Supplementary Material

Search strategy

Using MEDLINE, Embase, Cochrane (output pasted below):

Alteplase OR r-tPA OR tPA OR Tenecteplase AND stroke OR cerebrovascular accident OR myocardial infarction

• Ovid MEDLINE® and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily (1946 to November 12, 2021)

#	Query	Results from Nov 13, 2021
1	(tenecteplase or alteplase or tPA or r-tPA).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	26,600
2	(stroke or cerebrovascular accident or myocardial infarction).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	552,048
3	1 and 2	4,871
4	limit 3 to humans	3,756

• Embase (1974 to November 12, 2021)

#	Query	Results from Nov 13, 2021
1	(tenecteplase or alteplase or tPA or r-tPA).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	52,927
2	(stroke or cerebrovascular accident or myocardial infarction).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]	811,682
3	1 and 2	20,915
4	limit 3 to human	18,696

• Evidence-Based Medicine (EBM) Reviews - Cochrane Database of Systematic Reviews (2005 to November 11, 2021)

#	Query	Results from Nov 13, 2021
1	(tenecteplase or alteplase or tPA or r-tPA).mp. [mp=title, short title, abstract, full text, keywords, caption text]	41
2	(stroke or cerebrovascular accident or myocardial infarction).mp. [mp=title, short title, abstract, full text, keywords, caption text]	1,988
3	1 and 2	31

Of these results, we were especially interested in including randomized-controlled trials and meta-analyses examining the safety and efficacy of tenecteplase (TNK) or alteplase in acute ischemic stroke, as well as the use of TNK in cardiology. We also considered observational studies reporting safety and efficacy outcomes of TNK use in acute ischemic stroke. We did not use a grading criterion to assess quality of papers used. The review was conducted by two stroke neurologists. We finally included 33 papers in the main narrative review and an additional 15 papers in the Supplement.

Tenecteplase – ST-elevation myocardial infarction trials

In the cardiology domain, TNK was first used in clinical trials with comparison to alteplase, in addition to heparin and aspirin. ASSENT-2 (Assessment of the Safety of a New Thrombolytic) was the first large phase III double-blinded trial (n=16,949) which showed similar rates of mortality (7%), intracranial hemorrhage (0.9%), and significantly fewer non-cerebral bleeding complications with TNK (26% vs. 29%, *P*=0.0003).[1] There was no difference in rates of reinfarction between the two groups.[1] Over time as primary percutaneous coronary intervention (PCI) became the first line standard of treatment for ST-elevation myocardial infarction (STEMI), thrombolysis was administered in only those cases where PCI was unavailable in time (<90 min). The ASSENT-4 trial, which tried to test the use of adjunctive TNK in addition to primary PCI in patients with STEMI (n=1,667), showed that patients in the TNK arm had more than twice the chance of recanalization of the culprit artery; however, this was counter-balanced by higher rates of in-hospital death, intracranial hemorrhage, and reinfarction in the TNK arm. These conflicting findings could be due to the narrow window of potential benefit in STEMI as the time gain for reperfusion was truly short in the facilitated PCI arm (PCI+TNK) and the median time from onset to needle was much prolonged as compared to routine care.[2] This led to the Strategic Reperfusion Early After Myocardial Infarction (STREAM) trial (n=1,892) which compared prehospital intravenous TNK to primary-PCI in patients who were unable to access primary PCI within 60 minutes of first medical contact. The primary endpoint, a composite of death, shock, congestive heart failure or reinfarction up to 30 days, occurred in 12.4% in the fibrinolysis (TNK) group and in 14.3% in the primary PCI group; this was not statistically significant. The incidence of intracranial hemorrhage was elevated in the fibrinolysis group (1.0% vs. 0.2%), but after a protocol amendment reducing the TNK dose by half, the hemorrhage risk was comparable.[3] Current cardiology guidelines adapt this pharmaco-invasive approach and recommend starting fibrinolysis (with TNK) immediately in

STEMI patients if primary PCI cannot be achieved within 120 minutes after the first medical contact.[4] The similarly designed STREAM-2 trial is currently comparing the safety and efficacy of this approach in patients aged 60 years and older.[5] TNK achieved regulatory approval by the US Food and Drug Administration (FDA) and European Medical Agency in 2000 for weight-based treatment in STEMI. While robust data are lacking, a biosimilar variant of TNK in India and China for both STEMI and stroke (under different commercial names and different doses) is routinely used.

Historical overview of thrombolysis for acute ischemic stroke

Early trials from the 1980s were methodologically different from current practice and more recent trials in that they used intravenous thrombolysis infusions over several days as compared to current use of one-time bolus doses with/without brief hour-long infusions. Several agents like streptokinase, urokinase, and desmoteplase were tested until 1996 when alteplase was approved by the US FDA for treatment of acute ischemic stroke within 3 hours of symptom onset based on results from the NINDS (National Institute of Neurological Disorders and Stroke) trial; this trial showed that earlier administration of intravenous alteplase within 3 hours of symptom onset was associated with better clinical outcomes. The European Cooperative Acute Stroke Study (ECASS) III trial showed modest benefit of using alteplase in the 3- to 4.5-hour window which led to changes in guidelines in many countries; however, the US FDA approval is still only for alteplase use within 3 hours of symptom onset. For around a quarter of a century, alteplase has been the standard of care in acute stroke treatment. The major phase III historical clinical trials of intravenous thrombolysis are summarized in Supplementary Table 1.

Pharmacological advantages of tenecteplase over alteplase

Endogenous tPA (a subtype of tissue-type plasminogen activator) is a serine protease secreted by endothelial cells as a single chain 527 amino acid glycosylated protein and is further converted to two-chain form by plasmin. It then binds to fibrin within the thrombus to catalyze the conversion of fibrin bound inactive plasminogen to plasmin which in turn initiates local fibrinolysis. For clinical purposes, t-PA is genetically engineered into r-tPA (alteplase), which is synthesized from melanoma cells. Synthesis of wild-tPA enabled its use in clinical practice to target arterial thrombi in ischemic heart disease (STEMI), acute massive pulmonary embolism, and ischemic stroke. However, rapid clearance of r-tPA requiring post-bolus infusion, and increased rates of bleeding complications with higher doses motivated further development of newer generation thrombolytics. Like endogenous tPA and r-tPA, TNK is a genetically engineered t-PA mutant, which has several advantages over alteplase due to site mutagenesis leading to a 14-fold greater fibrin specificity, 80-fold resistance to inhibition by plasminogen activator inhibitor (PAI-1), more rapid thrombolysis, and decreased plasma clearance[6] (Supplementary Table 2).

Clinically, these properties translate into several desirable properties. One, TNK can be administered as a single 5-second bolus, while r-tPA is administered over 1 hour (initial 10% bolus

Supplementary Table 1. Historical overview of intravenous thrombolysis trials

Trial	Drug	Number (n)	Dose	Time from onset (hr)	Result	Symptomatic ICH rates (vs. placebo)
NINDS I[13]	Alteplase	291	0.9 mg/kg	≤3	No improvement in 24-h NIHSS	5.6% vs. 0%
NINDS II[13]	Alteplase	333	0.9 mg/kg	≤3	Better global outcome at 90 days	7.1% vs. 1.2%
NINDS I+II[13]	Alteplase	624	0.9 mg/kg	≤3	Better global outcome at 90 days	6.4% vs. 0.6%
ATLANTIS-A[14]	Alteplase	142	0.9 mg/kg	0–6	No benefit	11.3% vs. 0%
ATLANTIS-B[15]	Alteplase	613	0.9 mg/kg	3–5	No benefit	6.7% vs. 1.3%
ECASS I[16]	Alteplase	620	1.1 mg/kg	≤6	No benefit	19.8% vs. 6.8%
ECASS II[17]	Alteplase	800	0.9 mg/kg	≤6	No benefit	8.8% vs. 3.4%
ECASS III[18]	Alteplase	821	0.9 mg/kg	3–4.5	Better global outcome at 90 days	2.4% vs. 0.2%
ASK[19]	Streptokinase	340	1.5 MU	≤4	No benefit, increased mortality	13.2% vs. 3%
MAST-E[20]	Streptokinase	310	1.5 MU	≤6	No benefit, increased mortality	21.2% vs. 2.6%
MAST-I[21]	Streptokinase	622	1.5 MU	≤6	No benefit	6% vs. 0.6%
DIAS-3[22]	Desmoteplase	193	57 subjects—90 μg/kg 66 subjects—125 μg/kg	3–9	No benefit	3.5%–4.5% vs. 0%

ICH, intracerebral hemorrhage; NINDS, National Institute of Neurological Disorders and Stroke; NIHSS, National Institutes of Health Stroke Scale; ECASS, European Cooperative Acute Stroke Study; ATLANTIS, Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke; ASK, Australian Streptokinase Trial; MAST-E, Multicenter Acute Stroke Study-Europe; MAST-I, Multicenter Acute Stroke Trial-Italy; DIAS, Desmoteplase in Acute Stroke; MU, million units.

https://doi.org/10.5853/jos.2022.02880

Supplementary Table 2. Pharmacological comparisons between tenecteplase and alteplase

	Alteplase	Tenecteplase
Half-life (min)	4–9	17–24 (6-fold higher)
Fibrin specificity	+	++ (15-fold higher)
Inhibited by PA-1	+++	+ (80-fold resistant)
Pro-coagulant effect	Yes	No

PA-1, plasminogen activator inhibhitor-1.

and remaining 90% infusion). Thus, infusion for r-tPA requires a dedicated intravenous catheter insertion that may delay treatment initiation, in patients with difficult venous access. In addition, due to its short half-life, a gap between end of bolus and initiation of infusion may lead to underdosing. The initial plasma half-life of alteplase is 3–5 minutes and total plasma clearance ranges from 16 to 88 minutes. Alteplase is predominantly metabolized by the liver and the plasma clearance has been defined as 476–572 mL/min. Bolus to infusion delays or interruptions in the infusion of tPA after the bolus can occur in up to 8%–10% of patients receiving alteplase for stroke and this may significantly impact serum tPA levels, may reduce the efficacy of thrombolysis and even require repeat bolus of alteplase.[7-9] Two, TNK may have fewer adverse bleeding complications and greater potency in dissolving clots owing to its high fibrin specificity. Lower fibrin specificity is associated with increased levels of fibrin degradation products which are known to be predictive of parenchymal hematoma and systemic bleeding. Since TNK is more fibrin specific as compared to alteplase, the risk of bleeding may be lower with TNK.[9,10] Another key advantages of using TNK is that it potentially reduces the door to needle times (DNT) substantially. A retrospective analysis from a prospectively collected stroke registry showed that TNK was associated with significantly shorter DNT as compared to alteplase (median 41 min for TNK vs. 58 min for alteplase, $P<0.001$).[11] The one-time single bolus administration of TNK also improves the door-in-door-out times for patients who receive thrombolysis at a primary stroke center and need to be moved to a comprehensive stroke center for potential EVT, which in turn leads to faster reperfusion from onset and potentially increased likelihood of good clinical outcome.[12]

Supplementary References

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https://doi.org/10.5853/jos.2022.02880