The neutral results of the SAINT II study\(^1\) dashed the hopes for a second ischemic stroke drug in the near future. Although this is certainly disappointing for stroke neurologists, a careful analysis of the NXY-059 development program reveals several deficiencies, which if properly addressed, might revitalize and strengthen the field of neuroprotection for acute ischemic stroke. This critique points out several potential reasons for the failure of SAINT II to replicate the positive findings of SAINT 1,\(^2\) discusses the preclinical problems with NXY-059, and provides a perspective on the development of future neuroprotective agents for acute ischemic stroke.

Clinical Trial Problems
The SAINT II trial\(^1\) investigated the efficacy of the free-radical–trapping agent, NXY-059, for the treatment of acute ischemic stroke and failed to confirm the positive results of SAINT I.\(^2\) In the latter study with 1699 patients, NXY-059 showed for the first time in a large phase III trial that a drug other than tissue plasminogen activator (t-PA) might lead to significant improvement on the modified Rankin Scale (mRS) using a novel and controversial outcome analysis (Rankin shift analysis). The larger SAINT II trial with 3306 patients not only failed to replicate improvement on the same primary end point but all secondary end points (neurological deficits on the National Institutes of Health Stroke Scale (NIHSS) at 90 days and activities of daily living on the Barthel Index at 90 days) were identical between treatment groups. Besides the obvious possibility that the drug itself was inactive, which may be considered due to the known instability of free-radical–trapping agents, several additional problems were evident. Both trials were designed, in part, as combination studies, testing the addition of NXY-059 to standard therapy compared with t-PA alone in the 3-hour time window. SAINT II gave the combination to almost 50% of the enrolled patients (43.9 in the placebo group and 44.1 in the NXY group) with all the potential pitfalls of such a design. Significant detection of an additional improvement in neurological outcome caused by a neuroprotective drug on top of t-PA would likely be in the low single digit percent range and therefore very difficult to measure.\(^3\) However, patients treated with the combination were not better at all in SAINT II, and in fact, they performed slightly worse than those treated with t-PA. In SAINT I, patients receiving the combination did not improve on the primary outcome measure compared with NXY-059 alone.\(^1,2\) This is further supported by the finding of reduced asymptomatic and symptomatic hemorrhages in combined treated patients compared with t-PA treated patients alone (12.9% versus 20.9% and 2.5% versus 6.4%, respectively).\(^2\) A plausible explanation could be a t-PA neutralizing effect of NXY-059 on the microcirculation and blood rheology. A direct t-PA inhibiting effect appears less likely due to the fact that NXY-059 administration was delayed to 1 hour after induction of thrombolysis. Unfortunately, neither published pharmacological data on the interactions between both drugs nor experimental data on brain perfusion including the microcirculation are available. The lack of enhanced benefit from combination therapy is not surprising given prior preclinical studies in rabbits. The combination of NXY-059 and t-PA showed no synergistic effects in an embolic model.\(^4\)

Although often claimed to be modern and progressive, the SAINT trial design suffered from indiscriminate inclusion of all types of ischemic stroke patients. The study clearly missed the opportunity to homogenize the study population by using brain imaging to demonstrate the ischemic subtype and whether the infarcts involved cortical gray versus white matter. An estimated 25% of patients may have had a subcortical or white matter stroke which should have been a study exclusion criterion. This is important because white
matter ischemia is substantially different from gray matter, and free-radical–trapping agents have not been shown to exert a beneficial effect on the white matter. A drug aimed to treat white matter stroke should be tested for efficacy in appropriate animal models, a major deficiency in the preclinical NXY-059 program.

A third issue in the SAINT trials concerns the mechanism of action of NXY-059 and its effects on long-term outcome. As shown by the subgroup analysis of SAINT I, NXY-059 may have been effective in the early poststroke period, 7 days after stroke onset (mRS 1.31 odds ratios [OR] 1.09, 1.57, \( P=0.002 \); NIHSS 1.46 OR 1.13, 1.89, \( P=0.003 \); Barthel Index 1.55, OR 1.22, 198, \( P<0.0001 \)). This effect almost disappeared at 90 days after stroke (mRS 1.20, OR 1.01, 1.42, \( P=0.038 \); NIHSS 1.13, OR 0.9, 1.41, \( P=0.2 \); Barthel Index 1.16, OR 0.93, 1.45, \( P<0.14 \)) indicating transient protection of the drug acting only on the acute phase of ischemic stroke pathophysiology. Similar results were found for magnesium which showed a trend suggestive of a transient benefit on mortality and disability at 30 days after ischemia, but that disappeared at 90 days. Whether a similar transient effect was seen in SAINT II remains to be seen in a subgroup analysis. Sustained benefit may be better achieved with agents that not only target the ischemic cascade in the early stages of ischemic injury but also promote recovery over the ensuing weeks after stroke.

**Preclinical Drug Study Problems**

Although there are many divergent opinions on clinical trial design and the choice of therapeutic agents for acute stroke therapy trials, we need to take a step back and consider how robust was NXY-059 in reducing damage and improving outcome in the preclinical animal studies. A closer examination indicates that the drug was not rigorously tested and did not fully meet the STAIR recommendations as the authors of the SAINT II trial had claimed.

First, the overall methodological quality of the animal studies was not rigorous. Many studies did not monitor cerebral blood flow, without which it is difficult to know whether the animals actually had an acute vascular occlusion. For example, the first study to demonstrate efficacy reported that many drug-treated animals had no infarcts. The absence of an infarct in any study using the intraluminal suture middle cerebral artery occlusion model, especially the absence of striatal damage where ischemic injury initially occurs, should raise concern about the degree of blood flow reduction. Because blood flow was not monitored in this study, it is unknown whether animals with no infarcts had an middle cerebral artery occlusion. We estimate that over 50% of all the NXY-059 studies did not monitor cerebral blood flow.

Second, few studies reported blinding to outcome analysis and no studies reported blinding investigators to treatment administration, two pivotal features of clinical trial design and important components of the STAIR I guidelines for the preclinical evaluation of stroke therapeutics. The lack of blinded-unfortunately does reduce confidence in the validity of the data. But, it is also important to acknowledge that the absence of reporting whether investigators were blinded does not necessarily indicate that blinding was not performed.

Third, the collective work on the behavioral testing of NXY-059 was inadequate. Kuroda et al in the transient middle cerebral artery occlusion model reported behavioral outcome up to 1 week using the Bederson scale, which is a 4-point, crude evaluation of neurological deficits. Unfortunately, there remain no published studies in the rodent literature on the long-term outcome of NXY-059-treated animals after middle cerebral artery occlusion. The primate studies, on the other hand, did subject nongyrenecephalic animals at 10 weeks after stroke to a range of behavioral tests, which one of us conservatively argues merely showed an improvement in arm weakness and did not reliably give any information on cortical function. It remains unknown in animals if NXY-059 enhances recovery on neurological tests that might better match the clinical rating scales in humans.

Fourth, an important STAIR criteria is reproducibility of positive effects in multiple models in different laboratories and that both positive and negative data should be published. Some of the most impressive data on NXY-059 comes from a permanent model suture occlusion study in which the drug, when administered at 4 hours after stroke, substantially reduced infarct size with minimal variability. This data were generated by the parent pharmaceutical company and reproducibility in an independent laboratory should have been investigated. In the transient 2 hours model, however, reduction in infarct size was independently replicated when the drug was given at 3 hours after stroke but the SAINT trials enrolled patients up to 6 hours after stroke onset.

Lastly, there were warnings in the literature that NXY-059 might prove to be ineffective. The pivotal primate study, for example, found that the drug did not significantly reduce infarct volume except in the putamen. Even more worrisome, one study using an embolic model reported that NXY-059 was only protective when the drug was given at 5 minutes after clot injection but not when given at 3 hours after embolization.

**Summary**

The above discussion helps to illuminate, in part, why NXY-059 ultimately failed in clinical development. SAINT I may simply have been a false-positive study or the potential benefits of NXY-059 were ephemeral without any long-lasting effect on outcome. It is therefore important to consider whether the drug should have been brought forward to the clinical arena in the absence of further preclinical testing.

**A Perspective on the Future of Neuroprotection**

Admittedly, NXY-059 was one of the first neuroprotective drugs to be tested more thoroughly compared with prior agents and did follow some of the STAIR criteria. The deficiencies in its preclinical and clinical development should now galvanize the stroke community to apply more rigorous methods to evaluate future neuroprotective agents. We look forward to STAIR VI where we are eager to learn from our colleagues how best to develop better criteria for the development of future stroke therapies. We offer the following suggestions:
1. Animal studies should better adhere to quality controls including blinding, randomization, and cerebral blood flow monitoring. Reproducibility of efficacy in separate laboratories needs to be emphasized.

2. Which behavioral tests in rodents and higher species and at what time points after stroke should be performed to prove that a neuroprotective agent might lead to clinically meaningful and sustained benefit over time?

3. Homogenize patients with MRI to separate cortical gray matter from white matter strokes.

4. Neuroprotection should initially be studied separate from reperfusion in an initial proof of concept study.

5. Consider the use of drugs with neuroprotective plus regenerative mechanisms of action to achieve long lasting effects on functional neurological outcome.

Disclosures

None.

References


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