Urokinase, a promising candidate for fibrinolytic therapy for intracerebral hemorrhage

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OBJECTIVE Intracerebral hemorrhage (ICH) is associated with a high rate of mortality and severe disability, while fibrinolysis for ICH evacuation is a possible treatment. However, reported adverse effects can counteract the benefits of fibrinolysis and limit the use of tissue-type plasminogen activator (tPA). Identifying appropriate fibrinolytics is still needed. Therefore, the authors here compared the use of urokinase-type plasminogen activator (uPA), an alternate thrombolytic, with that of tPA in a preclinical study.

METHODS Intracerebral hemorrhage was induced in adult male Sprague-Dawley rats by injecting autologous blood into the caudate, followed by intraclot fibrinolysis without drainage. Rats were randomized to receive uPA, tPA, or saline within the clot. Hematoma and perihematomal edema, brain water content, Evans blue fluorescence and neurological scores, matrix metalloproteinases (MMPs), MMP mRNA, blood-brain barrier (BBB) tight junction proteins, and nuclear factor- κ B (NF- κ B) activation were measured to evaluate the effects of these 2 drugs in ICH.

RESULTS In comparison with tPA, uPA better ameliorated brain edema and promoted an improved outcome after ICH. In addition, uPA therapy more effectively upregulated BBB tight junction protein expression, which was partly attributed to the different effects of uPA and tPA on the regulation of MMPs and its related mRNA expression following ICH.

CONCLUSIONS This study provided evidence supporting the use of uPA for fibrinolytic therapy after ICH. Large animal experiments and clinical trials are required to further explore the efficacy and safety of uPA in ICH fibrinolysis.

<http://thejns.org/doi/abs/10.3171/2016.1.JNS152287>

KEY WORDS intracerebral hemorrhage; urokinase-type plasminogen activator; tissue-type plasminogen activator; fibrinolytic therapy; perihematomal edema; vascular disorders

SPONTANEOUS intracerebral hemorrhage (ICH) is the most devastating type of stroke worldwide, and currently there are few treatment strategies for ICH-induced brain injury.²⁰ The physical effects of hematoma (mass effect) as well as perihematomal edema (PHE) can worsen patient outcomes after ICH.^{6,7,36}

In recent years, stereotactic aspiration and subsequent fibrinolysis have been developed and, compared with the conventional surgical removal of ICH, have been considered a less invasive and more effective treatment modality for spontaneous ICH given their limited damage to peripheral normal brain tissues.^{25,26,39} So far, tissue-type plasminogen activator (tPA) is the most widely used agent for

ICH fibrinolysis.³⁹ However, beyond its beneficial effect of resolving hematoma, many other side effects, such as the proedema, proinflammatory, and proneurotoxic effects, have been observed in preclinical trials.^{12,14,21,29,33–35} Thus, the use of one or the other types of fibrinolytics remains empirical. In recent years, urokinase-type plasminogen activator (uPA), an older thrombolytic drug (not available in the United States since 1999), has gradually resurfaced as a possible fibrinolytic treatment.^{13,14} A growing number of clinical research studies have reported that uPA not only increases the rate of clot resolution but also improves patient outcome in ICH fibrinolysis.^{5,8,23,38} Nevertheless, no preclinical or clinical studies have evaluated the differen-

ABBREVIATIONS BBB = blood-brain barrier; EB = Evans blue; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; ICH = intracerebral hemorrhage; IVH = intraventricular hemorrhage; MISTIE = minimally invasive surgery plus tPA for ICH evacuation; MMP = matrix metalloproteinase; NF- κ B = nuclear factor- κ B; PHE = perihematomal edema; RBC = red blood cell; RT-PCR = real-time polymerase chain reaction; tPA = tissue-type plasminogen activator; uPA = urokinase-type plasminogen activator; vWF = von Willebrand factor.

SUBMITTED September 29, 2015. **ACCEPTED** January 29, 2016.

INCLUDE WHEN CITING Published online April 22, 2016; DOI: 10.3171/2016.1.JNS152287.

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tial effect of these 2 agents for fibrinolytic therapy following ICH. We hypothesized that uPA could provide greater benefits than tPA when used for ICH fibrinolysis. Thus, in the present study, we decided to compare the efficacy of these 2 fibrinolytics in an ICH model in rats.

Methods

Animals

One hundred thirty adult male Sprague-Dawley rats weighing 250–350 g were used in this study (Third Military Medical University). Animals were housed under controlled, pathogen-free conditions with free access to pellet food and water. Surgical procedures were performed aseptically, and standard microsurgical techniques were used with an operating microscope. At the study end point, while under deep anesthesia, animals were killed using an overdose of intraperitoneal pentobarbital. All efforts were made to minimize suffering and animal numbers, according to the *Guide for the Care and Use of Laboratory Animals*, and the study protocol was approved by the Animal Care and Use Committee at the Third Military Medical University.

Surgical Procedures

To induce ICH, animals were anesthetized with pentobarbital (40 mg/kg intraperitoneal), and the right femoral artery was catheterized to monitor arterial blood pressure, blood pH, PaO₂, PaCO₂, and glucose levels 1 hour before and 6 hours after ICH (Table 1). A feedback-controlled heating pad was used to maintain body temperature at 37°C. A cranial bur hole (1 mm) was drilled, and a 29-gauge needle was inserted stereotactically into the right caudate nucleus (coordinates: 0.2 mm anterior, 5.5 mm ventral, and 3.5 mm lateral to the bregma). In total, 100 µl of autologous arterial blood was infused at a rate of 10 µl/minute by using a microinfusion pump in each rat. The sham groups required only a needle injection into the right caudate nucleus.

Experimental Groups and Fibrinolytic Therapy

There were 2 parts to this study. First, 70 rats were randomly divided into 5 groups of 14 rats each: sham, ICH, ICH+saline, ICH+tPA, and ICH+uPA. Thirty minutes after hematoma placement, 2 µl of saline, 2 µl of tPA (10 µg/µl, Actilyse, Boehringer Ingelheim International), or 2 µl of uPA (100 IU/µl, urokinase for injection, No. H44020647, approved by state, Livzon Pharmaceutical Group Co.) was injected through an intracerebral catheter with a microinfusion pump, into the core of the hematoma. In the present study, the dose regimen of tPA (20 µg) was used, as described in a previous study²⁸ of a Wistar rat model of ICH in which Wistar rats received a 50-µl blood injection and a subsequent 12-µg recombinant tPA (rtPA) treatment. For uPA, no dose study in rat ICH fibrinolysis has been reported as yet. In a preclinical study of intraventricular hemorrhage (IVH) fibrinolysis, Gaberel et al. reviewed the relevant literature and proposed that 10,000 IU of uPA is equivalent to 1 mg of tPA.¹⁴ Thus, 200 IU of uPA was selected as an amount comparable to 20 µg of tPA in our study. Furthermore, according to the proportion between hematoma size and drug dosage, the established

TABLE 1. Physiological parameters of rat 1 hour before and 6 hours after ICH

Variable	Before ICH	ICH+tPA	ICH+uPA
No. of rats	4	4	4
MABP (mm Hg)	105.6 ± 7.3	109.4 ± 9.8	110.2 ± 11.5
pH	7.12 ± 0.07	7.24 ± 0.09	7.21 ± 0.08
PaO ₂ (mm Hg)	114.6 ± 9.4	108.5 ± 10.7	113.1 ± 12.4
PaCO ₂ (mm Hg)	45.2 ± 5.1	46.3 ± 3.2	47.5 ± 4.8
Glucose (mg/dl)	197.1 ± 15.4	212.3 ± 15.9	217.3 ± 17.5

MABP = mean arterial blood pressure.

dose of tPA and uPA are equivalent to the dosage in ICH patients.^{8,23,26} To avoid additional injury to the normal perihematomal brain tissue during intraclot drainage, we used fibrinolytic injection alone rather than fibrinolytic injection with intraclot drainage. The bur hole was sealed with bone wax, and the skin incision was closed with sutures after the needle was removed. Animals were allowed to recover in the conditions described above until the testing phase. All rats were killed on Day 3 after ICH for brain water content (8 rats/group) and Evans blue (EB) fluorescence (6 rats/group) after performing MRI and behavioral tests.

Second, 54 rats were randomly divided into 3 groups of 18 rats each: ICH+saline, ICH+tPA, or ICH+uPA. After fibrinolysis, as described above, all rats were killed on Day 3 after ICH for Western blot (9 rats/group: 4 for matrix metalloproteinases [MMPs] and tight junction protein ZO-1 and 5 for nuclear factor [NF]-κB), real-time polymerase chain reaction (RT-PCR; 4 rats/group) assay, and immunofluorescence (5 rats/group). A total of 6 rats died within 24 hours of the injection of autologous arterial blood.

Magnetic Resonance Imaging and Volume Measurement

Rats were anesthetized with a 2% isoflurane/air mixture throughout the MRI examination performed on Day 3 after ICH. Magnetic resonance imaging was performed in a 7.0-T Varian MR scanner (Bruker Corp.) with a T2 fast spin-echo sequence using an FOV 35 × 35 mm and 17 coronal slices (1.0-mm thickness). Volumes were calculated as previously described.^{6,10} Hematoma and PHE were outlined, and the areas were measured. Volumes were assessed by calculating the areas of all slices and multiplying by the section thickness. All image analyses were performed by 2 observers blinded to the rat treatment factors by using ImageJ (National Institutes of Health).

Brain Water Content

Three days after ICH and MRI, rats were killed. Brains were removed and divided into 2 hemispheres and the cerebellum, as previously described.¹¹ The wet weights were determined immediately. Then, the brain sections were dried at 100°C for 24 hours before obtaining the dry weights. The percentage of water content was calculated as follows: (wet weight – dry weight)/wet weight × 100%. The percentage of water content was calculated by 2 observers blinded to the rat treatment factors.

Evans Blue Fluorescence

Vascular permeability was determined by measuring

the fluorescence intensity of EB dye (Aladdin US) extravasation 3 days after blood infusion. Rats were injected with 2% EB dye (5 ml/kg) via the tail vein 60 minutes before perfusion and brain removal, as previously described.⁶ Then, the brains were removed in preparation for coronal brain sections. Red autofluorescence of EB was observed on the slides, as previously described.^{9,22} The mean red autofluorescence of EB was evaluated by 2 observers blinded to rat treatment factors.

Corner Turn Test

The corner turn test was performed 3 days after ICH and before MRI, as described previously.¹⁸ Briefly, the rat was allowed to proceed into a corner, whose angle was 30°. To exit the corner, the rat could turn to either the left or the right, and that direction was recorded. The test was repeated 10–15 times, with at least 30 seconds between trials, and the percentage of right turns was calculated. The mean neurological score was evaluated by 2 blinded observers.

Forelimb Placing Test

The forelimb-placing test was performed 3 days after ICH and before MRI, as described previously.¹⁸ Briefly, each rat was tested 10 times for each forelimb, and the percentage of trials in which the rat placed the appropriate forelimb on the edge of the countertop in response to vibrissae stimulation was determined. Testers were highly experienced and blinded to the condition of the animal. The mean neurological score was evaluated by 2 blinded observers.

Real-Time PCR

The PCR was performed and analyzed, as previously described.³⁰ For RT-PCR MMP gene expression analysis, rats (4/group) were sacrificed by decapitation 3 days after ICH. The brains were then dissected 2 mm anteriorly and 2 mm posteriorly to the needle entry site (easily identifiable on the brain surface) and were divided into separate hemispheres along the midline. Next, the striatum on each side of the brain was separated from the surrounding white matter and cortex and was used for RNA extraction. In the ICH animals, this area included most of the hematoma and a surrounding region of striatal tissue. Primers were designed with the Primer3 Output program (<http://primer3.ut.ee>; Table 2). Total RNA was extracted using TRIzol reagent (Invitrogen). A positive standard curve for each primer was obtained using a serially diluted cDNA (complementary DNA) sample mixture. Gene expression was quantified with standard samples and normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The data are expressed as normalized mRNA expression (fold mRNA [messenger RNA] increase). All data analyses were performed in a blinded manner by 2 observers.

Western Blot Analysis

Western blot analysis was performed, as previously described.^{16,31} The brains were perfused with saline before decapitation on Day 3 after injection. The perihematomal brain tissue (4-mm-thick brain tissue around the hema-

TABLE 2. Primers used for RT-PCR

Gene & GenBank Accession No.	Primer Sequences
MMP-2	
NM031054	AGAAGGCTGTGTTCTTCGCA (FP)
	AAAGGCAGCGTCTACTTGCT (RP)
MMP-9	
NM031055	CTGCCTGCACCACTAAAGG (FP)
	GAAGACGAAGGGGAAGACG (RP)
MMP-12	
NM053963	CTGGGCAACTGGACACCT (FP)
	CTACATCCGCACGCTTCA (RP)

FP = forward primer; RP = reverse primer.

toma) was sampled. The primary antibodies included anti-MMP-2 (1:1000, CST), anti-MMP-12 (1:1000, Abcam), anti-ZO-1 (1:500, Santa Cruz), anti-NF- κ B p65 (1:1000, CST), anti-phospho-NF- κ B p65 (1:1000, CST), anti-GAPDH (1:1000, Santa Cruz), anti- β -actin (1:1000, Beyotime). The relative densities of the bands were analyzed using NIH ImageJ software. All data analyses were performed in a blinded manner by 2 observers.

Immunofluorescence

Immunofluorescence staining of brain tissue was performed on fixed frozen sections, as previously described.⁶ Rats were anesthetized with pentobarbital (100 mg/kg intraperitoneal) and perfused with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (pH 7.4) on Day 3 after ICH. The brains were removed and kept in 4% paraformaldehyde for 4–6 hours and then immersed in 30% sucrose for 3–4 days at 4°C. The brains were embedded in an optimal cutting temperature compound (Sakura Finetek USA), and 18-mm-thick slices were cut using a cryostat. The following primary antibodies were used: goat anti-ZO-1 (1:200, Santa Cruz), mouse anti-von Willebrand factor (vWF; 1:200, Santa Cruz), and mouse anti-claudin-5 (1:200, Invitrogen). Appropriate secondary antibodies were incubated with the specimens for 3 hours at 37°C. Immunofluorescence was examined using a confocal fluorescence microscope (LSM780, Zeiss).

Statistical Analysis

Sample size estimations were made by power analysis using a Type I error rate of 0.05 and a power of 0.8 on a 2-sided test. Results are expressed as the means $\pm$ standard deviation. For each type of experiment, data were obtained from at least 3 independent measurements. Statistical analysis of the results was conducted using a 1-way ANOVA, followed by the least significant difference test or Newman-Keuls test. Differences were considered significant at $p < 0.05$. All data were strictly analyzed in a blinded manner by 2 observers.

Results

Effect of tPA and uPA on Hematoma Resolution and Brain Edema Following ICH

Three days after ICH, although both tPA and uPA treat-

ment reduced the volumes of PHE ($p < 0.01$ vs vehicle; Fig. 1A and B) and hematoma ($p < 0.05$ vs vehicle; Fig. 1C), uPA was more effective in reducing PHE (7.42 ± 3.31 vs 12.15 ± 4.64 mm³, $p < 0.05$ vs tPA). In addition, significantly decreased brain water content in the ipsilateral hemisphere was observed in the uPA group (79.84 ± 0.41 vs 80.89 ± 0.68 mm³, $p < 0.05$ vs vehicle; Fig. 1D) rather than the tPA group ($p > 0.05$ vs vehicle).

Effect of tPA and uPA on Blood-Brain Barrier Protection and Neurological Recovery After ICH

In comparison with tPA, uPA therapy more significantly attenuated blood-brain barrier (BBB) injury and promoted functional recovery on Day 3 following ICH. Quantification of perivascular EB fluorescence revealed that uPA, in comparison with tPA, more effectively prevented BBB destruction and decreased EB extravasation from the vasculature (uPA 69.34 ± 22.62 mm³ vs tPA 87.31 ± 26.78 mm³, $p < 0.05$; Fig. 2A and B). During the behavior tests on Day 3 post-ICH thrombolytic therapy, the tPA group had a trend toward a higher neurological score than did the vehicle group, but there was no significant difference (tPA vs vehicle, $p > 0.05$; Fig. 2C and D); the uPA group had a lower corner turn score (uPA 69.82 ± 23.95 vs vehicle 90.49 ± 21.54 , $p < 0.05$) and higher forelimb placing score (uPA 49.24 ± 28.17 vs vehicle 17.13 ± 13.80 , $p < 0.05$) than did the control groups.

Effect of tPA and uPA on MMP and BBB Tight Junction Protein Level Regulation After ICH

The uPA therapy more effectively upregulated BBB tight junction protein expression than did tPA, which was partly attributed to the different effects of uPA and tPA on the regulation of MMPs and related mRNA expression after ICH. As shown in Fig. 3A, both uPA and tPA effectively decreased the MMP-12 mRNA expression ($p < 0.05$ vs vehicle) on Day 3 after ICH. However, only uPA significantly reduced the MMP-2 mRNA expression level (uPA 0.87 ± 0.05 vs vehicle 1.03 ± 0.11 , $p < 0.05$). In addition, more remarkable MMP-9 mRNA expression was observed following treatment with tPA in comparison with uPA (tPA 1.03 ± 0.23 vs 0.87 ± 0.29 uPA, $p < 0.05$). Moreover, the corresponding Western blotting assays revealed trends in MMP expression levels similar to those in the mRNA assays (Fig. 3B). Despite the increased MMP-9 expression after ICH thrombolysis, both uPA and tPA effectively protected ZO-1 ($p < 0.05$ and 0.01 , respectively, vs vehicle; Fig. 4A and B) and claudin-5 ($p < 0.05$ and 0.01 , respectively, vs vehicle; Fig. 4C–E) from degeneration. In particular, uPA had a more prominent effect ($p < 0.05$, uPA vs tPA) in these processes.

Effect of tPA and uPA on NF- κ B Pathway Activation After ICH

To study the role of tPA and uPA on NF- κ B pathway

FIG. 1. Effect of tPA and uPA on hematoma evacuation and brain edema following ICH. **A:** Representative T2-weighted images obtained on Day 3 after ICH. Arrows denote the PHE area (high signal zone), and asterisks denote the ICH. Quantification of volumes of PHE (**B**) and hematoma (**C**) according to the related T2-weighted images (14 rats/group). Brain water content determination (**D**) in ipsilateral hemisphere 3 days after ICH (8 rats/group). Results are presented as the means $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$. NS = no significance; T2WI 3d = T2-weighted imaging Day 3 after ICH. Figure is available in color online only.

FIG. 2. Effect of tPA and uPA on BBB protection and neurological recovery after ICH. **A:** Representative confocal images display the perivascular EB dye leakage from vessels of the ipsilateral cortex. Each dashed white square represents the respective magnified inset. Asterisk in the brain section denotes the intracerebral hematoma; the solid white square in the brain section denotes the PHE. **B:** Quantification of perivascular EB fluorescence on Day 3 after ICH (6 rats/group). **C:** Results of corner turn test on Day 3 after post-ICH thrombolytic therapy (14 rats/group). **D:** Results of forelimb placing test on Day 3 after post-ICH thrombolytic therapy. Results presented as the means $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$. Figure is available in color online only.

activation after ICH, we performed Western blot analysis for phospho-p65 and total p65 (Fig. 5A). Although there was no significant difference among vehicle, tPA therapy, and uPA therapy on the expression of p65 (vehicle 1.290 ± 0.4106 , uPA 1.264 ± 0.4662 , tPA 1.325 ± 0.2809 , $p > 0.05$; Fig. 5B), we observed a significant exacerbation of phospho-p65 expression following treatment with tPA rather than with uPA or vehicle (tPA 0.7251 ± 0.2109 vs uPA 0.3991 ± 0.1332 , $p < 0.05$; tPA vs vehicle 0.2429 ± 0.08328 , $p < 0.01$). As phosphorylation of p65 has been seen as a key factor of the canonical NF- κ B pathway,^{15,17} we normalized phospho-p65 to p-65 and quantified the

NF- κ B pathway activation by the relative densities of each band (tPA 0.5474 ± 0.1592 vs uPA 0.3158 ± 0.1054 , $p < 0.05$; tPA vs vehicle 0.1884 ± 0.06457 , $p < 0.01$; Fig. 5C). Together, our data demonstrated that tPA resulted in a significant exacerbation of NF- κ B pathway activation for ICH fibrinolysis.

Discussion

We used a rat model of ICH, followed by intracerebral hematoma fibrinolysis with either uPA or tPA. Although both uPA and tPA displayed similar efficacy in reducing

FIG. 3. Effect of tPA and uPA on MMP and MMP mRNA levels regulation after ICH. **A:** Relative normalized expression levels of MMP mRNAs in the perihematomal area on Day 3 (3d) after ICH (4 rats/group). **B:** Representative Western blot analysis and quantification of the relative density of the band of MMPs (4 rats/group). Results are presented as the means $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$ vs vehicle group, # $p < 0.05$ vs the tPA group.

the hematoma volume, uPA-mediated fibrinolysis alleviated PHE and promoted functional recovery better than did tPA in ICH rats.

Although hematoma evacuation can effectively reduce PHE,²⁶ the surgical evacuation of deep hemorrhages is not always feasible, as sometimes it can require traversing a significant area of normal cerebral tissue. Thus, minimally invasive methods are essential. Minimally invasive surgery plus thrombolysis for ICH represents a minimally traumatic method of evacuating both superficial and deep hemorrhages. Over the last decade, several groups have reported significant results favoring accelerated clot thrombolysis with an acceptable safety profile.^{4,24,42} Recently, the minimally invasive surgery plus rtPA for ICH evacuation (MISTIE) Phase II trial tested the safety and efficacy of hematoma evacuation after ICH and demonstrated a significant reduction in hematoma volume as well as in PHE.²⁶ In agreement with results in the aforementioned clinical trials, we also observed a significant reduction in hematoma and PHE in rat models of ICH after thrombolytic therapy in the present study. Moreover, we exposed these thrombolysis-treated animals to behavioral tests and found a remarkable recovery of motor function, which is, to our knowledge, the first time the behavioral tests have been conducted. At present, the MISTIE Phase III trial is

under way to evaluate whether the reduction in hematoma size and edema translates to a reduced mortality and improved functional outcome.

During thrombolytic therapy in ICH rats in our study, without any attempts to aspirate the clot or the lysed material, as has been described elsewhere,²⁷ the fibrinolytics injection successfully accelerated intracerebral hematoma resolution. Although relatively little is known about the mechanisms involved in hematoma resolution and how they are regulated, phagocytosis of red blood cells (RBCs) by microglia and/or infiltrating macrophages has also been suggested to have a role in resolution.^{40,41} In this study, thrombolytics penetrated the clot and degraded the fibrin, which may produce more room for interactions between microglia and/or infiltrating macrophages and free RBCs. Thus, the greater RBC phagocytosis by macrophages in the thrombolytic group, in comparison with the control group, may contribute to accelerated clot evacuation. Future studies will need to delineate the mechanisms involved in this phenomenon. Because enhancing the endogenous mechanisms related to hematoma resolution is an alternative strategy in ICH treatment, it can be used to overcome the brain trauma associated with surgical clot removal.³⁹ The blood injection model used in the present study may not be the best model to assess the effects of

FIG. 4. Effect of tPA and uPA on BBB tight junction protein expression regulation after ICH. **A:** Representative photographs of immunofluorescence staining (arrows) for BBB tight junction protein (ZO-1, green) expression in endothelia (vWF, red) in the perihematomal area 3 days following ICH (5 rats/group). Western blot assay (**B**) for the ZO-1 level in the perihematomal area on Day 3 after ICH (4 rats/group). Immunofluorescence staining for claudin-5 (**C**) and relative fluorescence intensity assay to control group in the ipsilateral cortex (**D**) and perihematomal region (**E**, 5 rats/group). * $p < 0.05$, ** $p < 0.01$ vs the vehicle group, # $p < 0.05$ vs the tPA group. Bar = 10 μ m (**A**), 20 μ m (**C**). Figure is available in color online only.

fibrinolytics (especially without aspiration) on hematoma volume reduction, and other types of models are required in the future.

As noted above, many researchers have reported reduced PHE after tPA thrombolysis and aspiration either in large animal models or in humans with ICH.^{26,28,32,37,42} However, it should be noted that the adverse effects of tPA are still under debate. Preclinical studies have reported that tPA can have neurotoxic effects by inducing inflammation, disrupting the BBB, causing neuronal degeneration, and exacerbating cerebral edema.^{12,14,21,29,34,35} A recent

meta-analysis conducted by Gaberel et al. has suggested that uPA, an alternate thrombolytic, is superior to tPA for IVH clot evacuation.¹³ Subsequently, Gaberel et al. designed a preclinical experiment to evaluate the differential impact of uPA versus tPA in a rat model of IVH and found that only uPA significantly improved functional recovery.¹⁴ These findings provide further evidence that uPA is more efficacious than tPA for fibrinolysis in IVH. Furthermore, an increasing number of clinical studies have proposed that uPA carries a high fibrinolytic potential without excitotoxicity, thus representing a novel, promising candidate

FIG. 5. Effect of tPA and uPA on NF- κ B pathway activation after ICH. Representative Western blot analysis (**A**) and quantification of the relative density (**B**) of the band of total p65 and phospho-p65 (p-p65) in the perihematomal area on Day 3 after ICH. The p-p65 protein expression levels (**C**) were normalized to the p65 protein levels and quantification of the relative densities of each band (5 rats/group). * $p < 0.05$, ** $p < 0.01$ vs the vehicle group, # $p < 0.05$ vs the tPA group.

for fibrinolytic therapy for ICH.^{5,8,23,38} In agreement with the above publications, our results also revealed the greater efficacy of uPA, compared with tPA, in ameliorating PHE and motor function after ICH.

Claudin-5 and ZO-1 are important components of the BBB that maintain the normal function of the central nervous system. Matrix metalloproteinases are involved in the degradation of these proteins, thereby resulting in BBB disruption after ICH.² In our study, we observed the elevated expression of claudin-5 and ZO-1 (Fig. 4) following treatment with uPA and tPA in comparison with vehicle, which may be partly explained as more effectively down-regulating MMP (MMP-2 and MMP-12; Fig. 3) expression in the 2 fibrinolytic groups. The EB extravasation test also demonstrated that the fibrinolytic groups protected BBB integrity and that the uPA was more effective (Fig. 2A and B). Meanwhile, we found that tPA increased MMP-9 expression more than uPA and vehicle did. Note that MMP-9 has been implicated in ICH¹ and that tPA has been shown to activate MMPs through different mechanisms in which the NF- κ B pathway has a central role.^{3,19} Therefore, we hypothesized that tPA and uPA could result in the differential expression of MMP-9 via the differential effects on the NF- κ B pathway, thereby causing a different extent of BBB disruption. Then, we evaluated this hypothesis by Western blot analysis for NF- κ B activation and found that tPA showed a more remarkable activation of the NF- κ B pathway than did uPA (Fig. 5) for ICH fibrinolysis, which may partly explain the reduced MMP-9 expression and improved BBB integrity in the uPA group.

In summary, both tPA and uPA protected the BBB by downregulating the MMPs, in which uPA was shown to be more efficacious by reducing activation of the NF- κ B pathway. Because of limited evidence, more studies are needed to further validate the efficacy and safety of uPA for ICH fibrinolysis in larger preclinical research studies and clinical trials, particularly its comparison with tPA.

Conclusions

In the present study, we compared the use of 2 fibrinolytics, namely uPA versus tPA, in a rat ICH model. Our results showed that, despite showing a similar effect on hematoma reduction, uPA treatment better ameliorated PHE and improved the outcome following ICH than did tPA. Thus, uPA may be a promising candidate for fibrinolytic therapy for ICH.

Acknowledgments

We thank Dr. Fu Peishu from the pharmaceutical department of Southwest Hospital, Chongqing, for her diligent guidance in using the uPA and tPA during the whole study process. We are also grateful for the support from the Collaboration Innovation Center for Basic Science of China. This work was supported by Grant Nos. 81571130 (Z.C.), 81271281 (Z.C.), and 81371440 (Yang Yunfeng) from the National Natural Science Foundation of China and Grant No. 2014CB541606 (H.F.) from the National Key Basic Research Development Program (973 Program) of China.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Z Chen, Q Chen. Analysis and interpretation of data: Tan, Q Chen, Tao, Tang. Drafting the article: Tan, Q Chen. Critically revising the article: Z Chen, Tan, Niu, Tao, Yang, Guo, Zhu. Reviewed submitted version of manuscript: Z Chen, Li, H Feng, Zhu. Approved the final version of the manuscript on behalf of all authors: Z Chen. Statistical analysis: Z Feng, Li, Tang. Administrative/technical/material support: Tan, Niu, Z Feng, Yang, Guo, H Feng. Study supervision: Z Chen, H Feng.

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