Reviving Neuroprotection Using a New Approach
Targeting Postsynaptic Density-95 to Arrest Glutamate Excitotoxicity

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The quest to develop an effective neuroprotective agent for acute ischemic stroke (AIS) has never been more bleak. Hundreds of neuroprotective drugs have been shown to salvage ischemic brain tissue but none have been shown to reproducibly improve outcome when administered to AIS patients. The graveyard of drugs that looked promising in animal studies that later failed in clinical trials continues to grow, with the latest results of AXIS II showing no benefit in patients treated with granulocyte-colony stimulating factor. In the wake of yet another clinical study testing a drug that, compared with prior agents, had more rigorous and reproducible preclinical evidence supporting a robust effect in animal stroke models, why should we maintain hope that neuroprotection is feasible in patients with ischemic stroke? The results of a recent study published in Nature by Mike Tymianski’s group may provide a lifeline to rejuvenate the field.

Neuroprotection in Primates With a Postsynaptic Density-95 Inhibitor

In the study by Cook et al., the investigators studied the efficacy of Tat-NR2B09c (NA-1) in macaques monkeys with AIS. NA-1 is a peptide comprising the 9 C-terminal residues of the NR2B subunit of the N-Methyl-D-aspartate receptor fused to the 11-mer HIV-1 Tat protein transduction domain and inhibits postsynaptic density-95, a scaffolding protein which links N-Methyl-D-aspartate receptor activation to nitric oxide mediated neurotoxic signaling in neurons. Over 10 years ago, Tymianski’s group identified postsynaptic density-95 as a therapeutic target to arrest glutamate excitotoxicity and has developed postsynaptic density-95 inhibitors as a neuroprotective approach for stroke from cell culture models and now to primates. NA-1 was developed to selectively disrupt only N-Methyl-D-aspartate receptor neurotoxic signaling without affecting normal N-Methyl-D-aspartate currents or intracellular signaling pathways involved in normal neuronal functions.

Cook et al. showed for the first time in primates (n=20) that an intravenous bolus of NA-1 1 hour after severe ischemia reduced infarct volume and improved neurological outcome up to 30 days after stroke. Infarct volume was reduced up to 55% at 24 hours (measured on diffusion-weighted imaging) and up to 70% at 30 days (measured on T2 magnetic resonance imaging). Neurological function was assessed by using the Nonhuman Primate Stroke Scale (NHPSS), a variant of the National Institutes of Health Stroke Scale in nonhuman primates, and a sensorimotor battery for the evaluation of limb strength, fine motor function, cognitive deficits, and visual field defects. NA-1 improved neurological function, and importantly the improved outcome correlated with smaller infarcts at 24 hours and 30 days. In the second experiment, the authors modeled the clinical situation of recombinant tissue plasminogen activator induced reperfusion by treating 12 macaques (n=6 per group) 1 hour after a 4.5-hour duration of severe ischemia. NA-1 reduced infarct volume measured on magnetic resonance imaging at 7 days and improved neurological outcome on the NHPSS as early as 12 hours up to 7 days. In the third experiment, NA-1 was administered at 3 hours after stroke onset within a 3.5-hour interval of ischemia in a milder stroke model, preserving a substantial penumbra. In this model, the macaques (n=12 per group) had smaller infarct volumes at 48 hours (diffusion-weighted imaging) and 14 days (T2) and exhibited improved NHPSS scores over the observation period of the 14 days. Finally, the authors performed gene expression arrays and found preservation of differentially regulated cytoprotective genes in the NA-1–treated animals compared with the placebo group, suggesting that NA-1 preserves cellular functionality.

Promising but Translational Questions Remain

Overall, this study meets a number of standards for high quality research in therapy development including randomization, blinding, control of physiological parameters, assessment of short and long-term outcomes, and testing of efficacy in mild and severe models of ischemia. Altogether, 62 macaques were used, and for a nonhuman primate study, certainly this is one of the largest sample numbers ever reported. However, the sample sizes were as small as 6 animals per group, which at least raises the question whether there should be replication of results by independent groups. Such small group sizes intuitively can lead to superselection of very homogeneous groups of animals. Lack of heterogeneity is probably one of the most obvious differences when comparing bench and bedside results, and maybe a major reason for lack of reproducibility of preclinical results in humans in prior neuroprotection studies. The typical preclinical setting includes 6 to 20

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animals per group selected for identical age, sex, type of infarction, lack of illness, and absence of comorbidities such as diabetes, hypertension, atrial fibrillation, dyslipidemia, smoking, and sedentary lifestyle—the typical risk factors of stroke patients. Comorbid and aged species are more difficult to treat compared with young and healthy animals as recently shown for example for the growth factor granulocyte-colony stimulating factor.6 Before translation to a human stroke population, it is prudent to consider testing NA-1 in aged and comorbid rodents (primates may be too complicated) to estimate the decrease in effect size.

It is furthermore unclear how a prior neuroprotective agent that was effective in rodents but not in humans would fare in this primate model. A comparison with a drug such as NXY-059 would have been an excellent addition to these experiments. Such a study would have helped to address the question whether primate research is a missing prerequisite in the preclinical evaluation of a neuroprotective agent. This study, after all, is not the first to use primates with gyrencephalic brains to test a neuroprotective agent for stroke. FK-506 has been tested in this species with positive results7 but there are no clinical trials that have adequately assessed its effects in patients with AIS.

Last, the behavioral testing for this model using the NHPSS has not been validated, in contrast to the National Institutes of Health Stroke Scale. This may be an important issue requiring further investigation using this model to test neuroprotective agents in the future. If one examines the placebo groups comparing the 2 different models of 90 minutes of middle cerebral artery occlusion versus 4.5 hours of occlusion, the deficits on paring the 2 different models of 90 minutes of middle cerebral artery occlusion versus 4.5 hours of occlusion, the deficits on the placebo groups compared neuroprotection with NA-1 in the context of reperfusion, although not significantly, were actually substantially less in the more severe model.

Despite these translational issues, Cook et al1 should be credited for contributing a number of novel and important components to preclinical study research: (1) penumbral imaging was performed in the primate brain; (2) the animals were assessed longitudinally on a comprehensive primate stroke scale reminiscent of the National Institutes of Health Stroke Scale routinely performed in stroke patients; and (3) gene transcription was also assessed as an index of cellular function in the injured brain.

Ready for Translation Into Humans With Acute Stroke?

Based on the results from this study, is the logical next step a direct translation from primate acute stroke to human acute stroke? In such a situation, direct application of the animal data would translate to an acute stroke clinical trial with an overlapping treatment window for intravenous recombinant tissue plasminogen activator (t-PA). Although Cook et al studied neuroprotection with NA-1 in the context of reperfusion, it is important to evaluate a neuroprotective approach in combination with recombinant t-PA in an embolic stroke model and to test for synergistic or detrimental effects. The currently established acute stroke care guidelines leave few patients within the 4.5-hour window that could be tested with a neuroprotective drug alone versus placebo. Thus, testing NA-1 should be modeled in combination with intravenous t-PA as it was done for other compounds.8 Recent clinical trials using citicoline, erythropoietin, and granulocyte-colony stimulating factor9 in combination with t-PA failed to show any beneficial effect and even showed harm for the combination of erythropoietin and t-PA.9 Furthermore, a strict translation from animals to patients would also mean designing a clinical trial that involved young and previously healthy men with middle cerebral artery strokes. Thus, testing in more clinically relevant animal models is an important consideration.

Summary

Cook et al have conducted an impressive study with the scope and breadth that has never been published before in primate acute stroke research. They have provided proof of concept that a neuroprotective drug can salvage penumbral tissue in the largest primate study of its kind. The results illustrate that proving neuroprotection may be feasible for AIS but require testing in a narrow population at first as the authors have already demonstrated in the ENACT study.10 At the same time, we encourage further animal testing of NA-1 to encompass more clinically relevant scenarios to better understand the extent to which it might succeed in heterogeneous populations of stroke patients.

Disclosures

Dr Schäbitz received honoraria from speakers bureau (Trommsdorff GmbH & Co KG Arzneimittel).

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