Fibrinolysis therapy achieved with tissue plasminogen activator and aspiration of the liquefied clot after experimental intracerebral hemorrhage: rapid reduction in hematoma volume but intensification of delayed edema formation

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Object. Fibrinolysis therapy accomplished using tissue plasminogen activator (tPA) and aspiration is considered to be a viable alternative to microsurgery and medical therapy for the treatment of deep-seated spontaneous intracerebral hematomas (SICHs). Tissue plasminogen activator is a mediator of thrombin- and ischemia-related delayed edema. Because both thrombin release and ischemia occur after SICH, the authors planned to investigate the effect of fibrinolytic therapy on hematoma and delayed edema volume.

Methods. A spherical hematoma was created in the frontal white matter of 18 pigs. In the tPA-treated group (nine pigs), a mean of 1.55 ml tPA was injected into the clot and the resulting liquefied blood was aspirated. Magnetic resonance (MR) imaging was performed on Days 0 (after surgery), 4, and 10, and the volumes of hematoma and edema were determined. In the animals not treated with tPA (untreated group; nine pigs), the volume of hematoma dropped from 1.43 ± 0.42 ml on Day 0 to 0.85 ± 0.28 ml on Day 10. In the tPA-treated group, the volume of hematoma was reduced from 1.51 ± 0.28 ml on Day 0 to 0.52 ± 0.39 ml on Day 10. In comparison with the untreated group, the reduction in hematoma volume was significantly accelerated (p = 0.02). In the untreated group, perihematomal edema increased from 0.32 ± 0.61 ml to 1.73 ± 0.73 ml on Day 4, before dropping to 1.17 ± 0.92 ml on Day 10. In the tPA-treated group, the volume of the edema increased from 0.09 ± 0.21 ml on Day 0 to 1.93 ± 0.79 ml on Day 4, and further to 3.34 ± 3.21 ml on Day 10. The increase in edema volume was significantly more pronounced in the tPA-treated group (p = 0.04).

Conclusions. Despite a significantly accelerated reduction in hematoma volume, the development of delayed perifocal edema was intensified by fibrinolytic therapy, which is probably related to the function of tPA as a mediator of edema formation after thrombin release and ischemia. Further experimental and clinical investigations are required to establish the future role of fibrinolysis in the management of SICH.

Key Words • intracerebral hemorrhage • edema • ischemia • fibrinolysis • pig

Abbreviations used in this paper: FLAIR = fluid-attenuated inversion recovery; GE = gradient echo; ICP = intracranial pressure; MR = magnetic resonance; PAI-1 = plasminogen activator inhibitor–1; rtPA = recombinant tissue plasminogen activator; SICH = spontaneous intracerebral hematoma; TSE = turbo–spin echo.

Hypertension-associated hyaline degeneration of small arteries and arterioles and age-dependent amyloid deposition in vessel walls are the most frequent causes of SICH.8,11,17 Occurring in 11 to 15 persons per 100,000 population, the annual incidence of SICH is already substantial,5,17,61 and a further increase can be expected to accompany rising life expectancy. The incidence of mortality and severe morbidity following SICH is higher than that of other subtypes of stroke. An effective treatment of patients with SICH is thus urgently needed, but the optimal therapy—either medical or surgical—is not yet defined. In five prospective randomized studies, craniotomy and clot removal provided no better clinical results than the best medical management,1,8,23,41,46 possibly because of the additional brain injury that may occur during the transcortical approach to the clot. Stereotactic hematoma puncture, clot aspiration, and lysis of clot remnants with tPA allow us to reduce hematoma volume by 50 to 70%,56 with minimal associated brain damage. Promising clinical results in nonrandomized studies32,60 and experimental data showing a positive effect on the volume of early perihematomal edema and ischemia8,70 support this therapeutic concept.

Recently, there have been reports of tPA-mediated, thrombin- and ischemia-induced brain injury with delayed edema formation.13,66 Thus, it seems possible that injection of tPA for clot lysis promotes the development of delayed perihematomal edema, counteracting the beneficial effects of rapid hematoma volume decrease. In this experimental study in a porcine model of lobar hematoma,69 we planned to investigate whether fibrinolytic therapy consisting of tPA administration and subsequent aspiration of the liquefied clot contributes to the development of delayed perihematomal edema despite a rapid reduction in hematoma volume.

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Materials and Methods

Animal Preparation and Surgery

A total of 27 Deutsche Landrasse pigs, each weighing between 30 and 35 kg, were used for the experiment. All procedures were performed in strict accordance with the guidelines of the Animal Care and Use Committee of Aachen University. The experimental protocol was approved by the committee for animal research of Nordheim–Westfalia, Germany. The pigs were sedated with azaperone and ketamine (10–15 mg/kg) to allow placement of a venous line. Pentobarbital (15–20 mg/kg) was then administered and endotracheal intubation was performed. During the surgical and imaging procedures, anesthesia was maintained by administration of a barbiturate. The respiratory rate and tidal volume were adjusted to maintain arterial blood gas levels within physiological limits (PO2, 100–150 mm Hg; PCO2, 35–40 mm Hg). Each animal’s body temperature was kept at 38˚C. During surgery, ICP was monitored with the aid of an intraparenchymal pressure device (Codman, Johnson & Johnson Medical GmbH, Norderstedt, Germany), which was introduced via a specially designed bolted into the right frontal bone (0.7 cm lateral to the midline at the level of the coronal suture). Because the experiment was designed to include a 10-day survival period for the pigs, no approval was obtained for intraarterial measurement of blood pressure during the investigation period.

A 3-mm burr hole was drilled 1.7 cm anterior to the coronal suture and 0.7 cm to the right of the midline, and the dura mater was perforated. The tip of a Fogharty catheter was introduced into the parenchyma of the right frontal lobe and the balloon (volume 2 ml) was inflated for 2 seconds. A 1.2-cm-long catheter with an attached Rickham reservoir was then placed into the preformed cavity. In 18 pigs, 3.5 to 7.0 ml (mean 5 ml) of autologous venous blood was slowly injected through the Rickham reservoir and catheter into the right frontal lobe by using a modified double-injection method to prevent substantial blood reflux along the catheter into the subdural compartment.9 The injection volume was not kept constant, but was guided by the animal’s ICP measurements to create a hematoma that was at least 1 ml in volume (preliminary studies had indicated that the creation of a spherical intracerebral clot with a volume between 1 and 2 ml is indicated by a sharp, short-term increase in ICP to values of at least 28 mm Hg [unpublished data]). Immediately after removal of the ICP probe and skin closure, MR imaging was performed while the pigs remained intubated and ventilated. Of the 27 pigs, nine constituted a control group, in which only balloon inflation, catheter placement, and MR imaging were performed.

Magnetic Resonance Imaging

Magnetic resonance imaging was performed 30 minutes after intracerebral injection of blood, which followed balloon inflation and catheter placement. All pigs were investigated using a 1.5-tesla MR imaging system (Gyroscan ACS-NT, Philips Best, The Netherlands). In all animals, T1-weighted images (TR 500 msec, TE 25 msec), T2-weighted TSE images (TR 3000 msec, TE 120 msec, TSE factor 16), T2-weighted FLAIR TSE images (TR 6000 msec, TE 150 msec, TI 2000 msec, TSE factor 24), and T1-weighted GE images (TR 487 msec, TE 35 msec, flip angle 15°) were obtained. The volume of hematoma was calculated on T2-weighted GE images and the edema volume on T2-weighted FLAIR images.87 The areas of hemorrhage and edema were traced directly on the MR imaging screen for each slice. The total volumes of hematoma and edema were calculated by multiplying the 3-mm slice thickness by the measured areas; the calculation was performed using MR imaging software. In all pigs, MR imaging studies were acquired again on Days 4 and 10 after surgery to analyze variations in the volumes of hematoma and edema. In one pig, movement artifacts made calculation of the edema volume on Day 4 impossible. For the second and third MR imaging investigations, the pigs were anesthetized and again intubated to avoid the occurrence of movement artifacts during imaging.

Fibrinolytic Therapy

Because no information on the appropriate dosage of rtPA (Activase; Dr. Karl Thomae GmbH, Biberach, Germany) for fibrinolytic therapy exists,46 we decided to use the same formula for dosage calculation in this experimental study as we did in our patient series:50,60 maximum diameter of clot (in mm) × 0.1 mg rtPA in a solution of 0.1 ml (for example, 1.4 mg rtPA would be injected into a hematoma with a maximum diameter of 14 mm). Immediately after imaging with calculation of hematoma volume and maximum diameter, fibrinolytic therapy was initiated in nine of the 18 pigs with the frontal intraparenchymal clot (tPA-treated group). The operation wound was reopened and the calculated rtPA dosage was slowly injected into the clot through the Rickham reservoir and catheter. The plasma half-life time of rtPA is defined as ranging from 4 to 9 minutes,26 but the drug’s intrahematomat half-life time remains unknown. Following data provided by recent clinical and experimental trials on intravenous, intracisternal, and intracerebral clot lysis14,24,28,30,75 the Rickham reservoir was again punctured 20 minutes after injection and the liquefied blood was slowly aspirated. The Rickham reservoir and catheter were left in place and the operation wound was again closed.

Histopathological Examination

Immediately after the final MR imaging session on Day 10, the pigs were killed by injection of pentobarbital. The intact brains were removed and 25 of the 27 brains were fixed in 10% formalin for at least 7 days. The formalin-fixed brains were cut into 3-mm-thick coronal slices. For calculation of the approximate hematoma volume, the following formula was used: long diameter (x axis) × short diameter (y axis) × x number of coronal brain slices with hemorrhage × slice thickness (z axis).28 The hematoma volume observed during gross pathologic investigation was correlated with that furnished by the final T2-weighted GE images. Brain slices containing the needle track, hematoma, and edema were dehydrated, embedded in paraffin, and stained with hematoxylin and eosin and with Turnbull blue for evaluation of inflammatory infiltration, edema, and hemoglobin degradation. The extent of perihematomat edema and inflammatory infiltration was determined in a semiquantiative manner by using a three-point scale (Grade 1, minor edema or inflammation, in which edema or inflammatory cells are found directly adjacent to the hematoma and catheter track; Grade 2, moderate edema or inflammation, in which edema or inflammatory cells are not limited to the direct vicinity of the hematoma and catheter track, but do not reach the gyri; and Grade 3, major edema or inflammation, in which edema or inflammatory cells extend to the gyri). The histological findings of swollen astrocytes and/or spongiforme transformation of white matter due to enlargement of the intercellular space and formation of vacuoles were considered to indicate edema.10,11 The brains of two pigs with untreated hematoma were cut immediately at the level of the clotted hematoma and tissue samples of that region, which were seen as hyperintense areas on the final T2-weighted FLAIR sequences, were weighed. The tissue samples were then dried in an oven (80˚C) and weighed again to define the dry weight and to calculate water content as a percentage by using the formula (wet weight – dry weight)/wet weight × 100. This water content was compared with that of ipsilateral isointense white matter to prove that the hyperintensity displayed on the T2-weighted FLAIR images corresponds to perilesional edema.

Statistical Analysis

Hematoma and edema volumes as well as histological findings in the tPA-treated group and the untreated group (animals with hematoma that were not treated) were compared using the Student t-test. A probability value of 0.05 or less was considered to indicate a significant difference.

Results

Systemic and Neurological Parameters

After intubation, in all pigs the PO2 was greater than 100 mm Hg and the PCO2 lay between 35 to 40 mm Hg; this was maintained during surgery. Each animal’s body temperature was measured and kept constant at 38˚C through-
out the acute experimental period that included creation of the hematoma. At the beginning of each experiment, the ICP was 10 mm Hg or lower. In the 9-day period following surgery, systemic parameters were not measured again.

Immediately after surgery and daily during the follow-up period, the neurological status and behavior of each pig was observed and considered to be normal in every case.

**Hematoma Volume**

**Untreated Group.** As confirmed by GE sequences obtained immediately after surgery, intraparenchymal spherical hematomas with a mean volume of 1.43 ± 0.42 ml were created in the nine pigs in the untreated group by injecting 5 to 10 ml (mean 7.8 ml) of venous blood into the right frontal white matter. Magnetic resonance imaging demonstrated a mean hematoma volume of 1.16 ± 0.46 ml on Day 4 and a mean volume of 0.85 ± 0.28 ml on Day 10. The hematoma volume on Day 10 was significantly lower than that recorded on the day of surgery (p < 0.001). The total volume reduction of the created clot during the observation period was 0.58 ml (40.6%). The volume reduction by spontaneous clot degradation was 0.27 ml (18.9%) within the first 4 days and 0.31 ml (21.7%) between Days 4 and 10, suggesting a linear degradation process (Fig. 1).

**Animals Treated With tPA.** A right frontal spherical hematoma with a mean volume of 1.51 ± 0.28 ml was created in the nine pigs in the tPA-treated group. A 1- to 2-ml (mean 1.55 ml) injection of rtPA was administered into the core of the clot after initial MR imaging. Twenty minutes later, 0.1 to 2.1 ml (mean 0.85 ml) of lysed hematoma was gently aspirated. The mean hematoma volume on Day 4 was 0.65 ± 0.37 ml and that recorded on Day 10 was 0.52 ± 0.39 ml. Fibrinolytic therapy achieved a total hematoma volume reduction of 0.99 ± 0.38 ml (65.6%). The reduction in clot volume between the day of surgery and Day 4 (0.86 ml [57%]) was more pronounced than that recorded between Days 4 and 10 (0.13 ml [8.6%]). The volume of hematoma on Day 10 was significantly lower than that measured on the day of surgery (p < 0.001) (Fig. 2). Statistical analysis showed that injection of tPA and aspiration of the clot volume were significantly more effective in hematoma volume reduction than spontaneous clot resorption (p = 0.02). In accordance, clot volumes on Day 4 (p = 0.02) and on Day 10 (p = 0.05) were significantly lower in the tPA-treated group than in the untreated group.

The correlation coefficient of the hematoma volume as seen on the T2-weighted GE MR images obtained on Day 10 was correlated with that of findings of the gross pathological investigation. The correlation coefficient was 0.87 in the untreated group and 0.85 in the tPA-treated group, confirming that MR imaging is a reliable tool for the determination of clot volumes (Figs. 3 and 4).

**Control Group.** In nine pigs, no hematoma was created, but a balloon was inflated for a short time and a catheter was placed into the frontal brain parenchyma. Possibly induced by vessel rupture during balloon inflation, postsurgery MR images demonstrated the presence of a hematoma.
in two of the nine pigs. During the follow-up period, the initial hematoma volumes of 1.56 and 0.87 ml in these animals decreased to 1.4 and 0.51 ml, respectively. In the remaining seven pigs, no blood or minimal traces of blood could be seen on the MR images.

**Edema Volume**

In two pigs in the untreated group, the water content of brain tissue, which was harvested from an area of perihematoma hyperintensity on FLAIR sequences, was compared with that of ipsilateral isointense brain tissue. In both pigs, the water content of the hyperintense brain parenchyma was substantially higher than that of the isointense brain tissue (87.5% compared with 77%, respectively, and 78.6% compared with 71.6%, respectively). These results support the data from the literature that areas of hyperintensity on FLAIR sequences correspond to brain edema. Thus, $T_2$-weighted FLAIR images obtained on the day of surgery and on Days 4 and 10 postoperatively were used for calculation of edema volume.

**Untreated Group.** Immediately after surgery, the mean edema volume in animals with untreated hematoma was $0.32 \pm 0.61$ ml and this increased until Day 4 to a volume of $1.73 \pm 0.73$ ml. The mean edema volume measured on Day 10 ($1.17 \pm 0.92$ ml) was less than that recorded on Day 4, but higher than that recorded on the day of hematoma creation. Edema volumes measured postoperatively—especially those recorded on Day 4 ($p = 0.0001$), but also those recorded on Day 10 ($p = 0.04$)—were significantly higher than edema volumes measured directly after surgery. From Day 0 to Day 10, the mean edema volume increased by $0.86 \pm 1.09$ ml (Fig. 5).

**Animals Treated With tPA.** The mean edema volume recorded after creation of the hematoma, but before fibrinolytic therapy was $0.09 \pm 0.21$ ml. Magnetic resonance images obtained on Day 4 demonstrated a mean edema volume of $1.93 \pm 0.79$ ml. The highest edema volume ($3.34 \pm 3.21$ ml) was seen on Day 10 after surgery, which contrasts with the findings in the untreated group. The variability of edema volume on Day 10 was substantial, ranging between 0.49 and 9.56 ml. In seven of nine pigs, however, the increase in volume occurred from Day 0 to Day 10, underlining the authenticity of the observation, despite this variability (Fig. 6). In the remaining two pigs, the highest edema volume was reached on Day 4. Edema recorded on Day 4 ($p < 0.001$) and Day 10 ($p = 0.007$) was significantly more voluminous than edema measured on the day of hematoma creation and clot lysis. From the day of surgery until Day 10, the mean edema volume increased by $3.24 \pm 3.09$ ml. This increase in the edema volume was significantly more pronounced in the tPA-treated group ($p = 0.04$). Without reaching significance, a clear tendency to larger edema in the tPA-treated group, compared with edema measured...
in the untreated group on Days 4 and 10, could be demonstrated.

**Control Group.** In this group, a slight edema with a mean volume of $0.29 \pm 0.4$ ml could be seen shortly after balloon inflation and catheter placement. The edema volume on Day 4 ($1.7 \pm 0.74$ ml) was significantly higher than the initial edema volume ($p < 0.001$). On Day 10, the edema volume was $0.52 \pm 0.63$ ml. The mean edema volume increased $0.23 \pm 0.39$ from Day 0 to Day 10. A statistical difference between edema volumes measured on Day 10 and on the day of surgery could not be shown ($p = 0.12$). In two pigs, balloon inflation resulted in hematoma formation. To define more clearly the role of balloon inflation in edema development, we calculated the mean edema volume with these two pigs excluded. Directly after surgery, the mean edema volume was $0.22 \pm 0.29$ ml. On Day 4 the mean edema volume was $1.54 \pm 0.47$ ml and on Day 10 it was $0.36 \pm 0.17$ ml. The mean edema volume increased $0.14 \pm 0.28$ from Day 0 to Day 10. Again, the edema volumes on Days 0 and 10 were not statistically different.

**Histopathological Findings**

**Untreated Group.** The extension of edema and perifocal inflammatory infiltration was assessed semiquantitatively by applying a three-point scale to hematoxylin and eosin-stained slices. In all pigs there was a perihematomal spongiform transformation of white matter, which in eight of the nine pigs extended from the hematoma to the gyrus (Grade 3). Two pigs in the untreated group displayed a major and six pigs a moderate inflammatory reaction. In one pig, the perifocal inflammatory infiltration was limited to the close vicinity of the hematoma and the stitch canal. The inflammatory infiltration consisted of monocytes and leukocytes.

**Animals Treated With tPA.** In seven of nine pigs there was massive edema extending from the border of the clot to the cortical surface on Day 10 after surgery and fibrinolysis. In two pigs, a Grade 2 edema was found. Few astrocytes were slightly swollen, but the appearance of an enlargement of the intercellular space and formation of vacuoles suggested increased water content mainly in the extracellular compartment (Fig. 7). In four pigs in the tPA-treated group there was a severe inflammatory infiltration, which extended from the clot to the gyri. In four pigs a moderate inflammation and in one pig a minor inflammation was observed. The cellular composition of the infiltration did not differ from that of the untreated group.

**Control Group.** Balloon inflation without blood injection caused moderate edema in eight pigs and minor inflammatory infiltrations in seven pigs. Both edema and inflammation were less extensive in the control group than in the tPA-treated and untreated groups.
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Discussion

Incidences of mortality and morbidity attributed to intracerebral bleeding are closely related to the increase in ICP and the direct and irreversible destruction of neurons during the bleeding. Further risk of morbidity is added by the secondary destructive effect of the SICH. The hematoma produces a perifocal edema that results in delayed neuronal damage in initially intact perilesional brain parenchyma. Recent studies focusing on posthemorrhagic edema formation have elucidated some of the underlying pathophysiological mechanisms of edema formation. Within the first hours after bleeding, retraction of the clot, extravasation of plasma proteins, increase in sodium, and oncotically driven water accumulation seem to be the leading forces of edema formation. Beginning 24 hours after bleeding, activation of the coagulation cascade and thrombin release predominantly contribute to the perilesional edema. Thrombin causes edema by opening the blood–brain barrier directly, by vasodilation of cerebral vessels, and by induction of an inflammatory response. From 72 hours onward, lysis of red blood cells and hemoglobin- and hemoglobin degradation product–induced toxicity are the driving forces of edema formation. It is believed that hemoglobin release contributes to edema formation for up to 3 weeks. Nuclear factor-κB as well as complement activation with formation of a membrane attack complex and stimulation of inflammatory cells additionally contribute to late posthemorrhagic edema formation and cell death. Edema-related neurological deterioration of patients is often observed 1 to 4 days after hemorrhage, which underlines the more substantial contribution of thrombin and hemoglobin release to edema formation than early clot retraction and plasma protein extravasation.

Experiments in which balloons are inserted in the rat brain and inflated as well as experiments in which intracerebral injections of mineral oil are used have shown that edema formation is substantially but not exclusively related to the presence of an intracerebral blood clot. The mass effect, even if exerted only for minutes, causes a prolonged perifocal ischemia that initiates edema formation. The edema starts to resolve 5 to 7 days after bleeding; however, even substantially later edema resolution has been described. It was assumed that craniotomy and SICH evacuation contributes to a better outcome by effective reduction in the volume of ischemic perihematomal brain tissue by interrupting edema and ischemia development. In five prospective randomized trials, in which the best medical therapy given to patients with SICH was compared with craniotomy for SICH evacuation, however, the assumed superiority of the surgery could not be shown. The chance of death or dependency was even higher with craniotomy. These poor surgical results have been attributed to the additional surgical traumatization of intact brain overlying the clot and have stimulated the search for less invasive surgical techniques for hematoma removal. In recent years, considerable interest has focused on frame-based or frameless stereotactic hematoma puncture, aspiration, and fibrinolysis of portions of the hematoma that have already clotted and are not suitable for aspiration.

The results of several prospective, but nonrandomized clinical series suggest that the minor invasiveness of hematoma puncture, aspiration, and subsequent fibrinolytic therapy is superior to conservative management and craniotomy. Mortality rates as low as 6% and recovery rates as high as 71% were obtained by removing 50 to 84% of the initial clot volume. These promising results have been attributed partly to the positive effect of aspiration and fibrinolytic therapy on edema development and the ischemic cascade.

Data from animal studies seemed to support the therapeutic concept. Altumbabic and coworkers used a rodent model of collagenase-induced intracerebral hemorrhage. These authors observed a significantly reduced neuronal loss when the rats underwent clot lysis with streptokinase and aspiration. Deinsberger and coworkers created ganglionic hematomas in the rat. Hematoma puncture, fibrinolysis with tPA, and aspiration of the liquefied blood significantly reduced clot volume and the volume of ischemic perihematomal brain tissue. Wagner and coworkers used a lobar intracerebral hemorrhage model in 11 pigs. In six pigs, the clot was left untreated and in five pigs 0.3 ml of tPA was injected into the hematoma. The liquefied parts of the clot were then gently aspirated. Twenty-four hours after surgery, the mean volume of the clots was 1.25 ml and that of the perifocal edema was 1.5 ml in animals not treated with tPA. Both hematoma volume (0.4 ml) and edema volume (0.3 ml) were significantly reduced in the tPA-treated group compared with the untreated group.

In our experiment, injection of tPA into the newly created frontal hematoma reduced the initial clot volume by 65.6%, which is in line with reports of clinical series and animal experiments. Nonetheless, we did not observe a positive effect of fibrinolytic therapy on edema volume, but instead saw an increase in edema volume from the day of surgery to the day of death (Day 10). Edema volumes in the treatment group were higher than those measured in untreated pigs. The increase in the edema volume is not the mere result of the rapid, tPA-induced decrease in hematoma volume. If only clot volume reduction contributed to the edema, a constant or declining combined volume of edema and hematoma should have been seen on the follow-up MR images. Instead, the combined volumes on Days 4 and 10 were higher than the initial volumes of edema and hematoma. Thus, our results indicate an active role of tPA in the formation of delayed edema. Urokinase and tPA injected directly into brain parenchyma do not cause edema. It can be assumed that tPA only becomes an active, edema-promoting factor in the presence of an intraparenchymal clot. The half-life of tPA injected into an intracerebral clot is unknown. Because the estimated half-life in cerebrospinal fluid is 2 to 3 hours and the half-life in plasma is 5 to 8 minutes, an intrahematomatous half-life could be assumed to lie within this range. At first sight, the observation that the short-acting tPA causes edema for up to 10 days if injected into an intracerebral clot seems questionable; however, recent research gives possible explanations.

1) Thrombin triggers edema formation after SICH. Proteasemin-1 and PAI-1 act as inhibitors of thrombin and tPA. By injecting tPA for clot lysis and increasing the tPA concentration, less thrombin is inactivated by the enzymes. Brain endothelial cells exposed to tPA paradoxically diminish elaboration of PAI-1 messenger RNA, which increases the availability of clotting factors and promotes thrombin formation. The effect of tPA on thrombin concentration is further augmented by the positive feedback mechanism of tPA–tPA interaction. Thrombin concentrations and fibrinolytic activity are significantly increased in the tPA-treated group compared with the untreated group.
of reduced thrombin inhibition. The effect of reduced inhibition of thrombin is an increase in the thrombin concentration in the clot and the surrounding brain tissue. Some thrombin remains bound within the clot and is gradually released into adjacent brain tissue. With a higher concentration of clot-bound thrombin, a more prolonged thrombin release can be assumed, which would explain our finding that from Day 4 to Day 10 after blood injection the edema volume decreases in animals not treated with tPA and increases in those treated with tPA.

2) In many studies, perihematomal ischemia was a frequent finding after space-occupying intracerebral bleeding. Ischemia causes excessive and long-lasting glutamate release with subsequent glutamate receptor activation, which leads to pathological calcium influx and, finally, to cell death with edema formation. The finding of substantial edema on Day 4 in pigs in the control group, which only underwent repeated balloon inflation, is in accordance with the results of animal experiments conducted by Sinar and coworkers and underlines the role of ischemia in edema formation. Mice deficient in tPA are resistant to glutamate-mediated neuronal loss, which indicates that tPA acts as a mediator of this excitotoxic cell injury. It seems possible that additional tPA, which is injected for the lysis of the intracerebral clot, further activates the excitotoxic pathway with increased rates of cell death and subsequent edema. The PAI-1 level decreases if brain endothelial cells are exposed to exogenous tPA. Masuda and coworkers documented increased fibrinolytic activity, mediated by endogenous tPA, 7 to 10 days after creation of an experimental hematoma in the rat. It can be assumed that reduced PAI-1 levels caused by injecting tPA into the clot result in increased endogenous tPA levels on Days 7 to 10 with possible excitotoxic neural injury and edema formation. This could explain our observation of late intensification of edema in tPA-treated pigs. Erythrocyte lysis and hemoglobin formation is considered to be one major factor in delayed (4–21 days after hemorrhage) perifocal edema. The late excitotoxic cell injury due to PAI-1 level reduction and endogenous tPA release could be potentiated by the gradually released hemoglobin.

3) On Day 10 after surgery, no pig in the control group, two pigs in the untreated group, and four pigs in the tPA-treated group were found to have an extensive perihematomal inflammatory reaction. Inflammatory cells induce release of oxygen radicals and cytokines, which promote neurotoxicity and edema formation. Possibly, the provocation of intensive and long-lasting inflammation leads to formation of delayed perifocal edema in the pigs that underwent tPA clot lysis.

Our finding that tPA injected into the intraparenchymal clot did not reduce but aggravated and prolonged the perihematomal edema seems to contrast with the findings of Wagner, et al., who reported that fibrinolytic therapy decreases perilesional edema. As outlined earlier, edema formation after SICH involves several phases including an early phase (< 24 hours) of clot retraction and oncotically effective plasma protein extravasation, a second phase (> 24 hours) of coagulation cascade activation and thrombin release, and a late phase (> 72 hours) of red blood cell lysis and hemoglobin formation. Without fibrinolytic therapy, the edema often starts to resolve on Days 5 to 7. Wagner and coworkers killed the animals 24 hours after fibrinolytic therapy, with the consequence that only the effect of fibrinolytic therapy on early edema formation was investigated. We assessed edema volumes on Days 4 and 10 after induction of the hematoma and demonstrated the deleterious effect of fibrinolytic therapy on delayed edema. Thus, the findings of the two studies are not contradictory, but complementary; fibrinolytic therapy with tPA reduces early edema, but enhances and prolongs delayed edema.

The present study did not allow us to evaluate the potential clinical relevance of edema intensification following tPA-induced lysis of the intracerebral clot. The experiment was designed with a 10-day survival period for the pigs. Because a precondition for approval of this study design was avoidance of neurological deficits, we produced only medium-sized hematomas in a noneloquent brain area. It seems possible that edema formation in models of larger experimental hematomas, which, according to the protocol of fibrinolytic therapy, have to be treated with higher dosages of tPA, causes neurological deficits.

Deep-seated basal ganglia hematomas rarely require surgical therapy if their volumes do not exceed 20 to 30 ml. A hematoma with a volume of 30 ml occupies 2% of total human brain volume. A hematoma of a comparable dimension in the pig brain would range in volume between 1.2 and 2.5 ml. Clots ranging in volume between 1 and 2.3 ml were created in the present study and, therefore, the results that we have obtained probably could be transferred to humans. Even if the clinical relevance of massive delayed edema after fibrinolytic therapy is not defined, the known association between edema, demyelination, and cell death should stimulate additional experimental and clinical investigations before fibrinolysis and clot aspiration can be considered to be a better alternative to microsurgery and conservative management.

Conclusions

The injection of tPA into an experimental hematoma in the white matter of pigs and subsequent aspiration of liquefied portions of the clot allowed us to reduce hematoma volume by 65.6%. In comparison with the natural course of the disease, fibrinolytic therapy significantly accelerated the reduction of hematoma volume. This acceleration of clot degradation does not prevent the development of delayed perihematomal edema. In contrast, fibrinolysis with tPA significantly intensifies and prolongs the formation of delayed edema. After intracerebral bleeding, thrombin release and ischemia are common findings. The pathological mechanism that probably underlies the observed edema intensification is the function of tPA as mediator of thrombin- and ischemia-related edema formation. In the light of these findings, further experimental and clinical investigations are required to evaluate the future role of fibrinolytic therapy in the management of SICHs.

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