fibrin and interspersed with clusters of cells. The integrity of endothelium and the basal lamina was mostly preserved. (D) Diagrammatic drawing of the embolic stroke procedure.

paper, two novel rat thromboembolic models were used to evaluate the thrombolytic dosage and therapeutic time window of rPA through a series of evaluation indices.

## 2. Experimental procedures

### 2.1. Animal treatment

Male Sprague–Dawley (SD) rats (Vital River Laboratory Animal Technology Co., Beijing) weighing 250–300 g were acclimatized for 2 days and assigned to different groups randomly. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Peking Union Medical College and were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animal suffering and the number of animals used.

### 2.2. Drug administration and experimental group

rPA (Shandong Ehua Biopharmaceutical Co.) or rtPA (Boehringer Ingelheim) was dissolved and diluted in normal saline as the vehicle. All agents were administered into the external jugular vein (EJV) twice with a 30 min interval between injections, according to the rPA administration route in myocardial infarction clinically. Drug administration was predefined before the assignment into the various treatment groups: the normal saline and rPA (0.1, 0.2, 0.4 mg/kg) were injected at 2, 4, 6 and 12 h after ischemia (n = 10); rtPA (0.2, 0.4 mg/kg) was injected at 2, 4 and 6 h after ischemia (n = 10).

### 2.3. CCA (common carotid artery) embolism model

Rats were anesthetized with isoflurane (1–1.2%) in a 30% oxygen and 70% nitrous oxide mix. The body temperature of rats was controlled to approximately 37 °C during experiments.

The CCA embolism model was used for evaluating the efficiency of rPA or rtPA on reducing the weight of the thrombus. The surgery procedure was as follows: 2-cm-long midline incision was made on the neck, the surgical field was exposed, and the right CCA, external carotid artery (ECA), internal carotid artery (ICA), and pterygopalatine artery (PPA) were dissected from the surrounding nerves and fascia (without harming the vagal nerve).

The CCA was wiped dry with sterile cotton-wool mops and the distal end of which was closed with artery clamp. Then the CCA was placed in the clamp of a YLS-14B thrombus formation tester (Jinan Yiyan Science & Technology Development Co., Ltd), and the galvanic stimulation (1.00 mA) was initiated for 120 s. The thrombus could