Microplasmin and Tissue Plasminogen Activator:

Comparison of Therapeutic Effects in Rat Stroke Model at Multiparametric MR Imaging¹

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Purpose:

To prospectively compare therapeutic and hemorrhagic effects of microplasmin and tissue plasminogen activator (tPA) in stroke therapy by using multiparametric magnetic resonance (MR) imaging in a photothrombotic rat stroke model.

Materials and Methods:

The animal experiment complied with institutional regulations for laboratory animals. Stroke was induced in rats with photothrombotic occlusion of middle cerebral artery (MCA). T2-weighted, perfusion-weighted (PW), and diffusion-weighted (DW) MR imaging was performed 1 hour and 24 hours after occlusion. On the basis of PW and DW images at 1 hour, 49 rats with cortex and subcortex involvement and with perfusion-diffusion mismatch were randomly assigned into one of four groups: control group, group treated with 7.5 mg/kg microplasmin, group treated with 10 mg/kg microplasmin, or group treated with 10 mg/kg tPA. Agents were intravenously injected 1.5 hours after occlusion. Infarct size and hemorrhagic transformation were assessed with MR imaging and histomorphologic findings. Neurologic deficit was scored. Measurements were statistically analyzed.

Results:

There were 13 rats in the control group, 13 in the 7.5 mg/kg microplasmin group, nine in the 10 mg/kg microplasmin group, and 14 in the 10 mg/kg tPA group. Despite similar baseline perfusion-diffusion mismatch, histochemically defined total infarct volume was reduced from $25\% \pm 5$ (standard deviation) in control group to $21\% \pm 2$, $20\% \pm 4$, and $20\% \pm 5$ in 7.5 mg/kg microplasmin, 10 mg/kg microplasmin, and tPA groups, respectively, as similarly shown on T2-weighted, DW, and PW images at 24 hours ($P < .05$). Cerebral hemorrhage rate at 24 hours was higher in tPA group than in the other three groups. Bederson score of neurologic deficits was significantly reduced in treated groups compared with that in control group.

Conclusion:

Perfusion-diffusion mismatch appeared useful in selecting candidates for thrombolytic therapy. Multiparametric MR imaging allowed noninvasive assessment of effects of microplasmin and tPA in rats; microplasmin had a significantly lower hemorrhagic rate.

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Tissue plasminogen activator (tPA) has shown therapeutic effects for the treatment of acute ischemic stroke in both animals (1,2) and patients (3,4). Results of some studies (5–7) indicate that tPA may increase the risk of hemorrhagic transformation, especially in cases of blood-brain barrier perturbation before tPA administration (5,6) and in cases of delayed (4–6 hours) tPA treatment (5,8). In patients who received tPA, the incidence of symptomatic hemorrhage showed a 10-fold increase compared with that in a placebo group (3,9); however, there is still controversy about the adverse effects of tPA (10,11). Therefore, efforts have been made to develop new thrombolytic agents that may improve the benefit-to-risk ratio in the management of stroke.

Microplasmin, a truncated form of plasmin, has been developed as a new therapeutic agent that combines a direct thrombolytic activity with a neuroprotective property (12,13). Results of previous studies have shown that microplasmin reduces brain tissue damage in stroke models of the mouse (14) and the rat (15). However, to our knowledge, direct comparison of tPA and microplasmin for their therapeutic effects in rats, the most commonly used species in cerebral ischemic studies, has not been reported.

In the treatment of acute stroke, thrombolytic agents target the brain tissue at risk (or the ischemic penumbra) that has been clinically defined as the ischemic, but still viable and salvageable, tissue if local perfusion is rapidly restored (16,17).

Multiparametric magnetic resonance (MR) imaging, including perfusion-

weighted (PW) and diffusion-weighted (DW) imaging, has been widely applied for guiding and monitoring treatment in hyperacute stroke (2,8,9,11,16,18–20). The mismatch between relative lesion volume (rLV) on PW source images and that on apparent diffusion coefficient (ADC) maps (perfusion-diffusion mismatch) provides a practical and approximate measure of the tissue at risk and has been increasingly used for the evaluation of acute stroke. Therefore, the purpose of our study was to prospectively compare the therapeutic and hemorrhagic effects of microplasmin and tPA in stroke therapy by using multiparametric MR imaging in a photothrombotic stroke model of rats.

Materials and Methods

Animal Model

Our animal experiment complied with the guidelines of the International Committee on Thrombosis and Hemostasis (21) and our institutional regulations for the use of laboratory animals. Male Sprague-Dawley rats (JAMVIER, Leuven, Belgium) weighing 220–350 g were initially anesthetized with inhalation of 4% halothane (Zeneca, Destelbergen, Belgium) for 3 minutes; anesthesia was maintained with 2% halothane in a mixture of 20% oxygen and 80% room air. Body temperature was kept at $37.5^{\circ}\text{C} \pm 0.5$ (standard deviation) with a heating pad (TR-100; First Sensor Technology, Munich, Germany) during surgery. The middle cerebral artery (MCA) was occluded by using a photothrombotic approach, as described in detail elsewhere (19,20,22). A plastic catheter was placed in the right femoral vein for injection of the photosensitizer rose bengal, test drugs, and contrast agent.

Implication for Patient Care

■ Microplasmin may serve as a potential alternative to tissue plasminogen activator in the treatment of patients with ischemic stroke, with comparable effects and lower hemorrhage rate.

MR Imaging

MR imaging was performed with a 1.5-T unit (Sonata; Siemens, Erlangen, Germany) by using a commercially available 4-channel phased-array wrist coil. Rats were placed supinely into a plastic holder and were anesthetized with 2% isoflurane (Forene; Abbott, Louvain-la-Neuve, Belgium) by using the same breathing anesthesia system used at surgery. For each imaging sequence, three pilot images (sagittal, coronal, and transverse) were obtained first; subsequently, 12 coronal images were acquired with a section thickness of 2 mm and intersection gap of 0.2 mm.

Turbo spin-echo T2-weighted MR imaging was performed with a repetition time msec/echo time msec of 5860/100, field of view of 55×31 mm, and acquisition matrix size of 256×146 . DW MR imaging (3000/80) was performed by using a two-dimensional spin-echo echo-planar sequence with a field of view of 140×70 mm and an acquisition matrix size of 192×96 , resulting in an in-plane resolution of 0.7×0.7 mm. A parallel imaging technique—namely, a generalized autocalibrating partially parallel acquisition (GRAPPA; Siemens) with

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Abbreviations:

ADC = apparent diffusion coefficient
 DW = diffusion weighted
 MCA = middle cerebral artery
 PW = perfusion weighted
 rCBF = relative cerebral blood flow
 rCBV = relative cerebral blood volume
 rLV = relative lesion volume
 tPA = tissue plasminogen activator
 TTC = triphenyltetrazolium chloride

Author contributions:

Guarantors of integrity of entire study, F.C., N.N., G.M., Y.N.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, F.C., Y.S., N.N., Y.N.; experimental studies, F.C., Y.S., N.N., X.S., H.W., J.Y., Y.N.; statistical analysis, F.C., Y.S., N.N., H.W., Y.N.; and manuscript editing, F.C., Y.N.

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Advances in Knowledge

- Multiparametric MR imaging with a clinical imager can be applied for noninvasive therapeutic monitoring in rats with stroke.
- Microplasmin appears to be an alternative to tissue plasminogen activator for ischemic stroke treatment because of the significantly lower hemorrhage rate.

an acceleration factor of two was applied. ADC maps were calculated on the basis of three b values (0, 500, and 1000 sec/mm²). Dynamic susceptibility contrast material-enhanced PW MR imaging was performed by using a T2*-weighted (2000/40) echo-planar sequence in combination with the GRAPPA technique (Siemens) to increase spatial homogeneity. A dynamic image series of 100 measurements (12 images per measurement and 1200 images in total) resulted in an imaging time of 3 minutes 34 seconds, with a field of view of 156 × 43.5 mm and an image acquisition matrix size of 128 × 64, which led to an in-plane resolution of 0.7 × 0.7 mm. During the dynamic series, bolus injection of contrast material (gadodiamide, Omniscan; Amersham, Oslo, Norway) at a dose of 0.3 mmol per kilogram of body weight was started after the 30th imaging acquisition to ensure sufficient pre-contrast baseline images. The entire MR imaging protocol typically lasted 20 minutes for each animal at each time point. All MR imaging sequences were performed 1 hour and 24 hours after MCA occlusion.

Study Design and Procedure

This animal experiment had a randomized, blinded, and placebo-controlled study design. Animals with cortex involvement on PW source images at the central coronal section or the sixth section from the tentorium of the cerebellum and with perfusion-diffusion mismatch were entered into the study with the consensus of three observers (F.C., N.N., and Y.N., with 15, 4, and 16 years of experience, respectively) on the basis of visual evaluation, as previously described and validated in detail (15,19). The qualified rats were randomly assigned to one of the following treatment groups with blinded labels: a control group treated with solvent, a group treated with 7.5 mg/kg microplasmin, a group treated with 10 mg/kg microplasmin, and a group treated with 10 mg/kg tPA.

Both microplasmin and tPA were dissolved in saline with 33 mmol/L mannitol and 5 mmol/L citric acid (pH = 3.1). The control agent was prepared

with the same solvents adjusted at equal quantity and pH (14,23). With blinded labels, 0.2 mL of each group's agent was intravenously administered in rats 1.5 hours after MCA occlusion by using a single bolus. No heparin was given.

Before sacrifice, neurologic deficits of the rats were assessed (Y.S., with 10 years of experience) by using the ranking method of Bederson et al (24). After we tested wrist and elbow flexion and postural reflex and circling for determining paw dysfunction, hemiparesis, and obtundation, each score was summed and represented as a single neurologic score (0–10).

Histochemical Staining and Histologic Examination

After in vivo studies, animals were euthanized with overdose of pentobarbital (Nembutal; Sanofi Sante Animale, Brussels, Belgium). Brains were excised and sliced into six coronal slices of 2 mm thickness from the anterior 3.5–13.5 mm by using a brain matrix (Agar Scientific, Stansted, England). After hemorrhage was registered, slices were incubated at 37°C in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 15 minutes; normal brain tissue stained brick red, and cerebral infarct remained pale. The brain slices were subsequently fixed with 10% formalin and processed with hematoxylin-eosin staining for microscopy.

The cortical and subcortical infarct volumes at TTC staining were obtained with image analysis software (Image J, version 1.34s; National Institutes of Health, Bethesda, Md). With cross-referencing TTC staining, microscopic cellular changes, such as neuron necrosis, neuropil vacuolation, and inflammatory infiltration, were observed qualitatively by consensus (J.Y., with 16 years of experience; F.C.; Y.N.).

Image Analysis

Image analysis was performed off-line on a Linux workstation by using dedicated software (Biomap; Novartis, Basel, Switzerland). For quantification of rLV on T2-weighted, DW, and PW images, the areas of the lesion and the entire brain were delineated by three authors in consensus

(F.C., Y.N., Y.S.) with an operator-defined region of interest on all lesion-containing sections. For the delineation of ischemic lesions, ADC maps and PW source images were used because of the clearer lesion demarcation derived with the clinic MR imager. The lesion was contoured as a whole because it was difficult to separate the cortex and subcortex on rat brain images. The software automatically generated the lesion volume (LV) and brain volume (BV) for each animal, from which rLV was calculated as $rLV = (LV/BV) \cdot 100\%$.

The mismatch between rLV values on PW source images and rLV values on ADC maps at 1 hour after MCA occlusion was defined as $[(sPW - ADC)/sPW] > 10\%$, where sPW is PW source image, and was applied to justify animal inclusion on the basis of visual evaluation of PW and DW images before treatment.

The signal intensity changes on DW images and ADC maps were observed, and the relative ADC value of the total ischemic lesion (lesion ADC value divided by contralateral ADC value) on three representative sections was quantified at 24 hours to reflect the degree of cellular damage.

Software built into the MR unit (Siemens) was used to generate PW-derived parametric maps. A 25-mm² region of interest containing 49 pixels of 0.72 × 0.72 mm was placed on the region contralateral to the region that contained the lesion, which included the origin of the right MCA to allow measurement of the arterial input function, from which the pixels representing the right MCA branch were selected (20) with consensus of three authors (F.C., Y.N., Y.S.).

Relative cerebral blood volume (rCBV) and relative cerebral blood flow (rCBF) values were derived as described by the following steps: (a) one central lesion-containing section was chosen from PW source images, which provided a clearer image than PW-derived maps and enabled separate delineation of the cortex and subcortex; (b) the cortical and subcortical regions of interest thus obtained from images of the lesional and contralateral brain were transferred to rCBF and

rCBV maps; (c) readouts of rCBF and rCBV were automatically generated by the software; and (d) the percentage of lesional rCBF and rCBV in comparison to those in the contralateral normal sides was calculated (20).

To assess the extravasation of contrast agent at MR imaging as an in vivo indicator of disintegration of the blood-brain barrier, the spatial-temporal changes in $T2^*$ relaxation rate (ie, $\Delta R2^*$) on dynamic susceptibility contrast-enhanced PW images in the region of cerebral ischemia were calculated as follows: $\Delta R2^*(t) = \Delta 1/T2^*(t) = -\ln[SI(t)/SI_0]/TE$, where $SI(t)$ and SI_0 are signal intensities at time t and before injection of contrast agent, respectively (5), and TE is echo time in milliseconds.

The multifocal cerebral hemorrhages on macroscopic brain slices were visually determined by consensus (N.N., Y.N., Y.S.). The rate of hemorrhage (as a percentage) was calculated as the number of hemorrhagic cases divided by the total number of cases in each group. The volume of hemorrhage was not measured because of technical constraints at both MR imaging and histomorphologic analysis.

Statistical Analysis

Parametric data were reported as means $\pm$ standard deviations, and nonparametric data were expressed as medians and ranges. Statistical analysis was performed with software (SPSS for Windows, release 13.0; SPSS, Chicago, Ill). A general linear model of univariate

test was applied for global comparisons between groups and techniques, followed by least significant difference tests for multiple comparisons of lesion size and ratio. For comparisons of MR imaging measurements between 1 hour and 24 hours, repeat analysis of variance was performed. The χ^2 test was performed to compare rates of hemorrhage occurrence, and the Mann-Whitney U test was performed to compare behavior scores between groups. A P value of less than .05 was considered to indicate a significant difference. Adequate statistical power was ensured for our study by increasing the number of cases in each group in comparison with that in one of our previous studies, which showed a calculated statistical power of 0.90 (15).

Results

General Aspects

In total, 66 rats underwent MCA occlusion. Fourteen rats were excluded prior to randomization according to the selection criteria because of the absence of perfusion-diffusion mismatch at 1 hour; three rats died during the experiment. Finally, 49 rats qualified for our study. After data collection and analyses were completed, the actual treatment for each individual rat was unblinded. The distribution of animals among the groups was as follows: 13 rats in the control group, 13 in the 7.5 mg/kg microplasmin group, nine in the 10 mg/kg micro-

plasmin group, and 14 in the 10 mg/kg tPA group. For logistic reasons with the imager, MR imaging at 24 hours was not performed in eight of 49 rats (ie, two in each group).

Neurologic Symptom Scores

At 24 hours, all animals in the control group exhibited neurologic impairment, with a median Bederson score of 7 (range, 4–9). For the 7.5 mg/kg microplasmin, 10-mg/kg microplasmin, and 10 mg/kg tPA groups, there was a significant reduction in median score—to 5 (range, 3–8), 4 (range, 3–6), and 4 (range, 3–7), respectively ($P < .05$).

MR Imaging Findings and Perfusion-Diffusion Mismatch at 1 Hour

Lesions on T2-weighted images 1 hour after occlusion were not measured because of the difficulty of delineation. Before treatment, rLV values defined at 1 hour on ADC maps and PW source images, respectively, in the control group ($21\% \pm 4$ and $26\% \pm 5$) were not significantly different from those in the other groups, with rLV values in the 7.5 mg/kg microplasmin group of $22\% \pm 7$ and $25\% \pm 7$; in the 10 mg/kg microplasmin group of $19\% \pm 5$ and $24\% \pm 4$; and in the 10 mg/kg tPA group of $22\% \pm 4$ and $26\% \pm 6$ ($P > .05$).

The value of the perfusion-diffusion mismatch at 1 hour in rats of the control group ($23\% \pm 17$) was also similar to that in rats of both microplasmin-treated groups ($29\% \pm 14$ and $30\% \pm 14$ in 7.5 mg/kg and 10 mg/kg microplasmin groups, respectively) and the tPA-treated group ($22\% \pm 17$) ($P > .05$) (Fig 1).

MR Imaging Findings at 24 Hours

rLV values obtained from T2-weighted images, ADC maps, and PW source images, respectively, at 24 hours in the control group ($27\% \pm 3$, $27\% \pm 3$, and $30\% \pm 5$) were significantly higher than those in the groups treated with 7.5 mg/kg microplasmin ($23\% \pm 3$, $23\% \pm 3$, and $26\% \pm 2$), 10 mg/kg microplasmin ($20\% \pm 4$, $20\% \pm 4$, and $24\% \pm 5$), and 10 mg/kg tPA ($21\% \pm 6$, $21\% \pm 5$, and $24\% \pm 3$) ($P < .05$) (Fig 2).

Visually, an ADC reversion sign was observed on the subcortical lesion in all

Figure 1

Figure 1: Graph shows comparison of mismatch of rLV values on PW source images and those on ADC maps among four groups 1 hour before treatment. ns = No significant difference, μ Pli = microplasmin. Error bars = standard deviations.

animals at 24 hours; this sign appeared as a hyperintense region on ADC maps or a hypointense region with hazily defined margins on DW trace images. The area with ADC reversion always emerged at the core of the subcortical lesion or near the original site of the photochemical injury (Fig 3). Quantitatively, however, there was no significant difference in relative ADC values for the whole ischemic lesion between the control group ($76\% \pm 10$) and the 7.5 mg/kg microplasmin ($79\% \pm 9$), 10 mg/kg microplasmin ($85\% \pm 4$), and 10 mg/kg tPA ($80\% \pm 8$) groups ($P > .05$).

Comparison of MR Imaging Findings at 1 Hour and 24 Hours

rLV values determined with ADC maps between 1 hour and 24 hours altered from $21\% \pm 4$ to $27\% \pm 3$ in the control group, from $22\% \pm 7$ to $23\% \pm 3$ in the 7.5 mg/kg microplasmin group, from $19\% \pm 5$ to $20\% \pm 4$ in the 10 mg/kg microplasmin group, and from $22\% \pm 4$ to $21\% \pm 5$ in the 10 mg/kg tPA group. The increase in rLV values on ADC maps was significantly smaller in the three treated groups than in the control group ($P < .05$) (Fig 4).

rLV values determined on PW source images between 1 hour and 24 hours altered from $26\% \pm 5$ to $30\% \pm 5$ in the control group, from $25\% \pm 7$ to $26\% \pm 2$ in the 7.5 mg/kg microplasmin group, from $24\% \pm 4$ to $24\% \pm 5$ in the 10 mg/kg microplasmin group, and from $26\% \pm 6$ to $24\% \pm 3$ in the 10 mg/kg tPA group. The increase in rLV values on PW source images was significantly smaller in the three treated groups than in the control group ($P < .05$) (Fig 4).

TTC Staining Findings at 24 Hours

rLV values measured at TTC staining at 24 hours in the cortex and the total lesion, respectively, were significantly reduced in the 7.5 mg/kg microplasmin group ($8\% \pm 3$ and $21\% \pm 2$), 10 mg/kg microplasmin group ($7\% \pm 4$ and $20\% \pm 4$), and 10 mg/kg tPA group ($6\% \pm 3$ and $20\% \pm 5$) ($P < .05$) compared with those in the control group ($12\% \pm 5$ and $25\% \pm 5$). For rLV values from the subcortical lesion, there was no significant change

Figure 2

Figure 2: Graphs show rLV at MR imaging and TTC staining 24 hours after MCA occlusion. rLV was significantly reduced on T2-weighted (T2W) turbo spin-echo images, ADC maps, PW source images, and at TTC staining in microplasmin (μ Pli)- and tPA-treated groups compared with that in control group. * = $P < .05$, ** = $P < .01$ versus control group. rLV visualized at TTC staining was not significantly different from that on T2-weighted images, ADC maps, and PW source images in each group. ns = No significant difference ($P > .05$).

between the control group ($13\% \pm 3$) and the three treated groups ($12\% \pm 3$ for the 7.5 mg/kg microplasmin group, $11\% \pm 1$ for the 10 mg/kg microplasmin group, and $12\% \pm 3$ for the 10 mg/kg tPA group) ($P > .05$) (Fig 5).

Comparison of MR Imaging and TTC Staining Results at 24 Hours

Because it was not always feasible to separate the cortex and subcortex on MR images, only the total lesion was compared between MR imaging and TTC staining at 24 hours. rLV values at TTC staining were not significantly different from those on T2-weighted images, ADC maps, and PW source images for each group ($P > .05$) (Fig 2).

Comparison of rCBV and rCBF between 1 Hour and 24 Hours

The rCBV and rCBF of the ischemic lesion in the cortex at 24 hours were significantly increased compared with those at 1 hour for each group ($P < .05$). For the lesion in the subcortex, there were no significant changes in rCBV and rCBF at 24 hours compared with those at 1 hour for each group ($P > .05$), except for a

significant decrease in rCBF in the 7.5 mg/kg microplasmin group ($P < .05$). No significant changes for rCBV and rCBF between 1 hour and 24 hours at the cortex and subcortex were found between the three treated groups and the control group (Fig 6).

Intracerebral Hemorrhage

The incidence of hemorrhage was significantly higher in the tPA-treated group than in the control group and both microplasmin-treated groups ($P < .05$), with the lowest incidence found in the 10 mg/kg microplasmin group (Table).

As an indicator of blood-brain barrier breakdown, the value of $\Delta R2^*$ at 24 hours was significantly higher in the 10 mg/kg tPA group ($0.0014 \text{ sec}^{-1} \pm 0.0011$) than in the 7.5 mg/kg microplasmin group ($0.0005 \text{ sec}^{-1} \pm 0.0004$), the 10-mg/kg microplasmin group ($0.0002 \text{ sec}^{-1} \pm 0.0003$), and the control group ($0.0004 \text{ sec}^{-1} \pm 0.0004$) ($P < .001$). As indicated in Figure 7, the $\Delta R2^*$ curve from the tPA group showed the largest tail compared with that in the three other groups (Fig 7).

Histologic Findings

Results of cross-reference with TTC staining showed that the viable and nonviable areas depicted at micros-

copy were clearly defined in specimens from both the control and the treated groups. In the nonviable region, extensive neuron necrosis was found, with the characteristic appearance of eosinophilia and structural disintegration. Neuropil vacuolation and mild peripheral inflammatory infiltration commonly appeared (Fig 3). Except for the different lesion sizes as defined at TTC staining, no histomorphologic distinctions between the groups could be identified.

Discussion

Our study results demonstrated that both microplasmin and tPA were able to

reduce the cerebral infarct size, as evidenced with in vivo multiparametric MR imaging and postmortem TTC staining, and to improve neurologic deficits in rat stroke models; these results confirmed the results of previous experiments on different animal species (14,15,23,25). In comparison with results in the control group, early administration of microplasmin and tPA significantly attenuated infarct expansion, especially in the cortex, which is in line with previous observations (15). In addition, a higher tendency to intracerebral hemorrhage with tPA treatment than with microplasmin treatment was observed in tissue specimens and was supported with the MR imaging–derived parameter $\Delta R2^*$.

Comparison of Intracerebral Hemorrhage in Four Groups of Rats

Group	No. of Cases
Control (A)	5/13 (38)
7.5 mg/kg Microplasmin (B)	4/13 (31)
10 mg/kg Microplasmin (C)	0/9 (0)
10 mg/kg tPA (D)	11/14 (79)

Note.—Data in parentheses are percentages. *P* values between groups are as follows: A vs B, .593; A vs C, .039; A vs D, .038; B vs C, .084; B vs D, .009; and C vs D, .000.

Figure 3

Figure 3: MR findings on coronal PW source images (2000/40), DW images (3000/80), ADC maps (*b* value, 1000 sec/mm²), and T2-weighted (T2W) (5860/100) images and results of TTC staining in control group (first row), 10 mg/kg microplasmin (*μPli*) group (second row), and 10 mg/kg tPA group (third row), with corresponding histologic findings (fourth row). In control group, 1 hour after MCA occlusion, ischemic lesion was virtually invisible on T2-weighted image and could be defined on DW image and ADC map but was well delineated as hypoperfused area on first-pass PW source image involving cortex and subcortex. At 24 hours, ischemic lesion was expanded and final infarction involved cortex and subcortex, as verified at TTC staining. ADC reversion (arrows) occurred at subcortical lesion on DW image and ADC map. In microplasmin and tPA groups, at 1 hour after MCA occlusion, ischemic lesion was similar to that on T2-weighted images, DW images, ADC maps, and PW source images in control group. At 24 hours, expansion of infarction was much less on MR images than on those of control group and was limited mainly to the subcortex, as verified at TTC staining. ADC reversion (arrows) also occurred at subcortical lesion on DW image and ADC map. Fourth row shows typical histologic photographs. (Hematoxylin-eosin stain; original magnification, $\times 50$.) Histologic findings of part of TTC-stained specimens (rectangles in far right column) showed extensive neuron necrosis (*) with neuropil vacuolation and structural disintegration, mainly at subcortex in treated groups but at both cortex and subcortex in control group. C = cortex, S = subcortex.

In our study, we used the visual perfusion-diffusion mismatch as a tool to select appropriate animals for treatment. The calculation during imaging analysis showed that perfusion-diffusion mismatch occurred homogeneously among the four groups before treatment. The animals thus selected responded well to the therapy in the microplasmin and tPA groups. As reported for clinical studies (26), the use of perfusion-diffusion mismatch proved to be both feasible and helpful in this study in identifying potential responders to thrombolytic therapies.

Our study results also suggested that MR imaging findings could be used as a noninvasive end point to reflect the pathologic nature of the ischemic lesion. Although T2-weighted imaging alone was not sensitive at 1 hour, multiparametric MR imaging, including T2-weighted imaging, DW imaging, and PW imaging, at 24 hours accurately depicted infarction volume as proved at TTC staining for all groups of rats in this study, which is in line with results elsewhere (19,20). MR imaging outcomes in the control group support the findings of a previous invasive study that showed that ischemic infarction was fully developed at 24 hours in the same stroke model (27).

On ADC maps and PW source images, the expansion of the ischemic lesion between 1 hour and 24 hours was significantly lessened by using microplasmin and tPA, in contrast to findings in the control group. In animal research, it is important to determine whether the obtained difference is the result of different treatments or because of individual variations. In this study, despite the insignificant variation of initial lesion size between the control and treated groups, we documented the lesion by using MR imaging at 1 hour and 24 hours and subsequently compared the lesion expansion during this period; hence we used a more reliable method that minimized influence from individual variations. Therefore, the observed reduction in lesion expansion can be attributed to the vessel recanalization caused by thrombolytic agents (25,28).

With regard to the PW-derived

Figure 4

Figure 4: Graph shows comparison of rLV changes between 1 hour and 24 hours after MCA occlusion. Ischemic lesion expansion between 1 hour and 24 hours was significantly reduced on ADC maps and PW source images (sPWI) in treated groups compared with that in control group. * = $P < .05$ versus control group, μ Pli = microplasmin.

Figure 5

Figure 5: Graph shows rLV measured at TTC staining 24 hours after MCA occlusion. rLV of cortical and total lesion were significantly smaller in treated groups than in control group. In subcortex, there was no significant difference of rLV between control group and three treated groups. * = $P < .05$, ** = $P < .01$ versus control group, ns = no significant difference, μ Pli = microplasmin.

functional parameters, cortical rCBV and rCBF between 1 hour and 24 hours were significantly increased in each group; this increase was not significantly different between the control and treated groups. In control rats, this was likely because of spontaneous reperfusion and/or collateral circulation 3–8 hours after MCA occlusion, as observed elsewhere with the same stroke model (29–32). However, such late reperfusion due to endogenous thrombolysis may not be efficient enough to rescue the reversibly ischemic tissue from infarction (27). On the contrary, reperfusion induced by using microplasmin and tPA likely occurred much earlier in this study because of the more prompt thrombolytic intervention at 1.5

hours after occlusion and hence more efficient restoration of blood flow. Reperfusion and restored tissue viability were almost always found in the cortex.

However, subcortical rCBV and rCBF between 1 hour and 24 hours decreased in each group, but this decrease was not significantly different between the control and treated groups. The observed poorer postischemic perfusion may be because the subcortical region is more vulnerable to ischemic injury (15) than the cortical region. Our MR imaging results showed that the ADC reversion observed at 24 hours always occurred in the subcortical lesion of all groups, which is consistent with the overall lower perfusion in this region. No significant difference in lesional ADC was

found between the control and treated groups. At microscopy, the area with ADC reversion or increased water diffusion reflected colliquative necrosis with loss of cellular membranes as the diffusive barriers. All the above data suggest that an ischemic lesion in the subcortex is less affected by thrombolytic agents than one in the cortex.

It has been suggested that introduction of tPA aggravates degradation of microvascular barriers (ie, mainly basal lamina and blood-brain barrier) in ischemic injury (5). The breakdown of basal lamina—caused mainly by matrix metalloproteinase-9 induction by lipoprotein-receptor-relating protein (7,25,33,34)—is associated with hemorrhagic transformation (5). Consequently, exogenous contrast agents may leak from vessels and induce parametric alterations on MR images that are then exploitable as a tool for diagnosis of cerebral bleeding and blood-brain barrier disintegration. A high dose of intravenously administered paramagnetic gadopentetate dimeglumine induced T2* (1/R2*) shortening or the enhanced transverse relaxation rate R2* on dynamic susceptibility contrast-enhanced

PW images. Therefore, abnormal R2* changes ($\Delta R2^*$) reflect extravasation of gadopentetate dimeglumine or increased permeability of the blood-brain barrier (5). In our study, the $\Delta R2^*$ curve from the tPA group had the largest tail, which implied considerable leakage and contrast material retention in the brain tissue after the first pass (35), which in turn led to a significantly higher $\Delta R2^*$ than that in the microplasmin and control groups at 24 hours. Our results suggest that tPA treatment aggravated the blood-brain barrier damage that may herald a higher risk of hemorrhage.

The exact dose of thrombolytic drugs seems critical not only in regard to the antistroke effects but also in regard to the hemorrhagic side effect, and the optimal dose of each drug also varies among species of animals (15,23,25,36). For our study, we used 10 mg/kg tPA, which is approximately equivalent to its standard clinical dose of 1 mg/kg for patients. The doses of 7.5 and 10 mg/kg microplasmin were considered appropriate for depleting circulating α_2 -antiplasmin to exert therapeutic effects without resulting in much safety concern. Indeed, all rats in the three treated groups had

comparable antistroke effects. However, administration of 10 mg/kg tPA caused significant bleeding, as evidenced with histomorphologic findings, which were in agreement with findings of previous studies (5–8,23,36). Interestingly, there was a dose-dependent neural and/or vascular protective effect with microplasmin because a significantly lower hemorrhagic rate was found, particularly with a higher dose (10 mg/kg) of microplasmin. Such a potential benefit deserves further preclinical and clinical exploitations.

Although the detailed mechanisms are still unknown, the therapeutic effects of microplasmin may be realized on two levels. The first is the partial recanalization of the occluded vessels, as evidenced by the improved MR imaging parameters through direct thrombolysis (15) due to the neutralization of plasma α_2 -antiplasmin. The second is the nonthrombolytic neuroprotective effect, which is supported by the fact that administration of microplasmin decreased the infarct volume even in a mouse model with permanent MCA occlusion (14,25). Therefore, the improvement in neurologic deficits in our

Figure 6

Figure 6: Graphs show comparison of changes in (a) rCBV and (b) rCBF in ischemic lesion. Both rCBV and rCBF significantly increased between 1 hour and 24 hours in cortex in each group. ** = $P < .05$. In subcortex, rCBF decreased between 1 hour and 24 hours in each group. * = $P < .05$. No significant differences in rCBV and rCBF changes between 1 hour and 24 hours were observed between control and treated groups at cortex and subcortex ($P > .05$). ns = No significant difference, μ Pli = microplasmin.

Figure 7

Figure 7: Graph shows values for $\Delta R2^*$ (changes in $T2^*$ relaxation rate) as a PW source image-derived parameter associated with blood-brain barrier breakdown and intracerebral hemorrhage in region of cerebral ischemia. At 24 hours after MCA occlusion, $\Delta R2^*$ was significantly higher in tPA-treated group than in control group but was significantly lower in 10 mg/kg microplasmin group than in control group. Note that $\Delta R2^*$ curve for tPA group showed largest tail compared with that for the other three groups; this indicates considerable leakage and contrast material retention in brain tissue after first pass in tPA group. * = $P < .05$ versus control group, μPli = microplasmin.

study may be attributed to both of these therapeutic effects of microplasmin.

Certain limitations existed in our study. First, because of constraints of the clinical MR imager, the field of view as an imaging acquisition parameter was not identical between MR imaging sequences, which hampered direct coregistration for correcting geometric distortion. Therefore, point-to-point matching between MR images was not feasible. As an alternative, relative values were applied to facilitate the comparison between animals and imaging methods. Second, volumetric quantification of intracerebral hemorrhage was not attempted. Instead, the rate of hemorrhage was compared between control and treated groups. Third, because of the limited accessibility of the clinical imager, we were unable to monitor the therapeutic effects at more time points between 1 hour and 24 hours.

In conclusion, perfusion-diffusion mismatch is a useful tool in selecting appropriate animals for stroke therapy.

Both microplasmin and tPA shared a similar effect in preventing infarct lesion expansion with improved behavioral scores. However, the intracerebral hemorrhage rate was significantly lower in rats treated with microplasmin than in rats treated with tPA. A multiparametric MR imaging approach allows noninvasive assessment of the therapeutic effects and hemorrhagic transformation after administration of anti-stroke agents.

Practical application: Microplasmin may serve as a potential alternative to tPA in the treatment of patients with ischemic stroke, with comparable effects and lower hemorrhage rate.

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