HUS and them

By Lauren Martz, Staff Writer

Two groups have identified new strategies to treat or prevent hemolytic uremic syndrome, a potentially lethal complication of *Escherichia coli* infection. A team at the **University of Toronto** has suggested that the marketed CXC chemokine receptor 4 antagonist Mozobil plerixafor could be repurposed for the indication, whereas **Carnegie Mellon University** researchers have found that the natural metal ion manganese could neutralize the toxin that causes the condition.[1,2]

Hemolytic uremic syndrome (HUS) is caused by the release of Shiga toxins from pathogenic bacteria such as the O157:H7 strain of *E. coli* and triggers symptoms such as anemia, thrombocytopenia and acute renal injury.

There are no available tests to determine whether a patient with gastrointestinal *E. coli* infection will develop HUS, and there are no therapeutics that actually neutralize the toxin. Current treatments are palliative and include dialysis, blood transfusions and corticosteroids. The mortality rate is 5%–7%, and many patients who do recover have long-term renal problems.[3]

Alexion Pharmaceuticals Inc.'s Soliris eculizumab is approved to treat atypical HUS, a rare genetic form of the disease that is not caused by Shiga toxins. The company is running a Phase II trial of the drug in Shiga toxin–driven HUS and declined to comment.

Now, Philip Marsden and colleagues have used genetic studies to identify the specific genes that are dysregulated by exposure to Shiga toxins. Marsden is professor of medicine and director of nephrology at the University of Toronto and chair in medical research at **St. Michael's Hospital**.

The team's genetic profiling studies showed that the toxin led to greater expression of genes encoding CXC chemokine receptor 4 (CXCR4; NPY3R), CXCR7 and chemokine CXC motif ligand 12 (CXCL12; SDF-1) than expression in untreated controls. These data suggested antagonizing the chemokine pathway could prevent the toxin's effects.

CXCR4 and the chemokine pathway components are known primarily as players in vascular development and maintenance, as well as stem cell homing to the bone marrow.

In mice, Shiga toxin exposure caused weight loss and acute renal failure compared with no exposure. The toxin also altered *Cxcr4*, *Cxcr7* and *Cxcl12* mRNA and protein levels in tissues including the thymus, heart, liver and kidney.

Following exposure to the toxin, 42.3% of mice treated with 10 μg/g of Mozobil daily for 10 days beginning 1 day after toxin exposure survived compared with 23.1% of untreated animals. The drug also improved kidney function compared with control.

Sanofi markets Mozobil to increase mobilization of hematopoietic stem cells to the bloodstream for collection and autologous transplantation in patients with multiple myeloma (MM) or non-Hodgkin's lymphoma (NHL). The pharma declined to comment.

Maria Victoria Ramos, professor of immunology at the **National Academy of Medicine**'s Institute of Experimental Medicine, told *SciBX* the data reveal Mozobil has therapeutic and prophylactic potential in HUS.

"One point to be considered about this antagonist is that even though it is injected one day after Shiga toxin treatment, it still reduces renal damage. Considering that there is a period of time between when children are intoxicated with Shiga toxin *E. coli* and when they develop HUS, an effective drug within that window could reduce the risk of disease," she said.

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>
> ***—Maria Victoria Ramos, National Academy of Medicine***

The team also showed that CXCL12 levels were higher in plasma samples from children infected with *E. coli* O157:H7 who progressed to HUS than those in patients whose infections cleared without complication. Levels of the protein were greater in the blood prior to HUS symptoms, suggesting CXCL12 could be used as a biomarker and that the pathway could be targeted to prevent HUS before the onset of symptoms.

The findings were published in *The Journal of Clinical Investigation*. The paper also included researchers from **The Hospital for Sick Children**, the **University of Iowa Carver College of Medicine**, **Beth Israel Deaconess Medical Center** and the **Washington University in St. Louis School of Medicine**.

Marsden now wants to confirm the findings during an *E. coli* outbreak. He said that if they could measure CXCL12 levels in real time during an *E. coli* outbreak to confirm the findings, his team would have a strong case for a plerixafor trial in patients with Shiga toxin–producing *E. coli* to see if it prevents or improves HUS cases.

Marsden said the findings are unpatented and unlicensed and that his team is looking for partners.

Turning up the metal

Rather than targeting Shiga toxin's mechanism of action like the Toronto team, Carnegie Mellon's Adam Linstedt and Somshuvra Mukhopadhyay have proposed a different approach to prevent HUS: inducing degradation of the toxin by blocking its normal cell-processing pathway.

Linstedt is professor of biological sciences and Mukhopadhyay is a postdoctoral fellow in the Department of Biological Sciences at Carnegie Mellon.

The pair previously showed that manganese, a natural element

found in the human body and food sources, causes degradation of proteins involved in the lysosomal pathway of toxins.[4]

Building on that work, Linstedt and Mukhopadhyay monitored the trafficking of Shiga toxin using a fluorescently labeled form of its B subunit. In HeLa cells, the toxin was trafficked from the cell surface to the Golgi apparatus, bypassing the late endosomes and lysosomal degradation pathway. However, when the cells were cultured with manganese, the toxin accumulated in endosome-like structures and was degraded.

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—Alfredo Torres, The University of Texas Medical Branch

In the cells, treatment with manganese protected against a lethal dose of Shiga toxin compared with no treatment. In mice, daily intraperitoneal administration of manganese beginning 5 days before lethal toxin challenge protected all animals from renal damage and death, whereas all untreated mice died within 96 hours.

Linstedt told *SciBX* that his team does have plans to test whether treatment with manganese beginning once symptoms of HUS have presented could be effective therapeutically, "although our primary interest will be to see if we can effectively treat at the first sign of diarrhea symptoms before the onset of HUS."

Results were published in *Science*. Linstedt said a patent application has been filed by Carnegie Mellon.

Next steps

Going forward, both strategies could be impeded by a dearth of animal models of *E. coli* infection.

"A major disadvantage for drug developers in the field is that mouse models for *E. coli* infection are not very good to recapitulate diarrheal disease," said Alfredo Torres, associate professor in the Department of Microbiology and Immunology and the Department of Pathology at **The University of Texas Medical Branch**. "One option could be to see if the results hold true in humanized mice. These mice could give a better indication of how the treatments will function in humans."

Torres said another challenge of treating Shiga toxin "is that drug delivery is difficult. It will be ideal to target the toxin within the cell—the challenge is to get a high enough concentration of the drug inside the cells without side effects."

Linstedt agreed. "High-level exposure of manganese is a known hazard, causing neurological defects especially after prolonged exposure. For treatment, the time of exposure and dose must be minimized," he said.

Thus, he told *SciBX* the next steps for his team include optimizing the delivery and dosage of manganese in the mouse models.

He suggested that using manganese as part of a combination therapy might allow a lower dose of the ion for therapeutic efficacy. "The main motivation would be to combine manganese with antibiotic therapy so that both the bacteria that spread the toxin and the toxin itself are neutralized. In theory this might shorten the time period of the treatment so it could also reduce the total exposure of a patient to manganese," he said.

However, Torres noted that antibiotics are contraindicated for the indication. He said that antibiotic-induced bacterial death induces the release of the toxin and can increase the toxin load in a patient.

Linstedt suggested that developing a therapeutic against manganese's target, Golgi integral membrane protein 4 (GOLIM4; GPP130), could be an alternative to using manganese. "Whether it would be safer can't be known until it is tested," he said. "It would likely be expensive to develop and produce, especially in comparison to manganese."

For CXCR4 antagonism, Torres suggested the Canadian team should consider developing a more specific inhibitor. "Although they used an approved drug, we need to know what will happen to patients at the required dose when we block this important pathway." He added, "The inhibitor can always be optimized by drug design to increase binding or translocation to the cells or perhaps to increase potency, resulting in a lower dose that will be required to target the toxin."

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   e-mail: p.marsden@utoronto.ca
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   Contact: Adam D. Linstedt, Carnegie Mellon University, Pittsburgh, Pa.
   e-mail: linstedt@andrew.cmu.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Alexion Pharmaceuticals Inc. (NASDAQ:ALXN), Cheshire, Conn.
Beth Israel Deaconess Medical Center, Boston, Mass.
Carnegie Mellon University, Pittsburgh, Pa.
National Academy of Medicine, Buenos Aires, Argentina
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
The Hospital for Sick Children, Toronto, Ontario, Canada
St. Michael's Hospital, Toronto, Ontario, Canada
University of Iowa Carver College of Medicine, Iowa City, Iowa
The University of Texas Medical Branch, Galveston, Texas
University of Toronto, Toronto, Ontario, Canada
Washington University in St. Louis School of Medicine, St. Louis, Mo.