The SAINT studies on NXY-059\textsuperscript{1,2} have reinvigorated debate over the momentous issues of neuroprotection and the development of novel stroke therapeutics. Some may argue there is little new ground that NXY-059 has unearthed and, in fact, the clinical trials merely confirm that as a strategy, neuroprotection has not been shown to be effective. Editorials in high-profile journals have sent out the clarion call to terminate research on neuroprotection given decades of work, which has uniformly led to failure after failure in clinical studies.\textsuperscript{3} These sentiments are premature and ignore studies showing that hypothermia improves outcome in patients after cardiac arrest, a major cause of global cerebral ischemia.\textsuperscript{4,5} Proof for the neuroprotection concept has therefore already been established. Despite decades of neutral clinical trials, there still remains optimism among the stroke academic community. A majority of university-affiliated stroke neurologists in the United States in a recent survey still believe neuroprotection is a viable therapeutic strategy, although admittedly, that survey was conducted between the SAINT I and SAINT II publications.\textsuperscript{6} However, the question whether neuroprotection will ever work in stroke has been overshadowed by the larger issue of whether animal studies, which serve as the foundation for the development of stroke therapeutics, are even relevant to our patients with acute stroke. In the discussion section of the SAINT II article, the authors write, “it is possible that the animal models of acute focal infarction are not relevant to the patient population; they certainly are insufficient to guarantee a positive clinical-trial result.”

Are Animal Studies Relevant to Human Stroke?

Have all prior neuroprotective agents failed in clinical studies because rodent stroke is not relevant to human stroke? This is a key question. Substantial reductions in infarct size in rodents with stroke treated with various types of purported neuroprotective agents would seem to support this view. There is ample evidence, however, that rodent stroke shares similar features with human stroke. As one example, there is similarity in the cerebrovasculature between rodents and humans. Just recently, spreading depression has been shown to occur in human stroke,\textsuperscript{7} which validates an important phenomenon characterized in animals, including rodents, decades ago. The penumbra, as defined using cerebral blood flow, has also been shown in both rodents and humans. Mismatch imaging using diffusion-weighted imaging and perfusion-weighted imaging might potentially be used in both rodents and humans to select patients who might benefit from neuroprotection.\textsuperscript{8} Important molecular and biochemical mechanisms, including immediate early genes and caspase expression, that occur in rodent stroke have been found to occur in brain tissue from patients with stroke.\textsuperscript{9} Thrombolytic therapy with tissue plasminogen activator recanalizes occluded vessels in rodents and humans. There may even be similar time windows in humans and rodents to salvage the penumbra with thrombolysis under certain circumstances.\textsuperscript{10} Of course, it is important to point out that the discovery of the only approved therapy for acute ischemic stroke, tissue plasminogen activator, was conducted in the rabbit model, which is well suited to study embolism and test the effects of thrombolytic drugs.\textsuperscript{11} Those pivotal discoveries on tissue plasminogen activator provide support for the development of stroke therapeutics in animal models. However, at the same time, no animal model fully mimics all of the clinical conditions of human stroke and these limitations need to be considered in preclinical drug evaluation.

How Rigorous Was the Preclinical Testing on NXY-059?

A less explored area to explain the disconnect between positive animal studies and neutral clinical trials is the lack of rigor and quality of preclinical experiments. In 1999, investigators from academia and industry met with representatives from the National Institutes of Health and the US Food and Drug Administration to compile a list of guidelines for animal testing, known as the STAIR guidelines. NXY-059 has been hailed as the poster child for STAIR, but in fact, new studies have emerged showing that it did not meet many of the STAIR recommendations for high-quality testing,\textsuperscript{12–14} including randomization and blinding.

In addition, the original STAIR paper calls for physiological monitoring, including cerebral blood flow during the surgery to induce focal cerebral ischemia. The need for cerebral blood flow monitoring is best exemplified by the...
very first published study on NXY-059 in which profound reductions in infarct size were observed in drug-treated animals in a dose-dependent fashion. However, several of the animals in the drug-treated group had no infarctions, which raises the question to what extent was cerebral blood flow reduced in those animals.\textsuperscript{15} Cerebral blood flow was not monitored in that study nor in most of the other animal studies on NXY-059. These observations support what we already know: the suture model, on which most neuroprotective drugs have been tested, is imperfect in causing a sustained cerebral blood flow reduction in the middle cerebral artery. As illustrated in Figure 1, an animal that underwent insertion of a suture into the internal carotid artery and then intraluminal obstruction of the middle cerebral artery had a drop in blood flow as monitored by laser Doppler over a branch of the middle cerebral artery. However, over the next few minutes, the flow spontaneously increased with the suture in place. The suture was removed, another suture was advanced from the carotid artery to the middle cerebral artery, and the cerebral blood flow signal once again precipitously dropped and remained persistently low. This is an example of model failure, the incidence of which in the suture model has not been well characterized. There should therefore be strong concern that in the absence of cerebral blood flow monitoring, results based on the suture model are difficult to interpret.

**Has There Been Any Progress in Meeting High-Quality Testing Since NXY-059?**

The past decade since NXY-059 has seen the introduction of several further protective agents in clinical trials, most of which have preclinical support, but almost none of the drugs in current clinical studies have fared better than NXY-059 in meeting STAIR guidelines.\textsuperscript{13} There is extensive heterogeneity in the quality and completeness of preclinical packages for individual drugs. However, it should also be acknowledged that quality and completeness of drug studies are being held up against standards written in the STAIR publication. Others may differ on what constitutes high-quality testing and sufficiency of preclinical experiments.

**Are There Any Published Neutral Studies on Neuroprotection?**

Even if the lack of rigor or physiological monitoring would not be enough to explain the failures of neuroprotection, there were warning signs in the animal literature on NXY-059. The primate studies found no difference in infarct size,\textsuperscript{16} the drug was not effective when given beyond 5 minutes after occlusion in an embolic clot model,\textsuperscript{17} and several neutral studies have yet to be published as revealed by an abstract presented at the International Stroke Conference in 2008. Prior investigators have already clearly shown that there is a publication bias that favors positive studies of neuroprotective agents.\textsuperscript{18}

**Is STAIR Useful?**

Although retrospective critique might be helpful to examine the deficiencies in the NXY-059 program, it is also important to discuss whether the STAIR guidelines themselves are useful. After all, if SAINT II had confirmed the positive results of SAINT I, NXY-059 would have been seen as a validation of the STAIR criteria. In this respect, it must be stated that STAIR is not a recipe that will yield a positive drug. In other words, fulfilling STAIR is not predictive of a positive result in clinical trials. In fact, what our analysis has shown is that there is no consensus on which preclinical studies are necessary to perform and there is no consensus on which quality measures are important to build into our studies.\textsuperscript{13} We will not know which recommendations are
important until there is a positive drug. Therefore, STAIR is only guidelines and should not be considered criteria.

One Way Forward: Lessons Learned
It is not for a junior investigator to suggest to the world how to improve neuroprotection studies, but my own bias would be to give the guidelines a fair chance by conducting studies that do indeed follow the recommendations. The contributors to STAIR 6 hope to disseminate revisions that will further aid future studies in this area of research. Points of discussion should consider more rigorous testing that includes randomization and blinding. Blood flow monitoring would help to ensure the reliability of the animal model to cause vessel occlusion. It should be well established through reproducibility in multiple independent laboratories that a drug is protective before proceeding to clinical studies. Neutral and negative data need to be published and the entire preclinical package scrutinized before moving forward to a clinical trial. Perhaps, meta-analyses of all data collected from all laboratories should be completed to give a better estimate of the effect size.

Once there is uniform confidence in the preclinical efficacy of a neuroprotective agent, one is then confronted with the complexities of clinical trial design. There is much debate about which analytic end points are relevant to a neuroprotective strategy in contrast to a reperfusion strategy. However, what has been missing from prior neuroprotection work is a surrogate of activity. After the National Institute of Neurological Diseases and Stroke trial, investigators showed that tissue plasminogen activator induces recanalization and increases cerebral blood flow. Similar work proving a physiological effect of neuroprotective agents should be done before advancing to pivotal efficacy studies. The pharmacokinetics and pharmacodynamics of neuroprotective drugs may substantially differ in humans compared with rodents. Transporters along the blood–brain barrier that promote drug efflux may be one of several underlying reasons to account for those differences. Accordingly, it is important to demonstrate that a neuroprotective agent enters the human brain if cytoprotection is the goal. This may require imaging depending on the drug, but certain agents such as Caffeinol and glutamate antagonists can be monitored for central nervous system penetration on purely clinical grounds. However, in the advent of molecular imaging, we could go further and show that a drug acts on an important mechanism of action in the human brain. Lastly, it would be helpful to demonstrate that the drug reduces infarct size using conventional imaging before moving forward to pivotal efficacy studies.

Reverse Translation
These suggestions are merely to provide further bridges between the bench and the bedside by assessing similar end points in our study population of interest. At the same time, we can also strive to engage in reverse translation and better match our animals with our patients (Figure 2). Almost all neuroprotective work is based on the use of young animals, whereas our patient population is mostly middle-aged and senior adults. Many of our patients with stroke also have comorbidities such as diabetes and hypertension. Animals with baseline disease states are available and can be used for preclinical testing. Certain strains of spontaneously hypertensive rats, for example, are prone to small vessel stroke and hemorrhage. Diabetic and obese rodents are also commercially available. The expense of animals with diseased baselines will undoubtedly need to be taken into account as well as the mortality associated with stroke modeling in older animals.
Conclusion

Although NXY-059 may have been considered a failure, it has given us pause to reflect on how we can refine our bidirectional research efforts to design experiments that will better inform us whether a drug will be effective in the clinical setting. There are several gaps in the translational spectrum from bench to bedside that still need to be filled before concluding that neuroprotection should be abandoned.

Disclosures

None.

References


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