A NAC for TTP

By Kai-Iye Lou, Staff Writer

U.S. and Canadian researchers have found that the generic drug *N*-acetylcysteine could be repurposed for use in thrombotic thrombocytopenic purpura, a rare hematological disorder for which cumbersome plasma exchange therapy has been the standard of care for almost two decades.¹ The group is seeking a partner to sponsor clinical trials in the new indication.

Activated endothelial cells produce and secrete long polymers of von Willebrand factor (VWF) that are linked together with disulfide bonds. These long VWF polymers adhere to platelets and collagen, which can trigger thrombus formation.

In normal individuals, an enzyme called ADAMTS13 (ADAM metalloproteinase with thrombospondin type 1 motif 13) cleaves these long VWF polymers into smaller, less adherent multimers.

However, most patients with thrombotic thrombocytopenic purpura (TTP) have a deficiency in ADAMTS13 that prevents the enzyme from processing long VWF polymers into their proper size, resulting in spontaneous platelet aggregation and the formation of microvascular thrombi (see Figure 1, “Preventing thrombus formation in TTP with *N*-acetylcysteine”).

In the predominant, acquired form of the disease, ADAMTS13 is blocked by autoantibodies. The more rare, congenital form of the disease is caused by mutations in *ADAMTS13*. Plasma exchange therapy removes the autoantibodies from circulation and provides functional ADAMTS13.

Diagnosing patients with TTP can be difficult as many of the symptoms associated with the disorder overlap with those of other diseases. Early symptoms include low platelet counts and headaches, which can then progress to purpura and nervous system damage.

N-Acetylcysteine (NAC) has been used for clearing mucus in the airways for more than 50 years and works by disrupting the disulfide bonds in polymeric mucins, which are the main protein component in mucus.

NAC is also used in higher doses to manage acetaminophen overdose and is available as an over-the-counter antioxidant supplement.

Because VWF multimers are linked with disulfide bonds and have an overall structure that is similar to that of polymeric mucins, a research group led by José López hypothesized that NAC could mimic the VWF-cleaving function of ADAMTS13 and have a beneficial effect in TTP.

“Von Willebrand factor’s ability to bind to platelets and endothelial cells is related to its size,” said López, who is EVP of research at the **Puget Sound Blood Center**. “We found that we can treat the longest multimers of von Willebrand factor with *N*-acetylcysteine to make them smaller.”

In human plasma, NAC decreased the size of VWF multimers and diminished their ability to bind platelets and collagen compared with no treatment. In activated human endothelial cells, NAC lowered the

Figure 1. Preventing thrombus formation in TTP with *N*-acetylcysteine. Von Willebrand factor (VWF) is important for initiating platelet aggregation and thrombus formation. Activated endothelial cells produce and secrete VWF as long polymers (yellow circles) linked together with disulfide bonds [a].

In healthy individuals, ADAMTS13 (ADAM metalloproteinase with thrombospondin type 1 motif 13; blue triangles) cleaves the long VWF polymer into smaller multimers to maintain normal hemostasis [b]. The smaller VWF multimers have lower platelet-binding ability and less potential for spontaneous thrombus formation than the ultra-large multimers found in thrombotic thrombocytopenic purpura (TTP)—a rare blood clotting disorder. These smaller VWF multimers are still able to initiate platelet aggregation in response to injury.

Patients with TTP usually have an ADAMTS13 deficiency and high levels of ultra-large VWF multimers. Many platelets can bind to these ultra-large VWF multimers, thus leading to aggregation and spontaneous thrombus formation [c].

In *The Journal of Clinical Investigation*, U.S. and Canadian researchers have shown that *N*-acetylcysteine (NAC; purple diamonds) reduces the size of VWF multimers by disrupting the disulfide bonds [d]. In *Adams13*-deficient mice, the drug decreased platelet thrombus accumulation, time to thrombus resolution and thrombus size compared with saline control.

number of platelet-decorated VWF strings by 77% ($p < 0.001$).

In wild-type mice, NAC decreased the size of VWF multimers compared with saline. In *Adams13*-deficient mice, NAC lowered platelet thrombus accumulation, time to thrombus resolution and thrombus size compared with no treatment.

Results were published in *The Journal of Clinical Investigation*.

“The primary advantage of *N*-acetylcysteine would be its broad availability and ease of use compared with plasma exchange,” which involves the removal of a patient’s plasma through apheresis and replacement with donor plasma, said López, who is corresponding author on the paper. “The drug also is considered to be very safe.”

"The most interesting aspect to me is that the paper provides the rationale for repositioning a drug we've been using in humans for a long time—for which we have decades of safety data—and testing it for efficacy in the setting of TTP," said J. Evan Sadler, a professor in the Department of Medicine and chief of the hematology division at **Washington University in St. Louis**.

"Most of the activity and interest in the development of new therapies for TTP has been focused on the proteolytic enzyme ADAMTS13," noted James George, a professor in the Department of Medicine at **The University of Oklahoma Health Sciences Center**.

Baxter International Inc. has recombinant human ADAMTS13 in preclinical development for TTP. The most advanced compound in development for the indication is ALX-0081, an anti-VWF Nanobody from **Ablynx N.V.** that is in Phase II testing.

Although the companies declined to comment on the *JCI* paper, Ablynx CBO Eva-Lotta Allan noted that the company's Phase II anti-VWF Nanobody "has a suitable PK/PD profile for acute and maintenance 30-day therapy."

David Motto, an assistant professor in the Departments of Internal Medicine and Pediatrics at **The University of Iowa**, said he was surprised that NAC had an effect on VWF in mice at the tested doses, which were single boluses of 400 or 800 mg/kg.

"The tremendous reduction in the number of platelet aggregates that formed in the NAC-treated mice over this dosing range was surprising," he added.

The 400 mg/kg dose is comparable with that used in a protocol for treating acetaminophen poisoning, which recommends a 400 mg/kg total dose of NAC delivered as a 150 mg/kg bolus followed by continuous infusion over 36 hours.

Setting the standard

Based on its safety profile and broad availability, NAC is well positioned to be used as an adjunct treatment to plasma exchange in TTP or in cases for which plasma exchange is not available.

"Right now, TTP is a disorder where patients may present at a relatively late stage of illness, and current therapies do not rapidly address disease pathophysiology," Sadler told *SciBX*. "By the time patients get to the ER, they typically have very low platelet counts and even nervous system damage. Plasma exchange is the standard-of-care treatment for TTP, but this therapy sometimes takes weeks to work."

Plasma exchange therapy also has limited availability because specialized equipment and personnel are needed to administer the treatment. In contrast, oral and i.v. formulations of NAC are widely available.

"Perhaps the best use of NAC is when a diagnosis of TTP is suspected but the patient has not yet received plasma exchange or does not readily have access to the treatment," Motto told *SciBX*. "You could potentially give this drug to patients as a holdover until they are transported to a center where plasma exchange is available. If the drug is ultimately proven to be effective, its primary benefit is that it will be available at every clinic around the country."

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**—J. Evan Sadler,
Washington University
in St. Louis**

NAC's safety profile also suggests that it could potentially be used when initial diagnosis is uncertain.

"If there is some hesitancy on the initial diagnosis of TTP, *N*-acetylcysteine could be safely used while the doctor tries to get a definitive diagnosis," said López. "This could help reduce the likelihood of unnecessarily exposing patients to blood products and other risks associated with plasma exchange like reactions to the transfused blood products."

"NAC also could be a perfect adjunctive treatment that could be given before and in combination with existing treatments for TTP," George told *SciBX*. "It would be good to see if NAC could defer or reduce the need for plasma exchange and

possibly replace or be used in combination with other adjunctive treatments like corticosteroids and rituximab."

Rituxan rituximab, a chimeric anti-CD20 mAb from **Biogen Idec Inc.** and the **Genentech Inc.** unit of **Roche**, is used off-label in TTP. The drug is approved to treat non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL) and rheumatoid arthritis (RA).

"There are also TTP-like syndromes that don't respond to plasma exchange but are caused by the presence of ultra-large, hyperactive von Willebrand factor multimers, so *N*-acetylcysteine might be helpful in those settings as well," added López.

López said his group is now looking to translate the results into the clinic and is seeking government and not-for-profit sponsorship for clinical trials. "We are most interested in first looking at patients with TTP-like syndromes that don't respond to plasma exchange therapy and patients with the congenital form of the disease before going into patients with the acquired form of the disease," he told *SciBX*.

"I don't really think that there are many more preclinical studies that need to be done," he added. "I think the drug is ready to go into human testing, and we just need to figure out how to dose it for TTP."

Motto added that it could be a good idea to develop an assay to evaluate how long NAC stays in human circulation and its duration of effect on VWF. "Such information would be useful for figuring out how much and how often NAC would need to be dosed in patients."

The work is unpatented.

Lou, K.-J. *SciBX* 4(8); doi:10.1038/scibx.2011.214
Published online Feb. 24, 2011

REFERENCES

- Chen, J. *et al.* *J. Clin. Invest.*; published online Jan. 27, 2011; doi:10.1172/JCI41062
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COMPANIES AND INSTITUTIONS MENTIONED

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Baxter International Inc. (NYSE:BAX), Deerfield, Ill.
Biogen Idec Inc. (NASDAQ:BII), Weston, Mass.
Genentech Inc., South San Francisco, Calif.
Puget Sound Blood Center, Seattle, Wash.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
The University of Iowa, Iowa City, Iowa
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