NXY-059 is a nitrene compound with free radical trapping properties that appears to be neuroprotective in some animal models of stroke.\textsuperscript{1,2} The publication this month of SAINT-I trial in the *New England Journal of Medicine*\textsuperscript{3} demonstrates a small but statistically significant improvement of the primary outcome by NXY-059 treatment. The authors observed reduced disability at 90 days as assessed by a shift in the modified Rankin scale. Patients were treated within 4 hours from stroke onset with doses of NXY-059 compatible with the neuroprotective effects seen in some animal models.\textsuperscript{1,2}

The development of the drug is to be commended in that a number of the STAIR criteria\textsuperscript{4} were followed, but there are questions that are being discussed. Critically, the site of action of NXY-059 has still not been conclusively determined, and this dispute originates from the poor permeability of NXY-059 across the blood–brain barrier.\textsuperscript{2,5} Therefore, the question is if NXY-059 eventually exhibits any pharmacological protection by scavenging free radicals in the brain tissue. Alternatively, its mechanism of action might be a manifestation of physiological protection, mediated probably by improving cerebral blood flow.

Another consideration is whether the benefit of NXY-059 at 90 days, after stroke onset, is not a long-term effect, but rather a postponement of the injury. This is supported by the reduction of the odds for improved outcome by NXY-059 compared with placebo, from 7 to 30 to 90 days. Another criticism of the SAINT-I trial is that the modified Rankin scale is used in a nonconventional way, which is a nonvalidated concept. Finally, an important caveat of the study was that NXY-059 did not result in any significant National Institutes of Health Stroke Scale (NIHSS) score improvement.

**Effects of NXY-059 on Cerebral Blood Flow**

An unexpected result of the SAINT-I trial is the lack of improvement in the primary outcome when NXY-059 treatment is combined with thrombolysis.\textsuperscript{3} This would suggest that both interventions have the same mode of action, that is, improving cerebral blood flow, therefore resulting in the same clinical outcome.

It is important to note that the effect of NXY-059 on cerebral blood flow has not been addressed yet. As a result, speculation for the protective mechanism of NXY-059 in the animal models might be that in permanent ischemia at high concentrations, it is probably improving blood flow, therefore inhibiting the extension of the core. In transient ischemia, NXY-059 is probably reducing the breakdown in the blood–brain barrier. As a result, in patients, the risk of hemorrhage is reduced and in animal models, some reduction in infarct size is achieved.

It is clear that any physiological effects exerted by NXY-059 on cerebral blood flow would be detrimental for its classification as a neuroprotectant. This demonstrates that in future neuroprotective studies, it should be essential to carry out routine experiments in the animal models to determine the accompanying *New England Journal of Medicine* perspective article,\textsuperscript{6} at the level of the vasculature. The drug is known to have very poor permeability into the brain\textsuperscript{2,5} and therefore is not rescuing the tissue at risk from free radical injury, but is rather working at the level of the endothelium, which would be easily accessible.

In contrast to this notion, a recent study showed that in animal models of permanent focal ischemia, NXY-059 successfully, although to a very small extent, crosses the blood–brain barrier.\textsuperscript{7} However, the authors used brain homogenates without providing convincing methodological details to prove that the concentration of NXY-059 measured in the brain is actually in the brain parenchyma and not in the endothelium of the cerebral blood vessels.

**Is the Site of Action of NXY-059 the Brain or the Vessel?**

A significant question that remains to be answered is whether NXY-059 is truly neuroprotectant or whether it is acting at the blood–brain barrier and is protecting, as was suggested by the accompanying *New England Journal of Medicine* perspective article,\textsuperscript{6} at the level of the vasculature. The drug is known to have very poor permeability into the brain\textsuperscript{2,5} and therefore is not rescuing the tissue at risk from free radical injury, but is rather working at the level of the endothelium, which would be easily accessible.

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whether the drug has any effect on the blood flow at the site that is protected.

**Combining NXY-059 Treatment With Thrombolysis**

One of the exciting findings of the SAINT-I study is that patients receiving both tissue plasminogen activator (t-PA) and NXY-059 have a significantly lower risk of both symptomatic and asymptomatic hemorrhage. If this observation is indeed reproduced in the SAINT-II trial, rapid licensing for the constrained application of giving NXY-059 with t-PA would be an exciting development for stroke thrombolysis.

There can be different explanations for the reduction of the hemorrhagic action of t-PA by NXY-059. We would speculate that NXY-059 prevents matrix metalloproteinase activation or even tissue plasminogen-induced injury to the blood–brain barrier.

An alternative explanation is provided by the fact that in stroke patients who did not have thrombolysis, NXY-059 did not prevent intracranial hemorrhage more than the placebo. This could suggest that NXY-059 eventually inactivates t-PA, thereby offsetting the risk of hemorrhage induced by t-PA during thrombolysis. The proposed inhibition of t-PA by NXY-059 could also be another reason for the lack of interaction between the treatment effect of NXY-059 and thrombolysis.

**Lessons From Thrombolysis and Thrombostasis**

One way forward with neuroprotection has to be as a result of lessons learned from thrombolysis and thrombostasis. With thrombolysis, the surrogate of opening obstructed middle cerebral arteries or basilar arteries was used. The development of thrombostasis from laboratory to human trials was based on demonstrating that the target could be safely achieved, that is, the size of hemorrhage was not increased. Finally, the strength of thrombolysis was the improvement shown on the follow-up NIHSS score, compared with the baseline, in a case-by-case setting.

**The Way Forward**

The problem with neuroprotection is that there is no imaging surrogate that demonstrates mechanism of protection and ultimately proof of concept. We recommend that the STAIR criteria should contain confirmation of the pharmacological action of the compound by imaging in the in vivo models. This would confirm that the in vitro mechanism is also taking place in vivo.

In the case of NXY-059, it should have been requisite to image the production of free radicals in vivo and demonstrate that the infusion of NXY-059 trapped those radicals, leading to improved imaging outcome, which correlated with improved clinical outcome. This would truly allow the animal translation to human and for the efficacy data from trials to be translated into effective clinical care.

The challenge for NXY-059 now is to show that when combined with thrombolysis, it can consistently reduce hemorrhage, which would be a major step forward in stroke care, but more importantly to see whether the small but statistically significant improvement in neurologic outcome can be reproduced. The animal model data for NXY-059 are compelling, but there will be a need to see a powerful effect in the second trial before we can accept this as a true neuroprotectant rather than one that protects the vascular component of the neurovascular unit.

**References**


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NXY-059: Brain or Vessel Protection
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