Coagulation and Fibrinolytic Activity of Tenecteplase and Alteplase in Acute Ischemic Stroke

Xuya Huang, MRCP; Fiona Catherine Moreton, MRCP; Dheeraj Kalladka, MRCP; Bharath Kumar Cheripelli, MRCP; Rachael MacIsaac, PhD; R. Campbell Tait, MBChB; Keith W. Muir, MD

Background and Purpose—We compared the fibrinolytic activity of tenecteplase and alteplase in patients with acute ischemic stroke, and explored the association between hypofibrinogenaemia and intracerebral hemorrhage.

Methods—Venous blood samples from a subgroup of participants in the Alteplase–Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) study were obtained at pretreatment, 3 to 12 hours, and 24±3 hours post-intravenous thrombolysis for analyses of plasminogen, plasminogen activator inhibitor-1, d-dimer, factor V, fibrinogen, and fibrin(ogen) degradation products, in addition to routine coagulation assays. Related sample Wilcoxon signed-rank tests were used to test the within-group changes, and independent Mann–Whitney tests for between-group differences.

Results—Thirty patients were included (alteplase=14 and tenecteplase=16) with similar baseline demographics. Compared with baseline, alteplase caused significant hypofibrinogenaemia (P=0.002), prolonged prothrombin time (P=0.011), hypoplasminogenaemia (P=0.001), and lower factor V (P=0.002) at 3 to 12 hours after administration with persistent hypofibrinogenaemia at 24 hours (P=0.011), whereas only minor hypoplasminogenaemia (P=0.029) was seen in the tenecteplase group. Tenecetepase consumed less plasminogen (<0.001) and fibrinogen (P=0.002) compared with alteplase.

Conclusions—In patients with acute ischemic stroke, alteplase 0.9 mg/kg caused significant disruption of the fibrinolytic system, whereas tenecteplase 0.25 mg/kg did not, consistent with the trend toward lower intracerebral hemorrhage incidence with tenecteplase in the ATTEST study.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01472926.

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Key Words: alteplase ■ cerebral hemorrhage ■ factor V ■ prothrombin time ■ stroke ■ tenecteplase ■ thrombolysis

Intravenous thrombolysis with alteplase in acute ischemic stroke improves clinical outcome, but is associated with an absolute risk of fatal intracerebral hemorrhage (ICH) of around 2.7%, ≈7-fold greater odds compared with placebo (odds ratio [95% confidence interval], 7.14 [3.98–12.79]).¹ In 2 phase 2 trials in acute ischemic stroke,²³ tenecteplase was associated with a trend toward fewer ICH complications.

As a substudy of the Alteplase–Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) study, we compared the effects of the 2 agents on coagulation and the fibrinolytic system, and explored potential associations with ICH.

Methods

The study protocol of ATTEST has been detailed elsewhere.³ Eligible thrombolysis candidates within 4.5 hours of onset were randomized to receive a standard alteplase regime (0.9 mg/kg) or 0.25 mg/kg tenecteplase. This substudy was initiated partway through the main trial. All trial participants were approached after it commenced.

Venous blood samples were collected into citrate (final concentration 0.109 mol/L, Greiner Bio-One, Austria) at baseline (pretreatment), 3 to 12 hours (TP2), and 24±3 hours (TP3) after the initiation of thrombolysis. Plasma was harvested by centrifugation immediately after sampling and stored at −80°C until analysis. We measured prothrombin time, activated partial thromboplastin time (APTT), fibrinogen, fibrin(ogen) degradation products, plasminogen, d-dimer, factor V (FV), plasminogen activator inhibitor-1 activity, and prothrombin fragment 1+2 (F1+2) at 3 TPs, respectively (assay methods are shown in detail in Table I in the online-only Data Supplement).

Statistical Analysis

Baseline values were expressed as mean±SD, changes at TP2 and TP3 as mean±SD percent change from baseline. We used related...
sample Wilcoxon signed-rank tests to examine the within-groups differences (TP2 versus TP1 and TP3 versus TP1), using a Bonferroni correction to yield a significance level of $P<0.025$. Between-group effects were explored with independent Mann–Whitney test. An univariate binary logistic regression model was used to explore any association between the change of fibrinogen and ICH.

### Results

Of 104 participants in the main ATTEST trial, 30 participated in this substudy (alteplase=14 and tenecteplase=16; Figure I in the online-only Data Supplement). Key baseline characteristics were similar between groups (Table).

### Effects on Coagulation and Fibrinolysis

Alteplase was associated with prolongation of prothrombin time ($P=0.005$), reduced fibrinogen ($P=0.011$) and plasminogen ($P=0.001$), elevated fibrinogen degradation products ($P=0.002$) 24-hour post-thrombolysis, a transient drop of factor V ($P=0.002$), and increase of $\alpha$-dimer ($P=0.003$) at TP2 (Figure; Table II in the online-only Data Supplement). In contrast, tenecteplase resulted only in elevation of fibrinogen degradation products ($P=0.009$), $\alpha$-dimer ($P=0.008$) ≤24 hours and transient reduction of plasminogen ($P=0.029$) at TP2.

Compared with tenecteplase, alteplase induced greater change of prothrombin time ($P=0.037$), fibrinogen ($P=0.002$), plasminogen ($P=0.001$), and factor V ($P=0.002$) at TP2, with sustained differences in prothrombin time ($P=0.031$), fibrinogen ($P=0.011$), and plasminogen ($P=0.001$) at 24 hours.

### Association Between ICH and Depletion of Fibrinogen

Six patients had hemorrhage post-thrombolysis (4 with alteplase and 2 with tenecteplase), 4 classified as hemorrhagic infarction type 1, 1 as hemorrhagic infarction type 2, and 1 had a small subarachnoid hemorrhage. None was considered symptomatic or severe subarachnoid hemorrhage. Matosevic et al$^8$ reported that within 6-hours post-thrombolysis, a decrease of ≥2 g/L in fibrinogen level was an independent predictor for bleeding of all kinds. Significant hypofibrinogenaemia (a decrease of 2 g/L or 50% from baseline) occurs in about one fifth of those receiving intravenous alteplase, whereas a tenecteplase dose escalation study$^9$ showed no severe hypofibrinogenaemia (fibrinogen <1 g/dL) in any dose (0.1–0.5 mg/kg) tested. Similarly, in our sample, tenecteplase treatment did not cause hypofibrinogenaemia using either of these criteria. We could not replicate an association between hypofibrinogenaemia and ICH, probably because of the small sample.

Limitations of our study include small sample size, variable time of sampling at TP2, and the low incidence of serious ICH (none having parenchymal hemorrhage or symptomatic clinical deterioration attributable to hemorrhage). Nonetheless, we found significant changes in coagulation and fibrinolysis after intravenous alteplase consistent with the literature, and minimal disruption with tenecteplase, consistent with a potentially better safety profile for tenecteplase, with retained fibrinolytic efficacy.

### Conclusions

In acute ischemic stroke, tenecteplase caused significantly less disruption to the coagulation and fibrinolytic systems compared

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### Table. Key Demographic and Stroke Characteristics of the 30 Patients

<table>
<thead>
<tr>
<th></th>
<th>Alteplase, n=14</th>
<th>Tenecteplase, n=16</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y, mean±SD)</td>
<td>70±12</td>
<td>69±15</td>
<td>0.95</td>
</tr>
<tr>
<td>Male (n, %)</td>
<td>10 (71%)</td>
<td>10 (63%)</td>
<td>0.71</td>
</tr>
<tr>
<td>OTT min (mean±SD)</td>
<td>187±52</td>
<td>181±47</td>
<td>0.75</td>
</tr>
<tr>
<td>Baseline NIHSS (median, IQR)</td>
<td>10 (6–15)</td>
<td>11 (8–17)</td>
<td>0.58</td>
</tr>
<tr>
<td>Cardioembolic stroke (n, %)</td>
<td>8 (57%)</td>
<td>8 (50%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Baseline vessel occlusion (n, %)</td>
<td>9 (64%)</td>
<td>8 (50%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Large vessel occlusion, ICA, M1, (n, %)</td>
<td>6 (43%)</td>
<td>6 (38%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Sampling time for TP2, h (median, IQR; range)</td>
<td>5.3 (4.8–10.1; 3.7–11.6)</td>
<td>4.4 (3.9–11.8; 3–12.1)</td>
<td>0.27</td>
</tr>
<tr>
<td>Sampling time for TP3, h (median, IQR; range)</td>
<td>23.8 (23.1–24.6; 20.9–25.5)</td>
<td>23.9 (23.5–24.6; 21.2–25.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Diurnal sampling for TP2* (n, %)</td>
<td>1 (7%)</td>
<td>5 (31%)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

ICA indicates internal carotid artery; IQR, interquartile range; M1, middle cerebral artery M1 segment; NIHSS, National Institutes of Health Stroke Scale; OTT, onset to treatment time; TP, time point; TP2, 2–12 h post-thrombolysis; and TP3, 24±3 h post-thrombolysis.

*Sampling time between 7 and 9 am. Frequencies were compared using $\chi^2$ test and Fisher test; mean or median values were compared using independent t test and Mann–Whitney U test, respectively.
with alteplase. This finding was consistent with the trend toward reduced incidence of ICH observed in the ATTEST trial.

**Acknowledgments**

We thank Prof. Gary Ford and Dr M.J. MacLeod from the Trial Steering Committee; Prof. Kennedy R. Lees, Drs Mark Parsons, and Christopher Weir from Data Safety Monitoring Committee; and Prof. Michael Hill and Dr Andrew Demchuk from External adjudicators.

**Sources of Funding**

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**Disclosures**

Dr Muir received a personal fee from Boehringer Ingelheim for speaking at a sponsored satellite meeting at European Stroke Conference 2013 on acute stroke treatment. Boehringer Ingelheim manufactures both drugs used in this trial. The other authors report no conflicts.

**References**


**Figure**. The changes of coagulation and fibrinolytic variables in alteplase- and tenecteplase-treated stroke patients from baseline to 24-hour post-thrombolysis. APTT indicates activated partial thromboplastin time; F1+2, prothrombin fragment 1+2; FDP, fibrinogen degradation products; PAI-1, plasminogen activator inhibitor-1; and PT, prothrombin time. *Statistical significant difference within or between groups.


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The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/46/12/3543

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2015/11/20/STROKEAHA.115.011290.DC1

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Supplemental Material

Additional discussion

Fibrin specificity

tPA activates plasminogen to plasmin, which breaks fibrin down to fibrin-degradation products. Non-fibrin-selective agents, such as streptokinase or urokinase significantly affect haemostasis by breaking down circulating fibrinogen as well as the fibrin in thrombus. Recombinant tPA (rtPA) more selectively binds to fibrin on the surface of clot, and activates mainly fibrin-bound plasminogen, which results in relatively local fibrinolysis, hence fewer bleeding complication and more targeted lysis. By restricting the activation of plasminogen to the clot surface, systemic plasminogen and fibrinogen pools are preserved to ensure more effective lysis. This property of selective binding to clot fibrin is termed fibrin specificity or affinity, and is approximately 15 fold greater in tenecteplase than alteplase. The expectation that tenecteplase should decrease haemorrhage incidence without compromising efficacy was evidenced by MI thrombolysis studies.

Clauss method of fibrinogen assay

Clauss method, a clot rate assay is the recommended method to analyse fibrinogen level by British Society for Haematology. It is the most sensitive method in detecting low fibrinogen levels and provides the lowest value among the three common used assays by reliably detecting values as low as 0.55g/L with normal plasma.

It is sensitive to heparin therapy and high FDP levels, which can produce falsely lower levels, but can be corrected with simple modification. In an acute MI thrombolysis study using alteplase, compared to sulphite precipitation in fibrinogen measurement, Clauss method is superior and more reliable in detecting hypofibrinogenaemia.
Figure I. Coagulation and fibrinolysis sub-study CONSORT chart.

Sub-study recruitment started on 27/12/2012
(patient No.67)

8 not included in the sub-study to avoid treatment delay:
3 presented > 4 hrs from symptoms onset;
5 had poor access.

30 patients included in the sub-study
(A=14, T=16)

TP1 samples=30;
TP2 samples =28 (1 haemolysed, 1 not done);
TP3 samples=28 (2 not done).

30 patients included in analysis
(A=14, T=16)

A =Alteplase; T=Tenecteplase; TP=Time Point.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Assay method &amp; analyser</th>
<th>Assay Kit</th>
<th>Reference Limits</th>
<th>Assay CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin Time</td>
<td>Clot-based [ACL TOP700 CTS]</td>
<td>HemosIL® ReCombiPlasTin2G *</td>
<td>9-13s</td>
<td>1.8-2.6%</td>
</tr>
<tr>
<td>APTT</td>
<td>Clot-based [ACL TOP700 CTS]</td>
<td>HemosIL® SynthASil *</td>
<td>27-38s</td>
<td>2.3-2.5%</td>
</tr>
<tr>
<td>Fibrinogen [Clauss]</td>
<td>Clot-based [ACL TOP700 CTS]</td>
<td>HemosIL® Fibrinogen-C XL *</td>
<td>2.0-4.1 g/L</td>
<td>7.2-8.3%</td>
</tr>
<tr>
<td>Factor V</td>
<td>Clot-based [ACL TOP700 CTS]</td>
<td>HemosIL® Factor V deficient plasma *</td>
<td>66-167 iu/dL</td>
<td>6.8%</td>
</tr>
<tr>
<td>Fibrin D-dimer</td>
<td>Latex immunoassay [ACL TOP700 CTS]</td>
<td>HemosIL® D- Dimer HS *</td>
<td>&lt; 243 ng/mL</td>
<td>5-7%</td>
</tr>
<tr>
<td>Fibrin(ogen) degredation products (FDP)</td>
<td>Latex immunoassay [ACL TOP700 CTS]</td>
<td>HemosIL® FDP *</td>
<td>&lt; 2.01 ug/mL</td>
<td>3.4%</td>
</tr>
<tr>
<td>Plasminogen activity</td>
<td>Chromogenic assay [ACL TOP700 CTS]</td>
<td>HemosIL® Plasminogen *</td>
<td>73-140 u/dL</td>
<td>5%</td>
</tr>
<tr>
<td>PAI-1 activity</td>
<td>ELISA **</td>
<td>ZYMUSEST PAI-1 Activity, HYPHEN BioMed</td>
<td>&lt; 5ng/mL</td>
<td>6.6-11.2%</td>
</tr>
<tr>
<td>Prothrombin F1+2</td>
<td>ELISA **</td>
<td>Enzygnost® 1+2 [monoclonal], SIEMENS</td>
<td>62-229 pmol/L</td>
<td>2.3-12.5%</td>
</tr>
</tbody>
</table>

* Assay kits manufactured and supplied by Instrumentation Laboratory Company (Bedford, United States)

** ELISA assays carried out on a TECAN Sunrise spectrophotometer (Labtech International Ltd, United Kingdom)
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>% change TP 2 versus TP1</th>
<th>% change TP3 versus TP1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alteplase</td>
<td>TNK</td>
<td>φ</td>
</tr>
<tr>
<td><strong>PT</strong></td>
<td>12±1</td>
<td>12±2</td>
<td>0.47</td>
</tr>
<tr>
<td><em>9-13 secs</em></td>
<td><em>APTT</em></td>
<td>30±3</td>
<td>29±3</td>
</tr>
<tr>
<td><em>27-38 secs</em></td>
<td><strong>Fibrinogen</strong></td>
<td>3.1±0.6</td>
<td>3.3±0.7</td>
</tr>
<tr>
<td><em>2.0-4.1 g/L</em></td>
<td><strong>FDPs</strong></td>
<td>3.8±3.8</td>
<td>8.3±19.5</td>
</tr>
<tr>
<td><em>&lt; 2.01ug/mL</em></td>
<td><strong>Plasminogen</strong></td>
<td>84±16</td>
<td>79±11</td>
</tr>
<tr>
<td><em>73-140 U/dL</em></td>
<td><strong>PAI-1 activity</strong></td>
<td>1.2±1.3</td>
<td>0.9±0.5</td>
</tr>
<tr>
<td><em>&lt;5 ng/mL</em></td>
<td><strong>D Dimer</strong></td>
<td>570±631</td>
<td>934±2419</td>
</tr>
<tr>
<td><em>&lt;243 ng/mL</em></td>
<td><strong>Factor V</strong></td>
<td>88±21</td>
<td>89±18</td>
</tr>
<tr>
<td><em>66-167 IU/dL</em></td>
<td><strong>F1+2</strong></td>
<td>308±173</td>
<td>413±518</td>
</tr>
<tr>
<td><em>62-229 pmol/L</em></td>
<td>**Baseline values were expressed as mean ± SD, the changes at TP2 (3-12 hours post thrombolysis) and TP3 (24±2 hours post thrombolysis) were expressed as mean ± SD % changes from baseline; <em>laboratory reference value; <em>P value between the groups; φP value within the group; TP Time point; PT Prothrombin time; APTT Activated Partial Thromboplastin Time; FDP Fibrin(ogen) degradation products; PAI-1 Plasminogen Activator Inhibitor-1; F1+2 Prothrombin Fragment 1&amp;2.</em></em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References


7. Mischke R, Menzel D, Wolling H. Comparison of different methods to measure fibrinogen concentration in canine plasma with respect to their sensitivity towards the fibrinogen degradation products x, y and d. *Haemostasis*. 2000;30:131-138
