Efficacy of Alteplase in a Mouse Model of Acute Ischemic Stroke

A Retrospective Pooled Analysis

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Background and Purpose—The debate over the fact that experimental drugs proposed for the treatment of stroke fail in the translation to the clinical situation has attracted considerable attention in the literature. In this context, we present a retrospective pooled analysis of a large data set from preclinical studies, to examine the effects of early versus late administration of intravenous recombinant tissue-type plasminogen activator.

Methods—We collected data from 26 individual studies from 9 international centers (13 researchers; 716 animals) that compared recombinant tissue-type plasminogen activator with controls, in a unique mouse model of thromboembolic stroke induced by an in situ injection of thrombin into the middle cerebral artery. Studies were classified into early (<3 hours) versus late (≥3 hours) drug administration. Final infarct volumes, assessed by histology or magnetic resonance imaging, were compared in each study, and the absolute differences were pooled in a random-effect meta-analysis. The influence of time of administration was tested.

Results—When compared with saline controls, early recombinant tissue-type plasminogen activator administration was associated with a significant benefit (absolute difference, −6.63 mm^3; 95% confidence interval, −9.08 to −4.17; I^2=76%), whereas late recombinant tissue-type plasminogen activator treatment showed a deleterious effect (+5.06 mm^3; 95% confidence interval, +2.78 to +7.34; I^2=42%; P_{int}<0.00001). Results remained unchanged after subgroup analyses.

Conclusions—Our results provide the basis needed for the design of future preclinical studies on recanalization therapies using this model of thromboembolic stroke in mice. The power analysis reveals that a multicenter trial would require 123 animals per group instead of 40 for a single-center trial. **(*Stroke*. 2016;47:1312-1318. DOI: 10.1161/STROKEAHA.116.012238.)**

Key Words: magnetic resonance imaging ■ middle cerebral artery ■ stroke ■ thrombolytic therapy ■ tissue-type plasminogen activator

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Intravenous recombinant tissue-type plasminogen activator (r-tPA), administered within 4.5 hours after stroke onset, is the only pharmacological treatment approved for acute ischemic stroke.[1] However, it can only be administered to a minority of patients, achieves early arterial recanalization in <50% of cases, and has deleterious effects, including intracerebral hemorrhage, thus underlying the need for developing new acute strategies to be used for the treatment of stroke.

Testing potential acute therapies in animal models is presently the most common strategy for the development of new drugs for use in stroke. However, many approaches that showed efficacy in experimental stroke models have either not been translated or failed when tested in clinical trials.[2–6] This translational roadblock is commonly attributed to inherent weaknesses of preclinical studies that include, lack of clinical relevance of the stroke models,[7,8] monocentric design, and small sample sizes.[9] Thus, improving the validity and reproducibility of preclinical studies is warranted. Some authors advocate for the use of multicenter preclinical studies,[10] and much effort was expended to develop new experimental models that mimic more closely the pathophysiology of stroke.[11–13] Reporting of systematic reviews and meta-analysis of preclinical stroke studies from groups such as Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (http://www.dcn.ed.ac.uk/camarades/default.htm: About) has increased.[14–18] These approaches allow one to take into account the fact that individual studies may have used small sample sizes, and to compare data from >1 type of experimental stroke model.

In 2007, we developed a thromboembolic model of stroke in mice,[12] which seems physiologically relevant and is now used by several groups to evaluate the effects of r-tPA either alone or where necessary in combination with putative neuroprotective drugs.[19–21] However, no large-scale validation of this model is available to date. Therefore, we evaluated the effects of early (<3 hours) and late (≥3 hours) r-tPA administration in this stroke model in a retrospective pooled analysis of individual data from 9 international research centers.

Materials and Methods

Selection Criteria, Search Strategy, and Data Collection

Eligible studies for inclusion in this analysis were those that (1) used the thromboembolic stroke model described below according to a Standard Operating Procedure, (2) compared human r-tPA (Alteplase) treatment alone with a control saline group, whatever the time-window of treatment after stroke onset or the dose of r-tPA used, and (3) evaluated efficacy on lesion volumes measured either by magnetic resonance imaging (MRI) or histology at 24 hours post stroke onset. There were no restrictions on the strain of mice, or the dose of r-tPA used, during the protocol. Relevant studies were identified by a systematic search of the scientific literature of studies published from 2007 to 2013, and by collecting data from studies that we were aware of but not yet published. Thus, at the time of this meta-analysis, 9 international centers were identified and made their data available for this study. The inclusion criterion was a reduction of cerebral blood velocity to at least 60% of the baseline value before initiating treatment. No animals were excluded from the final analysis because of premature death—related to technical complication—or as a result of the drug itself, during or after administration. However, 85 animals were excluded from the global analysis (47 saline controls and 15 early and 31 late r-tPA treated) because either an excessively high-dose of thrombin was used (3 UI) in noncompliance with the Standard Operating Procedure or an unmatched control group was used (ie, early saline versus late r-tPA treated). For each study, we collected raw data that included the identification of each experimenter, mouse strain, sex, experimental treatment (including the dose of thrombin used), the dose of r-tPA administered, the time of administration of r-tPA after stroke onset, and the lesion volume (Table I in the online-only Data Supplement). Early r-tPA administration was defined when the injection was performed within the first 3 hours, and late r-tPA administration was defined when r-tPA was injected after 3 hours post stroke onset.

Animals and Ethics

Depending on the research centers, experiments were performed on groups of male mice (Swiss or C57/Bl6) weighing 25 to 40 g and 20 and 30 g, respectively (Charles Rivers Laboratory; Janvier Laboratory, Jackson Laboratories, Harland Laboratories, and the Center Universitaire de Resources Biologiques of Caen—Normandie Universitie). All animals were housed under standard conditions with a 12-hour light/dark cycle and access to food and water ad libitum.

Studies were performed in accordance with the mandate of either the European Community Council Directive of November 24, 1986 (86/609/EEC) or the National Institutes of Health guide for the Care and Use of Laboratory Animals. Animal procedures were approved by the regional Ethical Committees for Laboratory Animal Experiments for each center. All efforts were made to minimize the possible suffering of the animals.

Thromboembolic Stroke Model

Cerebral ischemia was induced as described previously,[12] and all centers followed a Standard Operating Procedure (Standard Operating Procedure; Materials and Methods in the online-only Data Supplement). In brief, the mice were anesthetized with isoflurane (induction 4%–5%, maintenance 1%–2%) in eight of nine centers. In 1 center, mice were anesthetized with a ketamine/xylazine mixture (IP, 50 and 6 mg/kg, respectively). A small craniotomy was performed, the dura was excised, and the middle cerebral artery was exposed. The pipette (glass micropipette, tip size 30–50 μm) was introduced into the lumen of the artery and 1 or 2 μL of murine α-thrombin (Hematologic Technologies) was injected to induce a clot, in situ. One center used human α-thrombin (0.75 UI/μL; Hematologic Technologies). Different doses of thrombin were used by the different centers (0.75–3 UI). However, we considered that a dose >2 U/μL of thrombin was too high to allow reperfusion after r-tPA treatment. Thus, 23 animals were excluded from the global analysis because 3 UI of thrombin was used. To allow stabilization of the clot, the pipette was removed 10 minutes after the injection of thrombin. Thrombolysis was initiated via the tail vein (200 μL) of human recombinant tPA (r-tPA; Boeringher Ingelheim, Alteplase; 10% in bolus, 90% in perfusion >40 minutes) at different doses (0.9, 5, and 10 mg/kg, IV) and at different times after stroke onset (from 20 minutes to 4 hours). Control mice received saline under identical conditions. Rectal temperature was maintained at 37±0.5°C throughout the surgical procedure using a feedback-regulated heating system. Cerebral blood flow velocity was used as an index of the occlusion and was measured using either laser Doppler within the middle cerebral artery territory or Doppler Speckle on the dorsal surface of the skull during 60 to 120 minutes.

Outcome Assessment

The primary outcome was the lesion volume measured 24 hours after stroke onset either by histological staining or MRI analysis. Brains were cryosectioned, and slices (20 μm) were stained interchangeably using cresyl violet, thionine, or hematoxylin/eosin. One section in 10 (10- or 20-μm thick) was stained and analyzed (covering the entire lesion). Regions of interest were determined through the use of a stereotaxic atlas for the mouse and an image analysis system (ImageJ software) was used to measure the infarct. MRI images were obtained from

T2-weighted RARE sequences with either a 7T Bruker pharmascan MRI (echo time [TE]/repetition time [TR]=51.3 ms/2500 ms) or a 9.4T Bruker biospec (TR/TE=3300/60 ms). Lesion areas were quantified on T2-weighted images with ImageJ software (version 1.45r; National Institutes of Health).

Statistical Analyses

Our primary analysis was to determine whether the efficacy of r-tPA differed according to the time-window of treatment and consisted of a pooled analysis of mean differences in infarct volume between r-tPA and saline (control), with stratification by treatment time-window (classified into <3 and ≥3 hours). For each experiment, we calculated the mean (±SD) difference in infarct volume between the r-tPA and the control group. The weighted mean difference was obtained using a random-effect meta-analysis; the weight given to each experiment being equal to the inverse of the variance of the difference. We then assessed whether the effect of r-tPA on infarct volumes differed between early and late r-tPA, using an interaction test. We also examined whether the result differed according to various experimental characteristics (eg, the mouse strain (Swiss versus C57Bl6), method of outcome assessment (MRI versus histology), and dose of thrombin used 0.75, 1, or 1.5 U/μL). This analysis was performed with RevMan 5.3 software. Finally, we performed a sensitivity analysis after exclusion of data from the largest center (Caen).

Results

We collected data from 26 experimental studies performed between 2007 and 2013 (from 13 experimenters in 9 different laboratories; Table I in the online-only Data Supplement). In total, data from 716 mice were available for the study. As previously explained, we excluded 85 animals. Thus, 623 animals (291 saline treated and 332 r-tPA treated) were included in the final analysis (Table I in the online-only Data Supplement). In the r-tPA group, 235 animals had early r-tPA treatment (<3 hours: from 20 to 40 minutes post ictus) and 97 late treatment (≥3 hours: 180 and 240 minutes post ictus; given 200 μL IV whatever the dose used 0.9, 5, or 10 mg/kg). As such, data were evaluated in 19 early administration studies and in 9 late r-tPA treatments.

In the pooled analysis, the early r-tPA was associated with a significant reduction in the final infarct volume (absolute difference, −6.63 mm^3; 95% confidence interval, −9.08 to −4.17; P_{sig}<0.0001; I^2=76%; Figure 1),[19–28] whereas the late r-tPA treatment showed a deleterious effect (+5.06 mm^3; 95% confidence interval, +2.78 to +7.34; P_{sig}<0.0001; I^2=42%; Figure 1), with a statistically significant qualitative interaction (P_{int}<0.00001).

A similar beneficial effect was observed for the early r-tPA treatment when considering the 7 studies performed outside the Caen laboratory: absolute difference=−10.61 mm^3; 95% confidence interval, −14.80 to −6.43; P_{sig}=0.008; I^2=66% (Figure 2).[20–25] Looking at the 2 studies performed outside of our laboratory that applied late r-tPA treatment, there was

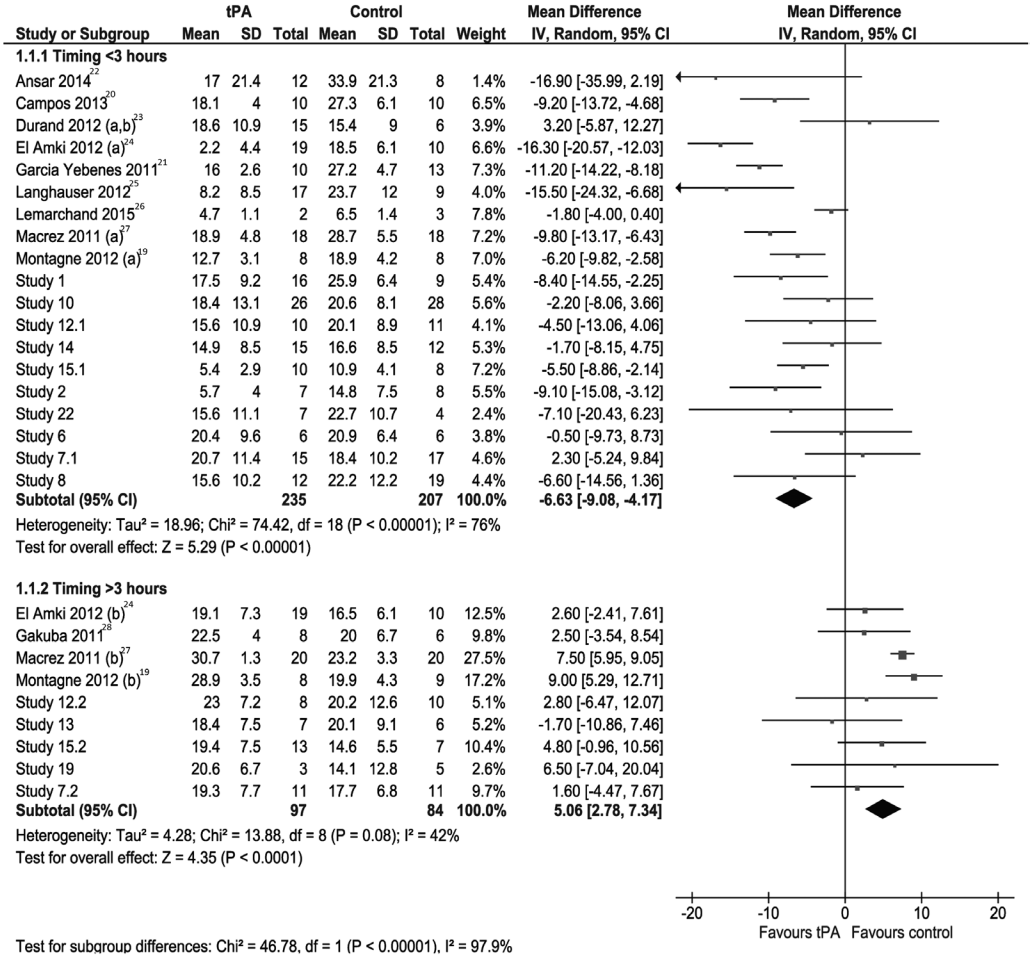

Figure 1. Pooled analysis of lesion volumes expressed in mm^3, comparing the mean values of saline (control) and recombinant tissue-type plasminogen activator (r-tPA)–treated animals. Total is the number of animals per group. CI indicates confidence interval.

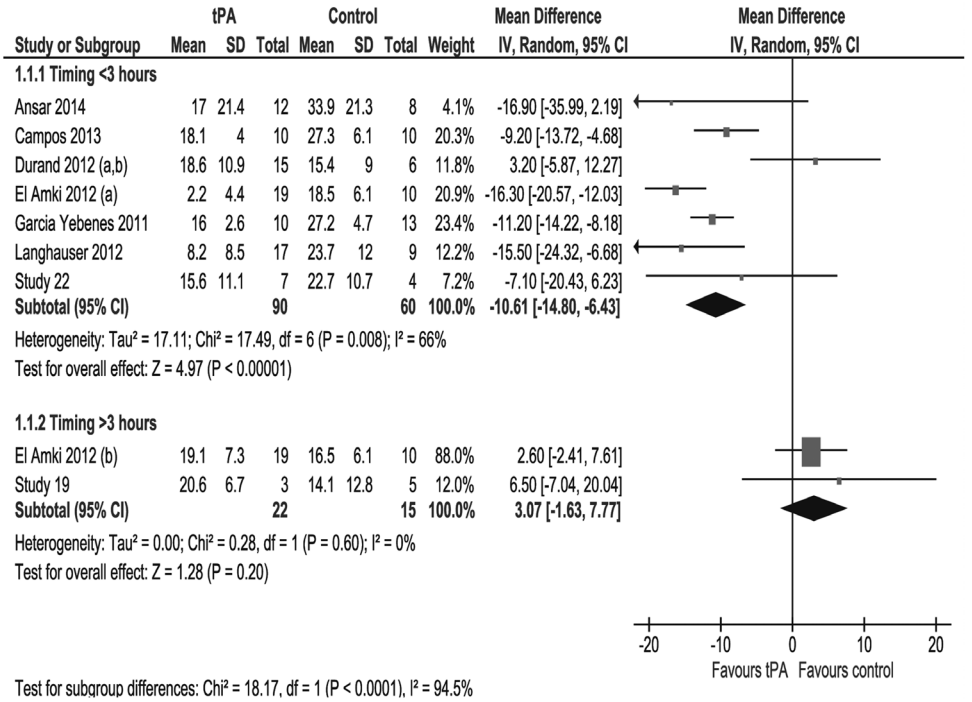

Figure 2. Pooled analysis of lesion volumes expressed in mm^3, comparing the mean values of saline (control) and recombinant tissue-type plasminogen activator (r-tPA)–treated animals after exclusion of Caen data. Total is the number of animals per group. CI indicates confidence interval.

still no beneficial effect (absolute difference=+3.07 mm^3; 95% confidence interval, −1.63 to +7.77; P_{sig}=0.6; I^2=0%; Figure 2). Again, the interaction with the time-window was still significant (P_{int}<0.0001).

Interaction at the subgroups level (Table)—which includes mouse strain (Swiss mice versus C57/Bl6 mice); method of evaluation to determine the lesion volume (ie, histology versus MRI analysis); whether studies were published, whether the studies were performed in a blinded manner; expertise of the experimenters; or whether the studies reporting hemorrhages are included; and dose of r-tPA administered—had no influence on the effects of r-tPA (Figure 1).

Discussion

In this retrospective study of a large pooled analysis of multicenter preclinical data (based on a thromboembolic stroke model), we demonstrated that early (<3 hours) administration of r-tPA after cerebral ischemia is associated with a significant reduction in lesion volume, whereas late administration (≥3 hours) has no, or a deleterious, effect.

Although pooled analyses of data are common in clinical studies, such analyses are rare in preclinical research and no pooled analysis exists on r-tPA in ischemic stroke in animals. Yet, such an approach is of major importance because most of therapeutic strategies with beneficial effects in experimental stroke models failed when evaluated in humans, or have not been translated into a clinical trial, because of lack of support from industry or clinicians. It is therefore crucial to provide drug companies and clinicians with reliable stroke models that represent the clinical situation as much as possible.

As the benefit of r-tPA is well established in humans, it appeared to us interesting to demonstrate that this benefit is also clear in an appropriate animal model of ischemic stroke. Usually, preclinical studies have small sample sizes, and there is often a substantial heterogeneity in the stroke models used. Although we focused on a specific model of thromboembolic stroke and increased the sample size, we still observed a certain degree of heterogeneity across studies. This heterogeneity may be explained by variations in the animal strain, in the method of assessment, or in interindividual technical aspects despite a well-standardized model. However, our sensitivity analyses were highly consistent with the main finding (ie, a time–effect relationship between r-tPA administration and infarct volume).

The inclusion of a large sample population (623 animals) may have helped contribute to the validation of the model. Our group developed and characterized an embolic stroke model in mice, in which cerebral ischemia is induced by a local injection of thrombin directly into the middle cerebral artery. This leads to the immediate formation of a clot, cerebral blood flow disruption, and subsequent cortical infarction.[12] Several other experimental stroke models exist and have been used for years in various animal species. However, those that use electrocoagulation, ligatures, or a filament are not appropriate in which to test thrombolytic drugs. Other researchers use the autologous injection of a clot, or microemboli, via the internal carotid artery to induce stroke, but such methods evince poor reproducibility and uniformity in the location of the lesion[29] and result in a high mortality rate.[30,31] Accordingly, despite successful r-tPA–induced reperfusion, it is not surprising to observe opposite effects of r-tPA treatment on infarct size depending on the extent to which the models reflect the contribution of fibrinolysis, blood–brain barrier alterations, or neurotoxicity.

Table. Interaction Between Early and Late r-tPA Treatments in Different Subgroups

	No. of Studies	Mean Difference (95% CI)	I^2 (%)	Test for Interaction, *P* Value
Mouse strains				
Swiss				
Early r-tPA	14	−6.31 (−9.03 to −3.58)	79	<0.00001
Late r-tPA	7	5.38 (2.93 to 7.83)	47	
C57Bl				
Early r-tPA	5	−8.18 (−14.90 to −1.46)	64	<0.02
Late r-tPA	2	2.42 (−3.12 to 7.96)	0	
Evaluation methods				
Histology				
Early r-tPA	9	−9.92 (−12.49 to −7.35)	52	<0.00001
Late r-tPA	2	5.65 (1.00 to 10.31)	70	
MRI				
Early r-tPA	10	−3.76 (−6.28 to −1.25)	52	<0.0001
Late r-tPA	7	4.41 (1.41 to 7.41)	31	
Published vs unpublished studies				
Published				
Early r-tPA	9	−8.77 (−12.74 to −4.80)	87	<0.00001
Late r-tPA	10	6.26 (3.52 to 9.00)	55	
Unpublished				
Early r-tPA	2	−4.70 (−6.78 to −2.62)	7	<0.0003
Late r-tPA	5	2.73 (−0.67 to 6.14)	0	
Blind vs not blind studies				
Blind				
Early r-tPA	17	−6.35 (−9.04 to −3.66)	78	<0.00001
Late r-tPA	8	4.19 (1.61 to 6.78)	26	
Not blind				
Early r-tPA	2	−8.76 (−13.05 to −4.47)	0	<0.00001
Late r-tPA	1	7.50 (5.95 to 9.05)	…	
Caen vs others				
Caen				
Early r-tPA	12	−4.89 (−7.09 to −2.69)	57	<0.00001
Late r-tPA	7	5.31 (2.76 to 7.85)	47	
Others				
Early r-tPA	7	−10.61 (−14.80 to −6.43)	66	<0.0001
Late r-tPA	2	3.07 (−1.63 to 7.77)	0	
Influence of training				
Upper 25% trained				
Early r-tPA	7	−6.45 (9.37 to −3.52)	47	<0.00001
Late r-tPA	3	6.98 (4.10 to 9.86)	53	
Lower 25% trained				
Early r-tPA	2	−8.98 (−13.26 to −4.70)	0	…
Late r-tPA	0	…	…	
Doses of tPA				
High doses (5–10 mg/kg)				
Early r-tPA	18	−5.98 (−8.22 to −3.74)	68	<0.00001
Late r-tPA	8	5.48 (3.12 to 7.84)	39	
Low dose (0.9 mg/kg)				
Early r-tPA	1	−16.30 (−20.57 to −12.03)	…	<0.00001
Late r-tPA	1	2.60 (−2.41 to 7.61)	…	
Influence of reported hemorrhages				
Studies with no reported hemorrhages				
Early r-tPA	17	−5.50 (−7.66 to −3.34)	59	<0.00001
Late r-tPA	8	5.48 (3.12 to 7.84)	39	
Studies with reported hemorrhages				
Early r-tPA	2	−13.52 (−18.50 to −8.54)	73	<0.00001
Late r-tPA	1	2.60 (−2.41 to 7.61)	…	

CI indicates confidence interval; MRI, magnetic resonance imaging; and r-tPA, recombinant tissue-type plasminogen activator.

Reporting of systematic reviews and meta-analysis of preclinical stroke studies is increasing.[14–18] In the present study, we evaluated the effects of r-tPA in a model of thromboembolic stroke with a large sample population and examined the effects of r-tPA dose, time of administration, animal strain, research center, and method for calculating the infarction volume in the mouse. Nonetheless, there are some potential limitations in our analysis. Inherent differences exist between animal and human studies and applying the same method of meta-analysis to preclinical data is not straightforward.[16] Although we had the individual data available, we finally opted for a pooled analysis of group (research center) studies. Indeed, in each study, there is no heterogeneity in the animal model that has the same characteristics at baseline and consequently excludes adjustment for confounding factors. The main source of nonuniformity was the experiment (the study) itself. However, we also performed the same analyses with generalized linear models and found the same interaction with time (data not shown). In addition, although we initially used 10 mg/kg r-tPA, as is usually recommended in rodents, the current analysis shows that a dose as low as 0.9 mg/kg (the dose used in clinical studies) is sufficient to produce a beneficial effect with early r-tPA treatment.

Although the original publication[12] was based on data obtained from Swiss mice, the present data show similar results when using C57/Bl6 animals. The use of different time-windows, different doses, and 2 strains of mice together

with histological analysis is in agreement with some of the recommendations made by the Stroke Treatment Academic Industry Roundtable (STAIR) group.[32] Furthermore, saline was used in all control groups instead of the vehicle containing L-arginine, which is used in clinical trials. Nevertheless, recent experimental studies demonstrated no significant effect of L-arginine when compared with saline in a stroke model in rabbits.[33,34] The outcome we used was infarct volume as functional recovery was not consistently assessed. Similarly, the influence of sex or comorbidities such as diabetes mellitus, hypertension, or age was not addressed. However, the main consequence of these factors is likely to increase heterogeneity and attenuate the effects, rather than invalidate the findings.

In conclusion, we demonstrated in a pooled multicenter analysis that in this experimental model of thromboembolic stroke, r-tPA treatment is beneficial when given early after stroke onset (<3 hours) and not beneficial when the administration is delayed (≥3 hours). On the global data, a power analysis revealed that for a single-center trial, considering a power of 0.8 and an α risk of 0.05 (2 sided), a mean infarct volume of 20.9 mm^3 in the control group and a SD of ±10 mm^3, 40 animals per group (drug treated and a control group) would be required to detect a 30% reduction with early tPA. In contrast, a multicenter trial would require 3 times more animals, ie, 123 animals per group (246 overall) if we assume the same heterogeneity across experiments that we observed in our meta-analysis (I^2=76%).

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Disclosures

None.

References

1. Jauch EC, Saver JL, Adams HP Jr, Bruno A, Connors JJ, Demaerschalk BM, et al; American Heart Association Stroke Council; Council on Cardiovascular Nursing; Council on Peripheral Vascular Disease; Council on Clinical Cardiology. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2013;44:870–947. doi: 10.1161/STR.0b013e318284056a.
2. Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. *Nature*. 2006;441:1094–1096. doi: 10.1038/nature04960.
3. Fisher M. Acute ischemic stroke therapy: current status and future directions. *Expert Rev Cardiovasc Ther*. 2013;11:1097–1099. doi: 10.1586/14779072.2013.827450.
4. Röther J, Ford GA, Thijs VN. Thrombolytics in acute ischaemic stroke: historical perspective and future opportunities. *Cerebrovasc Dis*. 2013;35:313–319. doi: 10.1159/000348705.
5. Howells DW, Sena ES, O'Collins V, Macleod MR. Improving the efficiency of the development of drugs for stroke. *Int J Stroke*. 2012;7:371–377. doi: 10.1111/j.1747-4949.2012.00805.x.
6. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol*. 2006;59:467–477. doi: 10.1002/ana.20741.
7. Hossmann KA. The two pathophysiologies of focal brain ischemia: implications for translational stroke research. *J Cereb Blood Flow Metab*. 2012;32:1310–1316. doi: 10.1038/jcbfm.2011.186.
8. Endres M, Engelhardt B, Koistinaho J, Lindvall O, Meairs S, Mohr JP, et al. Improving outcome after stroke: overcoming the translational roadblock. *Cerebrovasc Dis*. 2008;25:268–278. doi: 10.1159/000118039.
9. Mergenthaler P, Meisel A. Do stroke models model stroke? *Dis Model Mech*. 2012;5:718–725. doi: 10.1242/dmm.010033.
10. Dirnagl U, Fisher M. International, multicenter randomized preclinical trials in translational stroke research: it's time to act. *J Cereb Blood Flow Metab*. 2012;32:933–935. doi: 10.1038/jcbfm.2012.51.
11. Karatas H, Erdener SE, Gursoy-Ozdemir Y, Gurer G, Soylemezoglu F, Dunn AK, et al. Thrombotic distal middle cerebral artery occlusion produced by topical FeCl(3) application: a novel model suitable for intravital microscopy and thrombolysis studies. *J Cereb Blood Flow Metab*. 2011;31:1452–1460. doi: 10.1038/jcbfm.2011.8.
12. Orset C, Macrez R, Young AR, Panthou D, Angles-Cano E, Maubert E, et al. Mouse model of in situ thromboembolic stroke and reperfusion. *Stroke*. 2007;38:2771–2778. doi: 10.1161/STROKEAHA.107.487520.
13. Zhang Z, Zhang RL, Jiang Q, Raman SB, Cantwell L, Chopp M. A new rat model of thrombotic focal cerebral ischemia. *J Cereb Blood Flow Metab*. 1997;17:123–135. doi: 10.1097/00004647-199702000-00001.
14. Ström JO, Ingberg E, Theodorsson A, Theodorsson E. Method parameters' impact on mortality and variability in rat stroke experiments: a meta-analysis. *BMC Neurosci*. 2013;14:41. doi: 10.1186/1471-2202-14-41.
15. Moja L, Pecoraro V, Ciccolallo L, Dall'Olmo L, Virgili G, Garattini S. Flaws in animal studies exploring statins and impact on meta-analysis. *Eur J Clin Invest*. 2014;44:597–612. doi: 10.1111/eci.12264.
16. Vesterinen HM, Sena ES, Egan KJ, Hirst TC, Churolov L, Currie GL, et al. Meta-analysis of data from animal studies: a practical guide. *J Neurosci Methods*. 2014;221:92–102. doi: 10.1016/j.jneumeth.2013.09.010.
17. Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, et al. Evaluation of excess significance bias in animal studies of neurological diseases. *PLoS Biol*. 2013;11:e1001609. doi: 10.1371/journal.pbio.1001609.
18. Pedder H, Vesterinen HM, Macleod MR, Wardlaw JM. Systematic review and meta-analysis of interventions tested in animal models of lacunar stroke. *Stroke*. 2014;45:563–570. doi: 10.1161/STROKEAHA.113.003128.
19. Montagne A, Hébert M, Jullienne A, Lesept F, Le Béhot A, Louessard M, et al. Memantine improves safety of thrombolysis for stroke. *Stroke*. 2012;43:2774–2781. doi: 10.1161/STROKEAHA.112.669374.
20. Campos F, Qin T, Castillo J, Seo JH, Arai K, Lo EH, et al. Fingolimod reduces hemorrhagic transformation associated with delayed tissue plasminogen activator treatment in a mouse thromboembolic model. *Stroke*. 2013;44:505–511. doi: 10.1161/STROKEAHA.112.679043.
21. García-Yébenes I, Sobrado M, Zarruk JG, Castellanos M, Pérez de la Ossa N, Dávalos A, et al. A mouse model of hemorrhagic transformation by delayed tissue plasminogen activator administration after in situ thromboembolic stroke. *Stroke*. 2011;42:196–203. doi: 10.1161/STROKEAHA.110.600452.
22. Ansar S, Chatzikonstantinou E, Wistuba-Schier A, Mirau-Weber S, Fatar M, Hennerici MG, et al. Characterization of a new model of thromboembolic stroke in C57 black/6J mice. *Transl Stroke Res*. 2014;5:526–533. doi: 10.1007/s12975-013-0315-9.
23. Durand A, Chauveau F, Cho TH, Bolbos R, Langlois JB, Hermitte L, et al. Spontaneous reperfusion after in situ thromboembolic stroke in mice. *PLoS One*. 2012;7:e50083. doi: 10.1371/journal.pone.0050083.
24. El Amki M, Lerouet D, Coqueran B, Curis E, Orset C, Vivien D, et al. Experimental modeling of recombinant tissue plasminogen activator effects after ischemic stroke. *Exp Neurol*. 2012;238:138–144. doi: 10.1016/j.expneurol.2012.08.005.
25. Langhauser FL, Heiler PM, Grudzenski S, Lemke A, Alonso A, Schad LR, et al. Thromboembolic stroke in C57BL/6 mice monitored by 9.4 T MRI using a 1H cryo probe. *Exp Transl Stroke Med*. 2012;4:18. doi: 10.1186/2040-7378-4-18.
26. Lemarchand E, Gauberti M, Martinez de Lizarrondo S, Villain H, Repessé Y, Montagne A, et al. Impact of alcohol consumption on the outcome of ischemic stroke and thrombolysis: role of the hepatic clearance of tissue-type plasminogen activator. *Stroke*. 2015;46:1641–1650. doi: 10.1161/STROKEAHA.114.007143.
27. Macrez R, Obiang P, Gauberti M, Roussel B, Baron A, Parcq J, et al. Antibodies preventing the interaction of tissue-type plasminogen activator with N-methyl-D-aspartate receptors reduce stroke damages and extend the therapeutic window of thrombolysis. *Stroke*. 2011;42:2315–2322. doi: 10.1161/STROKEAHA.110.606293.

28. Gakuba C, Gauberti M, Mazighi M, Defer G, Hanouz JL, Vivien D. Preclinical evidence toward the use of ketamine for recombinant tissue-type plasminogen activator-mediated thrombolysis under anesthesia or sedation. *Stroke*. 2011;42:2947–2949. doi: 10.1161/STROKEAHA.111.620468.
29. Wang CX, Todd KG, Yang Y, Gordon T, Shuaib A. Patency of cerebral microvessels after focal embolic stroke in the rat. *J Cereb Blood Flow Metab*. 2001;21:413–421. doi: 10.1097/00004647-200104000-00010.
30. Romanos E, Planas AM, Amaro S, Chamorro A. Uric acid reduces brain damage and improves the benefits of rt-PA in a rat model of thromboembolic stroke. *J Cereb Blood Flow Metab*. 2007;27:14–20. doi: 10.1038/sj.jcbfm.9600312.
31. Carmichael ST. Rodent models of focal stroke: size, mechanism, and purpose. *NeuroRx*. 2005;2:396–409. doi: 10.1602/neurorx.2.3.396.
32. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, et al; STAIR Group. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009;40:2244–2250. doi: 10.1161/STROKEAHA.108.541128.
33. Harston GW, Sutherland BA, Kennedy J, Buchan AM. The contribution of L-arginine to the neurotoxicity of recombinant tissue plasminogen activator following cerebral ischemia: a review of rtPA neurotoxicity. *J Cereb Blood Flow Metab*. 2010;30:1804–1816. doi: 10.1038/jcbfm.2010.149.
34. Lapchak PA, Daley JT, Boitano PD. A blinded, randomized study of L-arginine in small clot embolized rabbits. *Exp Neurol*. 2015;266:143–146. doi: 10.1016/j.expneurol.2015.02.016.