Published in final edited form as:

Exp Neurol. 2015 April ; 266: 143–146. doi:10.1016/j.expneurol.2015.02.016.

A Blinded-Randomized Study of L-Arginine in Small Clot Embolized Rabbits

Paul A. Lapchak, Ph.D., FAHA¹ [Professor, Director of Translational Research], Jessica T. Daley, B.F.A.², and Paul D. Boitano, B.Sc.³

Cedars-Sinai Medical Center, Department of Neurology & Neurosurgery, Advanced Health Sciences Pavilion, Suite 8305, 127 S. San Vicente Blvd., Los Angeles, CA 90048, Office: 310-248-8188, Fax: 310-248-7568

Abstract

Objective—Tissue plasminogen activator (tPA) is administered to acute ischemic stroke victims in a vehicle formulation containing high concentrations of L-arginine (3.5 grams/100mg vial), a well-known nitric oxide synthase (NOS) substrate and precursor to nitric oxide (NO), as well as an enhancer of cerebral blood flow.

Methods—We studied the effects of tPA-vehicle compared to tPA (3.3mg/kg) formulated in the same vehicle containing L-arginine, normal saline or normal saline containing L-arginine, on behavioral function following small clot embolic strokes in rabbits using clinical rating scores and quantal analysis curves as the primary endpoint. Treatments were administered intravenously (1ml/kg; 20% bolus/80% infused over 30 minutes) starting 1 hour following the injection of small-sized blood clots into the brain vasculature and terminal behavior was measured 2 days following embolization. Behavioral rating scores were used to calculate the effective stroke dose (P_{50} in mg) that produces neurological deficits in 50% of the rabbits.

Results—In this study, tPA significantly ($p=0.001$) improved behavior compared to all other treatments including tPA-vehicle, saline and saline-L-arginine, increasing the P_{50} by 141% over tPA-vehicle. Saline-L-arginine was not significantly different from either saline or tPA-vehicle ($p>0.05$).

Conclusion—This study demonstrates that L-arginine component of the tPA vehicle does not contribute to the reproducible clinical improvement observed following tPA administration in rabbits. Moreover, the administration of L-arginine was not an effective method to promote behavioral recovery following embolic strokes in the stringent rabbit small clot stroke model, nor did L-arginine exacerbate behavioral deficits or intracerebral hemorrhage in embolized rabbits.

© 2015 Published by Elsevier Inc.

¹Please send correspondence to Paul A. Lapchak. Paul.Lapchak@cshs.org.

²Jessica T. Daley. Jessicadaley5@hotmail.com

³Paul D. Boitano. Paul.boitano@cshs.org

Conflict of Interest Statement: The authors have no study-related conflict of interest to report. PAL is Editor-in-Chief of the Journal of Neurology and Neurophysiology and Associate editor for Translational Stroke Research.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

nitric oxide; cerebral blood flow; toxicity; neuroprotection; thrombolytic

Introduction

In 1996 the FDA approved the thrombolytic, tissue plasminogen activator (tPA) for the treatment of stroke(1) based upon a randomized and blinded clinical trial comparing tPA to tPA-vehicle; tPA remains the only approved therapy for stroke in North America. tPA is a thrombolytic that promotes clot lysis, also known as a clot buster, which increases cerebral blood flow to the formerly clot-affected core and penumbra(2–10); this results in clinical and/or behavioral improvement.

In the clinic, tPA is administered to patients in a vehicle solution containing high concentrations of L-arginine (3.5 grams/100ml infusion)(11), a well-known nitric oxide synthase substrate and precursor to nitric oxide, as well as an enhancer of cerebral blood flow (CBF)(12, 13). As recent as October 2014(10), almost 20 years after the initial National Institute of Neurological Disorders and Stroke (NINDS) tPA trial(1), there remains controversy regarding the possible positive or negative physiological effects of intravenous(IV) administration of tPA-vehicle containing L-arginine, which to date, has not been studied in detail using a standardized embolic stroke model(14, 15). This is important for 3 reasons: 1) first to adhere to Stroke Therapy Academic Industry Roundtable (STAIR) recommendations(16) and study L-arginine in an experimental species other than a rodent, 2) because 10 previous studies, all conducted in rodents, have been inconclusive(13), or have not been conducted according to current RIGOR guidelines(17–19), and 3) to conduct the study randomized and blinded, with the inclusion of a “positive control” group as recommended in the literature(20).

Thus, we conducted a blinded and randomized study to investigate the potential effects of commercially supplied tPA vehicle containing L-arginine or normal saline containing L-arginine and compared these treatment groups to normal saline and a positive control using 3.3mg/kg tPA, which has previously been shown to effectively promote behavioral improvement in rabbits following embolic stroke(5, 21).

Methods

This study was conducted randomized and blinded according to current RIGOR guidelines(17, 18) with blinded conduct of the experiment and blinded reporting of outcome. We determined if vehicles containing L-arginine would alter behavioral function using the rabbit small clot embolic stroke model (RSCM)(14), the model originally used to provide tPA efficacy data 30 years ago(21).

Treatment Groups: All treatments were administered intravenous (IV) as 20% bolus injection with the remaining 80% infused over 30 minutes starting 1 hour following embolization. The study groups were:

1. saline (3.3 ml/kg; normal sterile injectable saline [Baxter Inc.]);

2. saline (3.3 ml/kg; normal sterile injectable saline [Baxter Inc.] containing 3.5grams/100ml L-arginine Sigma Inc.);
3. tPA-vehicle (3.3 ml/kg, which contains 3.5grams L-arginine grams/100ml. This was reconstituted in sterile water [Hospira Inc.] for injection);
4. Genentech clinical grade tPA (3.3 mg/kg, reconstituted in sterile water for injection [Hospira Inc.] at a dose of 1 mg/ml). Each 100mg vial of tPA contains: L-arginine (3.5 grams/100 mg vial), phosphoric acid (1g/100mg vial) nor Polysorbate80 (10mg/100mg vial).

Animals and animal welfare

This study utilized 2.2–2.5 kg male New Zealand white rabbits (*Oryctolagus cuniculus*) obtained from a commercial supplier in California. Rabbits were supplied food (alfalfa cubes) and water ad libitum while under quarantine in an enriched environment for at least 3 days prior to experimental use. All animals were purchased under an Institutional Animal Care and Use Committee (IACUC)-approved protocol for both surgical and treatment procedures used in this study. Throughout the study, veterinary staff monitored the health status of rabbits. Rabbits were humanely euthanized if they were in pain, showed extreme discomfort, or if they were unable to reach food or water after embolization. Studies were conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals.

Surgery and embolization

Surgical procedures were done as described previously(5, 22, 23), using isoflurane by mask (5% induction/3% maintenance) in a controlled environment with a room temperature between 22.4–23.2°C, with the following minor modifications. After placement of the carotid catheter, the catheter was filled with 0.2 ml of heparinized saline (33 units/ml) and plugged with an injection cap. The skin around the catheter was sealed tightly around the catheter using a combination purse string-Chinese finger trap suture. In this study, rabbits were allowed to recover from the effects of anesthesia for at least 2 hours before embolization. The absence of volatile anesthetics during the embolization procedure is beneficial when studying neuroprotection, since anesthetics have been shown to be neuroprotective, and can interfere with the ischemic cascade(22, 24) which can confound the interpretation of valuable scientific data due to drug-drug interactions and alteration of multiple components of the ischemic cascade. Analgesics are also not administered because of the potential to interfere with the ischemic stroke cascade(22).

For blood clot preparation prior to embolization, blood is drawn from one or more donor rabbits and allowed to clot for 3 h at 37°C. The single resulting large blood clot is then suspended in phosphate-buffered saline pH 7.4 (PBS) with 0.1% bovine serum albumin (BSA) and large Polytron-generated clot fragments are sequentially passed through sizing screens to result in a suspension of small-sized blood clots. The small-sized blood clots are then suspended in PBS containing 0.1% bovine serum albumin, and the clot suspension is then spiked with ⁵⁷Co-labeled microspheres to allow for tracking into the brain. An aliquot

of the solution is removed for the determination of specific activity (i.e. $^{57}\text{Co}/\text{mg}$ clot). This will aid in determination of clot burden in brain following embolization(5, 22, 23).

Behavioral Function and Quantal Dose-Response Analysis

The use of clinical rating scores and quantal analysis is a powerful statistical method to determine how a heterogeneous stroke population will respond to a treatment(5, 22, 23). To evaluate the quantitative relationship between clot burden in brain and clinical scores, logistic sigmoidal quantal analysis curves were constructed as originally described by Waud(25) and thereafter(5, 22, 23).

All RSCEM methods have been described in detail previously(5, 23). The primary endpoint used when assessing treatment efficacy in the RSCEM is behavioral functional, which is based upon motor function components of the National Institutes of Health Stroke Scale (NIHSS)(26). The use of clinical rating scores is a desirable primary endpoint to use when studying a novel therapeutic(14). Clinical scores in combination with quantal analysis is a sophisticated statistical analysis method to determine how a large population of stroke “patients”, in this study, rabbits, will respond to a treatment. To evaluate the quantitative relationship between clot dose lodged in the brain and behavioral deficits or clinical scores, logistic sigmoidal (S-shaped) quantal analysis curves were fit to dose-response data as originally described by Waud(25). To construct a quantal analysis curve, a wide range of clot doses were injected via the indwelling carotid catheter in order to produce a spectrum of behaviorally normal to abnormal animals, which included death on the continuum of embolization-induced effects(14). In the absence of a neuroprotective or thrombolytic treatment regimen, large clot doses invariably caused encephalopathy and behavioral deficits due to ischemia, neuronal degeneration, depletion of ATP and cell death(14). Small numbers of microclots lodged in the brain vasculature cause no grossly apparent neurologic dysfunction. In this study, less than 5% of animals died due to embolization, but in the event that embolization did result in a rapid death, there was a positive correlation with a high clot dose measured in brain and the animal was represented as an abnormal (or dead) on the quantal analysis curve if the rabbit received treatment, either vehicle or drug. A 3-tiered clinical scoring scale is used for analysis of embolized rabbits: normal, abnormal or dead. Embolized rabbits are scored as abnormal if they have one or more of the following symptoms: ataxia, leaning, circling, lethargy, nystagmus, loss of balance, loss of limb/facial sensation and occasionally, hind-limb paraplegia. Using a simple dichotomous rating system, with a reproducible composite result and low inter-rater variability (<5%), each surviving animal is rated as either behaviorally normal or abnormal by an observer naïve to the study design and/or treatment groups. Because this study was conducted randomized and blinded, there was no bias in behavioral scoring of embolized rabbits. For construction of quantal analysis curves, clot burden is plotted against behavior in order to define the P_{50} value. A separate quantal curve was generated for each treatment condition and a statistically significant increase in the P_{50} value (or the amount of blood clots in brain that produce neurologic dysfunction in 50% of a group of animals) compared to a control curve is indicative of neuroprotection or a behavioral improvement in the study population.

Behavior was measured 2 days following embolization in order to calculate; using an iterative curve fitting algorithm described previously, the effective stroke dose (P_{50} ; Mean $\pm$ SE in mg) that produces neurological deficits in 50% of the rabbits. After 48 hours, surviving animals were euthanized with 1–1.5 ml of Beuthanasia-D via the marginal ear vein.

Intracerebral hemorrhage analysis

Since tPA can cause intracerebral hemorrhage (ICH) in patients and embolized rabbits(27–35), we also determined the consequences of L-arginine and tPA on ICH incidence in embolized rabbits as described previously(27, 28, 36). After behavioral analysis, brains are removed, fixed in paraformaldehyde by immersion, and 5mm coronal slices are cut and examined for ICH by an observer blinded to the treatment regimen. The presence of ICH is defined according to published criteria(37).

Power and Statistical Analysis

Power analysis of quantal curves indicates that, assuming $\alpha=.05$ and $\beta=.90$, a coefficient of variation of 15% and a difference between means of 20%, a minimum sample size of 14 animals is required per group. In this study, 20 rabbits were enrolled in each group to ensure that sufficient numbers were included in each group for statistical analysis. Due to the complexity of the model, we do have some animals that are premature losses prior to and during embolization (i.e.: non-patent catheters or catheters that become dislodged from the carotid artery), extreme severe embolism response requiring immediate euthanasia per IACUC protocols, and some animals become excluded before treatment(s) can be fully administered. For statistical analysis, behavioral results were analyzed using the t-test (Graph Pad) and ICH incidence was analyzed using chi-square (Graph Pad) as described previously(36).

Results

In this systematic study, we compared the effects of administration of tPA-vehicle containing L-arginine to both normal saline, saline containing equivalent amounts of L-arginine and a tPA (3.3mg/kg) positive control group.

Behavioral Analysis

As shown in Figure 1A, the P_{50} value in the normal saline group was 1.15 ± 0.19 mg ($n=18$). Administration of saline containing L-arginine to simulate a major component of the tPA-vehicle did not affect P_{50} ($p>0.05$). However, tPA administration significantly increased the P_{50} values to 2.51 ± 0.35 mg ($n=19$; $p=0.0010$) compared to tPA vehicle, which had a P_{50} value of 1.04 ± 0.18 mg ($n=17$; $p=0.0079$) (Figure 1B), and $p=0.0019$ compared to saline.

Exclusions

In the saline, saline-L-arginine and tPA-vehicle-treated groups, 2 rabbits in each group died before 48 hours or were euthanized before 48 hours due to extreme embolism-induced

deficits. In the tPA-treated group, 3 rabbits were euthanized or died before 48 hours. 4 rabbits were excluded from the study due to technical issues with catheters or embolization.

Intracerebral Hemorrhage Analysis

As shown in Table 1, ICH was not significantly different when comparisons were done between groups. There was a baseline hemorrhage rate of 5.5–5.8% in the vehicle groups (punctate and hemorrhagic infarctions) and a 21% ICH incidence in the tPA-treated group, which included 2 parenchymal hemorrhages, 1 punctate hemorrhage and, 1 hemorrhagic infarction (2 rabbits died prior to 48 hours).

Discussion and Conclusions

This study demonstrates conclusively that tPA improved behavioral function and increased P₅₀ values compared to normal saline and two vehicles containing the same concentration of L-arginine that is a component of the tPA product. Neither vehicle which contained L-arginine altered behavior (protection or toxicity) when administered IV to rabbits at doses equivalent to 3.3mg/kg. It is possible that the already existing spike in nitric oxide observed in rabbits following embolism(38) attenuates any additional behavioral response to L-arginine/nitric oxide.

There are 2 caveats to the current study: 1) the study did not assess the effects phosphoric acid (1g/100mg vial) nor Polysorbate80 (10mg/100mg vial) a nonionic surfactant and emulsifier, which are the 2 other components of the lyophilized commercially available Alteplase product, and 2) We administered tPA/tPA vehicle at 3.3 mg/kg, higher than the clinical dose(0.9mg/kg)(1). This dose of tPA has repeatedly been shown to be necessary to produce statistically significant behavioral improvement in New Zealand white rabbits(5).

There is some important information in the literature(13) indicating that L-arginine may have either beneficial or detrimental effects, but the effects of L-arginine are controversial(13) and the results heterogeneous. As cited in the paper by Harston et al.(13), there have already been 10 studies of the effects of L-arginine on either behavior and/or infarct volume, and the results are heterogeneous and inconclusive. It has been speculated that the high level of L-arginine in the tPA formulation may be “fueling” rt-PA neurotoxicity, but the claim is unsubstantiated by scientific evidence in the peer-reviewed literature (see(13)).

In conclusion, the L-arginine component of the tPA vehicle does not appear contribute to the reproducible clinical improvement observed following tPA administration in either rabbits, since there was no effects of either L-arginine vehicle or L-arginine in saline compared to a saline control group. The administration of L-arginine, a NOS substrate and NO precursor was not an effective method to promote behavioral recovery following embolic strokes in the stringent rabbit model, nor did L-arginine exacerbate behavioral deficits or ICH.

Acknowledgments

This study was supported by donations to fund stroke research. This research was not directly supported by NIH or any other institutional funding source. PAL was partially supported by NS060685.

References

1. NINDS. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N Engl J Med.* 1995; 333(24):1581–7. [PubMed: 7477192]
2. del Zoppo G. Thrombolytic therapy in cerebrovascular disease. *Current Concepts of Cerebrovascular Disease.* 1988; 23:7.
3. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. *Lancet.* 1998; 352(9136):1245–51. [PubMed: 9788453]
4. Lapchak PA. Development of thrombolytic therapy for stroke: a perspective. *Expert Opin Investig Drugs.* 2002; 11(11):1623–32.
5. Lapchak PA, Araujo DM, Zivin JA. Comparison of Tenecteplase with Alteplase on clinical rating scores following small clot embolic strokes in rabbits. *Exp Neurol.* 2004; 185(1):154–9. [PubMed: 14697326]
6. Lyden PD. Should all stroke patients receive tissue plasminogen activator therapy, despite mild or improving symptoms? *Nat Clin Pract Cardiovasc Med.* 2006; 3(4):184–5. [PubMed: 16568123]
7. Lyden PD, Lees KR, Davis SM. Alteplase for acute stroke revisited: the first 10 years. *Lancet Neurol.* 2006; 5(9):722–4. [PubMed: 16914399]
8. Lyden PD, Zivin JA, Clark WA, Madden K, Sasse KC, Mazzarella VA, et al. Tissue plasminogen activator-mediated thrombolysis of cerebral emboli and its effect on hemorrhagic infarction in rabbits. *Neurology.* 1989; 39:703. [PubMed: 2496332]
9. Hacke W, Donnan G, Fieschi C, Kaste M, von Kummer R, Broderick JP, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *Lancet.* 2004; 363(9411):768–74. [PubMed: 15016487]
10. Hacke, W., editor. *Thrombolysis, Thrombectomy and Acute Stroke Therapy Conference; Thrombolysis, Thrombectomy and Acute Stroke Therapy Conference; 2014; Heidelberg - Mannheim.*
11. ACTIVASE Prescribing Information. Genentech Inc; 2012. http://www.gene.com/download/pdf/activase_prescribing.pdf updated 2012 Available from: http://www.gene.com/download/pdf/activase_prescribing.pdf [Last accessed October 28, 2014]
12. Willmot M, Gray L, Gibson C, Murphy S, Bath PM. A systematic review of nitric oxide donors and L-arginine in experimental stroke; effects on infarct size and cerebral blood flow. *Nitric oxide : biology and chemistry/official journal of the Nitric Oxide Society.* 2005; 12(3):141–9. [PubMed: 15797842]
13. 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(11):1804–16. [PubMed: 20736961]
14. Lapchak PA. Translational Stroke Research Using a Rabbit Embolic Stroke Model: A Correlative Analysis Hypothesis for Novel Therapy Development. *Transl Stroke Res.* 2010; 1(2):96–107. [PubMed: 20539748]
15. Turner R, Jickling G, Sharp F. Are underlying assumptions of current animal models of human stroke correct: from STAIRS to high hurdles? *Translational Stroke Research.* 2011; 2(2):138–43. [PubMed: 21654913]
16. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke.* 1999; 30(12):2752–8. [PubMed: 10583007]
17. Lapchak PA. Recommendations and practices to optimize stroke therapy: developing effective translational research programs. *Stroke.* 2013; 44(3):841–3. [PubMed: 23391768]
18. Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, et al. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature.* 2012; 490(7419):187–91. [PubMed: 23060188]
19. Lapchak PA, Zhang JH, Noble-Haeusslein LJ. RIGOR guidelines: escalating STAIR and STEPS for effective translational research. *Transl Stroke Res.* 2013; 4(3):279–85. [PubMed: 23658596]

20. Lapchak PA, Zhang JH. Resolving the Negative Data Publication Dilemma in Translational Stroke Research. *Transl Stroke Res.* 2011; 2(1):1–6. [PubMed: 21666822]
21. Zivin JA, Fisher M, DeGirolami U, Hemenway CC, Stashak JA. Tissue plasminogen activator reduces neurological damage after cerebral embolism. *Science.* 1985; 230(4731):1289. [PubMed: 3934754]
22. Lapchak, PA. A clinically relevant rabbit embolic stroke model for acute ischemic stroke therapy development: mechanisms & targets. In: Lapchak, PA.; Zhang, JH., editors. *Translational Stroke Research: From Target Selection to Clinical Trials.* Springer; USA: 2012. p. 541–84.
23. Lapchak PA, Wei J, Zivin JA. Transcranial infrared laser therapy improves clinical rating scores after embolic strokes in rabbits. *Stroke.* 2004; 35(8):1985–8. [PubMed: 15155955]
24. Kawaguchi M, Furuya H, Patel PM. Neuroprotective effects of anesthetic agents. *J Anesth.* 2005; 19(2):150–6. [PubMed: 15875133]
25. Waud DR. On biological assays involving quantal responses. *J Pharmacol Exp Ther.* 1972; 183(3): 577–607. [PubMed: 4636393]
26. Lyden P, Brott T, Tilley B, Welch KM, Mascha EJ, Levine S, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. *Stroke.* 1994; 25(11):2220–6. [PubMed: 7974549]
27. Lapchak PA. Effect of internal carotid artery reperfusion in combination with Tenecteplase on clinical scores and hemorrhage in a rabbit embolic stroke model. *Brain Res.* 2009; 1294:211–7. [PubMed: 19643097]
28. Lapchak PA, Song D, Wei J, Zivin JA. Pharmacology of caffeinol in embolized rabbits: clinical rating scores and intracerebral hemorrhage incidence. *Exp Neurol.* 2004; 188(2):286–91. [PubMed: 15246828]
29. Albers GW, Bates VE, Clark WM, Bell R, Verro P, Hamilton SA. Intravenous tissue-type plasminogen activator for treatment of acute stroke: the Standard Treatment with Alteplase to Reverse Stroke (STARS) study. *Jama.* 2000; 283(9):1145–50. [PubMed: 10703776]
30. Lapchak PA. Hemorrhagic transformation following ischemic stroke: significance, causes, and relationship to therapy and treatment. *Curr Neurol Neurosci Rep.* 2002; 2(1):38–43. [PubMed: 11898581]
31. Lapchak PA, Araujo DM. Advances in ischemic stroke treatment: neuroprotective and combination therapies. *Expert Opin Emerg Drugs.* 2007; 12(1):97–112. [PubMed: 17355216]
32. Lapchak PA, Araujo DM. Advances in hemorrhagic stroke therapy: conventional and novel approaches. *Expert Opin Emerg Drugs.* 2007; 12(3):389–406. [PubMed: 17874968]
33. Lyden PD, Madden KP, Clark WM, Sasse KC, Zivin JA. Incidence of cerebral hemorrhage after treatment with tissue plasminogen activator or streptokinase following embolic stroke in rabbits [corrected]. *Stroke.* 1990; 21(11):1589–93. [PubMed: 2122552]
34. Lyden PD, Zivin JA. Hemorrhagic transformation after cerebral ischemia: mechanisms and incidence. *Cerebrovasc Brain Metab Rev.* 1993; 5(1):1–16. [PubMed: 8452759]
35. Lyden PD, Zivin JA, Soll M, Sitzer M, Rothrock JF, Alksne J. Intracerebral hemorrhage after experimental embolic infarction. Anticoagulation. *Arch Neurol.* 1987; 44(8):848–50. [PubMed: 3632395]
36. Lapchak PA. Efficacy and safety profile of the carotenoid trans sodium crocetin administered to rabbits following multiple infarct ischemic strokes: a combination therapy study with tissue plasminogen activator. *Brain Res.* 2010; 1309:136–45. [PubMed: 19891959]
37. Lapchak PA, Chapman DF, Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. *Stroke.* 2000; 31(12): 3034–40. [PubMed: 11108768]
38. Silver JH, Lapchak PA. Continuous Monitoring of Changes in Plasma Nitrite following Cerebral Ischemia in a Rabbit Embolic Stroke Model. *Translational Stroke Research.* 2011; 2(2):218–26. [PubMed: 21625287]

Highlights

- We studied the effects of L-arginine and tPA in embolized rabbits
- L-arginine did not affect behavioral outcome or hemorrhage compared to saline
- tPA improved behavioral outcome compared to L-arginine vehicle or saline

Figure 1. Effect of treatment on behavioral outcome

For the superimposed graphs, behaviorally normal animals are plotted on the y-axis at 0 and abnormal animals are plotted at 100. The figure shows that there is positive correlation between the raw data and the statistically fit sigmoidal quantal curves. Fig 1A provides data for saline-treated and saline-L-arginine-treated rabbits. The curves are not statistically different ($p>0.05$). Figure 1B provides data and quantal analysis curves for the tPA-vehicle-treated group and the tPA (3.3mg/kg)-treated rabbits. tPA significantly increases P_{50} ($p=0.001$) compared to tPA-vehicle.

Table 1

Effect of treatment on intracerebral hemorrhage rate in embolized rabbits

	Treatment Group			
Measure	Saline	Saline + L-arginine	tPA vehicle	tPA (3.3 mg/kg)
ICH rate	1/18 (5.5%)	1/18 (5.5%)	1/17 (5.8%)	4/19 (21.0%)
p value	NA	P= 1	P= 1	P= 0.36

Statistical significance was determined using a 2×2 contingency table.