Intravenous thrombolysis with 0.9 mg/kg alteplase for acute ischaemic stroke: a network meta-analysis of treatment delay

Xi Chen,1 Yu Shen,1 Chengfang Huang,2 Yu Geng,3 Yunxian Yu1,4

ABSTRACT

Objectives The aim of this study was to evaluate the effect of alteplase in intravenous thrombolysis of acute ischaemic stroke (AIS) regarding the different time windows of treatment (<3 hours, 3–4.5 hours, >4.5 hours).

Methods A systematic literature search was conducted from PubMed, Cochrane Library and Embase. 12 clinical randomised controlled trials with 3402 patients with AIS met the inclusion criteria. The primary, secondary and tertiary outcomes were modified Rankin Scale (mRS) scores 0–1, mortality at 90th day after treatment and symptomatic intracerebral haemorrhage within 36 hours, respectively. Network meta-analysis and conventional meta-analysis were carried out for calculating odds ratio (OR), the surface under cumulative ranking curve (SUCRA) and the probabilities of being the best.

Results For mRS, alteplase regardless of time delay was significantly more effective than placebo (OR 1.33–2.17). However, alteplase used within 3 hours after AIS occurrence (SUCRA=98.3%) was significantly more effective (OR=1.64) than that at 3–4.5 hours (SUCRA=43%) and showed the trend of priority (OR=1.47) compared with that beyond 4.5 hours (SUCRA=58%). For the mortality, compared with placebo (SUCRA=64.7%), alteplase within 3 hours was similar to that of 3–4.5 hours whereas alteplase beyond 4.5 hours (SUCRA=7.3%) showed the trend of significantly increasing 85% mortality. For the tertiary outcome, alteplase within 3 hours (SUCRA=19.0%) was comparable with placebo (SUCRA=99.9%) whereas alteplase beyond 3 hours significantly increased (OR 5.89–6.67) the symptomatic intracerebral haemorrhage.

Conclusions Alteplase within 3 hours should be recommended as the best treatment delay for its best efficacy among all the intervention and equivalent safety compared with placebo. Alteplase beyond 3 hours was less effective compared with that within 3 hours and increased the risk of mortality on 3 months as well as symptomatic intracerebral haemorrhage at 36 hours. More head-to-head clinical trials are needed to confirm those findings.

INTRODUCTION

Acute ischaemic stroke (AIS) is the leading cause of disability and death in China.1 Intravenous thrombolysis with alteplase was a pivotal therapy for AIS. Current guidelines recommend 3 or 4.5 hours after the onset of stroke as the time window for intravenous thrombolysis.2 3 The recommendation was mainly established from the classical and famous international clinical trials including the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study (NINDs), European Cooperative Acute Stroke Study (ECASS), the Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke Study (ATLANTIS), Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET), as well as their serial trials.4–7 On top of that, more and more studies had verified the efficacy of alteplase in patients with stroke beyond 4.5 hours as well as wake-up stroke.8–10 Even though, a study showed no efficacy of alteplase exceeding 4.5 hours compared with placebo.11 Recently, a conventional meta-analysis of individual patient data from the aforementioned trials suggested that patients with ischaemic stroke 4.5–9 hours after onset treated with alteplase achieved better functional outcomes than placebo.12 Collectively, these data provided evidence supporting the hypothesis that patients with stroke after onset for less than 3 hours or 3–4.5 hours, or beyond 4.5 hours might probably benefit from alteplase.

However, to date, there was no clinical randomised controlled study nor cohort study comparing the effect and safety of those three different time delays of intravenous alteplase in AIS simultaneously, leaving the paucity of clinical reference. Therefore, in this network meta-analysis, we tried to illustrate the ranking probabilities and ORs in terms of the efficacy and safety profile of different time delays (in other words, time from onset to needle) of alteplase based on the comparison between alteplase and control (or placebo) as well as the pairwise comparison of different time delays (less than 3 hours or 3–4.5 hours, or beyond 4.5 hours).

METHODS

Searching strategy

We initiated our study by searching medical databases including PubMed, Cochrane Library and Embase with the following mesh terms: (‘alteplase’ or ‘rt-PA’ or ‘recombinant tissue plasminogen activator’) and (‘stroke’ or ‘infarction’) and (‘thrombolysis’ or ‘thrombolytic’). This meta-analysis was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guideline.

Inclusion criteria

Randomised controlled trials (RCT) investigating the effect and safety of intravenous alteplase as long as 3 months on thrombolysis for AIS were
The dosage of alteplase in each study should be 0.9 mg/kg. The treatment delay should apply 3 or 4.5 hours as cut-off or could be classified into less than 3 hours, 3–4.5 hours and beyond 4.5 hours from the baseline.

Exclusion criteria
The exclusion criteria were as follows: (1) alteplase was combined with other regimes in the thrombolysis, including mechanical recanalisation, and so on; (2) thrombolytic drugs were not exclusively alteplase and the data could not be separately extracted; (3) time of onset to needle was not recorded; (4) intra-arterial thrombolysis with alteplase; (5) proportion of patients with modified Rankin Scale (mRS) scores 0–1 on 3 months could not be obtained; (6) transient ischaemic attack; (7) non-adult patient studies; (8) patients in each arm were less than 20; (9) single arm study; and (10) non-English article.

Data extraction and quality assessment
Two investigators (XC and YS) independently searched studies and assessed the quality by using Jadad Scale for RCTs. Year of publication, gender distribution of participants, number of cases, age at stroke onset, baseline National Institutes of Health Stroke Scale (NIHSS) score, time of onset to needle and outcome measures (mRS scores 0–1 at 3 months (defined as favourable outcome), mortality at 3 months and symptomatic intracerebral haemorrhage (SICH) at 36 hours according to Safe Implementation of Thrombolysis in Stroke-Monitoring Study (STIS-MOST) criteria13) were collected. Any discrepancy was arbitrated by the senior reviewer (YY).

Endpoints
The primary endpoint was favourable outcome. The secondary endpoint was SICH at 3 months after AIS.

Statistical analysis
The OR and 95% CIs were used for evaluating the effect of outcomes. Conventional meta-analysis was performed for analysing all the endpoints from direct comparisons among each time delay of alteplase and control with statistical heterogeneity set as I²<50% and p>0.05 for fixed effect model, otherwise the random effect model would be applied. The implementations of network meta-analyses were carried out according to frequentist framework in Stata software V.15.0 by random effects model to compare the endpoints from pooled data (shown as forest plot) composed of direct and indirect comparisons among each time delay of alteplase and control. The surface under cumulative ranking curve (SUCRA) and the probabilities of being the best ranking curve (SUCRA) and the probabilities of being the best

RESULTS
Eventually, a total of 12 RCTs reporting 3402 patients were included (figure 1). Jadad score of RCTs ranged from 1 to 7. The mean age of patients ranged from 60 to 79 years. The proportion of male patients exceeded 50% in all studies except one. The mean NIHSS score ranged from 4 to 14.53 (online supplementary material).

Twelve of 12 studies reporting 3402 patients were eligible for analysing the primary endpoint. The network plot is shown in figure 2A, thrombolysis with alteplase within 3 hours compared with placebo was the most comparison. Most patients received placebo (n=1642), followed by alteplase in 3–4.5 hours (n=760), exceeding 4.5 hours (n=513) and within 3 hours (n=487). Thrombolysis with alteplase within 3 hours after stroke ranked best (SUCRA=98.3%) in mRS scores 0–1, followed by that exceeding 4.5 hours (SUCRA=58.0%) and at 3–4.5 hours (SUCRA=43.0%) (table 1). From the forest plot, all the thrombolytic therapies with alteplase regardless of the time delay were significantly more effective than placebo (OR 1.33–2.17).

Alteplase at 3–4.5 hours (OR=0.61, 95% CI 0.40 to 0.93) was significantly superior to alteplase within 3 hours whereas alteplase exceeding 4.5 hours (OR=0.68, 95% CI 0.43 to 1.06) showed the trend of significance of inferiority compared with alteplase within 3 hours (figure 3A).

Eleven of 12 studies reporting 3340 patients were eligible for analysing the secondary endpoint. The network plot is shown in figure 2B. Alteplase within 3 hours ranked best (SUCRA=86.7%) in mortality on 3 months, followed by control (SUCRA=64.7%) (table 1). However, only alteplase exceeding 4.5 hours showed the trend of significance compared with placebo (OR=0.54, 95% CI 0.29 to 1.00) in mortality on 3 months and alteplase within 4.5 hours was comparable with placebo (figure 3B).

Nine of 12 studies reporting 3137 patients were eligible for analysing the tertiary endpoint. The network plot is shown in figure 2C. Placebo ranked best (SUCRA=99.9%) in symptomatic intracranial haemorrhage within 36 hours, followed by alteplase at 3–4.5 hours (table 1). From the forest plot, thrombolysis with alteplase exceeding 3 hours significantly increased symptomatic intracranial haemorrhage by 4.89 to 5.67-fold compared with placebo whereas alteplase within 3 hours was comparable with placebo. This safety profile was comparable between alteplase at 3–4.5 hours and alteplase beyond 4.5 hours (figure 3C).

From the funnel plots, small sample effect was verified to be existing in the comparison between alteplase within 3 hours and control in analysing the primary endpoint (figure 4A); in the
comparison between alteplase at 3–4.5 hours and control in analysing the secondary endpoint (figure 4B); in the comparison between alteplase at 3–4.5 hours and control as well as alteplase exceeding 4.5 hours and control in analysing the tertiary endpoint (figure 4C).

No overall nor loops inconsistency existed in the analysis of each endpoint.

In the conventional meta-analysis, all the time delays of alteplase showed a significant benefit (OR 1.18–1.68) regarding the primary endpoint compared with placebo, alteplase at 3–4.5 hours was equivalent to that beyond 4.5 hours. Alteplase

<table>
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Mortality=mortality at 90th day; mRS, modified Rankin Scale scores 0–1 at 90th day; PrBest, probabilities of being the best; SICH, symptomatic intracerebral haemorrhage within 36 hours; SUCRA, surface under cumulative ranking curve.
exceeding 4.5 hours significantly increased the 90 days' mortality for 73%-fold and symptomatic intracranial haemorrhage within 36 hours for 4.84-fold compared with placebo whereas alteplase within 4.5 hours was safe regarding these secondary and tertiary endpoints (figure 5). The results resembled those from the network meta-analysis.

**DISCUSSION**

To our knowledge, this is the first network meta-analysis investigating the effect of different time delays of alteplase in patients with AIS. For the primary endpoint, we found that all the treatment delays of alteplase could significantly increase the proportion of patients with mRS scores 0–1 (that was independent of patients) on 3 months after stroke from both network meta-analysis and conventional meta-analysis, which was partly in accord with that from previous studies. However, we also found that alteplase within 3 hours was significantly more effective than alteplase at 3–4.5 hours, which was consistent with a cohort study. There was a trend that alteplase within 3 hours was more effective than alteplase beyond 4.5 hours (the latter vs the former: OR=0.68, 95% CI 0.43 to 1.06) though it did not reach the significance. We speculated it as the result of inadequate studies as well as the difference of the baseline characteristics (ie, NIHSS scores, age) from each included study. Another finding was that alteplase at 3–4.5 hours was equivalent to that beyond 4.5 hours, similar to Wang et al's study. Theoretically, the efficacy should be weakened as the treatment delay increases due to the following mechanisms. Although alteplase promotes the degradation of fibrin-based blood clots recovering the blood perfusion in cerebrum, the ensuing ischaemic/reperfusion injury results in the activation of several reactive oxygen species-generating enzymatic systems, impairing the neurovascular function. In addition, alteplase increases matrix metalloproteinase-9 (MMP-9), MMP-2 and MMP-3, and binds to platelet-derived growth factor receptor alpha (PDGFRα), which then collectively produces the disruption and increases the permeability of the blood–brain barrier (BBB). Even without alteplase, human study showed that serum MMP-9 increased early after stroke with a peak at 6–8 hours. On top of that, alteplase
demonstrated neurotoxicity through N-methyl-D-aspartate pathway. The disruption and opening of BBB could occur as early as 2 hours after stroke onset and persist for weeks. The later the alteplase was administrated, the weaker the BBB was, then the more harmful chemical substance was produced and infiltrates the cerebral parenchyma, causing severer neural damage, together with the less viable tissue in the ischaemic penumbra, eventually compromising the benefit of alteplase (abbreviated as benefit-harm theory).

For the secondary endpoint, both network meta-analysis and conventional meta-analysis verified that alteplase beyond 4.5 hours increased the mortality on 3 months whereas alteplase within 4.5 hours did not, compared with placebo. It was interesting that the mortality was indeed comparable between alteplase beyond 4.5 hours and placebo in each included study, however, when merged them together by conventional and network meta-analyses, significance emerged without heterogeneity. This result was similar to that of Lu et al.’s study; in that study, the mortality was 54.5% in reperfusion group compared with 18.1% in non-treatment group based on Sprague Dawley rats model with a middle cerebral artery occlusion. Although our result was inconsistent with the recent conventional meta-analysis, it is still possible that alteplase beyond 4.5 hours resulted in a higher frequency of favourable outcome but contemporarily higher frequency of mortality compared with placebo, especially in the pooled analysis from a larger sample size. A potential explanation to this paradoxical phenomenon is that most of the patients treated with alteplase beyond 4.5 hours in the included studies were within the time limit (assuming it was 12 hours) where the benefit of alteplase still outweighed its harm to the brain according to the benefit-harm theory referred above, but it is likely that the ratio of benefit/harm decreases as time goes longer (ie, >12 hours) and even inverses, namely more mortality and equivalent or even less favourable outcomes. Anyway, more studies are needed to verify this assumption.

For the tertiary endpoint, a previous cohort study showed that SICH within 36 hours following alteplase beyond 4.5 hours (OR=1.57, 95% CI 0.68 to 3.64) and at 3–4.5 hours (OR=1.22, 95% CI 0.92 to 1.61) was respectively comparable with alteplase within 3 hours after adjustment. The result was similar to that of the current study. However, in our study, we additionally found that the haemorrhage might be substantially increased fourfold to sixfold if alteplase was administrated beyond 3 hours compared with placebo; even in the conventional meta-analysis, the result of 4.84-fold of alteplase beyond 4.5 hours compared with placebo, these had never been reported in the previous literature. These results were verified in Susan et al.’s study, that SICH rates were 25%, 50%, 75% and 100%, respectively, in Wistar rats with reperfusion of 1.5, 2.5, 3.5 and 5 hours after modelling with middle cerebral artery occlusion. The mechanism of SICH was similar to the benefit-harm theory that alteplase induces ischaemic/reperfusion injury and elevates MMPs and activates PDGF-CC, leading the disruption of BBB.

In our study, the SUCRA value of mRS at 90th day in alteplase at 3–4.5 hours (SUCRA=43%) was lower than that beyond 4.5 hours (SUCRA=58%), but there was no significant difference between their ORs (the latter vs the former: OR=1.11, 95% CI 0.76 to 1.61). The SUCRA value between alteplase at 3–4.5 hours and beyond 4.5 hours seems unreasonable. However, this phenomenon was not rare and has been addressed in several previous studies. Therefore, indirect and direct pairwise comparison among the interventions or the sequential network matrix showed a network forest plot, as Mbuagbaw et al. proposed in his article, collectively contributing to a more precise interpretation of the network meta-analysis. Here we adopted the network forest plot as well as the conventional meta-analysis as the consolidation. Hence, we speculated that the primary outcome between alteplase at 3–4.5 hours and beyond 4.5 hours was similar.

There were several limitations to our study. First, we did not include any cohort studies, resulting in a relatively smaller sample size. In our primitive search, we tried to include cohort studies in the network meta-analysis, however the result was paradoxical: alteplase beyond 4.5 hours was the most effective whereas alteplase within 3 hours was comparable to placebo. We found the baseline of each arm in cohort studies was not always comparable (p<0.05), though they were adjusted in the final analysis in these studies; given the current methodology of network meta-analysis, the adjusted data could not be used. So we eventually included only RCTs, like the other meta-analysis did. Second, given the limited potential deviation of SUCRA especially in the process of calculation, because SUCRA does not consider the magnitude of differences in effects between treatments, therefore an intervention with wide CIs or small number of events could result in even a higher SUCRA value but non-significant ORs compared with certain interventions. Therefore, indirect and direct pairwise comparison among the interventions or the sequential network matrix showed a network forest plot, as Mbuagbaw et al. proposed in his article, collectively contributing to a more precise interpretation of the network meta-analysis. Here we adopted the network forest plot as well as the conventional meta-analysis as the consolidation. Hence, we speculated that the primary outcome between alteplase at 3–4.5 hours and beyond 4.5 hours was similar.

What is the potential mechanism accounting for the seeming increment of 90 days’ mortality and symptomatic intracerebral haemorrhage within 36 hours as the intervention of intravenous alteplase delays?
included trials, we could not make a subgroup analysis according to the age or baseline NIHSS scores, resulting in potential bias; nonetheless, the results in our research were closed to clinical perception. Third, we did not include ‘6 hours’ as a cut-off of treatment delay although the International Stroke Trial (IST) study26 investigated it, due to the overlap with ‘4.5 hours’ from other studies and no recommendation from the current guideline. Fourth, we did not include intra-arterial thrombolysis with alteplase since it is not widely used in the clinical practice.

CONCLUSION
In summary, thrombolysis with intravenous alteplase of 0.9 mg/kg for AIS should be within 3 hours from onset to needle for its best efficacy among all the intervention and equivalent safety compared with placebo. Alteplase beyond 3 hours was less effective compared with that within 3 hours and increased the risk of mortality on 3 months as well as SICH at 36 hours. More head-to-head clinical trials are needed to confirm those findings.

Contributors YY and YG designed the study and developed the retrieve strategy. XC and YG executed the systematic evaluation as the first and second reviewers, searching and screening the summaries and titles, assessing the inclusion and exclusion criteria, generating data collection forms and extracting data, and evaluating the quality of the study, and drafted the article. CH advised and revised the inclusion/exclusion criteria and screening the summaries and titles, assessing the inclusion and exclusion criteria, and performed the meta-analysis.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. The data used to support the findings of this study are available from the corresponding author upon request.

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REFERENCES
14. Ahmed Net al. Results of intravenous thrombolysis within 4.5 to 6 hours and updated results within 3 to 4.5 hours of onset of acute ischaemic stroke recorded in the safe implementation of treatment in stroke international stroke thrombolysis register (SITS-ISTR). JAMA Neurology 2013;70:837–44.
26. The IST-3 collaborative group. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. The Lancet 2012;379:2352–63.