Neurobiology of Disease 45 (2012) 804–809

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Subacute intranasal administration of tissue plasminogen activator increases functional recovery and axonal remodeling after stroke in rats

Zhongwu Liu [a], Yi Li [a], Li Zhang [a], Hongqi Xin [a], Yisheng Cui [a], Leah R. Hanson [b], William H. Frey II [b], Michael Chopp [a,c,*]

[a] Department of Neurology, Henry Ford Hospital, Detroit, MI, USA
[b] Alzheimer's Research Center at Regions Hospital, HealthPartners Research Foundation, St. Paul, MN, USA
[c] Department of Physics, Oakland University, Rochester, MI, USA

ARTICLE INFO

Article history:
Received 21 September 2011
Accepted 7 November 2011
Available online 15 November 2011

Keywords:
Functional recovery
Middle cerebral artery occlusion
Neuronal remodeling
Tissue plasminogen activator

ABSTRACT

As a thrombolytic agent, application of recombinant tissue plasminogen activator (tPA) to ischemic stroke is limited by the narrow time window and side effects on brain edema and hemorrhage. This study examined whether tPA, administered by intranasal delivery directly targeting the brain and spinal cord, provides therapeutic benefit during the subacute phase after stroke. Adult male Wistar rats were subjected to permanent right middle cerebral artery occlusion (MCAo). Animals were treated intranasally with saline, 60 μg or 600 μg recombinant human tPA at 7 and 14 days after MCAo (n = 8/group), respectively. An adhesive-removal test and a foot-fault test were used to monitor functional recovery. Biotinylated dextran amine (BDA) was injected into the left motor cortex to anterogradely label the corticorubral tract (CRT) and the corticospinal tract (CST). Naive rats (n = 6) were employed as normal control. Animals were euthanized 8 weeks after stroke. Compared with saline treated animals, significant functional improvements were evident in rats treated with 600 μg tPA ($p<0.05$), but not in 60 μg tPA treated rats. Furthermore, 600 μg tPA treatment significantly enhanced both CRT and CST sprouting originating from the contralesional cortex into the denervated side of the red nucleus and cervical gray matter compared with control group ($p<0.01$), respectively. The behavioral outcomes were highly correlated with CRT and CST axonal remodeling. Our data suggest that delayed tPA intranasal treatment provides therapeutic benefits for neurological recovery after stroke by, at least in part, promoting neuronal remodeling in the brain and spinal cord.

Introduction

Recombinant tissue plasminogen activator (tPA) is the only FDA approved thrombolytic agent for acute treatment of ischemic stroke; however, the efficacy and safety of its therapeutic application are limited by the narrow 4.5-h treatment time window and adverse side effects on brain edema and hemorrhagic transformation (Cronin, 2010). Presently, fewer than 5% of stroke patients in the United States receive tPA. For decades, the primary stratagem for stroke treatment has been focused on neuroprotection to reduce the cerebral infarction. Unfortunately, all neuroprotective agents developed in the laboratory have failed in clinical trials (Rother, 2008). There is therefore a compelling need to develop neurorestorative therapies to enhance neurological recovery, by primarily treating the intact cerebral hemisphere and the compromised cerebral tissue to promote neuronal plasticity to compensate for the damaged tissue during the subacute and chronic phases after stroke.

tPA has pleiotropic actions in the brain besides its well established fibrinolytic action. It induces neural injury in the setting of acute stroke (Benchenane et al., 2004). However, it is also involved in synaptic plasticity (Samson and Medcalf, 2006), dendritic remodeling (Mataga et al., 2004) and axonal outgrowth (Minor et al., 2009). We have demonstrated that endogenous tPA mediates bone marrow stromal cell-induced neurite outgrowth and functional recovery after stroke (Shen et al., 2011). Thus, we hypothesized that exogenous administration of tPA during the subacute phase may also provide beneficial effects on stroke recovery by promoting axonal remodeling.

The catalytic activity of tPA is rapidly inactivated through binding of protein inhibitors, primarily plasminogen activator inhibitor-I (PAI-1). The tPA/PAI-1 complex is cleared from the circulation by the liver. Therefore, tPA has a short half-life of 5 to 10 min in the bloodstream (Gravanis and Tsirka, 2008). Intranasal delivery method has been demonstrated to directly target the brain and spinal cord along olfactory and trigeminal nerves innervating the nasal passages

Abbreviations: tPA, tissue plasminogen activator; MCAo, middle cerebral artery occlusion; BDA, biotinylated dextran amine; CRT, corticorubral tract; CST, corticospinal tract; PAI-1, plasminogen activator inhibitor-I; MMP-9, matrix metalloproteinases-9.

* Corresponding author at: Neurology Research, E&R Bldg., Room 3056, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA. Fax: +1 313 916 1318.
E-mail address: chopp@neuro.hfh.edu (M. Chopp).

Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2011.11.004

to bypass the blood–brain barrier (Dhuria et al., 2010). To avoid the rapid inactivation and clearance of tPA from the circulation system, we for the first time examined the effect of tPA administered by intranasal delivery on sensorimotor functional recovery in adult rats during the subacute phase after ischemic stroke. In addition, to investigate the neuronal substrate of the behavioral recovery, we examined axonal remodeling of the corticorubral tract (CRT) and corticospinal tract (CST) originating from the intact cortex into the denervated side of the red nucleus and spinal cord.

Materials and methods

Adult male Wistar rats (n = 30, 2 month-old, weighing 250–275 g) were used throughout this study in a blinded fashion for treatment, behavioral measurements and tissue analysis. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Henry Ford Hospital.

Animal model

Permanent right side middle cerebral artery occlusion (MCAo) was induced using a method of intraluminal vascular occlusion, modified in our laboratory (Chen et al., 1992). Briefly, a length of 4–0 monofilament nylon suture (18.5–19.5 mm) with a heating rounded tip was advanced from the external carotid artery into the lumen of the internal carotid artery to block the origin of the MCA.

Functional measurements

A series of behavioral tests were performed before MCAo, 3 days after MCAo and weekly thereafter to evaluate the sensorimotor disability and recovery with an adhesive-removal test (Schallert and Whishaw, 1984), which measures the sensory and motor deficits by recording the time required to remove an adhesive tab from the stroke-impaired left forepaw, and a foot-fault test (Hernandez and Schallert, 1988), which measures the accuracy of forepaw placement on a non-equidistant grid as the percentage of foot-faults of the left forepaw to total steps.

Intranasal administration of tPA

The animals were randomly selected to receive saline (n = 8), low dose (60 μg, n = 8) or high dose (600 μg, n = 8) recombinant human tPA (Genentech Inc, San Francisco, CA) intranasally at 7 and 14 days after MCAo, respectively. The delivery method described by Thorne et al., (2004) was modified for intranasal treatment. Briefly, under Forane anesthesia, the rats were placed in a supine position with a rolled 2 × 2-in. gauze under the neck to maintain a horizontal head position. Ten 6-μl drops for a total volume of 60 μl of saline or tPA solution in saline were placed alternately onto each nostril with a 3-min interval between drops and naturally sniffed in by the rat. The animals were kept in supine position for an additional 10 min. An additional animal group without MCAo and treatment (n = 6) was employed as normal control. To validate the efficiency of intranasal delivery, a test performed in tPA−/− mice showed that the tPA was successfully delivered into the brain (Supplementary materials).

Anterograde axonal tracing

Fourteen days before euthanasia, the rats were restricted in a Kopf stereotaxic apparatus and a craniotomy was performed over the left frontal sensorimotor cortex with a high speed drill under ketamine anesthesia. Ten percent solution of biotinylated dextran amine (BDA, 10000 MW; Invitrogen, Carlsbad, CA) in saline was injected through a finely drawn glass capillary into 4 points (stereotaxic coordinates: 0.5 and 1.5 mm rostral to the bregma, 2 and 3 mm lateral to the midline, 1.5 mm deep from the cortical surface; 100 nl per injection) in the left forepaw motor cortex to anterogradely label the axons of CRT and CST originating from these areas.

Tissue preparation and data analysis

Animals were sacrificed under deep ketamine anesthesia at 8 weeks after MCAo. Rats were perfused transcardialy with saline, followed by 4% paraformaldehyde. The entire brain and spinal cord were immersed in 4% paraformaldehyde overnight. The brain was cut into 7 equally spaced (2 mm) coronal blocks. The brain blocks and tissue from the medulla and cervical cord were cut into 100 μm-thick coronal sections using a vibratome. The lesion volume was measured on sections from each brain block as percentage of the lesion area compared with the contralateral hemisphere, as previously described (Swanson et al., 1990). The remaining sections were incubated with avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA) at 4 °C for 48 h, and the BDA-labeling was visualized with 3,3′-diaminobenzidine-nickel (Vector).

The number of BDA-positive fibers in the pyramidal tract at the medulla level ipsilateral to the injection site was counted and averaged on 3 consecutive coronal sections for each animal. The NIH image software (Image J) was employed to measure the BDA-positive CRT density in the bilateral red nucleus on 5 continuous midbrain sections, and the BDA-positive CST length in the stroke-impaired side of the ventral gray matter on 40 consecutive cervical cord sections (C5-7). To avoid inter-animal variation in tracing efficiency, axonal remodeling of the CRT was estimated by the ratio of axonal density in the ipsilesional denervated side to the contralateral intact red nucleus measured on same section analyzed with, and the CST remodeling was estimated by the total BDA-labeled axonal length normalized with a quotient of individual BDA-labeled CST number in the pyramidal tract to the mean number calculated in all animal groups.

Statistics

All data are presented as mean ± SD. The experimental groups were compared statistically using the ANOVA test. A value of $p<0.05$ was taken as significant. Pearson's correlation coefficients were calculated between functional recovery and anatomical reorganization.

Results

Intranasal tPA administration enhances functional recovery

In the permanent suture MCAo model, the ischemic infarct included the forelimb area of the sensorimotor cortex, striatum, and the supraoptic area in the right cerebral hemisphere. As shown in Table 1, there was no significant difference on lesion volume among the control and different treatment groups. In addition, intranasal tPA administration did not induce animal death or brain hemorrhage (data not shown).

The motor performance of the stroke-impaired left forelimb was assessed with the adhesive-removal test and foot-fault test. All animals showed a remarkable functional deficit on postoperative day 3,

Table 1
Lesion volume among experimental groups.

Groups	Infarct volume (% of contralesional hemisphere)
Saline	38.2 ± 7.2
tPA 60 μg	38.6 ± 9.6
tPA 600 μg	37.4 ± 6.3