Plant-made hemophilia therapy shows promise, study finds

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Freeze-dried lettuce containing a protein of interest. Credit: University of Pennsylvania

People with hemophilia require regular infusions of clotting factor to prevent them from experiencing uncontrolled bleeding. But a significant fraction develop antibodies against the clotting factor, essentially experiencing an allergic reaction to the very treatment that can prolong their lives.

Researchers from the University of Pennsylvania School of Dental Medicine and University of Florida have worked to develop a therapy to prevent these antibodies from developing, using a protein drug produced

in plant cells to teach the body to tolerate rather than block the clotting factor.

Successful results from a new study of the treatment in dogs give hope for an eventual human treatment.

Henry Daniell a professor in Penn Dental Medicine's Department of Biochemistry and director of translational research, was the senior author on the study, collaborating on the work with his former advisee, Roland W. Herzog, a professor at the University of Florida and lead author on the paper. The work was published in the journal *Molecular Therapy*.

"The results were quite dramatic," Daniell said. "We corrected blood clotting time in each of the dogs and were able to suppress antibody formation as well. All signs point to this material being ready for the clinic."

The study made use of Daniell's patented plant-based drug-production platform, in which genetic modifications enable the growth of plants that have specified human proteins in their leaves. In the case of hemophilia, the researchers' aim was to prevent individuals with hemophilia from developing antibodies that would cause a rejection of life-saving clotting-factor infusions.

The researchers had the idea that ingesting a material containing the clotting factor, such as the transformed plant leaves, could promote oral tolerance to the factor protein, just as children fed peanuts early in life are less likely to develop an allergic reaction.

This technique had shown promise in previous experiments, in which the researchers demonstrated that feeding hemophilia A plant material containing the clotting factor VIII to mice greatly reduced the formation

of inhibitors against that factor.

In the new work, the team focused on hemophilia B, a rarer form of disease in which patients have deficiencies in clotting factor IX. The researchers produced lettuce that had been modified to produce a fusion protein containing human clotting factor IX and the cholera non-toxin B subunit. The latter component helps the fused protein cross the intestinal lining as the lettuce cells are digested by gut microbes while the plant cell walls protect the clotting factor from digestion in the stomach. The lettuce plants were grown in a hydroponic facility.

Because the researchers also wanted to ensure that the therapy would work in an animal model closer to humans, they pursued their trials in dogs with hemophilia B.

The researchers began with a pilot study of two dogs, headed by co-author Timothy Nichols of the University of North Carolina. Twice a week for 10 months, the dogs consumed the freeze-dried lettuce material, which was spiked with bacon flavor and sprinkled on their food.

Observing no negative effects of the treatment, the team went on to a more robust study, including four dogs that were fed the lettuce material and four others that served as controls. The four dogs in the experimental group were fed the lettuce material for four weeks. At that point, they also began receiving weekly injections of factor IX, which continued for eight weeks. The control dogs only received the injections.

All four of the dogs in the control group developed significant levels of antibodies aginst factor IX, and two had visible anaphylactic reactions that required the administration of antihistamine. In contrast, three of the four dogs in the experimental group had only minimal levels of one type of antibody, IgG2, and no detectable levels of IgG1 or IgE. The

fourth dog in the experimental group had only a partial response to the treatment, which the researchers believe to be due to a pre-existing antibody to human factor IX.

Overall, levels of IgG2 were 32 times lower in the treated dogs than in the controls.

In addition, the dogs showed no negative side effects from the treatment, and blood samples taken throughout the experiment revealed no signs of toxicity from the treatment.

Daniell said the results are encouraging.

"Looking at the dogs that were fed the lettuce materials, you can see it's quite effective," he said. "They either developed no antibodies to factor IX, or their antibodies went up just a little bit and then came down."

Though rarer than hemophilia A, more patients with hemophilia B develop antibodies against their treatment, thus making the need for a tolerance treatment all the more urgent.

The next steps for the research team include additional toxicology and pharmacokinetics studies before applying for an Investigational New Drug application with the FDA, a step they hope to take before the end of the year. A National Institutes of Health grant called Science Moving Towards Research Translation and Therapy and which uses the acronym SMARTT, is supporting IND-enabling studies. SMARTT's mission is to accelerate the progress of therapies that have shown promise in animal models to the stage of pursuing clinical trials in humans.

More information: *Molecular Therapy*, dx.doi.org/10.1016/j.ymthe.2016.11.009

Sherman et al. Suppression of inhibitor formation against FVIII in a murine model of hemophilia A by oral delivery of antigens bioencapsulated in plant cells, *Blood* (2014). DOI: 10.1182/blood-2013-10-528737