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Elevated plasminogen activators are associated with hematoma progression in spontaneous intracerebral hemorrhage

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ABSTRACT

Endogenous fibrinolysis might lead to hematoma progression in spontaneous intracerebral hemorrhage (ICH). We studied plasma biomarkers of fibrinolysis and hemostasis in twenty-two patients with ICH and nine healthy controls (HC) in a single-center study. Patients with ICH had significantly higher D-dimer and plasmin-alpha-2-antiplasmin complexes compared to HC. At baseline, patients with hematoma progression had higher urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) and lower plasminogen levels, compared to those with no progression. 24-hour and day-7 matrix metalloproteinase-9 (MMP-9) was significantly increased in patients with hematoma progression.

1. Introduction

Fibrinolysis is initiated by two major plasminogen activators, tissue-type plasminogen activator and urokinase-type plasminogen activator in response to tissue injury[1] and may contribute to hematoma expansion. Plasminogen activators had not been previously shown to be associated with hematoma expansion in spontaneous intracerebral hemorrhage (ICH) to the best of our knowledge.

In the Tranexamic acid for IntraCerebral Hemorrhage-2 (TICH-2) trial, patients who were treated with tranexamic acid had a significant reduction of hematoma expansion.[2] In this sub-study, we explored if fibrinolysis plays a role in hematoma expansion by studying several plasma biomarkers.

We explored if there were differences in plasma biomarkers between healthy controls (HC) and ICH patients; and between ICH patients with and without hematoma progression. We also assessed the effects of tranexamic acid on the plasma biomarkers.

2. Methods

This was a sub-study of the TICH-2 trial conducted in Nottingham City Hospital, United Kingdom. The TICH-2 was a randomized placebo-controlled trial, which examined the efficacy and safety of tranexamic acid in spontaneous ICH.[2] Patients recruited into the trial were eligible for the sub-study unless there was coagulopathy, systemic hypotension, or admission to intensive care unit. In addition, HC who were age- and sex-matched to the patients were recruited. HC were excluded if they had a stroke, ICH, venous thromboembolism or been hospitalised in the preceding three months, or taken tranexamic acid or an anticoagulant in the preceding seven days. Ethics approval was obtained prior to commencement. Informed consent was obtained from all participants.

Venous blood was collected using EDTA tubes at baseline (before the first dose of tranexamic acid or placebo), 24-hour and day-7. For HC, only one blood sample was obtained. Platelet-poor plasma samples were obtained by centrifuging blood samples at 2000*g* for 10 min at 4 °C within 30 min of venepuncture. Samples were aliquoted and stored at −80 °C, then analyzed in batches.

Assays assessed tissue-type plasminogen activator (tPA; ab190812, Abcam, Cambridge, UK), urokinase-type plasminogen activator (uPA; ab119611, Abcam, Cambridge, UK), plasminogen (As-

Abbreviations: HC, healthy controls; ICH, intracerebral hemorrhage; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; PAP, plasmin-alpha-2-antiplasmin complex; TAT, thrombin-antithrombin complex; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator.

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sayMax, St Charles, US), plasmin-alpha-2-antiplasmin complex (PAP; SKU:HPAPKT-COM, Molecular Innovations, Michigan, US), D-dimer (ab196269, Abcam, Cambridge, UK), fibrinogen antigen (ab208036, Abcam, Cambridge, UK), thrombin-antithrombin complex (TAT; SEA831Hu, CloudClone, Texas, US), matrix metalloproteinase-2 (MMP-2; KHC3082, Invitrogen, California, US) and matrix metalloproteinase-9 (MMP-9; RK00217, ABclonal, Massachusetts, US). All assays were ELISA assays and performed in duplicates according to suppliers' protocol.

Hematoma progression was defined as an increase in hematoma volume on follow-up scan of >33% or >6 mL from baseline hematoma, or when follow-up scans were not available, early neurological deterioration (≥4 points increase in NIHSS or drop in GCS of ≥2) or death before 24-hour clinical assessment.

Due to the small sample size, data were not normally distributed. Non-parametric tests (Mann-Whitney U test) are used for statistical analyses. Data are shown as median [interquartile range, IQR], $p < 0.05$ is considered significant and analyses were performed using SPSS version 24.

3. Results

Twenty-two patients and nine HC were recruited. Plasma samples were collected in all participants at baseline, 19/22 at 24-hour (86.4%, 3 patients died) and 16/22 on day-7 (72.7%, 5 died and one on end-of-life care). There were no significant differences in the baseline characteristics between ICH patients and HC (Table 1). Nine patients received tranexamic acid while 13 received placebo. There were no differences in baseline characteristics between treatment groups apart from a greater proportion of lobar hemorrhage in the tranexamic acid group (Table 1). There were higher baseline D-dimer and PAP levels in ICH patients compared to HC but no significant differences in other baseline biomarkers (Table 2).

Seven patients (31.8%) had hematoma progression including 3 (13.6%) with hematoma growth and 3 (13.6%) who died before 24-hours and one with neurological deterioration and died on day-5. Patients with hematoma progression had significantly higher baseline tPA and lower plasminogen levels (Table 2). Median uPA level was 2 times higher in the hematoma progression group ($p = 0.053$). At 24-hour, tPA and uPA levels in the hematoma progression group declined to levels similar to patients without hematoma progression and healthy controls (Fig. 1). Plasminogen levels in hematoma progression group decreased further at 24-hour before returning to baseline levels on day-7.

MMP-9 levels were similar between groups at baseline with levels in patients with hematoma progression gradually increase at 24-hour (270.8 [200.5–322.6] vs 97.3 [60.3–207.7] ng/mL; $p = 0.012$) and day-7 (656.6 vs 131.8 ng/mL, $p < 0.001$). There were no significant differences in other biomarkers between those who had hematoma progression and those had not at baseline, 24-hour and day-7.

There were no significant differences in biomarkers between tranexamic acid and placebo group apart from 24-hour plasminogen level, which was lower in tranexamic acid group (Fig. 2).

Table 1
Baseline characteristics of ICH patients who received tranexamic acid or placebo and healthy controls.

Variables	Tranexamic acid	Placebo	All ICH	Healthy controls	P^*
Participants (N)	9	13	22	9	
Age (years)	74.8 (13.9)	74.5 (9.5)	74.6 (11.2)	66.9 (13.7)	0.11
Sex (male)	3 (33.3	8 (61.5)	11 (50)	4 (44.4)	0.78
Medical history (%)					
Prior stroke	0 (0)	2 (15.4)	2 (9.1)	0 (0)	0.35
Hypertension	4 (44.4)	8 (61.5)	12 (54.5)	3 (33.3)	0.28
Diabetes mellitus	2 (22.2)	4 (30.8)	6 (27.3)	1 (11.1)	0.33
Hypercholesterolemia	1 (11.1)	3 (23.1)	4 (18.2)	1 (11.1)	0.63
Ischemic heart disease	2 (22.2)	3 (23.1)	5 (22.7)	0 (0)	0.12
Atrial fibrillation	1 (11.1)	1 (7.7)	2 (9.1)	0 (0)	0.35
Previous venous thromboembolism	1 (11.1)	0 (0)	1 (4.5)	0 (0)	0.52
Previous intracerebral hemorrhage	1 (11.1)	0 (0)	1 (4.5)	0 (0)	0.52
Peripheral artery disease	0 (0)	1 (7.7)	1 (4.5)	0 (0)	0.52
Prior antiplatelet therapy	2 (22.2)	7 (53.8)	9 (40.9)	2 (22.2)	0.32
Prior statin	2 (22.2)	6 (46.2)	8 (36.4)	1 (11.1)	0.16
Glasgow Coma Scale	15 [12.5, 15]	14 [14, 15]	15 [14, 15]	–	–
NIHSS	11 [2.5, 20]	14 [6.5, 21]	13.5 [5.75, 19.5]	–	–
Hematoma location (%) †				–	–
Supratentorial deep	2 (22.2)	10 (76.9)	12 (54.5)	–	–
Supratentorial superficial	6 (66.7)	3 (23.1)	9 (40.9)	–	–
Infratentorial	1 (11.1)	0 (0)	1 (4.5)	–	–
Intraventricular hemorrhage	1 (11.1)	3 (23.1)	4 (18.2)	–	–
Subarachnoid extension	0 (0)	4 (30.8)	4 (18.2)	–	–
Hematoma volume (mL)	31.8 (43.1)	33.0 (46.8)	32.5 (44.2)	–	–
Perihematomal edema volume (mL)	13.5 (19.0)	16.7 (17.2)	15.4 (17.6)	–	–

Data are number (%), median [interquartile range] or mean (standard deviation). NIHSS = National Institute of Health Stroke Scale.

* Comparison of ICH participants and volunteers by Chi-squared, Mann-Whitney U or Student t tests.

† Hematoma location differed between participants randomized to tranexamic acid and placebo, $p = 0.033$.

4. Discussion

Elevated tPA and uPA, and reduced plasminogen levels at baseline were associated with hematoma progression. Conversely, tPA, uPA and plasminogen levels in patients who did not have hematoma progression were similar to healthy controls. These results suggest plasminogen activators may play a role in hematoma progression in ICH.

Although ICH patients had higher D-dimer and PAP levels compared to HC, the levels were not increased in patients with hematoma progression. One possible explanation is that systemic activation of the fibrinolytic system only occurs when there is intraventricular or subarachnoid extension, as supported by two studies, which showed significantly higher D-dimer and PAP in patients with intraventricular or subarachnoid extension.[3,4] Unfortunately we only had 4 cases of intraventricular hemorrhage and subarachnoid extension so we cannot test this hypothesis. Alternatively, tPA and uPA may have acted via a plasmin-independent pathway, e.g. upregulation of MMP-9 leading to hematoma growth as shown in animal

Table 2
Comparison of baseline plasma biomarkers of patients with hematoma progression and no hematoma progression.

Biomarkers	Healthy controls (n = 9)	All ICH (n = 22)	P	Baseline		
				Hematoma progression (n = 7)	No hematoma progression (n = 15)	P^*
tPA, pg/mL	3217.6 [2547.1–4083.3]	3688.0 [2646.4–6263.4]	0.49	6234.2 [4229–9251]	2801.0 [2398.0–4351]	0.010
uPA, pg/mL	338.9 [97.0–518.0]	245.9 [99.6–550.0]	0.90	555.7 [205.1–706.4]	203.8 [93.6–430.6]	0.053
Plasminogen, μg/mL	212.5 [182.0–249.4]	211.5 [194.7–273.1]	0.90	199.0 [176.7–208.0]	218.2 [202.6–317.6]	0.032
PAP, ng/mL	1.2 [0.9–1.5]	1.8 [1.1–2.4]	0.026	1.4 [1.0–2.6]	1.8 [1.7–2.3]	0.75
TAT, ng/mL	174.5 [159.3–196.3]	183.5 [169.9–200.5]	0.46	184.7 [175.1–196.4]	182.3 [141.4–220.3]	0.75
D-dimer, ng/mL	357.0 [189.5–425.5]	809.5 [417–1294.5]	0.002	673 [411–1279]	1012 [419–1299]	0.50
Fibrinogen, mg/mL	5.2 [2.2–8.1]	4.8 [3.3–7.2]	0.73	4.8 [3.9–11.9]	4.5 [3.0–6.7]	0.55
MMP-2, ng/mL	27.0 [23.0–30.0]	26.0 [20.5–27.5]	0.47	26.0 [22.0–27.0]	24.0 [19.8–29.8]	0.63
MMP-9, ng/mL	97.0 [77.2–134.4]	142.0 [81.1–199.6]	0.28	104.1 [24.4–200.8]	148.3 [86.1–199.2]	0.50

Data are median [interquartile range].
* Mann-Whitney U test is used for analysis. tPA = tissue-type plasminogen activator; uPA = urokinase-type plasminogen activator; PAP = plasmin-α2antiplasmin complex; TAT = thrombin-antithrombin complex; MMP-2 & -9 = matrix metalloproteinases-2 & -9.

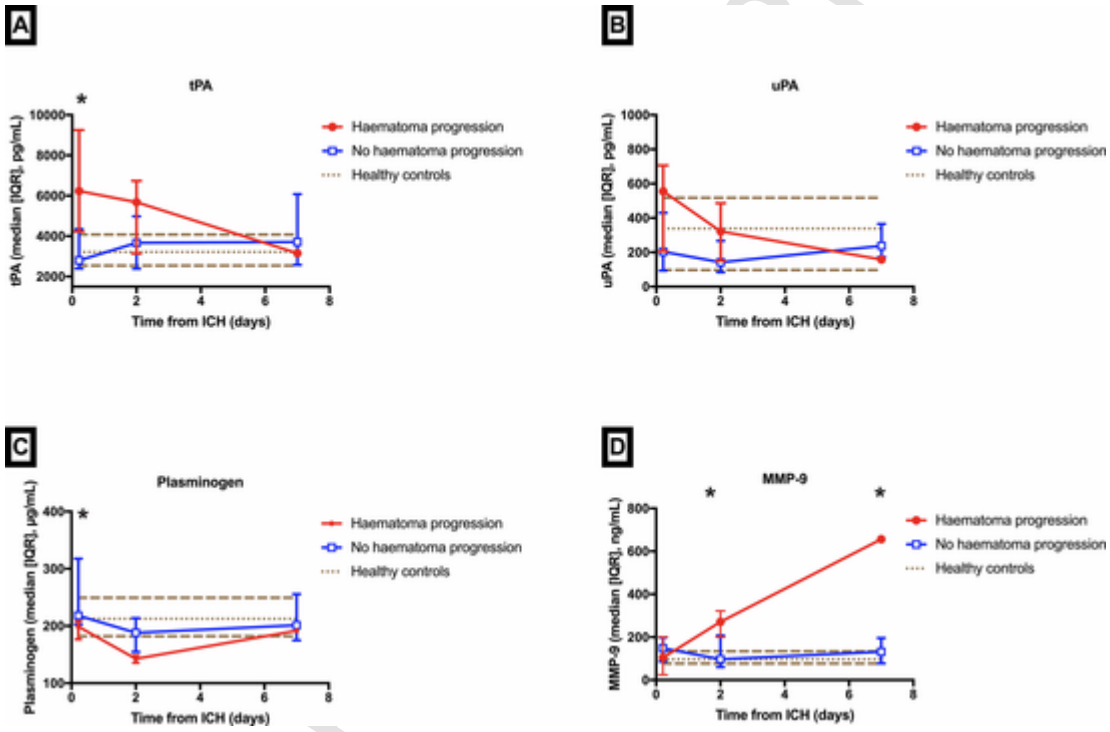

Fig. 1. Illustrates trend of tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), plasminogen and matrix metalloproteinase-9 (MMP-9) between baseline, 24-hour and day-7. *Indicates $p < 0.05$ on Mann-Whitney U test comparison between hematoma progression and no hematoma progression. There was no difference in all baseline, 24-hour and day-7 levels between the no hematoma progression group and healthy controls.

models.[5] The progressive rise of MMP-9 levels in patients with hematoma progression in the current study support this hypothesis.

Tranexamic acid inhibits tPA and uPA but higher doses of tranexamic acid paradoxically potentiate uPA action.[6] In our study, there was no difference in tPA and uPA levels at 24-hour, as the levels may have naturally declined in both tranexamic acid and placebo groups by then.

The strength of this study is its novel findings of increased plasminogen activators in hematoma progression. A main limitation is that it is exploratory in nature, and has a small sample size. Regression analysis, which would have allowed us to account for effects of variables such as hematoma volume, was not feasible due to the small sample size. Finally, due to the dynamic nature of spontaneous ICH, repeated sampling within the first 24 hours might have picked up more rapid changes in biomarker levels.

In conclusion, the role of uPA and tPA in hematoma progression in ICH needs to be further explored with larger studies. If the relation is proven significant, plasma biomarkers may have a role in targeting hemostatic treatment in ICH.

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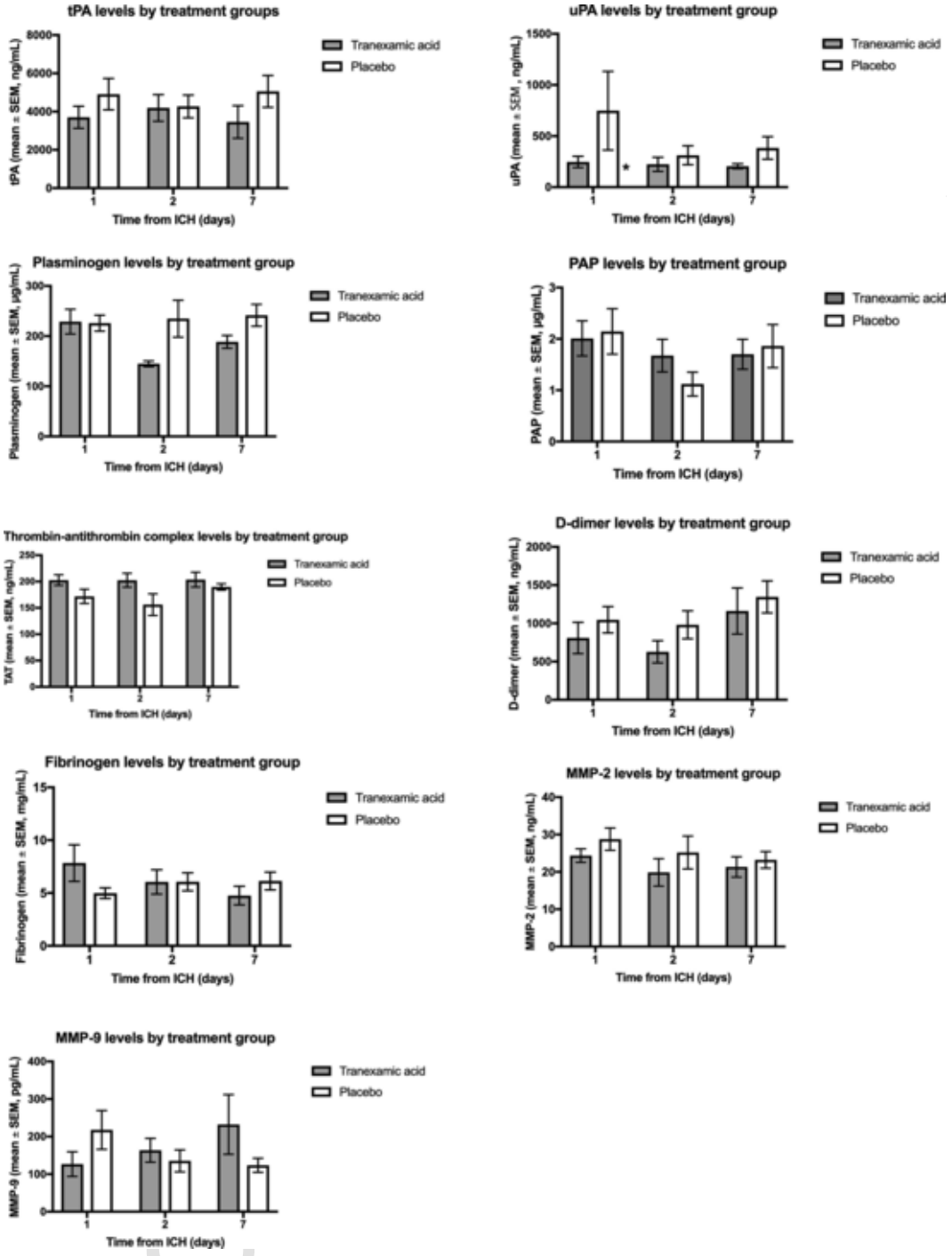

Fig. 2. Comparison of baseline, 24-hour and day-7 plasma biomarkers in patients who were treated with tranexamic acid and placebo. There was no significant difference (all $p > 0.05$) in all comparison except 24-hour plasminogen levels which were lower in tranexamic acid group ($p = 0.004$).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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