William M. Feinberg Award for Excellence in Clinical Stroke

High Explosive Treatment for Ultra-Acute Stroke: Hype of Hope

Philip M. Bath, DSc, FMedSci

Proven treatments for acute stroke can be categorized by target population (ischemic stroke [IS], intracerebral hemorrhage [ICH], or both), utility (proportion of patients who can be treated), magnitude of efficacy, and cost. In general, high-cost interventions need to have high efficacy for health economic reasons; examples include intravenous alteplase, mechanical thrombectomy, and hemicraniectomy. Conversely, low-cost interventions, such as aspirin, typically have low efficacy. Having a medium-to-high efficacy intervention that was inexpensive and could be used worldwide in both IS and ICH would be a major advantage.

High blood pressure (BP) is common in acute stroke and associated independently with poor functional outcome, increased death, and early recurrence (IS) and hematoma expansion (ICH).[1,2] Although debated since 1985,[3] it was only recently demonstrated in the INTERACT-2 (Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage Trial-2) trial that intensive BP lowering was beneficial,[4] as now promoted in international guidelines.[5,6] In contrast, whether BP should be lowered acutely in IS remains unclear.[7–9]

Nitric oxide (NO) is a diatomic gaseous molecule of nitrogen and oxygen that is highly reactive and short lived. However, it is a fundamental physiological control and defense molecule that may have been used by early organisms as far back as 3 billion years.[10] In human physiology, it has vascular regulatory, neurotransmitter, anti-infection, and reproductive roles. NO is synthesized from the amino acid L-arginine by 3 enzymes: endothelial, inducible, and neuronal NO synthase. Many effects of NO are mediated through the second messenger cGMP, which is catalyzed by guanylate cyclase from guanylate triphosphate. cGMP levels may be maintained by phosphodiesterase-5 inhibitors such as dipyridamole. NO is inactivated through oxidation to nitrite then nitrate; this reaction may be reversed and it now appears that NO may be synthesized from nitrate in humans (eg, from beetroot and green leafy vegetables).[11] The importance of NO in physiology was recognized in 1992 as molecule of the year,[12] and the awarding of Nobel prizes to Furchgott, Ignarro and Murad in 1998 for their contribution, along with that of Moncada, to the elucidation of NO as endothelium-derived relaxing factor and the mediation of its activity via cGMP.[13]

NO levels are low in acute IS and even more so in ICH.[14,15] In view of the multiple potentially beneficial actions of NO, supplementation might then be beneficial. Preclinical studies of experimental stroke, many of these now rather dated, found that NO donors reduced lesion size, but only if administered early after stroke induction[16]; NO also induced reperfusion in permanent models of ischemia. Clinically, NO can be administered as substrate (L-arginine or nitrate), as the gas (as done in neonatology), as a NO donating drug,[17] as a drug that stimulates endogenous NO synthesis (eg, statins), or as a drug that inhibits phosphodiesterase-5. Of these interventions, the most relevant for human study includes organic nitrates, such as glyceryl trinitrate (GTN, with transdermal, sublingual, or intravenous administration) and isosorbide mononitrate (oral), and the metal–NO complex, sodium nitroprusside (intravenous).

GTN is the medical name for nitroglycerin, a high explosive found in dynamite.[18] Alfred Nobel made his fortune in the late 1800s when he learnt to stabilize nitroglycerin and make its routine use feasible and safe; this fortune supports the prizes that are given his name.[19] Ironically, Nobel developed angina and used nitroglycerin for prophylaxis. Even more ironic, he died of a stroke (ICH)[19]; so, if the GTN-stroke story told here is correct, he might have been able to treat himself with his own invention!

Three phase II trials of transdermal GTN in patients with acute and subacute stroke found that this NO donor rapidly reduced peripheral and central BP, 24-hour BP, pulse pressure, and augmentation index (amounting to improved vascular compliance).[20–22] Although sodium nitroprusside, another NO donor, reduced platelet function,[23] GTN had no such effect[20]; as a result, GTN may be given in ICH. When given to lower systolic BP by ≈10%, neither sodium nitroprusside nor GTN reduced cerebral blood flow or altered cerebral blood flow velocity.[21–24]

On the basis of the above data, the safety and efficacy of GTN was tested in 4011 patients with acute IS or ICH (within 48 hours of ictus[25]) across 5 continents, 23 countries, and 173 hospital sites in the ENOS trial (Efficacy of Nitric

Received June 6, 2016; final revision received June 6, 2016; accepted June 8, 2016.

From the Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham, United Kingdom.

Presented as the William M. Feinberg Lecture at the International Stroke Conference, Los Angeles, CA, February 17–19, 2016.

Correspondence to Philip M. Bath, DSc, FMedSci, Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham, City Hospital Campus, Nottingham NG3 5LX, United Kingdom. E-mail philip.bath@nottingham.ac.uk

(*Stroke*. 2016;47:2423-2426. DOI: 10.1161/STROKEAHA.116.013243.)

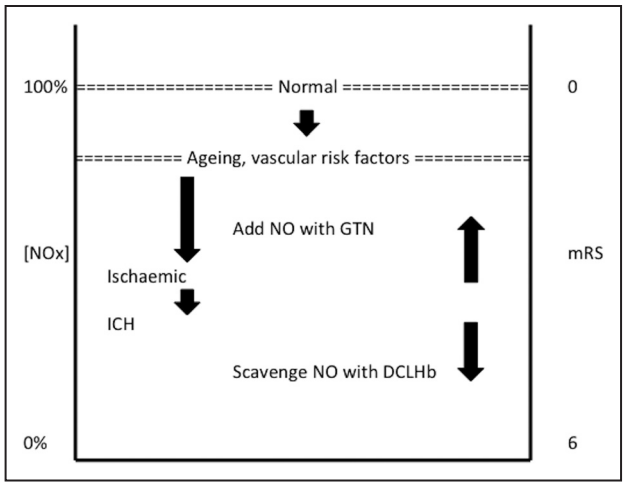

Figure 1. Hypothesized changes in vascular nitric oxide (NO) during aging, after stroke, and after treatment with a NO or scavenger. DCLHb indicates diaspirin cross-linked hemoglobin; GTN, glyceryl trinitrate; ICH, intracerebral hemorrhage; mRS, modified Rankin Scale; and NOx, nitrite+nitrate.

Oxide in Stroke).[26] GTN did not shift the modified Rankin Scale (adjusted common odds ratio 1.01; 95% confidence interval, 0.91–1.13).[9] However, GTN improved the modified Rankin Scale in patients randomized within 6 hours of stroke onset, a preplanned subgroup (called ENOS-early from here on).[9,27] Similarly, GTN improved the modified Rankin Scale in the small (n=41) phase II ambulance-based RIGHT (Rapid Intervention With Glyceryl Trinitrate in Hypertensive Stroke Trial) with paramedics performing screening, recruitment, consent, and treatment.[28,29]

Data from all 5 GTN trials (4197 patients) were aggregated in an individual patient data meta-analysis.[30] These confirmed that GTN improved functional outcome if given within 6 hours but not later; time-dependent benefits were also seen for death, disability (Barthel Index), cognition (telephone Mini-Mental State Examination, telephone interview cognition scale, and verbal fluency), mood (Zung Depression Scale), and quality of life (Euro-QoL health utility status and Visual Analogue Scale). When administered within 6 hours, GTN was effective in IS and, potentially more so, in ICH; in IS, the benefit was seen in patients who received intravenous alteplase (either before or after GTN), and potentially in those who were not given thrombolysis.[30]

The importance of low NO levels contributing to poor outcome after stroke is further highlighted by studies of diaspirin cross-linked hemoglobin, which increases levels of endothelin (a potent vasoconstrictor), and scavenges vascular NO.[31] In a small trial in patients with acute IS, treatment with diaspirin cross-linked hemoglobin was associated with a significant worsening of outcome, as measured using the modified Rankin Scale, Barthel Index, and National Institutes of Health Stroke Scale.[32] Hence, a model of vascular NO and stroke can be built whereby increasing age and vascular risk factors reduce NO levels, these falling further in acute IS, and particularly in acute ICH. Administering NO as GTN may improve outcome, whereas further lowering NO worsens outcome, as occurs through scavenging with diaspirin cross-linked hemoglobin (Figure 1).

Two parallel streams of research are now ongoing. First, the existing preclinical data[16] need to be expanded with modern high-quality multicentre studies[33] involving appropriate randomization and blinding, eg, through the Multi-PART international consortium (http://www.dcn.ed.ac.uk/multipart/, accessed 18 June 2016). And second, further clinical trials are needed in the ultra-acute prehospital period after stroke. One trial of GTN is ongoing (RIGHT-2, http://right-2.ac.uk), and others will start shortly or are seeking funding.

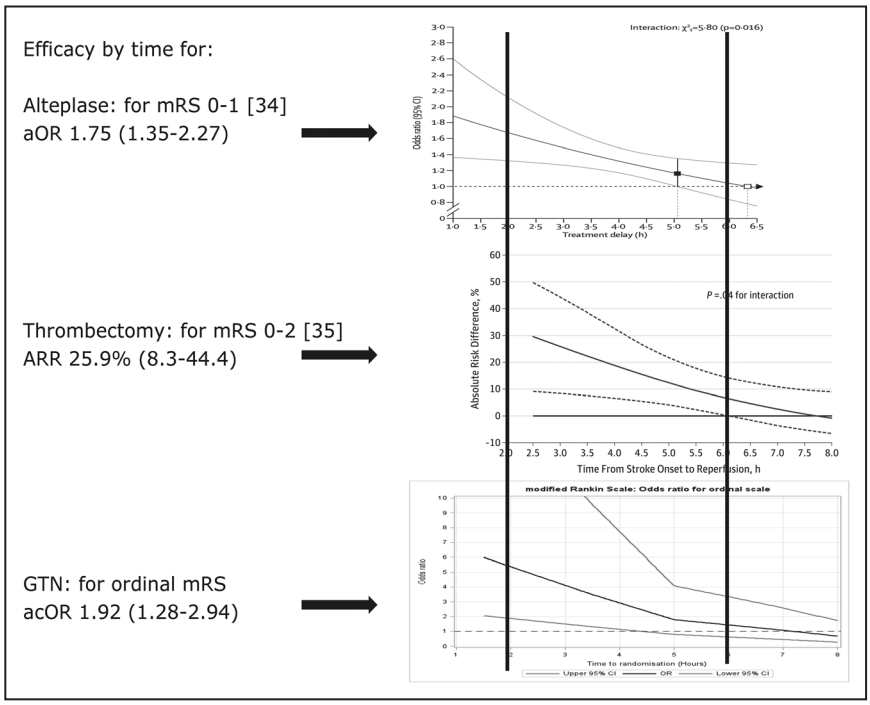

Figure 2. Efficacy by time to randomization, with high odds ratios (ORs) signifying improved functional outcome as measured using modified Rankin Scale (mRS). Figures are scaled to align randomization at 2 and 6 h (vertical black lines). aOR indicates adjusted OR; acOR, adjusted common OR; and GTN, glyceryl trinitrate. Adapted from Emberson et al[34] with permission of the publisher. Copyright ©2014, the Authors (see: https://creativecommons.org/licenses/by/4.0/). Adapted from Fransen et al[35] with permission of the publisher. Copyright ©2016, the American Medical Association. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

If NO, say given as transdermal GTN, is eventually shown to be effective, it is likely that its effects will be mediated through several mechanisms. First, the time course whereby efficacy is inversely related to the time from onset to treatment mirrors that seen for proven reperfusion strategies based on intravenous alteplase and mechanical thrombectomy (Figure 2).[34,35] That GTN might work through reperfusion is unsurprising in view of its known potent vasodilatory properties. Vasodilation might involve both enhancing collateral flow via surface pial arteries (ie, opening the backdoor[36]) and main cerebral arteries (front door). Second, GTN might amplify the effects of alteplase, both through preparing patients for thrombolysis by lowering their systolic BP below 185 mm Hg before hospitalization, and by opening the artery around the occluding clot so as to improve access by fibrinolytic agents, whether endogenous or exogenous. Nonsignificant trends to more and earlier thrombolysis were seen in the RIGHT pilot trial.[29] Third, lowering BP might reduce hematoma expansion after ICH,[4] and early recurrence after IS. Fourth, GTN has cytoprotective and antiapoptotic properties[37] and might prevent ischemic cells from dying. Finally, other properties that might be of relevance include sympathetic stimulation and plasma volume expansion.

The apparent positive effects of GTN on multiple clinical outcomes suggest that future trials of NO donors need to factor in such measures to give a more holistic view of its effects. Furthermore, ambulance-based trials should assess the effect of the intervention on in-hospital treatments such as use of alteplase, which might increase and be started earlier.[29] In contrast, thrombectomy, hemicraniectomy, intensive care, and therapy might each be less necessary. For example, ENOS-early suggested that involvement by a physiotherapist might be reduced in patients randomized to GTN.[27]

There is much to do to complete this story. Further trials of GTN (and possibly other NO donors) are needed because the existing data are small and largely come from a subgroup (ENOS-early) of a large neutral trial. Studies also need to be led by groups other than mine to expand the validity and diversity of data. Finally, stroke physicians may need to readjust their perspective that effective interventions have to be expensive; if GTN is effective, then it will break this link so that an easy to give intervention can be of relevance to both developed and developing countries, both major types of stroke, and cost <£5 (€6/$7) per patient. Ultimately, time will tell whether this developing story is hype or hope!

Acknowledgments

I thank my many mentors, research fellows, research colleagues, and partner for all their help and support during my career and this work on the nitric oxide-stroke journey.

Disclosures

P.M. Bath has been chief investigator of commercial trials, has provided advice to a number of pharmaceutical, medtech, and biotech companies as a member of scientific advisory boards, and has received honoraria for various presentations at international scientific meetings. He is Stroke Association Professor of Stroke Medicine.

References

1. Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PA; IST Collaborative Group. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke*. 2002;33:1315–1320.
2. Sprigg N, Gray LJ, Bath PM, Boysen G, De Deyn PP, Friis P, et al; TAIST Investigators. Relationship between outcome and baseline blood pressure and other haemodynamic measures in acute ischaemic stroke: data from the TAIST trial. *J Hypertens*. 2006;24:1413–1417. doi: 10.1097/01.hjh.0000234123.55895.12.
3. Hachinski V. Hypertension in acute ischemic strokes. *Arch Neurol*. 1985;42:1002.
4. Anderson CS, Heeley E, Huang Y, Wang J, Stapf C, Delcourt C, et al; INTERACT2 Investigators. Rapid blood-pressure lowering in patients with acute intracerebral hemorrhage. *N Engl J Med*. 2013;368:2355–2365. doi: 10.1056/NEJMoa1214609.
5. Steiner T, Al-Shahi Salman R, Beer R, Christensen H, Cordonnier C, Csiba L, et al; European Stroke Organisation. European Stroke Organisation (ESO) guidelines for the management of spontaneous intracerebral hemorrhage. *Int J Stroke*. 2014;9:840–855. doi: 10.1111/ijs.12309.
6. Hemphill JC 3rd, Greenberg SM, Anderson CS, Becker K, Bendok BR, Cushman M, et al; American Heart Association Stroke Council; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*. 2015;46:2032–2060. doi: 10.1161/STR.0000000000000069.
7. Sandset EC, Bath PM, Boysen G, Jatuzis D, Kõrv J, Lüders S, et al; SCAST Study Group. The angiotensin-receptor blocker candesartan for treatment of acute stroke (SCAST): a randomised, placebo-controlled, double-blind trial. *Lancet*. 2011;377:741–750. doi: 10.1016/S0140-6736(11)60104-9.
8. He J, Zhang Y, Xu T, Zhao Q, Wang D, Chen CS, et al; CATIS Investigators. Effects of immediate blood pressure reduction on death and major disability in patients with acute ischemic stroke: the CATIS randomized clinical trial. *JAMA*. 2014;311:479–489. doi: 10.1001/jama.2013.282543.
9. The ENOS Trial Investigators. Efficacy of nitric oxide, with or without continuing antihypertensive treatment, for management of high blood pressure in acute stroke (ENOS): a partial-factorial randomised controlled trial. *Lancet*. 2015;385:617–628.
10. Feelisch M, Martin JF. The early role of nitric oxide in evolution. *Trends Ecol Evol*. 1995;10:496–499.
11. Kapil V, Webb AJ, Ahluwalia A. Inorganic nitrate and the cardiovascular system. *Heart*. 2010;96:1703–1709. doi: 10.1136/hrt.2009.180372.
12. Koshland DJ. The molecule of the year. *Science*. 1992;258:1861.
13. Howlett R. Nobel award stirs up debate on nitric oxide breakthrough. *Nature*. 1998;395:625–626. doi: 10.1038/27019.
14. Ferlito S, Gallina M, Pitari GM, Bianchi A. Nitric oxide plasma levels in patients with chronic and acute cerebrovascular disorders. *Panminerva Med*. 1998;40:51–54.
15. Rashid PA, Whitehurst A, Lawson N, Bath PM. Plasma nitric oxide (nitrate/nitrite) levels in acute stroke and their relationship with severity and outcome. *J Stroke Cerebrovasc Dis*. 2003;12:82–87. doi: 10.1053/jscd.2003.9.
16. 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*. 2005;12:141–149. doi: 10.1016/j.niox.2005.01.003.
17. Wang PG, Xian M, Tang X, Wu X, Wen Z, Cai T, et al. Nitric oxide donors: chemical activities and biological applications. *Chem Rev*. 2002;102:1091–1134.
18. Akhavan J. *The Chemistry of Explosives*. Cambridge: The Royal Society of Chemistry; 2011.
19. Sohlman R. *The Legacy of Alfred Nobel, the Story Behind the Nobel Prizes*. London: The Bodley Head; 1983.
20. Bath PM, Pathansali R, Iddenden R, Bath FJ. The effect of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure and platelet function in acute stroke. *Cerebrovasc Dis*. 2001;11:265–272. doi: 10.1159/000047649.
21. Rashid P, Weaver C, Leonardi-Bee J, Bath F, Fletcher S, Bath P. The effects of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure, cerebral and cardiac hemodynamics, and plasma nitric oxide

levels in acute stroke. *J Stroke Cerebrovasc Dis*. 2003;12:143–151. doi: 10.1016/S1052-3057(03)00037-5.
22. Willmot M, Ghadami A, Whysall B, Clarke W, Wardlaw J, Bath PM. Transdermal glyceryl trinitrate lowers blood pressure and maintains cerebral blood flow in recent stroke. *Hypertension*. 2006;47:1209–1215. doi: 10.1161/01.HYP.0000223024.02939.1e.
23. Butterworth RJ, Cluckie A, Jackson SH, Buxton-Thomas M, Bath PM. Pathophysiological assessment of nitric oxide (given as sodium nitroprusside) in acute ischaemic stroke. *Cerebrovasc Dis*. 1998;8:158–165.
24. Sare GM, Gray LJ, Bath PM. Effect of antihypertensive agents on cerebral blood flow and flow velocity in acute ischaemic stroke: systematic review of controlled studies. *J Hypertens*. 2008;26:1058–1064. doi: 10.1097/HJH.0b013e3282fbd240.
25. The ENOS Trial Investigators. Glyceryl trinitrate vs. control, and continuing vs. stopping temporarily prior antihypertensive therapy, in acute stroke: rationale and design of the Efficacy of Nitric Oxide in Stroke (ENOS) trial (ISRCTN99414122). *Int J Stroke*. 2006;1:245–249.
26. ENOS Investigators. Baseline characteristics of the 4011 patients recruited into the 'Efficacy of Nitric Oxide in Stroke' (ENOS) trial. *Int J Stroke*. 2014;9:711–720.
27. Woodhouse L, Scutt P, Krishnan K, Berge E, Gommans J, Ntaios G, et al; ENOS Investigators. Effect of hyperacute administration (within 6 hours) of transdermal glyceryl trinitrate, a nitric oxide donor, on outcome after stroke: subgroup analysis of the Efficacy of Nitric Oxide in Stroke (ENOS) trial. *Stroke*. 2015;46:3194–3201. doi: 10.1161/STROKEAHA.115.009647.
28. Ankolekar S, Sare G, Geeganage C, Fuller M, Stokes L, Sprigg N, et al. Determining the feasibility of ambulance-based randomised controlled trials in patients with ultra-acute stroke: study protocol for the "Rapid Intervention With GTN in Hypertensive Stroke Trial" (RIGHT, ISRCTN66434824). *Stroke Res Treat*. 2012;2012:385753. doi: 10.1155/2012/385753.
29. Ankolekar S, Fuller M, Cross I, Renton C, Cox P, Sprigg N, et al. Feasibility of an ambulance-based stroke trial, and safety of glyceryl trinitrate in ultra-acute stroke: the Rapid Intervention With Glyceryl Trinitrate in Hypertensive Stroke Trial (RIGHT, ISRCTN66434824). *Stroke*. 2013;44:3120–3128. doi: 10.1161/STROKEAHA.113.001301.
30. Bath PM, Woodhouse L, Krishnan K, Anderson C, Berge E, Ford GA, et al. Effect of treatment delay, stroke type, and thrombolysis on the effect of glyceryl trinitrate, a nitric oxide donor, on outcome after acute stroke: a systematic review and meta-analysis of individual patient from randomised trials. *Stroke Res Treat*. 2016;2016:9706720. doi: 10.1155/2016/9706720.
31. Schultz SC, Grady B, Cole F, Hamilton I, Burhop K, Malcolm DS. A role for endothelin and nitric oxide in the pressor response to diaspirin cross-linked hemoglobin. *J Lab Clin Med*. 1993;122:301–308.
32. Saxena R, Wijnhoud AD, Carton H, Hacke W, Kaste M, Przybelski RJ, et al. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. *Stroke*. 1999;30:993–996.
33. Bath PM, Macleod MR, Green AR. Emulating multicentre clinical stroke trials: a new paradigm for studying novel interventions in experimental models of stroke. *Int J Stroke*. 2009;4:471–479. doi: 10.1111/j.1747-4949.2009.00386.x.
34. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al; Stroke Thrombolysis Trialists' Collaborative Group. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet*. 2014;384:1929–1935. doi: 10.1016/S0140-6736(14)60584-5.
35. Fransen P, Berkemer O, Lingsma H, Beumer D, van den Berg L, Yoo A, et al; for the Multicenter Randomized Clinical Trial of Endovascular Treatment of Acute Ischemic Stroke in the Netherlands (MR CLEAN) Investigators. Time to reperfusion and treatment effec for acute ischemic stroke: a randomized clinical trial. *JAMA Neurol*. 2016;73:190–196. doi: 10.1001/jamaneurol.2015.3886.
36. Morikawa E, Moskowitz MA, Huang Z, Yoshida T, Irikura K, Dalkara T. L-arginine infusion promotes nitric oxide-dependent vasodilation, increases regional cerebral blood flow, and reduces infarction volume in the rat. *Stroke*. 1994;25:429–435.
37. Kim YM, Talanian RV, Billiar TR. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. *J Biol Chem*. 1997;272:31138–31148.

KEY WORDS: blood pressure ■ cerebral hemorrhage ■ cerebral infarction ■ nitric oxide ■ nitroglycerin ■ stroke, acute

Stroke
Journal of the American Heart Association

William M. Feinberg Award for Excellence in Clinical Stroke: High Explosive Treatment for Ultra-Acute Stroke: Hype of Hope

Philip M. Bath

Stroke. 2016;47:2423-2426; originally published online July 21, 2016;
doi: 10.1161/STROKEAHA.116.013243
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628