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Marrying Activase with Gleevec

By Tim Fulmer, Senior Writer

Research by a team of Swedish and American scientists suggests that combining a tissue plasminogen activator with a platelet-derived growth factor receptor inhibitor may result in improved outcomes for stroke therapy. This may help widen the therapeutic window for **Genentech Inc.**'s Activase alteplase, the only recombinant tissue plasminogen activator approved to treat acute ischemic stroke, by combining it with **Novartis AG**'s Gleevec imatinib, a platelet-derived growth factor receptor-α inhibitor. But dosage and issues with side effects will have to be carefully worked out before implementing this potentially powerful therapeutic strategy.

The *Nature Medicine* paper provides insight into a plasminogen-independent signaling pathway in the CNS that may be partly responsible for poor stroke outcomes following late tissue plasminogen activator (tPA) treatment (*see* **Figure 1, "Advancing treatments for stroke"**).[1] The paper was coauthored by Daniel Lawrence, professor of cardiovascular medicine at the **University of Michigan Medical School**, and Ulf Eriksson, professor and head of the developmental biology group at the **Ludwig Institute for Cancer Research**, together with colleagues from both institutions.

In circulation, the serine protease tPA converts plasminogen to plasmin, an enzyme that breaks down fibrin-containing blood clots. In the CNS, however, excess tPA activity has been linked to neurotoxicity and increased vascular permeability, which can contribute to cerebral edema and, in some cases, hemorrhage.[2,3]

The clot buster Activase is approved only for patients who are treated within three hours of stroke symptom onset because of an increased risk of intracranial hemorrhage that may result from local vascular damage following ischemia.[4] Now, the paper published in *Nature Medicine* suggests that adjunct therapy using the cancer drug Gleevec could significantly lower the risk of bleeding and thus widen Activase's therapeutic window without compromising its activity.

Meanwhile, previous work by Lawrence and colleagues at the University of Michigan had found that genetic disruption or pharmacological inhibition of tPA in the CNS reduced blood-brain barrier permeability in murine stroke models.[5] Those data suggested that antagonizing tPA's activity in the CNS could lower edema and intracranial hemorrhage associated with ischemia and trauma.

Additional previous research by Eriksson and colleagues at the Ludwig Institute for Cancer Research had discovered a formerly unknown substrate for tPA in cultured fibroblasts—a dimeric precursor form of platelet-derived growth factor-C (PDGF-C), referred to as PDGF-CC.[6]

For the current paper, the two labs joined forces to test the hypothesis that tPA's ability to regulate blood-brain barrier permeability is mediated by activation of PDGF-CC. If so, antagonizing PDGF-CC might improve blood-brain barrier integrity and ameliorate stroke outcomes without interfering with tPA's clot-busting activity.

The researchers initially found that direct intraventricular injection of either tPA or PDGF-CC into nonischemic mice significantly increased cerebrovascular permeability compared with injection of saline control buffer ($p<0.01$). Conversely, co-injecting tPA with anti-PDGF-CC antibodies significantly lowered this permeability compared with injection of control IgG ($p<0.05$). Thus, tPA's effects on permeability required PDGF-CC, and preventing PDGF-CC from binding to its receptor (platelet-derived growth factor receptor-α (PDGFR-α)) might be a strategy for reducing cerebrovascular permeability during ischemia.

In murine ischemic stroke models, oral administration of Gleevec alone significantly lowered cerebrovascular permeability and infarct size compared with what was seen in mice that received vehicle control ($p<0.05$). In a separate set of experiments using the same models, administration of Gleevec following onset of ischemia but prior to administration of tPA substantially reduced intracerebral hemorrhage compared with that seen in mice that received saline vehicle and tPA alone ($p<0.05$).

"The *Nature Medicine* paper is a good first step toward realizing Gleevec as an adjunct therapy to tPA," said David Liebeskind, associate neurology director of the **University of California, Los Angeles Stroke Center** and neurology director of its Stroke Imaging Program. "In fact, the work may serve to shift research focus away from the often unsuccessful neuroprotectant approach and toward therapeutic strategies that focus on vascular aspects of stroke such as improving microcirculation and reperfusion."

He added: "The paper also points out that it's sometimes quite fruitful to look for therapies outside of your own field, in this case potentially repurposing a cancer drug as an adjunct stroke therapy."

Gleevec is marketed by Novartis to treat chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST).

Into man

As a result of the research, a clinical trial of Gleevec alone and in combination with tPA is expected to begin dosing within the next two months, corresponding author Lawrence told *SciBX*. The trial, which will enroll about 60 ischemic stroke patients, will be run by Nils Wahlgren and colleagues at the **Karolinska University Hospital**.

"I agree with moving the combination therapy straight into human trials—though with two caveats," said Geoffrey Donnan, professor of neurology at the **University of Melbourne** and director of the **National Stroke Research Institute**. "First, a leukemia drug like Gleevec could have some rather nasty side effects at doses necessary to get it into the brain. Second, if the drug is delivered orally and not intravenously, it may be difficult getting sufficient drug to the ischemic tissue within the short time window stroke often requires."

According to Gleevec's label, the most frequently reported drug-related adverse events are nausea, vomiting, edema and muscle cramps.

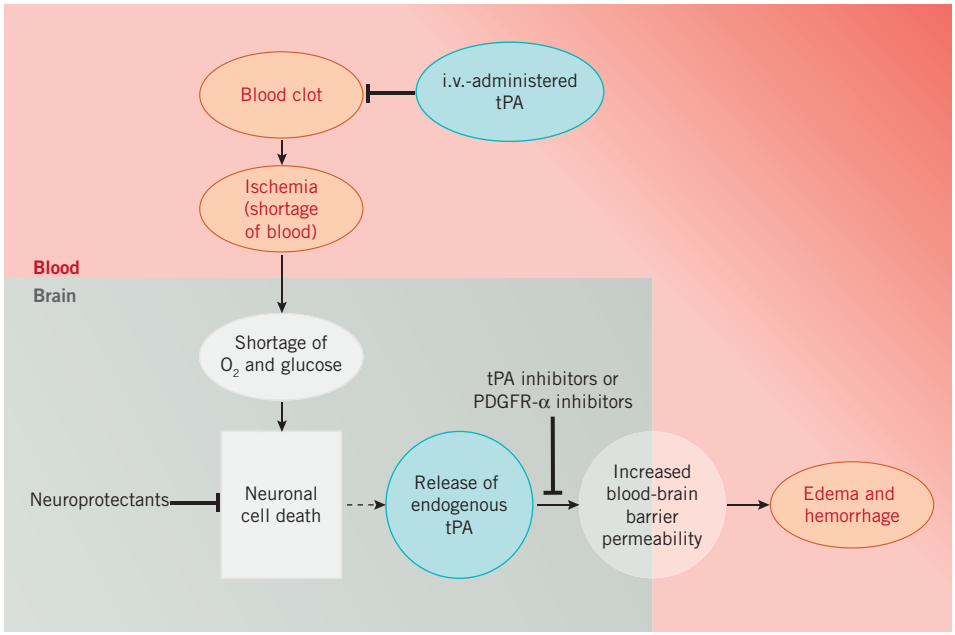

Figure 1. Advancing treatments for stroke. In acute ischemic stroke, a blood clot in the brain causes a shortage of local blood supply (ischemia), which leads to reduced local levels of O_2 and glucose in neural tissue. Metabolically stressed neurons then undergo an 'ischemic cascade' that can ultimately result in neuronal cell death and a localized area of necrotic neural tissue (infarction).

Two general strategies have been proposed to prevent or slow this process. One seeks to dissolve the clot using 'clot busters' like tissue plasminogen activator (tPA), whereas the other blocks the ischemic cascade (neuroprotectants). Both strategies have met with limited success.

According to Su *et al.* in *Nature Medicine*, one reason for the limited success may be that, under stimulus of ischemic stress, healthy or dying brain cells release endogenous tPA, which activates platelet-derived growth factor-CC (PDGF-CC) which, in turn, stimulates PDGF receptor-α (PDGFR-α) and leads to increased permeability of the blood-brain barrier and consequent risk of edema and hemorrhage. Blocking activation of this pathway using either PDGFR-α inhibitors like Gleevec imatinib from **Novartis AG** (NYSE:NVS; SWX:NOVN) or tPA inhibitors could thus protect the blood-brain barrier during tPA treatment and potentially improve the outcome of thrombolytic stroke therapies.

Nevertheless, said Donnan, "the *Nature Medicine* paper describes a potentially valuable way of widening tPA's therapeutic window, something that is certainly needed in the clinical setting. Besides risky off-label tPA administration, the only real options available to patients who present more than three hours post-ischemic onset are aspirin within 48 hours, management in a stroke unit or hemicraniectomy."

Hemicraniectomy is a procedure to reduce intracranial pressure by removing a portion of the skull.

Lawrence noted that the doses tested in the initial trial will not be considerably higher than standard doses used in cancer patients. He did say the drug will be given orally in the upcoming study, but noted that Gleevec has been formulated for i.v. administration in animal studies. "If there are encouraging data from the first trial, it may be possible to reformulate imatinib for i.v. administration in humans," he said.

Even without an i.v. version of Gleevec, Lawrence said the blood-brain barrier may not be a major obstacle.

"There's evidence that orally administered imatinib crosses the blood-brain barrier, even in healthy individuals," he said. "And in stroke patients, who typically have a compromised blood-brain barrier after stroke, imatinib will probably have even better access to the brain. In any case, even if doses have to be relatively high, they would be transient, lasting just long enough to ensure the thrombolytic can do its job properly, and not chronic."

Regardless of formulation, Lawrence told *SciBX* that using Gleevec as an adjunct to widen the therapeutic window of thrombolytics has a number of benefits. "First of all, it's a marketed drug supported by years of safety data. Second, since imatinib targets PDGF activity and not tPA, it leaves the clot-busting activity of tPA intact while counteracting tPA's negative effects on the blood-brain barrier."

Other strategies

Some companies in the stroke space are already exploring strategies that combine tPA with adjuncts. Other companies are seeking to avoid the drug's side effects in the first place by looking for safer clot busters.

Thrombotech Ltd. is trying the former approach. The company has developed a hexapeptide antagonist of tPA that could potentially be delivered with tPA. The peptide corresponds to six amino acids (EEIIMD) of an endogenous tPA antagonist called plasminogen activator inhibitor-1 (PAI-1).

The peptide does not bind to the enzyme's catalytic site. Thus, EEIIMD "blocks the downstream neurotoxic effects of tPA without compromising tPA's thrombolytic activity and can be delivered intravenously," said Abd Al-Roof Higazi, company founder and associate professor of pathology and laboratory medicine at the **University of Pennsylvania**.

In two different rat stroke models, i.v. EEIIMD in combination with i.v. tPA minimized infarct size and intracranial bleeding compared with tPA monotherapy. In piglets subjected to fluid percussion brain injury, the adjunct therapy resulted in less brain edema and neuronal loss compared with what was seen using tPA alone.[7]

"In addition to other peptides with improved potency over EEIIMD, Thrombotech also has in preclinical development compounds that inhibit the LDL receptor protein (LRP), which is required for tPA activity in the CNS," Higazi said. "We have synthesized chemically modified variants of receptor-associated protein (RAP) that bind and potentially prevent LRP from interacting with tPA and producing downstream neurotoxicity," he added.

Lawrence and colleagues also have considered directly targeting tPA, and earlier this year they received U.S. Patent No. 7,375,076 covering the use of tPA inhibitors to reduce vascular permeability in the brain and other

tissues. Inhibitors covered by the patent include wild-type and mutant forms of endogenous peptide tPA inhibitors (PAI-1 and neuroserpin), as well as anti-tPA antibodies.

Using an adjunct like Gleevec to mitigate the adverse effects of tPA on the blood-brain barrier is an acceptable strategy, but developing a plasminogen activator devoid of these effects in the first place would be preferable, said Karl-Uwe Petersen, head of preclinical development at **Paion AG**.

Paion has focused on developing a plasminogen activator isolated from lower mammals that has key structural differences from human tPA.

Previous work has shown that cleavage and activation of PDGF-CC by tPA occurs at an arginine residue and requires the presence of tPA's kringle 2 domain. "This reaction seems to have an important role in tPA-associated neurotoxicity," Petersen said. "Thus, kringle 2–dependent cleavage is implicated in both tPA-mediated neurotoxicity and blood-brain barrier damage."

Paion's desmoteplase, a genetically engineered salivary plasminogen activator from the vampire bat *Desmodus rotundus*, has no kringle 2 domain.

Intravenous desmoteplase resulted in significantly less neuronal death in a rat model of *N*-methyl-D-aspartic acid–induced neurotoxicity compared with i.v. tPA ($p<0.01$).[8] However, last year, desmoteplase missed the primary endpoint of clinical improvement at day 90 compared with placebo in the Phase III desmoteplase in acute ischemic stroke-2 trial.[9]

Paion said the outcome may have been due to a lack of clots in the brain arteries of patients in the study. Partner **H. Lundbeck A/S** has said it plans to begin a Phase III study to treat acute ischemic stroke in 2H08.

Genentech declined to comment on the *Nature Medicine* paper.

What's next

Lawrence told *SciBX* he hopes "to use the same murine stroke models to study the efficacy of imatinib in combination with other thrombolytics, including urokinase, streptokinase, staphylokinase and desmoteplase."

At the same time, his group plans to further characterize the Gleevec-tPA combination, focusing on the relative size and timing of the two doses necessary to optimize efficacy and safety.

Higazi said he would like to see a more definitive preclinical experiment evaluating Gleevec and thrombolytic adjunct therapies in ischemic stroke.

"Blood clots could be injected directly into the middle cerebral artery of mice, followed by intravenous injection of imatinib and tPA. Clot dissolution over time could then be monitored by MRI or ultrasound," he said. "This approach will presumably detect reperfusion injury following clot dissolution and also blood vessel disintegration, which is perhaps a better readout for cerebral hemorrhage than blood-brain barrier permeability."

But Lawrence noted such embolic stroke models "are very difficult in mice, as is monitoring reperfusion in real time by MRI or ultrasound, due to the small size of mice. Such experiments would likely be more informative if performed in larger species such as rat or even rabbit."

Donnan noted that other researchers are looking to expand tPA's therapeutic window with imaging technology rather than other drugs. "A number of practitioner-run trials are ongoing or planned to test whether MRI or CT [computated tomography] scans of the ischemic penumbra can select stroke patients who respond favorably to i.v. tPA beyond three hours of onset," he said.

The penumbra is a region of reduced blood supply that may be salvageable if blood flow is restored in time. Thus, said Donnan, "reduction in size of ischemic lesion would be a key efficacy outcome in these trials, and safety endpoints include hemorrhagic transformation. Perhaps such a trial design could also help determine if patients respond positively to tPA plus Gleevec beyond three hours."

In the longer term, Lawrence said he is interested in looking into how preserving blood-brain barrier integrity affects neuroprotectants.

"It may turn out that rescuing neurons from the ischemic cascade first of all requires that the blood-brain barrier remain intact. If so, neuroprotectants might have a wider window of opportunity and thus stand a better chance of success in the clinic if they were delivered with a compound that preserved blood-brain barrier integrity," he said.

However, Roger Simon, chair and director of the **Robert S. Dow Neurobiology Laboratories** and adjunct professor of neurology, physiology and pharmacology at **Oregon Health and Science University**, pointed out that, at least for some neuroprotectants, decreasing blood-brain barrier permeability in stroke could be a hindrance.

"Gleevec may expand the therapeutic window of a thrombolytic like tPA. However, it could have no effect or, even worse, the opposite effect on an intravenously delivered neuroprotectant that can take advantage of a permeable blood-brain barrier to reach ischemic tissue," said Simon, who is also a cofounding scientist at **NeuroProtect Inc.**

He added: "This would likely apply to our neuroprotectant NPI-505, a peptide inhibitor of calcium-permeable acid-sending ion channel-1."

According to Simon, the company's NPI-505 has shown efficacy in rodent models of stroke and will be tested in suture occlusion-reperfusion primate models of acute ischemic stroke in the near future.

REFERENCES

1. Su, E. *et al. Nat. Med.*; published online June 22, 2008; doi:10.1038/nm1787
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2. Pawlak, R. & Strickland, S. *J. Clin. Invest.* **109**, 1529–1531 (2002)
3. Zhang, Z. *et al. Circulation* **106**, 740–745 (2002)
4. Lansberg, M. *et al. Cerebrovasc. Dis.* **24**, 1–10 (2007)
5. Yepes, M. *et al. J. Clin. Invest.* **112**, 1533–1540 (2003)
6. Fredriksson, L. *et al. EMBO J.* **23**, 3793–3802 (2004)
7. Armstead, W. *et al. Nat. Neurosci.* **9**, 1150–1155 (2006)
8. Lopez-Atalaya, J. *et al. Stroke* **38**, 1036–1043 (2007)
9. Ku, A. *BioCentury* **15**(25), A14–A15; June 4, 2007

COMPANIES AND INSTIUTIONS MENTIONED

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