New study is proof-of-concept for low-cost drug made in lettuce

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Biopharmaceuticals, or drugs that are based on whole proteins, are expensive to make and require refrigeration to store. Insulin, for example, is unaffordable and inaccessible to most of the global population.

At the University of Pennsylvania School of Dental Medicine, Henry Daniell and colleagues have been working to overcome these obstacles by using a plant-based system to make shelf-stable drugs. In a study published in the journal *Biomaterials*, the researchers confirmed the viability of their method for FDA approval and human use, producing an effective drug that promotes tolerance to clotting factors, which could be taken by hemophilia patients, using freeze-dried lettuce leaves.

This is the first time a group has shown the commercial viability of producing a low-cost drug made from whole plants.

"This is a milestone in our field, to make a fully functional drug in plants, produce it at a large scale and in quantities sufficient for human clinical trials," Daniell said.

Daniell, professor and interim chair in Penn Dental Medicine's Department of Biochemistry, is senior author on the study. Collaborators from the University of Florida led by Roland Herzog conducted animal studies and Fraunhofer USA's Steve Streatfield facilitated large-scale production of lettuce in the company's FDA-compliant facility.

The study builds on previous work by Daniell's group demonstrating an ability to use genetically modified plants to introduce a protein into the body that would teach the immune system to tolerate clotting factors that are given as a treatment for hemophilia.

Normally, 20 to 30 percent of people who get infusions of clotting factor develop antibodies against them that interfere with treatment. The earlier study, published in the journal *Blood*, successfully stopped and even reversed the production of these clotting factor inhibitors by feeding the plant-based drug to mice with hemophilia A.

That study used a tobacco plant platform to "grow" the drug. To take this approach into humans, however, Daniell's team knew they needed to use a different plant species.

They launched work with lettuce, which required using a completely different genetic vector to introduce the therapeutic gene into the plant cell's DNA, as the tobacco construct would not work in a different species. After identifying a compatible vector, they used a similar protocol to their previous work, bombarding lettuce leaves with a fusion of the therapeutic protein, coagulation factor IX, or FIX, with cholera toxin B subunit, which allows the protein to reach the immune system. They then evaluated the resulting plants for those that took it up and then grew those plants to maturity.

The next step was to ensure that the drug would be shelf stable. To do that, they freeze dried the plant material, ground it and analyzed the resulting fine powder for expression levels of the fusion protein to determine the appropriate dose and to evaluate its efficacy.

Similar to their previous experiments, Herzog's lab fed hemophilia B mice with a suspension of plant cell containing clotting factor IX twice a week for eight weeks and then gave them the same clotting factor that human hemophilia patients take to encourage blood clotting. As before, their product was a success: mice given the drug had greatly suppressed inhibitor formation compared to untreated animals, even when various doses of the drug were tested.

"One of the key findings of our study was that we found our drug was efficacious across at least a

10-fold dose range," Daniell said.

Such flexibility is important for translation of the drug to humans, as there may be individual variations in how a drug is metabolized in the gut as plant cells are broken down by commensal bacteria.

In the work, the researchers used two different growing systems. One was in the greenhouse on Penn's Pennovation Works campus, a high-tech facility that grows the plant in soil and uses natural light. The second was the Fraunhofer USA facility, which more closely replicates how a commercial pharmaceutical production facility would run, using a hydroponic system and artificial lighting.

"Despite the fact that plants in the greenhouse were receiving 50 times more light, the Fraunhofer yield was quite close to ours and quite good," Daniell said. "In 1,000 square feet, they could produce 36,000 doses."

A hydroponic system could also easily be scaled up by adding racks and thus using vertical space, which a traditional greenhouse could not do. The researchers were able to harvest a new batch of pharmaceutical-containing lettuce every four to six weeks.

With this study, which confirms the viability of a plant-based biopharmaceutical production on a commercial scale, the researchers have eliminated several expensive obstacles that hamper the development of affordable traditional protein drugs. The method requires no fermenter, no purification to ensure sterility and no cold chain to keep the drug refrigerated. In addition, the researchers found that their capsules remained potent and effective for two years, ensuring the product is shelf-stable and patients could theoretically take the drug from home.

"Not only did we show a truly translational result for helping hemophilia patients," Daniell said, "but this also changes the way we think about delivering protein-based drugs to human patients.

"Current treatments for inhibitor formation in hemophiliacs cost almost a million dollars and are not affordable for a significant segment of the patient population," he said, "but the new drug is dramatically cheaper and may offer even a better solution for treating hemophilia patients. Most important, developing a low cost platform for protein drug delivery will make these drugs affordable for a large majority of the global population."

More information: *Biomaterials*, dx.doi.org/10.1016/j.biomaterials.2015.08.004

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