The Effects of Alteplase 3 to 6 Hours After Stroke in the
EPITHET–DEFUSE Combined Dataset
Post Hoc Case–Control Study

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Background and Purpose—Two phase 2 studies of alteplase in acute ischemic stroke 3 to 6 hours after onset, Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET; a randomized, controlled, double-blinded trial), and Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution Study (DEFUSE; open-label, treatment only) using MR imaging-based outcomes have been conducted. We have pooled individual patient data from these to assess the response to alteplase. The primary hypothesis was that alteplase would significantly attenuate infarct growth compared with placebo in mismatch-selected patients using coregistration techniques.

Methods—The EPITHET–DEFUSE study datasets were pooled while retaining the original inclusion and exclusion criteria. Significant hypoperfusion was defined as a Tmax delay >6 seconds), and coregistration techniques were used to define MR diffusion-weighted imaging/perfusion-weighted imaging mismatch. Neuroimaging, parameters including reperfusion, recanalization, symptomatic intracerebral hemorrhage, and clinical outcomes were assessed. Alteplase and placebo groups were compared for the primary outcome of infarct growth as well for secondary outcome measures.

Results—From 165 patients with adequate MR scans in the EPITHET–DEFUSE pooled data, 121 patients (73.3%) were found to have mismatch. For the primary outcome analysis, 60 patients received alteplase and 41 placebo. Mismatch patients receiving alteplase had significantly attenuated infarct growth compared with placebo \((P=0.025)\). The reperfusion rate was also increased (62.7\% vs 31.7\%; \(P=0.003)\). Mortality and clinical outcomes were not different between groups.

Conclusions—The data provide further evidence that alteplase significantly attenuates infarct growth and increases reperfusion compared with placebo in the 3- to 6-hour time window in patients selected based on MR penumbral imaging.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00238537 (Stroke. 2013;44:87-93.)

Key Words: diffusion-weighted image ■ intravenous tissue plasminogen activator ■ MR mismatch ■ perfusion-weighted image

Recombinant tissue plasminogen activator (alteplase) is the only approved therapy for treatment of acute ischemic stroke.\(^1\) Alteplase is of proven benefit in patients with acute ischemic stroke within 4.5 hours of onset, but there is uncertainty of its benefit beyond this time. The clinical efficacy of alteplase is known to decrease with time,\(^4\) hence the selection of patients would be important to identify those most likely to respond.

Penumbral tissue is potentially salvaged by reperfusion and arterial recanalization after thrombolysis,\(^5\) and may be a valid target for therapy in patients with acute ischemic stroke. However, the method to define the penumbral tissue more precisely still remains a work in progress. EPITHET (Echoplanar Imaging Thrombolytic Evaluation Trial)\(^6\) was a phase 2, randomized, double-blinded, placebo-controlled trial.
in which the primary outcome measure was attenuation of infarct growth. Although there was a trend toward a positive result, this was not significant. Importantly, the method of measuring the mismatch volume was the standard volumetric technique in which volumes are calculated by simple subtraction. We recently developed a more precise method of evaluating mismatch tissue volumes in patients with acute ischemic stroke. This is based on precise coregistration of diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI) images so that lesion topography is taken into consideration. Using this approach, larger mismatch volumes are identified and, as a result, a reanalysis of EPITHET dataset revealed a significant attenuation by alteplase of infarct growth.

The more appropriate hemodynamic parameter and threshold definition for hypoperfusion using MR PWI is still being determined. Based on knowledge at the time, Tmax 2 or more was selected for EPITHET. This may have created quite generous volumes and a high proportion of patients with apparent mismatch. However, our more recent work and that of others suggest that a threshold Tmax value of around 6 seconds may be improved with an acceptable symptomatic intracerebral hemorrhage (SICH) rate.

Methods

We combined the EPITHET and DEFUSE datasets with individual patient data to allow an overall analysis. In both trials, patients received alteplase or placebo 3 to 6 hours after stroke onset. Study design, including patient eligibility, treatment allocation, and imaging protocol, have been reported previously. Briefly, EPITHET was a prospective, double-blind, randomized, multicenter-controlled phase 2 trial in which 101 patients received alteplase or placebo 3 to 6 hours after stroke onset. DWI, PWI, and magnetic resonance angiography (MRA) were obtained before treatment and at 3 to 5 days. T2-weighted images at day 90 were obtained to measure final infarct volume. DEFUSE was a prospective, open-label, nonrandomized, multicenter study of 74 consecutive stroke patients treated with alteplase between 3 and 6 hours after symptom onset. MRI scans, including DWI, PWI, and MRA, were obtained immediately before treatment with alteplase and after 3 to 6 hours. MRI scans at day 30 included a fluid-attenuated inversion recovery sequence to measure final infarct growth. National Institutes of Health Stroke Scale (NIHSS) score and modified Rankin Scale (mRS) at day 90 were evaluated for the analyses of clinical outcome in both the studies.

The baseline characteristics, stroke risk factors as well as serum glucose level, and blood pressure at admission were recorded in both trials. Initial stroke severity evaluated by NIHSS score was measured in all patients. DWI, PWI, MRA, and T2-weighted or fluid-attenuated inversion recovery image were also pooled for the current analysis. To standardize image analysis, the determination of lesion volumes for both DWI and PWI was performed manually by 1 investigator (B.C.) who was not blinded to treatment. Regions of interest were manually drawn using careful windowing to outline the maximal visual extent of the acute DWI (B1000 trace-weighted) lesion with reference to the apparent diffusion coefficient image to avoid regions of T2 shine-through. The B1000 image was used as the primary template because quantitative apparent diffusion coefficient thresholds tend not to accurately outline the visually evident lesion and have been shown to vary with time after stroke onset and perfusion status. Manual regions of interest were also drawn to the maximal extent of the final infarct on the coregistered day 90 T2 (EPITHET) or day 30 fluid-attenuated inversion recovery (DEFUSE) images. This was done in DWI space to avoid the step artifact sometimes introduced by transforming binary regions of interest. Hypoperfusion volumes were defined as a Tmax delay >6 seconds.

Using the pooled dataset, our primary hypothesis was that alteplase compared with placebo would attenuate infarct growth. Our secondary hypotheses were that increased reperfusion and recanalization rates as well as clinical outcomes would be improved with an acceptable symptomatic intracerebral hemorrhage (SICH) rate.

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Volumetric Approach

The primary hypothesis that there would be greater attenuation of infarct growth in patients with an imaging mismatch who received alteplase than in those who received placebo, the study outcomes were compared between alteplase and placebo in the patients with apparent mismatch defined by the coregistration method as defined in the Methods section. Baseline imbalance between treatment groups was estimated using t test, Mann-Whitney U test, and Fisher exact test as appropriate. Various measures of infarct growths were compared between the treatments groups as per original EPITHET analysis using parametric or nonparametric tests as appropriate. Fisher exact test was used for comparison of categorical outcomes, that is, mRS 0 to 2, 0 to 1, good neurological outcome, reperfusion, recanalization, SICH, and mortality. The outcomes were then compared with those given alteplase or placebo with DWI lesion volumes of >5 mL.

Hence, there were 165 patients available for mismatch assessment. Median volumetric and coregistration mismatch volumes were 23.6 mL (interquartile range, 0.0–81.1) and 39.0 mL (interquartile range, 8.3–90.9), respectively. Although excellent agreement was achieved overall between volumetric and coregistration mismatch volumes with intraclais coefficient of 0.94 (95% CI, 0.92–0.96) and Lin concordance correlation coefficient of 0.94 (95% CI, 0.92–0.96), the volumetric method generally underestimated mismatch volumes compared with the coregistration method. This is well illustrated in Figure 2, in which reduced major axes regression analysis (slope, 1.04; intercept, −15.55) indicated that volumetric method produced estimations of mismatch volume up to 15.55 mL lower than the coregistration method for small mismatch volumes, but this difference diminishes as mismatch volume is enlarged.

Coregistration Mismatch: Outcome Analyses

From the 165 patients with coregistration mismatch volumes, 44 patients who did not have apparent mismatch (PWI > DWI within volume × 1.2 and ≥10 mL) and a further 20 patients who did not have images allowing final infarct growth assessment were excluded. The number of drop-out patients was 19 in alteplase and 1 in placebo group. Hence, 101 patients were available to test the primary hypothesis (Figure 1). Of the enrolled patients, 60 were from the alteplase and 41 from the placebo groups. The last observation carried forward was used in 16 patients who had subacute DWI when final scan was unavailable. Table 2 showed the baseline characteristics of the patients with alteplase and placebo. There were no statistically significant differences in the baseline characteristics between

Results

Mismatch Volumes: Coregistration Versus Volumetric Approach

From a total of 175 patients in the EPITHET–DEFUSE pooled data, we excluded 10 patients. One patient withdrew consent immediately, whereas 1 and 8 patients had poor quality of DWI and PWI, respectively (adequate for volumetric analysis in original studies but inadequate for coregistration; Figure 1).
the alteplase and placebo groups excepting for a trend toward a higher baseline NIHSS in the tissue plasminogen activator group. Alteplase significantly attenuated all measures of growth using the allowable statistical approaches. This included median relative, absolute, and any growth and difference in cube–root volumes compared with placebo (Table 3). The ratio of geometric means cannot be reliably used as an outcome measure because of the violation of underlying normality assumption. The reperfusion proportion was significantly higher in the alteplase compared with placebo groups ($P=0.003$). All patients with SICH had been treated with alteplase. When SITS-MOST definition was used, half of the patients with SICH had malignant profile. Mortality was not different between treatment and placebo groups. Alteplase did not increase the proportion of patients reaching mRS of 0 to 2 ($P=0.85$) or 0 to 1 ($P=0.40$) statistically or proportion of good neurological outcome ($P=0.13$), but the study was not powered to assess these outcomes. When DWI lesion volumes of 5 mL or less were excluded (as in EPITHET), the results were also unchanged.

Figure 1. Patient enrollment and subsequent availability for analysis based on MR diffusion-weighted imaging (DWI) / perfusion-weighted imaging (PWI) coregistration method to define significant mismatch. EPITHET indicates Echoplanar Imaging Thrombolysis Evaluation Trial; and DEFUSE, Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution.

Figure 2. A plot of individual vs patient mismatch volumes (MV) using the volumetric vs the coregistration techniques.
Table 2. Baseline Characteristics for Patients With Coregistration Mismatch (n=101)

<table>
<thead>
<tr>
<th></th>
<th>Alteplase (n=60)</th>
<th>Placebo (n=41)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR)</td>
<td>75 (62–81)</td>
<td>74 (64–83)</td>
<td>0.85*</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>27 (45.0%)</td>
<td>20 (48.8%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Hypertension</td>
<td>38 (63.3%)</td>
<td>25 (61.0%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14 (23.3%)</td>
<td>8 (19.5%)</td>
<td>0.81</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>25 (41.7%)</td>
<td>12 (29.3%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>21 (35.0%)</td>
<td>19 (46.3%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>22 (36.7%)</td>
<td>16 (39.0%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Median glucose (IQR)</td>
<td>6.8 (6–8)</td>
<td>6.3 (5.9–7.7)</td>
<td>0.64*</td>
</tr>
<tr>
<td>Mean systolic BP (±SD)</td>
<td>143.8±17.9</td>
<td>147.0±18.3</td>
<td>0.39†</td>
</tr>
<tr>
<td>Mean diastolic BP (±SD)</td>
<td>75.4±12.4</td>
<td>77.3±15.7</td>
<td>0.51†</td>
</tr>
<tr>
<td>Median time from onset to treatment (min, IQR)</td>
<td>310 (278–339)</td>
<td>307 (250–330)</td>
<td>0.20*</td>
</tr>
<tr>
<td>Median NIHSS (IQR)</td>
<td>14 (10–17)</td>
<td>11 (9–17)</td>
<td>0.31*</td>
</tr>
<tr>
<td>Malignant profile</td>
<td>28 (46.7%)</td>
<td>21 (51.2%)</td>
<td>0.69*</td>
</tr>
<tr>
<td>Median DWI volume (mL, IQR)</td>
<td>17.9 (10.8–38.5)</td>
<td>20.9 (9.7–46.5)</td>
<td>0.69*</td>
</tr>
<tr>
<td>Median Tmax6 volume (mL, IQR)</td>
<td>62.4 (42.2–126.6)</td>
<td>91.8 (51.1–149.8)</td>
<td>0.19*</td>
</tr>
<tr>
<td>Median Mismatch volume (mL, IQR)</td>
<td>50.8 (27.1–98.0)</td>
<td>59.1 (36.2–109.8)</td>
<td>0.21*</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; DWI, diffusion-weighted imaging; IQR, interquartile range; Tmax6, Time to peak of the residue function delay >6 s.
* Mann-Whitney U test.
† Unpaired t test.

Discussion

Our primary aim in this study in which individual patients’ data from both EPITHET and DEFUSE were pooled was to test the hypothesis that alteplase would attenuate infarct growth compared with placebo. This was substantiated using a variety of statistical approaches. Our main secondary hypothesis, that alteplase would increase reperfusion rates compared with placebo, was also supported. This adds to the mounting evidence that patients selected based on MR mismatch criteria beyond 3 hours and up to 6 hours may be an ideal group in which to mount a phase 3 trial of thrombolytic therapy.

Although the European Cooperative Acute Stroke Study III (ECASS III) results suggested that the time window for thrombolytic therapy could be extended from 3 to 4.5 hours after stroke onset,1 and the treatment effect appears to diminish further beyond 4.5 hours, the possibility that patients selected based on the presence of viable tissue remains.18 This may also increase the number of patients eligible for the potential benefits of thrombolysis.5 It is already established that the larger ischemic brain tissue at risk of infarction (penumbra) patients have, the greater the benefit of thrombolytic therapy obtained.19 This must be balanced by the knowledge that mismatch tissue fragments with time and may not be eloquently placed topographically.7 Hence, the need for of appropriate mismatch identification cannot be overemphasized.

As a part of this emphasis, the calculation of mismatch volume and hypoperfusion thresholds have been points of contention. Previously, we used Tmax ≥2 seconds in both EPITHET and DEFUSE; this seems likely to identify exceedingly large volumes of hypoperfused tissue and may include benign oligemia.20 Supportive data are also available from patients with acute stroke studied with PWI and positron emission tomography; here, a 5.5-second threshold of Tmax was found to be the optimal cut point for identification of the penumbral threshold.20,21 Our own published and unpublished data also suggest that a Tmax threshold of ~6 seconds may more accurately reflect the penumbral threshold.9

In our primary analysis, we used the coregistration approach and provided evidence that alteplase attenuated infarct growth in the patients with apparent mismatch within 6 hours of symptom onset. The main benefit of the coregistration method was to increase the number of eligible patients from 89 to 121. This is probably because the volumetric method is less precise and underestimates mismatch volume.7 Although more time-consuming, the coregistration method should become clinically useful with advances in automated imaging analysis.21

We have also shown the evidence that alteplase increases the reperfusion rate significantly compared with placebo. Alteplase, which is a strong driver of reperfusion, attenuates infarct growth in the patients with mismatch, supports the mismatch hypothesis, and suggests that selecting patients with PWI/DWI mismatch beyond 3 hours would be a useful approach to extend the time window for thrombolytic therapy.22 Although it has been reported that early recanalization can result in less infarct growth and more favorable clinical outcomes,23,24 unlike reperfusion, the recanalization rate in the current study was not increased by alteplase. This may be explained in part by the fact that MRA was performed in EPITHET at 3 to 5 days, a time at which spontaneous recanalization might have already occurred. Also, the number of patients who were able to have an MRA of adequate quality was small (52 cases and 27 controls), thus reducing the statistical power.

The rate of SICH for the pooled dataset was ~8%, consistent with other studies of thrombolysis.18 The similarity of mortality rates between treatment and placebo groups was reassuring. However, because SICH is associated with high mortality in patients treated by alteplase,25 there is clearly a need to refine further selection criteria to enable exclusion of patients at risk. Some progress is being made with identification of the malignant profile12 or very low cerebral blood flow,26 as potential markers of bleeding risk.

One limitation of this study was its relatively small sample size. Although adequate to test our primary hypothesis, the combined EPITHET–DEFUSE datasets were too small to adequately assess clinical outcomes. A number of phase 3 trials are in progress with time windows up to 9 hours after stroke that have larger sample sizes (up to 400) powered to test hypotheses based on clinical outcomes.27,28 Another potential limitation is the imperfection of MR coregistration, which may result in mismatch volume estimation errors. Also, we used manual method in a few patients, which makes the procedure of coregistration difficult and complicated. Although the technology required for coregistration is advancing quickly, manual intervention is still sometimes required. Moreover, the therapy was performed between 3 and 6 hours, which might be ethical now that alteplase was administered in all
the patients within 4.5 hours. Further study should be done to test the hypothesis that patients were well selected using coregistration method.

Because only a small proportion of patients with ischemic stroke currently benefit from thrombolysis because of the narrow 3-hour (or 4.5 hours with ECASS III) time window,\(^5\) we believe that to extend the time window further is essential if a larger number of patients are to benefit. Certainly, this is biologically plausible given the known duration of the ischemic penumbra up to even 48 hours after stroke.\(^5\)

Based on our current analysis of this combined dataset with patients selected with significant mismatch, the administration of alteplase within 3 to 6 hours of stroke onset was found to attenuate infarct growth and increase reperfusion compared with placebo up to at least 6 hours after stroke. Hence, a phase 3 trial of thrombolysis for extended time windows using MRI selection techniques would be appropriate.\(^{27,28}\)

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### Disclosures

Dr Donnans has been a member of the Boehringer Ingelheim, PAION, Servier, and Sanofi-Aventis advisory boards, and has accepted honoraria or consultancy payments from and has had the costs of participating in scientific meetings reimbursed by Boehringer Ingelheim, Sanofi-Aventis, and Servier. Dr Davis has been a member of the PAION, Servier, and Novo Nordisk advisory boards, and has received honoraria for lectures from Novo Nordisk, Sanofi-Aventis, Pfizer, and Boehringer Ingelheim. Dr Albers is a consultant for Lundbeck and a member of the DIAS 3/4 Steering Committee; he also has equity interest in iSchemaView. The other authors have no conflicts to report.

### References


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