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Researchers harness the power of plants to fight hemophilia

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(PhysOrg.com) -- Hemophilia, a disease linked with legends of European monarchs, frail heirs and one flamboyant charlatan called Rasputin, still afflicts many people today. And the very treatments that can help can also put patients' lives at risk.

The standard treatment is infusion with an expensively produced protein that helps the blood to clot. But in some patients the immune system fights the therapy, and in a subset of those, it sets off an allergic reaction that can result in death.

Now researchers at the University of Florida and the University of Central Florida have devised a way that potentially could help patients develop tolerance to the therapeutic protein before they are in need of treatment.

They genetically modified plants to encapsulate the tolerance-inducing protein within cell walls so that when ingested, it can travel unscathed through the stomach and be released into the small intestines where the immune system can act on it. The low-cost plant-based system, now being tested in mice, eventually could help improve the lives of many people who have hemophilia and dramatically reduce related health-care costs. The approach also has the potential for use with other conditions such as food allergies and autoimmune diseases.

“We’re hoping that our research will, in the future, result in better and more cost-effective therapies,” said Roland Herzog, an associate

professor of pediatrics, molecular genetics and microbiology in the UF College of Medicine and a member of the UF Genetics Institute, who was one of the study's leaders.

The findings were published Monday in the Proceedings of the National Academy of Sciences.

Hemophilia is characterized by defects in the gene that produces a protein required for blood to clot. People with hemophilia can suffer from spontaneous internal bleeding or severe bleeding resulting from minor injuries. Males get the disease, which is linked to the X chromosome, while females are "carriers" who rarely exhibit symptoms. The two forms of the disease — hemophilia A and B — are associated with the absence of proteins called factor VIII and factor IX, respectively.

Many people around the world have the disease — 1 in 5,000 boys are born with hemophilia A, the more common of the two forms.

Hemophilia treatment consists of infusing the missing protein into a patient's blood. But in 25 percent of patients, the immune system rejects the therapy and makes inhibitors that stop the clotting factor from taking effect.

In hemophilia B, up to 4 percent of patients develop inhibitors to the protein therapy and many develop severe systemic allergic reactions, called anaphylaxis, which can be life-threatening.

"If the very protein that you administer to the patient is neutralized, it's as if you haven't administered any protein at all," said Thierry Vandendriessche, an associate professor of medicine at the University of Leuven in Belgium, and president of the European Society of Gene Cell Therapy. He was not involved in the study.

Because treatment itself poses a potential threat to life, it has to be done in a hospital setting under supervision. That makes it an expensive enterprise that often includes blood transfusions and hospital stays costing up to $1 million. Average treatment costs are $60,000 to $150,000 a year, according to the National Hemophilia Foundation.

To help patients tolerate therapy, doctors try to exhaust patients' immune systems by administering the therapeutic protein intravenously at frequent intervals and for long periods until the body no longer responds by producing inhibitors. While that brute force approach works for hemophilia A, it often doesn't for hemophilia B, in which patients risk death from anaphylactic shock if exposed to the protein therapy.

To find a new, gentler approach to developing tolerance, Herzog teamed with Henry Daniell, a Pegasus professor and University Board of Trustees Chair in the College of Medicine at the University of Central Florida, who has spent the last two decades developing transgenic plants for producing and delivering oral vaccines and immune-tolerant therapies.

They inserted the gene responsible for producing the therapeutic protein into the genome of plants. To maximize the amount of protein produced, they inserted thousands of copies of the genes into chloroplasts — the energy-producing centers of plants — using a gene gun.

The research team, including first authors Dheeraj Verma, and Dr. Babak Moghimi, fed the encapsulated protein to hemophilic mice for an extended period. Surrounded by the hardy plant cell walls, the protein was protected from digestive acids and enzymes while traveling through the stomach. Once it arrived safely in the small intestines, however, surrounding bacteria chewed on the cell walls, causing the protein to be released and acted on by the immune system to induce tolerance.

When the mice were later treated intravenously with the clotting factor therapy, they produced little or no inhibitors, and none developed anaphylactic shock.
“We have made them develop tolerance, and removed the allergic part of this treatment,” Daniell said.

Not only did the pretreated mice survive the once-deadly treatment — they also had a greater positive effect from therapy than did other mice.

“You may wonder, ‘why hasn’t this happened before,’” Vandendriessche said. “It’s because it was difficult to administer a high amount of protein in the right place and at the right time. I think this is a milestone — nobody has previously achieved such levels of robust immune tolerance by any means using a noninvasive procedure.”

The researchers will continue to study how their method works, extend the approach to treating hemophilia A in mice and, ultimately, conduct trials in humans. Protein used in the human trials will be produced in lettuce and formulated to allow delivery of standard doses.

Provided by University of Florida