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Neuroprotective strategies for acute ischemic stroke: recent progress and future perspectives

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ABSTRACT

Stroke is one of the leading causes of death and most common cause of disability in adult. Despite of recent advances in the recanalization therapy for acute ischemic stroke, the need for neuroprotectants is substantial, to extend the window for recanalization therapy and to prevent neuronal death by ischemic brain injury or by reperfusion injury after successful therapy. Herein, the possible reasons for the failure of neuroprotectants trials during the past two decades and current status of neuroprotective strategies for acute ischemic stroke are discussed. This review will also address the recent advances and future perspectives in preclinical and clinical trials of neuroprotective agents, including the efforts of high quality of transition of preclinical results to clinical trial, genetic studies to trigger neuroprotectants development, application of neuroprotectants at optimal time and duration after stroke, adjuvant approaches, and biomarker-based triage.

Keywords: Ischemic stroke; Neuroprotective agents; Reperfusion injury; Stroke

INTRODUCTION

Stroke is one of the leading causes of death and most common cause of disability in adult. The approved treatment of acute ischemic stroke are intravenous thrombolysis (IVT) with tissue plasminogen activator and endovascular therapy (EVT) with stentriever. However, due to short time window (within 4.5 hours after onset of symptoms) for the use of IVT, only a minority (3% to 8%) of patients with acute ischemic stroke are eligible for thrombolysis. Thrombolysis does not always result in complete recanalization (20% to 66%), and even if recanalization is achieved with IVT, reocclusion with neurological deterioration can occur in >30% [1,2]. More recently, beneficial effect of EVT was confirmed in acute ischemic stroke [3-7]. However, a substantial patients are EVT-ineligible (only 7% to 13% of acute ischemic stroke were eligible to EVT) [8], failure (suboptimal or no reperfusion [TICI 0-2a] in 14% to 41% in five recent phase III randomized control trials [RCTs]) [3-7] or futile (26% to 49% showed poor outcome despite of successful recanalization) [9]. Although EVT improved outcome compared to standard medical treatment, a majority of patients treated with EVT have residual neurological impairments. In addition, preclinical and clinical studies have demonstrated infarct growth in the peri-infarct

areas after recanalization therapy by reperfusion injury. Therefore, there is substantial need for neuroprotective strategies to extend the window for recanalization therapy and to prevent neuronal death by ischemic brain injury or by reperfusion injury after successful therapy. This review article will discuss the current status and future perspectives of neuroprotective strategies for acute ischemic stroke.

SEARCH STRATEGY AND SELECTION CRITERIA

I identified references for this review by searching PubMed and ClinicalTrials.gov published in English up to May 2017, with the search terms stroke, cerebral infarction, and neuroprotection. Additionally, I searched references from the relevant articles and reviews. The final reference list was generated on the basis of originality and relevance to this topic. Because of space limitation, individual neuroprotective agents were not discussed in detail. In addition, several neuroprotective strategies, such as inflammation or stroke-induced immune responses, stem cells and microRNAs, and the strategies to enhancing restorative process were not dealt in this review because they were covered in other reviews and are out of scope of this topic.

RESULTS OF PRECLINICAL AND CLINICAL STUDIES OF NEUROPROTECTIVE AGENTS

Neuroprotection refers to the use of therapies that reduce brain injury during acute stroke, through actions on the brain rather than by improving blood flow. Over 1,000 neuroprotective agents have been studies in preclinical stroke research, many with promising results [10]. Nearly 200 neuroprotective clinical trials have been completed but very few have achieved success [10-12]. As a result, neuroprotection research has been perceived that everything works in animal but nothing works in people. In a rat stoke model, even single whisker stimulation could induce complete protection of rat cortex [13].

Why clinical stroke trials were unable to replicate bench findings? Possible reasons for lost in translation include (1) complexity of ischemic pathophysiology underestimated, (2) low quality of preclinical studies, underpowered, effect sizes overestimated, results not robust (low internal validity), (3) stroke models do not match with patient characteristics (age, sex, comorbidities, and polypharmacology), (4) negative publication bias (particularly in preclinical research), (5) heterogeneity of stroke patients, therapies not matched to individual pathophysiology, (6) super systemic effects (on immune, cardiovascular system, etc.) attributable to a substantial fraction of stroke morbidity and mortality, but little understood and under-researched, (7) timing of therapy wrong or clinically irrelevant, clinical trial design not matched to preclinical findings, and (8) significant species differences [14,15]. Early preclinical studies had quality problems [10]. Clinical trials were conducted in multicenter double blind endpoint design, evaluating hundreds to thousands patients, and performed under authorization by U.S. Food and Drug Administration. On the contrary, many of preclinical studies were performed by students in a single laboratory, and were measured by open evaluation of small numbers of animals. While most preclinical studies evaluated the effects of neuroprotective agents administrated at 24 hours after 90 minutes transient middle cerebral occlusion, treatment time was varied among studies in clinical trials and only a small number of patients had ischemic stroke caused by transient middle cerebral arteries.

The Stroke Treatment Academic Industry Roundtable (STAIR) criteria were offered as guidelines aimed at making preclinical results more reflective of human stroke. The Stroke-Acute Ischemic NXY Treatment (SAINT) trials [16,17], which tested a free radical scavenger, were reported to have fulfilled the criteria. After the failure of the SAINT II trial to show beneficial effect of NXY 059, subsequent studies have analyzed the preclinical studies leading up to the SAINT trials and showed that the STAIR criteria were not fulfilled in these preclinical studies [18]. As a result, while early STAIR recommendations emphasized careful design of the clinical trial that are similar to animal experiments, recent STAIR recommendations have raised the importance of the quality of preclinical studies [14,19]. The experts have suggested the need for international multicenter randomized animal trials [20].

At present, the American Heart Association/American Stroke Association guidelines do not recommend the use of pharmacological agents with putative neuroprotective actions, except for the research purposes [21].

RECENT ADVANCES AND FUTURE PERSPECTIVE

There have been several advances in the field of neuroprotective strategies (Table 1) [16,17,22-36].

First, more stringent qualification in preclinical study is re-

Table 1. Neuroprotective agents and results of clinical trials

Agents	Proposed mechanisms	Results of clinical studies
NXY-059	Free radical-trapping properties	Administration of NXY-059 within 6 hours after stroke onset improved the outcome (SAINT) [16], but failed to show the efficacy in a second, larger trial (SAINT II) [17].
Magnesium	Antagonist of calcium channel noncompetitive NMDA receptors and inhibit excitatory neurotransmitter	Prehospital initiation of magnesium sulfate therapy was safe, but did not improve disability outcome suggesting that a single neuroprotective agent may not interdict enough ischemic cascade pathway (FAST-MAG trial) [31].
Albumin	Hemodilution, antioxidant effects, metabolic benefits, collateral enhancing effects	High dose albumin therapy with/with IVT was not associated with improved outcome, and was associated increased rate of intracerebral bleeding and pulmonary edema (ALIAS trial) [39].
Minocycline	Anti-inflammatory and matrix metalloprotease inhibition effects	Early terminated because interim analysis showed futility (NeuMAST trial [NCT00930020])
Citicoline	Phosphatidylcholine precursor, and inhibition of free fatty acid release and free radical generation	Citicoline was not efficacious in treatment of moderate-to-severe acute ischemic stroke (ICTUS trial) [44].
Natalizumab	Antibodies against CD49d (leukocyte adhesion molecule α4 integrin) which inhibit the migration of leukocytes into the brain	Natalizumab administration up to 9 hours after stroke onset did not reduce infarct growth [24].
Edaravone	Free-radical scavenger	Edaravone combined with IVT were safe and may improve outcome (PROTECT4.5 trial) [36].
Uric acid	Antioxidant effects against singlet oxygen and free radicals	The addition of uric acid to thrombolytic therapy was safe but did not improve outcome (URICO-ICTUS trial) [37], but may be beneficial in a certain subgroup of stroke patients [45,46].
Glyceryl trinitrate	Nitric oxide donor, cerebral vasodilator, and lower blood pressure	Transdermal application of glyceryl trinitrate lowered blood pressure and was safe but did not improve outcome (ENOS trial) [33]. The RIGHT-2 trial is ongoing to test the safety and efficacy of transdermal glyceryl trinitrate in the prehospital setting.
NA-1	Postsynaptic density-95 protein inhibition to block NMDA-mediated excitotoxic signaling	Pre-interventional treatment of NA-1 prevented iatrogenic stroke (ENACT trial) [34]. The ESPACE-NA-1 (NCT02930018) and FRONTIER (NCT02315443) trials are ongoing.
Therapeutic hypothermia	Reduce oxygen demand, disruption of the blood-brain barrier, free radical formation, excitotoxicity, and inflammation	Intravascular cooling (ICTuS2 trial) [38] or surface cooling [47] in IVT-treated patients was safe despite the adverse events (e.g., pneumonia), but did not improve outcome. A multicenter phase III RCT (EuroHYP-1, NCT01833312) is ongoing to evaluate the effect of surface/endovascular cooling after IVT or EVT.
Remote ischemic conditioning	Humoral, neural, and systemic response pathway that induce ischemic tolerance and neurorestoration, and inhibit excitotoxicity	Remote ischemic postconditioning within 24 hours after stroke onset was safe and feasible, and may improve outcome (RECAST) [32]. Remote ischemic preconditioning was safe in patients undergoing carotid artery stenting, which may decrease iatrogenic ischemic brain injury [48].
Transcranial laser therapy	Near-infrared laser energy may modulate biochemical changes within neural cells and prevent apoptosis.	Early terminated because interim analysis showed futility (the NEST3 trial) [49].

SAINT, Stroke-Acute Ischemic NXY Treatment; NMDA, N-methyl-D-aspartate; FAST-MAG, Field Administration of Stroke Therapy-Magnesium; IVT, intravenous thrombolysis; ALIAS, Albumin in Acute Ischemic Stroke; NeuMAST, Neuroprotection With Minocycline Therapy for Acute Stroke Recovery Trial; ICTUS, International Citicoline Trial on acUte Stroke; PROTECT4.5, Postmarketing Registry On Treatment with Edaravone in acute Cerebral infarction by the Time window of 4.5 hours; URICO-ICTUS, Efficacy Study of Combined Treatment With Uric Acid and rtPA in Acute Ischemic Stroke; ENOS, Efficacy of Nitric Oxide in Stroke; RIGHT-2, Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2; ENACT, Evaluating Neuroprotection in Aneurysm Coiling Therapy; ESPACE-NA-1, Safety and Efficacy of NA-1 in Subjects Undergoing Endovascular Thrombectomy for Stroke; FRONTIER, Field Randomization of NA-1 Therapy in Early Responders; ICTuS2, Intravascular Cooling in the Treatment of Stroke 2; RCT, randomized control trial; EuroHYP-1, Cooling Plus Best Medical Treatment Versus Best Medical Treatment Alone for Acute Ischaemic Stroke; EVT, endovascular therapy; RECAST, remote ischemic conditioning after stroke trial; NEST3, NeuroThera® Efficacy and Safety Trial 3.

quired. Drugs should be taken forward to clinical trial only if data from animal experiments are valid and precise and in the public domain before clinical trials occur [37]. Recently, several neuroprotective agents have been tested under high quality of transition of preclinical results to clinical trial. For example, the first multicenter trial of experimental stroke research has been conducted to evaluate the effect of anti-CD49d antibodies which inhibit the migration of leukocytes into the brain on infarct volume in acute ischemic stroke [38]. The results showed the feasibility of performing preclinical multicenter RCTs and suggested that anti-CD49d treatment reduced infarct size in permanent stroke model but not in transient stroke model. Based on the preclinical results, a double-blind multicenter RCT of natalizumab (an antibody against the leukocyte adhesion molecule α4 integrin) has been performed in stroke patients which showed that natalizumab administration up to 9 hours after stroke onset did not reduce infarct growth [22]. Another example is NA-1, which targets postsynaptic density-95 protein to inhibit N-methyl-D-aspartate (NMDA)-mediated excitotoxic signaling following ischemia [39]. The effect of NA-1 has been tested in non-human primates (macaques) which bear genetic, anatomical and behavioral similarities to humans [40]. Both magnetic resonance imaging and behavioral assessments showed the beneficial effects of NA-1 in gyrencephalic non-human primates. The transition of these results to human is ongoing in a clinical trial (the ESCAPE-NA-1 trial, NCT02930018).

Second, bedside to bench approach using human genetic studies could be another way to increase the probability of success to find a novel neuroprotectant. Genetic findings have recently been used to trigger drug development. Typical example is monoclonal antibodies that inhibit proprotein convertase subtilisin-kexin type 9 (PCSK9) [41-43]. Similarly, genetic studies can be used to find a novel pathophysiologic target of ischemic brain injury as well as the risk factors of stroke (e.g., genetic risk score) and the response to drug (pharmacogenomics). Mutations in genes involved in ischemic brain injury might be expected to alter outcome after stroke. Therefore, by studying genetic variations and their target proteins in patients with stroke evolution (by infarct growth or reperfusion injury), one can find the mechanisms that involved in ischemic brain injury and candidate neuroprotectants (personal communication with Professor Jin-Moo Lee, Washington University). A large scale genome-wide association study (Genetics of Early Neurological Instability After Ischemic Stroke [GENISIS]) is ongoing to find the genetic variants and pathways associated with neurological instability.

Third, the appropriate time and duration of application of neuroprotective agents have been addressed in the recent clinical trials. Time of application is important. Among the several deficiencies in neuroprotective clinical design, the most important is that patients were being treated too late. About 92% of acute ischemic patients in the neuroprotective trials performed 2000 through 2005 were enrolled beyond 4 hours after stroke onset [44]. Recently, several clinical trials of neuroprotective agents evaluated the effects of neuroprotective agents at an earlier time points. Neuroprotection started in the field by paramedicine is possible and the effects of neuroprotection may be better with earlier treatment. Prehospital stroke trials of paramedic delivered therapy include the Field Administration of Stroke Therapy-Magnesium (FAST-MAG) [23], remote ischemic conditioning after stroke trial (RECAST) [24], Field Randomization of NA-1 Therapy in Early Responders (FRONTIER; NA-1, NCT02315443), and Efficacy of Nitric Oxide in Stroke (ENOS; Glyceryl trinitrate) [25] trials. Pre-intervention application has been tested in NA-1, which showed that pretreatment of NA-1 for endovascular aneurysm repair prevented iatrogenic stroke (Evaluating Neuroprotection in Aneurysm Coiling Therapy [ENACT] trial) [26]. In the FAST-MAG trial, accurate identification of stroke patients and elicitation of informed consent in the field was performed by paramedics, and prehospital initiation of magnesium sulfate therapy was possible and allowed the start of therapy within 2 hours after stroke onset [23]. The duration of treatment may also be important. Signals that mediate cell death during the acute stage of stroke, e.g., NMDA receptor, matrix metalloproteinase and CXCL12/CXCR4 (C-X-C motif chemokine 12/C-X-C chemokine receptor type 4) signaling, might promote repair during the recovery phase [45]. Therefore, more careful attention should be paid for the injury-repair transition time to decide the optimal duration of the use of neuroprotective agents.

Fourth, cotreatment of neuroprotective strategies could augment the value of recanalization therapy. The clinical trials of combination of neuroprotection with IVT or EVT have been completed or are ongoing, such as IVT-edaravone therapy (PROTECT4.5 [Postmarketing Registry On Treatment with Edaravone in acute Cerebral infarction by the Time window of 4.5 hours]) [27], IVT-uric acid (URICO-ICTUS [Efficacy Study of Combined Treatment With Uric Acid and rtPA in Acute Ischemic Stroke] study) [28], IVT-hypothermia (ICTuS2 [Intravascular Cooling in the Treatment of Stroke 2], Euro-HYP-1) [29], IVT/EVT-Magnesium (FAST-MAG) [23], IVT-Albumin (Albumin in Acute Ischemic Stroke [ALIAS]) [30], and EVT-

NA1 (ESCAPE-NA1). In addition, there is no single agent or target pathway for neuroprotection. The use of broad spectrum (pluripotential) neuroprotective therapies, such as therapeutic hypothermia and high dose albumin therapy, or simultaneous or serial (field and emergency room) administration of multiple neuroprotectants with different mechanisms may be more effective than neuroprotective agents with single mechanism [12]. The levels of natural antioxidants was associated with stroke outcome [46]. These natural antioxidants have multiple mechanisms of action and can be safety applied in a broad spectrum of patients with stroke, possibly including those with hemorrhagic events. The effects of albumin and uric acid have been tested in a large RCTs [28,30].

Lastly, biomarkers to select optimal candidate for neuroprotective agents are needed. The success of five pivotal RCTs of EVT and more recently the Clinical Mismatch in the Triage of Wake Up and Late Presenting Strokes Undergoing Neurointervention With Trevo (DAWN trial, NCT02142283) emphasized the importance of careful selection of candidate patients (e.g., small core and good collaterals) and application of EVT within an optimal time windows. Similarly, not all patients with ischemic stroke may have benefit from neuroprotective agents. In this context, further studies are needed on the serologic and neuroimaging biomarkers which can be used to triage optimal patients for neuroprotective strategies. Recently, the phase III clinical trials using biomarkers for predicting efficacy of new drugs are increasing.

CONCLUSION

Despite of numerous failure of neuroprotectants trials over two decades and recent success in revascularization therapies, research on neuroprotectants is worth continuing. The opportunity for neuroprotectants in stroke patients remains plausible. From the EVT trials, we have learn that success can be achieved by careful analyzing the causes of failures. With thorough analysis of the possible reasons for the failures of three RCTs published 2013 [47-49], the recent RCTs of EVTs conducted in 2015 could address the key points in the design in clinical trials (faster revascularization and selection of patients with a small core or presence of occlusion) and showed positive results [3-7]. Several key points are being addressed in the design of recent clinical and preclinical trials of neuroprotective agents, as aforementioned. Selection of candidate neuroprotective strategies with careful design of the clinical trials and conduct high-quality preclinical studies as well as selection of candidate patients using advanced neuroimaging techniques will decrease the likelihood of failure. It is hoped that with better understanding on the mechanisms of ischemic brain injury, better agents of pleiotropic effects on ischemic cascade with lesser side effects, and a novel agents with later or multiple targets are available in patients with acute ischemic stroke.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Ribo M, Alvarez-Sabin J, Montaner J, Romero F, Delgado P, Rubiera M, et al. Temporal profile of recanalization after intravenous tissue plasminogen activator: selecting patients for rescue reperfusion techniques. Stroke 2006;37:1000-4.
2. Heo JH, Lee KY, Kim SH, Kim DI. Immediate reocclusion following a successful thrombolysis in acute stroke: a pilot study. Neurology 2003;60:1684-7.
3. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 2015;372:11-20.
4. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 2015;372:1019-30.
5. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med 2015;372:1009-18.
6. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. N Engl J Med 2015;372:2285-95.
7. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. N Engl J Med 2015;372:2296-306.

8. Chia NH, Leyden JM, Newbury J, Jannes J, Kleinig TJ. Determining the number of ischemic strokes potentially eligible for endovascular thrombectomy: a population-based study. Stroke 2016;47:1377-80.
9. Molina CA. Futile recanalization in mechanical embolectomy trials: a call to improve selection of patients for revascularization. Stroke 2010;41:842-3.
10. 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;59:467-77.
11. Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. Nature 1999;399(6738 Suppl):A7-14.
12. Ginsberg MD. Neuroprotection for ischemic stroke: past, present and future. Neuropharmacology 2008;55:363-89.
13. Lay CC, Davis MF, Chen-Bee CH, Frostig RD. Mild sensory stimulation completely protects the adult rodent cortex from ischemic stroke. PLoS One 2010;5:e11270.
14. Dirnagl U, Hakim A, Macleod M, Fisher M, Howells D, Alan SM, et al. A concerted appeal for international cooperation in preclinical stroke research. Stroke 2013;44:1754-60.
15. Hossmann KA. The two pathophysiologies of focal brain ischemia: implications for translational stroke research. J Cereb Blood Flow Metab 2012;32:1310-6.
16. Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, et al. NXY-059 for acute ischemic stroke. N Engl J Med 2006;354:588-600.
17. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007;357:562-71.
18. Pundik S, Xu K, Sundararajan S. Reperfusion brain injury: focus on cellular bioenergetics. Neurology 2012;79(13 Suppl 1):S44-51.
19. Fisher M. New approaches to neuroprotective drug development. Stroke 2011;42(1 Suppl):S24-7.
20. Dirnagl U, Fisher M. REPRINT: international, multicenter randomized preclinical trials in translational stroke research: it is time to act. Stroke 2012;43:1453-4.
21. Jauch EC, Saver JL, Adams HP Jr, Bruno A, Connors JJ, Demaerschalk BM, et al. 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.
22. Elkins J, Veltkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, et al. Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): a randomised, placebo-controlled, double-blind phase 2 trial. Lancet Neurol 2017;16:217-26.
23. Saver JL, Starkman S, Eckstein M, Stratton SJ, Pratt FD, Hamilton S, et al. Prehospital use of magnesium sulfate as neuroprotection in acute stroke. N Engl J Med 2015;372:528-36.
24. England TJ, Hedstrom A, O'Sullivan S, Donnelly R, Barrett DA, Sarmad S, et al. RECAST (remote ischemic conditioning after stroke trial): a pilot randomized placebo controlled phase II trial in acute ischemic stroke. Stroke 2017;48:1412-5.
25. 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;385:617-28.
26. Hill MD, Martin RH, Mikulis D, Wong JH, Silver FL, Terbrugge KG, et al. Safety and efficacy of NA-1 in patients with iatrogenic stroke after endovascular aneurysm repair (ENACT): a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2012;11:942-50.
27. Yamaguchi T, Awano H, Matsuda H, Tanahashi N; PROTECT4.5 Investigators. Edaravone with and without .6 mg/kg alteplase within 4.5 hours after ischemic stroke: a prospective cohort study (PROTECT4.5). J Stroke Cerebrovasc Dis 2017;26:756-65.
28. Chamorro A, Amaro S, Castellanos M, Segura T, Arenillas J, Marti-Fabregas J, et al. Safety and efficacy of uric acid in patients with acute stroke (URICO-ICTUS): a randomised, double-blind phase 2b/3 trial. Lancet Neurol 2014;13:453-60.
29. Lyden P, Hemmen T, Grotta J, Rapp K, Ernstrom K, Rzesiewicz T, et al. Results of the ICTuS 2 trial (intravascular cooling in the treatment of stroke 2). Stroke 2016;47:2888-95.
30. Ginsberg MD, Palesch YY, Hill MD, Martin RH, Moy CS, Barsan WG, et al. High-dose albumin treatment for acute ischaemic stroke (ALIAS) part 2: a randomised, double-blind, phase 3, placebo-controlled trial. Lancet Neurol 2013;12:1049-58.
31. Davalos A, Alvarez-Sabin J, Castillo J, Diez-Tejedor E, Ferro J, Martinez-Vila E, et al. Citicoline in the treatment of acute ischaemic stroke: an international, randomised, multicentre, placebo-controlled study (ICTUS trial). Lancet 2012;380:349-57.
32. Llull L, Laredo C, Renu A, Perez B, Vila E, Obach V, et al.

Uric acid therapy improves clinical outcome in women with acute ischemic stroke. Stroke 2015;46:2162-7.
33. Amaro S, Llull L, Renu A, Laredo C, Perez B, Vila E, et al. Uric acid improves glucose-driven oxidative stress in human ischemic stroke. Ann Neurol 2015;77:775-83.
34. Piironen K, Tiainen M, Mustanoja S, Kaukonen KM, Meretoja A, Tatlisumak T, et al. Mild hypothermia after intravenous thrombolysis in patients with acute stroke: a randomized controlled trial. Stroke 2014;45:486-91.
35. Zhao W, Meng R, Ma C, Hou B, Jiao L, Zhu F, et al. Safety and efficacy of remote ischemic preconditioning in patients with severe carotid artery stenosis before carotid artery stenting: a proof-of-concept, randomized controlled trial. Circulation 2017;135:1325-35.
36. Hacke W, Schellinger PD, Albers GW, Bornstein NM, Dahlof BL, Fulton R, et al. Transcranial laser therapy in acute stroke treatment: results of neurothera effectiveness and safety trial 3, a phase III clinical end point device trial. Stroke 2014;45:3187-93.
37. Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I; Reviewing Animal Trials Systematically (RATS) Group. Where is the evidence that animal research benefits humans? BMJ 2004;328:514-7.
38. Llovera G, Hofmann K, Roth S, Salas-Perdomo A, Ferrer-Ferrer M, Perego C, et al. Results of a preclinical randomized controlled multicenter trial (pRCT): anti-CD49d treatment for acute brain ischemia. Sci Transl Med 2015;7:299ra121.
39. Tymianski M. Emerging mechanisms of disrupted cellular signaling in brain ischemia. Nat Neurosci 2011;14:1369-73.
40. Cook DJ, Teves L, Tymianski M. Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. Nature 2012;483:213-7.
41. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354:1264-72.
42. Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, Smith WB, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. N Engl J Med 2012;366:1108-18.
43. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med 2015;372:1489-99.
44. Saver JL. The 2012 Feinberg lecture: treatment swift and treatment sure. Stroke 2013;44:270-7.
45. Lo EH. A new penumbra: transitioning from injury into repair after stroke. Nat Med 2008;14:497-500.
46. Bang OY. Advances in biomarker for stroke patients: from marker to regulator. Precis Future Med 2017;1:32-42.
47. Broderick JP, Palesch YY, Demchuk AM, Yeatts SD, Khatri P, Hill MD, et al. Endovascular therapy after intravenous t-PA versus t-PA alone for stroke. N Engl J Med 2013;368:893-903.
48. Kidwell CS, Jahan R, Gornbein J, Alger JR, Nenov V, Ajani Z, et al. A trial of imaging selection and endovascular treatment for ischemic stroke. N Engl J Med 2013;368:914-23.
49. Ciccone A, Valvassori L, Nichelatti M, Sgoifo A, Ponzio M, Sterzi R, et al. Endovascular treatment for acute ischemic stroke. N Engl J Med 2013;368:904-13.