Factors Affecting Clot Lysis Rates in Patients With Spontaneous Intraventricular Hemorrhage

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Background and Purpose—In the treatment of severe intraventricular hemorrhage (IVH), thrombolytic use and clot size are known to influence clot lysis rates. We evaluated the effect of other variables on IVH clot lysis rates among patients treated with recombinant tissue-type plasminogen activator or placebo.

Methods—One hundred patients with IVH and intracerebral hemorrhage volume <30 mL requiring emergency external ventricular drainage from 2 multicenter trials were treated with intraventricular administration of recombinant tissue-type plasminogen activator (n=78; 53 males, 25 females) or placebo (n=22; 7 males, 15 females). IVH volume was quantified daily by head CT. A segmented linear regression using an optimized spline knot for each patient was fit. Random effects linear regression was used to estimate the effect of prespecified patient characteristics on clot lysis rates over the first 6 days.

Results—Stability IVH volumes were larger in males (N=60; 54±5 mL) than females (N=40; 36±5 mL; $P=0.01$). Intraventricular thrombolytic treatment was associated with an increase in clot lysis rate of 14.6% of stability IVH volume/day before the spline knot compared with the placebo group ($P<0.001$). After adjustment for thrombolytic, higher baseline serum plasminogen and lower baseline platelet count were independently associated with an increase in clot lysis of 1.28%/day per 10-g/dL increase ($P<0.001$) and 0.70% /day per 10×10^3/uL decrease ($P<0.001$) before the knot, respectively.

Conclusions—Although thrombolysis remains the major determinant of IVH clot lysis rate, higher baseline serum plasminogen and lower platelet count also predict faster clot lysis. Further studies are needed to confirm whether plasminogen availability and thrombus structure impact IVH clot removal.

Clinical Trial Registration—URL: http://clinicaltrials.gov. Unique identifier: NCT00650858.

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Key Words: acute care ■ critical care ■ drug trials ■ intracerebral hemorrhage ■ neurocritical care ■ randomized controlled trials ■ thrombolysis ■ treatment

The current therapy for intraventricular hemorrhage (IVH) causing obstructive hydrocephalus is drainage of blood and cerebrospinal fluid (CSF) through an external ventricular drain (EVD). Thrombolytic drugs, particularly recombinant tissue-type plasminogen activator (rtPA), can be administered safely into the ventricles of patients with IVH once IVH volume has stabilized and these drugs significantly shorten the time of blood clot resolution in both experimental models and humans.[1–5] The identification of other factors affecting clot lysis rates may be important for determining the optimal dosing regimen of rtPA, which may differ based on patient-specific characteristics.

In our initial prospective randomized trial using intraventricular urokinase, IVH clot resolution rate was favorably affected by female gender.[5] In this study, we explore the effect of gender and other variables on clot lysis rates in patients with acute IVH treated with either placebo or rtPA using completed phases of the CLEAR IVH studies. We hypothesize that intraventricular rtPA therapy results in faster clot lysis rates in females compared with males presenting with IVH.

Materials and Methods

This multicenter clinical study was performed under the approval of the Institutional Review Board committee of each participating center. Written consent was obtained from all participants or their legal representatives.

Study Design and Patient Selection

The CLEAR IVH trial study procedures have been published previously[4] (online-only Supplemental Figure I, http://stroke.ahajournals.org; supplemental material provides an overview of study designs). In the initial safety study, 48 patients (26 males, 22

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females) with severe IVH, and supratentorial intracerebral hemorrhage volume <30 mL, with EVD for treatment of obstructive hydrocephalus were randomized to receive 3.0 mg intraventricular rtPA in 1 mL of normal saline (N=26) or intraventricular placebo (placebo; 1 mL normal saline; N=22) every 12 hours. Fifty-two patients (34 males, 18 females) with the same indications participated in a 2-part open-label dose escalation trial of intraventricular rtPA at doses 0.3 or 1.0 mg every 12 hours or 1.0 mg every 8 hours. Patients were enrolled within 48 hours after the initial hemorrhage.

EVD Placement

Patients in these studies required emergency EVD. In the majority of patients (63%), EVDs were placed into the frontal horn of the lateral ventricle with the least blood. Seven patients had >1 catheter simultaneously. Intraventricular location of the catheter tip was confirmed by CT scan performed 6 hours after placement.

Documenting Clot Stability

The first CT scan performed at least 6 hours after EVD placement that demonstrated a stable IVH and intracerebral hemorrhage volume and either no or stable catheter tract hemorrhage was designated as the stability CT. Evidence of additional hemorrhage required delaying study agent administration for at least 12 hours and until clot stabilization was established.

Treatment Protocol

The study dose of rtPA or placebo was delivered after an effort was made to aspirate at least 4 mL of CSF. Isovolemic injection of the study agent was followed by a 2-mL flush. The EVD was then closed for 1 hour to allow adequate time for drug–clot interaction and reopened only if necessary to control medically refractory intracranial pressure elevations. After the 1-hour closure, the EVD was reopened to drain CSF at the gradient set by the treating physician. The first injection of study agent occurred no sooner than 12 hours but no later than 48 hours after the diagnostic CT scan and at least 6 hours after EVD placement. Study agent injections continued at the specified interval until clearance of hyperdense blood from the third and fourth ventricle was observed on daily head CT or for a maximum of 12 doses in the CLEAR A and B studies.

Evaluation of Clot Resolution

Head CT scans were obtained before the initial intraventricular injection and then daily for quantitative determination of IVH volume. Additional CT scans were performed in the event of acute neurological deterioration. The volumes of intraventricular and intraparenchymal hematomas were measured independently by a blinded experienced researcher using standard computerized volumetric analysis as described by Steiner et al.[6] Size of IVH clot was taken into account in the analysis of clot resolution by using IVH clot volume standardized as a percent of the IVH clot volume on the stability CT. The time of each scan with respect to the time of the stability CT scan was determined to the minute and then converted to 24-hour periods and proportions thereof.

Statistical Analysis

Comparison of Groups

Demographic and clinical characteristics were compared between rtPA- and placebo-treated patients and between male and female patients. Wilcoxon rank-sum test, test of medians, Student *t* test, and χ^2 or Fisher exact test were used for comparisons as appropriate. Summary data are presented as mean±SD unless otherwise indicated. "Baseline" refers to data at clinical presentation and "stability" to data at time of stability CT scan. Statistical analyses were performed using STATA 11.1 (STATA Corporation, College Station, TX).

Regression Models

Previous work with CT data of 5 to 7 days' duration[4] has depicted a nonlinear association between standardized IVH volumes and time. In the past, this has been dealt with by limiting linear models of the clot lysis process to the initial 72 hours after stability CT and by including quadratic terms when examining longer periods of time.[7] The distinct decrease in clot lysis rates after an initial period of lysis at a higher rate observed for most patients in this cohort led to the consideration of a spline or segmented linear regression model (Figure 1).[8]

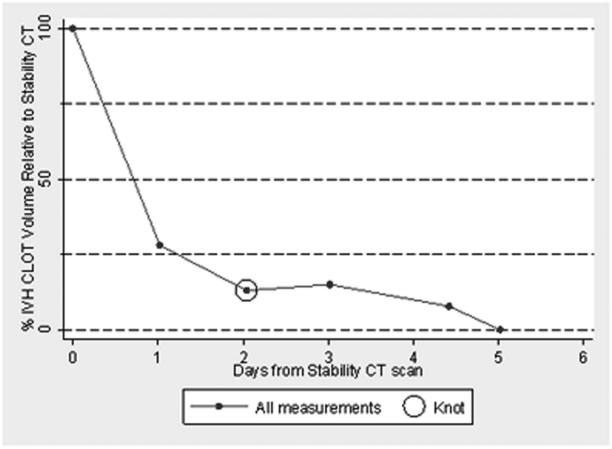

Figure 1. IVH clot volume remaining over time as a percentage of stability CT clot volume in 1 rtPA-treated patient showing the initial designation of the spline knot for this patient. IVH indicates intraventricular hemorrhage; rtPA, recombinant tissue-type plasminogen activator.

To implement the spline models, each patient's standardized IVH volumes were plotted against time and the plot was reviewed to identify the time at which a change in clot lysis rate (spline knot) seemed to take place if this phenomenon was present (initial time estimate). Alternative models in which the spline knot was shifted to either side of the initial time estimate were compared. The time associated with the highest likelihood (best fit) was selected for the spline knot. If there was a tie in the likelihood for 2 times, the earlier time was chosen. No spline knot was assigned for a patient's data if the relationship appeared linear.

A series of single candidate regression models was run to determine factors potentially affecting clot lysis for inclusion in a multivariate regression model. All analyses were performed with an offset of 100% instead of a constant term and included linear terms for time before and after the spline knot with crossproducts of each term with treatment with rtPA (base model). Crossproducts of these 2 linear terms with each of the candidate factors were then added to individual models. We used a random effects model, which accounted for multiple measurements per patient, and included clot volume measurements up to 6 days after the stability CT. We included rtPA dose as a variable in the analysis with dose groups as follows: Group 1=0.3 mg every 12 hours; Group 2=1.0 mg every 8 hours or every 12 hours; Group 2*=1.0 mg every 12 hours only; Group 3=3.0 mg every 12 hours; and Group 4=1.0 mg every 8 hours rtPA only. The effect of each candidate factor on clot lysis rates was adjusted for the effect of rtPA treatment. For each factor, its effect on rate of clot lysis before and after the spline knot, and the change between these 2, was estimated.

Several demographic and clinical characteristics were considered as the additional factors in the single candidate models: gender, stability IVH and intracerebral hemorrhage volume, time from symptom onset to first injection of study agent, side of EVD relative to side of greatest ventricular clot volume, and biological effectors/modulators of clotting including age, use of antiplatelet drugs, alcohol and cocaine, baseline platelet count, prothrombin time, partial thromboplastin time, serum fibrinogen, and plasminogen. Factors showing a strong relationship with clot lysis before the knot ($P<0.05$) were then combined into a multiple regression analysis with the basic model terms also included. We also evaluated baseline CSF level of plasminogen available in 44 rtPA-treated patients in a separate multivariate analysis of a spline model with log-transformed baseline CSF plasminogen.

The basic spline model was compared with a model with time treated as a linear variable and with a model with time treated as a quadratic variable using the log-likelihood ratio and Akaike Information Criterion as appropriate.

Results

For baseline clinical, laboratory, and CT characteristics of the patients in this study, refer to supplementary data (online-only Supplemental Tables I and II). There were significantly more males in the rtPA-treated groups compared with the placebo-treated group (68% versus 32%; $P=0.003$). Comparing variables by gender over both treatment groups, female patients were older than male patients, and males were more likely to have a history of seizures and significant alcohol and cocaine use. Stability IVH volumes were larger in males than females (median 48 versus 27 mL; $P=0.038$) and males had higher admission cerebral perfusion pressure than females (median 92 versus 78 mm Hg; $P=0.004$).

Four patients were removed from the analysis due to evidence of large early expansion of IVH, which would have detracted from the purpose of this study to assess factors affecting clot lysis under the assumption that clot volume decreases over time. Seventy-four patients were treated with intraventricular rtPA for a median of 1.8 days (range, 0–13.6 days). Twenty-two patients were treated with placebo for a median of 5.8 days (range, 0–14.6 days).

Regression Models

The mean time from diagnostic to stability CT was 0.64±0.39 days (range, 0.0–1.7 days). Median spline knot time was 2.5 days after the stability CT scan (range, 0.1–5.2 days). Twenty-four patients had linear clot resolution over the period (maximum, 6 days) and their data points were considered to be in the prespline knot period. Time to first dose of study agent from symptom onset was a median of 1.2 days (range, 0.5–2.6 days) and time to last dose was a median of 3.6 days (range, 1.1–15.7 days). The overall rate of clot resolution during the first 6 days after the stability CT was 20.92% lysis of stability clot volume/day before the spline knot and a rate of 1.34% clot volume/day after the spline knot. In both treatment groups, rates were significantly different from zero prespline knot (placebo 9.44%, $P<0.001$; rtPA 24.07%, $P<0.001$) but not postspline knot (Table 1).

The results of the spline models to evaluate the effect of individual factors on IVH clot volume resolved per day are presented in Table 2. Each model is adjusted using a base model, which takes treatment effect and time into account. In that model, the rate of clot resolution for any rtPA dose was estimated as 14.63% a day greater than for placebo ($P<0.001$). After this adjustment, lower IVH stability volume, higher baseline serum plasminogen, and lower baseline platelet count were significantly associated with faster rate of clot resolution prespline knot. For each 10-L decrease in IVH stability volume, clot resolution increased by 0.85% of stability volume/day. For each 10-g/L increase in serum plasminogen, clot lysis increased by 1.01% of stability volume/day. For each 10×10^3/uL lower platelet count, clot lysis increased 0.40% of stability volume/day. Analysis of side of EVD placement required removal of 11 patients whose inferior vena cava placement was ambiguous (crossing the midline) and 8 patients with simultaneous dual EVDs. The faster rate of clot lysis before the knot with an ipsilateral placed catheter compared with a contralateral placed catheter was marginally significant ($P=0.051$). Gender, stability intracerebral hemorrhage volume, and time from symptom onset to first injection of study agent were not significantly associated with rate of clot resolution. Each rtPA dose group was associated with significantly faster clot lysis rate before the knot compared with placebo (and no difference after the knot). Comparison of rtPA dose groups with each other (Group Models 1 and 2) showed no differences in clot lysis rates before or after the knot. Because clot lysis rates were not dependent on rtPA dose, rtPA treatment was considered as any rtPA versus placebo. Test for a plasminogen–rtPA interaction was not significant ($P=0.79$; data not shown). Baseline partial thromboplastin time was the only factor associated with clot lysis rate postspline knot ($P=0.025$). Factors associated with a significant change in the rate of clot resolution at the deflection point were rtPA treatment (all doses), baseline serum plasminogen, baseline partial thromboplastin time, and baseline platelet count. The first 2 variables had the effect of flattening the clot lysis rate after the knot, whereas higher baseline partial thromboplastin time and higher baseline platelet count were associated with an increase in clot lysis rate after the knot.

Table 1. Clot Lysis by Time Period and Treatment Group

Time Period/ Treatment Group	Clot Lysis Rate (% IVH Volume/d Resolved Relative to Stability; 95% CI)	*P* Value
Prespline knot		
All patients	20.92 (18.76–23.08)	<0.001
Placebo-treated	9.44 (4.91–13.96)	<0.001
rtPA-treated	24.07 (21.69–26.46)	<0.001
Postspline knot		
All patients	1.34 (−1.46 to 4.14)	0.349
Placebo-treated	4.11 (−1.61 to 9.82)	0.159
rtPA-treated	0.55 (−2.54 to 3.64)	0.728

IVH indicates intraventricular hemorrhage; rtPA, recombinant tissue-type plasminogen activator (any dose).

In the multivariate analysis, rtPA treatment (any dose) as well as higher baseline serum plasminogen and lower baseline platelet count remained significant predictors of faster clot lysis prespline knot and indicated a change in the slope of the clot lysis rate (Table 3).

The effect of rtPA and baseline plasminogen on estimated IVH resolution over time from this analysis is shown in Figure 2.

The likelihood ratio test statistics comparing the quadratic and spline models with the linear model were highly significant ($P<0.001$ for both models), indicating that the spline and quadratic models do perform better than the linear model over the first 6 days after stability. The Akaike Information Criterion values for the quadratic model (8150.641) and spline model (8028.537) suggest choosing the spline model. The spline model also provides an easier interpretability of the coefficients and seems to represent a more biologically plausible model over longer time periods.

In a separate analysis of baseline CSF plasminogen levels in 40 patients (44 original; excluded because of assay uncertainty—3 zeros and 1 outlier), median CSF plasminogen

Table 2. Analysis of Factors Associated With IVH Clot Lysis Rate in the Acute Phase (Over 6 d Post Stability) Using a Spline Model

Variable	No.†	No. of Patients	Rate of Lysis/D Before Knot*			Rate of Lysis/D After Knot*			Rate/D Change: After Knot–Before Knot*		
			Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value
Base model: treatment with rtPA	524	96	14.634	(9.52–19.75)	<0.001	−3.559	(−10.06 to 2.94)	0.283	−18.193	(−24.91 to −11.48)	<0.001
Additional model‡: serum plasminogen level§	458	84	0.101	(0.03–0.17)	0.005	−0.013	(−0.08 to 0.06)	0.722	−0.114	(−0.19 to −0.04)	0.003
IVH stability volume§	524	96	−0.085	(−0.15 to −0.02)	0.007	−0.021	(−0.10 to 0.06)	0.592	0.063	(−0.02 to 0.15)	0.144
Platelet level§	524	96	−0.04	(−0.07 to −0.01)	0.004	−0.007	(−0.04 to 0.03)	0.671	0.032	(0.00–0.06)	0.049
Antiplatelet medication	524	96	−3.129	(−10.44 to 4.18)	0.401	−2.454	(−12.66 to 7.75)	0.637	0.674	(−10.01 to 11.36)	0.902
Fibrinogen§	478	88	−0.004	(−0.02 to 0.01)	0.685	0	(−0.02 to 0.02)	0.968	0.004	(−0.02 to 0.03)	0.719
History of cocaine	524	96	−1.644	(−7.72 to 4.44)	0.596	−0.493	(−8.26 to 7.27)	0.901	1.151	(−6.60 to 8.90)	0.771
History of alcohol	524	96	1.229	(−4.30 to 6.76)	0.663	−4.485	(−11.60 to 2.63)	0.217	−5.714	(−12.98 to 1.55)	0.123
PT stability§	474	87	−0.732	(−1.81 to 0.34)	0.181	−0.359	(−1.79 to 1.07)	0.623	0.374	(−1.10 to 1.85)	0.619
PTT stability§	489	90	−0.064	(−0.50 to 0.37)	0.775	0.64	(0.08–1.20)	0.025	0.704	(0.16–1.24)	0.011
Old age (<60 y)	524	96	−1.691	(−6.17 to 2.79)	0.459	1.316	(−4.36 to 7.00)	0.65	3.008	(−2.75 to 8.76)	0.306
Gender	524	96	0.067	(−4.48 to 4.62)	0.977	3.258	(−2.69 to 9.21)	0.283	3.192	(−2.76 to 9.14)	0.293
Stability ICH volume	524	96	−0.019	(−0.23 to 0.19)	0.857	0.013	(−0.26 to 0.28)	0.926	0.032	(−0.24 to 0.31)	0.817
Symptom onset to first dose, h	524	96	0.234	(−4.16 to 4.63)	0.917	−0.501	(−6.62 to 5.62)	0.872	−0.735	(−6.94 to 5.47)	0.816
Ipsilateral EVD	419	77	6.044	(−0.03 to 12.12)	0.051	−0.05	(−8.07 to 7.97)	0.99	−6.093	(−14.48 to 2.29)	0.154
Group Model 1	524	96									
Group 1_S_t			17.01	(8.62–25.40)	<0.001	−9.231	(−20.58 to 2.12)	0.111	−26.241	(−37.36 to −15.12)	<0.001
Group 2_S_t			14.168	(8.64–19.70)	<0.001	−2.442	(−9.42 to 4.53)	0.492	−16.61	(−23.78 to −9.44)	<0.001
Group 3_S_t			14.57	(8.30–20.84)	<0.001	−4.146	(−12.21 to 3.92)	0.314	−18.717	(−26.91 to −10.52)	<0.001
Group 1–Group 2			2.842	(−4.88 to 10.57)	0.471	−6.789	(−17.37 to 3.79)	0.208			
Group 1–Group 3			2.44	(−5.83 to 10.71)	0.563	−5.085	(−16.41 to 6.24)	0.379			
Group 2–Group 3			−0.402	(−5.75 to 4.95)	0.883	1.704	(−5.23 to 8.64)	0.630			
Group Model 2	524	96									
Group 1_S_t			17.015	(8.62–25.41)	<0.001	−5.136	(−14.94 to 4.67)	0.304	−26.26	(−37.38 to −15.14)	<0.001
Group 2*_S_t			14.989	(6.29–23.69)	0.001	3.858	(−5.11 to 12.83)	0.399	−15.24	(−26.31 to −4.17)	0.007
Group 3_S_t			14.576	(8.30–20.85)	<0.001	−0.043	(−5.73 to 5.64)	0.988	−18.728	(−26.92 to −10.54)	<0.001
Group 4_S_t			13.975	(8.24–19.71)	<0.001	1.12	(−3.32 to 5.56)	0.621	−16.965	(−24.39 to −9.54)	<0.001
Group 1–Group 2*			2.026	(−8.21 to 12.27)	0.698	−8.994	(−22.28 to 4.29)	0.185			
Group 1–Group 4			3.04	(−4.83 to 10.91)	0.449	−6.255	(−17.02 to 4.51)	0.255			
Group 2*–Group 4			1.014	(−7.18 to 9.21)	0.808	2.738	(−7.27 to 12.75)	0.592			
Group 1–Group 3			2.439	(−5.84 to 10.72)	0.564	−5.092	(−16.42 to 6.24)	0.378			
Group 2*–Group 3			0.413	(−8.17 to 9.00)	0.925	3.902	(−6.72 to 14.52)	0.471			
Group 3–Group 4			0.601	(−4.95 to 6.15)	0.832	−1.163	(−8.38 to 6.05)	0.752			

Group 1=0.3 mg every 12 h rtPA; Group 2=1.0 mg every 8 h or every 12 h rtPA; Group 2*=1.0 mg every 12 h rtPA only; Group 3=3.0 mg every 12 h rtPA; Group 4=1.0 mg every 8 h rtPA only. For each dose group, the baseline is placebo and the estimates are slope differences from placebo. Group Model 1 combines 1 mg rtPA every 8 h and every 12 h groups; Group Model 2 considers these dose frequencies separately.

rtPA indicates recombinant tissue-type plasminogen activator; IVH, intraventricular hemorrhage; PT, prothrombin time; PTT, partial thromboplastin time; ICH, intracerebral hemorrhage; EVD, external ventricular drain.

*Each patient had a specific optimized spline knot to identify the deflection point in their lysis rate, if appropriate. Estimate is expressed as percent of the IVH volume on the stability CT scan resolved per day.

†No. of measurements across patients used in the model.

‡Each additional single candidate model had time and treatment with rtPA included with spline terms.

§Variable was centered by its median before a cross product with time relative to stability was obtained.

level was 8 g/L (range, 1–28 g/L), representing 7.1% of median serum plasminogen (range, 0.01%–24.9%). The correlation between baseline CSF plasminogen and baseline IVH volume was significant (Spearman $\rho=0.447$; $P=0.003$), whereas the correlations between serum and CSF plasminogen and between serum plasminogen and baseline IVH volume were not. A multivariate analysis of a spline model with log-transformed baseline CSF plasminogen in those treated with rtPA showed a trend between lower CSF plasminogen and faster clot lysis rate: 2.6%/day for each 2-fold decrease in plasminogen ($P=0.09$).

Discussion

Factors Affecting IVH Clot Lysis: Main Results

Based on these clot lysis rates from the CLEAR IVH studies, predictors of faster IVH volume resolved per day over the first 6 days of treatment from clot stability were intraventricular rtPA treatment, higher baseline serum plasminogen, and lower baseline platelet count. Our initial hypothesis, that female gender would be associated with improved clot lysis rates in rtPA-treated patients with IVH, was not observed. Initial IVH volume and ipsilateral EVD placement relative to

Table 3. Final Model of Factors Associated With IVH Clot Lysis Rate in the Acute Phase Using a Spline Model

Variable	No.†	No. of Patients	Rate of Lysis/D Before Knot*			Rate of Lysis/D After Knot*			Rate/d Change: After Knot–Before Knot*		
			Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value
Time relative to stability	458	84	10.05	(5.73–14.37)	<0.001	4.61	(−0.86 to 10.08)	0.099	−5.44	(−11.49 to 0.61)	0.078
Treatment with rtPA	458	84	13.88	(9.06–18.70)	<0.001	−2.949	(−9.29 to 3.39)	0.362	−16.828	(−23.74 to −9.92)	<0.001
Serum plasminogen level‡	458	84	0.128	(0.06–0.19)	<0.001	−0.009	(−0.08 to 0.06)	0.783	−0.138	(−0.21 to −0.06)	<0.001
Platelet level‡	458	84	−0.069	(−0.10 to −0.04)	<0.001	−0.001	(−0.04 to 0.04)	0.967	0.068	(0.03–0.11)	0.002

IVH indicates intraventricular hemorrhage; rtPA, recombinant tissue-type plasminogen activator (any dose).

*Each patient had a specific spline knot to identify the deflection point in their lysis rate. Estimate is expressed as percent of the IVH volume on the stability CT scan resolved per day.

†No. of measurements across patients used in the model.

‡Variable was centered by its median before a cross product was obtained.

side of greatest IVH volume were associated with faster clot lysis in the univariate but not in the multivariate analysis.

Fibrinolysis in IVH

Lysis of intraventricular clots could depend on the fibrinolytic activity of the CSF rather than the "fibrinolytic state of the circulation."[9] Normal CSF, however, contains very low levels of fibrinolytic enzymes, although fibrinolytic activity (as measured by fibrinogen, plasminogen, and total protein) in both CSF and serum is higher in acute brain-injured states and in older compared with younger patients.[10] Plasminogen is normally excluded from the CSF by the blood–brain barrier due to its high molecular weight (92 kDa).[11] In a study of neonatal IVH, CSF plasminogen levels were only 0.55% of normal adult plasma (and only 2% of neonatal plasma), remained very low during the weeks after IVH, and were not significantly different from reference infants without IVH.[12] Our findings of extremely low CSF:serum plasminogen ratios at baseline are consistent with these data. A relative deficiency of plasminogen at the thrombus site may explain the finding of only modest clot lysis despite high concentrations of plasminogen activator locally. The source of plasminogen in the CSF is likely 2-fold: from blood including the original hemorrhage and production by microglial cells with diffusion across the inflamed ependymal lining of the ventricles.[13,14] Low levels of CSF plasminogen after IVH may reflect

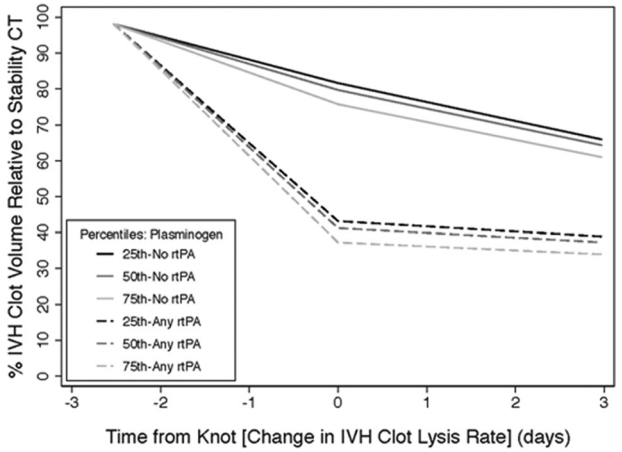

Figure 2. Estimated IVH clot volume remaining over time as a percentage of stability CT clot volume for placebo and rtPA patients by quartile of baseline serum plasminogen level. IVH indicates intraventricular hemorrhage; rtPA, recombinant tissue-type plasminogen activator.

that rtPA has activated all available plasminogen. Baseline CSF plasminogen was positively correlated with stability IVH volume suggesting that more serum plasminogen is incorporated into larger clots. The finding that higher serum plasminogen and possibly lower CSF plasminogen at the time of the first dose of study agent were associated with faster clot lysis rates supports the hypothesis that high serum concentrations of plasminogen in the initial hemorrhage provide more substrate for the plasminogen–rtPA interaction but quickly becomes depleted. Whether lower CSF plasminogen is a marker of more effective clot lysis or a lack of substrate is unknown. This analysis was independent of treatment effect because no study agent had yet been administered at the time of plasminogen measurement. Serial plasminogen levels in both CSF and plasma would be required to better understand this issue.

With a deficiency of thrombolytic factors in CSF, it has been postulated that, in untreated patients, plasminogen and rtPA within the clot are responsible for clot resolution and that this enzyme system is saturated by 24 to 48 hours leading to a constant percentage rate clot resolution thereafter.[7] Naff et al found that during the first 24 to 48 hours of untreated IVH, there was little if any clot resolution, whereas a substantial number of clots expanded during this time. In our study, this early latency period was not observed most likely because the first CT scan used for the analysis occurred at least 6 hours after EVD placement and approximately 18 hours after the diagnostic CT scan. In Naff's study, the rate of clot resolution over the first 10 days was 10.8% per day. This compares reasonably well with the prespline 9.4% clot resolution rate in placebo patients in this study. In rtPA-treated patients, administering thrombolytic agents hastened early clot resolution by 14.6%/day over treatment by placebo to achieve an average rate of 24%/day. After the spline knot, in rtPA-treated patients, the rate of clot resolution significantly decreased (to 0.55%/day) either because the CSF plasminogen/rtPA thrombolytic system became saturated or because many patients had stopped receiving intraventricular rtPA due to radiographic clearing of blood from third and fourth ventricles, which prompted stopping the study drug.

Implications of Clot Composition on IVH Lysis Rates

The finding that lower baseline platelet level is associated with faster clot lysis suggests that IVH clot composition influences clot dissolution independent of rtPA effect. In the current

understanding of fibrinolysis, plasminogen activation occurs on the clot surface and creates a lytic zone at the fluid solid interface, which is propagated to the core of the clot.[15] Dissolution of thrombus depends on diffusion and permeation, which are affected by clot composition. Specifically fibrinogen-to-fibrin conversion results in a heterogeneous gel phase mesh to which platelet attachment through glycoprotein IIb/IIIa to fibrin increases fiber density in platelet-rich areas.[16] Higher platelet concentration in clots is known to significantly reduce the rate of fibrinolysis under both static and flow conditions.[17,18]

Initial Hypothesis

Our initial hypothesis, that female gender would be associated with improved clot lysis rates in rtPA-treated patients with IVH, was not confirmed by these data. The only predictor related to female gender was initial IVH clot volumes, which were significantly smaller in females. Quantitative MRI studies of elderly volunteers (mean age, 75 ± 5 years) report a difference in CSF volume of the lateral ventricles between genders of approximately 10 mL (greater in males).[19] It is arguable that the anatomic difference in normal ventricular size may not fully explain the 19-mL difference in median IVH volume between genders.

Limitations

Although the population size is small, this represents the largest comprehensive data set of adult IVH analyzed to date and the effort to obtain daily CT scans permitted a much more detailed examination of rate of clot resolution. Serial measurements of fibrinolytic factors would have been optimal in this study and are therefore under investigation in upcoming trials. Moreover, many other factors regulating fibrinolysis including plasminogen activation, fibrin and thrombus structure, and EVD positioning are not accounted for in this analysis. Based on the inclusion criteria, results from this study cannot be generalized to patients with IVH and intracerebral hemorrhage >30 mL nor to patients whose clot volumes do not stabilize within 48 hours of diagnosis.

Conclusions

Future assessments of fibrinolysis in the cerebral ventricle will need to consider plasminogen availability and thrombus structure, especially the impact of platelets. A better understanding of the factors regulating fibrinolysis in IVH could lead to patient-specific dose adjustments of fibrinolytic agents and possibly to novel delivery agents such as plasminogen. Optimizing plasminogen activation through either improved matching of thrombolytic dose to plasminogen availability and/or mechanical dissolution of clot to increase clot surface area may lead to faster clot lysis and removal at the same time as reducing risk of rehemorrhage. If faster IVH removal translates into better clinical outcomes (the hypothesis of the ongoing CLEAR III IVH trial), then enhancing this process may improve recovery from severe IVH.

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References

1. Pang D, Sclabassi RJ, Horton JA. Lysis of intraventricular blood clot with urokinase in a canine model: part 2: in vivo safety study of intraventricular urokinase. *Neurosurgery*. 1986;19:547–552.
2. Wang YV, Lin CW, Shen CC, Lai SC, Kuo JS. Tissue plasminogen activator for the treatment of intraventricular hematoma: the dose effect relationship. *J Neurol Sci*. 2002;202:35–41.
3. Mayfrank L, Lippitz B, Groth M, Bertalanffy H, Gilsbach JM. Effect of recombinant tissue plasminogen activator on clot lysis and ventricular dilatation in the treatment of severe intraventricular hemorrhage. *Acta Neurochir*. 1993;122:32–38.
4. Naff N, Williams M, Keyl PM, Tuhrim S, Bullock RM, Mayer S, et al. Low-dose rtPA enhances clot resolution in brain hemorrhage: the intraventricular hemorrhage thrombolysis trial. *Stroke*. 2011;42:3009–3016.
5. Naff NJ, Hanley DF, Keyl PM, Tuhrim S, Kraut M, Bederson J, et al. Intraventricular thrombolysis speeds blood clot resolution: results of a randomized, double-blinded controlled clinical trial. *Neurosurgery*. 2004; 54:577–583.
6. Steiner L, Bergvall U, Zwetnow N. Quantitative estimation of intracerebral and intraventricular hematoma by computer tomography. *Acta Radiol Suppl*. 1975;346:143–154.
7. Naff NJ, Williams MA, Rigamonti D, Keyl PM, Hanley DF. Blood clot resolution in human cerebrospinal fluid: evidence of first-order kinetics. *Neurosurgery*. 2001;49:614–619.
8. Harrell FE. *Regression Modeling Strategies*. New York, NY: Springer; 2001.
9. Takashima S, Koga M, Tanaka K. Fibrinolytic activity of human brain and cerebrospinal fluid. *Br J Exp Pathol*. 1969;50:533–539.
10. Hansen AR. CNS fibrinolysis. a review of the literature with a pediatric emphasis. *Pediatr Neurol*. 1998;18:15–21.
11. Wu KK, Jacobsen CD, Hoak JC. Plasminogen in normal and abnormal human cerebrospinal fluid. *Arch Neurol*. 1973;28:64–66.
12. Whilelaw A, Mowinckel MC, Abildgaard U. Low levels of plasminogen in cerebrospinal fluid after intraventricular hemorrhage: a limiting factor for clot lysis? *Acta Paediatr*. 1995;84:933–936.
13. Nakajima K, Tauzaki N, Nagata K, Takemoto N, Kohsaka S. Production and secretion of plasminogen in cultured rat cell microglia. *FEBS Lett*. 1992;308:179–182.
14. Nakajima K, Nagata K, Hamanoue M, Takemoto N, Kohsaka S. Microglia derived elastase produces a low molecular-weight plasminogen that enhances neurite outgrowth in rat neocortical explant cultures. *J Neurochem*. 1993;61:2155–2163.
15. Kolev K, Longstaff C, Machovich R. Fibrinolysis at the fluid-solid interface of thrombi. *Curr Med Chem Cardiovasc Hematol Agents*. 2005;3:341–355.
16. Collet JP, Montalescot J, Lesty C, Weisel JW. A structural and dynamic investigation of the facilitating effect of glycoprotein IIb/IIIa inhibitors in dissolving platelet-rich clots. *Circ Res*. 2002;90:428–434.
17. Kunitada S, FitzGerald GA, Fitzgerald DJ. Inhibition of clot lysis and decreased binding of tissue-type plasminogen activator as a consequence of clot retraction. *Blood*. 1992;79:1420–1427.
18. Komorowicz E, Kolev K, Léránt I, Machovich R. Flow rate-modulated dissolution of fibrin with clot-embedded and circulating proteases. *Circ Res*. 1998;82:1102–1108.
19. Coffey CE, Saxton JA, Ratcliff G, Bryan RN, Lucke JF. Relation of education to brain size in normal aging: implications for the reserve hypothesis. *Neurology*. 1999;53:189–196.